THE GENETICS OF THE DOG

Edited by

A. Ruvinsky & J. Sampson

CABI *Publishing*

CABI *Publishing* is a division of CAB *International*

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Oxon OX10 8DE
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A catalogue record for this book is available from the British Library, London, UK.

Library of Congress Cataloging-in-Publication Data
The genetics of the dog / edited by A. Ruvinsky and J. Sampson.
p. cm.
Includes bibliographical references.
ISBN 0-85199-520-9 (alk. paper)
1. Dogs--Genetics. 2. Dogs--Breeding. I. Ruvinsky, Anatoly. II. Sampson, J. (Jeff)
SF427.2.G46 2001
636.7′08′21--dc21 00-054690

ISBN 0 85199 520 9

Typeset in Garamond by AMA DataSet Ltd
Printed and bound in the UK by Biddles Ltd, Guildford and King's Lynn

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Preface

According to the latest data, wolf domestication commenced more than 100,000 years ago and there were several independent cases of domestication. No other domesticated animal has had such a long history of close relationship with humans as the dog. It should also be noted that no other species has shown such an enormous range of phenotypic and genetic variation. Ancient, multiple domestication events certainly contributed to this phenomenon, but other factors such as intensive selection have led to a degree of variation unsurpassed in other species. Since Darwin, it has become apparent that the dog is the best species for studying domestication. Hopefully this book provides readers with comprehensive information about different aspects of domestication. Nevertheless, this fundamental problem still requires significant attention.

Tremendous progress in mammalian genetics, caused by both the genomic and biotechnological revolution, during the last decade has immensely accelerated dog genetics. This newly generated knowledge is very important from many points of view including breeding, selection, health, breeds differentiation and better understanding of the history of dog domestication. Just a few years ago the locations of only a few genes on dog chromosomes were known. At the time of publication of this book this number has reached several hundred mapped loci.

Previously separated, quantitative and molecular genetics are now taking a united approach toward identification of loci underlying important traits in domestic and laboratory animals. The dog is no exception and we shall witness progress in this field sooner rather than later.

The main purpose of this volume is to collect the available data concerning dog genetics and bring together previously separate areas of research. The book covers all major directions in dog genetics. The first five chapters discuss systematics and phylogeny of the dog, domestication and single gene traits. Chapters 6–12 present information about biochemical polymorphism, molecular genetics, immunogenetics and genetic aspects of disease, genome structure and gene mapping. The next section covers genetic

aspects of behaviour, reproduction and development. Finally, chapters 16–19 are devoted to genotype testing, pedigree analysis, quantitative genetics and the application of dog genetics in medical and forensic fields. Standard genetic nomenclature, a list of kennel clubs and some additional information are presented in the Appendix.

The authors of this book have made every attempt to highlight the most important publications in the area of dog genetics in recent decades with emphasis on the most up-to-date papers, reviews and books. However, we realize that omissions and errors are unavoidable and apologize for any possible mistakes. This book is addressed to a broad audience, which includes researchers, lecturers, students, dog breeders, veterinarians and all those who are interested in the dog's biology and genetics. *The Genetics of the Dog* is the fifth publication in the series on mammalian genetics published by CAB International. Four previous books, *The Genetics of Sheep* (1997), *The Genetics of the Pig* (1998), *The Genetics of Cattle* (1999) and *The Genetics of the Horse* (2000) are based on similar ideas and structure (http://ansc.une.edu.au/genpub/).

This book is a result of truly international cooperation. Scientists from several European countries, the USA and Australia contributed to this project. The editors are very grateful to all of them. It is our pleasure and debt to thank the people who helped in reviewing the book: M. Harvey, G. Montgomery, B. Tier, M. Willis, E. Bailey, M. Goddard, P. Thomas, M. Allen and K. Fowler.

It is our hope that the book will be useful for those who are interested in dog genetics. Possibly it will support consolidation and further progress in this field of science.

Anatoly Ruvinsky
Jeff Sampson
25 May 2001

Phylogeny and Origin of the Domestic Dog

1

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Introduction

The domestic dog (*Canis familiaris*) is the most phenotypically diverse mammal species known and ranges in size and conformation from the diminutive Chihuahua to the gargantuan Great Dane. The difference in size and conformation among dog breeds exceeds that among species in the dog family Canidae (Wayne, 1986a,b; see Chapter 3). Differences in behaviour and physiology are also considerable (Hart, 1995). An obvious question therefore is whether this diversity reflects a diverse ancestry. Darwin suggested that considering that great diversity of dogs, they were probably founded from more than one species (Darwin, 1871). This sentiment has been periodically revisited by researchers (e.g. Lorenz, 1954; Coppinger and Schneider, 1995). Knowledge of the evolutionary history of domestic dogs and of their relationships to wild canids provides insight into the mechanisms that have generated the extraordinary diversity of form and function in the dog. In this chapter, we discuss the evolutionary history of dogs and their relationship to other carnivores inferred from molecular genetic studies. Dogs belong to a unique

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and long distinct genetic lineage. Genetic data suggest that dogs were domesticated from wolves (*Canis lupus*) multiple times, beginning over 100,000 years ago.

Evolutionary Relationships of the Domestic Dog

The modern carnivore families originated over 40–50 million years ago (Flynn and Galiano, 1982). The domestic dog belongs to the family Canidae which, in turn, is classified within the superfamily Canoidea and order Carnivora. Therefore, seals, bears, weasels and raccoon-like carnivores are more closely related to canids than are cats, hyenas and mongooses (Fig. 1.1). The Canidae is the most phylogenetically ancient lineage within the superfamily Canoidea, diverging from other carnivores over 50 million years ago. The canine karyotype has little similarity to those in any other carnivore families (Wurster-Hill and Centerwall, 1982; Wayne *et al.*, 1987) suggesting that large chromosome blocks and linkage groups may not be conserved (but see O'Brien *et al.*, 1997; Lyons *et al.*, 1999). Because of the ancient divergence of canids from other carnivores, generalizations about gene structure and function from one carnivore family to another may be a questionable extrapolation (Wayne, 1993).

Three subfamilies of canids have been recognized. The subfamily Hesperocyoninae includes the oldest and most primitive members of the family (Wang, 1994). This Oligocene to Miocene Age subfamily includes small to medium sized predators and lasted for over 20 million years. In the Middle Miocene, the Hesperocyoninae were replaced by Borophaginae, large bone-crushing dogs, that are often the most common predators in late Tertiary deposits but were extinct by the mid-Pliocene, about 4 million years ago (Wang *et al.*, 1999). The third subfamily, Caninae, includes all living representatives of the family and first appears in the late Miocene.

Although canids belong to an ancient lineage, the 36 extant species (Table 1.1), are all very closely related and diverged only about 12–15 million years ago (Fig. 1.2). Based on mitochondrial DNA sequences, three distinct groups can be identified within the extant Canidae, including the red fox-like canids (e.g. red, kit and Arctic fox, among others), the South American foxes (e.g. grey and pampas foxes), and the wolf-like canids (the domestic dog, grey wolf, coyote, African hunting dog, dhole, Ethiopian wolf and jackals). Bush dog and maned wolf are two very divergent South American canids that cluster with wolf-like canids in some analyses (Fig. 1.2; Wayne *et al.*, 1997). The grey fox, raccoon dog and bat-eared fox represent long distinct lineages. Evolutionary relationships are also suggested by chromosome similarity. Chromosome number and structure vary widely among canid species, from 36 metacentric chromosomes in the red fox to 78 acrocentric chromosomes in wolves, coyotes and jackals (Fig. 1.2). However, the closely related wolf-like canids and South American canids all have high diploid numbers and acrocentric chromosomes (Fig. 1.2). Similarly, the closely related fox-like canids have low diploid numbers and metacentric chromosomes and share a common ancestry (Fig. 1.2).

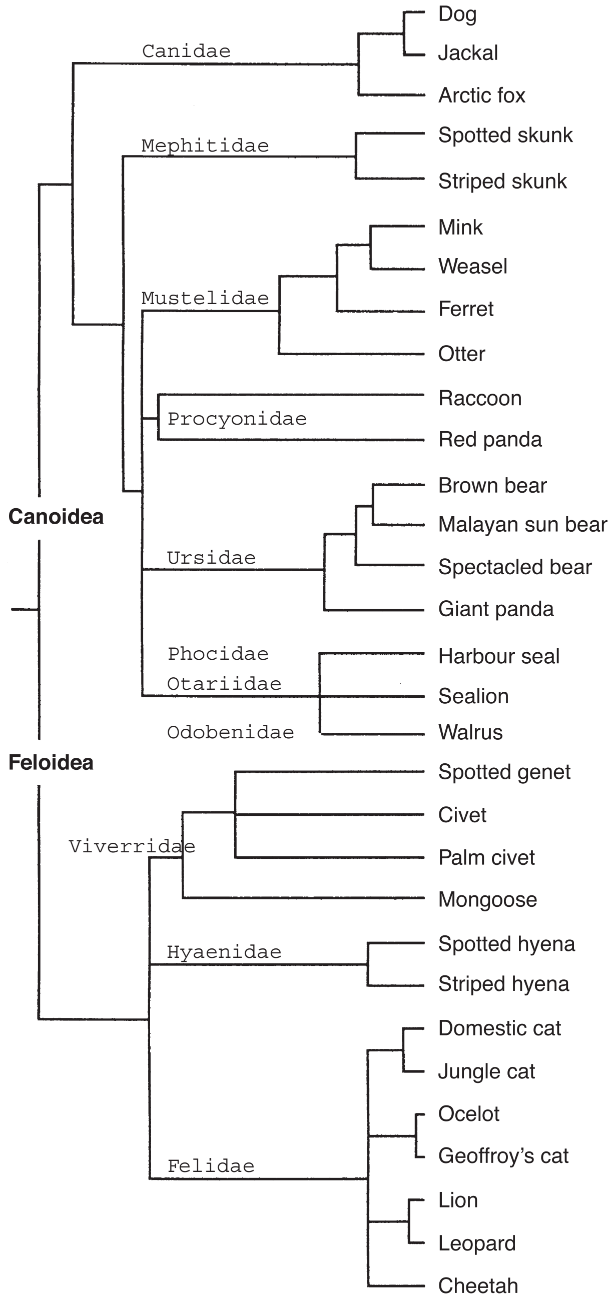

Fig. 1.1. Evolutionary tree of carnivores based on similarity in single copy DNA sequences as deduced by DNA hybridization (Wayne *et al*., 1989). Family and superfamily groupings are indicated.

Table 1.1. Extant species of the family Canidae based on Nowak (1999)

Taxon	Common name
Family Canidae	
Subfamily Caninae	
Genus: *Vulpes*	
V. bengalensis	Bengal fox
V. cana	Blanford's fox
V. chama	Cape fox
V. corsac	Corsac fox
V. ferrilata	Tibetan sand fox
V. macrotis	Kit fox
V. pallida	Pale fox
V. rueppellii	Sand fox
V. velox	Swift fox
V. vulpes	Red fox
Genus: *Fennecus*	
F. zerda	Fennec fox
Genus: *Alopex*	
A. lagopus	Arctic fox
Genus: *Urocyon*	
U. cinereoargenteus	Grey fox
U. littoralis	Island grey fox
Genus: *Lycalopex*	
L. vetulus	Hoary fox
Genus: *Pseudalopex*	
P. culpaeus	Culpeo fox
P. fulvipes	Darwin's fox
P. griseus	Argentine grey fox
P. gymnocercus	Pampas fox
P. sechurae	Sechuran fox
Genus: *Cerdocyon*	
C. thous	Crab-eating fox
Genus: *Nyctereutes*	
N. procyonoides	Raccoon dog
Genus: *Atelocynus*	
A. microtis	Small-eared dog
Genus: *Speothos*	
S. venaticus	Bush dog
Genus: *Canis*	
C. adustus	Side-striped jackal
C. aureus	Golden jackal
C. familiaris	Domestic dog
C. latrans	Coyote
C. lupus	Grey wolf
C. mesomelas	Black-backed jackal
C. rufus	Red wolf
C. simensis	Ethiopian wolf
Genus: *Chrysocyon*	
C. brachyurus	Maned wolf

Table 1.1. *Continued*

Taxon	Common name
Genus: *Otocyon*	
O. megalotis	Bat-eared fox
Genus: *Cuon*	
C. alpinus	Dhole
Genus: *Lycaon*	
L. pictus	African hunting dog

The raccoon dog appears to have the most primitive chromosome complement and may have some chromosome blocks that are homologous to those in cats (Wayne *et al.*, 1987). This high degree of variation contrasts with most other carnivore families in which chromosome number and structure are well conserved (Wurster-Hill and Centerwall, 1982).

Origin of the Domestic Dog

The origin of domesticated species is seldom well documented. The number, timing and geographic origin of founding events may be difficult to determine from the patchy archaeological record (Vilà *et al.*, 1997a,b). This problem is well exemplified by the domestic dog for which data are consistent with both single and multiple origins from the grey wolf alone or, additionally, the golden jackal, *Canis aureus* (Olsen, 1985; Clutton-Brock, 1995). However, the only criterion used to differentiate between dog and wolf remains from archaeological sites is skeletal morphology. Most modern dogs are morphologically differentiated from both wolves and jackals (Olsen, 1985). These differences were used to discriminate between species in archaeological sites, but consequently, only morphologically differentiated dogs could be distinguished and the initial stages of dog domestication, when the morphological differentiation was small, might have passed unnoticed. Even for the several hundred extant dog breeds that have been developed in the last few hundred years, the specific crosses that led to their establishment are often not known (Dennis-Bryan and Clutton-Brock, 1988). The genetic diversity of the founding population is essential knowledge for understanding the immense phenotypic diversity of dogs. A heterogeneous origin would suggest that gene diversity is critical to phenotypic evolution, whereas a limited founder population would imply that developmental variation is more important in breed diversity (e.g. Wayne, 1986a,b; see Chapter 3).

The ancestor of the dog

Molecular genetic data consistently support the origin of dogs from wolves. Dogs have allozyme alleles in common with wolves (Ferrell *et al.*, 1978;

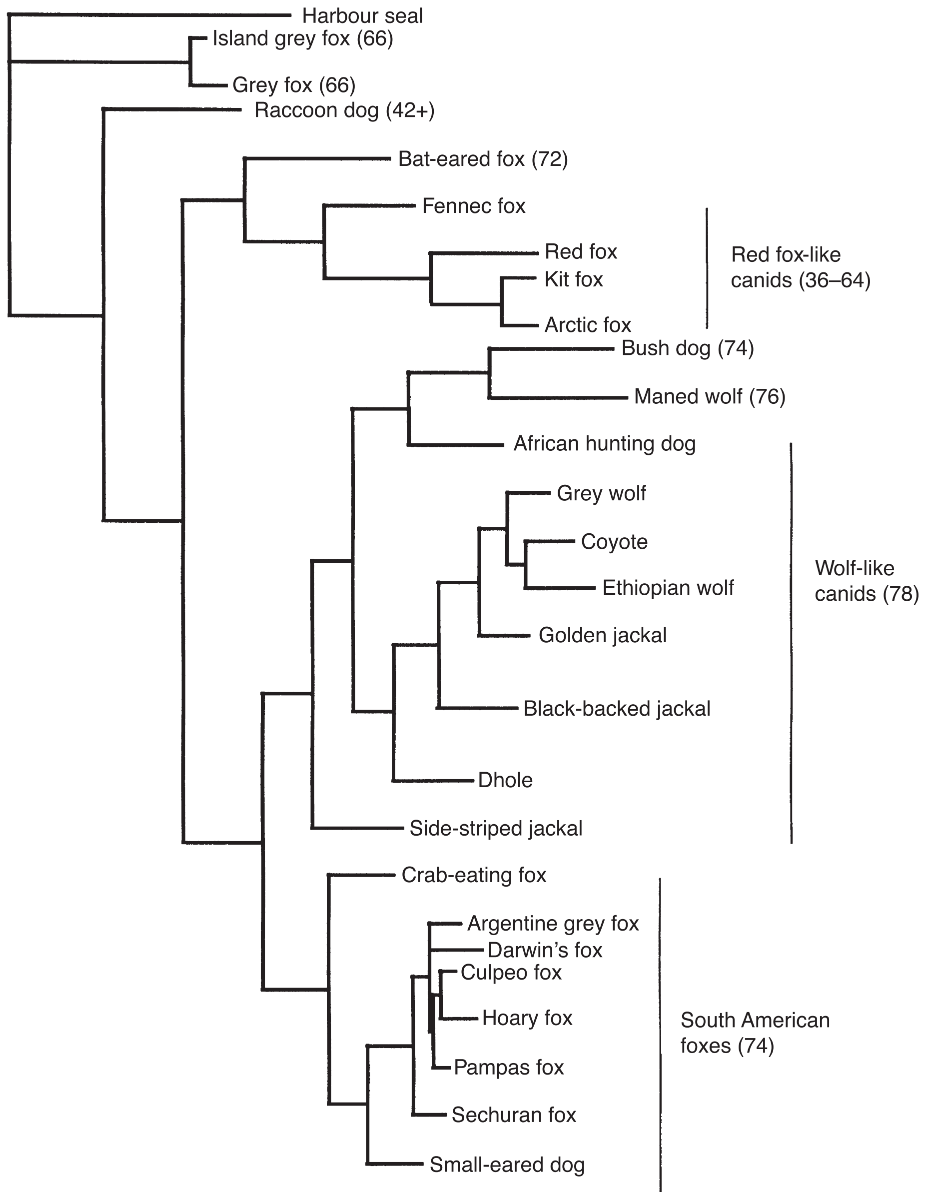

Fig. 1.2. Maximum parsimony tree of canids based on analysis of 2001 base pairs of protein coding mitochondrial DNA sequence (cytochrome *b*, cytochrome *c* oxidase I and cytochrome *c* oxidase II) from 27 canid species (Wayne *et al*., 1997). The harbour seal sequence is used as outgroup to root the tree. Diploid chromosome numbers are indicated in parentheses for species or groupings of canids (Wurster-Hill and Centerwall, 1982; Wayne *et al*., 1987).

Wayne and O'Brien, 1987), share highly polymorphic microsatellite alleles (García-Moreno *et al.*, 1996) and have mitochondrial DNA sequences similar or identical to those found in grey wolves (Wayne *et al.*, 1992; Gottelli *et al.*, 1994). An extensive survey of several hundred grey wolves and dogs found that the two species had only slightly divergent mitochondrial DNA control region sequences (Vilà *et al.*, 1997a). For example, the average divergence between dogs and wolves was about 2% compared with 7.5% between dogs and coyotes, their next closest kin. The average divergence between dogs and wolves is inside the range of genetic variability observed for wild wolves (Vilà *et al.*, 1997a, 1999a).

The domestication process

More controversial is the exact number of domestication events, their timing and location. The archaeological record suggests that the first domestic dogs were found in the Middle East about 12,000–14,000 years ago (Olsen, 1985; Clutton-Brock, 1995). However, very old remains are known also from North America and Europe (Nobis, 1979; Olsen, 1985; Pferd, 1987; Clutton-Brock, 1995; Schwartz, 1997) and morphological comparisons suggest that dogs are closest to Chinese wolves (Olsen and Olsen, 1977; Olsen, 1985). Moreover, the first appearance in the fossil record of domestic dogs, as indicated by their morphological divergence from wolves, may be misleading. Early dogs may have been morphologically similar to wolves for a considerable period of time (Vilà *et al.*, 1997a,b). Consequently, the appearance of distinct-looking dogs in the archaeological record may be due to a change in artificial selection associated with a cultural change in human societies (Vilà *et al.*, 1997a,b).

A genetic assessment of dog domestication based on mitochondrial control region sequence data finds four divergent sequence clades (Fig. 1.3). The most diverse of these clades contains sequences that differ by as much as 1% in DNA sequence (Fig. 1.3, clade I). Consequently, because wolves and coyotes diverged at least 1 million years ago and have control region sequences that are 7.5% different, dogs and grey wolves may have diverged 1/7.5 this value or about 135,000 years ago. These molecular results imply an ancient origin of domestic dogs from wolves. In fact, wolves and humans lived in the same habitats for as much as 500,000 years (Clutton-Brock, 1995) and domestication might not have been apparent until the nature of artificial selection and dog conformation changed with the shift from hunter–gatherer cultures to more agrarian societies about 12,000 years ago (Clutton-Brock, 1995). The role that dogs had in hunter–gatherer cultures was perhaps restricted more to protection and hunting, and dogs may have lived less closely with humans, resulting in more morphological similarity to their wild brethren.

At least four origination or interbreeding events are implied by the genetic results because dog sequences are found in four distinct groupings or clades, each with a unique ancestry to wolves (Fig. 1.3). In clade IV, a wolf sequence is identical to a dog sequence, suggesting a very recent interbreeding or

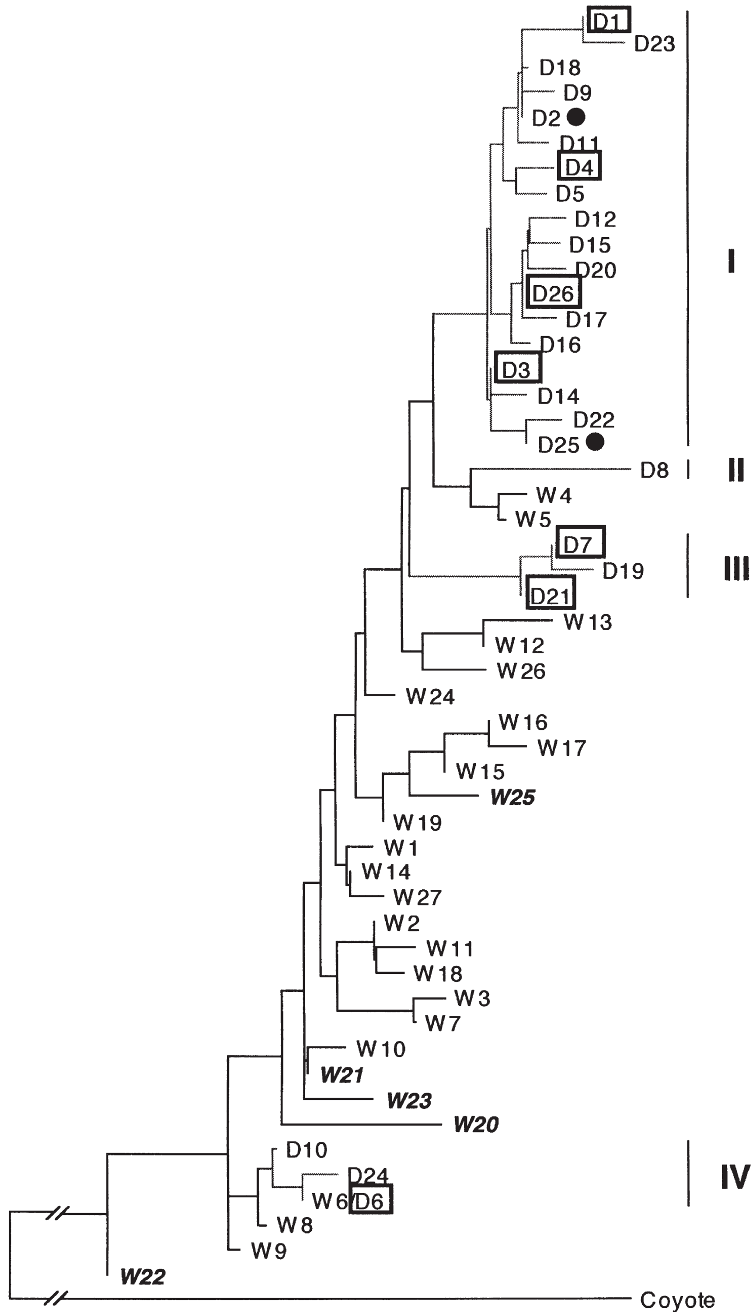

Fig. 1.3. Neighbour-joining relationship tree of wolf (W) and dog (D) mitochondrial DNA control region sequences (261 base pairs in length; Vilà *et al*., 1997a). Dog haplotypes are grouped in four clades, I to IV. Boxes indicate haplotypes found in the 19 Xoloitzcuintlis (Vilà *et al*., 1999b). Haplotypes found in two Chinese crested dogs, a presumed close relative of the xolo, are indicated with a black circle. Bold characters indicate haplotypes found in New World wolves (W20 to W25).

origination event (haplotypes W6 and D6). Interbreeding and origination events leave the same genetic signature, both transfer wolf mitochondrial DNA to the gene pool of dogs. Finally, the number of origination/interbreeding events is likely to be much more than that implied by the tree because of the maternal inheritance of mitochondrial DNA and the likelihood of stochastic loss of introgressed lineages (Vilà *et al.*, 1997a). The basic structure of the sequence tree has been independently confirmed (Okumura *et al.*, 1996; Tsuda *et al.*, 1997; Randi *et al.*, 2000).

Origin of breeds

Within breeds, the genetic diversity is high. Most breeds for which several individuals were sampled have at least 3–6 distinct sequences (Vilà *et al.*, 1997a). Because mitochondrial DNA is maternally inherited, this implies that multiple females were involved in the development of dog breeds (Vilà *et al.*, 1997a, 1999b). Few breeds have unique sequences and the relationship of sequences is not consistent with the genealogical relationships of breeds. The reason for this is that most breeds have originated too recently, within the past few hundred years, such that unique breed-defining control region mutations have not occurred. Ample genetic diversity within breeds is also supported by analysis of protein alleles (Simonsen, 1976; Ferrell *et al.*, 1978) and hypervariable microsatellite loci. Microsatellite loci have heterozygosity values ranging from 36% to 55% within breeds (Holmes *et al.*, 1993; Fredholm and Wintero, 1995; Pihkanen *et al.*, 1996; Zajc *et al.*, 1997; Morera *et al.*, 1999; Wilton *et al.*, 1999; Zajc and Sampson, 1999) whereas wild populations of wolves have an average value of 53% (Roy *et al.*, 1994; García-Moreno *et al.*, 1996). Consequently, the moderate to high genetic diversity of dog breeds indicates that they were derived from a diverse gene pool and generally are not severely inbred.

Ancient Dog Breeds

Molecular genetic studies suggest that the majority of breeds have moderate to high levels of genetic variability and the differentiation between them is mostly due to differences in the allelic frequencies (e.g. Pihkanen *et al.*, 1996; Vilà *et al.*, 1999b; Zajc and Sampson, 1999; but see Wilton *et al.*, 1999). These results reflect the recent origin of many breeds from a diverse founding stock and subsequent interbreeding among breeds. The small differentiation between breeds seems to be the result of their recent isolation in modern times. However, ancient breeds, such as the dingo and the New Guinea singing dog were developed when human populations and their domestic dogs were more isolated and founding populations were potentially more inbred. Dingoes and singing dogs were introduced into Australia and New Guinea by ancient travellers as early as 6000 years ago (Corbett, 1995), and this long period of isolation and small founding population size has translated into limited genetic

differentiation (Wilton *et al.*, 1999). The Romans were among the first to develop breeds of dogs that differed dramatically in conformation and size although some morphologically divergent dogs were depicted by the ancient Egyptians and in western Asia 4000 years ago (Clutton-Brock, 1999). Mastiffs and greyhounds were among these dogs; however, preliminary surveys fail to show lower diversity (Morera *et al.*, 1999; Zajc and Sampson, 1999). This suggests that these breeds might not have been isolated from each other since their origin. One control region clade (clade II, Fig. 1.3) was observed only in some Scandinavian dogs (Norwegian elkhound and jämthund; Vilà *et al.*, 1997a) and could represent a lineage independently domesticated from wolves and not extensively interbred with other dogs. In North America, the most ancient living breed is the Mexican hairless, or Xoloitzcuintli (Xolo) which is a hairless dog developed over 2000 years ago (Cordy-Collins, 1994). Because the Xolo is a pre-Columbian breed, the progenitors of the Xolo either migrated with the first Americans across the Bering land bridge over 10,000 years ago or were domesticated independently from North American wolves. A survey of 19 Xolos showed that they contained sequences identical or very similar to those found in Old World dog breeds rather than North American wolves (Vilà *et al.*, 1999b). Additionally, representatives of three of the four sequence clades were found in Xolos (Fig. 1.3), implying that the population of dogs that migrated with humans into the New World was large and diverse.

Wolf–Dog Hybridization

Wolves may still influence the genetic diversity of dogs. In the USA, there are thousands of wolf–dog hybrids of various proportions of wolf ancestry (García-Moreno *et al.*, 1996). Wolf–dog crosses are interbred with dogs and the progeny of hybrids and, by having a lower proportion of wolf genes, may be more docile. Consequently, wolf genes will diffuse into the dog gene pool. Gene flow may occur from dogs to wild wolf populations as well. In Italy, Israel and Spain, grey wolves interact and may interbreed with semi-feral populations of domestic dogs (Boitani, 1983). Wolf–dog hybridization can threaten the genetic integrity of wild wolf populations. Preliminary genetic analysis indicates that the frequency of wild hybrids is lower than previously thought (Vilà and Wayne, 1999). However, the most endangered living canid, the Ethiopian wolf, is clearly threatened by hybridization with domestic dogs (Gottelli *et al.*, 1994) and hybridization also occurs in eastern European wolves (Randi *et al.*, 2000).

Research Implications

Despite intense selection for phenotypic uniformity within breeds, the genetic diversity within dog breeds is similar to or slightly lower than that in wild grey wolf populations. Consequently, only breeds with a closely controlled history

of inbreeding should be considered genetically uniform. In most breeds, uniformity is likely only for genes affecting breed defining morphological, physiological or behavioural traits. In this regard, some breeds are useful genetic models for human inherited disorders (Wayne and Ostrander, 1999). Conversely, because of the high diversity within and among dog breeds, studies based on a limited sample of dogs will not adequately represent the variation among breeds. Efforts are currently being made to better characterize genetic differences between breeds (Pihkanen *et al.* 1996; Zajc *et al.*, 1997; Morera *et al.*, 1999; Wayne and Ostrander, 1999; Zajc and Sampson, 1999) Finally, because of the high degree of genetic similarity between dogs and wolf-like canids (Fig. 1.3), crosses between dogs and wild canids may not provide many new polymorphic loci for functional gene studies.

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2 Experimental Studies of Early Canid Domestication

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Introduction

A major evolutionary-genetic aspect of domestication has long been a debatable issue. The question was, how might the contemporary domestic dogs, so very diverse today, have evolved from a uniform wild-type ancestor? (Herre, 1959; Belyaev, 1969, 1979; Hemmer, 1990; Clutton-Brock, 1997; Coppinger and Schneider, 1997; Wayne and Ostrander, 1999). It is well known that certain dog breeds differ in body size and proportions much more than species, even genera. Putting it another way, domestication has given rise to drastic morphological and physiological changes in the dog at a rate exceeding genetic predictions. Accepting the classic notion of mutations as rare, small, chance alterations of individual genes, one casts serious doubt on the idea that the changes, which took place in the dog during a short span of time in evolutionary terms, were of a mutational nature. Even making allowance for saltatory events, leaps (Eldredge and Gould, 1972), it is, indeed, incomprehensible how all the mutations needed for the creation of the now existing diversity could be accommodated during the millennia that have elapsed since the time the earliest dog appeared (Coppinger and Schneider, 1997). It should be stipulated that mutations have been accumulating for

hundreds of millennia: an assessment of canid divergence based on the data for the highly polymorphic mitochondrial control region sequences suggests that the early dogs might have originated about 100,000, not just 10,000–15,000 years ago (Vilà *et al.*, 1997). Furthermore, there are data in the literature indicating that certain mutations, for example, those causing evolutionary changes in characteristics, which are under the pressure of sexual selection and which can eventually set up reproductive barriers, can possibly accumulate very rapidly (Civetta and Singh, 1999; Gavrilets, 2000). Certainly, new mutations have kept arising under conditions of domestication, too. However, circumstantial evidence indicates that their accumulation is not critical to morphological and physiological changes in dogs. In fact, an evolutionary consequence of dog domestication is the fundamental reorganization of the reproductive function, imperative for evolutionary survival. Dogs fulfil the primary biological task – to reproduce – differently from their wild counterparts. Dogs lost monoestricity and the seasonal breeding pattern, having acquired the capacity to breed any time of the year, biannually and more often. It appears that this change in the reproductive function, which is the integral result of the complex interaction of many neuroendocrine responses, might have occurred as a single mutation event. It is worth remembering that not only dogs, but also other domestic animals, have lost breeding seasonality. The parallelism of the morphological and physiological variability patterns is nowhere more conspicuous than in conditions of domestication. True, the species of domesticated animals are members of distant taxonomic groups (not only genera and families, even orders); however, variability in many of their characters is remarkably homologous. It appears unlikely that this variability was caused by homologous mutations in homologous genes in all the domesticates. There is more straightforward evidence that mutations did not accumulate rapidly in domestication conditions. Studies on the protein products of more than 50 loci have shown, for example, that dogs and wolves share alleles in common (Wayne and O'Brien, 1987).

The role of founder effects has been emphasized with reference to the evolutionary events occurring during domestication (Moray, 1994; Clutton-Brock, 1997; Coppinger and Schneider, 1997; Wayne and Ostrander, 1999). It has been suggested that there initially existed small founder groups, that they inbred and were repeatedly subjected to genetic drift. However, the diversity of the domestic dog is more often discussed in the light of neoteny as a major trend of changes in development brought about by domestication. It was frequently noted that many adult dogs are behaviourally and morphologically similar to wolf puppies. It has been even thought that characters arrested in a developmental stage may underlie the formation of breeds (Wayne, 1986; Coppinger *et al.*, 1987). In fact, it has been recognized that genetic variability in developmental patterns is the source of rapid and extensive changes at the organism level (Gould, 1982; Raff and Kaufman, 1983; McDonald, 1990; Pennisi, 1998). Because this variability is of importance, there must be a mechanism that safeguards it from the direct action of selection. For this reason, it is difficult to reconcile with the thought that retarded development of

the domestic dog is a consequence of selection for developmental rates. It has been suggested that neoteny, or retention of juvenile traits into adulthood, may be a sequel of direct selection for earlier sexual maturation (Clutton-Brock, 1997; Coppinger and Schneider, 1997; Wayne and Ostrander, 1999). However, the efficiency of this selection is very doubtful: all the reproductive traits, including sexual maturation timing, have minimum additive genetic variance (Bronson, 1988). Neoteny, however, might have arisen as a result of selection for traits that mark developmental rates. Such markers might have plausibly been infantile behavioural traits that have facilitated adaptation of animals to human company (Coppinger and Schneider, 1997). If this were the case, then, it must be conceded that delayed development of social behaviour is correlated with the developmental rates of other physiological and morphological characters. This means that the concession must be made that selection for traits of social behaviour is actually a case in point of selection for the regulatory mechanisms of temporal developmental parameters at the level of the whole organism.

The Russian geneticist-evolutionist, D. Belyaev, has pondered over the nature and origin of changes brought about by domestication and over the role of the regulatory developmental mechanisms in these changes (Belyaev, 1969, 1979). His vantage point for viewing evolutionary problems was out of the ordinary at that time. Belyaev believed that the rates of evolutionary transformations, in certain situations, depended not only on the force of selection pressure, but as much on its directionality or vector, i.e. on the intrinsic properties of the genetic systems on which the selection acts. When the key regulatory loci coordinating the entire process of development happened to be targeted by selection forces, selection perhaps became truly mutagenic. This might have created specific conditions at the organism level that gave rise to variability. The data in the literature supporting this idea have partly been reviewed in the Russian journal *Genetika* (Trut, 1993). The regulatory mechanisms were obviously subjected to the strongest selection when conditions became extremely challenging and demanded high tension of the general adaptive systems. The view was expressed that the genome, in such conditions, functions as a specific responsive system and evolves toward increasing genetic variability. The possible molecular mechanisms of this behaviour of the genome have also been discussed (Lenski and Mitler, 1993; Pennisi, 1998). Earliest domestication, when animals encountered a man-made environment for the first time, has been a drastic replacement of the surroundings. It was, indeed, a violent upheaval that produced a host of variations, such as the animal kingdom has never witnessed before. The historical start of domestication was blurred in retrospective. The significant fact remains that a new vector was brought into play – the combined action of natural and unconscious, artificial selection for particular behavioural traits, favouring the animals' ability to coexist with human beings, and to tolerate their settlements. Belyaev believed that the specificity of evolutionary events under these conditions was determined by selection of this kind. And the morphological and physiological transformations were primarily patterned by the genetic

changes taking place during behavioural reorganization. His unified view of the evolutionary past of the domestic dog needed experimental verification and support. This prompted him with the idea of reproducing a documental scenario of early domestication. The domestication experiment has been carried out at the Institute of Cytology and Genetics of the Siberian Department of the Russian Academy of Sciences for over 40 years. The species under domestication was the silver fox (*Vulpes vulpes*), a taxonomically close relative of the dog. The experiment recreated the evolutionary situation of strongest selection acting on behavioural traits conditioning success of adaptation to human beings.

The Domestic Fox in its Making During Selection

When the domestication experiment was started, the silver fox had been bred in fur farms for more than 50 years. It may be thought that the silver fox had overcome the barrier of natural selection during its alienation from nature and natural companions, caging and breeding in captivity. Nevertheless, the fox retained its standard phenotype, strict seasonality of biological functions and the relatively wild behavioural paradigm (Fig. 2.1). A genetically determined polymorphism for the expression of the aggressive and fear responses to humans was revealed in the farm-fox populations. There might have been, quite plausibly, such polymorphism for the type of defensive responses to humans in the initial natural populations of wolves. Some of the foxes manifested the responses particularly weakly. About 10% of the farm-bred foxes were such individuals (Fig. 2.2). The weak responders were selected to become the parental generation to start the experiment. The total number

Fig. 2.1. A strongly aggressive fox of the farm-bred population unselected for behaviour.

Fig. 2.2. This fox shows a weak aggressive response to attempts to touch it.

taken from fur farm populations to serve as the initial generation was 100 females and 30 males. The number of foxes of reproductive age was minimal (93) in the second generation, and maximal (600) throughout the twentieth to thirty-fifth generations. The selected foxes yielded more than 47,000 offspring that were tested for amenability to domestication (tameability). The capacity for domestication was tested at different times during development, from 2 weeks of age onwards. Pups interacted with humans for a scheduled time. The experimenter handed food to pups, and attempted to handle and fondle them. The behaviour of the tested pups was scored for parameters (Trut, 1999). The score for tameability, or amenability to domestication, was the major criterion for selecting animals. Selection was strict: only about 10% of females and not more than 3–5% of males were taken from a preceding generation to produce the next. The apparent effectiveness of selection, the selection process and everything relevant to the establishment of the experimental population have been dealt with elsewhere (Belyaev, 1979; Trut, 1980a, b, 1999). Selection was ongoing for more than 40 generations. Behaviour changed in the course of selection, illustrating its effectiveness. Most offspring of the selected population were assigned to the domestication elite. They behaved in many respects like domestic dogs. They did not flee from humans, they yearned for human companionship. When begging human condescension, they whined, wagged their tails and licked like dogs (Fig. 2.3). The early behaviour elites appeared at the sixth generation selected for tameness. Elite in this context means ‘impeccable’, tamed to the highest degree. Already 35% of offspring of the 20th generation selected for tameness were elites. At this time elite pups made up 70–80% of the experimental population. Many responded to their pet names. When competing for human attention, they growled and snarled at

each other (Fig. 2.4). When released from their cages for a while, they acted dog-like and submissively towards their mistress (Fig. 2.5). Thus, a unique population of silver foxes showing unusual, rather dog-like behaviour, was

Fig. 2.3. The dog-like behaviour of foxes is noteworthy. It is the result of breeding for tame behaviour.

Fig. 2.4. One fox driving another from its mistress and growling like a dog.

established through long-standing selection for tameability. This was one of the many effects of selection for domestication.

What could be the mechanisms of the domestication that made dogs and foxes feel more 'at home' in the new social surroundings near man? It is known that in dogs the sensitive period for this adaptation (or primary socialization) during postnatal development starts with the functional maturation of the sensory systems and locomotor activity providing awareness of the environment and response to it. The appearance of the fear response to unknown stimuli is thought to be a factor that does not arrest exploration of the environment and social adaptation, but rather complicates it (Scott, 1962; Serpell and Jagoe, 1997). It was found that selection of foxes for domestication accelerated full eye opening and the establishment of the early auditory response (Fig. 2.6). This selection concomitantly retarded the formation of the fear response during early postnatal development and, as a result, offspring of the domesticated population showed no attenuation of exploratory activity in an unfamiliar situation, as the offspring of the farm-bred population did (Fig. 2.7). In fox pups of the population unselected for behaviour, the fear response formed, on average, by 45 days of life. At this age, the parameters of exploratory activity decreased considerably. This did not occur even in tame

Fig. 2.5. When released from their cage, elite foxes follow their master/mistress faithfully.

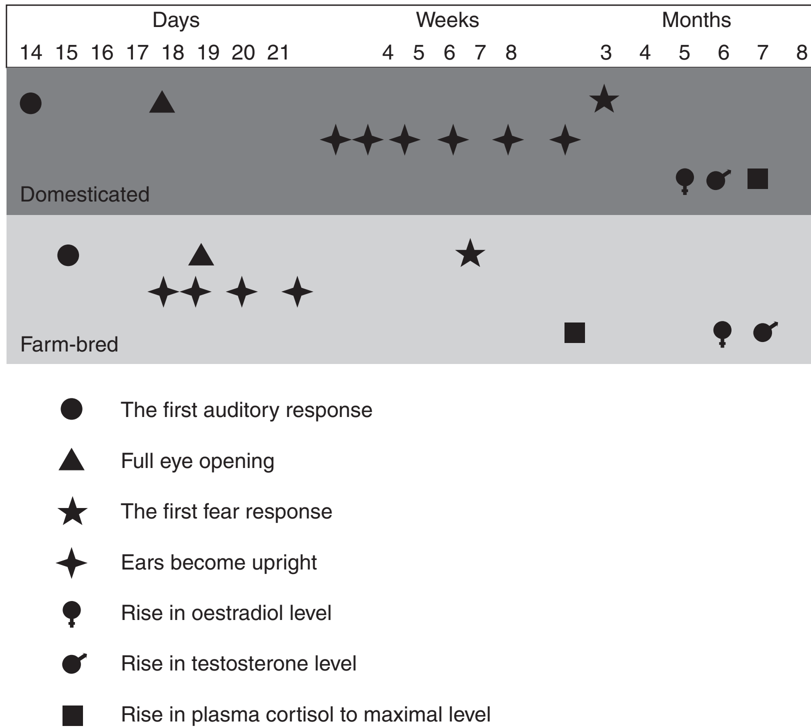

Fig. 2.6. Time appearance of certain characters during postnatal development.

pups aged 60 days because they did not exhibit the fear response at this age. These alterations in the rates of receptor-behavioural development prolonged the sensitive period of social adaptation and increased its efficiency (Belyaev *et al.*, 1984/1985). It is noteworthy that 45 days is not only when the sensitive socialization period ends, it is the age when glucocorticoids in the peripheral blood rise sharply in offspring of the farm-bred population (Fig. 2.7). In contrast, in offspring of the domesticated population not only the fear response was, as yet, not manifested and exploration not reduced, glucocorticoids also did not rise. Based on the above considerations, it may be inferred that selection for tame behaviour affected the genes for developmental rate and also that a function of genetic systems determining the activity of the pituitary–adrenal axis is involved in the regulation of the developmental rate. This inference will be examined below.

Phenotypic Novelties

As indicated in the Introduction, the view was generally held that the dog has been under domestication presumably from about 100,000 years ago (Vilà *et al.*, 1997). But phenotypic changes started to appear only 10,000–15,000 years ago. However, the authorative conclusion of domestication researchers

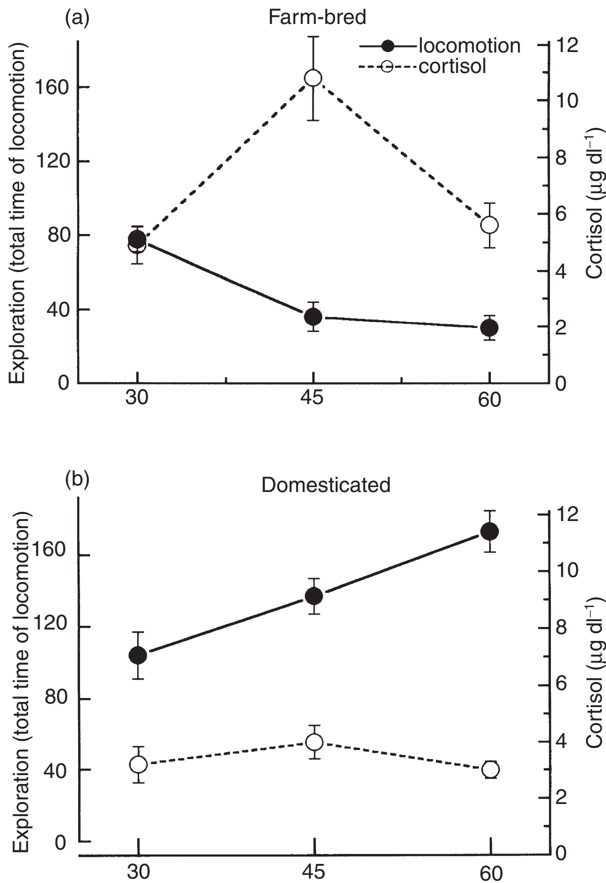

Fig. 2.7. Changes in exploratory activity (●) and plasma cortisol level (○) during the first 60 days of life in offspring of (a) farm-bred and (b) domesticated foxes.

(Herre, 1959; Zeuner, 1963) was that the primary increase in diversity was achieved very rapidly. Then a stasis followed and no changes occurred in the dog in the course of domestication history. The second step of increase in diversity came in more recent times with the development of breeding methods.

Morphological changes started to arise in foxes that had been subjected to selection for tameness for 8–10 generations. Many changes in characters were concordant with those not only of dogs, but also of other domestic animals (Figs 2.8–2.12). Changes in standard coat colour, to variegated coat colours, arose earliest, as in the dog (Hemmer, 1990). Seemingly distinct elements of animal biology, such as behaviour and pigmentation, altered in an integrated manner at the level of the organism. It is now known that the genetic systems of pigmentogenesis are, indeed, involved in neuro-endocrine physiology (Tsigas *et al.*, 1995; Barsh, 1996). Thus, there is evidence that the *E*-locus (*extension of black*) in mice encodes the receptor for the

Fig. 2.8. Specific loss of pigmentation determined by the homozygous state (*SS*) of the incompletely dominant autosomal *Star* (*S*) mutation. The *Star* is one of the earliest novelties.

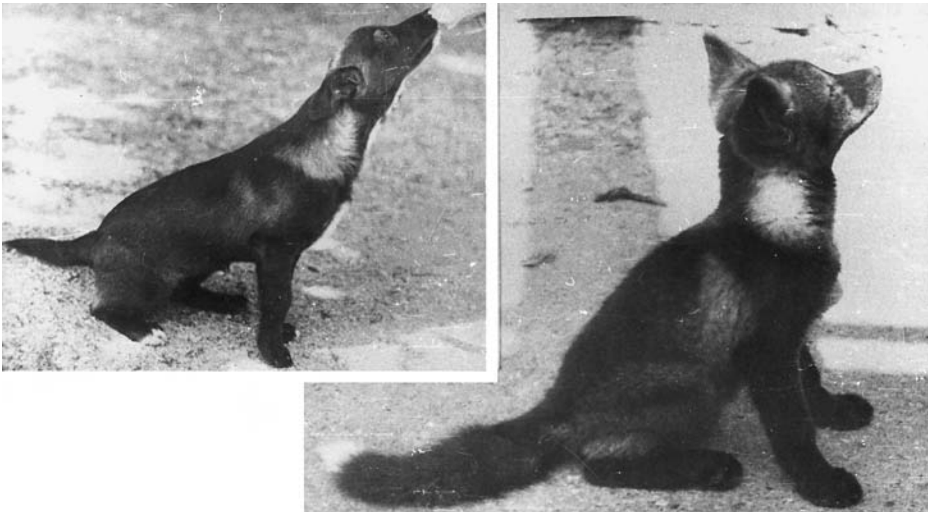

Fig. 2.9. Brown mottling (*bm*) is located on neck, shoulders, flank and hips. There is a phenotypic similarity between *bm* in foxes and the colour trait in dogs possibly caused by the allele of the *agouti* locus. The *bm* phenotype is determined by an autosomal recessive mutation.

melanocyte-stimulating hormone. There is reason to suggest that the *A*-locus (*agouti*) codes for its binding antagonist which in turn binds to the receptor (Jackson, 1993; Barsh, 1996). It is suggested that A-protein can act as an antagonist in other hormone–receptor interactions, for example, with ACTH

Fig. 2.10. Floppy ears. Ears remain floppy for the first months of life in some domestic foxes, rarer through life. This aberrant character does not show clear Mendelian segregation, although recurring in some lines.

(the adrenocorticotrophic hormone). It is also of interest that the melanocyte-stimulating hormone, which is involved in the regulation of melanin synthesis, has a receptor not only in the melanocytes. It has other kinds of receptors, one of which expresses exclusively in the brain tissues, at high concentrations in the hippocampus and the hypothalamus (Tsigas *et al.*, 1995), i.e. in the structures regulating exploratory and emotional behaviour. With this in mind, it is not at all surprising that selection for behaviour gave rise to primarily correlated changes in coat colour.

Aberrants with the *Star* white marking and curly tail were born at an impressively high frequency of 10^{-1}–10^{-2}. Short-tailed pups and those with floppy ears appeared at a frequency of a magnitude lower (10^{-3}). Some phenotypic changes, such as curly tail and piebaldness, started to arise in the

Fig. 2.11. Short tail. The number of tail vertebrae is normally 14 in foxes; their number is reduced to 8–9 in aberrant foxes. Its inheritance pattern is not clear.

Fig. 2.12. Tail carriage: tail rolled in a circle or a semicircle. Curly tail is the most frequently arising aberration. It does not show Mendelian segregation. The genetic basis of the character is, probably, different in different lines of domesticated foxes.

farm-bred fox populations some years later. It should be noted that, in farm populations bred under human control for about 100 years, both natural and artificial selection for domestication proceeded hand in hand. Surely, the intensity of this selection was not commensurate with that the experimental fox population was subjected to, nor were the occurrence frequencies of aberrants in the two fox populations similar.

What did the increased frequencies of phenotypic novelties in the domesticated population reflect? The answers may provide important clues. The increased frequency may be a consequence of stochastic processes and inbreeding whose roles have been highlighted in discussions of early canid domestication (Moray, 1994; Clutton-Brock, 1997; Wayne and Ostrander, 1999). In estimation of the role of inbreeding in the reorganizations brought about by domestication in foxes, it should be emphasized that most, if not all, the domesticated fox population from the start of its establishment was raised in an outbreeding regime. Moreover, efficient population size did not reduce to less than 93 individuals in the second selected generation, and it considerably increased in the successive generations. At this size of the reproductive part of the population, the occurrence probability of aberrant phenotypes due to homozygotization of recessives of the same origin appeared to be low (Falconer, 1981). The values of population inbreeding coefficients did not exceed the range 0.02–0.07. However, several fox lines were deliberately maintained in a regime of remote inbreeding. Homozygotization level in the representatives of these lines rose to 40–60% (Trut, 1980a). An important factor was that the occurrence frequencies of phenotypic changes in the offspring of these foxes did not exceed those in the offspring of the outbred foxes. It should be also noted that certain novel phenotypes (the *Star* white marking, for example) are determined by incompletely dominant mutations and the heterozygous phenotype is reliably marked (Belyaev *et al.*, 1981). In other words, there are grounds for believing that the emergence of phenotypic novelties was unrelated to inbreeding and stochastic processes in the domesticated fox population. In that case, may the changes that have arisen be regarded as classic correlated consequences of selection for just any quantitative character? In fact, it is known that strong selection pressure acting on a quantitative character, especially on one of adaptive significance, makes genetic systems less integrated (Falconer, 1981). The harmonious genetic system created by stabilizing selection is set out of balance: any increase in the value of the selected character is achieved at the expense of a breakdown of genetic homeostasis – the stability fixed by evolution. For this reason, selection for quantitative characters inevitably leads to the appearance of deviants from the stabilized phenotypical norm. However, such classic correlated responses to selection depend, as a rule, on the genetic pool of the starting population, and this renders their prediction and reproduction difficult. Each selection experiment is unique and none can be replicated in terms of the attendant correlated responses. As to the morphological and physiological consequences of domestication, their reproducibility is amazing. To illustrate, dogs and many animals have been repeatedly domesticated at different times and sites throughout their history. And each domestication event recurred and so did the same domestication changes. The changes in foxes in the experimental population are mirror reflections of the morphological novelties arising in other animals under domestication. Taking the remarkable concordance of evolutionary transformations into account, it is hard to regard the changes as trivial cases of correlated responses to selection for just any character.

Possibly, the specificities of the emergence of morphological and physiological novelties in domesticated foxes may shed light on the nature of the changes. Changes in various, more often unrelated, fox families arose, but these families belonged, as a rule, to the domestication elite. Various aberrant animals were recorded in the same litter of standard tame parents or parents showing a certain morphological change occasionally had offspring exhibiting quite different characters. These specificities are difficult to interpret, while conceding that each morphological novelty resulted from a single specific mutational change. The classic breeding studies also indicated that many of the morphological novelties observed in domestic foxes were not due to segregation in a simple Mendelian fashion. However, some morphological novelties were determined by single mutational events, such as the *Star* mutation, for example. However, this mutation showed peculiar behavioural features, suggesting that the phenomenon of genetic activation–inactivation was possibly behind its emergence and inheritance (Belyaev *et al.*, 1981). In the current literature, many cases of gene silencing, including the coat colour genes, have been adduced. Silencing was thought to have possibly resulted from the passage of a modified DNA methylation pattern through meiosis (Henkoff and Matzke 1997; John and Surani, 1999; Morgan *et al.*, 1999).

The phenomenon of inherited changes in genetic material activity might also have been involved in the morphological and physiological reorganizations in the dog. Hall (1984) has described a pertinent case in his review. As is known, the number of fingers on the foreleg typical for mammals is five; it is four for the hindleg in all representatives of the Canidae. The fifth hindleg finger has been lost some 10–15 million years ago. However, a result of wolf domestication was that the fifth finger, once missing, is now well developed in certain extant dog breeds. This is strongly suggestive that the phenotypic changes in dogs, which have arisen in the course of domestication, might have been due not only to specific mutational changes, but also (and to a greater extent) to changes in regulatory embryonic interactions and gene activity. The pattern of the genetic determination of phenotypic changes at the contemporary step of dog evolution may not reflect the genetic nature of their origination.

Craniological Changes

Changes in craniological dimensions shaping the skull may be assigned to a particular group of changes arising in foxes during domestication. During the past 20 years, developments in DNA-based research have contributed much to studies on variability. However, the traditional stronghold of craniological features in studies on evolution remains irrefutable (Hanken and Hall, 1993). In some foxes, the shape and size of the skull sharply deviates from normal (Fig. 2.13). In others, the upper jaw shortened and tooth bite became abnormal (Fig. 2.14). Comparative analysis of the farm-bred and domesticated populations revealed that changes in craniological dimensions were most

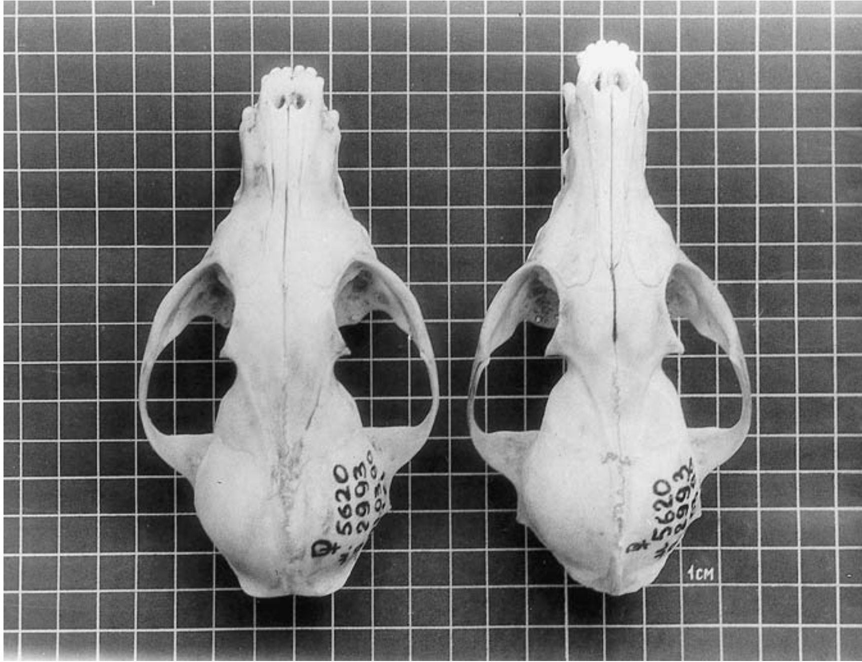

Fig. 2.13. The skulls of 8-month-old female foxes: normal (right), foxes with abnormally shortened and widened skulls (left).

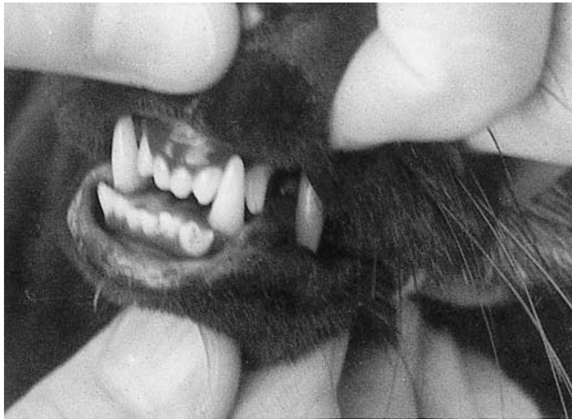

Fig. 2.14. The abnormal toothbite (underbite) in a fox with a shortened upper jaw.

prominent in males. The changes were associated with shortening and widening of the face skull and a decrease in the width and height of the cerebral skull. Moreover, tame males became smaller in almost all the cranial proportions and, as a result, the sexual dimorphism existing in the farm-bred population decreased in the domesticated foxes (Trut *et al.*, 1991). Similar changes in the sexual dimorphism pattern, judging by cranial measurements, were revealed also in farm-bred minks when compared with the wild mink

populations (Lynch and Hayden, 1995). Two mechanisms producing this effect have been implicated: abolition of sexual selection in farm conditions and enhancement of selection for increased total body size on males and females. These mechanisms can hardly account for the decrease in sexual dimorphism for craniological dimensions in the domesticated fox population. As to sexual selection, its effect was also abolished in the population unselected for behaviour (the control) with which the experimental population was compared. And selection for increased total body size was abolished precisely in the domesticated, not in the control, fox populations.

It is noteworthy that during early domestication the facial area of the skull became shorter and wider in dogs, like in foxes (Clutton-Brock, 1997; Wayne and Ostrander, 1999). Surely, evolutionary changes in craniological traits, as of any others, should be discussed in a genetic context. However, it is extremely difficult to relate the specific craniological to the specific genetic changes. To begin with, too little is known about the genetics of the shape and size of the skull. There are the traditional estimates of the heritability of certain dimensions in rats and mice (Atchley *et al.*, 1981). The quantitative genetics of the mandible has been studied in mice (Atchley, 1993), and the effects of certain pigmentation genes on the skull shape were identified in the American mink (Lynch and Hayden, 1995). Secondly, questions arising when studying craniological variability concern the developmental mechanisms even more (Atchley and Hall, 1991; Hanken and Hall, 1993). Thus, it is known that one of the sources of changes in the size and shape of the skull is alteration in the allometric interactions between growth rates. This proved that some changes in the craniological characters of foxes are explicable by precisely these alterations (Trut *et al.*, 1991). The genes controlling allometric interactions determine either the time when a structure appears, or its growth rate. Allometry changes during development. It seems its genetic determination also changes at different stages. A crucial role was assigned to developmental rate changes in surveys of the morphological evolution of the dog. In turn, the important role of selection for decreased body size and reproductive timing was recognized in discussions of the nature of changes in allometry (Moray, 1994; Clutton-Brock, 1997; Wayne and Ostrander, 1999). Possibly, the wolf was selected naturally or artificially for smaller body size during early domestication. But the sole selective criterion for modelling the domestication of foxes was behaviour. Total body size was an irrelevant character. Body size of domesticated foxes was compared with that of farm-bred foxes only at certain steps of selection (the F_{15-17} and the F_{25-26}). The comparisons revealed no correlated decrease in body size and, more than that, total body length tended to increase in tame males. It is precisely in these males that the decrease in craniological proportions and changes in the face skull were most expressed. As for the effect of direct selection for reproductive timing on the emergence of the described skull changes, its efficacy appeared very doubtful, as noted above. However, changes in these characters occurred in foxes as correlated responses to selection for behaviour (Logvinenko *et al.*, 1978, 1979). There is ample reason to believe that changes in the allometric interactions are

the correlated consequences of selection vectorized for domestication. This is evidence that profound genetic changes have occurred in the developmental regulatory processes under this selection.

Reorganization of the Seasonal Reproduction Pattern

It should be re-emphasized that a major evolutionary consequence of domestication was a fundamental reorganization of the vital function of reproduction. Dogs lost the reproduction seasonality pattern, and they became able to reproduce in any season and more than once a year. It is of importance that, in the domesticated fox population, the functional activity of the reproductive system was recorded both in females and males at times beyond the fox breeding season stabilized by natural selection (Belyaev and Trut, 1983). The mating season in foxes normally lasts from the beginning of January to the end of March. Males are in a state of sexual activity during all of this period. Mating entirely depends on when the females are in oestrus. Variability in the mating time during the seasonal time interval is determined mainly by environmental factors, and direct selection for this trait is ineffective. It is very important that some vixens showed oestral activity both in the autumn and spring, i.e. biannual oestricity tended to form. However, fertile extra-seasonal matings were extremely rare (Fig. 2.15). Pedigree analysis indicated that there indeed occurred an inherited reorganization of the seasonal rhythm of breeding: 300 females in which extra-seasonal sexual activity was recorded in the course of the experiment belonged to 20 unrelated families, i.e. extra-seasonal breeding arose in 20 female founders. Two of these, referred to the domestication elite, transmitted this ability to the numerous offspring of different generations. Seventy females showing extra-seasonal mating activity derived from one of the female founders and 49 came from the other. It is a matter of some importance that analysis of the degree of domestication in foxes tending to lose the strict seasonality of reproduction, and of their ancestors, indicated that this tendency can be regarded as a correlated response to selection for amenability to domestication.

Selection and Developmental Rates

Thus there are strong grounds for believing that the morphological and physiological reorganizations in silver foxes are the mirror images of the historical pathway of the domestic dog. As already noted, discussions on the nature of the reorganizations brought about by dog domestication centred on the developmental processes and their rates. Neoteny, the retention of the juvenile traits of morphology and behaviour into adulthood, is widely accepted as a mechanism by which the dogs became diversified. Neoteny as an evolutionary trend is an appealing notion. Thus, it has been postulated that certain breed-specific locomotor and behavioural features are actually retarded

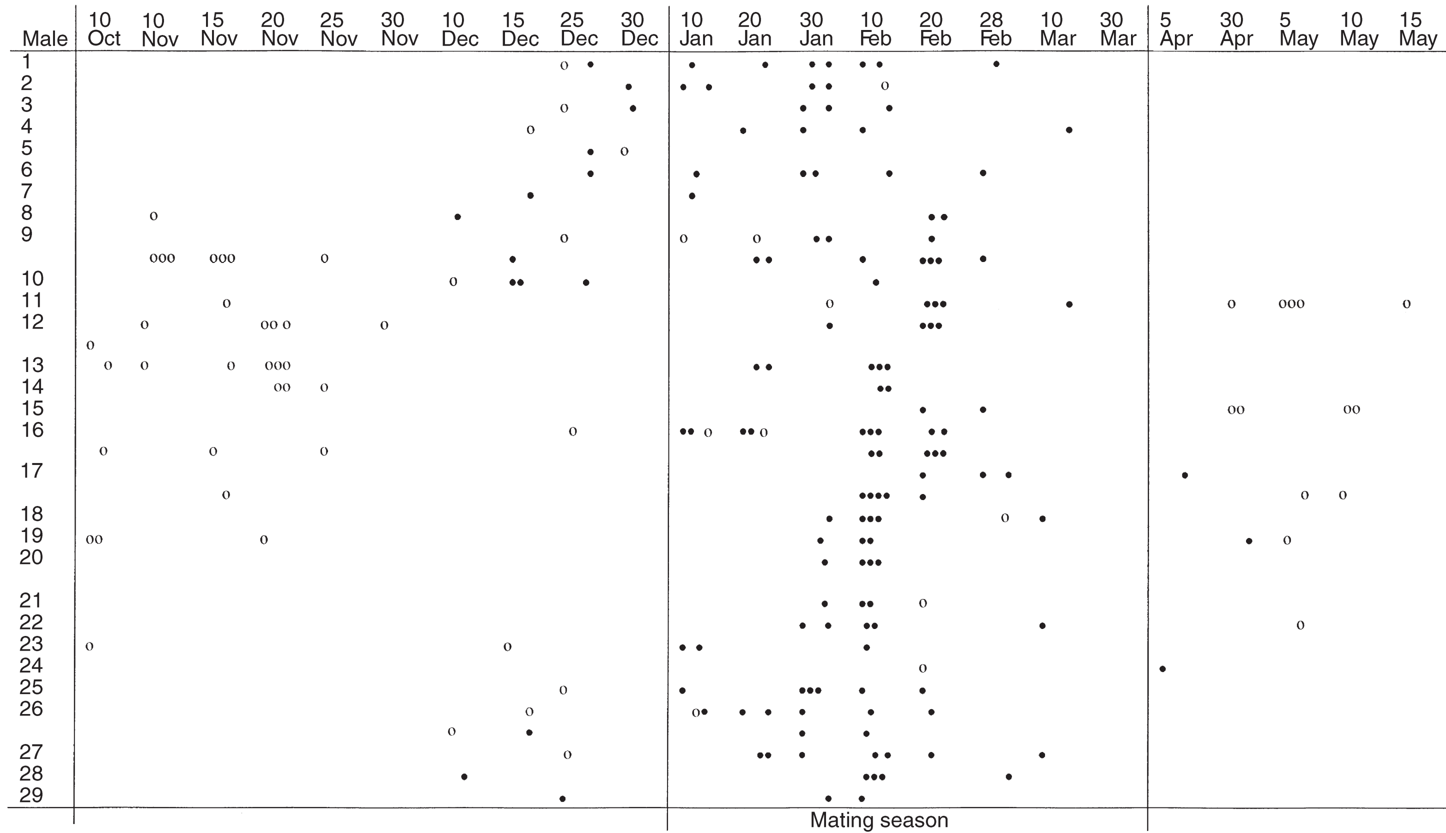

Fig. 2.15. The time course for matings in the tame foxes that showed extra-seasonal sexual activity. The circles next to the order number of males indicate their matings in years when they mated out of season. Designations: white circle – sterile matings; black circles – fertile matings.

juvenile responses (Coppinger *et al.*, 1987). Wayne (Wayne, 1986; Wayne and Ostrander, 1999) extended the idea to craniological characters. Furthermore, there is reason to suppose that some of the differences in the area distributions and amount of brain neurotransmitters (dopamine, for example) between breeds also reflect developmental differences in the neurotransmitter pattern (Arons and Shoemaker, 1992). To reiterate the crucial question posed in the Introduction: What genetic changes made domestic animals similar to the juvenile forms of their ancestors? Or in other words, what evolutionary processes have led to neoteny? Possibly the results obtained in the course of fox domestication may shed light on the primary cause of changes in developmental rates. The important fact was that changes in the rates of the corresponding processes underlied the emergence of many new characters in the domesticated foxes (Fig. 2.6). Thus, it has been suggested that reorganization from the relatively wild to more docile behaviour during selection was effected through changes in maturation timing that set the boundaries of the sensitive period of socialization. As a result, the duration of the period in foxes became prolonged and approached the one in dogs (Scott, 1962; Belyaev *et al.*, 1984/85). The developmental rates of certain morphological traits changed, too. A typical dog-like characteristic, such as floppy ears, is nothing else than a retained infantile feature. Ears are floppy during the early postnatal period in all fox pups. They become upright at the age of 2–3 weeks in offspring of the farm-bred population and at 3–4 weeks in that of the domesticated population. However, in some pups, ears were still not erect by the first 3–4 months of life and floppiness was lifelong in exceptional cases. Even certain changes in coat colour were due to shifts in developmental rates. As noted, the *Star* white marking was one of the earliest correlated responses to selection. It was found that the *Star* mutation causes piebaldness, affecting the developmental rate of the primary melanoblasts, the embryonic precursors of the melanocytes. The mutation delays their migration as they travel from the neural crest (the embryonic structure from which the melanoblasts derive) and their proliferation. The earliest melanoblasts normally appear in the epidermis of fox embryos on day 28 of development, while they appear on day 30 in carriers of the *Star* mutation (Prasolova and Trut, 1993). The melanoblasts arrive too late to the potentially unpigmented areas so that they cannot enter the hair follicle at the appropriate time. For this reason, there are no melanocytes in the areas devoid of pigment. The described changes in craniological dimensions in the domesticated foxes are also determined by shifts in the temporal parameters of development. Analysis of the pattern of intracranial allometry demonstrated that selection for behaviour shifts the time of the appearance of the cranial structures and their growth rates (Trut *et al.*, 1991).

The changes in the establishment rates of hormonal status in the experimental foxes appeared to be of importance. Thus, the pattern of embryonic and early postnatal establishment of the functional parameters of the pituitary–adrenal axis was altered in the tame foxes (Plyusnina *et al.*, 1991; Oskina, 1996). As is now well known, hormones have multiple tissue and function targets. A particular hormone at the right time and in the appropriate

concentration are the conditions necessary for their development and function. If all the conditions, or just one, are not met, a particular developmental process and its rate can become destabilized.

Effect of Selection on the Hormonal and Neurotransmitter Systems

Clearly, the rates of certain processes were shifted both during postnatal and embryonic development in the tame foxes. The question was, what genetic systems relevant to the regulation of development rate may possibly be affected by selection for behaviour? The set of genes controlling the functional state of the pitiutary–adrenal axis was probably implicated in the first place. An earliest correlated response to selection for tameness was attenuated activity of the axis. The common pool of circulating glucocorticoids, their *in vitro* production, the basal level of the adrenocorticotrophic hormone (ACTH), and the adrenal response to stress were all reduced in foxes subjected to domestication (Fig. 2.16) (Oskina, 1996). As a consequence of these changes, the entire development of the embryos of domesticated foxes enfolded at a decreased level of maternal glucocorticoids (Fig. 2.17). Such correlated changes in the activity of the pituitary–adrenal axis occurred not only in foxes. In fact, selection of rats for high- and low-anxiety related behaviour was also associated with changes in the functional state of the hypothalamic–pituitary–adrenal axis (Liebsch *et al.*, 1998; Neumann *et al.*, 1998; Ramos and Mormede, 1998). The role of these hormones in development is hard to overestimate. It suffices to note that the genes for the glucocorticoid receptors were found in the tissues of all the three germ layers (Kitraki *et al.*, 1997). Of particular concern presumably is that glucocorticoids have the functions of coordinators of temporal parameters of development (Bares *et al.*, 1994). In *in vitro* conditions, glucocorticoids control the time of cell proliferation and induce differentiation in the cells. The level of these hormones and the time of their appearance in *in vivo* conditions may be involved in the regulation of the duration of developmental processes.

The neurotransmitter genetic systems are primarily under the pressure of selection for behaviour. The activities of the serotonin, noradrenaline and dopamine transmitter systems in the specific brain regions, implicated in the regulation of the selected emotional defensive responses, were also altered in the tame foxes (Popova *et al.*, 1991, 1997; Trut *et al.*, 2000). This is most consequential because neurotransmitters perform the functions of morphogenes (Buznikov, 1987; Lauder, 1988; Lesch and Mossner, 1998). Not just early embryos, the reproductive cells contain the complete 'assembly' of the transmitter systems (the transmitters, the enzymes providing their synthesis, equivalents of their receptors). Each neurotransmitter has its own time course of changes and functions that alter during the stages of embryonic development. The serotonin transmitter system is thought to be the key in the inhibition of aggressive responses and its activity rose considerably during

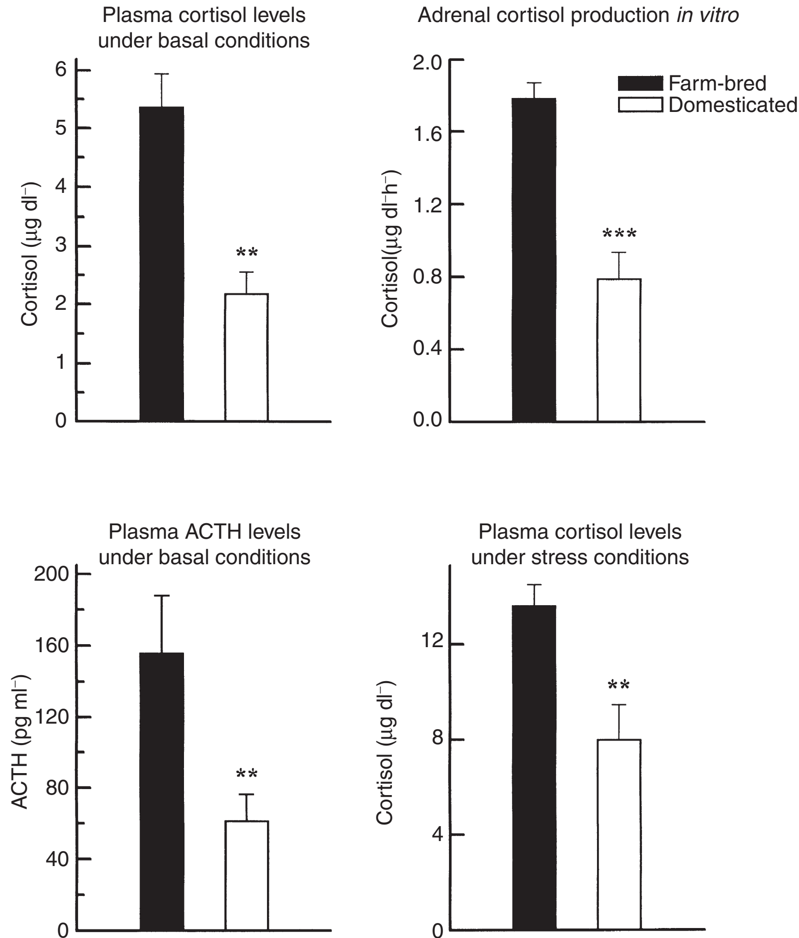

Fig. 2.16. Parameters of the functional activity of the pituitary–adrenal axis in farm-bred (■) and domesticated (□) foxes.

selection for domestication (Popova *et al.*, 1991, 1997; Trut *et al.*, 2000). This system is involved in triggering and regulating cell division, as well as in the migratory activity of embryonic cell material (Buznikov, 1987; Lesch and Mossner, 1998). Later during development, serotonin may cause deviations, for example, in the shape of the face and cerebral skull (Lauder, 1988). It is remarkable that selection of foxes for domestication not only enhanced the functioning of the serotonin system, but also, as described above, produced changes in the skull structures. Thus, it has been compellingly demonstrated that the neurohormonal and neurotransmitter systems are certainly involved in the regulation of development from its earliest onset, particularly in the regulation of its rate. Clearly, changes in the activities of these systems produce a destabilization of the temporal parameters of development. This is true for the experimental domesticated population of silver foxes.

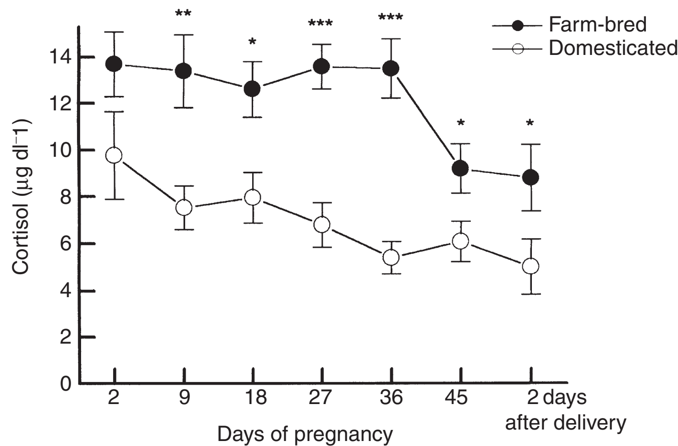

Fig. 2.17. Plasma cortisol level in farm-bred (●) and domesticated (○) vixens during pregnancy.

Implications for the Evolution of the Domestic Dog

It will probably never be guessed what course the evolving dogs might have followed. One can only come closer to a better understanding of the pathways and factors guiding evolving dogs by using approximations. It is hoped that the domestication experiments with foxes will shed some light on a long-disputed issue. Surely, the conditions of experimental re-creation of domestication in our days do not, even in rough outline, truly bring out the start of the ancient process. The task of the grand-scale experiment was to reproduce the major, as initially suggested, factor in the first steps of domestication – the strongest selection pressure on behaviour. All animals from the very start of domestication were challenged by the same evolutionary situation of the pressure of selection – primarily natural – on the specific behavioural traits favouring adaptation to the novel social factor – human beings.

What are the lessons of the long-term selection of foxes for tameness which are helping us to make clearer judgements about the evolutionary genetic mechanisms of dog domestication? In the light of the results, Hemmer's view (Hemmer, 1990) on the reorganization of dog behaviour becomes hardly tenable. He believed that selection for decreased sensitivity of the receptor systems, which started to act at the earliest stages of domestication, reorganized dog behaviour. As a result of the 'underreception', exploratory behaviour, stress responsiveness and the fear response all attenuated, and docility formed. On the other hand, foxes selected for domestication were characterized by an earlier establishment of the first auditory response, earlier opening of the eyes, and a higher level of exploratory behaviour. Later development of the fear response and, owing to this, lengthening of the sensitive period of socialization, were the mechanisms of adaptation to human and tame behaviour. To put it in another way, reorganization of behaviour towards domesticity

affected primarily the genes determining the rate of receptor-behavioural development, not the reception level. It was a different matter when morphological mutations exerting a pleiotropic effect on the reception level arose, but these were not the particular mutations that determined the formation of domestic behaviour and the success of social adaptation to a man-made environment.

The experiment with fox domestication demonstrated that, under conditions of strong selection pressure on the behavioural genetic systems, there occurred an increase, in the shortest time span (at the 8th–10th generations), in morphological and physiological changes. This disagrees with the view that the dog remained unaltered for a long time. This view was expressed when examining the possibility of reconsidering the timing of dog domestication (Vilà *et al.*, 1997). The data on fox domestication are consistent with the classic view that the first increase in diversity occurred explosively from the earliest step in the course of historical domestication of the dog (Herre, 1959; Zeuner, 1963). Our experimental data suggest that the accumulation of new chance mutations and their homozygotization due to inbreeding did not play a major role. Most probably, the phenotypic changes which have arisen in the course of domestication were caused by changes in a few genes. However, these genes have system effects. Their function (mission) was to integrate entire development as a whole and, for this reason, they occupied the highest level in the hierarchical structure of genome expression regulation. Even small genetic changes at this high level of regulators could produce a cascade of changes in gene activity and, as a consequence, rapid and extensive changes in the phenotype. Many changes in fox phenotype, under conditions of their experimental domestication, had resulted from shifts of the rates at which the relevant developmental processes proceeded. Developmental shifts in tame foxes, had, as in dogs, pedomorphic features: a trend to accelerated sexual maturation against the background of retarded development of somatic characters. The retarded development gave rise to adults showing characters arrested in a developmental stage (neoteny). The role of direct selection for accelerated sexual maturation as an evoluutionary mechanism of neoteny emergence has often been examined. Our data strongly suggest that this mechanism is selection for tameness, which affects the genetic systems with the function of development rate regulators at the level of the whole organism. As for acceleration of sexual maturation in foxes, it is also a correlated response to such selection.

Taken together, all the evidence suggests that the concordant behavioural-morphological and physiological reorganization in the fox and dog, as well as the similar changes in their developmental timing, may result from the same genetic changes provoked by addressed directional selection. Clearly, the experiment with fox domestication demonstrated what tremendous evolutionary potential may be released by selection for behaviour. Some important milestones in the evolutionary pathway of dogs under domestication were reproducible in the short span of 40 years by strongest selection for the genetic systems of the specific behavioural trait tameability. This selection

may be regarded as the key and universal mechanism of the evolutionary reorganization of animals during their historical domestication.

Acknowledgements

This work is supported by grants 99-04-499-28 and 00-15-97970 from the Russian Fund of Fundamental Research. The author expresses gratitude to A. Fadeeva for translation of this chapter from Russian into English. The author expresses her gratitude to I. Plyusnina, L. Kolesnikova, A. Kharlamova and E. Omelchenko for help during the preparation of the manuscript.

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3 Consequences of Domestication: Morphological Diversity of the Dog

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Introduction

Domestication commonly entails morphological divergence in size and conformation from wild progenitors. The underlying changes in skeletal size and proportion are the primary indicators of domestication in the archaeological record (see Zuener, 1963; Epstein, 1971; Clutton-Brock, 1987, 1995) and may indicate new modes of selection in captivity (Vilà *et al.*, 1997). Rather than function or fancy, many morphological changes may be a by-product of selection for docility (see Chapter 2). Regardless of this, morphological change under domestication often follows regular rules that are an expression of the underlying complexity of genetic and developmental systems. These regular rules are manifest by coordinated changes in proportion with a change in size.

Changes in proportion with size occur at a variety of levels from changes during development (ontogenetic), among adults of different breeds (intraspecific) and among adults of different species (interspecific). These levels of relative growth may be connected mechanistically, for example, by retarding growth rates in development, juvenilized adults may be formed, a process called neoteny (Gould, 1977). Consequently, ontogenetic and intraspecific

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allometry may be related. Neoteny, as a mechanism for creating evolutionary novelty and new species, extends this connection to interspecific scaling among species as well (Gould, 1977). In this chapter, allometric patterns among wild canids and among dogs will be reviewed to illustrate the morphological changes that have occurred under domestication. Similar changes that occur during development of individual dogs will then be discussed. A simple development model will be presented that attempts to account for the allometric patterns among adult dogs. The unusual allometry of dog development may explain, in part, the immense diversity in size and proportion of dog breeds. We currently do not have an adequate understanding of the genetic basis for differences in size and proportion between breeds despite extensive past breeding experiments (e.g. Stockard, 1941; Belyaev, 1979; Chapter 2). However, an understanding of the pattern of morphological differentiation between breeds provides direction for future genetic studies.

Measurements and Methods

A variety of cranial and limb measurements have been used to characterize proportional diversity in length and width of skeletal elements in wild and domestic canids. These have been chosen so as to assess locomotor and dental function in carnivores (e.g. Hildebrand, 1952; Radinsky, 1981; Van Valkenburgh, 1989; Van Valkenburgh and Koepfli, 1993), to improve taxonomy and systematics (e.g. Langguth, 1969; Clutton-Brock *et al.*, 1976; Nowak, 1979) or to understand the process of evolution and domestication (Wayne, 1986a,b,c; Morey, 1992, 1994; Wayne and Ruff, 1993). By and large, many of the same measurements are taken in all studies, although used to different ends, and involve the measurement of tooth length and width, cranial and zygomatic width, face length and width and limb bone length and mid-shaft width (Fig. 3.1). Measurements are often standardized to an index of body size, such as total skull length or femur length so that they can be compared among taxa of different sizes. Measurements are usually made on skeletons in museum collections and for multiple individuals from different sexes within each breed and species. If the sexes are dimorphic, they may often be treated separately or just one sex is examined (e.g. Gittleman and Van Valkenburgh, 1997). A subset of these measurements may be made from radiographs to determine proportions of living adults or developing juveniles (Wayne, 1986a,b).

The diversity in skeletal measurements can be portrayed by multivariate techniques that attempt to summarize the most significant components of variation in the data (Sokal and Rohlf, 1995). However, the interpretation of changes in form often are most readily visualized with bivariate or allometric plots expressing the change in one measure with that of another or with some index of body size (e.g. Fig. 3.2). These plots are generally log/log so that proportional change in a structure is indicated. If the slope of the relationship is 1, then the ratio between the two measurements does not change (the effect of photographic enlargement) and the relationship is isometric. If the slope is

less than 1 (negative allometry), the independent variable is relatively smaller with increasing size and if the slope is more than 1 (positive allometry), the independent variable is relatively larger with an increase in size. Allometric

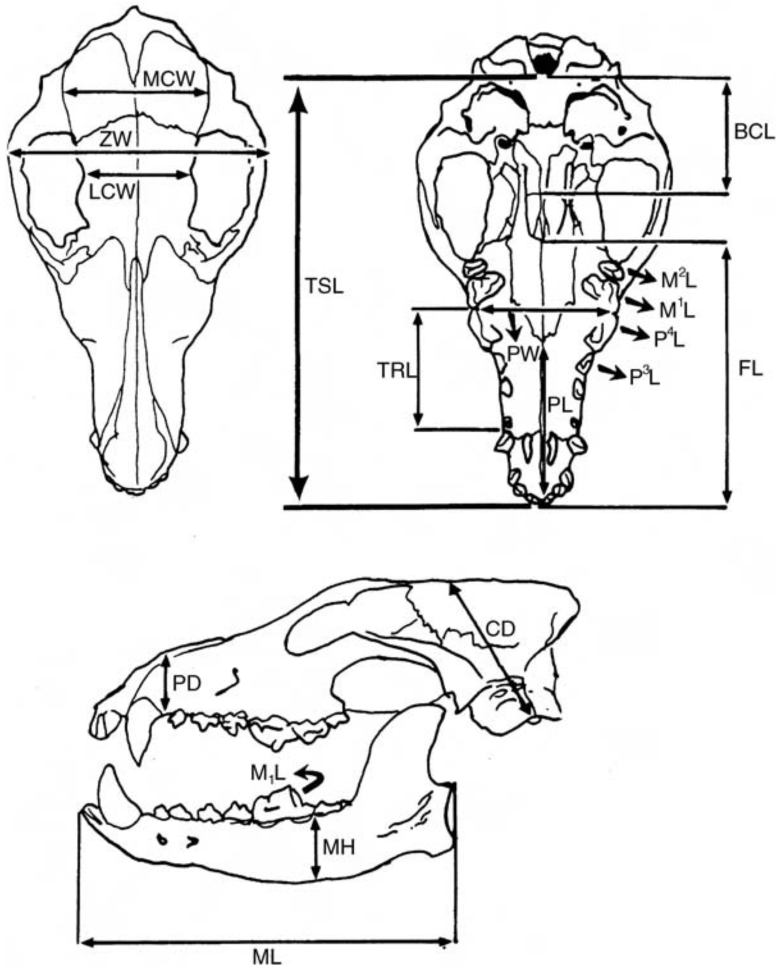


Fig. 3.1. Diagram of 21 cranial and dental measurements (*this page*) and 16 limb bone measurements (*next page*) made on domestic dogs and wild canids. Mandible width (MW) is not figured and is defined as the medial lateral width at the posterior end of the fourth premolar. Abbreviations and descriptions: *this page*, total skull length (TSL), face length (FL), palatal length (PL), basicranial length (BCL), upper premolar tooth row length (TRL), mandible length (ML), palatal width (PW), maximum cranial width (MCD), zygomatic width (ZW), least cranial width (LCW), cranial depth (CD), premaxilla depth (PD), mandible height (MH), P^3 length, (P^3L), P^4 length (P^4L), M^1 length (M^1L), M^2 length (M^2L), M^1 width (M^1W), M^2 width (M^2W), and M_1 length (M_1L); *next page*, femur length (LF), femur width (WF), metacarpal length (LMT), metacarpal width (WMC), radius length (LR), radius width (WR), ulna length (LU), humerus length (LH), tibia length (LT), tibia crest length (LTC), olecranon length (LO), metacarpal length (LMC), metacarpal width (WML), scapula length (LS), scapula width (WS), and width infraspinous fossa (WFO). See Wayne (1986a,b) for detailed descriptions of measurements and specimens.

relationships are commonly interpreted in functional terms, for example, with regard to biomechanical or physiological explanations of form and function (Brody, 1945; Kleiber, 1961; Taylor, 1970; McMahon, 1975; Schmidt-Nielsen, 1984; Alexander, 1992; Van Valkenburgh and Koepfli, 1993). However, regular changes in proportion with size (allometry) may also reveal developmental, mechanical or phylogenetic constraints on the way in which structures can change (Gould, 1977; Gould and Lewontin, 1979).

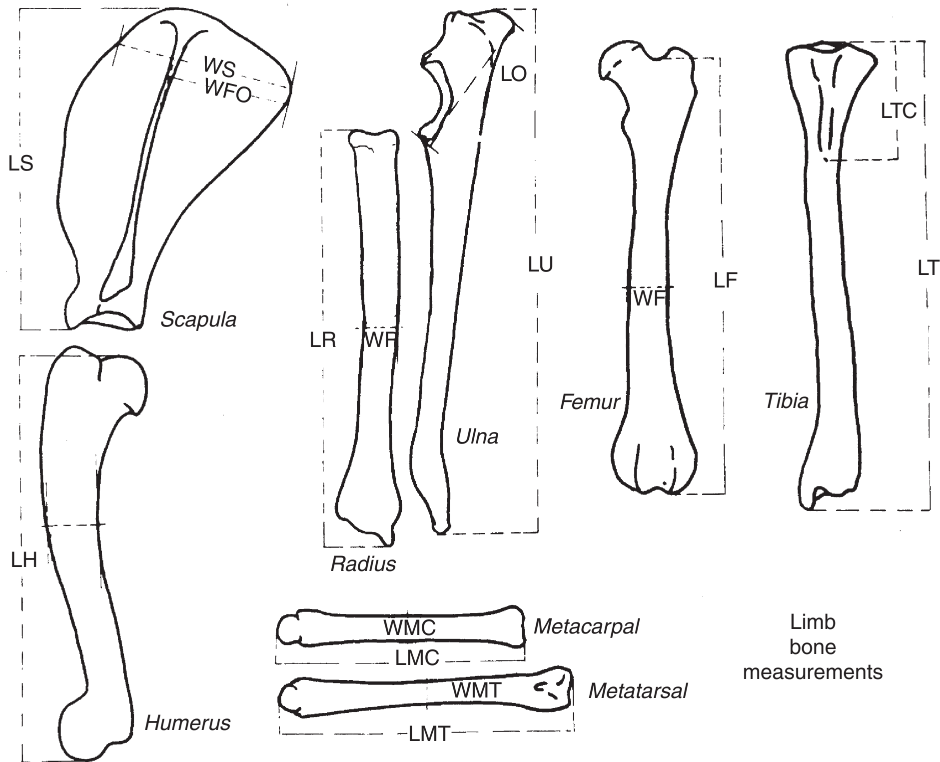
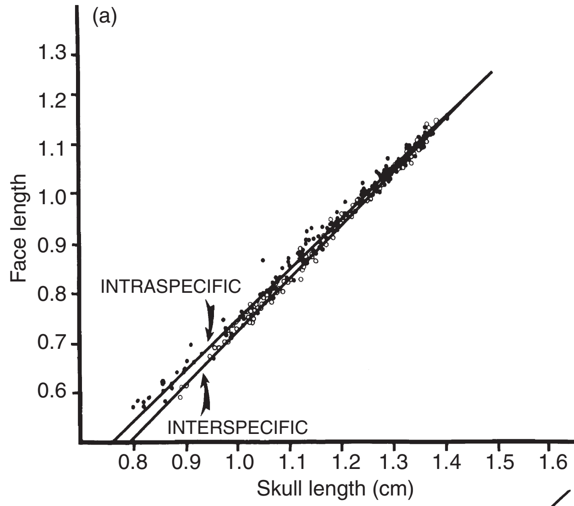
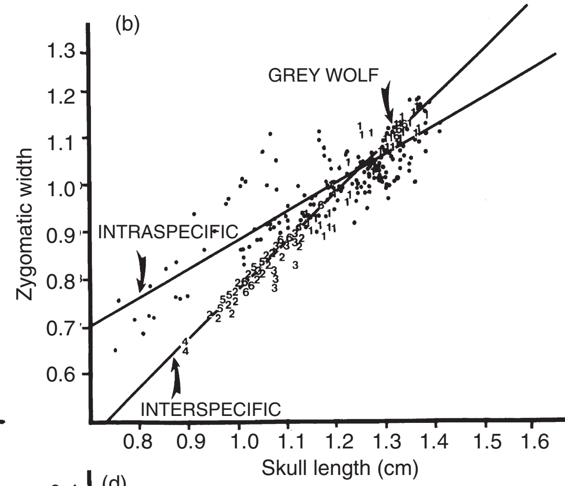
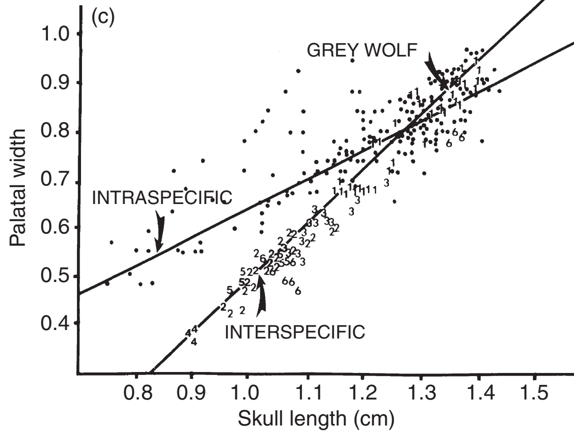


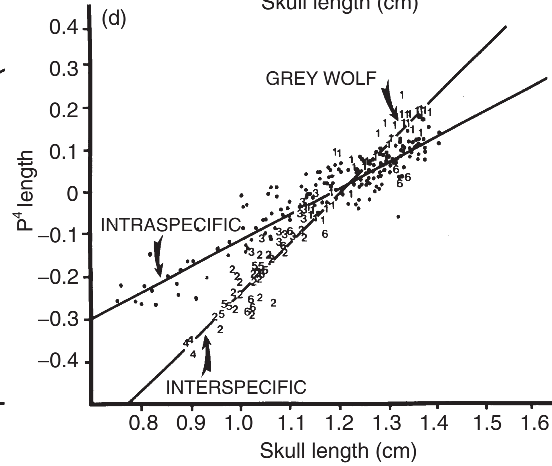
Fig. 3.1. *Continued*.

Fig. 3.2. Log/log plots of face length (a), zygomatic width (b), palatal width (c), and P[4] length (d) against skull length for domestic dogs (intraspecific) and wild canid species (interspecific). Measurement values of domestic dogs are represented by points and values of wild canids are represented by numbers. The position of the ancestor of the domestic dog, the grey wolf is indicated. See Table 3.1 for regression statistics. Measurements in centimetres. Numbers: 1, wolf-like canids, genus *Canis, Cuon, Lycaon*; 2, fox-like canids, genus *Vulpes* and *Alopex*; 3, South American foxes, genus *Cerdocyon* and *Dusicyon*; 4, the fennec, genus *Fennecus*; 5, the grey fox, genus *Urocyon*; 6 abberant canids, genus *Speothos* (bushdog), *Chrysocyon* (maned wolf), *Nyctereutes (*raccoon dog), *Otocyon* (bat-eared fox) and *Atelocynus* (short-eared zorro).


(a)
Face length
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
INTRASPECIFIC
INTERSPECIFIC
0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
Skull length (cm)


(b)
Zygomatic width
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
GREY WOLF
INTRASPECIFIC
INTERSPECIFIC
0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
Skull length (cm)


(c)
Palatal width
1.0
0.9
0.8
0.7
0.6
0.5
0.4
GREY WOLF
INTRASPECIFIC
INTERSPECIFIC
0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5
Skull length (cm)


(d)
P4 length
0.4
0.3
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
GREY WOLF
INTRASPECIFIC
INTERSPECIFIC
0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
Skull length (cm)

Morphological Diversity of Domestic Dogs

Multivariate analysis of 21 cranial and 16 limb bone measures on 202 domestic dogs and 3–10 individuals from each of 27 wild canids showed that diversity in dogs far exceeds that of their wild brethren (Fig. 3.3). However, taken together, dogs do not overlap the multivariate position of fox-like wild canids (numbers 2–5, Fig. 3.3). The variables on the cranial measurement axes are primarily skull width and tooth length rather than skull length (Wayne, 1986a; see also Morey, 1992). In limb bone measures, dogs and small wild canids are distinct with regard to differences in olecranon, metapodial and scapula morphology (Wayne, 1986b). These results show that, although dogs exceed the diversity in size and conformation found among wild canid species, no dogs have cranial or limb proportions similar to small fox-like wild canids.

These differences can be readily visualized on log/log plots with skull length and femur length as independent variables for cranial–dental and limb measurements, respectively. Both skull and femur length are well correlated with body weight (Wayne and Ruff, 1993). With regard to cranial width, dogs are negatively allometric with slopes much lower than wild canids (Table 3.1, Fig. 3.2). In contrast, measures of skull length are nearly isometric (slopes near 1) in both wild canids and domestic dogs. Consequently, small dogs have a wider cranium and face compared to large domestic dogs or wild canids of the same skull length. For example, the ratio of cranial width to total skull length in the Chihuahua is 0.59 whereas in the Great Dane it is 0.31. Small dogs and small wild canids such as foxes are morphologically dissimilar because the cranium and face of small foxes is more elongate and tapered (Wayne, 1986a). Similar negative allometry is evident in dental measurements (Table 3.1). Therefore, large dogs generally have smaller and less robust teeth than small dogs and all dogs generally have smaller teeth than wild canids of the same size. In fact, the generally smaller teeth of domestic dogs is one of the distinguishing characters separating wild wolves from equivalently sized domestic dogs and is one of the first indicators of domestication (Olsen, 1985; Clutton-Brock, 1995). Other characters include a prominent stop, toothrow crowding and a broad cranium and face (Epstein, 1971; Olsen, 1985; Clutton-Brock, 1995). Finally, dogs show much more variability in cranial and dental proportions than do wild canids. This conclusion is evidenced by the generally larger standard error of the regression for dog allometry (Table 3.1). For a given skull length, a great variety of proportions is found, supporting the obvious conformation diversity evident among dogs of similar size (Fig. 3.2).

The allometric scaling of limb measurements in domestic dogs and wild canids is more similar than that of cranial measurements (Tables 3.1, 3.2). Here, femur length is the standard index for body size rather than skull length (Fig. 3.4). In fact, the regressions for wild canids and domestic dogs are not significantly different for measures of ulna and humerus length. The greatest difference in regression lines of domestic dogs and wild canids occurs with measures of metacarpal length, olecranon length, scapula length and long bone width (Table 3.2, Fig. 3.4). With the latter three measurements, allometric

slopes are smaller and intercepts greater in domestic dogs than in wild canids. The reverse pattern is evident for metatarsal length. However, all allometric lines generally converge at large size (e.g. Fig. 3.4). Consequently small dogs

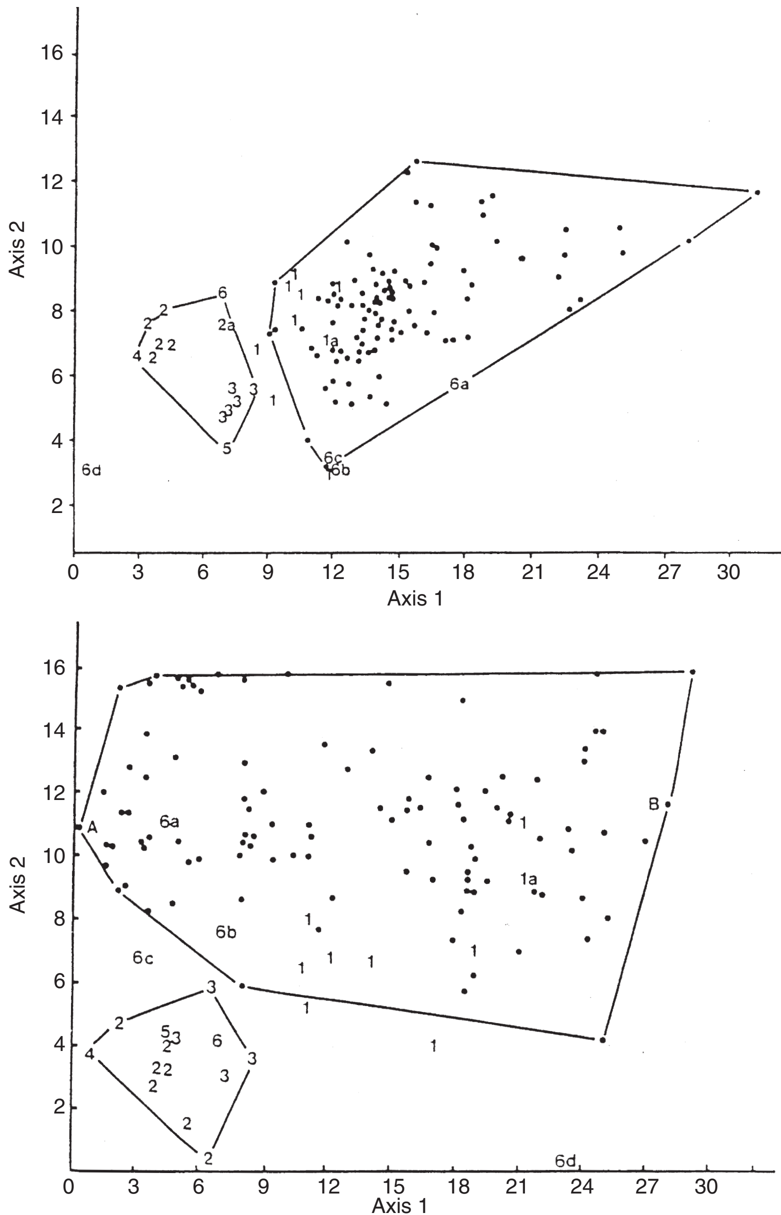


Fig. 3.3. Coordinate position of domestic dogs (dots) and wild canids (Nos 1–6) on the first two axes of a discriminant analysis of cranial (*top*) and skeletal (*bottom*) measurements. Numbers as in Fig. 3.2.

Table 3.1. Standard errors of the regression, slope (*b*), and intercept (*a*) for the regression of the log of the indicated variable on the log of total skull length

Dependent variable	Wild canids			Adult domestic dogs			Developing domestic dogs		
	SE	*b*	*a*	SE	*b*	*a*	SE	*b*	*a*
Cranial length									
FL	0.012	1.04	–0.32	0.014	1.00	–0.25	0.017	0.94	–0.17
PL	0.021	1.02	–0.47	0.027	0.97	–0.40	0.011	0.95	–0.38
BCL[b]	0.021	0.83	–0.26	0.034	0.88	–0.33	0.031	1.05	–0.48
TRL	0.034	1.02	–0.55	0.041	0.96	–0.48	–	–	–
ML	0.012	1.06	–0.16	0.022	0.96	–0.03	0.017	1.04	–0.11
Cranial width and depth									
PW[a,b]	0.033	1.10	–0.61	0.060	0.57	0.09	0.029	0.69	–0.08
MCW[a,b]	0.027	0.60	0.01	0.026	0.24	0.47	0.025	0.38	0.35
ZW[a,b]	0.028	1.03	–0.26	0.050	0.59	0.30	0.030	0.81	0.00
LCW[a,b]	0.050	0.76	–0.41	0.059	0.19	0.34	0.013	0.11	0.61
CD[a]	0.031	0.85	–0.22	0.037	0.52	0.24	0.023	0.47	0.25
PD[a]	0.046	1.24	–1.13	0.065	0.94	–0.70	0.041	0.86	–0.67
MW[a]	0.041	1.29	–1.60	0.064	0.78	–0.93	0.041	0.73	–0.83
MH[a,b]	0.047	1.39	–1.42	0.052	1.16	–1.08	0.069	0.95	–0.78
Dental length and width									
P^3L[a]	0.042	1.09	–1.29	0.050	0.67	–0.77	–	–	–
P^4L[a]	0.041	1.15	–1.18	0.035	0.58	–0.49	–	–	–
M^1L[a]	0.062	0.84	–1.00	0.054	0.64	–0.74	–	–	–
M^2L[a]	0.072	0.54	–0.86	0.082	0.72	–1.07	–	–	–
M^1W[a]	0.032	0.97	–0.96	0.038	0.67	–0.60	–	–	–
M^2W	0.048	0.74	–0.86	0.037	0.68	–0.82	–	–	–
M^1L[a]	0.042	1.17	–1.15	0.032	0.62	–0.46	–	–	–

[a]Regression lines of wild canids and adult domestic dogs are significantly different ($P < 0.05$).
[b]Regression lines of adult domestic dogs and developing domestic dogs are significantly different ($P < 0.05$).

have shorter metatarsals, longer olecrana and scapulas and wider bones than wild canids of equivalent femur length. In general, large dogs have similar limb proportions to wild canids except for a tendency to have wider bones (Wayne, 1986b). Finally, with the exception of olecranon, metapodial and scapula length and width and measurements of bone width, most allometric slopes approach 1. Hence, small dogs are similar in proportion to large ones except with regard to these negatively allometric measurements for which they are proportionally different.

In summary, the allometric scaling of cranial and dental length in dogs differs dramatically from that in wild canids. Because the allometry is often negative in dogs, small dogs have relatively larger measurement values and are more distinct than large dogs from wild canids of the same size. There is less divergence in limb bone allometry between wild and domestic canids,

Table 3.2. Standard errors of the regression, slope (b), and intercept (a) for the regression of the log of the indicated variable on the log of femur length

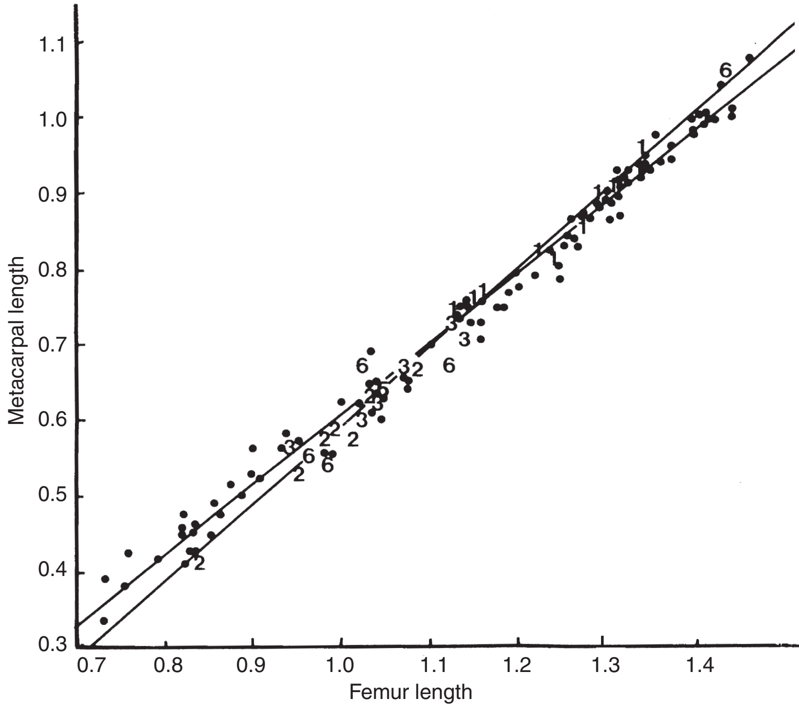
Dependent variable	Wild canids			Adult domestic dogs			Developing domestic dogs		
	SE	*b*	*a*	SE	*b*	*a*	SE	*b*	*a*
Long bone length									
LR[a]	0.029	1.08	–0.12	0.023	1.04	–0.07	0.012	1.00	–0.07
LU[b]	0.030	1.05	–0.02	0.017	1.02	0.02	0.024	0.98	0.04
LH	0.016	0.95	0.03	0.014	0.95	0.02	0.018	0.95	0.01
LT[a,b]	0.026	0.95	0.09	0.020	1.01	0.09	0.023	1.06	–0.11
LTC[a]	0.037	0.98	–0.61	0.060	1.03	–0.63	–	–	–
LO[a]	0.052	0.99	–0.62	0.045	0.83	–0.36	0.030	0.93	–0.31
LMC[a]	0.030	1.04	–0.45	0.032	0.90	–0.29	0.023	0.93	–0.27
LMT[a]	0.037	0.89	–0.21	0.207	0.93	–0.29	0.037	0.83	0.30
Long bone width									
WMC[a]	0.103	1.00	–1.50	0.120	0.85	–1.18	–	–	–
WH[a]	0.056	0.96	–1.10	0.088	0.85	–0.89	0.871	0.82	–0.77
WMT[a]	0.060	0.91	–1.32	0.075	0.76	–1.05	–	–	–
WF[a]	0.043	0.88	–1.00	0.074	0.76	–0.78	0.420	0.76	–0.73
WFE[a]	0.036	0.92	–0.59	0.066	0.89	–0.50	–	–	–
Relative width									
WMC : WMT	0.081	1.08	–0.02	0.108	1.08	–0.01	–	–	–
WH : WF	0.040	1.07	–0.02	0.050	1.09	–0.01	0.381	1.07	0.02
WMC : WH	0.098	1.00	–0.30	0.108	0.92	–0.29	–	–	–
WMT : WF	0.055	1.00	–0.28	0.057	0.95	–0.26	–	–	–
Scapula length and width									
LS[a,b]	0.03	1.06	–0.21	0.037	0.87	0.08	0.029	0.92	–0.09
WS[b]	0.04	0.89	–0.23	0.067	0.84	–0.17	0.053	0.93	–0.23
WFO[a]	0.04	0.94	–0.48	0.070	0.82	–0.33	–	–	–

[a]Regression lines of wild canids and adult domestic dogs are significantly different ($P < 0.05$).
[b]Regression lines of adult domestic dogs and developing domestic dogs are significantly different ($P < 0.05$).

but in all measures, the greatest dissimilarity is between small dogs and their wild counterparts. This makes sense considering that dogs were domesticated from wolves rather than small fox-size canids (Chapter 1). However, there are developmental reasons why there is so little similarity as well, a topic discussed next.

Ontogenetic Allometry of the Domestic Dog

Cranial and limb growth were measured in developing puppies from four dog breeds that differed dramatically in adult size and conformation including a


Metacarpal length
Femur length
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.7
0.8
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1.0
1.1
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1.3
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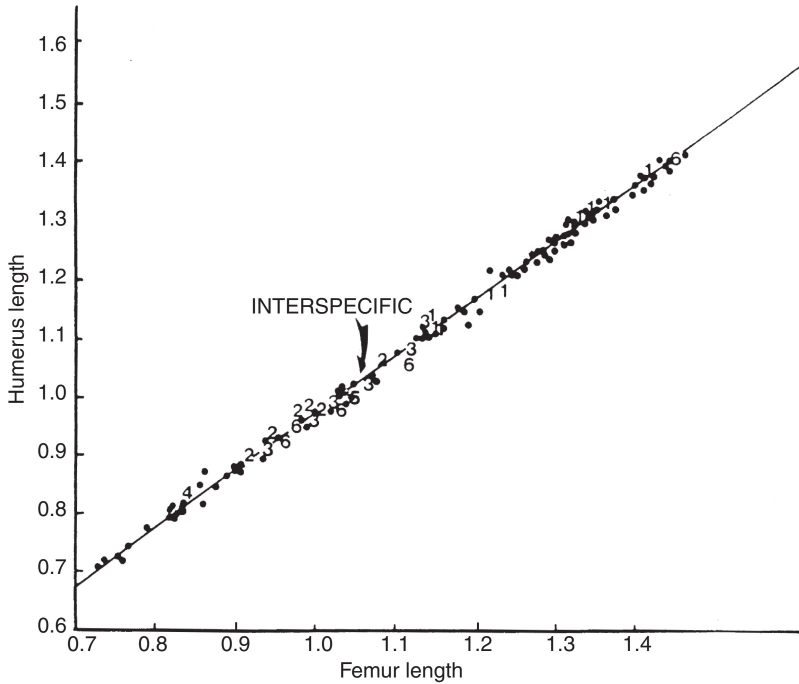
Humerus length
Femur length
INTERSPECIFIC
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4

Lhasa Apso, Cocker Spaniel, Labrador Retriever and Great Dane (Wayne, 1986a,b). Additionally, data on cranial growth from a German Shepherd obtained by Becker (1923) were used. In several measurements, allometric scaling of juvenile domestic dogs of various ages (ontogenetic) is similar or not significantly different from that among adults from different breeds (intraspecific) (Table 3.1). Those measurements that do show significant differences between ontogenetic and intraspecific allometry are generally those that measure aspects of cranial width (e.g. PW, MCW, LCW) as opposed to length (e.g. FL, PL, ML, TRL). However, these scaling differences are slight when compared to those between wild and domestic canids (e.g. Fig. 3.2). Consequently, there is a similarity between juveniles and adults of small breeds, many small breeds are therefore 'juvenilized' or neotenic (retaining juvenile proportions). In fact, some breeds are neotenic in the extreme, similar in proportion to neonate dogs of large breeds (Fig. 3.5). However, all breeds lying about the ontogenetic scaling line might be considered neotenic (Wayne, 1986a).

The allometric scaling similarly of growing dogs and of adults is more apparent with measurements of limb bone length (Table 3.2). Ontogenetic and intraspecific scaling does not differ for most measurements and those that differ significantly do not differ to a large degree (e.g. Fig. 3.5). The greatest differences are in measurements of limb bone width; puppies have proportionally wider bones than adults of the same size (growth is negatively allometric) (Table 3.2). In general, there is proportional similarity between growing dogs and adults of the same size from small breeds. So as with cranial measurements, dogs are neotenic.

Specific Growth Rates of Cranial and Limb Bone Measurements

The ratio of specific growth rates of measurements equals their allometric slope (see Wayne and Ruff, 1993). Consequently, changes in the specific growth rate of measurements will cause altered scaling coefficients and influence intraspecific allometric patterns. For example, in the Lhasa Apso and Great Dane, specific growth rates of zygomatic width are similar but skull length rates are lower in the Lhasa Apso (Fig. 3.6). As a result, the adult Lhasa Apso has a shorter and broader skull relative to the Great Dane. Such simple alterations in relative growth rates may explain, in part, the great diversity in the cranial conformation of dogs.

Postnatal limb bone growth appears more uniform in dogs (Wayne, 1986b). For example, specific growth rates do not differ for radius length of four breeds that are divergent in adult size (Fig. 3.7). This may explain the

---

Fig. 3.4. (opposite) Log/log plots of metacarpal length (*top*), and humerus length (*bottom*) against femur length for domestic dogs and wild canid species. Measurement values of domestic dogs are represented by points and values of wild canids are represented by numbers. The interspecific line is indicated for the regression of humerus length and femur length. See Table 3.2 for regression statistics. Measurements in centimetres. Numbers as in Fig. 3.2.

general isometric scaling in dogs with respect to limb length (Table 3.2; Wayne 1986b,c; Wayne and Ruff, 1993). Differences in limb proportion among breeds may therefore reflect proportional differences at birth more so than cranial

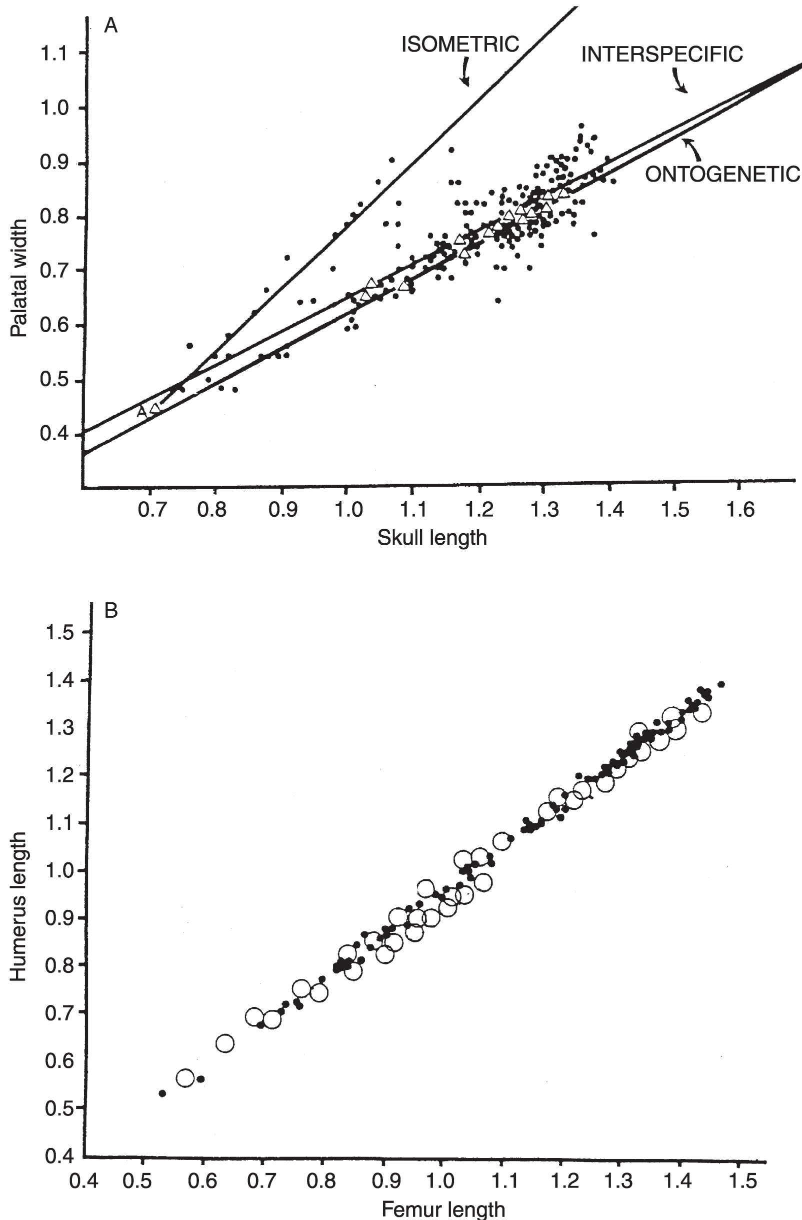

Fig. 3.5. (A) Log/log plots of palatal width against total skull length for juvenile German Shepherds (triangles, Becker, 1923) and adult domestic dogs (solid circles). The isometric line represents the proportional enlargement of a neonate German Shepherd. (B) Log/log plots of humerus length against femur length for juvenile dogs from four different breeds (circles) and adult domestic dogs (solid circles). Measurements in centimetres.

dimensions. Finally, when specific growth rates of measurements do not correspond, their scaling will not be isometric; for example, the relative growth rates of metatarsal and femur length change with age (Fig. 3.8). At first metatarsal growth rates are higher than that of the femur but after about 100 days of age metatarsal growth rates are lower. Consequently, puppies have larger feet than adults do and the allometric slope is negative (Table 3.2).

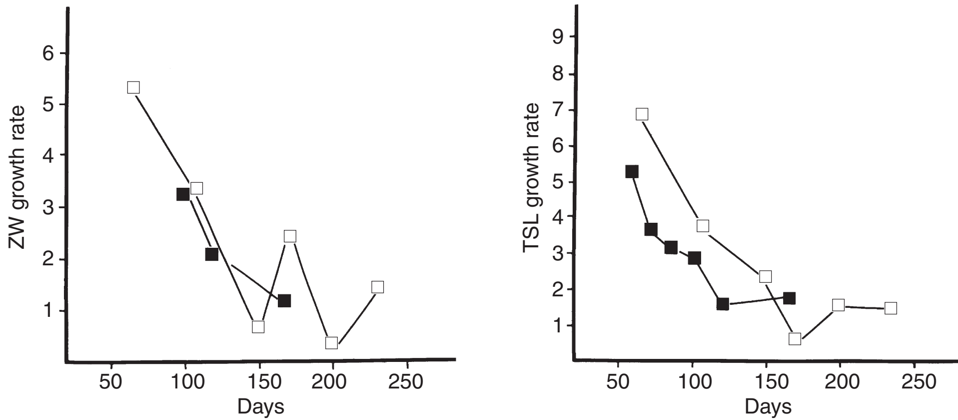

Fig. 3.6. Specific growth rates of zygomatic width (ZW, *left*) and total skull length (TSL, *right*) against time for the Great Dane (open squares) and the Lhasa Apso (solid squares). Specific growth rates in units of 1/day ×1000.

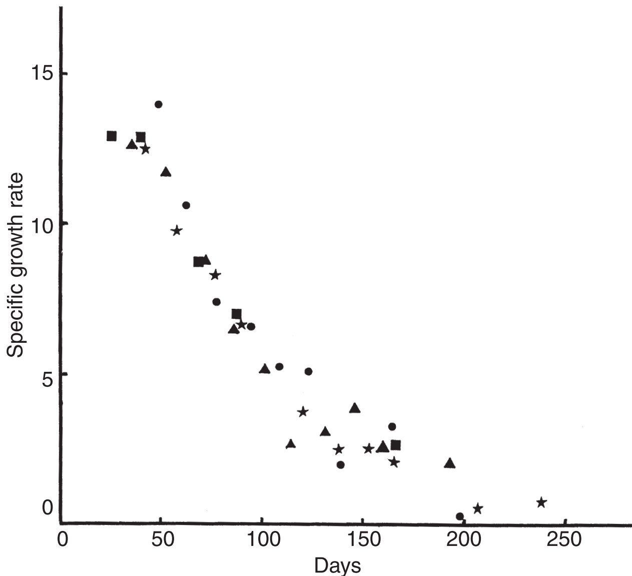

Fig. 3.7. Specific growth rate of radius length vs. time for growing domestic dogs. Great Dane (solid circles), Labrador Retriever (stars), Cocker Spaniel (triangles), and Lhasa Apso (solid squares). Specific growth rates in units of 1/day ×1000.

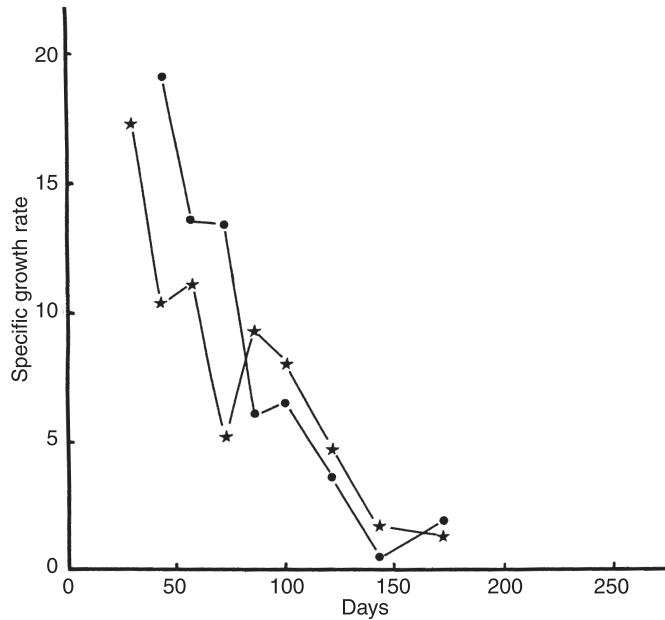

Fig. 3.8. Specific growth rates of metatarsal (solid circles) and femur length (stars) vs. time for the Lhasa Apso. Specific growth rates in units of 1/day ×1000.

A Theory of Diversity in the Dog

The diversity in cranial conformation of dogs may stem from the profound changes in size and form that occur during postnatal growth (Wayne, 1986a). Neonatal dogs have an extremely broad and foreshortened cranium, whereas a typical large dog, such as an adult German Shepherd, has a long tapered face and cranium (Fig. 3.9). Developmental alterations that truncate, accelerate, or retard aspects of this ontogenetic transformation create dramatically divergent skull morphologies that can readily be selected by breeders. Puppy-like morphological features in adult animals are often cultivated directly by humans (Gould, 1979). Alternatively, selection for docility and puppy-like behaviour may alter developmental processes affecting both morphology and behaviour (see Chapter 2). For example, our preliminary results suggested alterations of growth rate may explain the wider skulls of small breeds (Fig. 3.6). Because dogs exhibit strong allometric scaling in development (Table 3.1), dramatically new cranial proportions can evolve through simple changes in the timing and rate of postnatal growth. However, such postnatal growth rate changes are not apparent in limb bone measurements.

Postnatal allometric scaling in cranial growth is not as dramatic in other domestic mammals and, consequently, simple alterations in timing and rates of growth will not translate into dramatic differences among adults. For example, neonatal and adult domestic cats vary little in proportion (Fig. 3.9) and ontogenetic scaling in skull length and width is isometric, hence cat breeds

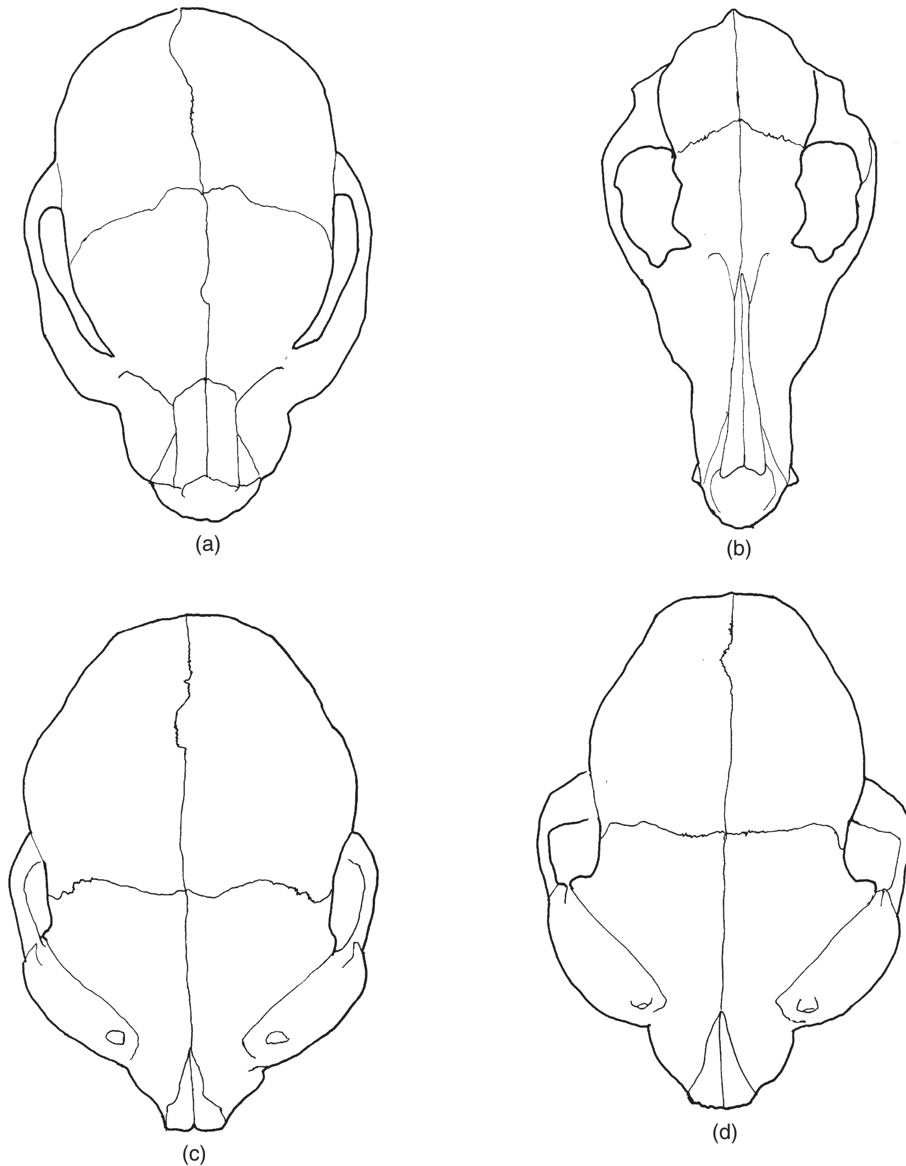

Fig. 3.9. Dorsal view of a skull of (a) a domestic dog neonate and (b) an adult dog, contrasted with that of (c) a domestic cat neonate and (d) an adult cat. All skulls are drawn to the same length.

are relatively similar in cranial proportions. Cranial diversity among adults from different breeds is reflected by ontogenetic diversity in other domestic mammals as well (Wayne, 1986a). This result implies that the difference in diversity between dogs and other domestic animals reflects the degree to which neonates and adults differ in conformation. Because of the strong

allometric scaling of cranial growth in dogs, the action of developmental genes that prolong or truncate juvenile patterns of growth will cause more dramatic change in adult dogs than in other domestic animals that have isometric patterns of growth throughout development (e.g. Fig. 3.9). Consequently, a focus of molecular genetic studies should be toward identifying genes that influence growth rate and timing of skeletal development in breeds of differing size and conformation. Molecular techniques that have been applied to bone disorders might well be applied to understanding skeletal differences among breeds (e.g. Everts *et al.*, 2000).

These observations on scaling among developing dogs, adult domestic dogs and wild canids suggest an explanation for the limits to diversity as well. No small domestic dogs have cranial proportions similar to that of wild fox-like canids of similar body size. The reason for this pattern of non-overlap may be that dogs are born with extremely wide crania that through negative scaling narrow in the typical adult dog skull (Fig. 3.9). A domestic dog neonate is approximately 50% the length of an adult fox but has a skull 90% of the width. Thus, the growth rate of skull width would need to be nearly nil to develop into a fox-sized adult with the appropriate fox-like proportions. It has been suggested that differences in neonate proportions of foxes and dogs may explain the difference in proportion between adults foxes and small dogs (Wayne, 1986a). This difference arises because foxes are born premature relative to dogs, having a gestation time of only 52 days vs. 63 days in the domestic dog. Consequently, neonate foxes are born with a different skull conformation and begin postnatal patterns of scaling relatively earlier. To bridge the morphological gap between the two species, artificial selection needs to produce more precocial dogs (Wayne, 1986a,c).

Summary Comments

Domestic dogs are remarkably diverse in size and conformation. This diversity exceeds that within the family Canidae. However, the overlap between wild canids and dogs is restricted to the large canids, mostly from the genus *Canis*, the close relatives of the domestic dog (Fig. 3.3; see Chapter 1). Small dogs are evolutionarily unique in conformation and tend to have juvenilized or puppy-like traits. This may be due to simple alterations of growth rate acting on a strongly allometric pattern of ontogenetic growth. Artificial selection may have been directed on the cranial traits themselves or on behaviour that also alters development mechanisms affecting morphology (see Chapter 2). The great diversity of domestic dogs may stem from postnatal ontogenetic diversity which is lacking in other domestic species. However, the length of the gestation period may be a critical variable explaining morphological differences between small dogs and small fox-like canids. Precise observations on pre- and postnatal skeletal development in wild and domestic canids are needed to test these hypotheses.

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4 Genetics of Coat Colour and Hair Texture

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Introduction

Dog colour variations have been studied for many decades, beginning with early studies in the infancy of genetics as a science. Identification and study of dog colour have been complicated by differences in terminology among different breeders and also by various hair structure variants in many breeds that can complicate accurate identification of colour phenotypes. The number of well designed experiments to determine coat colour genetics has been few. Only recently has the molecular biology underlying some coat colour mutants been documented (generally in mice, but also in several livestock species), and these details may ultimately help decipher the intricate genetic details behind dog colour. Much of the work on dog coat colour genetics is published in books rather than journal articles (Little, 1957; Burns and Fraser, 1966; Whitney, 1980; Robinson, 1990). Some books include actual segregation data on which some conclusions are based, while others are more speculative. Attempts at standardizing genetic nomenclature have occurred relatively late, and nomenclature may be different and incomplete in regard to homologies with other species. A list of dog coat colour loci and alleles is given in Table 4.1.

General Considerations of Pigmentation

Colour in mammals depends on the presence of melanins in skin and hair (Searle, 1968). Melanins occur in cellular organelles called melanosomes that are produced in melanocytes. Melanosomes reside in melanocyte cytoplasm and are deposited into epidermis and hair by exocytosis which transfers melanosomes from dendritic processes of the melanocytes into these other (generally epidermal) cells. Melanocytes migrate from the neural crest during embryological development, and have close ties to cells of the neurological system. Pigmentation of skin and hair depends on melanocytes being present, as well as on their relative level of melanogenic and exocytotic activity. The two main mechanisms by which pigmentation is reduced or absent are absence of melanocytes, or relative inactivity of melanocytes. Decrease or elimination of pigment by either of these basic mechanisms can be regional, or can involve the entire animal.

Melanins are large polymers that are formed of varying amounts of tyrosine and cysteine (Prota, 1992; Jackson, 1994). Melanins occur as two types: eumelanin and phaeomelanin. Eumelanin contains mainly tyrosine. It is black or a derivative of black such as blue grey or chocolate brown (referred to as ‘liver’ by most dog breeders, but as ‘red’ by many). Phaeomelanin has varying amounts of cysteine in addition to tyrosine and is generally reddish brown or yellowish tan. The existence of two types of melanin is important visually and genetically. These two are usually considered as two discrete classes of pigment. Some overlap occurs between the two, but this overlap usually plays only a minor role in understanding the genetic control of colour.

Table 4.1. Loci and alleles affecting dog colour

Locus	Symbol	Allele	Symbol
Agouti	*A*	*solid black*	A^S
		sable	A^y
		grey	A^g
		saddle	A^s
		black and tan	A^t
		no pattern	A^a
Albino	*C*	*wild type*	C^+
		chinchilla	C^{ch}
		dondo	C^d
		cornaz	C^b
Brindle	*Br*	*brindle*	Br^B
		wild type	Br^+
Brown	*B*	*wild type*	B^+
		brown	B^b
Cyclic Neutropenia	*Cn*	*wild type*	Cn^+
		cyclic neutropenia	Cn^{cn}
Dilution	*D*	*wild type*	D^+
		maltese dilution	D^d
Extension (MC1R)	*E*	*dominant black*	E^D
		wild type	E^+
		fawn	E^e
Flecking	*F*	*Flecked*	F^F
		nonflecked	F^f
Grey	*G*	*grey*	G^G
		wild type	G^+
Grey Points	*Grp*	*wild type*	Grp^+
		grey points	Grp^g
Harlequin	*H*	*harlequin*	H^H
		wild type	H^+
Intense	*Int*	*cream*	Int^C
		fawn	Int^f
		tan	Int^t
Mask	*Ma*	*masked*	Ma^M
		wild type	Ma^+
Merle	*M*	*merle*	M^M
		wild type	M^+
Piebald	*S*	*wild type*	S^+
		irish	S^i
		piebald	S^p
		extreme piebald	S^w
Pink-Eyed Dilute	*P*	*wild type*	P^+
		pink eyed dilute	P^p
Powder Puff	*Pp*	*wild type*	Pp^+
		powder puff	Pp^{pp}
Slate Grey	*Sg*	*slate grey*	Sg^S
		wild type	Sg^+

Continued

Table 4.1. *Continued*

Locus	Symbol	Allele	Symbol
Ticking	*T*	*ticked*	T^T
		wild type	T^+
Tweed	*Tw*	*tweed*	Tw^T
		wild type	Tw^+
White	*Wh*	*wild type*	Wh^+
		white	Wh^w

Overlap can also occur visually, with very dark phaeomelanin resembling lighter forms of eumelanin, but usually the two classes are distinct (Sponenberg *et al.*, 1988a). Dark phaeomelanin and brown eumelanin are both likely to be classed as 'red' by some dog breeders, contributing to confusion.

Melanins are formed from tyrosine and cysteine by catalysis, and the main enzyme involved in this process is tyrosinase (Jackson, 1994). Tyrosinase activity is essential for melanin synthesis. Two other proteins, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2), are also important for melanogenesis. The exact function of TRP-1 is uncertain, but it is well documented to have an important role in the synthesis of eumelanin and has little or no role in the synthesis of phaeomelanin. TRP-2 has dopachrome tautomerase activity.

Melanocytes are capable of forming both eumelanin and phaeomelanin, but they produce only one or the other at any one time. Dedication of melanocytes for eumelanin production depends on the presence of *alpha* melanocyte stimulating hormone (αMSH) secreted by the pituitary gland (Jackson, 1994). Melanocytes have surface receptors that bind this hormone. When αMSH binds to these surface receptors a cascade of events is initiated that eventually activates adenylate cyclase. This activation in turn stimulates the melanocyte to produce eumelanin. In the absence of this signal, which is dependent on αMSH as well as the surface receptors, melanocytes produce phaeomelanin. The switch between eumelanogenesis and phaeomelanogensis depends on the function of the receptors of αMSH.

Control of melanocyte function is intricate, and many loci have mutants which affect different components of melanogenic control mechanisms (Jackson, 1994). Some loci affect cell differentiation or migration from the neural crest. Other loci affect morphology of the melanocyte or its ability to deposit melanosomes in hair and epidermis. Other loci directly affect various enzymes and related proteins that are responsible for melanogenesis. A few loci have mutants that affect interaction of αMSH with the target melanocytes. All of these loci interact to produce the final colour phenotype.

White, as distinct from colour, arises from either white spotting or dilution (albino) mechanisms (Searle, 1968). White spotting in mammals occurs in regions where skin or hair follicles lack melanocytes. This absence of melanocytes can result from several mechanisms: failure of melanoblasts

to differentiate from the neural crest, failure to migrate to skin regions, or perishing once migration has taken place (Searle, 1968).

A second general mechanism for producing white is achieved through dilution of pigment mediated by decreased effectiveness of melanin production by melanocytes. Melanocytes are present, but are either ineffective at forming melanins or placing them in hair fibres, or both. Ineffective melanosome production or transfer results in unpigmented or weakly pigmented hair and skin even though melanocytes are present. Multiple mechanisms for dilution of colour, each under separate genetic control, are common among mammalian species.

Wild type colour in dogs must be considered as the standard type if the genetics of colour is to make sense. Wild type colour is that of wolves, whose colours are unfortunately more variable than those of many other wild species. Overall variation in wolf colour includes phenotypes varying from nearly black to very pale silver. The most common wolf colour is a pale sable where basal portions of most hairs are pale phaeomelanic, and distal portions are black eumelanic. The facial region to behind the eyes, the belly, and the legs are generally free of black pigment. The overall effect is of a silver colour with a black overlay over the topline.

Control of Colour

The **Agouti** *locus*

Mechanism of action and overview

The *Agouti* locus is responsible for formation of a protein that acts to nullify the action of αMSH on melanocytes (Jackson, 1994). In body regions where this protein is present, hair follicle melanocytes fail to respond to αMSH and therefore form phaeomelanin and not eumelanin. Melanocytes in regions lacking this protein have full capability for stimulation by αMSH and therefore form eumelanin (Jackson, 1994). In regions with a pulsatile formation of agouti protein the result is banded hairs.

In mice the *Agouti* locus has been extensively studied and implications of homology suggest that its biology is similar in dogs, although the *solid black* allele usually assigned as a dominant at the canine locus raises some questions that are discussed below. The series of murine *Agouti* alleles is a consistent array as it progresses from the most recessive to the most dominant allele (Jackson, 1994). The most recessive murine *Agouti* allele allows for no agouti protein production in the skin and hair follicles and therefore results in a completely eumelanic phenotype. The more recessive alleles, above the one coding for a completely eumelanic phenotype, allow for agouti protein in ventral body regions; phaeomelanic phenotype results in these regions. Successive alleles then add phaeomelanic colour to additional body regions to those of lower alleles, such that phaeomelanic areas of the coat are added successively as the series of alleles progresses from more recessive to more

dominant. In a few intermediate alleles, dorsal regions have pulsatile agouti protein production; banded hairs typical of the wild pattern in mice result. These hairs have a eumelanic tip and base, and a phaeomelanic middle.

Murine *Agouti* locus patterns exhibit no reversal of pigment type among alleles, thus regions which have become phaeomelanic from the action of more recessive alleles retain this pigment type in all patterns determined by more dominant alleles. In this regard the murine *Agouti* locus acts as though various alleles were simply shifting melanin production in an orderly stepwise progression from one extreme to the other, adding phaeomelanic areas body region by body region. More dominant alleles result in phenotypes that are entirely phaeomelanic, while the most recessive alleles have phenotypes that are entirely eumelanic. The stepwise progress of *Agouti* locus patterns gave rise to early hypotheses that the locus was a complex of small loci that each controlled pigment type in only a specific body region. Recent studies have shown that the complex hypotheses are mistaken and that the locus is indeed single and simple rather than an array of miniloci (Jackson, 1994).

Canine alleles

An allelic series at *Agouti* has long been postulated in dogs. This locus certainly involves at least three alleles, and perhaps additional ones. The four with most general acceptance are *solid black* (A^S), *sable* (A^y), *black and tan* (A^t) and *no pattern* (A^a) (Little, 1957; Burns and Fraser, 1966).

The solid black phenotype is entirely eumelanic, giving black in most dogs and liver or blue grey when conditioned by other loci. A dominant allele for solid black is a very common allele in dogs and accounts for most black dogs of most breeds. Considerations of homology raise questions that this allele may not belong at the *Agouti* locus. Data tend to support that in some instances a dominant black is indeed at *Agouti*, although other data suggest its residence at other loci. This issue is discussed in more detail below. While details of its action and location are somewhat unclear, *dominant black* has long been considered as a legitimate *Agouti* allele and so is included here but with some reservations. Whatever its locus, its dominance is very complete, masking other *Agouti* alleles.

Sable phenotype is basically phaeomelanic, with eumelanic tips on some hairs (Little, 1957; Burns and Fraser, 1966). Generally these black tipped hairs are on the body and ear tips. At one extreme, sables are almost entirely phaeomelanic, with only eumelanic ear tips. At the other extreme, sables are very dark with most body hairs heavily tipped in black. A distinctive mask occurs on most dark sables; the black tips sparing the phaeomelanic facial hairs and beginning abruptly a short distance behind the eyes and extend forward on the midline to form a distinctive peak of dark colour. Lower legs are usually entirely phaeomelanic. Some authors point to incomplete dominance of the *sable* (A^y) allele over the *black and tan* (A^t) allele, with heterozygotes being darker than homozygous sable dogs. Other authors have found that at least some homozygous sable dogs are indeed very dark, suggesting that incomplete dominance does not account for all dark sable

dogs. Sable is common in Collies and German Shepherds and many other breeds. Figure 4.1 illustrates a relatively pale sable phenotype.

Black and tan phenotype is very distinctive and repeatable and is illustrated in Fig. 4.2. The body is eumelanic: phaeomelanic regions occur over each eye, on sides of the muzzle, on the chest, belly, around the anus, and on the lower legs. Black and tan is common among hounds and is the standard phenotype of Doberman Pinschers and Rottweilers. Black and tan is also common in herding breeds, including Australian Shepherds and German Shepherds.

The 'no pattern' (A^aA^a) phenotype, as suggested by the name, is entirely eumelanic. This A^a allele is fairly rare among dog breeds but does occur in German Shepherds, Shetland Sheepdogs, and Australian Shepherds to name a few (Carver, 1984). This is phenotypically entirely eumelanic (black in most breeds) and is easily confused with dominant black.

Other suggested *Agouti* locus alleles include *wild type* (A^+ or A^w in some texts). This allele is similar to sable, but generally causes a pale phenotype with moderate to minimal phaeomelanin expression, and a pronounced

Fig. 4.1. Mountain Feist dog with pale sable phenotype in which the body is phaeomelanic, with residual eumelanin only on back, ears and tail.

Fig. 4.2. Australian Shepherd Dog with black and tan, liver, spotted phenotype. This degree of spotting segregates from the more usual Irish spotting in this breed.

eumelanic tip pattern similar to that on dark sables. Some workers referred to this as *grey*, because this is the wild type of grey wolves (Little, 1957; Burns and Fraser, 1966; Willis, 1976), and suggest it may be associated with the grey in Schnauzers. This allele appears to be very rare and is easily confused with sable. It does occur in Malamutes, Siberian Huskies, Borzois and German Shepherds.

Another controversial allele is *saddle* (A^s) that results in more extensive tan regions than does the black and tan pattern, as illustrated in Figs 4.3 and 4.4. Saddle dogs are born with a black and tan pattern, but tan regions expand until at maturity the eumelanic regions are limited to the back and sides (hence the name, saddle). Some early workers proposed that this is merely a modification of black and tan, and not a legitimate *Agouti* allele, although other evidence supports that this is indeed a legitimate allele in this series (Little, 1957; Burns and Fraser, 1966). Whatever the genetics behind this phenotype, it is common in Airedale and Welsh Terriers, as well as Beagles.

Observations of tan pointed dogs with eumelanic bellies, instead of the usual phaeomelanic bellies conditioned by the *black and tan* allele, suggest that yet another *Agouti* locus allele does exist. Segregation data are not available (D.P. Sponenberg, unpublished observation, 1992).

The Extension *locus*

Mechanism of action and overview

The *Extension* locus encodes one member of a group of seven αMSH receptor proteins (Jackson, 1994) and has been established as identical to the melanocortin 1 receptor locus (MC1R) (Newton *et al.*, 2000). This protein, when activated by αMSH, switches melanocytes from phaeomelanin production to eumelanin production. Activated receptor protein results in increased

Fig. 4.3. Treeing Walker Coonhound with saddle and piebald spotted phenotype. The tan and black regions of the saddle pattern have been removed from body regions by white areas that are typical of the *piebald* allele.

Fig. 4.4. Catahoula Leopard Dog with the saddle pattern combined with brindle. The distinctive brindle striping is only expressed in the regions that are phaeomelanic as conditioned by the *Agouti* locus.

cyclic adenosine monophosphate (cAMP) levels and increased protein kinase activity. The wild type *Extension* locus allele in most species allows for control of eumelanin and phaeomelanin production to be governed by the *Agouti* locus as determined by the regional distribution of the agouti protein which inhibits eumelanin formation.

Many species have dominant alleles at the *Extension* locus that act to increase adenylate cyclase either spontaneously or in response to αMSH. Specific mechanisms are different for different alleles in the mouse, and involve either responsiveness to αMSH or autonomous adenylate cyclase production (Jackson, 1994). Either mechanism results in an entirely eumelanic phenotype, and these alleles are usually considered as 'dominant black' regardless of the mechanism underlying the expression of this phenotype.

Recessive alleles at the *Extension* locus consistently result in an inactive receptor that is unresponsive to stimulation by αMSH (Robbins *et al.*, 1993). The result is a completely phaeomelanic phenotype. In this regard the *Extension* locus acts opposite to the *Agouti* locus of most species in that the more dominant phenotypes are completely eumelanic, and more recessive ones are completely phaeomelanic.

While dominant and recessive *Extension* alleles are usually epistatic to the *Agouti* locus, in the fox the dominant alleles tend to be incompletely epistatic so that the interactions of the two loci yield a number of distinct phenotypes rather than the few expected of a completely epistatic system (Adalsteinsson *et al.*, 1987; Våge *et al.*, 1997). Those homozygous for the dominant *Extension* alleles are black, as are those homozygous for the recessive *Agouti* allele. Animals heterozygous for the dominant *Extension* allele are intermediate between red and black, as are those heterozygous for the recessive *Agouti* allele although these two different intermediates are visually distinct. Such an effect has not been noted in dogs, but does have implications for the molecular biology of these loci.

Extension locus alleles tend to affect the entire hair coat, so that this locus does not produce patterns as are typical of the *Agouti* alleles, with interplay of the two pigment types. The *Extension* locus is usually responsible for completely eumelanic or completely phaeomelanic phenotypes, although exceptions do occur in some species such as rabbits (Searle, 1968). In rabbits, an intermediate allele behaves as a mosaic of the recessive allele for phaeomelanin production and the wild type allele. The result is generally a striped, or brindle, interplay of phaeomelanic regions and eumelanic areas as determined by the *Agouti* locus. These phenotypes tend to be mosaics of eumelanic and phaeomelanic areas. The patterns are striped in nonspotted animals but the colours tend to enlarge into discrete patches when combined with white spotting patterns. Mosaic genotypes in dogs are rare, and resemble those produced by the *Merle* locus (Fig. 4.5).

The *Extension* locus in dogs is linked to the plasma esterase locus (*Es-1*) (Arnold and Bouw, 1990). The distance in dogs is 34.4 cM. This linkage is homologous to other mammalian species.

Fig. 4.5. Labrador Retriever that is a mosaic for *Extension* locus alleles. This dog reproduced as if homozygous *fawn* at *Extension*, even though the phenotype is a mosaic of yellow (fawn) and black areas. The merle pattern in some breeds is a similar mosaic, but conditioned by a single allele rather than some accident of development as in this dog.

Canine alleles

The canine *Extension* locus has a few well-documented alleles and some which are more controversial as to locus assignment (Little, 1957; Burns and Fraser, 1966; Templeton *et al.*, 1977; Carver, 1984).

The *wild type* allele, (E^{+}), allows expression of the *Agouti* locus alleles. The recessive allele (E^{e}) is usually termed *fawn* (or yellow by phenotype) in dogs, and results in an entirely phaeomelanic hair coat with dark skin and eyes. These two alleles almost certainly belong to the *Extension* locus. Fawn (yellow) phenotype varies from very dark to cream coloured, but consistently lacks eumelanic hairs. This distinction makes it possible to distinguish between fawn and very minimally eumelanic sable dogs in most cases.

A proposed dominant allele, *mask* (E^{M}) results in a eumelanic mask over the muzzle. The masking pattern can be superimposed over any of the *Agouti* patterns, so the action of the allele is simply to supply a eumelanic mask over any other pattern. Robinson (1990) puts the *mask* (Ma^{M}) allele at its own locus, independent of the *Extension* locus, although extensive data are not given for this suggestion. Neither are data extant for including it at the *Extension* locus as is assumed by most authors. While most authors have concluded it is probably not at *Extension*, the exact location is unknown.

The *brindle* (E^{br}) allele is assumed by most authors to be at the *Extension* locus, but some investigators doubt this assignment. The canine brindle

pattern consists of vertical stripes of eumelanin superimposed over any region of phaeomelanin. Eumelanic regions of *Agouti* patterns remain completely eumelanic. Combining brindle and black and tan, for example, results in a dog in which the black areas remain black, but the tan points exhibit the brindle striping. This is fundamentally different from brindling of cattle or rabbits in which striping occurs only over regions destined to be eumelanic by the *Agouti* locus. Another distinction is that in cattle and rabbits brindling appears to be a mosaic phenotype that organizes into larger patches when combined with white spotting. In dogs this is not true, and white spotting does not change the expression of brindling in any way.

The brindle pattern in dogs varies from extensively striped to minimally striped. Extensively striped dogs are likely to be misclassified as black, as frequently occurs in the Boston Terrier. Relatively lightly striped dogs are likely to be misclassified as non-brindles, especially when the brindling is superimposed over one of the darker *Agouti* locus patterns.

The *brindle* allele is unlikely to be an *Extension* allele. One litter of purebred Labrador Retrievers sired by a black dog (E^+E^e) and out of a yellow bitch (E^eE^e) included four black pups, two brindle pups, and two yellow pups. This cannot result from *brindle* being at *Extension*; it is likely to be at its own locus as a separate modification lying over any phaeomelanic region as conditioned by *Agouti* or *Extension.* Brindle is common in Mastiffs, Boxers, Great Danes, Plott Hounds, Greyhounds, Boston Terriers and Whippets.

Dominant black in dogs

This dominant and completely eumelanic phenotype, which is very common among dogs, is perplexing as to its locus of action. This allele has been discussed above with the *Agouti* locus, and also with *Extension*, but deserves more discussion in light of the action of these two loci. Most workers assign the canine allele responsible for dominant black to the *Agouti* locus. This is at variance with the *Agouti* alleles of other domesticated mammals (Little, 1957; Burns and Fraser, 1966). It is difficult to imagine the mechanism of action of a dominant *Agouti* allele resulting in a eumelanic phenotype, because dominant expression at *Agouti* yields phaeomelanic phenotypes in all other species so far studied (Searle, 1968).

In most other domesticates the dominant black allele is at the *Extension* locus. This, however, is certainly not the case in all dogs. It is routine for crosses of fawn dogs (E^eE^e) to either sable or black and tan dogs (E^+-) to yield litters of only black pups (E^+E^e). The fawn parent must therefore be supplying the dominant black allele, and can only do this if the allele is not at *Extension.* While it could be at *Agouti*, it could also be at some other locus, although candidates are not readily apparent in other species. Regardless of its location, *dominant black* is one of the most common alleles in dogs, to the extent that it is nearly invariably present in fawn dogs, and is only certainly lacking in dogs expressing intermediate *Agouti* alleles because these are unable to mask its

presence. Recent work clearly demonstrates a dominant black at *Extension* in the Labrador Retriever, but still leaves unexplained the results of the above crosses (Newton *et al.*, 2000). This suggests that multiple genetic mechanisms in dogs can result in a black that masks the *Agouti* locus.

Red

Laukner (1998) proposes a locus, *Rot* (*Red*) at which a recessive allele conditions a completely phaeomelanic hair coat with no eumelanic hairs. This is similar to the action of the recessive *Extension* allele, and further documentation will be required to establish this allelic action at a separate locus.

Modifications of Basic Patterns

Basic coat colours of dogs are conditioned by the *Agouti* and *Extension* loci, along with the mask and brindle phenomena. These basic coat colours are modified by many other loci, nearly all of which act to change (generally reduce) the intensity of pigmentation. The effects of these loci can all be classed together as dilution of colour. Some of these loci are well documented and their action is straightforward. Others are less well documented, but are still important contributors to the overall variation in dog coat colour.

The **Brown** *locus*

In mice, the *Brown* locus controls tyrosinase-related protein 1 (TRP-1), which has important but poorly documented action within melanocytes (Jackson, 1994). The recessive *brown* allele reduces activity of TRP-1, and results in all regions that are eumelanic having the black form replaced by a brown form. Brown melanosomes are structurally different from black ones. In brown phenotypes the catalase normally involved in melanogenesis fails to protect eumelanin from the action of hydrogen peroxide which is produced during melanogenesis.

The murine *Brown* locus has several dominant alleles with the exact dominance relationship depending on the allele. These usually result in phenotypes with apical pigmentation and basal pallor on hair shafts, and similar mutants have not been documented in dogs. Banding on hairs that is caused by these murine alleles is due to accumulation of toxic metabolites within the melanocytes which increase as the hair growth cycle progresses. The result is that apical portions are pigmented due to functional melanocytes. As the hair grows, the melanocytes undergo toxicosis and pigmentation is therefore diminished as the base of a hair is produced.

In dogs it is well established that the *wild type* (B^+) allele allows for normal expression of eumelanin, resulting in black eumelanin. The *brown* (B^b) allele

is recessive and results in brown eumelanin (Little, 1957; Burns and Fraser, 1966). The resulting colour is commonly referred to as liver or chocolate in most dog breeds, although nomenclature describes it as red in some breeds such as the Doberman Pinscher or Australian Shepherd. The brown variant is distributed widely in gun and bird dog breeds (Retrievers, Pointers, Spaniels) and in herding dogs (Australian Shepherd, Kelpie), and is rare among the hounds.

The *brown* allele changes skin colour from the usual black to a liver or fleshy colour. Eye colour is also generally lighter in brown dogs than in black dogs. Many dog breed standards specifically fault pale skin or light eyes, and in those breeds the *brown* allele is especially rare because it lightens skin and eyes as part of its action.

The Albino *locus*

The *Albino* locus codes for the tyrosinase enzyme that is essential for melanogenesis. Recessive alleles at this locus are responsible for production of abnormal forms of tyrosinase that have either reduced or nearly completely absent activity (Jackson, 1994). Melanocytes are present but are incapable of full levels of melanogenesis, resulting in pale phenotypes.

Canine *Albino* alleles are reasonably well established (Little, 1957; Burns and Fraser, 1966; Carver, 1984). The dominant allele *full colour* (C^+) is the wild type allele, and tyrosinase is not affected. Coat colour in these dogs is determined by other loci. This is the most common allele in most dog breeds.

A few other alleles are more hypothetical, but are reported frequently in the literature. The *chinchilla* (C^{ch}) allele reduces phaeomelanin to a cream colour but leaves eumelanin unaffected. This allele acts as a recessive, and is one cause of cream coat colour in dogs when combined with fawn or sable genotypes at *Agouti* and *Extension*. This colour in dogs is found on occasion in Norwegian Elk Hounds.

Two other alleles are rare and poorly documented. These are *dondo* (C^d) and *cornaz* (C^b). Dondo is white with dark eyes, cornaz is white with light or blue eyes and has been called a 'blue eyed' albino in some previous reports. These two forms of 'albino' are reported in Pekinese and Pomeranian dogs. A fully albino allele in dogs has not been reported.

The Dilution *locus*

This locus acts to reduce pigmentation by clumping melanin within the hair shaft. It also lightens eyes and skin. It affects both eumelanin and phaeomelanin. Only two alleles occur at this well established locus: *intense* (D^+) is the wild type allele allowing for full colour, and *dilute* (D^d) is an autosomal recessive that reduces black to blue-grey, liver to light fawn, and phaoemelanin to cream (Little, 1957; Burns and Fraser, 1966). The resulting

colour is evident at birth. In some breeds the dilute phenotype is accompanied by hair coat abnormalities, though this relationship is not absolute even in breeds in which it is common, and many dilute dogs have normal hair coats. Dilute dogs tend to have a somewhat metallic sheen to the hair (Little, 1957). Nose leather colour is also more bluish on animals with the D^dD^d. Dilute phenotype is uniform in Weimaraners, and is also common in Poodles, Bearded Collies, Doberman Pinschers, and occurs more rarely in several other breeds.

The Grey *locus*

This autosomal locus controls a progressive lightening or silvering of the coat (Little, 1957; Burns and Fraser, 1966). Evidence of the locus is limited but it is believed that a dominant or partially dominant allele, *grey* (G^G) causes the birth coat to become progressively lighter until maturity, when the process stabilizes. Resulting colours include the blue-grey of the Kerry Blue Terrier and some silvers in Poodles. The recessive allele is the common allele in most breeds.

The Pink-eyed dilution *locus*

This autosomal locus has a rare recessive allele, *pink eyed dilute* (P^p), which may occur only in the Pekinese (Little, 1957). The result is a more profound dilution of eumelanin than of phaeomelanin, generally accompanied by very light eyes. This creates the so-called 'lilac' colour from black or blue colours while liver animals may become more yellowish.

The Intense *locus*

This autosomal locus affects the intensity of phaeomelanic areas but leaves eumelanic areas relatively unchanged (Iljin, 1932; Carver, 1984). The dominant allele, *cream* (Int^c), reduces intensity of phaeomelanin to a cream colour. An intermediate allele, *fawn* (Int^f), reduces it only to a fawn colour, and the recessive allele, *tan* (Int^t), does not reduce it. This is a common source of variation in the German Shepherd dog. It is different from most other canine loci that diminish pigment in that the paler phenotypes are caused by the more dominant alleles.

Other dilution alleles

A few other dilute phenotypes are seen in dogs, and are unlikely to occur at the previously documented loci. One is a dominant allele, *slate grey* (Sg^{Sg}), in Collies and other herding breeds (Ford, 1969). This allele lightens black to

dark grey, and generally lightens eyes to a greenish colour. Dogs of this colour are only subtly lighter than their littermates at birth, but become more obviously pale as they age. This occurs as a rare variant in Collies and Australian Shepherds.

A recessive dilution gene (Cn^{cn}) is responsible for cyclic neutropenia of Collies and related breeds (Ford, 1969; Lund *et al.*, 1970). The coats of these dogs have extreme dilution of phaeomelanic areas and less but still obvious dilution of eumelanic areas. Dogs homozygous for this allele suffer recurring bouts of low neutrophil numbers, and generally succumb to opportunistic infections early in life. This genotype rarely survives beyond a few months of age.

A variant called ‘powder puff’ affects black areas only (Lund *et al.*, 1970). This is due to a recessive allele, (Pp^{pp}), and the phenotypic effect is basal pallor to black areas, with apical portions of hair unaffected. Action of this allele is transitory, and is noticed only in pups and not in mature dogs.

In some breeds, intermating of dilute dogs that are phenotypically considered as D^dD^d dogs will produce dark-coloured pups. This result is impossible if both dilute parents are homozygous for alleles at the same locus. Such occurrences are rare. These litters demonstrate that not all details concerning dilution of dog colour have been documented, and that multiple loci are involved in producing very similar colour phenotypes. Investigations in the fox demonstrate that multiple loci can be involved in relatively similar end results, although the fine structure of the pigment granules within the hair shafts is likely to reveal subtle differences in the mechanism of the dilution (Bradbury and Fabricant, 1988).

Grey in the points

One subtle modification observed as a recessive variant is the incursion of grey areas into the phaeomelanic points of black and tan dogs (Carver, 1984). Grey areas extend into tan areas on some dogs. This variant was not noticed to modify any other *Agouti* locus pattern.

Merle *and its derivatives*

The *Merle* locus is autosomal with a dominant mutation, *merle* (M^M), that causes eumelanic areas in the coat to become pale with fully intense pigmented patches scattered throughout (Little, 1957; Burns and Fraser, 1966) (Fig. 4.6). These intense patches are usually discontinuous at the midline, suggesting that the patchwork is related to dermatomes. The *merle* allele does not affect phaeomelanic areas. The *merle* mutation is due to a transposable element, and the reversion rate is on the order of 3–4% in the germ-cell line (Sponenberg, 1984). Reversions are stable, with no further change back to *merle*. Homozygous merle dogs are usually very pale with defective hearing

Fig. 4.6. Australian Shepherd puppy with the merle pattern. This mosaic of intense and dilute areas includes distinct patches of varying sizes.

and visually defective microphthalmic eyes. This disorder is limited to homozygous merles, and occurs only in breeds in which merle is a colour: Collies, Shetland Sheepdogs, Australian Shepherds, and rarely Dachshunds and other hounds.

A few modifications of merle have been documented. A relatively common one is the harlequin variant in Great Danes. In this variant the generally blue background of the merle pattern is changed to nearly white, having black patches standing in stark contrast, with occasional small blue patches. Sponenberg (1985) concluded that a modifier at a locus other than *Merle* conditions the harlequin pattern. This modifier, *harlequin* (H^H), is dominant and is lethal to homozygotes. The dogs heterozygous for both the modifier and for *merle* are harlequin. A portion of the embryos that are homozygous for *merle* and heterozygous for the modifier are lethal. O'Sullivan and Robinson (1989) confirmed this hypothesis with a more extensive data set, and also demonstrated that non-merle dogs can supply the harlequin modifier, but with no visible evidence in their own colour.

Another phenotypic variant of merle is conditioned by an autosomal locus which has a dominant allele called *tweed* (Tw^T), illustrated in Fig. 4.7 (Sponenberg and Lamoreux, 1985). This variant causes merle patches to be larger

and smoother than in unmodified merles, and also causes more patches to be intermediate between the light merle background colour and the fully intense eumelanic patches.

Loci that Control White Spotting

Dogs are somewhat unusual among domesticated mammals in that few patterns of white spotting are well documented and these all fall within the patterns expected of the *Piebald* (*S*) locus. Other patterns are very rare, and the genetic basis of none of them is documented. The result is that other loci resulting in different spotting patterns are not characterized in dogs.

The Piebald *locus*

Mechanism of action

In mice the *Piebald* (*S*) locus has been documented to act on differentiation of melanocytes at the neural crest, as well as on their migration from the neural crest to the remaining body (Jackson, 1994). This results in an array of white regions on otherwise pigmented mice. The specific colour of the pigmented regions is governed by other coat colour loci: white spotting can be

Fig. 4.7. Australian Shepherd puppy with the tweed variation of the merle pattern in which the patches are larger and more distinct than in non-tweed merles, and in which dilute, intense, and intermediate areas occur.

superimposed over any colour or pattern. In all domesticated species the extent of spotting can be increased or decreased by modifier genes at other loci.

Canine alleles

The *Piebald* locus in dogs is analogous to the *Piebald* locus in mice (Little, 1957; Burns and Fraser, 1966). In dogs the dominant allele, *nonspotted* (S^+), is usual in dogs lacking white spots. Some few dogs that are S^+S^+ will have very minor white on the feet or chest. These are considered serious flaws in some whole-coloured breeds, and appear to be due to residual modifiers at undetermined loci. Minimally white-marked animals are also frequent among dogs that are heterozygous for the *nonspotted* allele and any one of the recessive alleles coding for spotting at this locus. Many dogs that are minimally marked at birth lose the white marks by about 8 weeks old.

Observations of most dog breeds fit the hypothesis that a series of mutants occurs at the *Piebald* locus. Each of these causes a median level of white spotting around which individual dogs will vary, and the darkest dogs of a more extreme allele will overlap the most extensively spotted dogs of a less extreme allele. The alleles, from more dominant to more recessive (and more coloured to more white), include *irish* (S^i), *piebald* (S^p), and *extreme piebald* (S^w) (Figs 4.2, 4.3, 4.8). The irish pattern has generally minor white trim including the feet, facial blaze, and sometimes a collar around the neck. The piebald pattern generally has white legs, a consistent and broad collar, and a facial blaze. The extreme piebald pattern usually has residual regions of colour over the ears, and sometimes at the base of the tail, with or without a few coloured

Fig. 4.8. Dalmatian bitch with litter. This breed's distinctive pattern is consistent and is the result of the *extreme piebald* allele along with *ticked* and *nonflecked*. The result is dark round spots on a white background. This intricate mechanism produces white puppies whose spots grow in with age, as can be seen by the varying stages of spotting on the members of this litter.

spots remaining on the body. Examples of breeds with the different genotypes include Basenji (S^iS^i), Beagle (S^pS^p), and white Bull Terriers (S^wS^w).

That these are individual alleles, rather than phenotypes conditioned by a single allele with modification at other loci, is supported by results in many breeds with low grades of spotting that occasionally produce puppies with very high grades of spotting. These extensively spotted pups have degrees of spotting discontinuous with the usual range of spotting for the breed producing them. This is more consistent with the segregation of a major allele than it is of segregation of modifiers. Some incomplete dominance occurs among the alleles at this locus, so that dogs which are heterozygous for alleles causing more extensive spotting are more extensively white than those not heterozygous for such alleles.

There is some evidence that an interaction between spotting and background colour occurs (Little, 1957). Black Cocker Spaniels with spotting are less extensively white than are red ones with spotting, and liver dogs are intermediate in degree of spotting between red and black dogs.

The Ticking *and* Flecking *loci*

The *Ticking* locus has a dominant allele, *ticked* (T^T), which causes small pigmented areas to grow into white areas caused by the *Piebald* locus (Little, 1957; Burns and Fraser, 1966) (Fig. 4.8). These small pigmented areas vary in number from few to many. When very few they can easily be overlooked. When numerous they can cause a dog to nearly resemble a non-spotted dog. Such dogs are usually called 'roan' due to residual white hairs among the small tick spots.

The colour of tick spots is that expected of *Agouti* or *Extension*, and depends upon underlying genotype and body region. Ticking is absent from the birth coat, but usually begins to appear at several weeks of age. Ticking is responsible for the distinctive colour pattern of Dalmatian dogs, although the final dramatic phenotype depends on modifiers in addition to the *ticked* allele. Ticking is also common in English Setters (in which it is called 'belton'), German Shorthaired and German Wirehaired Pointers, and is invariably present in the Australian Cattle Dog.

A related locus, *Roan*, has sometimes been proposed as causing intermixture of white and coloured hairs in regions conditioned to be white by *Piebald* locus alleles. It is likely that this is merely a manifestation of extreme ticking in which the small tick marks are so numerous and confluent as to more resemble roan than coloured spots in a white field.

Flecking is a modification of ticking. A recessive allele at this locus, *nonflecked* (F^f), acts to remove any white hairs from the dark tick marks. This variant is important in the Dalmatian breed where clean and crisp spots, caused by ticking, are the desirable phenotype (Schaible, 1973, 1981; Schaible and Brumbaugh, 1976). In the absence of this recessive modifier, the ticking spots are less distinct and have a roan appearance.

Other white spotting loci

In some dogs ticking is restricted to only certain white spots, with others lacking them (Whitney, 1980). This is evidence that multiple types of white spotting, possibly at multiple loci, may be operative in some breeds of dogs. Some of these alleles (notably those at the *Piebald* locus) are subject to infiltration by ticking, while others are not necessarily so.

White spotting in some breeds can take forms unexpected of *Piebald* type spotting which is observational evidence that multiple loci may be responsible. Some breeds, especially Bull Terriers and Border Collies, include dogs with relatively low grades of white spotting that have large white areas over the face and including the ocular orbits. This is unusual for *Piebald* spotting patterns because the periorbital area is among the last to become unpigmented. The repeatability of such patterns makes them candidates for other spotting loci, or for single-locus modifiers of the *Piebald* alleles.

White dogs

White coat colour occurs as a variant in many multicoloured breeds (Bull Terrier, Borzoi), is a breed requirement for others (West Highland White Terrier, Maremma, Komondor, Kuvasz), and occurs as a controversial (and generally shunned) variant in still others (Boxer, German Shepherd). White coat colour can result from either white spotting or dilution, and multiple mechanisms appear to have been used to generate white dogs in different breeds. Within most breeds the white dogs arise from a similar genetic mechanism, but across breeds these mechanisms vary.

Dogs of some breeds are white from combinations of extensive piebald spotting and relatively pale background colours. This mechanism is typical of many hound breeds (Borzoi), as well as most white livestock guardians (Great Pyrenees). It is sometimes possible in puppies of these breeds to see pale spots in regions expected of extreme piebaldness, and then for these to fade with age. The result is generally a white coated dog with dark lips, nose, and eye rims, and dark eyes. In extremely spotted individuals some of this residual skin pigment is weak or absent, although this is a fault in most breeds. White breeds that are white from a mechanism involving white spotting generally produce obviously spotted pups when mated to more solid coloured dogs.

A second mechanism responsible for whiteness arises from combinations of dilution with fawn. These dogs usually retain full skin pigment. This mechanism usually results in very pale rather than starkly white dogs. This mechanism is purported to account for some of the white dogs in otherwise coloured breeds, such as the German Shepherd. This is unlikely to explain all of these, though, because litters containing white pups do not generally also contain the fawns from which the whites are purported to be a modification.

Some non-spotted breeds that are generally dark-coloured produce occasional white dogs. These include the German Shepherd, Doberman

Pinscher and Boxer. In these breeds, a recessive gene segregates for white, with skin pigment and normally coloured eyes. White dogs in these breeds are controversial with some breeders, usually because the white colour is assumed to be correlated with skin, eye or ear defects. A few studies have failed to demonstrate that relationship, but even so, white dogs in these breeds remain unpopular. The locus at which this recessive white resides is controversial, although it appears not to be at the *Albino* locus and is symbolized Wh^w (Carver, 1984).

Many older works cite a dominant white in some Russian breeds, but without segregation data. Therefore confirmation is impossible (Whitney, 1980).

Single Gene Effects on Hair Structure

Several discrete alleles, at separate loci, determine many of the coat type variants in dogs. In most breeds these have been limited to a single combination, with a few breeds having more variation (Burns and Fraser, 1966). These are listed in Table 4.2.

Long hair is generally recessive to short hair, although the degree to which this occurs is subject to modification from additional genes. The recessive long hair allele, (L^l), results in longer hair on the body and legs than the dominant allele, and leaves the facial region unaffected. In some breeds the hair is distinctly longer on the ears and legs, and is referred to as 'feathering'. This coat variant is common in Setters and Spaniels.

Wire hair (Wh^W) is dominant to smooth hair and results in stiff bristly hair throughout the coat (Whitney, 1947; Winge, 1950). This coat is typical for

Table 4.2. Loci and alleles for hair structure

Locus	Symbol	Allele	Symbol
Curly	*Cu*	*curly*	Cu^C
		wild type	Cu^+
Kinky	*K*	*wild type*	K^+
		kinky	K^k
Hairless	*Hr*	*hairless*	Hr^{Hr}
		wild type	Hr^+
Hairless, American	*Ha*	*wild type*	Ha^+
		hairless, american	Ha^{ha}
Long Hair	*L*	*wild type*	L^+
		long hair	L^l
Ripple coat	*Rp*	*wild type*	Rp^+
		ripple	Rp^r
Wavy	*Wa*	*wild type*	Wa^+
		wavy	Wa^{wa}
Wire Hair	*Wh*	*wire hair*	Wh^W
		wild type	Wh^+

many terriers, and also the German Wirehaired Pointer. When combined with long hair the result is the coat typical of the Bearded Collie. Wire haired dogs vary greatly by coat texture and coat length.

Wavy coats can be somewhat subtle on short haired dogs, but in general waviness is recessive to straight coats (Whitney, 1947). Waviness is more obvious on long haired dogs, and is typical of Spaniels of various breeds in which long, wavy hair is desired.

The curly coat typical of the Curly Coated Retriever is dominant to smooth hair. This is in contrast to the kinky coat of the Irish Water Spaniel that is recessive to smooth hair (Whitney, 1947). This is a reminder that similar phenotypes can be produced by different genotypes. The poodle type coat is dominant.

Whitney (1947) noted a transitory variant in Bloodhound puppies that resulted in a ripple coat. This is due to a recessive gene, and the ripples or waves are regular across the coat, but disappear by 7 days of age.

Hairlessness can occur as a result of two different mutations. A dominant mutation coding for hairlessness is lethal to homozygotes *in utero* (Robinson, 1990). Heterozygotes are generally hairless, having only a crest of hair remaining on the head and some hair remaining on the feet. Abnormalities of dentition are also typical of heterozygotes.

A recessive hairlessness is also present in a few breeds and causes more complete hairlessness, but causes no dental abnormalities (Sponenberg *et al.*, 1988b). It is typical of the American Hairless breed, so the locus is designated *Hairless, American* to distinguish it from the dominant hairless locus.

Follicular dysplasias

The defining line between coat type variation and coat abnormality can be arbitrary. Most of the above variants are not considered abnormal, and are the source of definition for many dog breeds. In contrast, the follicular dysplasias are considered to be abnormalities. These result in changed hair texture as well as in hair loss (Gross *et al.*, 1992). The resulting coat is usually thin, dry and brittle.

Multiple forms of follicular dysplasia are documented, and while all are thought to be genetic the specific mode of inheritance for many is undocumented (Hargis *et al.*, 1991; Gross *et al.*, 1992; Schmutz *et al.*, 1998). Colour mutant alopecia is one type, and is generally limited to dogs with colour dilution presumed to be from the *Dilution* locus. In these dogs the typical hair abnormalities only occur in the dilute eumelanic regions, and not in the phaeomelanic regions. This syndrome is common in dilute Dobermans, so much so that one synonym for the syndrome is ‘blue doberman syndrome’.

Not all dilute dogs exhibit the hair abnormalities, although many do (Langebaek, 1986). The defect is common among dilute Doberman Pinschers to the extent that dilute animals with normal coats are rare in this breed. This is evidence that the colour mutant allele is responsible for the coat abnormality

as well. In other breeds, such as the Chow Chow, coat abnormalities are rare in dilute dogs. This phenomenon may point to the existence of different alleles resulting in dilution in these various breeds. Whether these are all at the same locus is undetermined.

Black hair follicular dysplasia has distinctions from colour mutant alopecia (Hargis *et al.*, 1991). This condition is seen in dogs with black regions, and usually in dogs with white spotting in which black regions remain. Only the black regions are abnormal, leaving the white coated areas normal or nearly so. The coat in the black regions is dry, lacks lustre, and is brittle. Histologically the hair follicles are dilated with keratin, and within the hair shafts the melanosomes are large and irregularly shaped. Similar findings are present in colour mutant alopecia, although clumped macromelanosomes are more common in that condition than in black hair follicular dysplasia. Black hair follicular dysplasia is documented to be due to an autosomal recessive gene (Schmutz *et al.*, 1998).

The distinctions between these two follicular dysplasias are not always appreciated, and in some instances it is difficult to be certain which of the two is present.

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5 Genetics of Morphological Traits and Inherited Disorders

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Introduction

The dog has been well served by cataloguers of inherited traits, especially inherited disorders. From the pioneering work of Hutt (1934, 1968), Robinson (1968, 1982) and Patterson (Patterson and Medway, 1966; Patterson, 1977) to the contemporary reviews by Mostoskey *et al.* (2000), Ostrander *et al.* (2000) and Patterson (2000), there have been many detailed summaries of the state of play with inherited disorders/traits. Moreover, at the time of writing, the definitive CD-ROM compiled by Patterson and colleagues (Patterson, 2001a) and the companion hard-cover volume (Patterson, 2001b) are in press. Together with this wealth of published information, there are two extensive reviews in the present volume: Chapter 9, covering all types of disorders and Chapter 10, covering hip dysplasia and other orthopaedic disorders. In addition, Chapter 18 provides a discussion of canine disorders as models for human disorders.

From 1978, the author of the present chapter has been compiling a catalogue of inherited disorders and traits in a wide range of animals, including dogs. This catalogue, available on the Internet as *Online Mendelian Inheritance in Animals* (OMIA) (http://www.angis.org.au/Databases/BIRX/omia/) is modelled on, complementary to, and reciprocally hyperlinked to the definitive catalogue of inherited disorders in humans, namely McKusick's *Online Mendelian Inheritance in Man* (OMIM) (McKusick, 1998, http://www.ncbi.nlm.nih.gov/omim/)

OMIA includes entries for all inherited disorders in dogs, together with other traits in dogs for which single-locus inheritance has been claimed,

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however dubiously. Each entry comprises a list of references arranged chronologically, so as to present a convenient history of the advance of knowledge about each disorder/trait. For some entries, there is additional information on inheritance or molecular genetics. If the disorder or trait has a human homologue, this is indicated by including the relevant MIM number(s) from the McKusick human catalogue. In the online version of OMIA, these MIM numbers provide a direct hyperlink to the relevant entry in OMIM. Finally, OMIA contains a full list of reviews of inherited disorders in dogs.

The Present Chapter

Given the comprehensive nature of the other chapters of this book, as described above, there is no need to produce a full list of entries from OMIA in the present chapter. It is, however, useful to produce a list that complements the discussion in Chapter 18 on canine models for human disorders. In that chapter, the authors note that it was not their intention to present a catalogue of inherited canine disorders with human counterparts. Since OMIA contains MIM numbers, it was a simple task to export a file from OMIA comprising all canine disorders for which MIM numbers have been allocated. In presenting this list, Table 5.1 provides a summary of canine disorders that may be useful as models of human disorders. Inevitably, there are errors of omission and commission in the table – some supposed homologues will be incorrect, and some actual homologues will not yet be in the list. The author would be very pleased to hear from readers who spot errors, as these can be corrected easily in the database. Another caveat concerns the evidence for claiming homology. In some cases, homology has been claimed on the basis of a single clinical report that contains no genetic data at all, and hence no evidence of inheritance in dogs. The justification for claiming such a disorder as a homologue is that in the majority of cases, if a particular syndrome is inherited in one species, it is inherited in other species. Of course, there are some notable exceptions to this generalization. But it is sufficiently true to provide an invaluable starting point: the inclusion of a MIM number can be regarded as a null hypothesis that this canine disorder is homologous to an inherited human disorder. In some cases the hypothesis will be disproved subsequently. But more often than not, additional evidence will support the initial hypothesis of homology.

Also included in Table 5.1 for each entry are the earliest and most recent published reference for that disorder, as an introduction to the relevant literature. A full list of references for each entry, together with hyperlinks to OMIM, is available by accessing OMIA on the Internet at the location given above.

For those disorders that have been characterized down to the peptide level, the relevant peptide is also included in Table 5.1.

Inevitably, there are discrepancies among the various published lists of disorders – there are even discrepancies among the lists in this book. There are several reasons for this: there is no simple way to standardize the terminology

Table 5.1. A list of canine inherited disorders that may be homologous to inherited human disorders. This list has been extracted from *Online Mendelian Inheritance in Animals* (OMIA), from which full details are available. MIM numbers indicate the relevant entries in McKusick's *Mendelian Inheritance in Man* (MIM), which is avaliable online at the address shown at the head of the last column

Disorder or trait	Earliest reference; latest reference (if different from earliest reference)	Peptide	MIM number(s) for homologous human disorders/traits [http://www3.ncbi.nlm.nih.gov/Omim/]
Addison disease	Volz (1991), Kelch *et al*. (1998)		300100, 104300, 269200, 103230, 300200
Afibrinogenaemia	Kammermann *et al*. (1971)	Fibrinogen	202400
Alexander disease	Weissenbock *et al*. (1996)		203450
Alopecia	Muller and Jones (1973), Cerundolo *et al*. (2000)		203600, 203650, 104110, 104130, 147770, 104100, 104000, 203655
Alpha-1-antitrypsin deficiency	Sevelius *et al*. (1994)	alpha-1-antitrypsin	107400
Alzheimer disease	Cummings *et al*. (1993), Lim *et al*. (1997)		104300, 104311, 104760, 104310, 163890, 502500, 600759
Amyloidosis	Dibartola *et al*. (1990), Loeven (1994)		105200, 105150, 105120, 105250, 105210, 137440, 176300, 191900, 204850, 204870, 204900, 254500, 301220
Amyloidosis, Aa	Johnson *et al*. (1995), Gruys (1996)		134610
Ankylosing spondylitis	Eichelberg *et al*. (1998)		106300
Arnold–Chiari malformation	Akker (1962), Churcher and Child (2000)		207950
Arthritis	Carr (1997), Michels and Carr (1997)		108100, 208250
Arthritis, rheumatoid	Spreng *et al*. (1993), Carter *et al*. (1999)		180300
Ascites	Otto *et al*. (1990), Rallis *et al*. (1998)		208300
Ataxia	Bjorck *et al*. (1957), Sumano *et al*. (2000)		208750, 301835, 108500, 108600, 108650
Atherosclerosis	Kagawa *et al*. (1998)		108725, 209010
Atopy	Wittich (1940), Masuda *et al*. (2000)		147050
Atrial fibrillation	Wang *et al*. (1995), Gut *et al*. (1998)		194200
Atrial septal defect	Kirberger and Berry (1992), Glardon and Amberger (1998)		108800, 108900, 113301, 178650
Budd–Chiari syndrome	Otto *et al*. (1990), Fine *et al*. (1998)		600880
Bullous pemphigoid antigen	Iwasaki *et al*. (1995), Xu *et al*. (2000)		113810, 113811
C3 Deficiency	Johnson *et al*. (1986), Ameratunga *et al*. (1998)	Complement component 3	120700
Cardiomyopathy, dilated	Keene *et al*. (1991), Calvert and Jacobs (2000)		600884, 115200, 300069, 107970, 212110, 302045, 601494
Cataract	Host and Sreinson (1936), Lohmann and Klesen (1997)		116200, 212600, 212700
Cerebellar hypoplasia	Cordy and Snelbaker (1952)		213002, 213000, 600545

Continued

Table 5.1. *Continued*

Disorder or trait	Earliest reference; latest reference (if different from earliest reference)	Peptide	MIM number(s) for homologous human disorders/traits [http://www3.ncbi.nlm.nih.gov/Omim/]
Ceroid lipofuscinosis	Hagen (1953), Franks *et al*. (1999)		204200, 204300, 204500, 204600
Ceroid lipofuscinosis, juvenile onset	Koppang (1965), Shibuya *et al*. (1998)		204200, 204300, 204500, 204600
Cleft lip	Setty (1958), Edmonds *et al*. (1972)		119530, 113620, 600625, 129810, 225000, 201180, 119300, 119580, 129400, 119500, 129900, 120433, 179400, 155145, 216100, 218090, 174300, 242840, 244300, 268300, 277170, 301815, 106260
Cleft palate	Steiniger (1940–42), Elwood and Colquhoun (1997)		119530, 201180, 258320, 129810, 225000, 303400, 119540, 129400, 260150, 119580, 106260, 119550, 120433, 119300, 129900, 119500, 179400, 119570, 129830, 216100, 216300, 218090, 164220, 231060, 241850, 242840, 244300, 255995, 114300, 181180, 261800, 268300, 277170, 301815, 106250, 600331, 600460
Coat colour, albinism	Pearson and Usher (1929), Gwin *et al*. (1981)	Tyrosinase	203100
Coeliac sprue	Kaneko *et al*. (1965)		212750
Coloboma	Gelatt and McGill (1973), Gelatt *et al*. (1981)		243910, 216820, 120433, 120200, 120300, 216800, 120400, 120430, 120330
Comedo syndrome	Kimura and Doi (1996), Hannigan (1997)		120450
Conotruncal heart malformations	Patterson *et al*. (1974), Patterson *et al*. (1993)		217095, 245210
Cryptorchidism	Hartl (1938), Schulz *et al*. (1996)		100100, 219050, 314300
Cushing disease	Diener and Langham (1961), Meij (1998)		219080, 219090, 160980, 185800
Dermatofibrosis	Perry (1995), Castellano *et al*. (2000)		166700
Dermatosis	Theaker and Rest (1992), Colombini (1999)		125600, 181600
Diabetes insipidus	Reidarson *et al*. (1990), Schwedes (1999)		125700, 304800, 125800, 222000, 221995, 241540, 222300, 304900
Diabetes mellitus	Krook *et al*. (1960), Hess *et al*. (2000)		125850, 125851, 222100, 125852, 125853, 176730, 520000, 222300
Dwarfism, pituitary	Andresen *et al*. (1974), Leeuwen *et al*. (2000)		262400
Dysautonomia	Schrauwen (1993), Schulze *et al*. (1997)		223900, 224000, 252320, 256800
Eclampsia	Drobatz and Casey (2000)		189800
Ectodermal dysplasia, X-linked	Thomsett (1961), Casal *et al*. (1997)		305100
Ectropion	Dreyfus (1953), Hamilton *et al*. (1998)		119580, 209885, 242510

Ehlers–Danlos syndrome	Ronchese (1936), Jelinek and Karban (1998)	Procollagen	130000, 130010, 130020, 130050, 130060, 130070, 130080, 130090, 225320, 128180, 225350, 225360, 305200, 225400, 153454, 120160, 225410, 120150, 130080, 225310, 147900, 229200
Encephalomyelopathy	Podell *et al*. (1996)		161700, 270900
Epidermolysis bullosa	Xu *et al*. (1998b), Olivry *et al*. (2000b)		131800, 131760, 226450, 131960, 131850, 131900, 131880, 226440, 226670, 226730, 226735, 302000, 601001
Epidermolysis bullosa, dystrophic	Nagata *et al*. (1995), Palazzi *et al*. (2000)		226500, 131750, 131950, 132000, 131705, 226600
Epidermolysis bullosa, junctionalis	Scott and Schultz (1977), Olivry *et al*. (1997)		226650, 226700
Epilepsy	Eberhart (1951), Thomas (2000)		117100, 254800, 226750, 601068, 203600, 600143, 132300, 121200, 121201, 125370, 208700, 220300, 159600, 226800, 132090, 182610, 254770, 254780, 104130, 266270, 267740, 270805, 301900, 310370, 545000, 600131, 226810, 600512, 600513, 600669, 226850, 601085, 132100
Exophthalmos with strabismus	Boroffka (1996)		185100, 136480, 141350, 178330, 182875
Exostosis, multiple	Owen and Bostock (1971), Jacobsen and Kirberger (1996)		133700, 133701, 158345, 175450, 600209, 156250
Factor VII deficiency	Mustard *et al*. (1962), Macpherson *et al*. (1999)	Factor VII	227500
Factor X deficiency	Dodds (1973)	Factor X	227600
Factor XI deficiency	Dodds and Kull (1971), Knowler *et al*. (1994)	Factor XI	264900
Factor XII deficiency	Otto *et al*. (1991)	Factor XII	234000
Fanconi syndrome	Bovee *et al*. (1978), Darrigrandhaag *et al*. (1996)		227200, 227800, 227810
Fragile site	Stone *et al*. (1991), Stone and Stephens (1993)		136630, 136620, 136580, 136570, 136560, 136540, 136590, 136610, 136660, 136670, 136640, 136650, 600651, 600819
Fucosidosis, alpha	Hartley *et al*. (1982), Skelly *et al*. (1999a)	Alpha-fucosidase	230000
Galactosialidosis	Knowles *et al*. (1993)		256540
Gangliosidosis, GM1	Karbe (1973), Yamato *et al*. (2000)	Galactosidase, beta	230500, 230600, 230650
Gangliosidosis, GM2	Ribelin and Kintner (1956), Singer and Cork (1989)	Hexosaminidase B	268800
Gaucher disease, Type I	Hartley and Blakemore (1973), Farrow *et al*. (1982)	Glucocerebrosidase	230800
Genu valgum	Hach and Lenehan (1995)		137370, 273050
Glaucoma	Magrane (1957), Ruhli and Spiess (1996)		137600, 200970, 137750, 137760, 137763, 137765, 156700, 157100, 187501, 137700, 229310, 231300, 231400, 231500, 267760, 270850, 308500, 600510, 600975
Glomerulonephritis	Wilcock and Patterson (1979), Rivers *et al*. (1997)		305800, 137940, 247800, 248760
Gluten-sensitive enteropathy	Daminet (1996), Garden *et al*. (2000)		212750

Table 5.1. *Continued*

Disorder or trait	Earliest reference; latest reference (if different from earliest reference)	Peptide	MIM number(s) for homologous human disorders/traits [http://www3.ncbi.nlm.nih.gov/Omim/]
Glycogen storage disease I	Bardens *et al*. (1961), Kishnani *et al*. (1997)	Glucose-6-phosphatase	232200
Glycogen storage disease II	Mostafa (1970), Reuser (1993)	Glucosidase, alpha-1,4	232300
Glycogen storage disease VII	Ewing (1969), McCully *et al*. (1999)	Phosphofructo-kinase, muscle	232800
Goitre, familial	Brouwers (1950), Verschueren and Belshaw (1989)	Thyroglobulin	188450, 138790, 138800, 274600, 274500
Goniodysplasia, mesodermal	Kellner (1996), Ruhli and Spiess (1996)		137600, 138770, 137750
Haemolytic anaemia, autoimmune	Bull *et al*. (1971), Burgess *et al*. (2000)		205700
Haemophagocytic syndrome	Walton *et al*. (1996)		267700
Haemophilia A	Taskin (1935), Clark *et al*. (2000)	Factor VIII	306700
Haemophilia B	Field *et al*. (1946), Gu *et al*. (1999a)	Factor IX	306900
Hemeralopia	Rubin *et al*. (1967), Chaudieu and Molonnoblot (1995)		163500, 257270, 268100, 310500
Hemivertebrae	Morgan (1968), Done *et al*. (1975)		207620
Hepatic fibrosis, idiopathic	Rutgers *et al*. (1993)		263200, 216360
Hernia, diaphragmatic	Butler (1960), Williams *et al*. (1998)		142340, 222448, 226735, 229850, 306950
Hernia, hiatal	Bright *et al*. (1990), Holt *et al*. (1998)		142400, 137270
Hernia, inguinal	North (1959), David *et al*. (1998)		142350, 245550
Hip dysplasia	Schnelle (1954), Wood *et al*. (2000)		142669, 142700, 244510, 265050
Hodgkin disease	Thanikachalam *et al*. (1993)		236000
Horner syndrome	Boydell (1995), van Hagen *et al*. (1999)		143000
Hydrocephalus	Brunetti *et al*. (1993), Cantile *et al*. (1997)		307000, 112240, 109400, 123155, 209970, 236635, 236600, 236660, 236670, 236640, 273730, 276950, 236690, 307010, 314390, 600256, 600257, 600559, 600991
Hydromelia	Kirberger *et al*. (1997)		109500, 211990, 304340, 220200, 220210, 220219, 220220, 267010, 123155, 118420, 207950, 600880
Hyperadrenocorticism	Blaxter and Gruffydd-Jones (1990), Zerbe (2000)		174800, 169170, 201710
Hyperbilirubinaemia, unclassified	Rothuizen *et al*. (1989), Kass *et al*. (1998)		237450, 237550, 237800, 237900
Hypercholesterolaemia	Jorge *et al*. (1996), Kocabatmaz *et al*. (1997)	Low density lipoprotein receptor	143890
Hypergammaglobulinaemia	Michels *et al*. (1995), Cerundolo *et al*. (1998)		247800
Hyperkeratosis, palmoplantar	Binder *et al*. (2000)		144200

Hyperlipidaemia	Jones and Manella (1990), Bauer (1995)		144250, 238400, 238500
Hyperlipoproteinaemia	Baum *et al*. (1969), Ford (1993)	Lipoprotein lipase	238400, 238500, 238600, 144250
Hyperparathyroidism	Persson *et al*. (1961), Reusch *et al*. (1999)		145001, 145000, 239199, 239200, 256120, 600166
Hyperphosphatasaemia	Lawler *et al*. (1996)		171720, 239100
Hypoadrenocorticism	Pascoe (1993), Greco (2000)		240200, 240300
Hypolipoproteinaemia	Whitney *et al*. (1993)		278100
Hypoparathyroidism	Sinke and Kooistra (1996)		247410, 146255, 146200, 241400, 240300, 241410, 256340, 307700, 600780
Hypoprothrombinaemia	Dodds (1977)	Factor II	176930
Hypothyroidism	Clark and Meier (1958), Plotnick (1999)		225050, 225250, 241850, 243800, 275120
Hypotrichosis	Anon. (1917), Cerundolo *et al*. (2000)		146520, 146550, 250460, 211370, 146530, 183849, 246500, 241900, 278200, 600077
Intestinal cobalamin malabsorption	Fyfe *et al*. (1991), Morgan and McConnell (1999)	Intrinsic factor receptor	261100
Intussusception	Greenfield *et al*. (1997), Sivasankar (2000)		147710
Kartagener syndrome	Edwards *et al*. (1989), Watson *et al*. (1999)		244400
Keratitis	Clerc and Jegou (1996)		148190, 148200, 148210, 242150
Krabbe disease	Fankhauser *et al*. (1963), McGowan *et al*. (2000)	Galactosylcera-midase	245200
Legg–Calve–Perthes disease	Moltzen-Nielsen (1937), Brenig *et al*. (1999)		150600
Leucocyte adhesion deficiency	Trowaldwigh *et al*. (1992), Kijas *et al*. (1999)		116920
Linear IgA disease	Olivry *et al*. (2000a)		602341
Malignant hyperthermia syndrome	Short (1973), Xu *et al*. (1998a)		145600, 180901
Masticatory muscle myositis	Vilafranca *et al*. (1995)		154850
Menkes syndrome	Guevara-Fujita *et al*. (1996)	Cu^{2+}-transporting ATPase, alpha	309400, 600468, 300011
Mitochondrial myopathy	Olby *et al*. (1997)		251900, 157650, 251945, 160550, 255140
Motor neurone disease	Pinter *et al*. (1997), Kent *et al*. (1999)		253500, 158700
Mucopolysaccharidosis I	Shull *et al*. (1982), Lutzko *et al*. (1999)	Iduronidase, alpha-l	252800
Mucopolysaccharidosis II	Wilkerson *et al*. (1998)	Iduronate-2-sulphatase	309900
Mucopolysaccharidosis IIIA	Fischer *et al*. (1998)	Sulphamidase	252900
Mucopolysaccharidosis VI	Haskins *et al*. (1992)	Arylsulphatase B	253200
Mucopolysaccharidosis VII	Haskins *et al*. (1984), Sammarco *et al*. (2000)	Glucuronidase, beta	253220

Table 5.1. *Continued*

Disorder or trait	Earliest reference; latest reference (if different from earliest reference)	Peptide	MIM number(s) for homologous human disorders/traits [http://www3.ncbi.nlm.nih.gov/Omim/]
Muscular dystrophy, Duchenne and Becker types	Valentine *et al*. (1988), Wetterman *et al*. (2000)	Dystrophin	310200
Myasthenia gravis	Jenkins *et al*. (1976), Yoshioka *et al*. (1999)		254210, 254200
Mycosis fungoides	Olivry *et al*. (1995), Magnol *et al*. (1996)		254400
Myoclonus epilepsy of Lafora	Hegreberg and Padgett (1976), Jian *et al*. (1990)		254780
Narcolepsy	Dean *et al*. (1989), Reilly (1999)	Orexin (hypocretin) receptor 2	161400, 223300
Necrotizing encephalopathy, subacute, of Leigh	Sawashima *et al*. (1996), Brenner *et al*. (2000)		256000, 161700
Nephritis, X-linked	Thorner *et al*. (1989), Lees *et al*. (1997)	Collagen, type IV, alpha-5 chain	301050, 303630
Nephritis, autosomal	Hood *et al*. (1990), Lees *et al*. (1998)		104200
Nephrotic syndrome	Vilafranca *et al*. (1993), Ritt *et al*. (1997)		215250, 251300, 256350, 256370, 256020
Neuronal abiotrophy	Sandefeldt *et al*. (1973), Delahunta and Averill (1976)		253300
Neuropathy, giant axonal	Duncan *et al*. (1981)		256850, 256851
Neuropathy, peripheral	Furuoka *et al*. (1992)		551550, 162370, 201300, 256800, 256810, 256850, 256851, 162375, 162500, 118220, 162375, 256855, 162380, 256860, 256840, 310470, 162400, 310490, 256870, 162600
Neutropenia, cyclic	Conklin (1957), Allen *et al*. (1996)		162800
Niemann–Pick disease	Bundza *et al*. (1979)	Sphingomyelinase	257200
Onychodystrophy	Scott *et al*. (1995), Verde and Basurco (2000)		106990, 106995, 107000
Osteochondromatosis	Caporn and Read (1996)		127820, 133700, 208230
Osteochondrosis	Bergsten and Nordin (1986), Necas *et al*. (1999)		181440, 188700, 259200
Osteochondrosis dissecans	Fox and Walker (1993), Johnston (1998)		181440, 188700, 259200
Osteodystrophy	Woodard (1982), Abeles *et al*. (1999)		600430, 103581, 103580, 300800, 203330
Osteogenesis imperfecta	Calkins *et al*. (1956), Lazar *et al*. (2000)		120150, 120160, 166260, 259770, 259420, 259440, 166240, 259400, 259410, 166210, 166200, 259450, 166220, 166230, 166260
Otitis media, susceptibility to	Holt and Walker (1997), Dvir *et al*. (2000)		166760
Pancreatic insufficiency, exocrine	Westermarck (1980), Wiberg *et al*. (1999)		260450
Patent ductus arteriosus	Patterson and Detweiler (1967), Kosztolich *et al*. (2000)		169100, 233500, 243185
Pelger–Huet anomaly	Feldman (1975), Mott *et al*. (1997)		260570

Pemphigus	Shinya *et al*. (1996), Marsella (2000)		169600, 169610, 169615
Persistent Müllerian duct syndrome	Meyers-Wallen *et al*. (1989), Jonen and Nickel (1996)		261550, 235255
Platelet delta-storage pool disease	Callan *et al*. (1995)		185050, 600515
Pneumothorax	Holtsinger *et al*. (1993), Valentine *et al*. (1996)		173600
Polycystic kidney disease	Liu *et al*. (1998), O'Leary *et al*. (1999)		263200, 600666, 600273, 263210, 600330, 600595, 263100
Polycythaemia	Donovan and Loeb (1959), Faissler *et al*. (1998)		133100, 263300, 263400
Polyglandular autoimmune syndrome, type II	Smallwood and Barsanti (1995)		269200
Polyuria	Belshaw (1995)		239350
Portosystemic shunt	Rothuizen *et al*. (1982), Hunt *et al*. (2000)		601466
Prekallikrein deficiency	Otto *et al*. (1991)		229000
Prognathism	Gruneberg and Lea (1940), Phillips (1945)		176700
Pyruvate kinase deficiency of erythrocyte	Tasker *et al*. (1969), Skelly *et al*. (1999b)	Pyruvate kinase	266200
Renal dysplasia	Murphy (1989), Olenick (1999)		266920, 266900, 266910
Respiratory distress syndrome	Jarvinen *et al*. (1995)		267450
Retinal and skeletal dysplasia	Carrig *et al*. (1977), Pugh and Miller (1995)		266920
Retinal degeneration II	Parry (1953a), Akhmedov *et al*. (1997)	Rhodopsin	180380
Retinal pigment epithelial dystrophy	Lightfoot *et al*. (1996), Veske *et al*. (1999)		136550, 179840
Retinoschisis	Schuh (1995)		180270, 268080, 268100, 312700
Rhabdomyolysis	Amberger (1995), Jacobson and Lobetti (1996)		268200
Right ventricular cardiomyopathy	Simpson *et al*. (1994), Bright and McEntee (1995)		107970
Rod–cone degeneration, progressive	Aguirre and Acland (1988), Gu *et al*. (1999b)		600852
Rod–cone dysplasia-1	Parry (1953b), Aguirre *et al*. (1999)	Retinal rod photoreceptor cGMP phosphodiesterase, beta subunit	180072
Rod–cone dysplasia-3	Petersen-Jones *et al*. (1999), Petersen-Jones and Zhu (2000)	Cyclic guanosine monophosphate phosphodiesterase, alpha subunit	180071
Scoliosis	Bagley *et al*. (1997)		181800, 182210, 555000

Table 5.1. *Continued*

Disorder or trait	Earliest reference; latest reference (if different from earliest reference)	Peptide	MIM number(s) for homologous human disorders/traits [http://www3.ncbi.nlm.nih.gov/Omim/]
Severe combined immunodeficiency disease, X-linked	Jezyk *et al*. (1989), Hartnett *et al*. (2000)	Interleukin-2 receptor, Gamma	308380, 300400, 312863
Sex reversal: XX male	Hare *et al*. (1974), Meyers-Wallen *et al*. (1999)		154230
Spina bifida	Parker *et al*. (1973)		182940, 183802, 301410
Spondylosis deformans	Glenney (1956), Langeland and Lingaas (1995)		184300
Stomatocytosis	Pinkerton *et al*. (1974), Slappendel *et al*. (1991)		185000, 185010, 185020
Subaortic stenosis	Patterson and Detweiler (1963), Orton *et al*. (2000)		192600, 271950, 271960
Syndactyly	Anon. (1961), Renoy and Balligand (1991)		212780
Syringomyelia	Itoh *et al*. (1996), Rusbridge *et al*. (2000)		186700, 272480
Systemic lupus erythematosus	Goudswaard *et al*. (1993), Chabanne *et al*. (1999)		152700
Tachycardia	Gilmour and Moise (1996), Moise *et al*. (1997)		192605, 272550
Testicular feminization	Meyers-Wallen (1993)	Androgen receptor	313700
Tetralogy of Fallot	Patterson (1972), Oguchi *et al*. (1999)		187500, 187501, 239711
Thrombasthenia	Brooks and Catalfamo (1993), Boudreaux *et al*. (1996)		273800, 187800, 187900
Thrombocytopenia	Eksell *et al*. (1994), LeGrange *et al*. (2000)		274000, 188030, 600588, 147750, 187900, 188000, 188025, 141000, 235400, 188020, 273900, 223340, 274150, 301000, 313900, 314000, 314050, 137560
Thrombocytopathy	Dodds (1967), Degopegui and Feldman (1998)		185070
Thrombocytopenic purpura, autoimmune	Brodey and Schalm (1963), Lewis and Meyers (1996)		188030
Thrombopathia	Patterson *et al*. (1989), Brooks and Catalfamo (1993)		185050
Tremor	Kollarits (1924), Wagner *et al*. (1997)		190200, 190300, 190310, 214380
Tremor, X-linked	Griffiths *et al*. (1981), Cuddon *et al*. (1998)	Proteolipid protein	312080
Urolithiasis	Keeler (1940), Osborne *et al*. (1999)		220150
Vasculopathy	Rest *et al*. (1996), Fondati *et al*. (1998)		192315, 225790
Vitiligo	Wisselink (1993)		193200, 221350, 270680, 277465
Von Willebrand disease	Dodds (1971), Riehl *et al*. (2000)	Von Willebrand factor	193400, 177820, 231200, 277480, 314560
Von Willebrand disease I	Brooks and Catalfamo (1993), Johnstone (1999)		193400, 277480
Von Willebrand disease III	Brooks and Catalfamo (1993), Venta *et al*. (2000)		193400, 277480
Wilms tumour	Shibuya *et al*. (1996), Pearson *et al*. (1997)		194070, 194071, 194090, 194080, 194072
Wilson disease	Hardy *et al*. (1975), Holmes *et al*. (2000)		277900
Xanthinuria	Vanzuilen *et al*. (1996), Flegel *et al*. (1998)	Xanthine oxidase	278300

of phenotypes, there is always room for differences in interpretation of data for certain phenotypes, and authors differ in their levels of expertise, experience and time that has been devoted to canine disorders. In covering all species of domesticated animals, the present author, for example, lacks the detailed clinical knowledge and canine-specific expertise put to such good use by the authors of Chapters 9, 10 and 18. It is to be hoped that sometime in the future, the resources of cataloguers can be pooled to provide online access to up-to-date, detailed information on all inherited disorders/traits in dogs.

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6 Biochemical Genetics and Blood Groups

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Introduction

During 1940–1960, a number of innovative techniques were described that enabled the detection of diverse qualitative genetic variations at the biochemical level within a species. These variations, reported first mostly in humans, were mainly of the following categories: (i) Blood groups (red cell antigens), studied by serological methods, (ii) inherited variants of proteins, detected by gel electrophoresis. A red cell antigen is termed as a blood group if it is proved to be present in some, but not all, individuals of a species. The electrophoresis methods brought about a revolution for revealing and typing

the different allelic variants of a given protein in a population. In this context, a polymorphic genetic system was defined as one in which there are two or more allelic variants, no one of which has a frequency of more than 99% in the population. In dogs, relatively few highly polymorphic proteins were reported until 1980. Notable among these were three plasma proteins (albumin, transferrin and esterase) and two red cell enzymes (superoxide dismutase and acid phosphatase). A breakthrough was achieved in the early 1980s when the dog plasma samples were analysed by improved methods using one- and two-dimensional polyacrylamide gel electrophoresis. This revealed five additional, highly polymorphic plasma proteins and also resulted in a better resolution of the transferrin variants. All of these proteins were clearly visible by an easy and inexpensive general protein staining technique (see Juneja *et al.*, 1987a). This showed that, as in some other economically important domestic animals, a number of easily available plasma protein markers were present in purebred dogs and these were thus used for parentage testing and different genetic studies in this species. Subsequently, by using immunoblotting, polymorphisms of different canine plasma complement proteins have also been described. The initial interest in characterizing the blood groups of dogs was to develop an animal model for studying the immune destruction of erythrocytes which occurs during incompatible blood transfusion. The first systematic study on canine blood groups was conducted by Swisher, Young and co-workers during 1949–1961. In all, 13 different blood group systems have so far been described. Canine blood grouping is now routinely performed in service laboratories in order to choose suitable donor blood for the purpose of blood transfusion within this species.

We give here a review on the different canine biochemical and serological genetic markers (biochemical polymorphisms and blood groups) and their significance in dog genetics and medicine. Previous reviews on the canine blood groups have been presented by Swisher and Young (1961), Bell (1983), Hale (1995) and Giger (2000a). A detailed description of several canine biochemical polymorphisms was given, in monograph form, by Tanabe (1980). Data on gene frequencies at several blood protein loci in various dog breeds of Asia and Europe were reported by Tanabe (1980) and Tanabe *et al.* (1991). A detailed compilation of canine blood protein and blood group gene frequency data has been given by Lachmann (1993).

Biochemical Polymorphisms

The biochemical polymorphisms studied in dogs comprise mainly the proteins of blood plasma (serum) and erythrocytes and also a few in leucocytes.

Plasma proteins

The different known plasma protein polymorphisms of dogs are listed in Table 6.1. Many of the canine plasma proteins show a high degree of polymorphism

Table 6.1. List of reported plasma protein systems in dogs

Plasma protein	Locus	Alleles	Methods[a]	References
Albumin	*ALB*	2	Acid StGE	1
Alpha1B-glycoprotein	*A1BG*	4	1D PAGE or 2D-AgGE-PAGE	2,3
Alpha1-protease inhibitor	*PI*	4	1D PAGE or 2D-PAGE or IEF	2,3,4
Apolipoprotein A4	*APOA4*	4	ID PAGE or 2D-AgGE-PAGE	3,5
Arylesterase	*ARE*	7	IEF	6
Esterase-1	*ES-1*	3	Alkaline StGE	7
Leucine amino peptidase	*LAP*	2	Alkaline StGE	8
Postalbumin 1	*PA1*	2	2D-AgGE-PAGE	3
Postalbumin 3	*PA3*	2	2D-AgGE-PAGE	3
Postalbumin 4	*PA4*	2	2D-AgGE-PAGE	3
Pretransferrin 1	*PRT1*	4	2D-AgGE-PAGE	3
Pretransferrin 2	*PRT2*	2	2D-AgGE- PAGE	3
Transferrin	*TF*	5	1D-PAGE	2
Complement C3	*C3*	2	AgGE and immunofixation	9
Complement C4	*C4*	11	AgGE and immunoblotting	10
Complement C6	*C6*	8	IEF and immunoblotting	11,12
Complement $C7^1$	$C7^1$	3	IEF and haemolytic detection	11
Complement $C7^2$	$C7^2$	3	IEF and haemolytic detection	11

[a]1D, one-dimensional; 2D, two-dimensional; StGE/AgGE/PAGE, starch gel/agarose gel/polyacrylamide gel electrophoresis; IEF, isoelectric focusing. The 2D-AgGE-PAGE consists of first-dimensional separation by agarose gel electrophoresis (pH 5.4) and the second one by horizontal PAGE, pH 9.0 (see text).
References: 1 – Christensen *et al*. (1985); Scherer and Kluge (1993); 2 – Juneja *et al*. (1981a); 3 – Juneja *et al*. (1987a); 4 – Braend (1988a); Sevelius *et al*. (1994); Federoff and Kueppers (2000); 5 – Juneja *et al*. (1989); 6 – Braend and Andersen (1987); 7 – Sugiura *et al*. (1977); 8 – Tanabe *et al*. (1974); 9 – Kay *et al*. (1985); 10 – Doxiadis *et al*. (1987); 11 – Eldridge *et al*. (1983); 12 – Shibata *et al*. (1995). The references given here are with regards to the method of analysis; for all other references, see text.
Note: Preliminary data on polymorphism of dog plasma haptoglobin (HP), GC protein and an unidentified acid glycoprotein(ACG) have also been reported (see text).

and are revealed by inexpensive protein staining procedures. Thus the phenotypes of some of these proteins have been studied in several breeds. We give here some salient features of the different polymorphisms.

Albumin (ALB)

By using acidic starch gel electrophoresis (pH 4.0), two *ALB* alleles have been reported. Both of the alleles are present in almost equal frequencies in many breeds (Day *et al*., 1971; Blirup-Jensen, 1976; Christensen *et al*., 1985; Tanabe *et al*., 1991; Jordana *et al*., 1992; Lachmann, 1993; Scherer and Kluge, 1993). Christensen *et al*. (1985) indicated a possible association between the ALB^S allele frequencies and the bone length of the dog breeds. It is, in general, difficult to get a clear separation of the dog ALB variants.

Alpha1B-glycoprotein (A1BG) and apolipoprotein A4 (APOA4)
Genetic polymorphism of two unidentified dog plasma proteins (called postalbumin, Pa, and postalbumin 2, Pa2) was reported by Juneja *et al.* (1981b, 1987a). These were later identified, respectively, as A1BG and APOA4 by conducting immunoblotting with antiserum specific to human plasma proteins (Juneja *et al.*, 1987b, 1989). The two common alleles, $A1BG^F$ and $A1BG^S$, are present in many breeds. Later $A1BG^D$ was found in breeds such as Golden Retriever and Dalmatian and $A1BG^I$, in Flat Coated Retriever. The A1BG D variant moves slightly ahead of the F variant while the I variant is located between the F and S variants (Juneja *et al.*, 1992). Three common alleles of *APOA4* ($APOA4^D$, $APOA4^F$ and $APOA4^S$) are present in several breeds (Juneja *et al.*, 1987a, 1989). A fourth variant, called APOA4T, with mobility slower than the S variant, was found in Border Terriers (R.K. Juneja, unpublished results). The function of the mammalian A1BG is as yet unknown. However, in the opossum, a marsupial, the homologue of A1BG was shown to be a metalloproteinase inhibitor that partially accounted for the resistance of that marsupial to the effects of the rattlesnake venom metalloproteinase (Catanese and Kress, 1992).

Alpha1-protease inhibitor (PI)/alpha1-antitrypsin (AAT)
Three common alleles PI^F, PI^I and PI^S in the PI system (then called PI I) were reported by Juneja *et al.* (1981b). Later a fourth allele, PI^M, was observed in Siberian Husky (Juneja *et al.*, 1992) and in the Akbash breed, native to Turkey (Morris and Lemon, 1992). The PI M variant has a migration between the PI I and PI S variants. The 1-D PAGE technique enables scoring of all of the four PI variants. Improved separation of dog PI variants was obtained by isoelectric focusing followed by immunofixation or immunoblotting with antiserum specific to canine PI protein (Kueppers *et al.*, 1993; Sevelius *et al.*, 1994). Braend (1988a) and Federoff and Kueppers (2000), using isoelectric focusing in both studies, reported three PI variants in Alaskan wolves, Grey and Mexican wolves with mobility identical respectively to the F, I and S variants of dogs. Vatne *et al.* (1996) reported that the elastase-inhibitory capacity of the isolated canine PI I variant, at pH 6.8, was significantly less than that of PI F and PI S variants. They hypothesized that in the case PI II genotype dogs, the PI protein may not be sufficiently effective to inhibit the proteases released in the liver as a result of some disease in that organ and this can then subsequently lead to liver cirrhosis. Melgarejo *et al.* (1996), on the basis of amino acid composition, provided evidence that the polymorphic PI protein of dogs is homologous to the human α1-antitrypsin (PI).

Eserine-resistant esterase (ES-1) and arylesterase (ARE)/paraoxonase (PON1)
Three alleles of *ES-1* were reported by using starch gel electrophoresis. The $ES\text{-}1^C$ allele was observed with a low frequency only in some Japanese breeds. Most of the European breeds have two common alleles (Sugiura *et al.*, 1977; Tanabe, 1980). Braend (1984), using isoelectric focusing (pH 4.2–4.9), obtained an improved resolution of a group of esterase fractions and

characterized that enzyme as arylesterase. A total of seven alleles were reported. Most of the breeds showed two common alleles and some rare alleles (Braend and Andersen, 1987; Piette, 1988). Braend and Roed (1987) reported four *ARE* alleles in Alaskan wolves and three of those alleles also occurred in dogs. Both in human and mouse, plasma arylesterase is called paraoxonase with locus symbol *PON1*. This enzyme is known to have important anti-atherogenic functions (PrimoParmo *et al.*, 1996). It is, in general, difficult to get a reproducible separation of the canine plasma ARE fractions.

Complement components C3, C4, C6, $C7^1$ and $C7^2$

Genetic polymorphisms of canine C3, C4 and C6 proteins were reported by conducting immunoblotting using antiserum specific to human, canine or rabbit complement components. Two alleles of *C3*, present with almost equal frequencies in a random sample of dogs, were described by Kay *et al.* (1985). In addition, a null allele of *C3* in a colony of Brittany Spaniels has also been reported. The C3-deficient dogs show a predisposition to recurrent bacterial infections and to type 1 glomerulonephritis (Ameratunga *et al.*, 1998). At the *C4* locus, eight different alleles were detected by agarose gel electrophoresis of plasma samples and three additional alleles by SDS-PAGE of the immunoprecipitates. Three of the alleles occurred with considerable frequency in a sample of various breeds (Doxiadis *et al.*, 1987). Shibata *et al.* (1995) described at the *C6* locus, a total of seven structural alleles and a null allele in Asian and European dog breeds. Among the three European breeds studied, two (Beagle and Pointer) showed three common alleles each, while the third (German Shepherd) was monomorphic. In the case of *C7*, there are two tightly-linked loci ($C7^1$ and $C7^2$), each with three alleles. There was some indication of a null allele or locus deletion at one of the loci. *C7* polymorphisms were studied by using isoelectric focusing and a haemolytic detection method (Eldridge *et al.*, 1983).

Transferrin (TF)

In all, five alleles have been reported in a vast number of dog breeds studied. The TF^B and TF^C alleles are the most frequent ones in all European and Asian breeds examined. TF^A was observed in a few breeds including German Shepherd and Poodle, TF^D in only two European breeds (Cocker Spaniel and Mastin Espanol) and in some Japanese breeds and TF^E exclusively in Arctic breeds like Siberian Husky, Borzoi, Alaskan Husky, Eskimo Dog, Nova Scotia Duck Tolling Retreivers and Samoyed. TF variants B and C have very similar mobilities but these are separated clearly from one another by using a 12% acrylamide concentration in the separation gel (Stevens and Townsley, 1970; Juneja *et al.*, 1981a; Reetz, 1981; Tanabe *et al.*, 1991; Jordana *et al.*, 1992; R.K. Juneja, unpublished results). Isoelectric focusing resulted in improved separation of canine TF variants but no further subtypes were detected (Braend and Andersen, 1987). Braend and Roed (1987) reported two common *TF* alleles in 146 Alaskan wolves; the two alleles were indistinguishable from the TF^B and TF^C alleles of dogs.

Haptoglobin (HP) and GC protein (GC)

The HP fractions in plasma are generally identified by their ability to form a complex with haemoglobin. Some authors have described the absence of this complex in certain dogs and have hypothesized the occurrence of an *HP* null allele (see Scherer and Kluge, 1993). A similar conclusion was drawn by Yoshida and Ikemoto (1986) who used a method of 2-D electrophoresis. This HP variation needs to be considered tentative since very limited inheritance data have been given for it. Recently Andersson *et al.* (1998) studied the HP fractions by isoelectric focusing, followed by immunoblotting and observed only one phenotype in a random sample of 50 dogs. They, however, observed disease-related variations in the glycosylation of HP fractions. A brief report indicating GC polymorphism in mongrel dogs was given by Yoshida and Ikemoto (1986). Clear evidence of GC polymorphism was observed by us in one family of Chihuahua breed. One sire and two of its offspring were heterrozygotes. The new GC variant moved ahead of the common variant in alkaline PAGE (Juneja *et al.*, 1992).

Alkaline phosphatase (ALP) and leucine aminopeptidase (LAP)

Tanabe (1980) reported six different phenotpes of alkaline phosphatase (ALP) and proposed the control by three codominant alleles. Symons and Bell (1992b) observed three different ALP phenotypes in various European breeds. The genetic control, however, was not clear. Assuming genetic control of two codominant alleles, the distributions of types in families differed significantly from expectations. Two alleles of leucine aminopeptidase (LAP), present in several breeds, have been described (Tanabe *et al.*, 1974; Tanabe, 1980; Jan and Bouw, 1983).

Unidentified plasma proteins

Polymorphism of five as yet unidentified plasma proteins called postalbumin 1, 3 and 4 (PA1, PA3 and PA4) and pretransferrin 1 and 2 (PRT1 and PRT2) was reported by Juneja *et al.* (1987a) and Juneja and Shibata (1992). PA1, PA3 and PRT1 show a high degree of polymorphism in almost all breeds. PRT1 B variant migrating ahead of PRT1 D was later observed in Greyhound, Whippet and in Finnish Lapphund (Juneja *et al.*, 1992; R.K. Juneja, unpublished results). PA3 fractions were not observed in electrophoretograms of fresh plasma but only in those plasma samples stored at −20°C for more than a year. It is thus probable that PA3 is the canine apolipoprotein A1(APOA1) that gets gradually released from the plasma lipid particles during the storage of plasma samples. This is supported by the results of Miller *et al.* (1994) who observed intensely stained fractions of APOA1 in dog plasma using a method of high-resolution 2D-electrophoresis, conducted under denaturing conditions. Miller *et al.* (1994), using immunoblotting with human specific antisera or by amino acid microsequencing, identified apo A4, complement factor B, GC protein, β-haptoglobin, β- and γ-fibrinogen and IgG heavy chain fractions in the electropherogram of the dog plasma samples. Yoshida and Ikemoto (1982) reported a polymorphic acid glycoprotein (ACG) with two phenotypes

(presence or absence of the ACG fraction) by SDS–polyacrylamide gel electrophoresis. The protein was presumed as an acid glycoprotein with PAS staining positive; its molecular weight was 34 kDa and its isoelectrofocusing point about 4.5. It is probable that ACG may be identical to PA3 protein of Juneja *et al.* (1987a).

Red cell enzymes

Genetic polymorphism has been described for canine haemoglobin and a number of red cell enzymes. The phenotypes at some of the following loci have been studied to a considerable extent.

Haemoglobin (HB)
Tanabe *et al.* (1978) first reported *HB* polymorphism in dogs; the variant allele, HB^{A} was present only in some Japanese breeds with a frequency of about 0.08. Later HB^{A} was found to be the predominant allele in some other Japanese and Korean breeds although it was totally absent in the European breeds (Tanabe *et al.*, 1991). Braend (1988b), using isoelectric focusing with immobiline pH gradient gels (pH 7.0–7.6), described another type of *HB* polymorphism; two common *HB* alleles were observed in most of the European breeds studied. It is not yet known if the above variations are in the genes controlling the alpha or the beta chain of the HB molecule.

Superoxide dismutase (SOD)
Two alleles of *SOD* (previously called tetrazolium oxidase) have been reported in several breeds. The variant allele is present with a considerable frequency (0.2–0.3) in Poodle, Boxer, Terrier, German Shepherd and Collie breeds (Baur and Schorr, 1969; Reetz, 1981; Tanabe *et al.*, 1991; Jordana *et al.*, 1992; Lachmann, 1993).

Glucose phosphate isomerase (GPI)
Tanabe *et al.* (1977) reported two alleles and the variant allele, GPI^{B}, was present almost exclusively in some of the Japanese breeds. Among the European breeds, they reported one out of 14 Dalmatians to be a heterozygote. GPI B variant has been reported also in Bangladesh Native Dog and Caucasian Sheepdog (Knyazev *et al.*, 1993) and in Azawakh and Sloughi, both breeds of African origin (Scherer and Kluge, 1993). Further work has shown that the GPI^{B} allele is present with a considerable frequency in the dogs of the Dalmatian breed in Sweden and Finland (Juneja *et al.*, 1994; R.K. Juneja, unpublished results). This may indicate that the Dalmatian breed most probably originated in Asia or Africa and not in Europe. A third allele, GPI^{C}, was reported to be present as a rare allele in the Chow Chow breed by Arnold and Bouw (1989). We used agarose gel electrophoresis (pH 8.6–barbital buffer system) of haemolysate samples for the scoring of dog GPI and SOD variants on the same gel (R.K. Juneja, unpublished results).

Acid phosphatase (ACP), peptidase D (PEPD) and catalase (CAT)
Two alleles of red cell acid phosphatase have been described. The variant allele has been reported to be present with considerable frequency (0.1–0.3) in many Japanese and European breeds (Braend and Austad, 1973; Tanabe, 1980; Rehm, 1988).

Two common alleles of *PEPD* have been reported in several European breeds (Saison, 1973; Burghoff, 1982; Jan and Bouw, 1983). A third rare allele was observed in the Beagle breed (Vriesendorp *et al.*, 1976). Tanabe (1980) provided evidence for two *CAT* alleles in various breeds. In addition, a null allele in the homozygous form leading to hereditary acatalasaemia has been described in some reports. The null allele was present with considerable frequency in, for instance, Pointer, Maltese and Beagle breeds (Allison *et al.*, 1957; Simonsen, 1976; Tanabe, 1980; Fukuda *et al.*, 1982). It is, in general, difficult to get a suitable resolution of the canine PEPD and CAT variants.

Miscellaneous enzymes
Genetic polymorphism was reported, in brief, of some other red cell enzymes as given below: red cell esterase 2 and 3 (Tanabe, 1980); esterase D, ESD in the Basenji breed (Weiden *et al.*, 1980); glutamate oxalacetate transaminase, GOT1 (Weiden *et al.*, 1974); phosphoglucomutase (PGM) 1, 2 and 4 (Khan *et al.*, 1973; Wong *et al.*, 1974; Pretorius *et al.*, 1975); glucose-6-phosphate dehydrogenase, G6PD (Vriesendorp *et al.*, 1976). In addition, polymorphism of two leucocyte enzymes, PGM3 (Vriesendorp *et al.*, 1976) and mannose phosphate isomerase, MPI, has also been reported (Jan and Bouw, 1983). A detailed study on the polymorphism of the sialic acid species of haematoside, which is the major glycolipid of the erythrocyte membrane, was reported in various dog breeds (Hashimoto *et al.*, 1984). Polymorphism of red cell potassium and glutathione levels in the Japanese Shiba Dog was reported by Fujise *et al.* (1997).

Significance of Blood Genetic Markers

The canine blood genetic markers have been used in different aspects of genetics, breeding and medicine as given below. While the biochemical variants have been used for parentage testing and population studies, the application of blood groups has mainly been in the context of blood-transfusion.

Plasma protein loci heterozygosity and parentage testing

Many of the dog plasma proteins (ALB, TF, A1BG, APOA4, ARE, ES-1, PI, PA1, PRT1, C4, C6 and C7) show a high degree of polymorphism (Table 6.1). There are generally two or three common alleles present for each of the above loci in most of the European breeds. Dog C4 shows an extensive degree

of polymorphism with eight considerably frequent and three less frequent alleles (Doxiadis *et al.*, 1987). The average proportion of heterozygotes at each of the following plasma protein loci (*TF*, *PI*, *PA1*, *PRT1*, *A1BG*, *C6* and *APOA4*) is 30–35% in most of the common European dog breeds (Aarskoug *et al.*, 1992; Lachmann, 1993; Shibata *et al.*, 1995; R.K. Juneja, unpublished results). This is in contrast to the lack of polymorphism observed for many of the dog red cell enzymes (Weiden *et al.*,1974; Fisher *et al.*, 1976; Simonsen, 1976; Jordana *et al.*, 1992). Some of the above plasma protein markers were thus found to be highly useful for the purpose of parentage testing. In our laboratory in Uppsala, we have used a scheme that involves only two tests as follows: (1) analysis of plasma samples by one-dimensional horizontal PAGE, followed by protein staining of the gel; (2) analysis of plasma samples by a simple method of 2-D electrophoresis – first dimension separation by agarose gel electrophoresis (pH 5.4) and the second one by horizontal PAGE (pH 9.0), followed by protein staining of the gel. These two tests allow the simultaneous phenotyping of six highly polymorphic proteins (TF, PI1, AIBG, APOA4, PA1 and PRT1) and two others, PA4 and PRT2 (Juneja *et al.*, 1987a; Juneja and Shibata, 1992; for general details of these methods, see Juneja and Gahne, 1987). The theoretical probability of excluding a wrongly assigned parentage by this scheme is 80–90% in most breeds, when the number of offspring tested of the given litter ranged from one to four (R.K. Juneja, unpublished results; Gundel and Reetz, 1981). Many laboratories are now exclusively using canine DNA microsatellite polymorphisms for parentage testing in dogs.

Linkage data

The following genetic linkages, involving the blood genetic markers, have been reported in dogs.

1. Linkage of the *C4* gene with the genes of the *MHC* (see Doxiadis *et al.*, 1987).
2. Tight linkage between the *C6*, $C7^1$ and $C7^2$ genes. Dog is the only species in which a duplication of the *C7* locus has been proposed (Eldridge *et al.*, 1983; Hobart, 1998).
3. Linkage between the gene for coat colour extension locus (*E*) and the plasma esterase locus *ES-1*, by studying a large sire-family of Beagle breed. The recombination distance between the two loci was about 34 cM. A homologous linkage was reported earlier in horse, rabbit and mouse (Arnold and Bouw, 1990).
4. By typing the samples of the DogMap reference panel, the linkage relations of four plasma protein genes were described; *APOA4* and *A1BG* were assigned respectively to chromosomes 5 and 1; *TF* to the linkage group L05 and *PI* to L06 (Jonasdottir *et al.*, 1999; Lingaas *et al.*, 2001).
5. Juneja *et al.* (1994), by studying a large sire-family of Dalmatian dogs, reported linkage between the loci for glucose phosphate isomerase (*GPI*) and plasma alpha1B-glycoprotein (*A1BG*). Four recombinants were observed

among a total of 35 informative offspring, giving a recombination frequency of about 11% between the two loci. In pigs, the MHS (malignant hyperthermia susceptibility) gene (halothane gene, *HAL*/calcium release channel gene, *RYR1*) is closely linked to *GPI* and *A1BG* genes (see Juneja *et al.*, 1987b). In dogs, *RYR1* has been mapped to chromosome 1 and recently it was indicated that the canine MHS gene co-segregates with a presumed mutation in *RYR1* (Roberts *et al.*, 2000). In humans, the loci *A1BG-RYR1-GPI* are located on chromosome 19q while in dog, these three loci are localized to chromosome 1. Thus linkage between these three loci has been observed in all the three species examined so far (pig, human and dog).

6. Juneja and Shibata (1992) reported tight linkage between the loci for two as yet unidentified plasma proteins PA4 and PRT1. The *PA4/PRT1* haplotype frequencies in several breeds were reported.

Population studies

Genetic relationships between different dog breeds, on the basis of blood protein gene frequencies, have been given in some reports. Jordana *et al.* (1992) studied genetic distance between ten different Spanish breeds. Tanabe *et al.* (1991) estimated gene frequencies at 14 variable loci and genetic distances in 40 different dog breeds of Asia and Europe. They have, in particular, elucidated the probable origins of the Japanese native dogs. Knyazev *et al.* (1993) indicated that the native dog breeds of central Eurasia occupy an intermediate position between the East Asian and the European breeds. Several marked differences in gene frequencies at different loci between the Asian and the European dogs have been reported. For instance, the frequency of HB^{A} ranged from 0.1–1.0 in many of the Japanese and Korean dogs but this allele was totally absent in the European breeds (Tanabe *et al.*, 1991). In the plasma *PA4* system, while many of the breeds of middle and north-eastern Asia showed a substantial frequency (0.1 to 0.6) of the *S* allele, a majority of the European breeds had only the *F* allele (Juneja and Shibata, 1992). Tanabe *et al.* (1991) reported that the degree of heterozygosity was higher in Korean and Japanese native breeds than in the European breeds. Lachmann (1993), by analysis of five polymorphic plasma protein loci, classified the race dogs Saluki, Whippet and Sloughi to one group and Greyhound, Windspiel and Afghan Hound to another group. Juneja *et al.* (1981b) compared the observed gene frequencies at *TF* and *PI* loci in a set of five breeds from Sweden and from Germany. In all cases, each breed from the two countries showed very similar gene frequencies.

Blood Groups

The first systematic study on canine blood groups was by Swisher, Young and co-workers, conducted between 1949 and 1961, when they defined seven

blood group systems. Their work still remains the most prominent one with respect to the roll of canine blood groups in transfusion (Swisher and Young, 1961; Swisher *et al.*, 1962). An additional six blood group systems were identified in subsequent studies (Bowdler *et al.*, 1971; Suzuki *et al.*, 1975; Colling and Saison, 1980a; Symons and Bell, 1992a). A comprehensive review on the history, methodology and genetic aspects of the canine blood groups was given by Bell (1983). The role of canine blood groups in transfusion medicine was discussed in detail in the excellent reviews of Hale (1995) and Giger (2000a). The data on canine blood group gene frequencies in different breeds were given by Ejima *et al.* (1986, 1994), Symons and Bell (1992b), Usui *et al.* (1995) and Giger *et al.* (1995).

We have given here a brief review on the canine blood groups. Red blood cell antigens in the dog are largely recognized through agglutination with polyclonal antisera, produced by canine alloimmunization. Currently, antisera to recognize five DEA (dog erythrocyte antigen) systems, is available through commercial typing services. Table 6.2 contains data on population incidence, relative significance and the alternate nomenclature systems used.

DEA 1.0 system

The DEA 1.0 system has been identified independently by groups in the United States, Australia and Canada. This system has multiple alleles, including 1.1, 1.2, 1.3 and a null allele. Only one of the four phenotypes (1.1, 1.2, 1.3 and null type) may be demonstrated in an individual. Autosomal dominance (Mendelian) has been demonstrated in family studies performed by Young *et al.* (1951), Colling and Saison (1980a) and Symons and Bell (1991). There is a linear order of dominance with 1.1 being the most dominant of the four alleles. Thus phenotype 1.1 can have one of the four possible genotypes: 1.1/1.1, 1.1/1.2, 1.1/1.3, 1.1/–.

Table 6.2. Dog erythrocyte antigen nomenclature, population incidence and significance

DEA group	'Old' nomenclature	Population incidence (%)	Natural occurrence of antibody	Transfusion significance
1.1	A_1	42	No	Acute haemolytic
1.2	A_2	20	No	Acute haemolytic
3	B	6	Yes	Delayed, non-haemolytic
4	C	98	No	None
5	D	23	Yes	Delayed, non-haemolytic
7	Tr	45	Yes	Delayed, non-haemolytic

Note: Only limited surveys on the incidence (frequency) of canine blood types have been reported. The incidence values given here are adopted from Dodds (1985) and are based on data from random dog populations in different countries. These values are very similar to the estimates compiled by Giger (2000a).

Gene frequencies in various breeds were reported by Ejima *et al.* (1986) and Symons and Bell (1991). Naturally occurring antibodies to the 1.0 system have not been documented. However, repeated sensitization of negative individuals with positive cells leads to potent IgM and IgG production. Prior sensitization leads to an acute haemolytic reaction and premature removal of cells in the recipient. In this context, the DEA 1.1 is the most antigenic blood type. Thus, a previously sensitized DEA 1.1-negative dog will develop an acute haemolytic reaction after transfusion of DEA 1.1-positive blood (Young *et al.*, 1952; Giger *et al.*, 1995). A monoclonal murine antibody recognizing DEA 1.1 has been successfully produced and maintained by Andrews *et al.* (1992). This has led to the recent development of a blood-typing card for classifying dogs as DEA 1.1 positive or negative as a simple in-practice kit. The test is based on an agglutination reaction that occurs within 2 minutes when erythrocytes that are DEA 1.1 positive interact with the monoclonal antibody specific to DEA 1.1 (for details see Kohn *et al.*, 1998; Giger, 2000a).

A known association exists between DEA 1.1 and neonatal isoerythrolysis (NI) in the dog. DEA 1.1-negative bitches bred to DEA 1.1-positive male dogs may produce DEA 1.1-positive puppies. If the bitch has been mated in this way once before, or has had a red blood cell transfusion, then she is sensitized to DEA 1.1 antigen. Antibody to DEA 1.1 is transferred to the puppies through the colostrum. The DEA 1. 1-positive puppies, normal at birth, show immune-mediated haemolytic anaemia in 3–10 days (Young *et al.*, 1952; for details, see Hale, 1995. Giger (2000b) concluded that NI is not a clinical problem in dogs unless the bitch has previously received blood products and acute haemolytic transfusion reactions are encountered only when a dog receives a blood transfusion 4 or more days after the first transfusion.

DEA 3.0 system

The DEA 3.0 system consists of two antigens: 3.0 and the null type (Swisher *et al.*, 1962; Colling and Saison, 1980a; Symons and Bell, 1992b). Simple autosomal dominance was documented in family studies performed earlier by Young *et al.* (1951) and by Colling and Saison (1980a). A monoclonal antibody was defined for DEA 3 by Hara *et al.* (1991). However, due to a laboratory accident, this monoclonal antibody no longer exists. Population indices in the US suggested that DEA 3 is a rare antigen found in 5–6% of the population (Swisher *et al.*, 1962). In Greyhounds, the frequency of DEA3-positive was about 23% (Hale, 1995). Ejima *et al.* (1986) reported a much higher incidence of DEA 3-positive samples in Japanese native dogs. Naturally occurring antibodies to the 3.0 system have been documented. Survival studies suggest that incompatible red blood cell transfusions with DEA 3-positive cells in sensitized individuals leads to removal of erythrocytes in 3–5 days (Young *et al.*, 1951).

DEA 4.0 system

The DEA 4.0 system consists of two antigens: 4.0 and the null type. This system has been identified independently by groups in the US, Australia, Canada, Brazil and Germany. Population indices performed in the US, Canada, Australia and Brazil suggest that DEA 4 is a common antigen occurring in 87–95% of the dogs tested (Swisher *et al.*, 1962; Giger *et al.*, 1995). Natural occurring antibody to the 4.0 system has been identified. Red blood cell survival studies using DEA 4.0-positive erythrocytes in sensitized individuals do not indicate clinical importance (Swisher *et al.*, 1962). DEA 4.0-positive dogs that are negative to all other blood group systems are considered 'universal donors'.

DEA 5.0 system

Two antigens comprise the DEA 5.0 system: 5.0 and the null type. Groups in the US, Australia and Canada have confirmed the presence of this system independently. To date, only serological evidence of this system exists. Dogs have been identified with naturally occurring antibody to DEA 5.0. Young *et al.* (1951) suggested a red blood cell survival curve similar to that found in the DEA 3.0 system. DEA 5.0 is a rare antigen occurring in about 15% of the dogs tested in the US and the presence of the antibody in a recipient is reported to shorten the survival of incompatible cells (Swisher *et al.*, 1962).

DEA 7.0 system

Two alleles, 7.0 and the null allele make up the DEA 7.0 system. Groups in the US, Canada and Australia have made independent confirmation by serological tests. Evidence for a third allele in this system was provided by Colling and Saison (1980b). DEA 7 is not a true red cell antigen but is produced in the tissues and absorbed on to the red cells. This soluble membrane antigen has been found in saliva and related to human blood group 'A' antigen (Bowdler *et al.*, 1971; Swisher *et al.*, 1973). Bull *et al.* (1975) showed that DEA 7.0 status does not change with bone marrow transplantation. A naturally occurring IgM has been identified in 20–40% of the population within the US. Sensitized individuals have demonstrated variable strength antibody with delayed red blood cell removal after incompatible transfusion (Bull, 1976). A relationship between the plasma alkaline phosphate (ALP) variants was demonstrated, with DEA 7.0 type dogs having a significant deficiency of ALP S type (Symons and Bell, 1992b).

There is, so far, very little known on the biochemical characterization of canine blood groups. Andrews *et al.* (1992) identified molecular weight bands of 200 kDa and 50 kDa for DEA 1.1 antigen. Hara *et al.* (1991) identified five different bands of molecular weight 34 kDa to 71 kDa for the DEA-3 antigen.

Corato *et al.* (1997) assigned molecular weight of 85 kDa to DEA 1.2 antigen, 32–40 kDa to DEA 4 and 50–66 kDa to DEA 7. Corato *et al.* (1997) also identified a 'human rhesus blood-group like' molecule on the dog erythrocytes. The presence of a Rh-30-like polypeptide in the erythrocyte membrane of different mammals, including the dog, was recently reported also by Apoil and Blancher (1999). In humans, the Rh blood group antigens are of crucial importance in transfusion-medicine because of their strong immunogenicity. Thus further work on the canine homologue of Rh blood group is of much interest. The human Lewis blood group antigen was shown to be the receptor for *Helicobacter pyroli* by Boren *et al.* (1993). Different studies have shown the presence of human blood group antigens ABH- and Lewis-like activities in the gastric and intestinal tissue of dogs (Horowitz *et al.*, 1961; McKibbin *et al.*, 1982). Miller-Podraza *et al.* (1997) reported that while the polyglycosylceramides (PCGs) of human erythrocytes showed strong binding affinity for *Helicobacter pyroli*, the PCG fractions from dog intestine were receptor-inactive for this bacterium or displayed only weak and poorly reproducible binding. The PCGs isolated from canine intestine showed ABH and Lewis blood group activity, and they might also be carriers of other biological specificities.

Conclusions

The studies conducted during the past five decades have revealed genetic polymorphism of about 30 different blood proteins in dogs. The canine plasma proteins, in contrast with the tissue enzymes, showed a remarkably high degree of polymorphism in the common purebred dogs. The general notion that the dog protein loci show an unusually low degree of polymorphism is thus not appropriate. Many of the plasma protein systems showed about or more than 30% heterozygotes in different breeds, which is quite near to the corresponding estimate of 40–60% reported for several dog microsatellite loci. The typing of a battery of six considerably polymorphic plasma proteins – A1BG, APOA4, PA1, PI, PRT1 and TF – by 1D- and 2D-electrophoresis and protein staining, provided a formidable test for parentage testing in dogs.

It was by virtue of genetic polymorphism that it became easy to identify alpha1-protease inhibitor (PI), alpha1B-glycoprotein (A1BG) and apolipoprotein A4 (APOA4) in dog plasma. As shown for human ApoA4 variants (see Fisher *et al.*, 1999), the reported dog ApoA4 variants may also have a significant effect on lipid metabolism. Polymorphism of four complement proteins, C3, C4, C6 and C7, were documented in canine plasma. The null alleles detected in dog complement systems may be associated with specific diseases as shown in the case of canine C3 (see Ameratunga *et al.*, 1998). The extensive studies by Tanabe and associates have revealed several significant differences in gene frequencies between the Asian and European breeds of dogs. The Asian breeds of dogs, in general, showed a higher degree of polymorphism than the European breeds (Tanabe *et al.*, 1991). The linkage assignment of

different protein loci is obviously of much importance for the purpose of comparative gene mapping..

In all, 13 different canine blood group systems have been reported. However, commercially available antiserum exists only for five systems. The significance of various dog blood groups in blood transfusion remains an integral part of canine internal medicine and genetics. It is obvious that with the present increase in the average lifespan of our pet dogs, repeated blood transfusions in the same animal are more likely and thus the blood group determination is extremely important in those cases. The development of PCR-based DNA methodology for typing of canine blood groups will allow more rapid and uniform testing. Further studies on canine blood groups are of much significance since some of the human blood groups have been found to be of much physiological importance. The Duffy blood group antigen was identified as the receptor for the malaria parasite *Plasmodium vivax* and, as stated before, the Lewis blood group antigen as receptor for *Helicobacter pyroli* (Boren *et al.*, 1993; Horuk *et al.*, 1993). Thus further data on the conventional genetic markers will continue to enrich our knowledge of the basic and applied genetics of the dog.

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Molecular Genetics of the Dog

7

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Introduction

The growing information on the canine genome shows it to be very similar to the genomes of other mammals. There are an estimated 3000 megabases (Mb) of DNA distributed between 38 pairs of autosomes and the X and Y

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chromosomes. Estimates on the number of genes in the dog have been based largely on similar estimates in other mammals and suggest somewhere in the region of 35,000–40,000 genes. The recent results from the human genome project support this estimate. However, there are data indicating that the real number of genes may be higher. Whatever the final number of genes, the majority of the canine genome is composed of non-coding DNA that is largely made up of repeated sequences. As in other mammals, these repeat sequences comprise many different types that have been subclassified into satellite, minisatellite, microsatellite, SINE, and LINE, amongst others. These repeat sequences have proven invaluable for genetic mapping studies, individual identification and parentage testing, and for studying the domestication of the dog from the wolf (along with mitochondrial DNA).

This chapter starts with a description and discussion of the major types of repeat sequence DNA in the canine genome. Mitochondrial DNA is also discussed as is what is becoming an invaluable resource for canine molecular geneticists, the canine BAC library. Although individual canine genes will not be discussed, the chapter provides a description of one multigene family in the dog, the genes for olfactory receptors. Olfaction has been one of the characteristics that breeders in the past have selected for when producing individual dog breeds. Today we have a group of different breeds that are collectively known as the ‘scent hounds’ that have been selected over the years for their exquisite scent differentiation. The chapter concludes with a discussion of parentage analysis in the dog. The world of pedigree dogs relies on having extensive and reliable pedigree data for all breeds that certainly goes back unbroken for over a century. Modern molecular genetics offers the opportunity to verify entries into pedigree databases, adding to both their integrity and value.

Structure of the Dog Genome

LINE elements

Long interspersed repetitive DNA elements (LINEs) are a major class of repetitive sequence in most mammalian genomes being present in about 10^4 copies per haploid genome. Full-length LINEs are more than 5 kb long and contain two open reading frames (ORFs), one of which shows sequence homology with the reverse transcriptase enzyme of retroviruses. LINE elements, however, lack the long terminal repeats required for viral DNA synthesis in retroviruses.

In a recent study of the major repetitive sequences present in the dog, LINEs were identified in 13.8% of clones from a *Sau3*A library sequenced on the basis of their hybridization to a total dog DNA probe, which was assumed to select clones containing repetitive sequences (Bentolila *et al.*, 1999). The majority of sequence homologies observed were to human L1 repeats, and specifically to the last 1000 base pairs at the 3′-end of this class of repeats. Truncation of the 5′ end of LINE elements has frequently been observed in

other species and in dogs no sequences with homology to the 5′ region of prototype LINEs were observed. In addition, these authors also noted an unusual class of LINE elements truncated at their 3′ ends by 350 base pairs, resulting from the insertion of a class of SINE elements (see below). On examining the sequence similarities of the canine LINE elements, two different consensus sequences were observed which shared 62% sequence homology over a 600 bp region.

A LINE element insertion in a rearranged *c-myc* gene has recently been associated with canine transmissible venereal tumour (Amariglio *et al.*, 1991; Choi *et al.*, 1999). The insertion of repetitive elements into new regions of the genome has the potential to disrupt gene expression or function and lead to disease.

SINE elements

Short interspersed repetitive DNA elements (SINEs), the most abundant repetitive sequences observed in the dog, are between about 130–150 base pairs (Minnick *et al.*, 1992; Das *et al.*, 1998). In the study described in the section on LINE elements, Bentolila *et al.* (1999) estimate that there are approximately 400,000 copies of the SINE element per haploid dog genome, whilst other estimates range between 360,000 and 600,000 (Das *et al.*, 1998). Two major classes of SINE elements have been observed in different mammalian species, both of which are derived from RNA polymerase III transcripts. One class of SINEs evolved from tRNA genes whilst the other class evolved from the 7SL RNA gene. Only tRNA-SINEs have been identified in the dog genome, which contrasts with the situation in humans where only 7SL-SINEs (*Alu* sequences) are found, and in mouse where both classes of element are present. SINEs appear to be widely dispersed in the dog genome and are frequently associated with microsatellite repeats. Whilst it is not certain from which tRNA gene the canine SINEs evolved, greatest similarity is shown to $tRNA^{Gln}$.

tRNA-SINE elements have a number of structural features which are conserved in the dog repeats, including two potential RNA polymerase III promoters, called the A box and the B box (Minnick *et al.*, 1992; Coltman and Wright, 1994). SINE elements are frequently flanked by direct repeats of 8–17 base pairs, thought to reflect the duplication of target sequences at the site of insertion.

A novel repeat, with some similarity to other SINEs, such as a 3′ terminal polyA tract, but lacking RNA polymerase III promoters, has been identified (Bentolila *et al.*, 1999). The DNA sequence of these elements does not share homology with either tRNA or 7SL-derived SINEs. The majority of these elements were inserted into the 3′ end of LINE sequences, have been called ELA-SINEs, and 63,000 copies are estimated to be present in the dog genome.

Sequences similar to the dog tRNA-SINE have been identified in a range of other carnivore species including the wolf, jackal, fox, mink and seal genomes, where the DNA homology in the latter two species is approximately 70%.

Microsatellites

Microsatellites are short tandem repeat sequences of 2–6 base pairs which have been extensively used in genetic linkage studies in many species due to their high levels of polymorphism and the ease with which the variation observed can be scored following PCR amplification (see Chapter 12). Microsatellite polymorphism results from variation in the number of tandem repeats at a given microsatellite locus.

A canine genomic phage library with an average insert size of 16 kb was screened with a range of microsatellite sequences to determine the relative frequencies of these repeats (Rothuizen *et al.*, 1994). The most frequent repeats were $(CA)_n.(GT)_n$ which were present approximately every 43 kb. In contrast, the distance between microsatellites of the tri- or tetra-nucleotide classes averaged about 320 kb. Interestingly, the GAAA class of tetranucleotide repeats was found to be extremely polymorphic in the dog, an important factor in trying to map traits in a species such as the dog with its stratified breed populations (Francisco *et al.*, 1996). These authors reported that tetranucleotide repeats were present about once every 100–200 kb in the dog genome. Canine microsatellites have been extensively used to examine breed structure (Fredholm and Wintero, 1995; Pihkanen *et al.*, 1996; Zajc *et al.*, 1997), and are proving invaluable markers for use in parentage analysis.

Minisatellites

Minisatellite DNA sequences were first identified in the human genome and shown to consist of arrays containing tandemly repeated units ranging in size from 2 to 250 bp (Paulsson *et al.*, 1992; Brereton *et al.*, 1993). The finding of a short minisatellite consisting of a 33 bp repeating monomer in an intron of the human myoglobin gene by Weller *et al.* (1984) was followed by the detection of multiple polymorphic loci by a pure repeat probe prepared from the myoglobin minisatellite (Jeffreys *et al.*, 1985). The resultant individual-specific DNA banding pattern was termed a DNA 'fingerprint'. The generation and subsequent sequencing of λ clones containing human minisatellite regions showed that individual repeats shared a core region with an almost invariant sequence. Polymorphism has been shown to result from variation in the number of these core sequence repeats at a given minisatellite locus in individuals.

Canine minisatellites were first detected using the human probes 33.15 and 33.6 (cloned DNA sequences based on the human minisatellite core repeat sequence), which produced individual-specific DNA fingerprints by cross-hybridization (Jeffreys and Morton, 1987). Cross-reaction of dog DNA with the minisatellite sequence present in the M13 bacteriophage was subsequently demonstrated (Vassart *et al.*, 1987). Canine minisatellite sequences were isolated and characterized by hybridization of the 33.15 and 33.6 probes to a dog genomic library (Joseph and Sampson, 1994). Seven polymorphic

minisatellites with heterozygosities between 20 and 88% were isolated. The most polymorphic minisatellite identified, cCfaMP5, detected 35 alleles in 25 unrelated individuals of 17 different breeds when used as a single locus probe. The minisatellite present in this clone was based on four variants of an 11 base pair repeat with homology to the 33.15 and 33.6 probes originally used to isolate the clone. Screening of the dog genome with eight minisatellite sequences revealed hybridization to all except D17S74, with a mean incidence of one locus every 1400 kb (Rothuizen *et al.*, 1994).

Satellite DNA

Two satellite sequences have been identified in the dog genome. The major α satellite repeat in the dog is 737 bp pairs long (Fanning, 1989). An internal 324 bp fragment amplified by PCR has been used in defining the canine karyotype by orientating the centromeres on the canine autosomes which are all acrocentric (Reimann *et al.*, 1996). A second satellite sequence based around a 37 bp subunit, which hybridized to 2% of the repeat clones examined, has also been identified (Bentolila *et al.*, 1999). This repeat shows no homology to the major α satellite repeat.

Mitochondrial DNA

Mitochondria possess small DNA genomes which are inherited maternally. The simple genome encodes 22 tRNAs, 2 rRNAs and several components of the oxidative phosphorylation system. Approximately 90% of the DNA represents coding sequence and several thousand molecules of mitochondrial DNA are present in each cell. Mitochondrial DNA sequences have been used to examine the origins of the domestic dog, the molecular phylogeny of the Canidae and to assist in the conservation of endangered species (Wayne, 1993; Gottelli *et al.*, 1994; Vilà *et al.*, 1997). The complete mitochondrial genome of the dog has recently been determined and found to be 16,727 bp (Kim *et al.*, 1998). Together with sequences from the Y chromosome, the maternally inherited mitochondrial sequences will be useful in determining the origins of the different pedigree dog breeds.

Resources

Canine BAC library

A large insert canine genomic bacterial artificial chromosome library, comprising 166,000 clones with an average insert size of 155 kb has been constructed (Li *et al.*, 1999). The library, constructed from Doberman Pinscher DNA, is predicted to have 8.1-fold coverage of the dog genome. Fluorescence *in situ*

hybridization of 50 clones from the library revealed no evidence of chimaerism. Value of the library is demonstrated in the mapping of the canine narcolepsy mutation to the hypocretin receptor 2 gene (Lin *et al.*, 1999). In this work, a 1.8 Mb contig comprising 77 BAC clones was constructed to flank the mapped location of the narcolepsy mutation, by hybridizing to human ESTs mapping to the appropriate region.

A Multigene Family – Olfactory Receptors in the Dog

The canine genome, in common with that of all vertebrates, contains a large number of transcribed multigene families. Those encoding proteins include the histone genes; a number of other structural molecule families such as the collagens; many small multigene families of enzymes; several families of transmembrane transport genes such as ion channels and solute carriers; and a variety of membrane receptor gene families, including those with immune system involvement and a variety of other types of receptor each with a specialized function. Many of the multigene families have not received systematic attention in the dog (although many individual genes within them may have been studied), whilst others such as the immunoglobulin (Ig) superfamily and the MHC complex are dealt with elsewhere in this book. Perhaps the largest of the specialized transmembrane receptor gene superfamilies, containing more genes than the Ig and T-cell receptor gene families combined, is the olfactory receptor gene family.

The olfactory system has developed as a means of general molecular recognition, a function which is also thought to be required in embryonic development (in cell migration and tissue recognition), and in the sperm and perhaps other motile cells. Olfaction has been widely recognized as particularly sensitive in dogs, with studies showing discrimination sensitivity for many odours to be one hundred to several thousand times greater than that of humans (Davis, 1973; Krestel *et al.*, 1984; Myers and Pugh, 1985). Odour sensitivity is one of the most important attributes put to use in working dogs (reviewed in Willis, 1989). The trait's heritability has been estimated as close to 0.4 (Geiger, 1972), and many hunting breeds have responded well to selection for outstanding tracking ability. The odour sensitivity of the dog has led to quite intensive study of olfaction in this species, which has included study of the molecular biology of the olfactory receptors.

Gene structure and gene numbers

Olfactory receptors have been cloned from many species including *Drosophila* and *Caenorhabditis*, as well as fish, amphibia, birds and mammals. Olfactory receptors (OLFRs) are seven-transmembrane G-protein-coupled receptors with a wide variety of sequences, which are expressed on the surface of olfactory neurons, as well as elsewhere (gametes, vemoronasal organ). The genes

lack introns in the coding sequence, which is approximately 1 kb in length. They are distinguished from other seven-transmembrane (TM) receptors by a set of conserved sequence motifs: LHTPMY in intracellular loop (IC) 1; MAYDRYVAIC at the end of TM domain 3 and start of IC 2; SY at the end of TM5; FSTCSSH at the beginning of TM6 and PMLNPF in TM7. There is hypervariability in the remainder of TM 3, 4 and 5. Binding of the odorant molecule is thought to occur in a pocket in the membrane created by the transmembrane domains of the OLFR, with tight contacts occurring to the hypervariable domains (reviewed in Mombaerts, 1999). The presence of conserved sequence elements in the OLFR proteins has allowed the design of degenerate oligonucleotides predicted to be present in all OLFR genes. PCR using these oligonucleotides on genomic DNA has been used in rapid cloning of many OLFR genes or part genes from mouse, rat and human, as well as a smaller number from dog.

The families of genes encoding olfactory receptors are amongst the largest known: with at least 1000 olfactory receptor genes in rat and mouse: or more than 1% of all genes. Similar numbers are believed to be present in man and other mammals. This large number of different receptors, as well as the ability of different receptors to work in combination, gives the olfactory system the capacity to bind a huge variety of chemicals and to discriminate a vast variety of odours (probably tens or hundreds of thousands), allowing an appropriate behavioural response.

In the mouse and rat (and so far in the dog), no pseudogenes have been reported amongst some 200 sequenced OLFR genes. In contrast, in man more than 50% of over 300 sequenced OLFR genes do not appear to encode functional receptors. The same trend to pseudogene formation is seen to a lesser degree in Old World monkeys, but not in New World monkeys, perhaps reflecting a recent loss of evolutionary pressure on olfaction (Buettner *et al.*, 1998; Rouquier *et al.*, 1998, 2000).

Signal transduction after OLFR stimulation

Olfactory sensory neurons (OSN) form part of the mucous olfactory epithelium lining the nasal trabeculae. The mechanisms of signal transduction in response to odorants have recently been reviewed by Schild and Restrepo (1998). An OSN is a bipolar cell, with an external dendrite ending in cilia and a single axon terminating directly in a glomerulus of the olfactory bulb of the brain. Any one type of odorant molecule transported to OSNs in the mucus will bind a sub-group of different OLFRs with differing affinities. Odorant binding to an OLFR mediates OSN signalling through secondary messenger pathways, the best characterized of which activates adenylyl cyclase through the G-protein Golf. This results in an increase in adenosine 3′,5′-cyclic monophosphate (cAMP), which elicits opening of cation (Ca^{2+}) channels directly gated by cAMP, depolarizing the olfactory neuron, and leading to the generation of an action potential. The binding of an odorant molecule to a particular grouping

of olfactory neurons is transmitted to the olfactory bulb in the brain, where processing of the pattern of signals allows identification of the odorant.

OLFR genes: families and chromosomal locations

Although the basic structure of OLFR genes is of 7-transmembrane receptors, there is a great amount of sequence diversity between different receptors – so that there is no sequence homology between the receptors of *Drosophila* and those of mammals. Olfactory receptor genes in vertebrates have been classified depending on whether they are activated by aqueous (class I) or airborne (class II) odorants (Freitag *et al.*, 1998). The mammals have only class II genes. Within species, olfactory genes have been classified based on sequence homology. Those that share at least 40% amino acid identity are considered members of the same family, whilst those that share 60% amino acid identity are members of the same subfamily. Functionally, subfamilies are defined by cloning and blotting: a single OLFR gene used as a hybridization probe will hybridize to other members of its subfamily. Individual members of a subfamily share 65–90% nucleic acid sequence identity, whilst other subfamilies share only about 40–60%. Individual subfamilies have paralogues in different mammalian species. In the dog, representatives of four OLFR subfamilies have been cloned. These subfamilies range in size from 2 to 20 individual genes, as judged by Southern blotting (Issel-Tarver and Rine, 1996). All members of the three small subfamilies, *CfOLF1*, 2 and 3, were clustered at single sites in the dog genome, with the sites of the *CfOLF1* and 2 subfamilies closely linked. *CfOLF4* subfamily members occurred at a maximum of five cluster sites. Numbers of transcript types identified for each subfamily in olfactory epithelium correspond closely to total numbers of genes, suggesting that there are few, if any, untranscribed pseudogenes in these families.

The canine OLFR genes have been used to select homologous genes representing the same four subfamilies in the human and mouse. The mapping relationships between cloned OLFR genes in dog, human and mouse are given in Table 7.1. The locations of two canine OLFR genes (*CfOLF1* and *CfOLF2*) have been mapped in dog/hamster radiation hybrids by Priat *et al.* (1998) to RH group 30a, corresponding to canine chromosome 18. Canine OLFR1 has also been directly assigned to chromosome 18 by PCR of flow-sorted canine chromosomes (Sargan, unpublished data). This location is in agreement with published comparative maps showing homology between dog chromosome 18 and human chromosome 11p14–q13 (Breen *et al.*, 1999; Yang *et al.*, 1999).

It is clear that most, if not all, OLFR genes occur in clusters, with each gene within a cluster separated by 5–50 kb. The total number of OLFR gene clusters located in the mouse is at least 12 (Sullivan *et al.*, 1996). These clusters probably represent less than 10% of the >1000 OLFR genes, so more cluster locations undoubtedly remain to be found. In man, more extensive surveys have been undertaken, locating a minimum of 25 OLFR gene clusters by PCR or *in situ* hybridization to at least 16 autosomes (Rouquier *et al.*, 1998). In the

Table 7.1. Chromosomal locations of canine OLFR

	Chromosomal location		
Canine gene	Dog	Mouse[a]	Human[a,b]
CfOLF1	18 RH30a[c]	2	11p11
CfOLF2	18 RH30a[c]	2	11p11
CfOLF3	NA	6	7q35
CfOLF4	NA	9	19q13.1
		9	19q13.2
		10	Unknown

Notes:
[a]Carver *et al*. (1998).
[b]Issel-Tarver and Rine (1997).
[c]Priat *et al*. (1998).

mouse, members of the same OLFR gene subfamily, as well as highly related subfamilies, often map to the same chromosomal locus. Thus, if recognition of a particular odorant is dependent on interactions with receptors of one particular subfamily, damage at a single locus could cause a specific anosmia. In man, recent evidence suggests that the situation may be more complex. One cluster mapped in detail had OLFR genes from two families and seven subfamilies represented amongst 17 functional genes in the cluster (Glusman *et al.*, 2000).

Regulation of OLFR gene expression

The nasal olfactory epithelium can be divided into several zones (four in the mouse), and in this species, expression of each OLFR is largely restricted to a single zone. On the other hand, genes in a single chromosomal cluster are sometimes expressed in more than one zone (Sullivan *et al.*, 1996). Within a given zone, OSN expressing different OLFRs intermingle, but all OSNs expressing the same OLFR converge their axons on to a few defined glomeruli, with the OLFR itself probably playing a role in axon guidance during development. (For review, see Mori *et al.*, 1999.) Only one OLFR allele (or possibly a small number in some cells) is expressed in each OSN. Evidence for this comes from *in situ* hybridization with OLFR gene probes, from single cell RT-PCR and most recently, a combination of RT-PCR and calcium imaging (Nef *et al.*, 1992; Ressler *et al.*, 1993; Vassar *et al.*, 1993; Chess *et al.*, 1994; Malnic *et al.*, 1999). Several mechanisms have been suggested to account for this restriction (see also Mombaerts, 1999). These include: (i) a stochastic process in the activation of the genes during olfactory neuron development in which low concentrations of transcription factors are bound to promoters at random; (ii) a DNA looping mechanism in which only a single OLFR at any one locus (cluster) can come into a transcriptionally active position; and (iii) a gene conversion or rearrangement process analogous to that seen in the variable

surface glycoprotein genes of trypanosomes, in which a single OLFR gene is moved to an active site.

There is little or no decisive evidence on which to judge these mechanisms. The large number of gene clusters ensures that there must be much less than one transcriptionally active gene copy per locus, so that DNA looping cannot be the sole mechanism of transcriptional regulation. However, all OLFR genes appear to be equipped with promoters that can be activated in transfected cells. No unique transcriptionally active site has been recognized or proved necessary, whilst a number of transcription factors known to bind OLFR promoters (including all members of the NFAT-1 family) are abundant in olfactory neurons, and so unlikely to regulate transcription through a titration mechanism. On the other hand, the *Rag1* and *Rag2* genes, which are responsible for DNA rearrangement in the immune system, are also active in olfactory neurons (at least in zebrafish, Jensen *et al.*, 1999), making genetic rearrangement to a single active conformation or site an attractive idea.

Expression of canine genes has been noted on male germ cells in several species including the dog (Vanderhaeghen *et al.*, 1993, 1997), where more than 14 different OLFRs are expressed. OLFR protein molecules are found on late spermatids and on the tail midpiece of sperm cells. These OLFRs show no specific clustering in sequence features, and some are homologous with previously reported olfactory epithelium OLFR genes. Expression studies using one abundant canine OLFR gene product showed a high level of mRNA in testis, but also (using RNase protection) the presence of a very closely homologous RNA in olfactory epithelium. In other species, OLFR genes may be transcribed in both olfactory epithelium and testis with either tissue showing the higher level of transcripts of any one gene. However only a small and evolutionarily conserved subset of OLFR genes are expressed in the germ line. Potential roles for these OLFRs have been postulated in signal transduction through Ca^{2+} homeostasis, in the control of sperm maturation, in migration (chemotaxis) or in fertilization.

Olfaction and dog breeds

Issel-Tarver and Rine (1996) used Southern blotting to compare the number of gene fragments hybridizing to each of four cloned canine OLFRs for ten breeds of scent hound, ten breeds of sight hound and six toy/companion breeds. They showed that there were no consistent differences in gene arrangement in any of the four subfamilies represented by *CfOLF1-4*, and suggested that there are unlikely to be major differences in OLFR repertoire between breeds.

A larger area of nasal epithelium should increase the probability of contact of an odorant with an appropriate receptor. This, as an alternative to differences in OLFR repertoire, might account for a proportion of the increased olfactory sensitivity demonstrated behaviourally in scent-hound breeds. Some authors have indeed suggested a major difference between breeds in the

surface area of the nasal mucosae, but no systematic study of nasal epithelium area in different breeds seems to have been published, so it is not possible to confirm this suggestion. It has also been argued that superiority in tracking demonstrated by scent-hound breeds may be accounted for purely in behavioural terms (Willis, 1989, see also Scott and Fuller, 1965), rather than by differences in olfactory acuity. In our current state of knowledge, this argument appears to be a convincing one.

Parentage Analysis

Parentage testing relies on the use of genetic systems that reveal polymorphisms within the species being studied; the greater the polymorphism, (i.e. the larger the number of alleles within the population), the more informative a locus is for parentage analysis. Early attempts at parentage testing in the dog used biochemical polymorphisms displayed by a variety of proteins present in blood (Chapter 6). As our understanding of the canine genome has increased, systems based on DNA polymorphisms have been developed, particularly utilizing the minisatellite and microsatellite sequences described above.

The alleles that characterize an individual originate from its parents, one allele being maternal and the other paternal in origin. Thus, for any given locus, provided that one of the parents is known (and this is usually the mother), the allele necessarily transmitted by the other parent (usually the paternal allele) can be easily deduced. Parentage testing involves determining the presence or absence of those alleles that must be paternal in origin in the genotype of a putative father. An absence of one or more alleles would suggest incorrectly assigned parentage. On the other hand, if all paternal alleles can be shown to be present in the putative father's genotype, then there is no evidence for incorrectly assigned parentage.

These decisions assume that allele sharing is not simply coincidental. The pedigree dog, and most cases of disputed canine parentage originate from the pedigree world where registration on kennel club databases requires that both parents are known, presents a serious challenge to this assumption. This stems from the genetic structure of the various breeds of dog. Over the centuries, dog breeders have been particularly successful at establishing new breeds of dog, so that today there are in excess of 400 different breeds recognized throughout the world (Padgett, 1998); there is no other species that shows such variation of morphological type. Probably all of these breeds are based on a relatively small number of founding animals and continued inbreeding is required to maintain breed type and characteristics. Further genetic bottlenecks can be introduced into breeds by the near total use of a particularly successful stud dog, or small number of successful stud dogs; the so-called 'popular sire effect'. These restricted breeding patterns mean that most pedigree dog breeds, even the most numerically large, are increasingly

homogeneous genetically. One of the consequences is that polymorphic loci tend to demonstrate reduced levels of polymorphism within a particular breed.

Testing based on protein polymorphisms

Early cases of disputed parentage have been solved successfully using a variety of biochemical markers including blood proteins and enzymes (Juneja *et al.*, 1987) and the major histocompatibility region (Vriesendorp *et al.*, 1974). Although the polymorphisms of individual markers are low, relatively high levels of exclusion probability have been achieved using an extended range of marker systems. So for example, Juneja *et al.* (1987) reported a probability of exclusion of ±80% in dogs using 11 different biochemical marker systems. The possibility of studying genetic variation at the DNA level provided a new set of markers that have been invaluable for parentage testing.

Testing based on minisatellite polymorphisms

The first DNA marker system to be used in determining canine parentage involved the use of canine minisatellite loci and the comparison of DNA fingerprints generated from the dam, sire and puppy (Jeffreys and Morton, 1987). The technique of DNA fingerprinting involves digesting DNA produced from white blood cells, or other tissue, with a suitable restriction enzyme and separating the fragments thus produced by gel electrophoresis. The separated fragments are then transferred on to a nylon membrane by Southern blotting which is then hybridized with radiolabelled probes (in this case 33.6 and 33.15). Autoradiography of the washed Southern blot reveals a ladder of fragments that constitutes the DNA fingerprint. DNA fingerprinting can be used to resolve cases of disputed parentage since approximately half of the fingerprint bands in an offspring are inherited from the dam or the sire. Thus, all of the bands in an offspring's fingerprint will have counterparts in its dam's or sire's DNA fingerprint. In cases of disputed parentage the question being asked is whether bands in an offspring's DNA fingerprint that are absent from the dam's DNA fingerprint have counterparts of similar intensity in the DNA fingerprint of a suspected sire.

The first reported use of DNA fingerprinting to establish definitive parentage (Morton *et al.*, 1987) cited two examples. The first concerned a family of Siberian Huskies; comparison of DNA fingerprints identified 14 bands in the offspring's fingerprint that were not detected in that of her dam (Fig. 7.1a). These clearly must have been inherited from the offspring's biological sire. All 14 of these bands were found in the DNA fingerprint of just one of the potential sires, showing that he was the true sire of the offspring. Previous analysis of band sharing in canine DNA fingerprints (Jeffreys and Morton, 1987) had shown that the probability that any band in one dog is, by chance, found in a second unrelated dog is on average 46%. Thus the probability that

this identified sire had by chance all 14 of these non-maternal bands present in the puppy's DNA fingerprint was calculated to be 100,000 to 1 against.

The second case reported involved a greyhound stud dog who over a 15-month period had mated 27 bitches and whose semen, on examination, revealed the absence of spermatozoa. On two occasions the mating successfully produced offspring and one of these two conceptions was investigated further. The DNA fingerprints of the dam, two puppies and the supposed sterile stud were compared (Fig. 7.1b, c). Nine bands that were present in one or both puppies, and absent from the dam's fingerprint, were not found in the

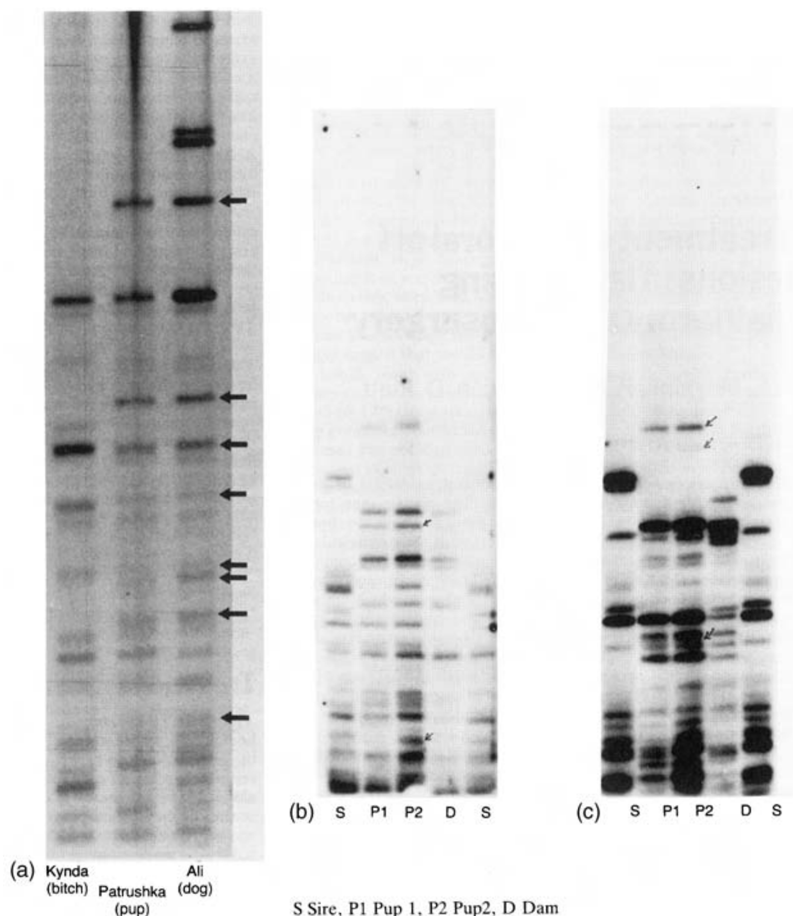

Fig. 7.1. The use of DNA fingerprints to establish parentage. (a) Siberian Husky; the arrows on the left hand side indicate eight of the fingerprint bands (identified using probe 33.15) present in the pup (Patrushka) and the putative sire (Ali), but not the dam (Kynda), that were used to establish that Ali was the biological sire of Patrushka. (b), (c) Greyhound; ((b) 33.15 DNA fingerprint; (c) 33.6 DNA fingerprint). Both pups (P1 and P2) have a number of bands that are not present in either the known dam (D) or the putative sire (S), establishing that this sire was not the biological sire of these pups. (Data taken from Morton *et al*. (1987), reproduced with kind permission of *The Veterinary Record*.)

DNA fingerprint of the sire tested. These multiple exclusions clearly establish that the stud dog could not have been the natural sire of the puppies.

Another report on the use of DNA fingerprinting was published in 1988 by Georges *et al.* In this case a Shih Tzu bitch was mated by a dog of the same breed and subsequently inseminated by a Coton de Tulear dog. Two puppies were produced and because the Shih Tzu and the Coton de Tulear are phenotypically similar, it was not possible to say whether these puppies were purebred or of mixed breeding. DNA fingerprinting showed clearly that one of the puppies was purebred and the other was of mixed breeding. Thus each had a different sire, showing that superfecundation had occurred.

The use of DNA fingerprinting is not without difficulties: Fingerprints are extremely difficult to standardize and it is virtually impossible to compare fingerprints unless they are run side by side on the same gel. Furthermore, it is very difficult to sort out allelic relationships between the bands that make up a fingerprint. It is also very time-consuming and requires μg quantities of DNA. Cloned canine polymorphic minisatellites (see above) have been used successfully as single-locus probes to establish parentage (Joseph and Sampson, 1994). Their use simplifies the analysis because there are just two alleles present on an individual's Southern blot, rather than the multi-allelic banding pattern observed in DNA fingerprints. The use of a bank of single-locus minisatellite loci would certainly provide high exclusion probabilities in parentage analysis.

Testing based on microsatellite polymorphisms

The practical problems of systems based on minisatellites have now been largely overcome by switching to microsatellites as an alternative source of DNA variation for canine parentage analyses. Although canine microsatellites have a lower level of polymorphism compared with minisatellites, the canine genome project has generated hundreds of microsatellite loci (see Chapter 12) that can be used in parentage analysis. Two papers by Zajc *et al.* (1994) and Binns *et al.* (1995) demonstrate the effectiveness of canine microsatellites as tools for parentage analysis. This has opened up a relatively simple, PCR-based system that can be easily adapted for automated analysis on DNA sequencing machines.

DNA samples from the dam, pup(s) and potential sires are used as templates in PCR reactions using oligonucleotide primers that are microsatellite locus specific. The amplified products of these reactions represent the allele sizes at this locus. In one system (Zajc *et al.*, 1994) the primers were unlabelled and the amplified products, separated on polyacrylamide gels, were detected using ethidium bromide. The more usual system is that reported by Binns *et al.* (1995) where the oligonucleotide primers carry a fluorescent tag that allows allele sizes to be determined on an automated DNA sequencer.

Figure 7.2 demonstrates the use of microsatellite loci in parentage analysis. The analysis involves the use of a number of different microsatellite loci. Allele

size comparison of the dam and pup's DNA identifies the paternal allele in the pup. All potential sires can then be analysed to see if this allele is present or not. In this way, potential sires can be excluded from being the biological sire. Usually sires are not excluded on the basis of just one microsatellite locus because this could be the result of a mutation or a null allele. Multiple exclusions are normally recorded before a sire is eliminated as the biological sire. The simplest form of analysis involves screening the DNA from all potential sires and eliminating all but one, who must be the biological sire.

Great progress has been made toward multiplexing a large number of microsatellites, thus reducing the number of individual PCR reactions required and simplifying the analysis. Commercial kits of locus-specific microsatellite

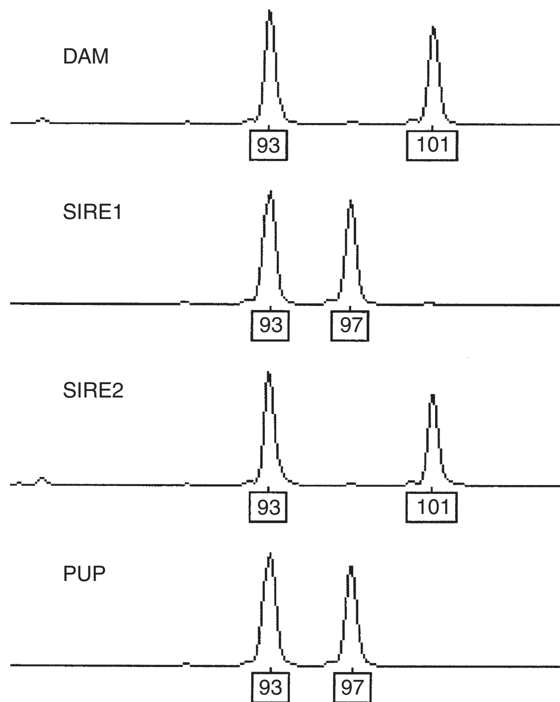

Fig. 7.2. The use of microsatellites to establish parentage. These data represent the analysis of canine parentage using a single microsatellite locus. The locus-specific primers were fluorescently labelled to allow analysis on an ABI automated sequencer. The dam has two different alleles of this locus (93 and 101), the pup also has two different alleles (93 and 97). Clearly the pup's 93 allele is maternal, so the other allele in the pup (97) must have originated from its sire. Of the two potential sires, only sire 1 has a 97 allele. In this particular case, additional microsatellite loci were analysed and multiple exclusions were obtained for sire 2; all of the allele data were compatible with sire 1 being the biological sire. Provided these were the only two potential sires, the multiple exclusions of sire 2, and the total agreement of sire 1, identifies sire 1 as the biological father. (Data kindly provided by Dr Nigel Holmes, Animal Health Trust, Lanwades Park, Kennett, Newmarket, Suffolk, CB8 7DW, UK.)

primers are now available for parentage testing. As mentioned above, the genetic structure of pedigree breeds tends to reduce the degree of polymorphism within a breed and, in some breeds (e.g. the Miniature Bull Terrier in the UK), a significant proportion of canine microsatellites fail to show heterozygosity (M. Binns, personal observation). A careful investigation and selection of microsatellite loci will therefore be necessary in order that a high enough exclusion probability can easily be achieved in most parentage cases. It is likely that at least two panels of microsatellite loci will be required to provide sufficient power of exclusion in all breeds and family lines of dogs.

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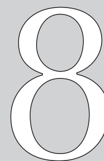
Immunogenetics



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Introduction

Knowledge of the canine immune system is valuable not only for understanding the pathogenesis of several human diseases, but also for improving canine health. The dog serves as an important model for drug toxicity trials and for a variety of human diseases such as cyclic neutropenia (Weiden *et al.*, 1974), X-linked severe combined immunodeficiency syndrome (SCID) (reviewed in Felsburg *et al.*, 1999), von Willebrand's disease (reviewed in Thomas, 1996), severe hereditary haemolytic anaemia (Weiden *et al.*, 1976), haemophilia (reviewed in Fogh *et al.*, 1984), gluten-sensitive enteropathy (Hall and Batt, 1990), rheumatoid arthritis (Halliwell *et al.*, 1972), systemic lupus erythematous (Lewis and Schwartz, 1971) and narcolepsy (Baker *et al.*, 1982). Dogs have high rates of spontaneous malignancies and thus have served as models for a variety of cancers including breast cancer (Mol *et al.*, 1999), non-Hodgkin's lymphoma (Weiden *et al.*, 1979) and prostate cancer (Navone *et al.*, 1998–1999). For over 40 years the dog has served as a valuable model for haematopoietic stem cell transplants (reviewed in Thomas and Storb, 1999).

The immune system of dogs protects them from a variety of infectious agents. In dogs, as in other mammals, the immune system has a complex series of functions including distinguishing self from non-self as well as the ability to 'recall' previous foreign antigen exposure. The major components of the immune system include the T cells and the B cells which are polymorphic because of gene segment rearrangements, point mutations and gene conversion leading to a variety of T cell receptor (TCR) and B cell receptor (a membrane immunoglobulin) phenotypes. Another important genetic element in the immune system is the major histocompatibility complex (MHC) which is complex due to a variety of allelic variants at several loci.

Despite the complexities of the mammalian immune system, different components act in a coordinated manner. B cells recognize antigens in their native form and depend on T cells to expand clonally to become plasma cells and produce antibodies (immunoglobulins). Similarly T cells depend on specialized cells such as B cells which process peptide antigens and present these peptides bound to MHC molecules (Klein, 1982).

There are several similarities between T cell receptors, immunoglobulin molecules, and MHC class I and class II molecules. These genes exhibit a precise correlation between exons and the protein domains they encode. The sizes of the external domains and the placement of disulphide bridges are similar. During gene expression, RNA splicing usually occurs between the first and second base of the junctional codon. The DNA sequences of the MHC class I exon 3, the class II exon 2 and the exon sequences encoding the immunoglobulin constant regions as well as parts of the T cell receptor

are homologous. These similarities suggest that perhaps these 'receptors' of the immune system have descended from a common ancestral gene and are members of one supergene family.

Major Histocompatibility Complex

Overview of the canine major histocompatibility complex

An understanding of the general structure and function of the MHC is helpful in order to comprehend its importance in transplantation and disease. The MHC is a linked cluster of genes and gene families and is one of the most extensively studied regions of the genome in several species, including humans and mice. In normal physiology, MHC gene products interact with bound peptide ligands and with products of rearranged TCR genes in the thymus that result in positive and negative selection of the peripheral T cell repertoire. In other words, this region of tightly linked genes is responsible for the presentation of self and non-self antigens to the immune system and, thus, is fundamental in the recognition and regulation of the immune response. MHC molecules perform these roles by binding and presenting peptide antigens to T cells. This antigen presentation can lead to several events, including elimination of infected cells or cellular rejection of transplanted organs.

The genes within the MHC are divided into at least three types: class I, class II and class III. The class I and class II molecules are the cell surface glycoproteins of similar structure involved in antigen presentation to T cells. Allelic variation occurs typically because of polymorphism in or around the peptide binding site, and in some species there may be more than 100 alleles for a given locus. Class III molecules are structurally unrelated to class I and class II molecules and are not relevant to antigen presentation but can be important in other aspects of the immune system, such as complement activation. The complement pathway is an effector mechanism activated by the humoral (antibody) immune response. Class I antigens are expressed on all somatic cells, whereas class II molecules are expressed on antigen-presenting cells such as macrophages. Interestingly, in contrast to mice and humans, canine class II gene products are present on almost all lymphocytes (Doxiadis *et al.*, 1989).

Genomic organization of the canine major histocompatibility complex

Gene mapping and chromosome assignment in dogs has lagged behind that in several other mammalian species. Part of the reason is that dog chromosomes are numerous and many of them are too small and similar to be identified unambiguously by Giemsa banding alone. Using the technique of fluorescence *in situ* hybridization (FISH), the dog leukocyte antigen (DLA) complex has been localized to canine chromosome (CFA) 12 (Dutra *et al.*, 1996). The

probes for the FISH study were made from genomic clones of class I and class II loci (see later discussion). Recently two tools have become available to aid in mapping the canine genome including a canine radiation hybrid cell line (Priat *et al.*, 1998) and a bacterial artificial chromosome (BAC) library (Li *et al.*, 1999). While most DLA loci have been assigned to CFA12, one gene, *DLA-79* (which structurally resembles a class I gene), appears to be located on CFA18 (Mellersh *et al.*, 2000). *TNF* (both α and β genes), which in humans are located in the class III region, also map to CFA12 (Mellersh *et al.*, 2000). While several genes within the canine MHC have been cloned and sequenced, the genetic distance between them and the ordering of genes on the chromosome is not currently known in the dog. However, information about the DLA can be inferred from information about other mammalian species since the MHC has been highly conserved throughout mammalian evolution. In all mammals the complement system (class III gene family) is present along with a class I gene family and a class II gene family, although the gene families do not have a contiguous organization in all mammals.

Cellular characterization

Early understanding of the canine MHC involved primarily cellular and serological analyses. Based on evidence from early transplantation experiments in various animals, it became apparent that histocompatibility genes could be analysed by determining their gene products or antigens on the cell surface membrane. One common method of this determination was the microcytotoxicity test in which lymphocytes from one animal are incubated with sera and complement from a different animal and if the animals are histoincompatible the cells are damaged and can take up dye. The performance of the test depended on a variety of standardized serological reagents each of which correlated with different allelic forms of various class I or class II molecules. Different class I or class II proteins on the cell surface (based on the allelic polymorphism) will react with different antisera. These serological reagents were obtained by immunizing dogs with lymphocytes from histoincompatible dogs and collecting the serum from the immunized animals.

Another method of cellular testing is known as the mixed lymphocyte culture (MLC). In this procedure, lymphocytes are taken from two genetically dissimilar mammals of the same species and cultured together for a number of days. Quantitation of the incorporation of radiolabelled thymidine correlates somewhat with the amount of genetic disparity between the two animals. In dogs, as in humans, specificities as defined by the MLC correlate with class II antigens (J.L. Wagner, Seattle, WA, 1999, personal communication).

Many early experimental histocompatibility studies in the dog have been oriented towards exploration of transplantation immunology. The production of antisera and evidence for the DLA was first demonstrated in 1971 (Vriesendorp *et al.*, 1971). Evidence for two closely linked DLA loci was described in 1972 (Vriesendorp *et al.*, 1972). Through the 1970s and the 1980s

a series of international workshops led to a better definition of DLA antigens (Vriesendorp *et al.*, 1973; Deeg *et al.*, 1986; Bull *et al.*, 1987). In the third workshop, the dog leucocyte antigen complex (DLA) was divided into three serologically defined loci *DLA-A* (with five specificities), *DLA-B* (with four specificities) and *DLA-C* (with three specificities) (Bull *et al.*, 1987). A fourth locus, *DLA-D* (with ten specificities), was defined by the mixed leukocyte culture (MLC) (Deeg *et al.*, 1986).

Biochemical analyses

Biochemical analysis of the DLA in the 1980s followed the serological characterization. The *DLA-A* loci are characterized as class I molecules by their association with β2-microglobulin (Krumbacher *et al.*, 1986). An immunochemical analysis of glycosylated and non-glycosylated DLA molecules suggests that the products of one predominant DLA class I locus (corresponding to *DLA-A*) are present on the surface of peripheral blood leucocytes and *DLA-C* gene products are thought to be weakly expressed class I antigens (Van der Feltz and Ploegh, 1984; Doxiadis *et al.*, 1986).

Conversely, *DLA-B* gene products when studied by two-dimensional gel electrophoresis and lysostrip experiments exhibit typical class II properties with a high level of serological polymorphism in the beta chain and no serological polymorphism in the alpha chain (Doxiadis *et al.*, 1989). One-dimensional isoelectric focusing and immunoblotting have defined *DLA-A* and *DLA-B* gene products and there is a high degree of correlation between the biochemically defined antigens and the serological specificities (Kubens *et al.*, 1995).

The fourth component of complement (*C4*) homologous to the human HLA class III protein of the same name, has been found to be linked to the MHC region in the dog (Grosse-Wilde *et al.*, 1983). In a survey of 291 dogs, five different variants were found and biochemical analysis suggested that there are two different genes encoding for *C4* (Doxiadis *et al.*, 1985). *TNF*α (Zucker *et al.*, 1994), a cytokine, is another class III molecule that has been characterized in the dog. In summary, biochemical characterization of serologically defined antigens demonstrated class I, II and III proteins homologous to better-characterized proteins in other mammalian species such as humans.

Molecular analyses – genes and polymorphism

Class I molecules such as HLA-A, -B and -C are heterodimeric glycoproteins that were initially defined by antisera. The molecules contain a polymorphic membrane-bound α chain (45 kDa) that is non-covalently associated with β2-microglobulin (a non-polymorphic product of a non-MHC-linked gene). Classical class I (Ia) loci, in contradistinction to 1b loci, tends to be transcribed at higher levels in more tissues and tend to be more polymorphic than class Ib

loci. Class Ia loci are more important in transplantation biology because of their greater contribution to alloreactivity and organ rejection.

The structure of class II loci is well conserved among mammalian species, and an orthologous relationship exists between class II loci of different mammalian orders. Thus, class II loci from different mammalian orders may have similar names such as *DLA-DRB1* and *HLA-DRB1*. Class II molecules in humans such as HLA-DR, -DQ, -DM and -DP, exist as αβ heterodimers composed of an α chain (34 kDa) and a β chain (29 kDa). In contrast, for class I loci, where gene families within each mammalian order have arisen independently, there is no orthologous relationship. Thus, while among primates there is a gene designated 'A' (e.g. *HLA-A*), there is no gene designated *DLA-A* in carnivores.

Beginning in the late 1980s a molecular analysis of the canine MHC began. Sarmiento and Storb used various human class II probes to study the number of class IIA and IIB genes on Southern blots (Sarmiento and Storb, 1988a,b). The same group used a similar approach with an HLA-B7 (a human class I) cDNA probe. By studying the patterns on Southern blots from 40 dogs, Sarmiento and Storb concluded that there are approximately eight canine class I loci (Sarmiento and Storb, 1989).

Unfortunately, banding patterns from Southern analysis did not provide definitive information on the number of loci present nor the degree of polymorphism. Therefore, other molecular methods were used. Using reverse transcriptase–polymerase chain reaction (RT–PCR) and primer sequences obtained from corresponding HLA class II loci, Sarmiento *et al.* found that at least three class II loci, *DRB*, *DQA*, and *DQB*, are polymorphic (Sarmiento *et al.*, 1990a, 1992, 1993). The DRA locus appears to be monomorphic (Wagner *et al.*, 1995).

The next step was to clone and sequence all the genes that were hypothesized to exist based on banding patterns from Southern analyses as well as the RT–PCR work. In order to do this, human probes were used to screen a canine cDNA library. Then the canine cDNA clones were used to probe a canine genomic DNA library. In the case of the class I loci, a canine cDNA library was screened with an *HLA-B7* probe, and only one distinct clone was isolated, designated *I16* (Sarmiento and Storb, 1990a). Using *I16* as a probe to screen a genomic library, over 15 clones were found which implied that at least seven distinct class I loci exist (Burnett and Geraghty, 1995; Burnett *et al.*, 1997).

One locus designated *DLA-79* has shown limited polymorphism, relatively low mRNA expression (in a variety of tissues except skeletal muscle) and, thus, has been designated a class Ib gene (Burnett and Geraghty, 1995). The gene was isolated a second time from a genomic library using *HLA-E* as a probe and, like *HLA-E*, it has a long hydrophobic signal sequence (Burnett and Geraghty, 1995). Interestingly *DLA-79* is located on a separate chromosome from the other DLA loci and such a genetic division of the MHC has not been seen in other mammals. Current studies are involved in elucidating the function of this gene.

Three other class I loci (besides *DLA-79*), termed *DLA-88*, *DLA-12*, and *DLA-64*, appear to be complete genes by sequence analysis, and all three are transcribed in canine peripheral blood leucocytes (Burnett *et al.*, 1997). DLA-88 appears to be more polymorphic than *DLA-12*, *DLA-79* or *DLA-64* (Graumann *et al.*, 1998). Two other genes, termed *DLA-53* and *DLA-12a*, are truncated class I pseudogenes (Burnett *et al.*, 1997). *C1pg-26* is a processed gene located outside the DLA (Burnett *et al.* 1997). Neither the tissue expression nor the function of any of the class I genes are known at present, although one could infer by analogy from other species that DLA class I gene products could serve as cytotoxic T lymphocyte targets.

Using methods similar to those described above for class I loci, several class II loci have been characterized. Using a human *DRB* cDNA probe, a canine *DRB* clone called *DRB5* was isolated (Sarmiento and Storb, 1990b). This canine cDNA clone was used to screen a genomic library. From these experiments one highly polymorphic *DRB* locus designated *DLA-DRB1* and a pseudogene termed *DLA-DRB2* were cloned and sequenced (Wagner *et al.*, 1996a, b). Similar strategies were used for the *DQ* loci. There is one *DQA* gene with a limited amount of polymorphism (Wagner *et al.*, 1996c). There is one polymorphic *DQB* gene and one *DQB* pseudogene (Wagner *et al.*, 1998a). A summary of known DLA loci and the number of alleles defined to date is shown in Table 8.1. For all the loci listed in Table 8.1, a group of random mixed-breed dogs was used to determine the degree of polymorphism (by DNA sequence) present at each locus. With the exception of *DLA-64*, almost all of these polymorphisms resulted in changes in the amino acid sequence, and the location of most of these substitutions was in the putative peptide binding site (see references beneath Table 8.1). The number of amino acid differences between alleles varied from one to five.

Table 8.2 shows the relationship of various class II genes among several different mammalian species. Most of the differences are due to allelic variations.

Table 8.1. Known DLA loci and their polymorphism

Locus name	Gene type	Class	Number of alleles known
DLA-DRA1	Complete	II	1
DLA-DRB1	Complete	II	43
DLA-DRB2	Pseudo	II	–
DLA-DQA1	Complete	II	11
DLA-DQB1	Complete	II	31
DLA-DQB2	Pseudo	II	–
DLA-88	Complete	I?a	48
DLA-79	Complete	Ib	11
DLA-12	Complete	I ?a or b	3
DLA-64	Complete	I ?a or b	3
DLA-53	Pseudo	I	–
DLA-12a	Pseudo	I	–

References: Wagner *et al*., 1995, 1996a,c, 1998a,b, 2000a; Burnett and Geraghty, 1995; Burnett *et al*., 1997; Graumann *et al*., 1998; Kennedy *et al*., 1998, 1999b,c, 2000.

Table 8.2. Cloned complete canine class II genes and amino acid identity (%) with other species

Gene	Mouse	Cow	Sheep	Pig	Horse	Cat	Human
DLA-DRA1	75	83	80	83	86	91	87
DLA-DRB1	79	81	80	–	–	83	81
DLA-DQA1	78	80	80	81	80	–	82
DLA-DQB1	70	81	80	76	78	–	79

– Protein sequence not in Genbank.

Histocompatibility typing

Histocompatibility typing is the process of determining which allele(s) are present for a particular locus. The process involves the application of immunogenetics of the MHC to the field of transplantation. While much work has been done with the dog outside of transplantation, the driving force for a large amount of research into the DLA was/is for transplantation experiments. Each advancement in understanding the DLA was accompanied by a refinement in the techniques of histocompatibility typing. Histocompatibility typing of dogs has several important uses. Not only can it be helpful for identifying the degree of histoincompatibility between a donor and recipient, but also can be used to identify the linkage between a genetic disease and the DLA region. Formerly histocompatibility typing relied on a combination of cellular and serological methods (Albert *et al.*, 1973; Deeg *et al.*, 1986). Later, restriction fragment length polymorphism (RFLP) analysis (Williamson *et al.*, 1989; Lu *et al.*, 1991; Burnett *et al.*, 1994) became popular.

Intrafamilial histocompatibility typing can be done using two polymorphic microsatellite markers – one located in the class I region (C.2200) near DLA-53 and one located in the class II region (C.2202) near DLA-DRB2 (Wagner *et al.*, 1996d). Both of these markers are tetranucleotide repeats of $(GAAA)_n$. Both markers were identified during the sequencing of genomic clones. A polymorphism informational content (PIC) value of 0.804 (Burnett *et al.*, 1995) was obtained for C.2200 and a value of 0.947 (Wagner *et al.*, 1996d) for C.2202. Analysis of both markers in over 100 families has shown that each is stable for following Mendelian inheritance through multigenerational families. Because of the high polymorphic index of these markers and their stability, they are useful for determining the inheritance of DLA haplotypes within families. DLA matching of dogs within families determines which littermates are best suited for organ transplantation experiments. While a polymorphic marker anywhere on any dog chromosome could potentially be used as a marker, only those within the DLA region will define DLA-matched dogs within families.

Microsatellite markers are not always suitable for finding DLA-matched unrelated dogs for transplantation experiments because they do not identify the genotype of any loci. Genotyping unrelated dogs at each polymorphic locus is an area of current investigation. Some investigators have used PCR-restriction fragment length polymorphism analysis (RFLP) for *DLA-DRB1* genotyping (He *et al.*, 1994a,b; Francino *et al.* 1997). Current typing methods

are similar to those used for HLA loci and are based on molecular differences to determine which alleles are present. These methods include PCR-single stranded conformational polymorphism (SSCP) (Wagner *et al.*, 1998b, 2000a), sequence-specific oligonucleotide probes (SSOP) (Kennedy *et al.*, 1998, 1999a, 1999b, 2000) and reverse transcriptase (RT)-nested PCR and cycle sequencing (Happ *et al.*, 1999). From large-scale typing studies it has become apparent that allelic frequencies of genes within the class II region vary from breed to breed (Kennedy *et al.*, 1999a). Correlation between molecular typing and cellular typing will help elucidate the functional importance of various loci.

Canine major histocompatibility complex and disease

As histocompatibility typing systems have evolved, so have efforts to determine the relationship between canine diseases and the MHC, particularly in cases in which the canine disease serves as a model for a human disease which has some association with the HLA. An early study suggested that systemic lupus erythematosus in dogs is associated with the class I serological specificity *DLA-A7* although no attempt to correlate this finding with a specific class I locus or molecular based typing has been published (Teichner *et al.*, 1990). Conversely, canine narcolepsy (Wagner *et al.*, 2000b) and gluten sensitive enteropathy (Polvi *et al.*, 1998) do not appear to be associated with the MHC as they are in humans. Whether the DLA plays a different role in the development of these diseases than in humans or whether the expression of the DLA molecules in dogs is different from that in humans awaits further studies.

T Cell Receptors and Other T Cell Surface Proteins

Thymus-derived (T) lymphocytes play an important role in the immune system. T cells not only control antibody production by B cells but can also regulate cellular immune responses. T cells can recognize antigen-derived peptides (e.g. from virus infected or tumour cells) in the context of self-MHC molecules and are capable of killing infected cells (Janeway and Travers, 1997).

T cells – overview

In the vertebrate immune system, there are three major types of T cells: (1) T helper cells (T_H), (2) cytotoxic T lymphocytes and (3) $\gamma\delta$ T cells (Janeway and Travers, 1997). The T cells interact with antigen-presenting cells via their T cell receptor (TCR). The TCR of $CD4^+$ T cells (MHC class II restricted) and the TCR of $CD8^+$ T cells (MHC class I restricted) consist of an $\alpha\beta$ heterodimer encoded by two separate genes. The TCR γ and δ chains are also encoded by separate

genes. In humans the α, β, γ, and δ chains are composed of variable (V) regions and constant regions (C) separated by a joining (J) region just like immunoglobulin molecules (Janeway and Travers, 1997).

T cell receptor

Knowledge of the canine T cell receptor is limited. The constant regions (C) of the α and the β chains have been cloned and sequenced (Ito *et al.*, 1993; Takano *et al.*, 1994). These constant regions have 46% and 84% amino acid homology, respectively, with the corresponding human sequences. Recently the variable regions (V) of the TCR β chain have been cloned and sequenced and seven distinct genes have been identified (Dreitz *et al.*, 1999). The partial mRNA sequence of the TCR V–J regions of the γ chains have been cloned and submitted to Genbank (Avery and Burnett, 1999; unpublished Genbank data). The distribution and expression of canine α and β TCRs are just starting to be studied (German *et al.*, 1999). Rearranged TCR genes can be used as markers for malignant T cells (Dreitz *et al.*, 1999).

In humans, associated with the TCR at the cell surface, are CD3 polypeptides. The CD3 glycoprotein in humans is comprised of at least five polypeptide chains called γ, δ, ε, ζ, and η. In dogs one part of the CD3 complex has been cloned – the ε chain – and this has 58% amino acid identity to the human and to the mouse (Nash *et al.*, 1991a).

Other T cell surface proteins

There are other cell surface proteins on T cells besides the TCR that are of immunological importance that have been cloned. CD4, which is an accessory molecule for TCR-MHC-antigen recognition, has been cloned and sequenced in the dog (Gorman *et al.*, 1994). The molecule has 57% amino acid identity to the human. Unlike in humans the CD4 antigen is expressed not only on T helper cells but also on canine neutrophils (Williams, 1997). The CD8 antigen, which has the same function as CD4, but is expressed on cytotoxic or suppressor T cells has also been cloned in the dog (Gorman *et al.*, 1994). Other canine T cell surface proteins that have been cloned include CD28 (Pastori *et al.*, 1994) that binds CD80, CD38 (Uribe *et al.*, 1995), and CD44 (Milde *et al.*, 1994).

In order to study the distribution and expression of these receptors, monoclonal antibodies are useful. Monoclonal antibody CA 15.8G7 (Moore and Rossitto, 1993) recognizes TCRαβ and CA20.8H1 recognizes TCRγ (Moore *et al.*, 1994). Analysis of T cell receptor proteins as well as other proteins can aid in the study and diagnosis of canine leukaemias and lymphomas (Vernau and Moore, 1999). Monoclonal antibodies that recognize several other canine T cell surface proteins are available and have been summarized in various workshops (Cobbold and Metcalfe, 1994; Williams, 1997). Many antibodies

were found by screening monoclonal antibodies that recognize human proteins for cross-reactivity with canine proteins (Chabane *et al.*, 1994).

Immunoglobulins

Immunoglobulins – overview

Immunoglobulins (Ig), also called antibodies, are glycoproteins mediating humoral immunity and are produced by B lymphocytes. Activated B cells differentiate into immunoglobulin producing plasma cells. Immunoglobulins produced by one plasma cell are normally specific for a single antigen. The basic structure of all immunoglobulin molecules is a Y-like unit consisting of two identical light chains and two identical heavy chains linked together by disulphide bonds. Heavy and light chains have N-terminal variable (V) and C-terminal constant (C) regions. Each immunoglobulin molecule is bifunctional: the V region of the molecule binds to the antigen while the C region mediates binding of the immunoglobulin to host tissues, including various cells of the immune system and the first component of the classical complement system. The class and subclass (also called isotype) of an immunoglobulin molecule is determined by its heavy chain type. The different isotypes are associated with different immunoglobulin functions. Mammals express some or all of the five known immunoglobulin classes, IgM, IgD, IgG, IgA and IgE. Furthermore, different subclasses of immunoglobulins are often found, such as, for example, IgG1, IgG2, IgG3 and IgG4 in humans. The immunoglobulin subclasses can have various biological activities. The differences between the various subclasses within an immunoglobulin class are less than the differences between the different classes. Unlike immunoglobulin classes, the number and properties of subclasses vary greatly between species.

IgG is the major immunoglobulin in serum, accounting for about 70–75% of the total immunoglobulin pool in humans. In the dog four IgG subclasses, IgG1, IgG2, IgG3 and IgG4, have been defined based on their electrophoretic mobilities and on data from chromatography (Mazza and Whiting, 1994).

IgM accounts for about 10% of the immunoglobulin pool in man and is the class that predominates in a primary immune response, i.e. after the first contact of the immune system with an antigen.

IgA is the predominant immunoglobulin in seromucous secretions such as saliva, tracheobronchial secretions, colostrum, milk and genito-urinary secretions. It represents 15–20% of the human serum immunoglobulin pool. A selective IgA deficiency has been described in the dog (Felsburg *et al.*, 1987).

IgE is only present in traces in serum and is mainly bound on the surface of basophils and mast cells. IgE plays an important role in the defence against endoparasites and in the pathogenesis of allergic diseases, which are rather frequent in the dog, explaining the comparatively good knowledge about canine IgE. A study by Peng *et al.* (1997) shows functional and physical heterogeneity of canine IgE, suggesting that dogs may have two IgE subclasses, IgE1

and IgE2. A canine IgE monoclonal antibody specific for a filarial antigen has been produced (Gebhard *et al.*, 1995), as well as recombinant fragments of the constant region of the IgE heavy chain (Griot-Wenk *et al.*, 1998). These reagents will facilitate the study of allergic diseases in the dog.

IgD accounts for less than 1% of total plasma immunoglobulins in humans but is present in large quantities on the membrane of many circulating B lymphocytes. It plays a role in antigen-triggered lymphocyte differentiation. IgD is not found in all species. IgD has been demonstrated in humans, rats and mice and is probably present in dogs (Yang *et al.*, 1995). It is absent from pigs and rabbits.

All immunoglobulin classes can be detected in the dog, as summarized in Table 8.3.

The genetic organization and regulation of immunoglobulins is among the most complex systems yet known. Antibodies have to be so diverse that they can recognize millions of antigens. Furthermore, the class of antibody changes during the course of an antibody response (class switch), although the antigen-binding ability does not alter. Thus, a B cell will first make IgM and in some species IgD. Eventually, the responding B cell switches to synthesizing either IgG or IgA or IgE. The unwanted heavy chain constant region (C_H) genes are excised, and the required C_H gene is spliced directly to the *V* genes (Esser and Radbruch, 1990).

Immunoglobulin heavy chain genes

The immunoglobulin heavy chain results from the expression of different variable and constant region genes on the immunoglobulin heavy chain locus

Table 8.3. Immunoglobulin isotypes in the dog and reagents currently available for their detection

Class	Subclasses	Detected with:	References
IgG	IgG1, IgG2, IgG3, IgG4	Polyclonal Ab* Monoclonal Ab	German *et al*., 1998 Perez *et al*., 1998 Mazza and Whiting, 1994
IgA	–	Polyclonal Ab Monoclonal Ab	German *et al*., 1998 Perez *et al*., 1998
IgM	–	Polyclonal Ab	German *et al*., 1998 Perez *et al*., 1998
IgE	IgE1, IgE2?	Polyclonal Ab Monoclonal Ab α-chain of IgE receptor (human)	Halliwell and Longino, 1985 DeBoer *et al*., 1993 Wassom and Grieve, 1998
IgD	–	Monoclonal Ab	Yang *et al*., 1995

*Ab = antibody.

(*IgH* locus) after recombination has occurred. The genes coding for the variable region consist of *V* (variability), *D* (diversity) and *J* (joining) genes. About 100 different V_H genes have been characterized in humans. They are located on the 5′ end of the *IgH* locus. They are followed in the 3′ direction by *D* genes (four in humans) and by *J* genes (nine in humans). As far as is known at present, the organization of the *VDJ* genes is similar between species, but the number of *V*, *D* and *J* genes at the *IgH* locus varies from species to species. The genes coding for the constant region of the immunoglobulin heavy chain, also called C_H genes, follow the *VDJ* genes in the 3′ direction. The heavy chain from each immunoglobulin isotype is coded by its particular C_H gene (Esser and Radbruch, 1990).

The canine C_H genes for IgA and IgE have been mapped to chromosome 8 (Priat *et al.*, 1998; Mellersh *et al.*, 2000). We anticipate that, as in other species, the canine heavy chain genes for the other immunoglobulin isotypes and for the heavy chain variable region are also located on chromosome 8. In contrast with other mammals, the genetic organization of the C_H genes has not been published for the dog to date.

The canine IgA and IgE C_H genes have been cloned and sequenced (Patel *et al.*, 1995). The canine IgA C_H gene codes for a protein of 343 amino acids which displays 57–82% identity to the corresponding human sequence, depending on subclass and allotype of the human IgA, 72% identity to bovine, 70% to ovine, 69% to pig, 61% to mouse and 57% to rabbit IgA.

The constant region of the canine IgE heavy chain is 426 amino acids long. Comparison with the corresponding amino acid sequences of other species shows that dog IgE C_H has the highest identity with cat IgE C_H (76%) followed by horse (64%), pig (60%), human (55%), sheep (54%), bovine (53%) and mouse (48%). The canine high affinity receptor for IgE has been cloned and sequenced (Goitsuka *et al.*, 1999). Knowledge of the DNA sequence of the canine IgE high affinity receptor gene may be useful for the search of genetic markers associated with the genetic predisposition for IgE-mediated allergic diseases (atopy) in the dog (de Weck *et al.*, 1997). In humans, genetic linkage between atopy and the beta subunit of the high-affinity IgE receptor has been demonstrated (Sandford *et al.*, 1993).

Partial sequences of the canine VDJ region of the *IgH* locus (44–119 amino acids long, Genbank No. AAC25182–AAC25185) and of the unchangeable region of dog immunoglobulin gamma chain are available in the Genbank.

Although the amino acid sequence of the constant heavy chain region of canine IgM was published long ago (Wasserman and Capra, 1978; McCumber and Capra, 1979), its nucleotide sequence is not known. The nucleotide sequence of the canine IgD C_H gene is not yet available.

Immunoglobulin light chain genes

Immunoglobulin light chains are common to all classes of immunoglobulins. They also consist of a variable region, coded by *V* and *J* genes, and a constant

region, coded by *kappa* (κ) or *lambda* (λ) genes. All species possess two classes of light chains, κ and λ. DNA sequences from the κ and λ chains of the dog have been determined. As shown immunohistochemically, tissue from the tonsils, spleen and cervical lymph nodes from normal dogs express mainly λ (>91%) and rarely κ light chains (9%) (Arun *et al.*, 1996). Conversely in pigs and in humans, the κ/λ ratio is more or less balanced (Arun *et al.*, 1996).

In humans and in other species like cattle, immunoglobulin light chains are encoded on chromosomes other than those encloding the heavy chain genes. In humans, the genes coding for the κ and λ chains are located on two different chromosomes, chromosomes 2 and 22, respectively. The genomic localization of these genes has not yet been determined in the dog.

Cytokines and Cytokine Receptors

Cytokines – overview

Cytokines are low molecular weight proteins that mediate much of the intercellular signalling involved in the response to a variety of external stimuli including infections and inflammation. Cytokines are involved in the regulation of such important events as cell differentiation and growth, especially in the areas of lymphopoiesis and haematopoiesis. Cytokines are generally produced by more than one cell type and generally affect more than one cell type.

The experimental models for which dogs are used have often dictated the characterization of cytokines. For example, characterization of many of the canine cytokines involved in haematopoiesis was driven by the dog's importance as a model for haematopoietic stem cell transplants (reviewed in Thomas and Storb, 1999). Therefore characterization of the cytokines has not been systematic and much of what has been published describes the measurement of the cytokines. Table 8.4 summarizes the currently characterized canine cytokines, their function in humans (as the specific function in the dog is often unknown) and references for their sequence data.

Canine cytokines can be quantified using protein-based assays or mRNA-based assays. Quantitative reverse transcription–polymerase chain reaction (RT–PCR) is frequently used to detect or quantify cytokine. Protein-based assays include bioassays that often take advantage of the cross-reactivity between dog and human cytokines. Other protein-based assays that have been developed for some canine cytokines include enzyme-linked immunosorbent assay (ELISA), radioactive immunoabsorbent assay (RIA) and immunohistochemistry.

Table 8.5 summarizes the degree of similarity of canine cytokine amino acid sequences with those of other species. While the canine sequences most closely resemble those of the cat, another carnivore, the canine sequences are also typically closer to the human than the murine sequences, which underscores the dog's important contribution to understanding several human pathological states.

Table 8.4. Currently available detection methods for canine cytokines

Cytokine	Function	Reference
Granulocyte-macrophage colony stimulating factor	Causes maturation of granulocytes	Nash *et al.*, 1991b
Granulocyte colony stimulating factor	Causes maturation of granulocytes	Lovejoy *et al.*, 1993
Stem cell factor	Causes maturation of a variety of haematopoietic cells	Shull *et al.*, 1992
Erythropoietin	Stimulates production of red cells	Wen *et al.*, 1993
Interleukin-1α	Proinflammatory molecule	Straubinger *et al.*, 1999
Interleukin-1β	Proinflammatory molecule	Gilmore *et al.*, 1996; unpublished Genbank data
Interleukin-2	Growth and differentiation factor for B cells and T cells	Dunham *et al.*, 1995
Interleukin-3	Growth and differentiation factor for eosinophils	R.A. Nash, 2000, Seattle, WA, personal communication
Interleukin-4	B cell stimulating factor	van der Kaaij *et al.*, 1999
Interleukin-5	Stimulates proliferation and antibody production by B cells	German *et al.*, 1998; unpublished Genbank data
Interleukin-6	Acute phase reactant	Kukielka *et al.*, 1995
Interleukin-8	Neutrophil chemotactic factor	Ishikawa *et al.*, 1993
Interleukin-10	T cell ‘inhibitory’ cytokine	Lu *et al.*, 1995
Interleukin-12	Induces production of IFN-γ	Okano *et al.*, 1997
Interleukin-18	Induces production of IFN-γ	Argyle *et al.*, 1999
Interferon-α	Stimulates NK cell activity and enhances MHC class I expression	Himmler *et al.*, 1987
Interferon-β	Same as IFN-α	Iwata *et al.*, 1996
Interferon-γ	Induces MHC class I and class II expression	Zucker *et al.*, 1992
Insulin-like growth factor-1	Induces DNA synthesis	Delafontaine *et al.*, 1993
Transforming growth factor-β1	Regulates cell growth and immune responses	Manning *et al.*, 1995
Tumour necrosis factor-α	Proinflammatory mediator	Zucker *et al.*, 1994
Fibroblast growth factor-8	Promotes angiogenesis	Quinkler *et al.*, 1989
Acidic fibroblast growth factor-1*	Promotes angiogenesis	Canatan and Lin, 1998
Basic fibroblast growth factor*	Promotes angiogenesis	Trochta *et al.*, 1998; unpublished Genbank data

*Indicates the full molecule has not been cloned and/or no information is available on detection methods.

Cytokines for haematopoiesis

Several cytokines for haematopoiesis have been characterized, including granulocyte macrophage-colony stimulating factor (GM-CSF) (Nash *et al.*, 1991b), granulocyte colony stimulating factor (GCSF) (Lovejoy *et al.*, 1993), stem cell factor (SCF) (Shull *et al.*, 1992) and erythropoietin (EPO) (Wen *et al.*, 1993).

Some haematopoietic growth factors such as EPO, which is an erythrocyte growth factor, have been cloned and expressed in order to treat canine

Table 8.5. Cloned canine cytokines and amino acid identity (%) with other species

Cytokine	Mouse	Cow	Sheep	Pig	Horse	Cat	Human
GM-CSF	53	71	74	72	–	75	70
GCSF	66	75	77	74	–	86	75
SCF[a]	81	89	89	91	91	95	85
EPO[b]	NA	NA	NA	NA	NA	NA	NA
IL-1α	58	75	76	73	79	83	70
IL-1β	44	50	50	56	57	65	45
IL-2	55	62	62	72	70	81	74
IL-3[b]	NA	NA	NA	NA	NA	NA	NA
IL-4	39	66	66	73	59	81	50
IL-5[a]	56	75	75	79	79	80	58
IL-6	39	56	55	69	68	75	58
IL-8	50	88	89	88	76	89	73
IL-10	72	80	78	75	84	85	80
IL-12 p35 subunit	59	84	85	86	88	93	86
p40 subunit	64	83	83	80	87	91	85
IL-18	62	80	–	86	84	–	73
IFN-α	46	54	50	52	60	61	55
IFN-β	44	52	–	62	59	–	60
IFN-γ	40	78	80	72	83	87	65
IGF-1	89	90	90	93	93	–	92
TGF-β1	89	93	93	93	91	–	94
TNF-α	77	81	80	85	83	95	93
FGF-8	98	–	–	–	–	–	98
FGF-1	86	80	–	86	–	–	86
BFGF[a]	98	99	99	–	–	–	97

References for canine sequences as listed in the text. All other species' sequences were taken from Genbank and amino acid sequences were aligned using the BLASTP program (Altschul *et al.*, 1997).
[a]Full canine cDNA sequence not in Genbank.
[b]Canine sequence not in Genbank.

anaemia, as the recombinant human form of the molecule can lead to the development of antibodies in dogs because of differences in the protein sequence (Randolph *et al.*, 1999). EPO can be detected by a bioassay (MacLeod *et al.*, 1998). Canine GCSF which causes growth of granulocyte progenitor cells (Obradovich *et al.*, 1991) has been used in several canine haematopoietic stem cell transplantation experiments (Schuening *et al.*, 1993; Storb *et al.*, 1994; Sandmaier *et al.*, 1996) as well as a canine model of cyclic neutropenia (Hammond *et al.*, 1990). Canine GCSF can be detected by RT–PCR (Lovejoy *et al.*, 1993). Canine SCF has been found to contribute to the proliferation and differentiation of canine liver mast cells (Morimoto *et al.*, 1998) as well as speeding up haematopoietic recovery after myeloablative conditioning (Schuening *et al.*, 1993, 1997). Canine SCF can be detected by RT–PCR (Shull *et al.*, 1992) and by immunochemistry (Huss *et al.*, 1995). GM-CSF in humans primarily affects the growth of monocyte and macrophage progenitor cells and

has been widely used in humans to accelerate the recovery of granulocyte counts after chemotherapy. Canine GM-CSF can be detected by RT–PCR or by a bioassay (Nash *et al.*, 1991b).

While the crystal structure of canine GCSF has been determined and is similar to the human and bovine structures (Lovejoy *et al.*, 1993), the full amino acid sequences of some canine 'haematopoietic cytokines' such as IL-3, IL-5, and monocyte-colony stimulating factor (M-CSF) have not been published. IL-5 is a cytokine that causes the differentiation of eosinophils in humans. The molecule has been partially cloned and sequenced (German *et al.*, 1998; unpublished Genbank data). IL-3 is a cytokine that has profound effects on the growth of all haematopoietic lineages and the canine molecule has recently been cloned (R.A. Nash, Seattle, 2000, personal communication).

Interleukins

A variety of other canine cytokines including several interleukins (IL) have been cloned and expressed, including IL-1, which has a variety of proinflammatory effects including the induction of fever. IL-1 exists as two functionally active forms – IL-1α and IL-1β. Two distinct cDNA fragments of *IL-1*α have been found in the dog (Straubinger *et al.*, 1999). The sequence of *IL-1*β has been determined (Gilmore *et al.*, 1996; unpublished Genbank data) and an RT–PCR assay (Grone *et al.*, 1998) for the transcript has been developed as well as a bioassay (Bravo *et al.*, 1996). Polyclonal antisera raised against the human molecule can also be used to detect canine IL-1 (Day, 1996).

Human IL-2 is produced by T cells and can induce the proliferation of T cells, B cells, and natural killer (NK) cells. A canine *IL-2* cDNA has been isolated and sequenced (Dunham *et al.*, 1995; Knapp *et al.*, 1995). IL-2 can be detected by a bioassay (Helfand *et al.*, 1992) as well as RT–PCR (Rottman *et al.*, 1996; Grone *et al.*, 1999; Pinelli *et al.*, 1999). *IL-2* has been mapped to CFA13 or 19 (Mellersh *et al.*, 2000).

Human IL-4 is produced by activated T cells and can induce the secretion of immunoglobulins by B cells and also has a critical role in immunoglobulin (Ig) class switching. Canine *IL-4* has been recently cloned and sequenced (van der Kaaij *et al.*, 1999). *IL-4* can be detected by RT–PCR (Rottman *et al.*, 1996; Pinelli *et al.*, 1999).

Human IL-6, like IL-1, is produced by many cell types and affects a great variety of target cells. IL-6 can influence the immune response, acute phase reactants, and haematopoiesis. The complete canine *IL-6* cDNA sequence has been published (Kukielka *et al.*, 1995). Canine IL-6 can be detected by bioassay and immunohistochemistry (Rivas *et al.*, 1992; Yamashita *et al.*, 1994; HogenEsch *et al.*, 1995; Carter *et al.*, 1999; Kearns *et al.*, 1999) as well as RT–PCR (Rottman *et al.*, 1996). Canine IL-6 has been found to be elevated in the synovial fluid of a significant proportion of dogs with rheumatoid and osteoarthritis (Carter *et al.*, 1999). *IL-6* has been mapped to syntenic group 4 which is comparable to human chromosome 7p21 (Mellersh *et al.*, 2000).

Human IL-8 is an inflammatory cytokine produced by a variety of cells and promotes the adhesion of neutrophils to vascular endothelium. Canine *IL-8* has been cloned by two separate groups (Ishikawa *et al.*, 1993; Matsumoto *et al.*, 1994). IL-8 can be monitored by RT–PCR (Matsumoto *et al.*, 1994) or by a dog-specific ELISA (Maisson *et al.*, 1995). Monitoring levels of this molecule are important for studying canine models of bacteremia and other infections (Maisson *et al.*, 1995).

Human IL-10 is expressed by a variety of cells and is often considered an 'inhibitory' cytokine. IL-10 can cause an inhibition of an allogeneic lymphoproliferative response. Canine *IL-10* has been cloned and the recombinant protein expressed in Chinese hamster ovary cells and dog kidney cells (Lu *et al.*, 1995). Levels of the transcript can be measured by RT–PCR (Rottman *et al.*, 1996; Grone *et al.*, 1999; Pinelli *et al.*, 1999). Canine IL-10 has been mapped to CFA7 which is comparable to human chromosome 1q31–32 (Mellersh *et al.*, 2000).

Human IL-12 induces the production of interferon-gamma (IFN-γ) in T cells and NK cells and has a major regulatory role in the immune response. The canine form has been cloned by two groups (Okano *et al.*, 1997; Buttner *et al.*, 1998) and can be detected by RT–PCR (Rottman *et al.*, 1996; Buttner *et al.*, 1998; Grone *et al.*, 1999). The canine molecule, like other mammalian forms, has two subunits – p35 and p40 (Buttner *et al.*, 1998).

Another IFN-γ inducing factor, *IL-18*, has recently been cloned and sequenced in the dog (Argyle *et al.*, 1999; Okano *et al.*, 1999). Canine IL-12 and IL-18 had a synergistic effect on the production of canine IFN-γ and suppressed the growth of tumour cells implanted in SCID mice (Okano *et al.*, 1999). *IL-18* transcripts can be detected by RT–PCR (Okano *et al.*, 1999).

Interferons

Interferons were identified by their ability to prevent viral replication. Interferon-alpha (IFN-α) was originally isolated from leucocytes following a viral infection while interferon-beta (IFN-β) was isolated from fibroblasts after a viral infection. Activated T cells produce IFN-γ. The alpha interferons are actually a group of secreted proteins that inhibit cell proliferation and regulate MHC class I expression. The canine interferon genes have been mapped to syntenic group 4 which is comparable to human chromosome 9p22 (Mellersh *et al.*, 2000).

In dogs there are three *IFN-α* genes, two of which have identical coding sequences and the third of which is only slightly different from the other two (Himmler *et al.*, 1987). *IFN-α* can be detected by RT–PCR (Himmler *et al.*, 1987). *IFN-β* has been cloned and expressed and can be detected by RT–PCR (Iwata *et al.*, 1996). *IFN-γ* has been cloned by two separate groups (Devos *et al.*, 1992; Zucker *et al.*, 1992). IFN-γ can be detected by RT–PCR (Devos *et al.*, 1992; Grone *et al.*, 1999; Pinella *et al.*, 1999), a bioassay (Zucker *et al.*, 1993) or ELISA (Fuller *et al.*, 1994).

Other cytokines

Other canine cytokines that have been cloned to date include insulin-like growth factor 1 (IGF-1) that induces DNA synthesis in multiple cell types (Delafontaine *et al.*, 1993). *IGF-1* has been mapped to syntenic group 6 which corresponds to human chromosome 12q23 (Mellersh *et al.*, 2000). *IGF-1* can be detected by RT–PCR (Delafontaine *et al.*, 1993) or RIA (Maxwell *et al.*, 1998). Transforming growth factor-beta 1(TGF-β1) has many functions such as regulating cell growth and modulating immune responses. *TGF-β1* has been cloned and sequenced (Manning *et al.*, 1995) and can be detected by RT–PCR (Manning *et al.*, 1995) or use of polyclonal antisera generated against the human protein (Vilafranca *et al.*, 1995).

Tumour necrosis factor-alpha (TNF-α), an MHC class III gene, is another important immunoregulatory molecule that has been cloned and expressed (Zucker *et al.*, 1994). TNF-α can be detected by RT–PCR (Zucker *et al.*, 1994; Rottman *et al.*, 1996), bioassays (Yamashita *et al.*, 1994; Carter *et al.*, 1999; Kearns *et al.*, 1999) or by immunohistochemistry (Day, 1996). *TNFα* has been mapped to CFA12 and is located between the MHC class I genes and the class II genes as it is in the human on chromosome 6p (Mellersh *et al.*, 2000). Less well known cytokine genes have been cloned including *fibroblast growth factor-8 (FGF-8)*, which is felt to be involved in the development of prostatic hypertrophy in the dog (Canatan and Lin, 1998), *acidic fibroblast growth factor-1 (FGF-1)* (Quinkler *et al.*, 1989) located on CFA2 (Mellersh *et al.*, 2000) and *canine basic fibroblast growth factor* (*BFGF*) that promotes angiogenesis (Trochta *et al.*, 1998; unpublished Genbank data).

Cytokine receptors

Cytokine receptors are classified on the basis of the domains their tertiary structures form, such as the immunoglobulin superfamily, the interferon receptor superfamily, the haematopoietic superfamily, etc. Relatively little has been published about canine cytokine receptors. The most widely studied canine cytokine receptor is the IL-2 receptor. In the human, the IL-2 receptor (IL-2R) has three IL-2 binding chains: IL-2Rα, which is recognized by the monoclonal antibody Tac and is classified as a member of the immunoglobulin superfamily; IL-2Rβ; and IL-2Rγ, which is located on the X chromosome and which has a structure that classifies it as a member of both the haematopoietic superfamily and the fibronectin type III superfamily (reviewed in Nelson and Kurman, 1994). The common γ subunit also forms part of the IL-4, IL-7, IL-9, and IL-15 receptors (reviewed in Felsburg *et al.*, 1999). The canine *IL-2Rγ* chain has been cloned and sequenced (Henthorn *et al.*, 1994). Various mutations including a frame-shift with a premature termination codon (Henthorn *et al.*, 1994) as well as a single nucleotide insertion (Somberg *et al.*, 1995) can cause an X-linked severe-combined immunodeficiency that is very similar to the human disease in terms of its clinical features and which

receptors are affected. The IL-2Rγ chain (CD132) has 71% amino acid identity to the mouse, 81% to the cow, and 84% to the human. The canine *IL-2R*α (CD25) chain has also been cloned and sequenced (Dickerson *et al.*, 1998; unpublished Genbank data). This receptor has 52% amino acid identity to the mouse, 54% to the cow and 61% to the human. Because canine IL-2 receptors bind human IL-2, IL-2 receptors in dogs can be detected by flow cytometry (phycoerythrin-labelling human IL-2) (Somberg *et al.*, 1992) or radiolabelling human IL-2 (Helfand *et al.*, 1995). The only other canine cytokine receptor that has been cloned to date is canine IL-8R (Chang *et al.*, 1999). The cDNA encodes for a protein of 356 amino acids which has 75% identity to the human sequence, 75% identity to the cow sequence and 41% to the mouse.

Summary

In conclusion, exploration of the canine immune system has been driven by the dog's importance as a model for several human diseases or for drug toxicity and transplantation experiments. In certain areas, such as mapping the canine MHC, the dog has lagged behind other species such as the pig, but in other areas, such as histocompatibility typing, the techniques have been developed to a greater extent in the dog than in other species. In fact, the dog was the first species in which the value of *in vitro* histocompatibility matching for the outcome of haematopoietic stem cell transplantation was demonstrated in the late 1960s (Epstein *et al.*, 1968). The last 10 years have seen a rapid growth of molecular genetic analyses of the canine immune system, but much more work needs to be done, especially detailed functional studies of known and soon to be characterized immune system molecules. A better understanding of the canine immune system could lead to a better understanding of a diverse variety of human and canine diseases.

Acknowledgements

This work was supported in part by a grant to J.L.W. from the National Institutes of Health (NIH)-National Center for Research Resources (RR 12558) and in part by a grant to R.S. from the NIH-National Cancer Institute (CA78902). E.M. was supported by a grant from the Hans-Sigrist Foundation of the University of Berne, Switzerland.

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Genetic Aspects of Disease in Dogs

9

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Introduction to Canine Genetic Disease

The domestic dog is second only to human beings in the number of reported naturally occurring hereditary disorders. More than 370 canine diseases are recognized as inherited or having major inherited components (Patterson,

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2000a), with new defects identified each year. This total includes a number of diseases considered to have 'breed predisposition' or increased prevalence within certain breeds. These diseases clearly depend on genotype, but are difficult to observe in outbred populations perhaps due to masking by heterozygosity in modifier genes, or co-selection with breed type (e.g. osteosarcoma in giant breed dogs). More than 200 canine genetic diseases are believed to be simple Mendelian (monogenic) traits, with approximately 70% demonstrating autosomal recessive inheritance patterns (Patterson, 1980). This percentage is somewhat higher than in human medicine, where just under 60% of diseases with monogenic inheritance patterns are autosomal recessive.

The study of canine genetic disease has a strong bearing on human medicine. At the time of writing, more than 215 canine disorders display close homology to specific human disease counterparts. The same gene product has been shown to be abnormal in dogs and humans in 41 of these diseases (Ostrander *et al.*, 2000). Early progress in canine mutation detection was accomplished by studying candidate genes already characterized in the corresponding human or rodent disease. The first disease-causing mutation in dogs was found to be a point missense mutation in clotting Factor IX. The resultant transcript was unstable, causing the disease phenotype of haemophilia B (Evans *et al.*, 1989). Undoubtedly, the development and availability of a canine genome map will speed localization and identification of canine disease genes. Dog gene mappers will be able to repay their debt to human molecular geneticists through discovery of disease-causing canine mutations applicable to human medicine. This process has begun in the recent identification of a mutation in the hypocretin type 2 receptor gene in narcoleptic Dobermann Pinschers (Lin *et al.*, 1999).

Emergence of genetic disease-population structure

Dogs have been heavily selected for particular traits of body size, conformation, pelage, temperament and behaviour over a period of at least 15 millennia, and they display greater phenotypic diversity than any other mammalian species. Particular specialized dog types became established through relative inbreeding, and for at least 150 years, breed-lines recognized for show purposes have often been deliberately genetically isolated. The prestige and monetary value of 'show champions' have contributed to further shrinkage of the gene pool through popular sire or founder effect. For instance, in the last 30 years, only 3–5% of registered dogs were used to produce the present purebred dog populations in the Netherlands (Ubbink *et al.*, 1998a). The resulting high levels of inbreeding and homozygosity contribute to the propagation of autosomal recessive and other inherited diseases in purebred dogs. In some breeds, it is likely that unwitting co-selection of a disease gene with desired traits has been important for establishing high prevalence of a

particular disease. Finally, the predominance of recessive disease conditions may result from the relative ease of selection against dominant disease traits.

Even within a breed, considerable genetic isolation often develops through line breeding. Studies of elbow dysplasia in Labrador Retrievers, portosystemic shunts in Irish Wolfhounds, and hepatic copper toxicosis in Bedlington Terriers showed that disease occurred in clusters of highly related animals and other clusters were unaffected (Ubbink *et al.*, 1998a, b). These authors have produced accurate models of risk for (presumed) complex disease traits by estimating relatedness of dogs to particular ancestors (Ubbink *et al.*, 1998b, c). Many genetic diseases, however, are not restricted to purebred dog populations. In clinical practice, veterinarians commonly encounter cases of hereditary congenital and skeletal defects, and monogenic disorders in crossbred ‘mutts’. In fact, the occurrence of a monogenic defect in unrelated crossbred dogs suggests that the defect is mutationally heterogeneous.

Complex traits: polygenic disease and major gene effect

Hereditary diseases having quantitative phenotypic variables with continuous distribution, or complex phenotypic expressions are often found to be polygenic traits. Compared with monogenic disorders, polygenic traits often have low heritability, i.e. the proportion of the total population variance in phenotype attributable to genetic causes is fairly low. Many common disease conditions of dogs (hip dysplasia and other skeletal abnormalities, congenital cardiac malformations and cardiomyopathies) were assumed to be polygenic and to have relatively low heritability. Recently, major gene effects on some presumed polygenic disorders have been demonstrated in controlled breeding conditions. Unlike clinical studies, the experimental pedigree studies reduced environmental influence and increased genetic homogeneity for weak modifier loci. The complex phenotype of cardiac conotruncal defect was shown to segregate as a single recessive locus through studies of F_2 crosses and backcrosses of a Keeshond line (with high disease expression) bred with unaffected Beagles (Patterson *et al.*, 1993). The disease gene is now being mapped, after exclusion of an obvious human homologue (Werner *et al.*, 1999). Single genes may also have major effects in some skeletal abnormalities (see Chapter 10). The identification of major gene effects on complex traits has often been hampered by the apparent poor response of selection to reduce disease prevalence (see Willis, 1997, on hip dysplasia in the UK). Features that complicate studies of voluntary or clinical screening programmes include conflicting interests for selection on the basis of many traits, biased sample selection, and inaccurate phenotypic ascertainment. Although many complex traits will prove to be polygenic, and a major gene locus in one breed may be unimportant in other breeds, experimental controlled mating studies can greatly simplify detection of major gene effects.

The Burden of Genetic Disease

Domestic dogs, as companion animals, receive a high level of individualized veterinary care. As a result of this scrutiny, reports of hereditary disease in dogs are common. Purebred dogs are popular throughout the developed world, thereby enhancing the recognition of hereditary disease in geographically distant, but related, individuals. Studies of disease prevalence in recent years suggest that a high proportion of dogs suffer from genetic diseases. Most such studies have been non-randomized and concentrated on a single disease or a single breed. Many breeds have more than 30 reported genetic diseases, although most defects are probably rare in the breed overall. The number of clinically relevant genetic defects (those that cause significant morbidity or mortality) may be around 4–8 in each breed, although accurate estimates, especially of complex disease traits, are difficult to develop.

Inherited prenatal and congenital defects

The burden of genetic disease begins before birth. Reproductive defects and embryonic and early fetal mortality are recognized by reductions in litter size. A number of studies have shown a strong correlation ($r = 0.83$) between litter size and dams' body weight (Robinson, 1973; Willis, 1989). The actual litter size for certain breeds (Pomeranian, Cavalier King Charles Spaniel, Newfoundland) deviates from predictions based on body weight, indicating a genetic basis for reduced litter size.

The prevalence of a number of congenital (although not exclusively hereditary) abnormalities has been estimated through case review. A prevalence of 7 cardiac malformations per 1000 cases was found in a comprehensive review of more than 35,000 case records from the veterinary clinic of the University of Pennsylvania over the period 1953–1965 (Patterson, 1971). Anatomical subclassification of the various defects revealed strong breed associations, subsequently confirmed by other survey studies and discussed in more detail in the following section on hereditary cardiac defects. A recent study of subaortic stenosis (SAS) found that breeds at significantly increased relative risk included the Newfoundland (odds ratio, 88.1), Rottweiler (odds ratio, 19.3), Boxer (odds ratio, 8.6), and Golden Retriever (odds ratio, 5.5) (Kienle *et al.*, 1994). Of the dogs available for follow-up, mortality attributed to SAS was 21% (primarily within 3 years of diagnosis), whilst morbidity was 33%. For Newfoundlands, this data represents a breed incidence of SAS of greater than 10%.

General studies on the heritability of perinatal mortality have been reviewed (Willis, 1989). In most surveys, heritable effects are modest. Demonstrable effects of sire on litter size were found for Airedale Terriers, Cocker Spaniels and Elkhounds, with no effects for other breeds in the same survey (Lyngset, 1973). Sire effect was also found on 1-week mortality rates in the Beagle (Gaines and Van Vleck, 1976). More recent estimates of the total

burden of congenital defects include a mortality estimate of 6.3% (Walter and Kirchhoff, 1995) and an estimated prevalence of congenital defects of 16.5% in puppies offered for sale (Ruble and Hird, 1993).

Breed specific neoplasia

Neoplasia is a major cause of death in all breeds of dog, but some breeds show marked predisposition to particular tumour types. Unfortunately, few heritability or prevalence estimates are available. Heritability for histiocytosis in the Bernese Mountain Dog was estimated at 0.298 (Padgett *et al.*, 1995). Population mortality statistics reveal large differences in age specific cancer rates between different breeds. In one survey the Boxer (highest incidence) has been shown to be 35 times more likely to suffer cancer than the Dunker (lowest incidence) (Arnesen *et al.*, 1995). Several attempts have been made to identify genes controlling breed susceptibility to cancer, generally by looking at tumour suppressor genes as candidates. Recently, a locus conferring susceptibility to hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis (RCND) was identified in German Shepherd dogs. This tumour is inherited as a rare autosomal dominant condition with high penetrance but late onset (Lium and Moe, 1985). In a large pedigree segregating for the tumour, the causative gene was recently mapped to canine chromosome 5q12–q13. Interestingly, no known homologues of human tumour suppressor genes are present at this location (Jónasdóttir *et al.*, 2000).

Simple and complex traits

Incidence rates of genetic disease with simple Mendelian inheritance vary greatly. Many disease reports are based on single families, but some diseases have apparent high prevalence. For example, prevalence of copper toxicosis in Bedlington Terriers was 0.46 in Holland (Ubbink *et al.*, 2000) and 0.34 in Britain (Herrtage *et al.*, 1987). Collie eye anomaly affected over 64% of Border Collies and 72% of Shetland Sheepdogs in the UK (Bedford, 1982). These diseases are both considered autosomal recessive, suggesting disease allele frequencies of 60–85% in the populations surveyed. Such high prevalence rates suggest selection either for a closely linked trait, or for an epistatic effect of the disease allele itself. Studies of data collected in testing for progressive retinal atrophy and for fucosidosis suggest that, in the absence of specific selection efforts against the defect, disease alleles have frequencies of 6–20% or more in different breeds (D. Sargan, unpublished data). These frequencies may be typical of the maintenance of an allele through genetic drift in a relatively inbred population. Bias introduced through self-selection by breeders for participation in test programmes will affect estimates of prevalence. Nevertheless, if five unlinked, monogenic defects are present within a breed (each of which has a disease allele frequency of 10%) then less than 1 in 20 dogs of that

breed will actually display signs of any of these genetic diseases. The likelihood that an individual dog will NOT carry ANY disease allele, however, is only 0.35. This consideration has profound effects as carrier detection tests are developed for increasing numbers of different disease traits (see Chapter 16).

Skeletal defects such as hip dysplasia have heritabilities of 0.2–0.6, and contribute heavily to inherited morbidity. Screening schemes for these defects give reliable estimates of total prevalence. Thus 14% of all screened dogs showed hip dysplasia (Kaneene *et al.*, 1997), with breed specific prevalence of up to 73% for the Bulldog. Further details are given in Chapter 10.

A Compilation of Genetic Disease Traits

In the remainder of this chapter we review the genetic diseases of dogs having a probable monogenic basis. We summarize clinical signs, pedigree studies and the biochemical and/or molecular genetic defects associated with the various disease phenotypes. Space limitations preclude discussion of breed predisposition for neoplasia, polygenic disease and diseases with low heritability. Detailed treatments of behavioural, developmental, morphological, hormonal and orthopaedic disorders, and of some immune mediated diseases will be found elsewhere in this book. Additional information on these and other defects may be found in the list of canine genetic defects by Patterson (2001).

Inherited diseases affecting eyes and ears

Vision defects, and to a lesser extent hearing defects, are often obvious to dog owners and inherited deficiencies in these senses have been reported in dogs for more than 100 years. This section of inherited diseases of the eye emphasizes intra-ocular defects. We omit conditions affecting the eyelid and lashes, and many complex hereditary congenital multi-ocular defects for which the mode of inheritance is unknown. Several syndromic conditions are dealt with later in this chapter, including diseases affecting both eyes and ears.

Progressive retinal atrophies (Table 9.1)

Progressive retinal atrophies (PRA) have been studied more intensively by molecular geneticists than any other group of canine hereditary diseases. One reason for this scrutiny is that the canine diseases are paralogues of the retinitis pigmentosas, the most common inherited human blindness of the developed world. Further enhancing the value of the canine disease models are thorough studies (by Rubin, Aguirre and co-workers in the USA, and Barnett and others in the UK) that led to early identification of these defects in many breeds, including ultrastructural and in some cases biochemical descriptions (reviewed in Gellatt, 1999). Progressive retinal atrophies are progressive degenerations of the retina. Their onset is characterized by bilateral nyctalopia (night blindness),

Table 9.1. Canine hereditary eye defects

Disease	Affected breeds	Biochemical/ phenotype/ progression comments	Causative mutation	References	Human homologous locus/chromosome
Progressive retinal atrophies					
PRA – rod–cone dystrophy type 1 (rcd-1)	Irish Setter, Sloughi (heteroallelic)	Setter: Elevated cGMP in retina. Failure of outer segment development Sloughi: early onset PRA	Setter: Trp > stop: codon 807 of *PDE6B* Sloughi: 8 bp insertion, exon 21, *PDE6B*	Hodgman *et al*., 1949; Aguirre & Rubin 1975; Suber *et al*., 1993; Clements *et al*., 1993; Dekomien *et al*., 2000	4p16.3
PRA – rod–cone dystrophy type 2 (rcd-2)	Collie	Elevated cGMP in retina. Nyctalopia by 6 weeks, blind by 1 year	Non allelic with *rcd*-1, *erd* No mutations found in any cGMP-PDE subunit	Wolf *et al*., 1978; Acland *et al*., 1989; Wang *et al*., 1999	
PRA – rod–cone dystrophy type 3 (rcd-3)	Cardigan Welsh Corgi	Nyctalopia by 8 weeks	Single base deletion in *PDE6A*	Keep, 1972; Petersen-Jones *et al*., 1999	5q31.2–q34
PRA – early retinal degeneration (erd)	Norwegian Elkhound	Nyctalopia by 6 weeks. Rods and cones have abnormal synaptic termini	Non-allelic with *rcd*-1, *rcd*-2 Linkage group L11 (Neff *et al*.,1999)	Acland & Aguirre, 1987; Acland *et al*., 1989; Acland *et al*., 1999	12p13–12q13
PRA – progressive rod–cone degeneration	Labrador Retriever, Chesapeake Bay Retriever, Portuguese Waterdog, Miniature & Toy Poodles, American & English Cocker Spaniels	Late disease, but age of onset varies with breed. Photoreceptors appear to develop normally, then develop irregularities. Disc transport up outer segment reduced	Linked to centromeric region of canine chromosome 9	Aguirre & Rubin, 1972; Aguirre & O'Brien, 1986; Acland *et al*., 1998	Allelic with human RP17 17q22–q25
PRA – progressive retinal atrophy (retinal pigment epithelium dystrophy)	Briard	Referred to as congenital stationary night blindness in early reports, subsequently seen to be progressive	4bp deletion in *RPE65*	Narfstrom *et al*., 1989; Anderson *et al*., 1997; Aguirre *et al*., 1998; Veske *et al*., 1999	1p31
PRA – X-linked progressive retinal atrophy	Siberian Husky	X-linked recessive	Linkage to canine X at *RPRG* locus. No abnormalities in *RPRG*	Acland *et al*., 1994; Zeiss *et al*., 2000	Homologue of RP3 : Xp21.1
PRA – photoreceptor dysplasia (pd)	Miniature Schnauzer	Photoreceptors abnormal from 24 days, yet vision maintained until late in degeneration (>2 years)	A number of candidate genes rejected	Parshall *et al*., 1991; Zhang *et al*., 1999	n.a.
PRA – autosomal recessive progressive retinal atrophy	Miniature Long Haired Dachshund	Photoreceptor outer segments abnormal from 6 weeks, advanced degeneration by 25 weeks. Blind 1–2 years. Recent work suggests this may be CORD (i.e. cone led)	Linkage studies show mutation on canine chromosome 15	Curtis & Barnett 1993; Ryder, 2000	12q24.1–q24.3 or short regions of 1p or q

Table 9.1. *Continued*

Disease	Affected breeds	Biochemical/ phenotype/ progression comments	Causative mutation	References	Human homologous locus/chromosome
PRA – autosomal recessive progressive retinal atrophy	Tibetan Terrier	Outer segment abnormalities from 9 weeks, ERG and ophthalmoscopic changes by 10 months–1 year. Distinct from lipofuscinosis in same breed	A number of candidate genes rejected	Garmer 1974; Barnett & Curtis, 1978; Millichamp *et al*., 1988	n.a.
PRA - autosomal recessive progressive retinal atrophy – (other/unclassified)	Many breeds	Breed specific onset and progression	A number of candidate genes rejected in some breeds	See Genetics Comm. of Am. College of Ophthalmol. (1999) 'Ocular disorders presumed to be inherited in dogs'	n.a.
Other retinal dysplasia/degenerations					
Cone degeneration	Alaskan Malamute	Cone-led retinal degeneration. ERG has no rod responses. Early loss of beta-3 subunit of transducin	No mutations found in any transducin subunit. No changes in mRNA amount	Rubin, 1971; Ray *et al*., 1997; Akmedov *et al*., 1998	
Multifocal retinal dysplasia with skeletal abnormalities	Labrador Retriever	Abnormal retinal development associated with cataract, corneal pigmentation; retarded growth in the long bones; un-united/hypoplastic anconeal and coronoid processes; hip dysplasia, and delayed development of epiphyses	Retinal abnormalities incomplete dominant; skeletal abnormalities recessive	Carrig *et al*., 1988	
Multifocal retinal dysplasia (no skeletal deformities)	Golden Retriever, Labrador Retriever, American & English Cocker, others	Diseases of retinal hyperproliferation/ incomplete differentiation	Autosomal recessive or incomplete dominant	MacMillan & Lipton, 1978; Dietz, 1985; Long and Crispin, 1999	
Collie eye anomaly (CEA)	Rough & Smooth Collie, Shetland Sheepdog, Lancashire Heeler	Bilateral choroidal hypoplasia Papillary or peripapillary coloboma and neuroretinal non-attachment	Autosomal recessive Incidence up to 70%. Not linked to *PAX3*	Barnett, 1979 Bedford, 1982, 1998; Bedford, personal communication	
Ceroid lipofuscinosis	English Setter	Autofluorescent lipopigments in neurons and neuronal derivatives – marked neurological signs	Not linked to *CLN2* or *CLN3*. Canine linkage group defined	Lingaas *et al*., 1998; Shibuya *et al*., 1998	

Ceroid lipofuscinosis	Tibetan Terrier, Miniature Long Haired Dachshund, Miniature Schnauzer, Australian Cattle Dog, Border Collie, others	Nyctolopia from 2 months precedes fundoscopic changes – distinct from PRA in all breeds – less marked neurological signs than in Setter. Many biochemical features investigated	Autosomal recessive	Riis *et al*., 1992; Vandevelde & Fatzer, 1980; Jolly *et al*., 1997; Wood *et al*., 1987; Studdert & Mitten 1991
Cataracts		**Progression**		
Posterior polar sub-capsular	Golden Retriever, Labrador Retriever, Chesapeake Bay, Flatcoat Retriever, Munsterlander (Irish Red & White Setter)	None or slow after first examination (usually at 1 year)	Dominant, incomplete penetrance	Curtis & Barnett, 1989; Bedford, personal communication
Central, sub-capsular	German Shepherd Dog	Progressive from 8 weeks, slows after 1–2 years, total blindness rare	Autosomal recessive	Barnett, 1986
Central (some projections to exterior)	German Shepherd Dog	Congenital, not progressive	Dominant, incomplete penetrance	von Hippel, 1930
Nuclear progressing to total	Boston & Staffordshire Bull Terrier, Miniature Schnauzer	Commences 2–6 months. Rapid progress to blind at 2 years	Autosomal recessive	Barnett, 1978
Radial spoke-like opacities	Boston Terrier	Commences 4–5 years, variably progressive	Unknown	Curtis, 1984
Mainly nuclear, with microphthalmos	Miniature Schnauzer (Cavalier King Charles, West Highland White & Russian Terriers, Old English Sheepdog)	Congenital, variable or no progression	Autosomal recessive (Cavalier K.C. and others in brackets: unknown)	Gelatt *et al*., 1983; Barnett, 1985; Narfstrom, 1981; Lohmann & Klesen, 1997
Nuclear, pulverulent	Norwegian Buhund	From 6 weeks, progressive to whole nucleus by 4 years	Dominant, incomplete penetrance	Bjerkas & Haaland,1995
Cortical to total	Afghan Hound, Welsh Springer Spaniel	From 4 months to total by 4 years From 8 weeks, to total by 2 years	Autosomal recessive	Roberts & Helper, 1972; Barnett, 1980

with subsequent loss of peripheral day vision and eventual total blindness. Typically, initial degeneration of the rod photoreceptors is followed by loss of cones, and eventual apoptotic cell death of the whole neural retina. Ophthalmoscopically, thinning of the retina leads to tapetal hyper-reflectivity and prominent retinal blood vessel attenuation. These changes, however, are usually preceded by easily measurable reductions in ERG sensitivity. The diseases affect more than 108 breeds (reviewed by Whitley *et al.*, 1995 and by the Genetics Committee of the American College of Ophthalmologists (1999)), but the age at onset and rate of progression varies between breeds. Broadly, the diseases are classified as those of early onset, in which the ERG is never normal and photoreceptors never develop outer segments; those with manifest abnormality of ERG by a few months of age, in which photoreceptors show some development of abnormal outer segments, but degenerate rapidly; and those of late onset (from 1 year to 8 years) in which, at least in some cases, eyesight appears to develop normally and photoreceptor outer segments appear normal, but then degenerate. Finally, several breeds with retinal degenerations superficially rather similar to PRA have now been shown to be suffering from ceroid lipofuscinoses.

The differences in time course between breeds reflect genetic heterogeneity. With the exception of X-linked recessive PRA of the Siberian Husky (Acland *et al.*, 1994), all diseases for which the mode of inheritance has been described are autosomal recessive. Autosomal dominant or more complex patterns of inheritance may be uncovered in some breeds with additional study. Autosomal dominant RP accounts for more than 20% of all human RP, and digenic forms have also been described (RetNet at http://www.sph.uth.tmc.edu/Retnet/sum-dis.htm gives a summary of 30 known human RP loci). The autosomal recessive forms of canine PRA are genetically diverse. Complementation experiments showed that at least three gene loci were involved in early onset PRAs, and that another caused later onset PRA (Acland *et al.*, 1989). Recently, at least four more genes or gene loci for PRA have been reported, with many more yet to be discovered.

Initially, a candidate gene approach was used to define the mutations causing PRA. The earliest PRA described was in the Gordon Setter by Magnusson in 1911. The same disease found in Irish Red Setters (Hodgman *et al.*, 1949) was later given the name rod–cone dysplasia type 1 (rcd-1) and fully characterized by Aguirre and Rubin (1975). As early as 1978, Aguirre and co-workers showed that cGMP accumulated in the retina of affected setters at the normal time of outer segment development (Aguirre *et al.*, 1978, 1982; Barbehenn *et al.*, 1988). In 1990, a similar retinal degeneration of mice (*rd* or *rd-1*) was shown to be due to mutation in the gene encoding the beta subunit of rod specific cGMP-phosphodiesterase, *PDE6B* (Bowes *et al.*, 1990; Pittler and Baehr, 1991). In 1993 Suber *et al.*, and Clements *et al.* showed that the rcd-1 form of canine PRA was caused by a nonsense mutation (Trp > stop) at codon 807 of the *PDE6B* gene. This mutation is unique to the Setter breed, but a different mutation in the same gene occurs in Sloughi PRA. Mutations in the *PDE6B* gene were subsequently found in some human RP families.

PDE6B is a member of the visual transduction cascade, a G-protein cascade that transduces the signal caused by photopic isomerization of retinal at the optic disc of the photoreceptor, leading to hyperpolarization of the cell membrane. Evidence that other visual transduction cascade genes were mutated in severe and early onset RP families initiated the cloning of essentially all canine visual transduction cascade genes over the next few years. Most of these genes have now been screened for mutations in breeds of dog affected with early or first year onset PRAs (reviewed by Petersen-Jones, 1998). A mutation has been identified only in a single breed, the Cardigan Welsh Corgi, which suffers an early onset PRA (Keep, 1972). This disease has recently been shown to arise from a single base deletion in the *PDE6A* locus, encoding the alpha-subunit of the cGMP-PDE implicated in setter PRA (Petersen-Jones *et al.*, 1999). The Corgi degeneration has been called rcd-3 to distinguish it from rcd-1 and another early onset PRA with elevated cGMP, rcd-2 in the Collie (Wolf *et al.*, 1978). The latter has no mutations in any cGMP-PDE subunit.

A number of other specific photoreceptor structural or transport proteins have been implicated in human and rodent retinal degenerations; however, these have been investigated as candidate genes for PRA without success. In the case of X-linked PRA of the Siberian Husky (Acland *et al.*, 1994), mapping guided by the human RP3 region showed close linkage to the retinitis pigmentosa GTPase regulator (*RPGR*) (Zeiss *et al.*, 2000). The *RPGR* gene is mutated in about 70% of human X-linked RP patients. The causative mutation for PRA in the Husky, however, has not yet been found.

The earliest and most severe retinal dystrophies of humans are associated with combined rod-cone degenerations known as Leber's congenital amaurosis. Some of these cases are associated with mutations in the *RPE65* gene, which encodes a 61 kDa protein expressed at high levels in the retinal pigment epithelium. This protein may have a role in production of 11-*cis*-retinol (Choo *et al.*, 1998; Redmond *et al.*, 1998) and has been shown to be mutated in a slowly progressive retinal degeneration of the Briard. The defect in Briards has been described both as a congenital stationary night blindness and a PRA (Narfstrom *et al.*, 1989; Veske *et al.*, 1999).

As the canine genetic map has advanced, more attempts have been made to map PRAs by linkage to polymorphic microsatellite or RAPD markers. This led to the mapping of the most common and widespread PRA, progressive rod–cone dystrophy (prcd). In prcd, photoreceptors form normally but the rate of outer segment renewal is reduced and finally the photoreceptors degenerate. Apoptotic cell death may be seen by 2 months, but the process is gradual and may not be complete until 3 years of age or more. Markers have now been found at zero genetic distance from the mutation allowing the development of a DNA based test for prcd. The *PRCD* locus maps to the centromeric region of canine chromosome 9, homologous to human 17q, where a dominant form of RP, RP17, is located (Acland *et al.*, 1998). Several genes mapping in the area of the mutation have been ruled out as candidates, including *APOH*. The RP17 gene is also unidentified as yet. PRA maps to the *prcd* locus in Labrador and Chesapeake Bay Retrievers, Portuguese Water

Dogs, American Cocker Spaniels, and at least some Miniature and Toy Poodles. The variety of these affected breeds suggests that the mutation is ancient.

Early retinal degeneration (erd) is an early onset PRA of Norwegian Elkhounds. The defect maps to a group corresponding to RH34a and RH40a of (Priat *et al.*, 1998), which in turn corresponds to a region showing synteny with human 12p13–12q13 (Acland *et al.*, 1999). This region has not been associated with retinal disease in humans. Mapping of the PRA locus has recently been successful in the Miniature Longhaired Dachshund (Curtis and Barnett, 1993, Ryder, 2000). This PRA is associated with outer segment optical disc stacking, and moderate to severe barrel width and orientation abnormalities. The disease maps to canine chromosome 15, in a region not associated with human or murine retinal disease.

Other retinal diseases

A cone-led retinal degeneration of Alaskan Malamutes is characterized immunochemically by the early loss of the beta 3 subunit of cone transducin (Rubin, 1971; Gropp *et al.*, 1996). This loss of transducin is not caused by mutations in the cDNA of any cone-expressed subunit of transducin, nor with changes in the amounts of any cognate mRNA (Ray *et al.*, 1997; Akmedov *et al.*, 1998).

Inherited 'multifocal retinal dysplasias' have been noted in a number of breeds, including Golden and Labrador Retrievers, and American Cocker and Springer Spaniels. The defects are present at birth and range from local folding of the retina, through geographically limited areas of abnormal development, to severe retinal disorganization and detachment of the retina. These dysplasias are often classified as diseases of hyperproliferation and inadequate differentiation. Apparent recessive and incomplete dominant forms occur. In Labrador Retrievers, the severe retinal dysplasia phenotype may be associated with cataracts and corneal pigmentation, and with skeletal abnormalities (retarded growth in the radius, ulna, and tibia; un-united and hypoplastic anconeal and coronoid processes; hip dysplasia, and delayed development of epiphyses). These skeletal defects show recessive inheritance, whilst the retinal defects are dominant with incomplete penetrance (Carrig *et al.*, 1988).

The 'Collie Eye Anomaly' (CEA) consists of bilateral choroidal hypoplasia, papillary or peripapillary coloboma, and neuroretinal non-attachment (Barnett, 1979). It is seen in the rough and smooth Collies, Shetland Sheepdog and Lancashire Heeler (Bedford, 1998). In many cases, the disease is not sight threatening, and retinal folds occurring in the puppy are lost in the adult. Incidence of CEA may reach 70% in the Collie breeds. Results of pedigree analyses and test matings are consistent with autosomal recessive inheritance (Bedford, 1982). Affected breeds have not responded to selection against coloboma (Wallin-Hakanson *et al.*, 2000), perhaps because the CEA gene is closely linked to a desirable Collie trait. Coloboma has been noted in a number of breeds, but few systematic studies have been made. In Australian Shepherd dogs it is associated with microphthalmia and merle coat colour and is inherited as an autosomal recessive trait with incomplete penetrance.

The accumulation of autofluorescent lipopigments in neurons and neuronal derivatives is the defining characteristic of ceroid lipofuscinoses. These diseases, although eventually syndromic, often have their earliest effects on vision. They have been well described in several dog breeds, including the Tibetan Terrier. In this breed, nyctolopia is apparent from 2 months or earlier, and substantially precedes fundoscopic or more widespread changes. The disease has been distinguished from PRA (which also occurs in Tibetan Terriers) as 'retinal degeneration with inclusions' (Riis *et al.*, 1992). In the English Setter, ceroid lipofuscinosis is accompanied by marked neurological signs. The human gene mutated in Batten disease (*CLN3*) has been investigated in the English Setter, but the Setter mutation is not linked to this gene (Lingaas *et al.*, 1998; Shibuya *et al.*, 1998).

Cataract

The lens is avascular, receiving both nutrition and oxygen from the aqueous. It contains a concentrated sol of proteins that maintain a refractive index of 1.38–1.41 without precipitating. The lens is susceptible to metabolic disturbances or infections that cause glycolation, dehydration or nutritional deficit. Whitley *et al.* (1995) list 110 breeds with pre-dispositions to cataract. However, the mode of inheritance is unknown in many breeds, and in some cases the inherited nature of the disorder unproven. Cataracts may form secondary to inherited defects having other primary manifestations (e.g. hereditary diabetes and PRA). Primary cataracts have been described in some detail in about a dozen breeds. Although hereditary cataracts may cause mild clinical signs in some breeds, the number of affected dogs may be high. For example, approximately 4.7% of Golden Retrievers and 5.5% of Labrador Retrievers examined (under the BVA/Kennel club eye examination scheme) were suffering from hereditary cataracts, accounting for 63–83% of all cataracts in the two breeds (Curtis and Barnett, 1989).

An unusual feature of the inheritance of cataracts is a fairly high proportion of dominant inheritance pattern. This might be expected if the ability of a lens protein to precipitate or a cell to acquire a light scattering character is seen as a novel acquired characteristic. These two modes of cataract formation are often recognized in murine models of non-syndromic primary cataract (see Hejtmancik, 1998). The molecular genetics underlying a number of human and rodent cataracts is now understood. Many of the mutated genes are either crystallins of the lens, or membrane proteins found at thin junctions and gap junctions. Hejtmancik suggests that cataracts fall into two groups. One group involves disruption of lens development and uncontrolled cell division, leading to loss of cellular order and light scattering. A second group results from aberrations of the lens crystallins or the intracellular reducing environment, disrupting orderly interactions between crystallins and causing them to aggregate or precipitate and scatter light.

Table 9.1 lists canine hereditary cataracts and their modes of inheritance. The different opacity patterns and rates of progression suggest that different genetic origins underlie cataracts in different breeds. Several breeds show

more than one form of primary cataract, based either on mode of inheritance, age of onset, or clinical signs (Table 9.1; Von Hippel, 1930; Barnett, 1986). The classification in Table 9.1 summarizes the best-defined defects, although the gene(s) and mutations causing cataracts have not been identified in any breed. In human beings, mutations in as many as 30 loci are estimated to cause autosomal dominant cataracts.

Cataracts in Miniature Schnauzers have been particularly well studied. Characteristic features include swelling of lens fibres leading to their rupture, and regions of granulation and liquefaction in the lens (Monaco *et al.*, 1984). A number of candidate genes were screened in affected dogs, including alpha, beta and gamma crystallins, *MP26* (a thin junction protein, mutations in which cause cataracts in the *lop* mouse), myotonic dystrophy and *PAX6*, but no mutations were found (Zhang *et al.*, 1991; Shastry and Reddy, 1994).

Other inherited eye defects

Spontaneous glaucoma is common in domestic dogs. Gelatt (1991) lists 42 breeds having a predisposition to glaucoma. The breeds include 14 Terriers, 5 Spaniels, and a striking number of miniatures amongst the remaining breeds. In the Terrier breeds, glaucoma is often associated with lens luxation, which is recessively inherited in the Tibetan Terrier (Willis *et al.*, 1979). In only a few breeds has the mode of inheritance of glaucoma been studied: recessive inheritance is found in the Beagle (Gelatt and Gum, 1981), and inheritance is thought to be autosomal dominant (with variable expression) in both the Welsh Springer Spaniel and the Great Dane (Cotrell and Barnett, 1988). Although several glaucoma-associated genes have been established in humans, no studies have been published of molecular genetics of canine glaucoma.

Persistent hyperplastic tunica vasculosa lentis and primary vitreous are defects resulting from failure of normal canine ocular development. This defect is best characterized in the Dobermann, where it may be associated with cataract. Anterior segment dysgenesis in this breed is described as autosomal recessive (Lewis *et al.*, 1986). Persistent pupillary membranes may represent a later arrest of normal development, and are seen as an autosomal recessive trait of Basenjis (Roberts and Bistner, 1968). A defect of tapetum formation in Beagles has been associated with the absence of zinc in this tissue (Wen *et al.*, 1982). Defects of eye development associated with the merle series coat colour gene have been studied most thoroughly in the Dachshund. The merle allele M^M reduces pigment in some areas, leaving a flecked or dappled appearance in $M^M M^+$ and $M^M M^M$ dogs. Unfortunately, this allele also affects eye development both in superficial appearance (heterochromia iridis) and in more major ways: (absence of tapetum lucidum, lack of retinal pigment, microphthalmia, microcornia or lens defect) (Klinckmann *et al.*, 1986). There is also evidence for effects on the ears, and the defect was likened to Klein-Waardenburg syndrome in man.

Deafness (Table 9.2)
In contrast to blindness, hearing deficits are typically of less concern to dog owners, and are likely to be under-reported (especially partial or unilateral deafness). None the less, deafness in Dalmatians first received serious study in 1896 (Rawitz). About 20% of Dalmatians are unilaterally deaf and 5% are bilaterally deaf (Wood and Lakhani, 1997). Inherited deafness associated with coat colour has been studied in several breeds (Table 9.2). Deafness has been clearly associated with deficient melanocyte distribution in the developing ear (review, Tachibana, 1999). The piebald (S^p) and extreme piebald (S^w) genes suppress pigmentation in Dalmatians and several other breeds. In these breeds, the Ticking allele (T^T) opens up the coat to spotting. Weaker expression of S^w allows patches of colour, and reduces deafness rates (Strain, 1992). There is no association with spot or patch colour, but brown eye colour is strongly associated with normal hearing, whilst blue-eyed dogs are often deaf, showing the effect of pigment distribution (Strain *et al.*, 1992). Unilaterally deaf dogs are twice as likely to have deaf offspring as normal dogs and there is a slight predominance of female over male deafness (Strain, 1992). The merle coat colour series (described above) also exerts epistatic effects on ear development in many breeds (Strain, 1996), whilst a syndrome of deafness associated with multiple ocular defects and partial albinism has been reported, possibly similar to human Wardenburg syndrome type I (Gwim *et al.*, 1981). Bilateral deafness has also been observed to segregate as an autosomal recessive trait, independent of coat colour (Table 9.2).

Lysosomal storage diseases (Table 9.3)

Storage diseases are defects of incomplete catabolism. Partial breakdown products of macromolecules including glycoproteins, glycolipids, sphingolipids, lipoproteins and glycogen accumulate within cells, destroying their architecture and function. In many (but not all) storage diseases, the most severe consequence of this accumulation is found in non-dividing cells, often those of the central nervous system (CNS). The breakdown of glycoproteins, glycolipids, sphingolipids, lipoproteins and glycogen occurs in a specialized but universal cellular organelle, the lysosome. This organelle creates an acidic environment and a safe compartment for degradative enzyme action. More than 30 different lysosomal storage diseases have been described in humans, and at least 19 of these have orthologues in dogs (Table 9.3). Fortunately many of these diseases appear to be quite rare with only a single case report, or a case cluster. Others have been reported many times, leading to the development of enzyme or DNA test based breeding programmes. For example, prior to the advent of a mutation detection test, 22–37% of UK Springer Spaniels screened at different shows (using enzyme activity tests) were carriers of fucosidosis (B.J. Skelly, unpublished data).

Table 9.2. Canine hereditary deafness

Disease phenotype	Affected breeds	Phenotype	Inheritance/mutation	References
Congenital sensorineural deafness	Dalmation, Bull Terrier, Samoyed, Great Pyrenees, Greyhound, Sealyham Terrier, Beagle, Bulldog, English Setter	Cochleo-saccular degeneration beginning with loss of stria vascularis, then collapse of Reissners membrane and the cochlear duct, then loss of hair cells in organ of Corti. Hearing loss starts at 4 weeks (normal development before this). Subsequent reduction in auditory cortex	Piebald coat colour series: S^P and S^W are associated with deafness – but n.b. in Dalmation and other piebalds, all individuals are S^w/S^w. Heritability on this background about 0.2. No mutations detected in *PAX3*	Rawitz, 1896, reviewed in Strain, 1996
Congenital sensorineural deafness	Collie, Shetland Sheepdog, Dappled Dachshund, Harlequin, Great Dane, American Foxhound, Old English Sheepdog, Norwegian Dunkerhound	Often syndromic deafness, including microphthalmia and other eye defects, also impairment of sperm function. Deafness is cochleo-saccular as above	Incomplete dominant with incomplete penetrance. Merle colour coat series: a proportion of M^+M^m and M^+M^+ dogs are deaf. Proportion varies with breed: up to 75% of white (M^+M^+) dunkerhounds are deaf	Reetz *et al*., 1977, reviewed in Strain, 1996
Sensorineural deafness	Pointer	In dogs bred for timidity, but segregates independently	Autosomal recessive	Steinberg *et al*., 1994
Bilateral sensorineural deafness	Dobermann, Shropshire Terrier	Loss of cochlear hair cells without any effects on stria vascularis	Autosomal recessive	Wilkes and Palmer, 1987; Igarashi *et al*., 1972
Black hair follicular dysplasia	Large Munsterhound	One report of deafness associated with melanocyte abnormality. See Table 9.9		Schmutz *et al*., 1988

Table 9.3. Canine lysosomal storage diseases

Disease	Affected breeds	Biochemical/ phenotype/ comments	Causative mutation	References	Human homologous locus/chromosome
Ceroid lipofuscinosis	Tibetan Terrier, Miniature Long Haired Dachshund, Miniature Schnauzer, Australian Cattle Dog, Border Collie, others		See Table 9.5		
Fucosidosis (alpha)	Springer Spaniel	Inability to catabolize glycoproteins. Delayed onset (recognized 12–18 months). Motor and mental function impaired: visual impairment, muscle wasting, lethargy, coma. No measureable plasma fucosidase activity	Autosomal recessive. 14-bp deletion at end of exon 1 of *FUCA1* gene creates frameshift, 25 novel codons in exon 2, and stop	Hartley *et al*., 1982; Kelly *et al*., 1983; Skelly *et al*., 1996; Occhiodoro & Anson, 1996	1p36
Galactocerebrosidosis (Krabbe disease, globoid cell leukodystrophy)	West Highland White Terrier, Cairn Terrier	Inability to remove galactoside moiety from galactolipids galactosylceramide, psychosine, myelin, due to lack of galactosylceramidase (aka galactocerebrosidase,GALC) activity	(A473C) transversion in galactosylceramidase (*GALC*) mRNA, gives (Tyr158Ser) in enzyme sequence	Fletcher *et al*., 1966; Victoria *et al*., 1996	14q31
Galactocerebrosidosis (Krabbe disease, globoid cell leukodystrophy)	Beagle, Bluetick Hound, Dalmation, Miniature Poodle	GALC enzyme deficiency: start 3–6 months visual deficiency, progressive locomotive abnormalities. Lesions bilateral in centrum semiovale, optic nerves	Unknown	Boysen *et al*., 1974; Johnson *et al*., 1975; Bjerkas, 1977; Zaki and Kay, 1973	14q31
Galactosialidosis	Schipperke	Probable deficiency in PPC, the protective protein for beta-D-galactosidase and neuraminidase. Adult onset (5 year) cerebellar atrophy. Lipid bound sialic acid twice normal	Unknown	Knowles *et al*., 1993	20q13.1?

Table 9.3. *Continued*

Disease	Affected breeds	Biochemical/ phenotype/ comments	Causative mutation	References	Human homologous locus/chromosome
Gaucher's disease I	Sydney Silky Terrier	Beta-glucocerebrosidase (glucosidase, beta, acid; glucosyl-ceramidase) deficiency: storage of glucocerebroside in various tissues associated with large foamy macro-phages (Gaucher's cells), progressive neurological signs from 8 months	Unknown	Hartley & Blakemore, 1973	1q21
Glycogen storage disease Ia (von Gierke disease)	Maltese	Glucose-6-phosphatase deficiency: necropsy at 47 days, small body size; enlarged pale livers with 7-fold increase in glycogen. Hepatocytes, renal tubule epithelium vacuolated, G-6-Pase activity >10% normal	(G450C) transversion in *G6PC* gene gives (M121I) in mutant enzyme with 6–7% of control activity on transfection	Brix *et al*., 1995; Kishnani *et al*., 1997	17q21
Glycogen storage disease II (Pompe disease)	Lapland Spitz	Acid-alpha-glucosidase deficiency: vomiting due to megaoesophagus, progressive muscle weakness leading to exhaustion and death before 2 years. Glycogen storage esp. in muscles. Catalytically inactive AAGD protein can be immunoprecipitated	Probable structural mutation in AAGD enzyme. (Disease not complemented by other AAGD deficient cell lines: not activating factor)	Walvoort *et al*., 1982, 1984	17q25.2–25.3
Glycogen storage disease VII	English Springer Spaniel, American Cocker Spaniel	6-phosphofructo-1-kinase (M form) deficiency: glycogen storage myopathy, with exertional haemolytic crises. Erythrocyte PFK activity 8–22% normal.	Autosomal recessive. (G2228A) transition in cDNA of *PFKM* results in (Trp>*)	Vora *et al*., 1985; Giger *et al*., 1992; Mhaskar *et al*., 1992; Smith *et al*.,1996	12q13.3
GM1 gangliosidosis	English Springer Spaniel, Portuguese Water Dog, Alaskan Husky	Beta-galactosidase deficiency: skeletal dysplasia from 2 months; neurological impairment from 4.5 months. ESS shows dwarfism, PWD does not. Both have BGAL activator protein	In Portuguese Water Dog, acid β-galactosidase G200>A (R60H) is causative. Other breeds unknown	Read *et al*., 1976; Alroy *et al*., 1985; Saunders *et al*., 1988; Muller *et al*., 1998; Wang *et al*., 2000	3p21.33

GM2 gangliosidosis	German Short-Haired Pointer	Hexosaminidase-B normal (?): but massive GM2 accumulation progressive neuromuscular dysfunction and impaired growth from a few weeks. Possible juvenile form Tay-Sachs? (caused in human by hexaminidase-A partial activity)	Unknown	Karbe & Schiefer, 1967; Singer & Cork, 1989	
GM2 gangliosidosis (type AB) (Tay-Sachs disease, AB variant)	Japanese Spaniel	Later onset, High beta-hexosaminidase. Activator protein inactive	Unknown	Cummings *et al*., 1983; Ishikawa *et al*., 1987	5q31.3–q33.1(?)
Mucopolysaccharidosis I (Hurler-Scheie syndrome)	Plott Hound	Alpha-L-iduronidase deficiency: dermatan sulphate and heparin sulphate accumulation in neural tissues and liver	G>A transition, intron 1 splice donor site: retention of intron 1 in *IDUA* mRNA, protein termination at stop codon at intron–exon junction	Shull *et al*., 1982; Spellacy *et al*., 1983; Menon *et al*., 1992	4p16.3
Mucopolysaccharidosis II (Hunter syndrome)	Labrador Retriever	Iduronate-2 sulphatase deficiency: exercise intolerance, visual impairment, progressive neurological signs: acid mucopolysaccharides accumulate in CNS. Vacuolation also in epithelial & endothelial cells, histiocytes of liver, kidney, spleen, thyroid	Recessive	Wilkerson *et al*., 1998	Xq28
Mucopolysaccharidosis IIIa (Sanfilippo syndrome A)	Wire Haired Dachshund	*N*-sulphoglucosamine sulpho-hydrolase (heperan sulphate sulphatase, sulphamidase) deficiency: progressive neurological disease without obvious somatic involvement: ataxia progressive from 3 years. Mucopolysaccharides accumulate in CNS. Vacuolation of fibroblasts, hepatocytes, epithelial cells	Unknown	Fischer *et al*., 1998	17q25.3
Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome)	Miniature Pinscher	*N*-acetylgalactosamine-4-sulphatase (arylsulphatase B) deficiency	Unknown	Neer *et al*., 1995	5p11–q13

Table 9.3. *Continued*

Disease	Affected breeds	Biochemical/ phenotype/ comments	Causative mutation	References	Human homologous locus/chromosome
Mucopolysaccharidosis VII (Sly syndrome)	German Shepherd (?)	Beta-glucuronidase deficiency: weakness in rear legs from 8 weeks, progressive. Joint laxity, large head, shortened maxilla, skeletal disease: precipitation of glyosaminoglycans, predominantly chondroitin sulphate in neurons, joint capsule, hepatocytes, keratocytes, fibroblasts, chondrocytes	(G559A) transition in beta-glucuronidase (*GUSB*) cDNA, giving (Arg166His), leading to *c.* 1% of normal liver & 15% of normal serum enzyme activity	Haskins *et al*., 1984; Schuchman *et al*., 1989; Ray *et al*., 1998	7q21.11
Neimann-Pick disease Type A/B	Poodle	Sphingomyelinase deficiency accumulated sphingomyelin and cholesterol in CNS and macrophages of lungs, lymph node, liver, kidney. Progressive neurological abnormalities in young dog (5 months)	Unknown	Bundza *et al*., 1979	11p15.4–p15.1
Neimann-Pick disease Type C	Boxer	Accumulates unesterified cholesterol (in human, major form of disease is mutant in *NPC1* gene which esterifies exogenously acquired cholesterol). Lactosylceramide and gangliosides GM3 & GM2 stored in neurones in brain, cholesterol in histiocytes of liver, spleen. Onset 9 months	Unknown	Kuwamura *et al*., 1993	18q11–q12?
Wolman disease	Fox Terrier	Presumed lysosomal acid lipase (acid cholesteryl ester hydrolase, *LIPA*) deficiency. Cholesterol ester storage as crystal deposits. High level of visceral involvement and rapid progression	Unknown	von Sandersleben *et al*., 1986.	10q24–q25?

Lysosomal storage diseases are recognized at microscopic level by the presence of intracellular waste products. The typical appearance is distended vacuoles containing crystalline or lipidaceous material, or sometimes whorls of membrane. Cells may have a foamy appearance if multiple small vacuoles are present. The most severely affected tissues vary with the disease, and often include the CNS (neurons), liver (Kupffer cells), kidney (epithelia) and immuno-reticular system (macrophages). Figure 9.1 (see Frontispiece) shows the appearance of the spleen and cerebellum of a Springer Spaniel with fucosidosis.

In general, storage diseases result from the absence or inactivity of one particular enzyme. Theoretically, replacement of that enzyme would restore cell function and return the organism to health. The lysosome is able to recruit enzyme precursors using an endocytic pathway. This is probably through a mannose-6-phosphate receptor mediated mechanism (as has been shown *in vitro* for β-glucuronidase (Kaplan *et al.* (1977), and for many other enzymes). It may be feasible to restore enzyme function and induce disease remission by producing enzymes in other tissues (or *ex vivo*), allowing the recombinant proteins to be taken up by the relevant cell population.

Storage diseases are usually progressive, as metabolic end products accumulate over time. This fact, together with metabolic differences between rodents and man, limits the utility of murine models of human storage diseases. The availability of naturally occurring canine models has been exploited for designing and testing therapies of the human disease. Strategies include direct enzyme replacement (Shull *et al.*, 1994, Kakkis *et al.*, 1996), bone marrow replacement/transplantation therapy (Taylor *et al.*, 1986, 1987, 1988, 1989) and more recently direct gene therapy (Taylor and Wolfe, 1994; Shull *et al.*, 1996; Ferrara *et al.*, 1997; Ray *et al.*, 1998).

Hereditary central nervous system defects (Table 9.4)

Introduction

Many congenital and developmental neurological defects have been described in dogs (Hoskins and Taboada, 1992; Summers *et al.,* 1995). Neurodegenerative disorders caused by metabolic storage diseases (discussed above) are among the best characterized canine hereditary defects. The biochemical basis of disease phenotype and candidate genes for many other canine neurological disorders are not yet defined. Some reports of a single case (or litter) may represent acquired disorders, because clinical and pathological findings of acquired and hereditary defects overlap. Signs of neurological dysfunction typically accompany structural or developmental defects of the skull and spinal column (see Chapter 5). Table 9.4 records diseases with clinical signs or pathological findings restricted to the central nervous system (CNS) consisting of the brain and spinal cord.

Hereditary neurological disorders may cause local lesions or diffuse neurological signs representing involvement of white matter or grey matter

Table 9.4. Canine hereditary central nervous system defects

Neuroanatomical location/ disease classification	Affected breeds	Disease characterization	References	Human disease counterpart
Epilepsy	Keeshond	Grand mal seizures after 12 months, predominantly in males. Autosomal recessive inheritance (?)	Hall & Wallace, 1996	Epilepsy
Cerebrum				
Narcolepsy	Doberman Pinscher	Signs of abnormal sleep/arousal patterns Autosomal recessive inheritance Mutation (insertion) in hypocretin receptor gene (*HCRTR2* locus)	Lin *et al*., 1999	Narcolepsy
	Labrador Retriever	Signs of abnormal sleep/arousal patterns Autosomal recessive inheritance Mutation (deletion) in hypocretin receptor gene (*HCRTR2* locus)	Lin *et al*., 1999	Narcolepsy
Lissencephaly	Lhasa Apso	Dull mentation, seizures Reduction or absence of cerebral gyri No other anatomical sites involved	Greene *et al*., 1976	Lissencephaly 1
Cerebellum				
Cerebellar cortical abiotrophies	Australian Kelpie, Airedale, Beagle, Chow, Finnish Harrier, Labrador Retriever, Rhodesian Ridgeback	Ataxia and tremors Purkinje and granule cell degeneration	Summers *et al*., 1995	Cerebellar ataxias
Hepatocerebellar degeneration	Bernese Mountain Dogs	Ataxia and head tremors at 1–2 months Purkinje cell degeneration, granular cell layer depletion Hepatic degeneration and fibrosis Autosomal recessive inheritance	Carmichael *et al*., 1996	Olivopontocerebellar atrophy
Cerebellar degeneration	Gordon Setter	Hypermetria, ataxia, nystagmus at 6–24 months Purkinje and granule cell degeneration Autosomal recessive inheritance	Steinberg *et al*., 1981	Cerebellar ataxias
Extrapyramidal abiotrophy	Kerry Blue Terrier	Ataxia, spasticity at 3–4 months Purkinje cell, olivary nuclei, and substantia nigra lesions Autosomal recessive inheritance	de Lahunta and Averill, 1976	Cerebellar ataxias
Cerebellar degeneration	Rough-Coated Collie	Hypermetria, ataxia, tremor at 2–4 months Purkinje cell degeneration, Wallerian degeneration in brain stem and spinal cord Autosomal recessive inheritance	Hartley *et al*., 1978	Cerebellar ataxias

Multisystem neuronal defects				
Grey matter spongy degeneration	Bull Mastiff, Saluki	Ataxia, tremors, visual defects, behavioural abnormalities Vacuolation and gliosis cerebellum, may involve cerebrum and white matter	Summers *et al*., 1995	
Neurodegeneration	Cairn Terrier	Progressive paraparesis, tetraparesis, collapse, tremors by 5 months of age Widespread chromatolytic degeneration	Zaal *et al*., 1997	
Neurodegeneration	Cocker Spaniel	Ataxia, hypermetria, behaviour change by 10–14 months Nerve cell degeneration and loss cerebrum, cerebellum, midbrain	Jaggy and Vandevelde, 1988	
Neuronal abiotrophy	Swedish Lapland Dogs	Rapidly progressive weakness, muscle wasting by 1–2 months Neuron degeneration cerebellum, spinal cord Autosomal recessive	Sandefeldt *et al*., 1976	
Spinal cerebellar degeneration	Brittany Spaniel	Ataxia, tremors, hind limb hypermetria with adult onset Purkinje cell loss with neurofilament accumulation, neuronal degeneration spinal cord	Higgins *et al*., 1998	
Neuronal vacuolation	Rottweiler	Hind limb weakness, ataxia, tremor Vacuolation Purkinje cells, brain stem nuclei, axonal degeneration brain stem, cerebellum, spinal cord	Pumarola *et al*., 1999	
Polioencephalomyelo-pathy	Australian Cattle Dog	Seizures, spastic paresis by 5–6 months of age Vacuolar degeneration cerebellum, brain stem, spinal cord	Brenner *et al*., 1997	
Neuroaxonal dystrophies				
	Rottweiler	Ataxia with progressive hypermetria by 1–2 years Axonal swellings throughout grey matter Autosomal recessive inheritance	Chrisman *et al*., 1984	
	Jack Russell Terrier	Ataxia, behavioural abnormalities, hypermetria by 2 months Axonal swellings of thalamus, medulla and spinal cord	Sacre *et al*., 1993	Seitelbergers disease
	Papillon	Hind limb ataxia by 3 months with rapid progression. Widespread axonal swellings with predominant involvement of dorsolateral spinal cord white matter	Franklin *et al*., 1995	
Giant axonal neuropathy	German Shepherd	Posterior paresis and ataxia by 15 months, progressive hypalgesia, megaoesophagus, hyporeflexia. Large axonal swellings, neurofilaments in CNS and PNS Autosomal recessive inheritance	Duncan *et al*., 1981	Giant axonal neuropathy (GAN)

Table 9.4. *Continued*

Neuroanatomical location/ disease classification	Affected breeds	Disease characterization	References	Human disease counterpart
Leukodystrophies				
	Dalmatian	Visual deficit, pelvic limb ataxia at 3–5 months Loss of myelin cerebrum and spinal cord, sparing of midbrain, cerebellum Autosomal recessive inheritance	Bjerkas, 1977	
Fibrinoid leukodystrophy	Labrador Retriever Scottish Terrier	Ataxia, weakness, altered sensorium by 6 months of age Loss of myelin of cerebrum and spinal cord, Rosenthal fibres present	McGrath, 1979; Sorjonen *et al*., 1987	Alexander disease
Hereditary myelopathy	Afghan	Rapidly progressive paraparesis to spastic paraplegia at 3–13 months Demyelination and myelomalacia Autosomal recessive inheritance	Cummings and de Lahunta, 1978	
Hereditary necrotizing myelopathy	Dutch Kooiker	Progressive hind limb paresis by 12 months of age Malacia of spinal cord with Wallerian degeneration Autosomal recessive	Mandigers *et al*., 1993	
Leukoencephalomyelo-pathy	Rottweiler	Progressive ataxia hind limbs by 1–3 years of age Demyelination of brain stem, cerebellum, spinal cord	Wouda and Van Ness, 1986	
Hypomyelinating diseases				
Dysmyelination	Chow	Congenital tremors with signs of improvement by 1 year Lack of myelin development and delayed gliogenesis	Vandevelde *et al*., 1978	
	Weimeraner	Generalized tremors by 1 week of age Widespread hypomyelination, low numbers of oligodendrocytes	Kornegay *et al*., 1987	
	Bernese Mountain Dog	Tremors by 9 weeks of age, may improve by adulthood Abnormal oligodendrocyte morphology Autosomal recessive	Palmer *et al*., 1987	
Shaking pup	Springer Spaniel	Severe tremor that prevents ambulation, early death by 3–4 months Single base change in proteolipid protein (*PLP*) gene results in overexpression of immature (DM-20) isoform. X-linked recessive	Nadon *et al*., 1990	Pelizaeus Merzbacher disease
White matter spongy degeneration	Labrador Retriever Samoyed Silkie Terrier	Tremor, ataxia Diffuse white matter vacuolation	Zachary & O'Brien, 1985; Summers *et al*., 1995	Canavan disease

Lower motor neuron disease				
Hereditary canine spinal muscular atrophy	Brittany Spaniels	Progressive muscle weakness and wasting by 1 month (homozygotes) or 12 months (heterozygotes) Degeneration of motor neurons with neurofilaments in axonal internodes Autosomal dominant	Blazej *et al*., 1998	Spinal muscular atrophy
Spinal muscular atrophy	German Shepherd	Weakness of forelimbs by 3 months, progressive limb contracture Degeneration of somatic motor neurons at cervical intumescence	Cummings *et al*., 1989	
Neurogenic muscular atrophy	Pointer	Hind limb paresis by 6 months of age, progressive to recumbency by 1 year Axonal degeneration with nuclear, axonal, and dendritic lipid granules Autosomal recessive	Izumo *et al*., 1983	
	Rottweiler	Hind limb paresis by 1 month of age, progressive muscle atrophy, megaoesophagus Degeneration of lower motor neurons of spinal cord and brain stem nuclei	Shell *et al*., 1987	
Spinal ataxias				
Degenerative myelopathy	German Shepherd	Slow progressive posterior paresis from 5 years of age Diffuse myelopathy of spinal cord	Summers *et al*., 1995	
	Ibizan Hound	Progressive spastic ataxia from birth, trunkal ataxia, depressed reflexes, seizures Axon degeneration, proliferative astrocytosis especially in ventral roots Autosomal recessive inheritance	Summers *et al*., 1995	
	Labrador Retriever	Progressive ataxia, hypermetria, spasticity by 3 months of age Bilateral degeneration of spinal cord white matter with swollen axonal spheroids, olivary nucleus neuropathy Autosomal recessive inheritance	de Lahunta *et al*., 1994	
Ataxia and myelopathy	Smooth Fox Terrier Jack Russell Terrier	Ataxia, dysmetria, intention tremor by 6 months of age Myelopathy with vacuolation, patchy Wallerian degeneration throughout CNS Autosomal recessive inheritance	Hartley & Palmer, 1973	

throughout the CNS. Neurological and histopathological examination findings, rather than biochemical or molecular defects, define most disease phenotypes.

CNS diseases

Signs suggestive of cerebral lesions include seizures, changes in mentation, and visual deficits. The primary seizure disorder, idiopathic epilepsy, is common in dogs (Quesnel, 2000). Although there are no gross or microscopic neurological lesions, a hereditary basis for this defect is suspected because certain breeds (Beagle, German Shepherd, Tervueren, Collie) are consistently over-represented in case series or family studies (Hoskins and Taboada, 1992). The inheritance pattern in the Keeshond in the UK is consistent with monogenic recessive disease (Hall and Wallace, 1996) whilst in several breeds (Bernese Mountain Dog, Labrador Retriever) polygenic recessive disease is suspected. No major gene locus has been identified. In contrast, the molecular basis of narcolepsy, an uncommon defect of sleep and arousal, has been well-characterized in two breeds (Table 9.4; Lin *et al.*, 1999). A hereditary basis for lissencephaly, a gross defect of cerebral conformation, is suspected in Lhasa Apsos (Greene *et al.*, 1976).

Diseases of the cerebellum cause signs of ataxia, hypermetria, and tremors. Cerebellar cortical abiotrophies (neuronal and glial cell degenerations) are the most commonly reported hereditary cerebellar defects in dogs. Characteristic histopathological findings of these defects include Purkinje cell degeneration, with apparent secondary loss of granule cell layer (Summers *et al.*, 1995). Different breeds show differences in age of disease onset and extent and location of neuron types involved, suggestive of an underlying molecular heterogeneity. Grey matter disorders affecting neurons throughout the brain cause more severe and complex signs, and are listed as multi-system neuronal defects (Table 9.4). Combined degenerative defects, affecting primarily the Purkinje cells of the cerebellum, but including neurons of the medulla and spinal cord, have been described in several breeds (Table 9.4).

The histopathological lesions of axonal swelling are characteristic of neuroaxonal dystrophies. Clinical signs of these defects include progressive gait abnormalities and reflect diffuse and multifocal brain involvement (Sacre *et al.*, 1993). Several canine axonal dystrophies share signs and laboratory features with human disease counterparts (Table 9.4), however the molecular defects responsible for canine phenotypes have not yet been identified.

Disorders of myelin synthesis and repair (leukodystrophies) impair neuron function of the brain and spinal cord, often causing signs of progressive vision loss, ataxia and weakness, with secondary muscle atrophy. The biochemical basis of canine leukodystrophies include some of the lysosomal storage diseases discussed earlier. In all other leukodystrophies, the biochemical and molecular pathologies are unknown, so differentiation is based on anatomical distribution of lesions and on histological review (Table 9.4). Hypomyelinating defects are caused by quantitative or functional defects of oligodendrocytes, the cell type responsible for myelin synthesis. Tremors apparent from birth, or soon after, are characteristic signs. An X-linked hypomyelinating disease,

comparable to a hypomyelinating defect in human beings, has been well-characterized in Springer Spaniels (Nadon *et al.*, 1990). Other canine hypomyelinating disorders appear to be autosomal traits (Table 9.4). Spongy degenerative disorders are a third category of myelin defects that typically involve the brain and spinal cord. These defects have only been described in single individuals or littermates (Summers *et al.*, 1995).

Spinal cord defects can be classified as primarily involving grey matter (lower motor neuron disorders) or white matter (myelopathies). Signs of lower motor neuron disorders include weakness and muscle atrophy, whereas early signs of myelopathies include spinal ataxia with hypermetria. Spinal muscular atrophy, a lower motor neuron defect in Brittany Spaniels, is phenotypically similar to human spinal muscular atrophy. Mutations in the survival motor neuron (*SMN*) gene (causative for an autosomal dominant form of the human disease) have been ruled out as the cause of the canine disease (Cork, 1991; Blazej *et al.*, 1998). German Shepherd degenerative myelopathy is a common, slowly progressive myelopathy of possible hereditary basis. Disease onset is typically seen in middle-aged or old dogs, and there is some evidence for an immune-mediated component to disease pathogenesis (Barclay and Haines, 1994). Degenerative myelopathies in Ibizan Hounds, Labrador Retrievers and Terriers (Table 9.4) are autosomal recessive defects with early (less than 1 year) ages of onset.

A poorly defined idiopathic tremor disorder occurs in small breed, white coat colour dogs (Maltese, West Highland White Terrier, Bichon Frise and Poodle). Disease onset is acute and usually self-limiting. A diffuse CNS defect is suspected, although histopathologic, biochemical, and molecular characterizations have not yet been reported.

Neuromuscular disorders (Table 9.5)

Neuromuscular disorders include defects of peripheral nerve, neuromuscular junction, and skeletal muscle. Weakness is the predominant clinical feature and signs usually become apparent within the first few months of life. Many diseases progress over time and affected dogs die because of neuromuscular failure or secondary complications of dysphagia, pneumonia and systemic infection (Shell and Inzana, 2000; Shelton, 2000).

Peripheral nerve defects

Peripheral nerve defects are classified on the basis of neurological examination findings, electrophysiological studies, and histological review of peripheral nerve biopsies (Inzana, 2000). All are autosomal traits, usually with recessive expression pattern. Numerous hereditary polyneuropathies occur in human beings; however, homology between the various canine and human defects is not yet well defined. Degenerative defects of canine peripheral nerve axons typically cause progressive weakness, apparent first as hind limb weakness (paraparesis), with secondary muscle atrophy. Differences between breed

Table 9.5. Canine hereditary peripheral nervous system, neuromuscular junction defects and myopathies

Disease	Affected breeds	Disease characteristics	Inheritance/mutation	References	Human homologous disease/locus
Axonopathies					Polyneuropathy
Hereditary polyneuropathy	Alaskan Malamute	Progressive (with remissions) posterior weakness with onset at 6–18 months of age. Prominent megaoesophagus and muscle atrophy. Demyelination and nerve fibre degeneration	Autosomal recessive	Moe, 1992	
Idiopathic polyneuropathy	Alaskan Malamute	Progressive posterior paresis with onset at 10–18 months of age	Axonal degeneration	Braund *et al*., 1997	
Progressive axonopathy	Boxer	Progressive signs of ataxia, weakness, and hyporeflexia from 2–3 months of age. Axonal swellings dorsal and ventral nerve roots and lumbar nerves. Electron dense granules appear in myelin sheath	Autosomal recessive	Griffiths *et al*., 1987	
Distal polyneuropathy	Rottweiler	Adult onset weakness progressing to paralysis and prominent muscle atrophy. Distal medium and large fibre axonal degeneration		Braund *et al*., 1994a	
Laryngeal paralysis-polyneuropathy	Dalmatian, Rottweiler	Laryngeal dysfunction, megaoesophagus, weakness at 2–6 months of age. Extensive distal axonal degeneration	Autosomal recessive	Braund, *et al*., 1994b; Mahoney *et al*.,1998	
Laryngeal paralysis	Bouvier des Flandres	Laryngeal dysfunction, inspiratory stridor. Axonal degeneration restricted to intrinsic laryngeal muscles	Autosomal dominant	Venker-van Haagen, 1981	Laryngeal abductor paralysis
Myelin defects					Charcot-Marie-Tooth demyelinating neuropathy
Hypomyelinating neuropathy	Golden Retriever	Mild pelvic limb atrophy and weakness at 5–7 weeks of age. Marked reduction in peripheral nerve myelin	Autosomal recessive	Matz *et al*., 1990	
Hypertrophic neuropathy	Tibetan Mastiff	Rapid progression of weakness and hyporeflexia at 8–10 weeks of age, with some improvement over time. Demyelination of peripheral nerves and roots, with little axon degeneration	Autosomal recessive	Cummings *et al*., 1981	

Myopathy and neuropathy	Doberman Pinscher (Dancing Doberman disease)	Adult onset, slowly progressive pelvic limb flexure, hyperreflexia, muscle atrophy. Multifocal muscle fibre atrophy and hypertrophy, peripheral nerve axonopathy		Braund, 1995	
Sensory neuropathies					Hereditary sensory neuropathy
Acral mutilation	English and German Shorthaired Pointer	Loss of pain sensation with self-mutilation of the paws at 3–8 months of age. Decrease in dorsal root ganglion cell body number and sensory axons	Autosomal recessive	Cummings *et al*., 1983	
Primary sensory neuropathy	Longhaired Dachshund	Hind limb weakness. loss of sensation, and urinary/faecal incontinence at 8–10 weeks of age. Reflexes intact Distal loss of large myelinated and unmyelinated sensory nerves		Duncan & Griffiths, 1982	
Neuromuscular junction defect					
Congenital myasthenia gravis	Gammel Dansk Honsehund	Non-progressive, episodic weakness No response to anticholinesterase drugs	Autosomal recessive	Flagstad, 1989	Familial infantile myasthenia gravis
Congenital myasthenia gravis	Jack Russell Terrier	Episodic weakness apparent by 5–8 weeks of age. Responds to anticholinesterase drugs		Wallace & Palmer, 1984	Autosomal recessive myasthenia gravis
Congenital myasthenia gravis	Smooth Fox Terrier	Weakness apparent by 5 weeks of age. Transient response to anticholinesterase drugs, low number of postsynaptic acetylcholinesterse receptors	Autosomal recessive	Jenkins *et al*., 1976	Autosomal recessive myasthenia gravis
X-linked myopathy	German Shorthaired Pointer	Progressive skeletal myopathy and cardio-myopathy with complete absence of skeletal muscle dystrophin	Complete dystrophin (*DMD*) gene deletion	Schatzberg *et al*., 1999	Duchenne muscular dystrophy/Xp21.2
	Golden Retriever	Progressive skeletal myopathy and cardiomyopathy with truncated dystrophin protein	Splice site mutation resulting in premature stop codon in exon 8, *DMD*. (In-frame transcripts lack either exons 3–9 or 8–12)	Schatzberg *et al*., 1998	Duchenne muscular dystrophy/Xp21.2
	Belgian Groenendaeler Shepherd, Irish Terrier, Miniature Schnauzer, Rottweiler, Samoyed	Progressive skeletal myopathy, elevated creatine kinase, cardiomyopathy, low muscle dystrophin		Ham *et al*., 1993; Presthus & Nordstoga, 1993; Paola *et al*., 1993	Duchenne muscular dystrophy/Xp21.2

Table 9.5. *Continued*

Disease	Affected breeds	Disease characteristics	Inheritance/mutation	References	Human homologous disease/locus
Hereditary myopathy	Labrador Retriever	Skeletal myopathy with megaoesophagus, stable disease after 1 year	Autosomal recessive	McKerrell & Braund, 1987; Amman *et al*., 1988	Congenital myopathy
Central core myopathy	Great Dane	Exercise intolerance, high serum creatine kinase, proximal muscle atrophy, central core structures within muscle fibres		Newsholme & Gaskell, 1987; Targett *et al*., 1994	
Nemaline rod myopathy	Border Collie, Schipperke	Exercise intolerance, rod bodies within type 1 muscle fibres		Delauche *et al*., 1998	Nemaline rod myopathy
Myotonia	Chow	Muscle hypertrophy, stiff gait, dyspnoea by 10–12 weeks of age, myotonic discharge on EMG	Autosomal recessive	Jones *et al*., 1977; Farrow & Malik, 1981	Myotonia congenita
Hypertonic myopathies	Dalmatian, King Charles Spaniel, Scottish Terrier (Scotty Cramp)	Exercise-induced muscle hypertonia Sarcotubular abnormalities (King Charles Spaniel)		Woods, 1977; Wright *et al*., 1987; Peters & Meyers, 1977	
Mitochondrial myopathy	Clumber, Sussex Spaniel	Exercise intolerance, exercise-induced metabolic acidosis. Low pyruvate dehydrogenase activity		Shelton, 2000	Mitochondrial myopathy
Mitochondrial myopathy	Old English Sheepdog	Exercise intolerance, exercise-induced metabolic acidosis. Low cytochrome C oxidase, ferricyanide reductase, and ATPase activities		Breitschwerdt *et al*., 1992; Vijayasarthy *et al*., 1994	Mitochondrial myopathy
Dermatomyositis	Collie, Shetland Sheepdog	Dermatitis and masticatory myositis at 3–6 months of age, remissions and relapses, inflammatory cell infiltrates, high serum IgG and circulating immune complexes	Autosomal dominant	Hargis & Mundell, 1992	Dermatomyositis

variants include the rate of disease progression and the extent of peripheral nerve involvement (Table 9.5). Prominent or exclusive laryngeal nerve degeneration has been described in Dalmatians, Rottweilers and Bouviers (Table 9.5; Inzana, 2000).

Defects of nerve myelin sheaths have been reported in Golden Retrievers and Tibetan Mastiffs. In Golden Retrievers, signs were mild and static over time (Matz *et al*., 1990). Tibetan Mastiffs had a more fulminant disease course, with weakness progressing to paralysis; however, dogs regained some motor function after 2–4 months (Cummings *et al*., 1981). Adult Dobermanns have a progressive neuromuscular disorder of the hind limbs. The defect has histological features of both neuropathy and myopathy (Braund, 1995).

Neuropathies affecting sensory, rather than motor, neurons have been reported in Dachshunds and Pointers. Affected dogs display intact spinal reflexes with diminished pain sensation, and are prone to self-mutilation (Inzana, 2000).

Neuromuscular junction

Myasthenia gravis is a defect of the neuromuscular junction, characterized by a functional or quantitative defect of acetylcholine receptors on the postsynaptic muscle membrane. Acquired and congenital forms occur in dogs (Shelton, 2000). Clinical features of congenital myasthenia have been described for three breed variants (Table 9.5). Differences between breeds are apparent in rate of disease progression and response to anticholinergic drugs (Miller *et al*., 1984; Wallace and Palmer, 1984; Flagstad, 1989).

Myopathies

Canine hereditary myopathies are characterized phenotypically on the basis of physical examination, electromyography, serum muscle enzyme (creatine kinase) concentration, and histopathology with immunostains to detect specific muscle proteins (Shelton, 2000; Table 9.5). The best defined and most common hereditary canine myopathy is X-linked recessive muscular dystrophy. Like Duchenne muscular dystrophy in human beings, canine X-linked muscular dystrophy is caused by mutations in the dystrophin gene (*DMD*) that lead to muscle degeneration, regeneration, and ultimately muscle fibrosis (Shelton, 2000). The defect has been reported in many different breeds and is mutationally heterogeneous (Table 9.5). Clinical signs typically include weakness, muscular atrophy, dysphagia due to megaoesophagus, arrhythmia due to cardiomyopathy, and high serum muscle creatine kinase (Cooper *et al*., 1988).

Other forms of canine myopathy are not defined on the protein (or gene) level. An autosomal recessive skeletal muscle myopathy has been described in Labrador Retrievers in North America, Europe and Australia (McKerrell and Braund, 1987; Amman *et al*., 1988; Gortel *et al*., 1996). Affected dogs have signs of generalized weakness, muscle atrophy, hyporeflexia, and megaoesophagus, with disease stabilization by 6–12 months of age. A myopathy characterized by proximal muscle atrophy, progressive weakness, and unique histological abnormalities has been described in Great Danes (Newsholme and

Gaskell, 1987). Affected Great Danes have central cylindrical cores within muscle fibres that, on electron microscopy, consist of glycogen granules, lysosomal whorls and mitochondrial aggregates (Targett and Anderson, 1993). These features have not been described in any human hereditary myopathy. Nemaline rod myopathies have been described as a congenital defect in Border Collies and an adult-onset defect in Schipperkes (Delauche *et al.*, 1998). The pathognomonic histological feature of this defect is rod bodies within type 1 muscle fibres. Inheritance pattern is unknown.

Myotonias are myopathies caused by sarcolemmal ion channel dysfunction. Clinical features of myotonia include stiff gait, muscle hypertrophy and characteristic decremental discharges on electromyography (Shelton, 2000). An autosomal recessive myotonia occurs in Chows (Jones *et al.*, 1977; Farrow and Malik, 1981). Poorly defined myopathies, associated with exercise or excitement-induced muscle hypertonicity, have been reported in Scottish Terriers, Dalmatians and King Charles Spaniels (Peters and Meyers, 1977; Woods, 1977; Wright *et al.*, 1987).

Mitochondrial myopathies have been reported in Clumber and Sussex Spaniels and Old English Sheepdogs (Shelton, 2000). The characteristic clinical signs are exercise-induced weakness and exertional lactic acidosis. Affected Spaniels had deficiency of pyruvate dehydrogenase (Shelton, 2000). Low activity of mitochondrial enzymes (cytochrome C oxidase, ATPase, ferricyanide reductase) were present in affected Old English Sheepdogs (Vijayasarthy *et al.*, 1994).

Dermatomyositis is a hereditary inflammatory disorder of skin and muscle reported in Collies and Shetland Sheepdogs (Hargis and Mundell, 1992). Clinical features include dermatitis of the face, ears and bony prominences, and myositis primarily affecting the muscles of mastication and the distal extremities. Histopathological findings include inflammatory cell infiltration and muscle atrophy with regeneration. The defect is an autosomal dominant trait with variable expressivity (Haupt *et al.*, 1985).

Cardiovascular defects (Table 9.6)

Canine hereditary cardiovascular disease encompasses congenital malformations, with signs present at birth or soon after, and developmental defects that may not be apparent until dogs reach adulthood (Kittleson and Kienle, 1998). Complex, polygenic inheritance patterns have been proposed for many of these disorders with apparent 'breed predisposition' (Patterson, 1989). But on the basis of detailed and consistent criteria for phenotypic classification, breeding experiments, risk estimates derived from pedigree analyses, and cohort studies, major gene effects of a single locus are now believed to underlie some of these traits (Patterson *et al.*, 1993; Meurs, 1998; Ubbink *et al.*, 1998b). Although specific protein or gene defects have not been discovered, the addition of comparative mapping and linkage analyses will help define the

Table 9.6. Canine hereditary cardiac and vascular defects

Disease classification	Affected breeds	Disease characterization	References	Human disease counterpart
Valvular defects				
Aortic stenosis	Newfoundland (subaortic stenosis)	Fibrous ring develops below aortic valve. Pressure gradient and heart murmur develop over 3–12 months Autosomal dominant	Patterson, 1976; Jacobs *et al*., 1990	Aortic stenosis
	Boxer, German Shepherd, Golden Retriever, Rottweiler (subaortic stenosis)	Fibrous ring develops below aortic valve. Signs and heart murmur may progress or remain static	O'Grady *et al*., 1989; Tidholm, 1997	
Pulmonic stenosis	Beagle, Boykin Spaniel, Bull Mastiff	Pulmonary valve dysplasia and stenosis	Patterson *et al*., 1981; Malik *et al*., 1993	Pulmonic stenosis
	English Bulldog, Boxer,	Subvalvular stenosis with left coronary artery anomaly	Buchanan, 1990	
	American Cocker Spaniel, Chow, Mastiff, Miniature Schnauzer, Samoyed, West Highland White Terrier	Breeds at increased risk on the basis of case series (increased odds ratios)	Buchanan, 1992	
Mitral (left atrio-ventricular) valve defects	King Charles Spaniels (chronic valvular disease)	Valvular disease and heart murmur by 5 years, polygenic trait, more severe in males	Swenson *et al*., 1996	
	Great Dane, German Shepherd, Rottweiler (mitral dysplasia)	Abnormally formed valve with regurgitation Breeds at increased risk on the basis of case series	Kittleson & Kienle, 1998	
	Newfoundland (mitral stenosis)	Combined defect with subaortic stenosis	Kittleson & Kienle, 1998	
Tricuspid (right atrio-ventricular) valve dysplasia	German Shepherd, Great Dane, Irish Setter, Labrador Retriever	Thickened tricuspid leaflets with fenestrations and attached papillary muscles, regurgitant blood flow. Breeds at increased risk on the basis of case series	Liu & Tilley, 1976; Buchanan, 1993	
Ventricular septal defect	English Springer Spaniel	Large defects associated with early heart failure, neonatal death Autosomal dominant inheritance	Brown, 1995	Ventricular septal defect
Conotruncal defect	Keeshond	Defects may include ventricular septal defect, pulmonic valve stenosis, dextroaorta, and right ventricular hypertrophy. Autosomal, major gene locus	Werner *et al*., 1999	Isolated (non-syndromic) Tetralogy of Fallot
Vascular defects				
Patent ductus arteriosus	American Cocker Spaniel, Bichon Frise, Collie, Springer Spaniel, German Shepherd, Keeshond, Maltese, Miniature & Toy Poodle, Pomeranian, Shetland Sheepdog, Yorkshire Terrier	Blood shunting of variable severity depending on extent of vessel patency. Clinical signs of left side or right side heart failure. Polygenic inheritance with threshold for expression (Poodle). Breeds at increased risk on the basis of case series	Patterson, 1989; Hunt, *et al*., 1990; Buchanan, 1993; Tidholm, 1997	Patent ductus arteriosus

Table 9.6. *Continued*

Disease classification	Affected breeds	Disease characterization	References	Human disease counterpart
Persistent right aortic arch:	German Shepherd, Great Dane, Irish Setter	Aortic arch abnormality producing an encircling ring around trachea and oesophagus, May be associated with persistent left cranial vena cava. Clinical signs of regurgitation, aspiration pneumonia, and failure to thrive due to esophageal compression	Patterson, 1989; Buchanan, 1993	
Portosystemic shunt	Cairn Terrier, Golden Retriever, Irish Wolfhound, Labrador Retriever, Maltese, Miniature Schnauzer, Yorkshire Terrier	Vascular shunt bypassing liver by connecting portal vein with systemic venous circulation Breeds at increased risk on the basis of case series and pedigree analyses	Johnson *et al*., 1987; Center & Magne, 1990; Ubbink *et al*., 1998b	Patent ductus venosus
Cardiomyopathies				
Dilated cardiomyopathy	Boxer	Dilated cardiomyopathy with low myocardial carnitine concentration	Harpster, 1983; Keene *et al*., 1991	Dilated cardiomyopathy
	Dalmatian, American Cocker Spaniel	Dilated cardiomyopathy may be responsive to taurine and carnitine supplementation	Freeman *et al*., 1996; Kittleson *et al*., 1997	
	Doberman Pinscher	High breed prevalence, many secondary abnormalities of myocardium present (low carnitine, LDH, ATP, CK). Autosomal dominant with reduced penetrance	Smucker *et al*., 1990; Hammer *et al*., 1996; Minors & O'Grady, 1998	
	Portuguese Water Dog	Early onset at 2–30 weeks of age, rapid progression, males and females affected	Dambach *et al*., 1999	
	English Cocker Spaniel, German Shepherd, Great Dane, Irish Wolfhound, Newfoundland	Adult onset congestive heart failure with dilation of all cardiac chambers. Males affected at earlier age and/or more severe signs Breeds at increased risk on the basis of case series	Fox, 1988; Tidholm *et al*., 1996; Sisson *et al*., 2000	
Hypertrophic cardiomyopathy	Boxer, German Shepherd, Rottweiler	Left ventricular concentric hypertrophy	Sisson *et al*., 2000	Hypertrophic cardiomyopathy
Impulse conduction and formation disorders				
Sick sinus syndrome	American Cocker Spaniel, Dachshund, Miniature Schnauzer, West Highland White Terrier	Arryhthmias (sinus arrest, sinus bradycardia) associated with fibrosis of the sinus node. Breeds at risk on the basis of case series	Miller *et al*., 2000	Familial sinus node disease
Stenosis of the bundle of His	Pug	2° atrioventricular heart block, cardiac standstill, syncope and sudden death associated with narrowing of the AV bundle	Branch *et al*., 1977	Cardiac conduction defect
Hereditary ventricular tachycardia	German Shepherd	Ventricular arrhythmias (premature ventricular contraction, ventricular tachycardia) esp. during REM sleep and sudden death, associated with deficiency of myocardial sympathetic innervation	Dae *et al*., 1997	Idiopathic ventricular tachy-arrhythmias
Peripheral (femoral) artery occlusive disease	King Charles Spaniel	Diminished or absent femoral pulse caused by intimal thickening and vessel thrombosis	Buchanan *et al*., 1997	

molecular basis of the proposed hereditary canine cardiovascular disorders, and hence we have included them here.

Cardiac malformations are common, affecting atrial or ventricular valves, septa, or outflow tracts (Table 9.6). Aortic stenosis, pulmonic stenosis, and patent ductus arteriosus are consistently listed among the most common hereditary cardiac malformations of dogs (Patterson, 1989; Hunt *et al.*, 1990; Tidholm, 1997). Aortic stenosis in dogs is almost invariably subvalvular (Kittleson and Kienle, 1998). An autosomal dominant mode of inheritance has been reported for subaortic stenosis of Newfoundlands (Patterson, 1976). Pulmonic stenosis is typically a combined valvular and subvalvular lesion. Mitral (left atrioventricular) valve defects include dysplasia or malformed valves and stenotic valves (Table 9.6). An apparent hereditary form of mitral valve insufficiency with early onset of signs at 3–5 years of age occurs in Cavalier King Charles Spaniels (Swenson *et al.*, 1996). Tricuspid (right atrioventricular) valve dysplasia typically affects large breeds.

Congenital atrial and ventricular septal defects are relatively uncommon in dogs, although prevalent in human pediatric medicine. English Springer Spaniels appear to be a breed at risk for ventricular septal defect, with a proposed polygenic or autosomal dominant (incompletely penetrant) mode of inheritance and expression (Brown, 1995).

Patent ductus arteriosus (PDA) is a failure of closure of a fetal vessel that antenatally shunts blood from the pulmonary artery to the descending aorta, thereby bypassing the pulmonary circulation. Canine hereditary PDA is characterized by a variable extent and severity of smooth muscle hypoplasia along the ductus (Patterson, 1989). The prevalence of hereditary PDA is highest in small breeds (Table 9.6) and, unlike most hereditary cardiac defects, females are over-represented in case series (Patterson, 1989). Hereditary persistent right aortic arch has been reported in a few large breed dogs (Table 9.6). Portosystemic vascular anomalies (PSVA) are direct vascular communications between the portal system and systemic venous circulation that bypass the hepatic vasculature. Hereditary PSVA of large breeds is usually caused by failure of closure of the fetal ductus venosis. Extrahepatic shunts are more commonly reported in small breeds (Center and Magne, 1990). Signs of PSVA include hyperammonaemia, hepatic encephalopathy, episodic weakness and failure to thrive. The inheritance and expression patterns of PSVA are complex, but pedigree analyses have been used to develop risk estimates within a breed population (Ubbink *et al.*, 1998b).

Defects associated with primary myocardial failure are classified as either dilated (idiopathic) cardiomyopathies or hypertrophic cardiomyopathies (Kittleson and Kienle, 1998). In contrast to human beings (and cats), dilated cardiomyopathy is by far the most common familial form of cardiomyopathy in dogs. The time course of disease progression to refractory heart failure, arrhythmia, and sudden death varies between breeds. Dobermanns consistently have the highest prevalence of dilated cardiomyopathy in case series (Fox, 1988; Tidholm and Jonsson 1997). In most breeds, males tend to have more severe signs at an earlier age, although females are also affected (Calvert

et al., 1997; Kittleson and Kienle, 1998). Hypertrophic cardiomyopathy (left ventricular hypertrophy in the absence of outflow obstruction, hypertension, or metabolic disease) is uncommon in dogs. Breeds with hereditary forms of subaortic stenosis, are over-represented in case series (Sisson *et al.*, 2000). In human beings, mutations in the dystrophin gene and in various sarcomeric genes have been associated with familial dilated and hypertrophic cardiomyopathies, respectively. To date, the loci responsible for canine cardiomyopathies are unknown (Kittleson and Kienle, 1998).

Disorders of electrical impulse conduction and formation have been reported in a few breeds (Table 9.6). These defects are associated with cardiac arrhythmia, ECG abnormalities, and syncope.

Hereditary defects of peripheral vessels are uncommon. Femoral artery thrombosis, associated with intimal thickening, has been reported in male and female King Charles Spaniels. The relationship of femoral artery and mitral valve diseases in this breed is unknown (Buchanan *et al.*, 1997).

Haematological defects (Table 9.7)

Introduction

Hereditary haematological disorders encompass defects of cellular elements (red blood cells, white blood cells and platelets) and defects of plasma constituents (haemostatic proteins, immunoglobulins and complement). Phenotypic characterization of these disorders ranges from compilation of typical clinical signs to the identification of specific defective pathways or proteins. This section focuses on haematological disorders known to be single gene defects or disorders with likely candidate genes.

Red cell disorders

Non-pathological variations in red cell volume and intracellular potassium content, with a presumed hereditary basis, have been described in several breeds of dog. Macrocytosis (high red cell volume) occurs in Miniature and Toy Poodles (Schalm, 1976) and Greyhounds (Sullivan *et al.*, 1994). Microcytic (low volume) red cells with high intracellular potassium concentration have been described in two Japanese breeds: Akita and Shiba Inu (Degen, 1987; Nishida and Kanaya, 1990; Gookin *et al.*, 1998).

Pathological red cell disorders cause anaemia. The mode of inheritance of all the hereditary red cell disorders (identified to date) is autosomal recessive. Table 9.7 lists the phenotypic and molecular characterization of hereditary red cell defects in dogs, along with any known homologous human gene loci and chromosomal locations. Hereditary haemolytic anaemias occur in many breeds (and mixed breed dogs) due to intrinsic erythroenzymopathies and erythrocyte membrane defects. Pyruvate kinase (PK) deficiency has been reported in the largest number of breeds. Affected dogs lack the isoenzyme R-PK. Haemolysis is accompanied by a vigorous regenerative response, however osteosclerosis, progressive anaemia, and hepatic failure typically cause death by 5 years of

Table 9.7. Canine hereditary blood disorders

Disease	Affected breeds	Biochemical phenotype	Causative mutation	References	Human homologous locus/chromosome
Erythrocyte defects					
Membranopathies					
Elliptocytosis	Mixed-breed dog	Truncated erythrocyte membrane protein 4.1	Gene deletion (63 bp) of spectrin-actin binding domain of *EPB41*	Conboy *et al*., 1991	*EPB41*/1p36.2–34
Non-spherocytic haemolysis	Beagle	Increased osmotic fragility, high RBC K^+	Unknown	Pekow *et al*., 1992	
Stomatocytosis with chondrodysplasia	Alaskan Malamute	High MCV, high RBC Na^+	Unknown	Fletch *et al*., 1975	
Stomatocytosis with gastritis	Drentse Partrijshond	High RBC cholesterol, increased osmotic fragility	Unknown	Slappendel *et al*., 1994	
Enzymopathies					
Pyruvate kinase deficiency	Basenji	Aberrant M_2-type PK activity in RBC, absent R-PK activity	Single bp deletion; mutant *PKLR* transcript lacks catalytic site	Whitney and Lothrop, 1995	*PKLR*/1q21
Pyruvate kinase deficiency	West Highland White Terrier (& Cairn Terrier)	Low RBC R-PK activity	Insertion (6 bp) in *PKLR* C domain	Skelly *et al*., 1999	*PKLR*/1q21
Pyruvate kinase deficiency	Beagle, Miniature Poodle, Dachshund	Low RBC R-PK activity	Unknown	Giger, 2000	
Phospho-fructokinase deficiency	English Springer Spaniel, American Cocker Spaniel	Low RBC M-type PFK activity	Missense (G-A) mutation with premature stop codon	Giger *et al*., 1992	*PFKM*/12q13.3
Catalase deficiency	Beagle	Catalase deficiency in reticulocytes and tissues – oral ulceration, gangrene	G > A transition (A327T) destabilizes catalase enzyme	Nakamura *et al*., 2000	11p13
Maturation defect					
Intestinal cobalamin malabsorption	Giant Schnauzer	Low serum B12, high urine methylmalonic acid	Unknown (see Table 9.9)	Fyfe *et al*., 1991a,b	
Immunodeficiencies					
Severe combined immunodeficiency (SCID):					
X-linked SCID	Basset Hound	Depressed T-cell blastogenic response. Low serum IgG, IgA	4 bp deletion in *IL2RG* chain; mutant transcript lacks transmembrane domain	Henthorn *et al*., 1994	*IL2RG*/Xq13
X-linked SCID	Cardigan Welsh Corgi	Depressed T-cell blastogenic response. Low serum IgG, IgA	Insertion (1 bp) produces premature stop codon;mutant *IL2RG* transcript lacks transmembrane domain	Somberg *et al*., 1995	*IL2RG*/Xq13

Table 9.7. *Continued*

Disease	Affected breeds	Biochemical phenotype	Causative mutation	References	Human homologous locus/chromosome
Canine leucocyte adhesion deficiency (CLAD)	Irish Setter	Neutrophil phagocytic failure, low leucocyte CD 11/CD18 expression	Missense mutation in leucocyte integrin beta 2 subunit results in low CD18 expression	Kijas *et al*., 1999	*ITGB2*/21q22.3
Complement (C3) deficiency	Brittany Spaniels	Low serum C3, glomerulonephritis	Deletion (1 bp) generates premature stop codon, minimal transcript produced	Ameratunga *et al*., 1998	*C3*/19p13.3–p13.2
Cyclic haematopoiesis	Collie	Dilute coat colour, cyclic (10–12 day) neutropenia	Unknown	Yang, 1987; Avalos *et al*., 1994	
Neutrophil defect	Weimeraner Doberman Pinscher	Neutrophilia, impaired neutrophil bactericidal activity. Low serum IgG, IgM (Weimaraner)	Unknown	Couto *et al*., 1989; Breitschwerdt *et al*., 1987	
Common variable immunodeficiency	Miniature Dachshunds	Low serum IgA, IgG, IgM, decreased lymphocyte transformation	Unknown	Lobetti, 2000	
Immunodeficiency syndrome	Shar Pei	Low serum IgA, IgM, depressed lymphocyte response to mitogens	Unknown	Rivas *et al*., 1995	
IgA deficiency	Beagle, German Shepherd, Shar Pei	Low serum IgA	Unknown	Guilford, 1987	
Platelet					
Membrane glycoprotein defects					
Glanzmann's-type thrombasthenia	Otterhound	Abnormal clot retraction, aggregation, low expression platelet GpIIbIIIa	Missense mutation in calcium binding domain GpIIb	Catalfamo & Dodds, 1988; Boudreaux and Catalfamo, 2001	*ITGA2B*/17q21.32
Glanzmann's-type thrombasthenia	Great Pyrenees	Abnormal clot retraction, aggregation, low expression platelet GpIIbIIIa	Insertion (14 bp) and splice site defect in GpIIb	Boudreaux *et al*., 1996; Lipscomb *et al*., 2000	*ITGA2B*/17q21.32
Storage pool and signal transduction defects					
Delta storage pool defect	American Cocker Spaniel	Abnormal aggregation to ADP/collagen, abnormal ADP secretion	Unknown	Callan *et al*., 1995	
Storage pool defect (with cyclic neutropenia)	Collie	Defective uptake and release of serotonin, abnormal aggregation to all agonists but ADP	Unknown	Lothrop *et al*., 1991	

Intrinsic platelet function defect	Spitz	Abnormal aggregation to ADP/ collagen; low serotonin release	Unknown	Boudreaux *et al*., 1994	
Basset thrombopathia	Basset Hound	Abnormal aggregation to ADP/ collagen, abnormal cAMP metabolism	Unknown	Catalfamo *et al*., 1986	
Haemostatic protein disorders					
von Willebrand disease (vWD)					
Type 1 vWD	Doberman Pinscher, Manchester Terrier, Pembroke Welsh Corgi, Poodle	Low plasma vWF concentration, normal vWF multimer composition	Splice site defect *VWF* gene (location not specified)	Brewer *et al*., 1998	*VWF*/12p13.3
Type 1 vWD	Airedale Terrier, Akita, Dachshund, German Shepherd, Golden Retriever, Greyhound, Irish Wolfhound, Schnauzer, Shetland Sheepdog	Low plasma vWF concentration, normal vWF multimer composition	Unknown	Dodds *et al*., 1981; Brooks, 1999	
Type 2 vWD	German Shorthaired Pointer, German Wirehaired Pointer	Low plasma vWF concentration, high MW vWF multimers absent	Unknown	Johnson *et al*., 1988; Brooks *et al*., 1996	
Type 3 vWD	Dutch Kooiker	No detectable plasma vWF protein	Splice site defect introducing stop codon at vWF propeptide	Rieger *et al*., 1998	*VWF*/12p13.3
Type 3 vWD	Scottish Terrier	No detectable plasma vWF protein	Deletion (1 bp) in pre-pro region *VWF*; resultant transcript is truncated	Venta *et al*., 2000	*VWF*/12p13.3
Type 3 vWD	Chesapeake Retriever, Shetland Sheepdog	No detectable plasma vWF protein	Unknown	Johnson *et al*., 1988; Raymond *et al*., 1990	
X-linked coagulation factor deficiencies					
Haemophilia A	Sporadic cases in more than 50 breeds and mixed-breed dogs; common familial in German Shepherds	Low or undetectable Factor VIII coagulant activity	Unknown	Fogh & Fogh, 1988; Brooks, 1999	*F8C*/Xq28
Haemophilia B	Mixed-breed dog	Undetectable Factor IX coagulant activity and protein	1 bp substitution at catalytic site	Evans *et al*., 1989	*F9*/Xq27.1–q27.2
Haemophilia B	Lhasa Apso	Undetectable Factor IX coagulant activity and protein	4 bp deletion and transition; resultant transcript is unstable	Mauser *et al*., 1996	*F9*/Xq27.1–q27.2

Table 9.7. *Continued*

Disease	Affected breeds	Biochemical phenotype	Causative mutation	References	Human homologous locus/chromosome
Haemophilia B	Labrador Retriever	Undetectable Factor IX coagulant activity and protein	Complete gene deletion	Brooks *et al*., 1997	*F9*/Xq27.1–q27.2
Haemophilia B	Airedale Terrier	Undetectable Factor IX coagulant activity and protein	Insertion (5 kb); resultant transcript lacks catalytic site	Gu *et al*., 1999	*F9*/Xq27.1–q27.2
Haemophilia B	Pit Bull, mixed-breed	Undetectable Factor IX coagulant activity and protein	Partial (5' region) gene deletion	Gu *et al*., 1999	*F9*/Xq27.1–q27.2
Haemophilia B	Sporadic cases in more than 20 breeds and mixed-breed dogs	Low Factor IX coagulant activity; protein present or absent	Unknown	Fogh & Fogh, 1988; Brooks & Catalfamo 2000b	*F9*/Xq27.1–q27.2
Autosomal coagulation factor deficiencies					
Factor I (Fibrinogen)	Bernese Mountain Dog, Borzoi, Collie, Vizsla	Low plasma fibrinogen concentration	Unknown	Kammerman *et al*., 1971; Dodds, 1989	*FGA, FGB, FGG*/ 4q28
Factor II (Prothrombin)	Boxer	Low Factor II coagulant activity	Unknown	Dodds, 1989	*F2*/11p11–q12
Factor VII (Proconvertin)	Beagle, Malamute	Low Factor VII coagulant activity	Unknown	Spurling *et al*., 1972; Mills *et al*., 1997	*F7*/13q34
Factor X (Stuart-Prower factor)	American Cocker Spaniel, Jack Russell Terrier	Low Factor X coagulant activity	Unknown	Dodds 1973; Cook *et al*., 1993	*F10*/13q34
Factor XI (Plasma thromboplastin antecedent)	English Springer Spaniel, Kerry Blue Terrier	Low Factor XI coagulant activity	Unknown	Dodds & Kull, 1971; Knowler *et al*., 1994	*F11*/4q35
Factor XII (Hageman factor)	Miniature Poodle, Shar Pei	Low Factor XII coagulant activity	Unknown	Randolph *et al*., 1986; Otto *et al*., 1991	*F12*/5q33–qter

age (Giger, 2000). A defect of muscle-type phosphofructokinase (M-PFK) causes a regenerative haemolytic anaemia in two related Spaniel breeds (English Springer Spaniel, American Cocker Spaniel). This defect is associated with haemolytic crises and persistent reticulocytosis. In contrast to PK deficiency, PFK deficiency is rarely fatal (Giger *et al.*, 1992). Abnormal red cell morphology and increased osmotic fragility are characteristics of erythrocyte membrane defects. The specific biochemical and molecular basis of these defects are not yet well defined. A non-regenerative anaemia, caused by cobalamin deficiency secondary to intestinal malabsorption, has been identified in Giant Schnauzers (Table 9.7).

Immunodeficiency disorders (white cell, immunoglobulin, complement defects)

Pelger-Huët anomaly, found in many different breeds, is a non-pathological disorder of neutrophil development, characterized by nuclear hyposegmentation, (Latimer, 2000). More typically, white cell disorders cause immunodeficiency states. Affected dogs are recognized because of recurrent fever and signs of systemic infection. Definitive diagnoses of canine immunodeficiencies are difficult because the characterization of these defects is beyond the scope of most clinical veterinary laboratories. Nevertheless, recent studies have identified the biochemical and molecular basis for immunodeficiency syndromes in several different breeds (Table 9.7). The disease phenotypes are heterogeneous, caused by a lack of cytokine receptor, leucocyte surface integrin, or complement component (Felsburg, 2000). A complex immunodeficiency in colour-dilute Collies is associated with cyclic cytopenias and intrinsic abnormalities of neutrophil and platelet function (Yang, 1987). Many immunodeficiency disorders are not well defined, characterized only by *in vitro* abnormalities of leucocyte function and/or low serum immunoglobulin concentration within a breed or litter of dogs. Some abnormalities may represent the effects of systemic infection, rather than primary causes of immunodeficiency (Studdert *et al.*, 1984).

Severe combined immunodeficiency is an X-linked recessive trait. This defect occurs in two breeds (Basset Hound, Cardigan Corgi) as the result of two different mutations in the same gene: interleukin 2-γ receptor (Henthorn *et al.*, 1994; Somberg *et al.*, 1995). The inheritance and expression of all other canine defects characterized at the molecular level and cyclic neutropenia of colour-dilute Collies are autosomal recessive.

Platelet disorders

Non-pathological macrothrombocytosis has been reported in King Charles Spaniels (Brown *et al.*, 1994). Periodic thrombocytosis and platelet dysfunction are features of the cyclic neutropenia syndrome of colour-dilute Collies (Lothrop *et al.*, 1991; Table 9.7). All of the other reported hereditary platelet disorders of dogs are functional defects (thrombopathias) and affected dogs have normal platelet counts. Typical clinical signs of platelet dysfunction

include mucosal bleeding, cutaneous bruising, and prolonged bleeding from injured tissues.

Phenotypic characterizations of thrombopathia are based on panels of assays that measure the platelets' ability to support clot retraction, aggregation response to a series of physiological agonist compounds, release of alpha or delta granule contents, the presence and density of surface glycoproteins, and platelet ultrastructure (Brooks and Catalfamo, 2000a). As a result of these studies, thrombopathias are broadly classified as defects of platelet membrane glycoproteins (integrins), deficiencies of intracellular storage granule contents, or signal transduction defects (Table 9.7). Functional studies must be performed within a few hours of blood sample collection, and it is likely that platelet function defects of dogs are under-diagnosed.

All platelet disorders identified to date are autosomal traits. Pedigree studies of Glanzmann's thrombasthenia and Collie cyclic neutropenia are compatible with recessive expression; other defects are likely to be recessive; however, confirmatory studies have not been reported.

Haemostatic protein disorders

Defects of plasma haemostatic proteins (von Willebrand disease and coagulation factor deficiencies) are among the most commonly reported canine hereditary disorders (Dodds *et al.*, 1981). Clinical signs of haemorrhage are obvious indications for owners to seek veterinary care and definitive diagnoses are accomplished through sample submission to a testing laboratory, rather than case referral. In addition to diagnosis bias, studies in human beings (and preliminary studies in dogs) indicate that many cases of vWD and haemophilia occur as the result of frequent *de novo* mutations in the corresponding genes (Thompson, 1994; Sadler *et al.*, 1995). On-line databases of these mutations have been compiled from human cases diagnosed worldwide (hhtp://mmg2.im.med.umich.edu/vWF/; http://www.umds.ac.uk/molgen/haemBdatabase.htm; http://europium.csc.mrc.ac.uk/).

Typical signs of von Willebrand disease (vWD) are similar to those of thrombopathia (i.e. mucosal bleeding, bruising, and prolonged bleeding from injured tissues). Coagulation factor deficiencies cause a failure of fibrin clot formation, with resultant haemarthrosis, haematoma formation and prolonged bleeding from even minor wounds.

Most of the hereditary haemostatic defects identified in human beings have been reported in dogs (Table 9.7). In both species, vWD and haemophilia are phenotypically heterogeneous and are by far the most common traits (Dodds, 1988). The phenotypic characterizations of haemostatic protein defects are based on specific functional and quantitative factor assays (Brooks, 1999). Although a vWD phenotype unlinked to the vWF gene has been reported in a mouse model (Nichols *et al.*, 1994), it is likely that most of the haemostatic protein defects in dogs are caused by mutations in their corresponding genes (Table 9.7).

Haemophilia A (Factor VIII deficiency) and haemophilia B (Factor IX deficiency) are X-linked recessive traits. Both genes are located on the long

arm of the X chromosome, but they are unlinked to each other (Dutra *et al.*, 1996). Characteristic features of haemophilia A and B include familial transmission within breeds, and frequent sporadic cases in mixed-breed dogs and many different purebreds. Analogous to the disease in human beings, the case ratio of haemophilia A to haemophilia B is about 4 : 1 (Brooks, 1999). Phenotypic characterization of canine haemophilia A and B, and preliminary molecular analyses of haemophilia B (Table 9.7) indicate that these diseases are mutationally heterogeneous, and the occurrence of haemophilia within each breed or family is the probable result of a *de novo* mutation event.

Von Willebrand disease and all remaining coagulation factor deficiencies are autosomal traits. Recessive inheritance and expression have been documented in type 2 and type 3 vWD (Johnson *et al.*, 1988). On the basis of protein concentration, there is evidence for both incomplete dominant and recessive inheritance of type 1 vWD (Moser *et al.*, 1996; Riehl *et al.*, 2000). A common splice-site mutation apparently occurs in several breeds affected with type 1 vWD (Brewer *et al.*, 1998); however, it is unclear how this mutation is related to expression of a bleeding diathesis, and whether an identical mutation occurred *de novo* in different breeds.

Renal disorders (Table 9.8)

The canine hereditary nephropathies with proven modes of inheritance are listed in Table 9.8. The hereditary nature of nephropathies is suspected but not proven in many breeds. Canine hereditary nephropathies were reviewed by Picut and Lewis (1987). They group the disorders under renal agenesis, renal dysplasias, primary cystic diseases, glomerulopathies, tubulo-interstitial nephropathies and tubular transport dysfunctions. Juvenile renal disease was described in more than 20 breeds by Nash (1989). This name has been used to cover several lesions including nephritis (with inflammatory features) and unilateral renal agenesis (Dobermann Pinscher, Beagle, etc.). The defect appears to be distinct from renal dysplasia. X-linked forms of glomerular nephritis in humans and dogs are often linked to defects in Col4a5 or Col4a6 proteins. In humans, these defects cause renal failure accompanied by sensorineural deafness (Alport syndrome). Hearing deficits have not been identified in the corresponding canine disease. In the Bernese Mountain dog, females with nephritis outnumber males by 4 to 1: the disease is hereditary, almost certainly autosomal recessive, but modified by a sex responsive second locus (Minkus *et al.*, 1994).

Renal dysplasias as defined by Picut and Lewis (1987) are diseases that mimic a failure of differentiation: asynchronous nephron development; persistent fetal mesenchyme; persistent metanephric ducts; atypical tubular epithelium and dysontogenic metaplasia. The kidneys of affected dogs are small and pale, with low numbers of immature glomerulae. A linkage-based test is available for screening for this disease in Soft-coated Wheaten Terrier as well as Lhasa Apso and Shih Tzu (see Chapter 5). The disease in the

Table 9.8. Canine hereditary renal and hepatic defects

Defect	Affected breeds	Phenotype	Inheritance/mutation	References
Renal				
Polycystic kidney disease	Bull Terrier	Multiple bilateral renal cysts lined with cells of nephron origin. Proteinurea	Autosomal dominant.	O'Leary *et al*., 1999
Hereditary nephritis	Bull Terrier	Proteinurea from a few months. Death of renal failure up to 10 years. Glomerular basement membranes (GBM) thickened, laminated, ruptured. Hearing normal	Autosomal dominant	Hood *et al*., 1990, 1995
Hereditary nephritis	Samoyed	Proteinurea in both sexes from 3–4 months, males show juvenile onset renal failure leading to death at 8–15 months, females show milder renal disease. GBM thickened, laminated, ruptured. Hearing normal	X-linked dominant. G > T transversion (Gly>*)in codon 1027 of *COL4A5*	Jansen *et al*., 1984; Zheng *et al*., 1994
Hereditary nephritis	Cross-breed	Proteinurea in both sexes from 3–4 months, males show juvenile onset renal failure leading to death at 8–15 months, females show more slowly progressive renal failure. GBM thickened, laminated, ruptured	X-linked dominant. Complete absence of both Col4a5 and Col4a6 proteins. Mutation unknown but not that of Samoyed nephritis.	Lees *et al*., 1999
Hereditary nephritis	Cocker Spaniel	Proteinurea from 5 months. Death of renal failure from 8–27 months GBM thickened, laminated, ruptured	Autosomal recessive. Col4a5 and 6 protein chains present in GBM, but Col4a3 & 4 absent.	Lees *et al*., 1997, 1998
Renal dysplasia	Lhasa Apso Shih Tzu, Soft Coated Wheaten Terrier	Onset of renal failure before 12 months. See text.	Probably not simple monogenic with complete penetrance. A linkage based test is offered for these three breeds, (http://www.vetgen.com/renaldys.html) although basis for this is unpublished	O'Brien *et al*., 1982; Eriksen & Grondalen 1984; Nash *et al*., 1984
Tubular transport dysfunction 'Fanconi's syndrome'	Basenji	Signs appear 2 months – 6 years. Urine concentrating defect, polyurea, polydipsia: associated with elevated membrane cholesterol. No obvious changes of bone density or other skeletal abnormalities	Familial, presumed autosomal recessive	Easley & Breitschwerdt, 1976; Bovee *et al*., 1978; Hsu *et al*., 1994

Renal amyloidosis	Beagle, Foxhound	Glomerular and interstitial amyloidosis, proteinurea, onset 5–11 years	Familial	Bowles and Mosier, 1992; Mason & Day, 1996; Rha *et al*., 2000
Cystinuria	Newfoundland, Irish Terrier	Renal tubular malabsorption of cystine leads to formation of cysteine uroliths which may cause renal failure	Autosomal recessive. A nonsense mutation (C > T in exon 2 of *SLC3A1* (R1817>*)) truncates the protein in Newfoundlands but not six other breeds, including the Irish Terrier	Casal *et al*., 1995; Henthorn *et al*., 2000
Urate urolithiasis	Dalmatian	Defective purine metabolism (or urate transport?) results in elevated urate in serum and hyperuricosuria. Correctable by hepatocyte transplantation	Autosomal recessive trait shared by all Dalmatians	Keeler, 1940
Cystine and urate uroliths	Bulldog, Irish Terrier, Dachshund, Mastiff, others	Renal tubular malabsorption of cystine and up to 5 other amino acids. Leads to formation of cysteine and urate uroliths which may cause renal failure	Probable autosomal recessive in some cases	Bartges *et al*., 1994; Osborne *et al*., 1999
Renal cystadeno-carcinoma and nodular dermatofibrosis	German Shepherd	Bilateral, multifocal tumours in kidneys and numerous firm collagenous nodules in the skin and subcutis	Autosomal dominant. Canine chromosome 5 corresponding to small portion of human 1p or 17q	Lium & Moe, 1985; Moe & Lium,1997; Jónasdóttir *et al*., 2000
Hepatic				
Copper toxicosis	Bedlington Terrier	Copper accumulation in zone 3 hepatocytes throughout life. Inflammation /degeneration in area of Cu accumulation	Autosomal recessive. Linked marker available. Mapped to canine autosome 10q26 (homologous to 2p21–p13)	Hardy *et al*., 1975; Twedt *et al*., 1979; Yuzbasiyan-Gurkan *et al*., 1997; Dagenais *et al*., 1999; van Wolferen *et al*., 1999; Van de Sluis *et al*., 2000
Renal + hepatic				
Polycystic kidney and liver disease	Cairn Terrier, West-Highland White Terrier	Liver failure by 5 weeks. Multiple bilateral cysts of biliary and ductal origin in liver and kidneys. Resembles human AR polycystic kidney disease	Probable autosomal recessive	McKenna and Carpenter, 1980; McAloose *et al*., 1998
Amyloidosis (Shar-Pei fever)	Shar-Pei	Febrile episodes, amyloid deposition in kidneys and liver, arthritic changes: kidney disease onset by 2 years of age. Similar to human Mediterranean fever	Probable autosomal recessive. Canine orthologue of human *MEFV* gene has been cloned. No mutation yet found	DiBartola *et al*., 1990; Loeven, 1994

Soft-Coated Wheaten Terrier is distinct from the recently recorded familial protein losing enteropathy and nephropathy in the same breed (Littman *et al.*, 2000).

Tubular transport defects, reported in several breeds, result in the failure to clear particular metabolic intermediates from urine (Table 9.8). In general, these diseases lead to an inability to concentrate urine as in Fanconi's syndrome (Basenji) or to urolith deposition. Renal and cystic calculi are more likely to be diagnosed in male than female dogs because of anatomical differences in the urinary outflow tract.

Renal cystadenocarcinoma and nodular dermatofibrosis is an autosomal dominant disease of German Shepherds for which a linked marker has recently been found. Polycystic kidney and kidney and liver diseases are described in Table 9.8.

Hepatic/liver disorders (Table 9.8)

Copper toxicosis

Copper toxicosis in the Bedlington Terrier has a special place in the recent history of canine disease genetics. It was the first disease for which a carrier test based on a linked polymorphic marker was announced. This common disease, with measured prevalence up to 46% in some Bedlington Terrier populations (Ubbink *et al.*, 2000), results in the accumulation of copper complexed to metallothionein in the lysosomes of liver hepatocytes (Johnson *et al.*, 1981; Lerch *et al.*, 1985). The disease responds to zinc acetate administration (Brewer *et al.*, 1992). Yuzbasiyan-Gurkan *et al.* (1997) identified a microsatellite marker tightly linked to the disease locus, with a maximum LOD score at a recombination fraction of zero in their sample. This marker has proved useful in identifying affected and carrier Bedlington Terriers, although there have been one or two examples of change of phase, in testing programmes based on the marker (see Chapter 16). Several candidate genes have been examined based on the similarity of the disease to the human copper transport defect Wilson's disease. These studies have shown that the defect is neither in, nor linked to, the ATP7B gene mutated in Wilson's disease (Dagenais *et al.*, 1999; Nanji and Cox, 1999). The mutation has now been located to a short region of canine chromosome 10 homologous to part of human 2p21–2p13 (Dagenais *et al.*, 1999; van Wolferen *et al.*, 1999; Van de Sluis *et al.*, 2000). The disease is distinct from copper toxicosis in the West Highland White Terrier, a familial disease in which copper accumulation is not related to age, and the distribution of inflammatory foci differs from that seen in the Bedlington (Thornburg *et al.*, 1986). The disease in 'Westies' has more features in common with chronic active hepatitis (CAH) in Dobermann Pinschers. The defect in Dobermanns primarily affects females and copper accumulation may be secondary to a failure of biliary excretion (Johnson *et al.*, 1982; Thornburg *et al.*, 1996).

Other hepatic diseases
Hepatic encephalopathy (a degenerative disease of the CNS induced by a failure of the liver to clear circulating ammonia, and abnormal modulation of excitatory and inhibitory amino acids) is inherited as a consequence of portosystemic shunts in the Irish Wolfhound (Meyer *et al.*, 1996), see PSVA above. Amyloidosis is an autosomal recessive disease of Shar Peis in which amyloid is deposited in the kidneys and liver.

Skin disorders (Table 9.9)

Many immune mediated familial diseases of skin (some with systemic involvement) have been reported in the dog (e.g. junctional and dystrophic epidermolysis bullosa, dermatomyositis, pemphigus, atopies). Non-immune and non-endocrine mediated, breed specific dermatoses, such as the zinc-responsive dermatosis of Alaskan Malamutes and Siberian Huskies, also occur (Colombini, 1999). Characteristic lesions of this defect include erythema, alopecia, scales and crusts that primarily affect the head. Lethal acrodermatitis is a rare inherited disorder of Bull Terriers that does not respond to zinc supplementation and is invariably fatal. Table 9.9 lists additional non-immune mediated dermatoses and systemic disorders having monogenic inheritance.

Enteric disorders (Table 9.9)

Inherited inflammatory enteropathies have been noted and studied in several breeds, including the Lundehund (Flesja and Yri, 1977; Landsverk and Gamlem, 1984); German Shepherd (Batt *et al.*, 1983), Basenji (De Buysscher *et al.*, 1988) and Soft Coated Wheaten Terrier (Littman *et al.*, 2000; Vaden *et al.*, 2000). All are not gluten-dependent, and there has been no complete description of their inheritance. The predominant clinical signs are vomiting, diarrhoea, weight loss, pleural and peritoneal effusions, with hypotrophy of the intestinal microvilli. More complete studies have been performed on the gluten-dependent enteropathy of the Irish Setter by Batt and co-workers starting in 1984. Recently they have shown conclusively that the inheritance of this defect is monogenic and autosomal recessive (Garden *et al.*, 2000; Table 9.9).

Exocrine pancreatic insufficiency, associated with acinar cell degeneration, is found sporadically in many breeds of dog. The defect is familial, with possible autosomal recessive inheritance in German Shepherd dogs (Weber and Freudiger, 1977). Some authors have suggested an autoimmune involvement, but this has not been confirmed by others.

Histiocytic ulcerative colitis (inflammatory bowel disease) is an inflammation of the mucosa and submucosa of colon and rectum. It is familial, but details of inheritance are unclear.

Hereditary oesophageal achalasia has been shown to be inherited in a manner consistent with a monogenic autosomal disorder in a line of Miniature

Table 9.9. Miscellaneous hereditary conditions of the dog

Defect	Affected breeds	Phenotype	Inheritance/mutation	References
Enteric				
Gluten sensitive enteropathy	Irish Setter	Gluten sensitivity. Reduction in villus length and functional capacity of small intestine	Autosomal recessive. Not MHC linked	Batt *et al*., 1984; Polvi *et al*., 1998; Garden *et al*., 2000
Selective intestinal cobalamin malabsorption	Giant Schnauzer	Inappetance and failure to thrive from 6 weeks, low serum B12, neutropenia with hypersegmentation, anaemia, megaloblastic changes. Cbl malabsorption. Receptor absent from ileal apical brush border microvilli	Autosomal recessive. Amino acid sequencing shows abnormality in intrinsic factor – cobalamin receptor (*CUBN*)	Fyfe *et al*., 1991a,b
Hypertrophic gastritis with stomatocytosis	Drentse Partrijshond	See Table 9.7		
Skin/connective tissue				
Lethal acrodermatitis	Bull Terrier	Non zinc responsive progressive lethal acrodermatitis, tail chasing behaviour, growth retardation, bronchopneumonia. Reduced plasma zinc and copper	Autosomal recessive	Jezyk *et al*., 1986; Uchida *et al*., 1997
Cutaneous asthenia (Ehlers-Danlos syndrome)	Springer Spaniel, (other breeds	Skin is hyperelastic and has pendulous folds. Dogs prone to wounding	Autosomal dominant	Hegreberg *et al*., 1969
Cutaneous vasculopathy	German Shepherd	Footpad swelling/depigmentation: necrosis of basal cells in depigmented lesions, nodular dermatitis; pyrexia lethargy, ulceration of extremities	Autosomal recessive	Weir *et al*., 1994
Black hair folicular dysplasia	Bearded Collie, Large Munsterlander, Saluki, Jack Russell	Underdevelopment and subsequent loss of black coat hairs. Yellow or pale irises. One report of deafness associated with the condition	Autosomal recessive. Not linked to melanocyte stimulating hormone receptor gene (*MSHR*) in the Munsterlander	Selmanowitz *et al*., 1972; Lewis, 1995; Harper, 1978; Schmutz *et al*., 1998
Nodular dermatofibrosis with renal cystadenoma	German Shepherd	See Table 9.8		

Pulmonary and pulmonary/systemic				
Cyliary dyskinesia (Kartagener's syndrome)	Golden Retreiver, English Springer Spaniel, Old Enlish Sheepdog, Shar-Pei, Chow Chow, Bichon Frise, Border Collie, English Setter, Rottweiller, Doberman, Dalmatian, Chihuahua, Miniature Poodle, Newfoundland	Immotile cilia, showing abnormalities of ultrastructure and/or irregularities of arrangement. May include immotile sperm. Dogs contract recurrent pneumocid disease. May be situs invertus viscerum in 50% of affected animals (description as Kartagener's syndrome applies). In Chow Chow, Kartagener's has been described without ciliary abnormality	Autosomal recessive where looked at	Edwards *et al*., 1992; Watson *et al*., 1999; Edwards *et al*., 1989
Respiratory distress syndrome	Dalmatian	Severe respiratory failure/death at 3 weeks. Diffuse densities in lungs, alveolar arterial oxygen difference, inflammatory changes in alveolar epithelium: associated with congenital abnormalities	Probable autosomal recessive	Jarvinen *et al*., 1995
Reproductive				
Sry –ve sex reversal	American Cocker Spaniel, German Short-Haired Pointer, Norwegian Elkhound (Sporadic cases in 11 other breeds summarized in Melniczek *et al*., 1999 and 12 others in Meyers-Wallen *et al*., 1999)	In at least the first three breeds dogs may be either XX male (aspermatogenic) or true hermaphrodite (with ovotestes)	No Y chromosome. *SRY*-ve by PCR and RT-PCR. Autosomal recessive in first 3 breeds	Selden *et al*., 1984 Meyers-Wallen & Patterson, 1988 Meyers-Wallen *et al*., 1999
Persistent Müllerian duct syndrome (PMDS)	Miniature Schnauzer, Bassett Hound	Persistence of complete Müllerian ducts (oviducts, uterus, cervix and cranial vagina) in XY, bilateral testes bearing males	Autosomal recessive. Müllerian inhibiting substance (MIS) is normal in embryo and post-birth: suggests MIS receptor defect	Marshall *et al*., 1982 Meyers-Wallen *et al*., 1989 Nickel *et al*., 1992

Table 9.9. *Continued*.

Defect	Affected breeds	Phenotype	Inheritance/mutation	References
Endocrine				
Diabetes mellitus	Keeshond	Insulin dependent diabetes mellitus with hypoplasia of Islets of Langerhans. Onset before 6 months	Probable autosomal recessive	Kramer *et al*., 1988
Juvenile hyperparathyroidism	German Shepherd	Hyperplasia of parathyroid chief cells, nodular hyperplasia of thyroid C-cells, fibrous osteodystrophy, hypercalcaemic nephropathy, and extensive mineralization of lungs and gastric mucosa	Probable autosomal recessive	Thompson *et al*., 1984
Pituitary dwarfism (combined pituitary hormone deficiency)	German Shepherd Karelian Bear Dog	Dwarfism. Depressed or zero circulating growth hormone and IGF-1. No response to GHRH. Responsive to GH	Autosomal recessive Not linked to Pit-1 transcription factor locus (*PIT1*)	Andresen & Willeberg, 1976, 1977; Lantinga-van Leeuwen *et al*., 2000

Schnauzer dogs, although the inheritance is more complex when bred outside this line (Cox *et al.*, 1980).

Respiratory disorders (Table 9.9)

There are few reports of clearly monogenic disorders with primary involvement of lungs or respiratory system. A progressive pulmonary disease resulting in severe respiratory failure and death has been reported to segregate as a probable autosomal recessive disease in Dalmatians (Jarvinen *et al.*, 1995). Primary ciliary dyskinesias have been reported in many breeds (Table 9.9). These defects impair the clearance of bacterial infections of the lungs and affect sperm motility. Some ciliary dyskinesias (described in Chapter 5) cause major morphological defects of situs invertus viscerum.

Reproductive disorders (Table 9.9)

Anomalies of sexual differentiation, including chromosomal abnormalities, have been reviewed by Meyers-Wallen (1993). Sex reversal of Sry negative XX dogs has been noted in many breeds. This defect is inherited as an autosomal recessive. Unexpectedly, the sex-reversed dogs have no Sry gene. In other species, the Sry gene product is normally needed for differentiation of testes, and in sex reversed XX females it is usually found transposed to an autosome. A similar condition has been reported in goats, where the autosomal recessive sex reversing mutation was shown to be closely linked to the *polled* locus. (Just *et al.*, 1994). Persistent Müllerian duct syndrome (PMDS) has also been studied (Table 9.9). Testicular feminization has not been reported in dogs. Familial forms of azoospermia are probably present, but are poorly documented.

Endocrine disorders (Table 9.9)

A number of endocrine disorders show increased prevalence in certain breeds, but the mode of inheritance has not been established. In some cases, these diseases are associated with breed prevalence of particular tumours or result from apparent autoimmunity. Examples include hyper- and hypoadrenocorticism (Cushing's and Addison's diseases) and hypothyroidism. Although diabetes mellitus and diabetes insipidus appear to be familial (in some cases), the heritability of the condition is low, and studies are restricted to few breeds (Kramer *et al.*, 1988; Post *et al.*, 1989). Diabetes mellitus in the Keeshond can be inherited in an autosomal recessive mode. Pituitary dwarfism and juvenile hyperparathyroidism have also been demonstrated to have probable autosomal recessive inheritance in some breeds (Table 9.9).

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10 Genetics of Canine Hip Dysplasia and Other Orthopaedic Traits

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Introduction

In a multicentre referral population, at least 30% of all canine patients presenting with a musculoskeletal problem are diagnosed with a disease with a genetic aetiology (Johnson *et al.*, 1994). Although many orthopaedic diseases with a genetic aetiology (traits) are already present at birth, others become apparent or increase in severity later in life (delayed onset). In this chapter, orthopaedic diseases will be classified as *constitutional* or *acquired* bone and cartilage diseases. Constitutional bone and cartilage diseases can be identified at or shortly after birth, while acquired diseases will develop during adolescence or later in life. Orthopaedic conditions with a genetic aetiology will be discussed, with an emphasis on aetiology, known breed predispositions, heritability, mode of inheritance, and the biochemical and genetic defects causing the disease. Because of its prominence in the list of orthopaedic acquired and developmental canine traits, hip dysplasia will be discussed in most detail. Complete descriptions of the clinical, radiographic and pathological signs of other traits described here can be found in the cited references or in canine orthopaedic handbooks (Olmstead, 1995; Piermattei and Flo, 1997).

Canine Hip Dysplasia

Canine hip dysplasia (CHD) is a developmental orthopaedic disease marked by hip laxity and disconformity that leads to hip osteoarthritis (OA) during and after maturity (Schnelle, 1935; Henricson *et al.*, 1966; Leighton *et al.*, 1977; Hedhammar *et al.*, 1979). The pattern of inheritance indicates that CHD is a complex trait controlled by the interaction of several genes located at quantitative trait loci (QTLs) and environmental factors. Several major and many minor QTLs are probably involved in phenotypic expression. A major locus may influence this trait based on biometric methods outlined by Leighton (1997). Most diagnostic features rely on the presence of laxity in the supporting structures of the hip (mostly capsular tissue in the passive state) and subluxation of the femoral head which can occur due to chondro-osseous malformation and laxity.

Intensive studies have defined the cartilage and soft tissue changes characteristic of the OA that occurs in dysplastic hips (Burton-Wurster *et al.*, 1993; Lust, 1997). The initial cartilage lesion occurs perifoveally, suggesting that abnormal magnitude or direction of load results in increased stress in this focal area (Farese *et al.*, 1998; Burton-Wurster *et al.*, 1999), leading to pain, articular cartilage degeneration, and bony remodelling characteristic of hip OA. There may be inductive or protective QTLs that control expression of hip OA that are independent of those controlling the dysplastic phenotype (Vingsbo-Lundberg *et al.*, 1998; Chapman *et al.*, 1999). Indeed, some breeds may develop radiographically detectable hip OA as a result of antecedent CHD faster than other breeds (Popovitch *et al.*, 1995).

Larger breeds share a heritable tendency to hip OA as a result of CHD (Kaneene *et al.*, 1997; Leighton *et al.*, 1977; Hedhammer *et al.*, 1979; Willis, 1989; Cardinet *et al.*, 1983; Tables 10.1 and 10.4). Yet, many dogs with genetic susceptibility do not show the clinical phenotype (Willis, 1989). Affected dogs may have abnormalities in other joints (Olsewski *et al.*, 1983; Farquhar *et al.*, 1997; Kealy *et al.*, 1997; Morgan *et al.*, 1999).

Phenotypes

Traditional

Mature dogs are positioned in dorsal recumbency with the hips extended and a ventrodorsal radiograph is taken (Fig. 10.1). In North America, radiographs are scored based on the degree of subluxation and OA according to the seven-point scale set out by the Orthopedic Foundation for Animals (OFA) (Henry, 1992) although other scales are used in Europe (Kealy *et al.*, 1992; Leighton, 1997). The OFA scale ranges from excellent, good and fair hip conformation through to borderline and mild, moderate, and severe CHD. Radiographic signs of hip OA include osteophytes on the acetabular rim and femoral neck, flattening of the acetabulum and femoral head, and subchondral sclerosis (Fig. 10.1). Heritability estimates for CHD based on the standard hip-extended, ventrodorsal radiographic projection range from 0.1 to 0.68 (see Table 10.1).

Femoral capital ossification

As assessed radiographically, dysplastic German Shepherds (Madsen *et al.*, 1991) and Labrador Retrievers (Todhunter *et al.*, 1997) were significantly older at the onset of capital femoral ossification (17 days of age for Labrador Retrievers) than their non-dysplastic counterparts (13 days). Based on quantitative computed tomography, the volume of the secondary centre of ossification in the femoral head is smaller at 4 weeks of age but larger by 6 weeks of age in genetically dysplastic Labrador Retrievers compared to genetically non-dysplastic Labrador Retrievers. This divergence in volume continues at least to 12 weeks of age (Foels *et al.*, 2000).

Distraction index (DI)

The maximum amount of passive lateral hip laxity can be measured on a distraction radiographic projection (PennHIP, Synbiotics, Malvern, Pennsylvania; Smith, 1997) (Fig. 10.2). Labrador Retrievers with DI values of less than 0.3 at 8 months of age have a greater than 80% probability of not developing hip OA. Those with DIs greater than 0.7 have a high probability of developing hip OA (Lust *et al.*, 1993). Some breeds appear to display different susceptibilities to CHD based on their DIs and some breeds may tolerate more passive hip laxity than other breeds (Popovitch *et al.*, 1995; Smith *et al.*, 1995). Hip laxity as measured by the DI has at least as high or higher heritability than estimates based on the standard OFA-type scoring. At the Seeing Eye,

Table 10.1. Heritability of hip dysplasia in natural canine pedigrees

Breed	Years	No. offspring	No. sires	No. dams	No. litters	Age (mo) at evaluation	Hip score scale[a]	Incidence of hip dysplasia	Heritability model	Heritability	Reference
German Shepherd	1969–1975	1186	28	127	258	5–11	9 point	27%	Mixed	0.22	Leighton *et al*., 1977
German Shepherd	1965–1973	2404	65	205	401	15	5 point	46%	Mixed	0.4	Hedhammer *et al*., 1979
German Shepherd	1976–1989	31951	NA	NA	NA	12–24	4 point	26%	Regression[b]	0.49	Swensen *et al*., 1997a
Golden Retriever	1976–1989	9347	NA	NA	NA	12–24	4 point	20%	Regression[b]	0.44	Swensen *et al*., 1997a
Labrador Retriever	1976–1989	16915	NA	NA	NA	12–24	4 point	20%	Regression[b]	0.57	Swensen *et al*., 1997a
Newfoundland	1976–1989	2087	NA	NA	NA	12–24	4 point	41%	Regression[b]	0.49	Swensen *et al*., 1997a
Rottweiler	1976–1989	2302	NA	NA	NA	12–24	4 point	18%	Regression[c]	0.33	Swensen *et al*., 1997a
Bernese Mt. Dog	1976–1989	1699	NA	NA	NA	12–24	4 point	23%	Regression[b]	0.68	Swensen *et al*., 1997a
St Bernard	1976–1989	812	NA	NA	NA	12–24	4 point	55%	Regression[c]	0.40	Swensen *et al*., 1997a
German Shepherd	1980–1996	2037	23	NA	NA	12–16	9 point	55–24%[d]	Mixed	0.35	Leighton, 1997
Labrador Retriever	1980–1996	1821	19	NA	NA	12–16	9 point	30–10%[d]	Mixed	0.35	Leighton, 1997
German Shepherd	1982–1984	10595	990	3036	NA	12–15	–[e]	53–58%	Mixed	0.11–0.48[f]	Distl *et al*., 1991

[a]All dogs were evaluated in hip-extended position.
[b]Realized heritabilities from regression of son's phenotype on the sire's phenotype or daughter's phenotype on dam's phenotype. The heritability is the average heritability derived from both regressions.
[c]Heritability from regression of all offspring on sires and all offspring on dams. The heritability is the average heritability derived from both regressions.
[d]Change over five generations in those years.
[e]Not described, but used scale of German Shepherd Centre of Germany.
[f]Heritability dependent on hierarchical design and sibship–parent relationships in model.

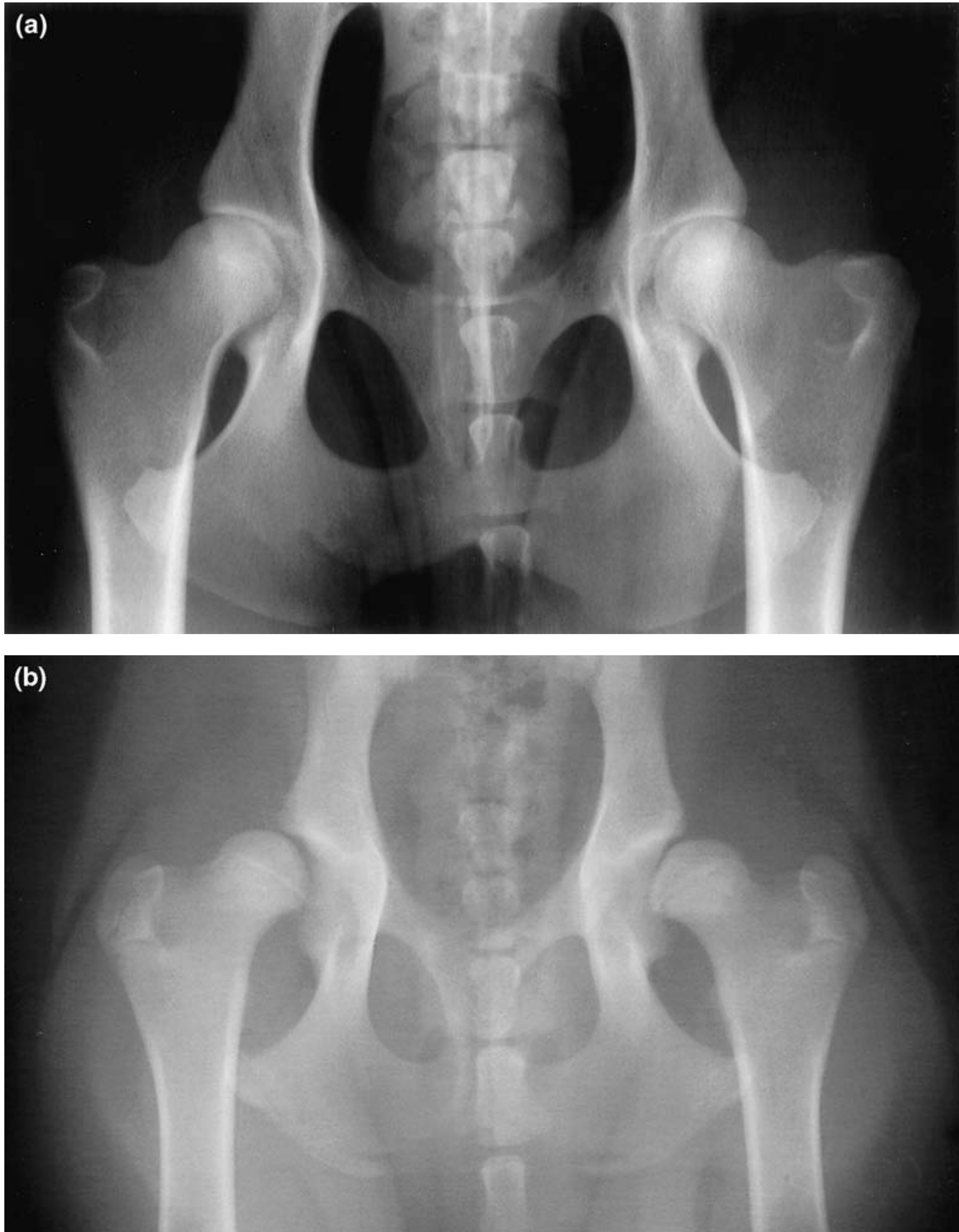
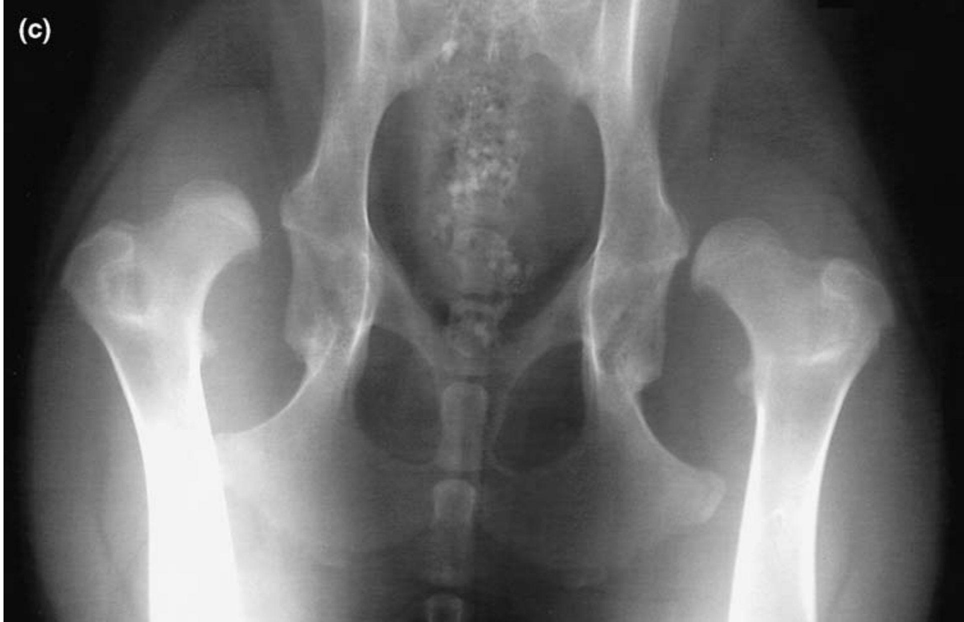


Fig. 10.1. (a) Photograph of a ventrodorsal, hip-extended radiograph of a dog with normal hip conformation. Note the good congruency between the femoral heads and acetabulae. (b) Photograph of a ventrodorsal radiograph of a dog with CHD. Note the subluxation of the femoral heads from the acetabulae. (c) Dysplastic hip showing extreme subluxation of the femoral heads from the acetabulae. (d) Photograph of a ventrodorsal radiograph of a dog with hip OA as a result of CHD. Note new bone formation in and around the hip joints as a result of the OA process.

Morristown, New Jersey, heritability for the OAF-type hip score was about 0.35 but for the DI it was 0.45 in both German Shepherds and Labrador Retrievers (Leighton, 1997).



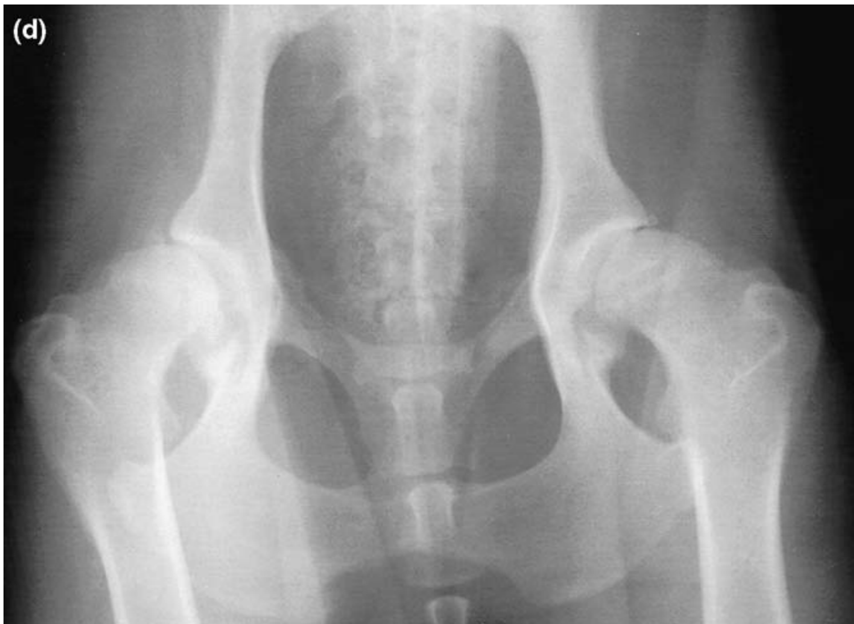


Fig. 10.1. *Continued*.

Dorsolateral subluxation (DLS)

This test elicits DLS of the femoral head from the acetabulum with the hips in a load-bearing position. The stifles are flexed and the dog is placed in a kneeling position so that natural load-bearing forces are transmitted to the hips by flexing and adducting the stifles that are in contact with the table-top. On the dorsoventral radiograph, the DLS score is measured as the percentage of the femoral head covered by the craniodorsal acetabulum (Fig. 10.3). The DLS

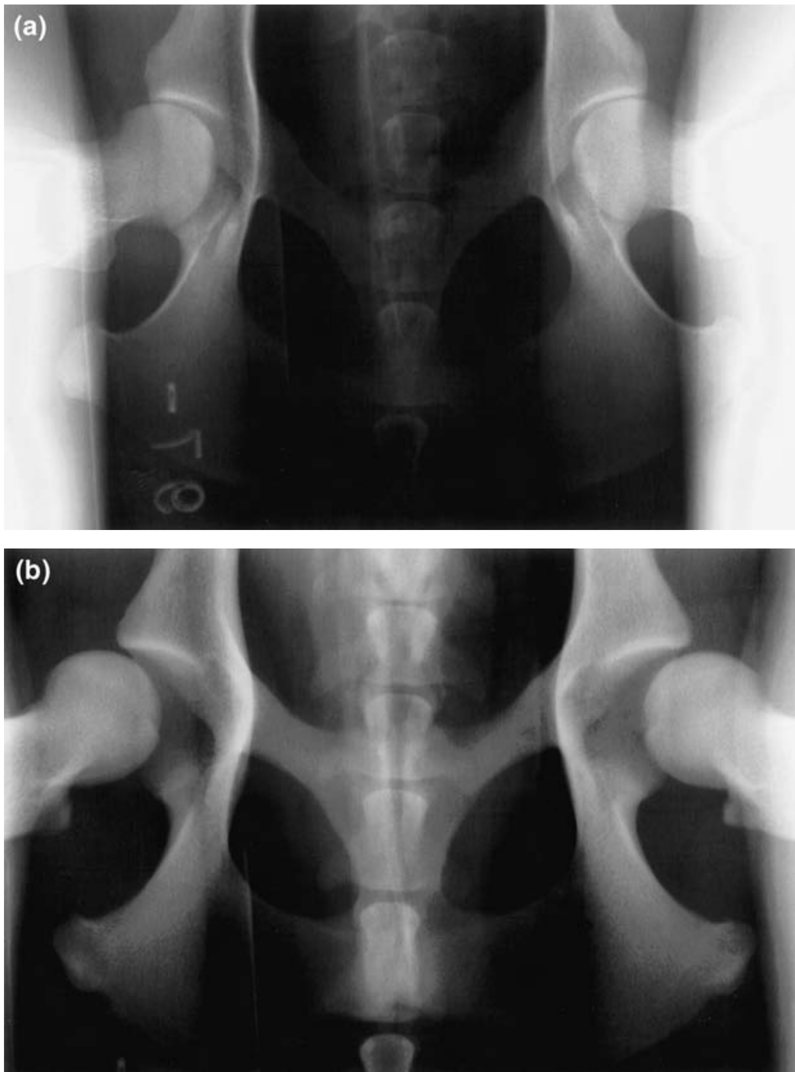


Fig. 10.2. Photographs of radiographs showing the ventrodorsal distraction projection used to measure the distraction index. (a) Hips with low laxity; the femoral heads do not distract from the acetabulae. (b) Hips with high laxity; the femoral heads distract from the acetabulae.

method has many similarities to the Fluckiger method (1999), both produce more hip subluxation (if it is present) than the hip-extended position. In an experimental outcrossed pedigree, all but two dogs with greater than or equal to 50% Greyhound genes had high DLS scores, indicating good osseous conformation with no CHD (Todhunter *et al.*, 1999). The mean DLS score for dysplastic Labrador Retrievers with hip OA at 8–12 months of age was $42 \pm 8\%$.

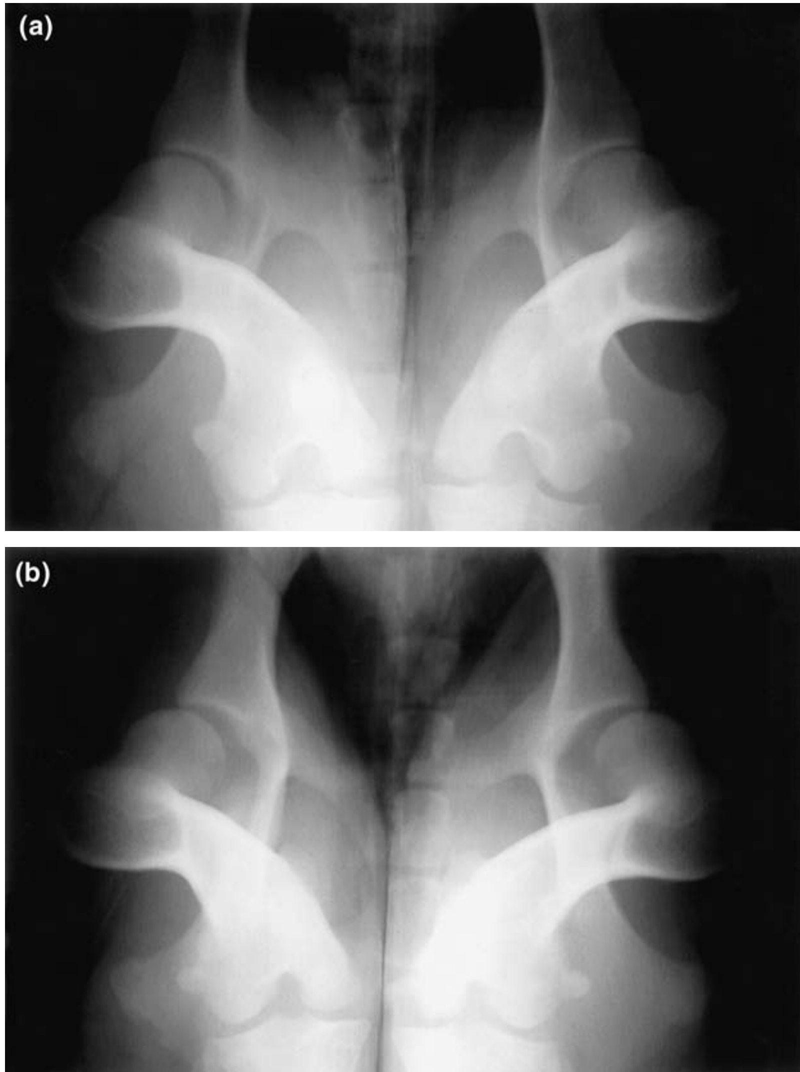


Fig. 10.3. Photographs of a radiograph showing the dorsolateral subluxation, dorsoventral projection. (a) Normal hips with no subluxation. (b) Dysplastic hips showing subluxation of the femoral heads from the acetabulae.

Environmental factors (nutrition)

Hip dysplasia develops during a pup's rapid growth spurt in the first 6–7 months of life. Overfeeding itself does not cause CHD; but it maximizes trait expression in genetically predisposed individuals (Lust *et al.*, 1973; Hedhammar *et al.*, 1974). The frequency and severity of CHD and OA were reduced markedly in Labrador Retrievers by limiting food consumption by 25% during growth (Kealy *et al.*, 1992, 1997). Dogs on the restricted diet had significantly decreased OA in their hips, shoulders, and stifles.

Hormonal influences and nutrition

Hormones and growth-promoting peptides such as insulin, cortisol, epidermal growth factor, insulin-like growth factors (IGFs), parathyroid-hormone related peptide, relaxin, oestrogen and oestrogen precursors are present in colostrum and milk (Ballard *et al.*, 1995; Xu, 1996) and are absorbed from the gastrointestinal tract in the postnatal period (Xu and Wang, 1996). These proteins could influence hip joint development in genetically susceptible tissues. Relaxin, oestrogen, and oestrogen precursors abound in the milk of Labrador Retrievers and are transported via suckling into the circulation of pups. While total serum oestrogens were similar in pups born of dysplastic and normal matings, testosterone was detected only in the milk of dysplastic Labrador Retriever bitches, and oestradiol-17β appeared only in the serum of pups born of dysplastic matings. An aromatase inhibitor (prevents conversion of testosterone to 17β-oestradiol) injected from birth throughout the lactation period significantly reduced hip joint laxity at maturity in dysplastic-bred Labrador Retrievers (Steinetz *et al.*, 1997).

Relaxin is a potent inducer of neutral matrix metalloproteinases I (procollagenase) and III (prostromelysin) and plasminogen activator expression. Relaxin significantly increased the length and weight of interpubic fibrocartilage in oestrogen-primed rats and decreased the total collagen content (Samuel *et al.*, 1996). Interestingly, the only treatment that has been shown to significantly reduce the expression of OA in CHD (besides reduced food intake) is systemic polysulphated glycosaminoglycan (Adequan) given to dysplasia-prone Labrador Retrievers from 6 weeks to 8 months of age (Lust *et al.*, 1992). Polysulphated glycosaminoglycan is an inhibitor of neutral matrix metalloproteinase activity. Polysulphated glycosaminoglycan also significantly reduced pubic symphyseal relaxation in oestrogen-primed tissues (Steinetz and Lust, 1994). Repetitive oestrogen dosing in the growing period can induce CHD (Henricson *et al.*, 1966; Beling *et al.*, 1975). Relaxin alters the proportion of collagen types secreted by fibroblasts and chondrocytes in culture, causing them to express a dedifferentiated phenotype (Bonaventure *et al.*, 1988). The joint capsule contributes substantially to the stability of the hip (Smith *et al.*, 1990) and laxity plays a role in the development of the dysplastic hip (Lust *et al.*, 1980). The round ligament of the femoral head may maintain hip

stability for the first 4 weeks of life (Riser, 1975). The proportion of types III to I collagen increased in hip joint capsules from adult dogs with CHD and OA (Madsen *et al.*, 1994; Madsen, 1997) but these changes could reflect a remodelling response to injury, as the tissues were collected at maturity.

Genetic linkage analysis

In the absence of candidate loci, pedigrees can be used to find linkage between a marker and a heritable trait (Lander and Schork, 1994; Risch and Merikangas, 1996; Darvasi, 1998). Later, the genes that colocalize with those QTLs can be sought through comparative mapping and positional cloning strategies. Then the total number of trait loci controlling the detectable genetic variance in the trait can be identified (Daw *et al.*, 2000). A three-generation, informative canine pedigree has been constructed for linkage analysis of CHD by outcrossing dysplastic Labrador Retrievers with unaffected Greyhounds (Todhunter *et al.*, 1999, 2000). Alleles at loci controlling age at onset of capital femoral ossification, DLS score and DI resegregated in the backcross generation so that backcrosses to the Labrador Retriever showed a wide range of phenotypes from unaffected to dysplastic and OA hips. In other pedigrees, 8 out of 19 German Shepherd–Greyhound crossbred offspring were dysplastic (Cardinet *et al.*, 1983). The crossbred offspring of Lust *et al.* (1973) from normal or dysplastic German Shepherds and Golden Retrievers and normal Beagles also had CHD in over 50% of dogs. Only 1 dog out of 34 was dysplastic in the F_1 generation of our outcrossed pedigree, similar to the results of Gustafsson *et al.* (1972).

As a prelude to genome-wide scanning and based on a single marker analysis, we have calculated that this outcrossed Greyhound/Labrador Retriever pedigree has a power of 0.99 to detect linkage to age at femoral capital ossification with the 58 dogs already bred in the backcross generations. Both the backcross to the Labrador Retriever and the Greyhound breeds have similar power to detect linkage to ossification and DLS score. For the best case (i.e. if recombination fraction, $\theta = 0$ and heterozygosity = 1), 50 dogs would give us a power of 0.98 to detect linkage to DLS in the backcross to the Greyhound at the observed effect size (a function of the difference in parental means and the standard deviation). For the worst case scenario, where $\theta = 0.1$ for a genome-wide scan at 10 cM intervals and marker heterozygosity = 0.75 (as determined for this pedigree based on microsatellite genotyping), the power would drop to 0.7 for 150 dogs in the backcross to the Greyhound. The backcross to the Greyhound generation has the most power to detect linkage to DI, in that 50 dogs would provide a power of 1.0 to detect linkage for the best case at the observed effect size while 100 dogs would still provide a power of 0.86 for the worst case. For composite interval mapping or Markov Chain Monte Carlo estimation methods, in which several markers are examined simultaneously, the power to detect linkage would be greater than that calculated for single marker analysis.

Prevention

A dog's phenotype is no guarantee of its genotype. Progeny of dogs with CHD tend to be dysplastic and sound dogs are more likely to be born to parents with trait-free hips. Yet a dog with nondysplastic hips may have one or more major loci that promote CHD and when they occur in concert with certain other loci in a mate, CHD will occur in the offspring. Genetic selection to improve the phenotype has been achieved in controlled populations like the Seeing Eye, by combining individual phenotypic information with parental and offspring information to obtain estimated breeding values (Leighton, 1997). Similarly, broad screening programmes through open registries as conducted in Sweden have resulted in improvement in hip quality (Swenson *et al.*, 1997a), but in Finland (Leppanen and Saloniemi, 1999), the US (Kaneene *et al.*, 1997) and in the UK (Willis, 1997), improvement in phenotypes has been disappointing.

Use of the DI or a subluxation index may be an improvement over the hip-extended methods in detection of subtle cases of CHD. Breeding dogs based on low laxity alone may decrease the incidence or severity of CHD. Yet several methods for hip evaluation may be necessary to fully define hip quality (Puerto *et al.*, 1999). Strict application of these methods with progeny testing seems to be more important than the method of hip evaluation itself in the reduction of trait incidence. Simply breeding animals with better hips than the average for the breed will never eliminate the trait (Leppanen and Saloniemi, 1999). Until genetic screening is available, the best indication of a dog's genetic makeup is the phenotype of its parents and grandparents, its offspring, and its siblings or half siblings. Prospective purchasers of puppies should request from breeders phenotypic information on these relatives. In the end, the best solution to eliminating the trait from a breed is to know the linked markers and then the mutations at the major controlling loci in that breed. That could lead to genetic screening of pups before purchase and breeding.

Other Orthopaedic Traits: Constitutional Bone and Cartilage Diseases

Orthopaedic diseases may be classified as *constitutional* or *acquired* bone and cartilage diseases. Constitutional bone and cartilage diseases are *genetic* bone diseases identifiable at or shortly after birth, and consist of dysostoses (Table 10.2) and osteochondrodysplasias (Table 10.3; Anon., 1983, 1992, 1998; Sande and Bingel, 1983; Jezyk, 1985). Dysostoses are malformations of individual bones or part of bones, resulting from an intrinsically abnormal developmental process (Spranger *et al.*, 1982). The malformations are caused by a failure in anlagen formation or failure of the transformation of the anlagen into cartilage or bone (Noden and de Lahunta, 1985). Dysostoses are further classified as: (i) dysostoses with cranial and facial involvement (not discussed here),

Table 10.2. Canine dysostoses

Trait	Breed	Mode of inheritance
Hemivertebra	German Short-haired Pointer	Simple autosomal recessive
Spina bifida	Bulldog	Unknown
Anury	Cairn Terrier	Unknown
	Cocker Spaniel	Autosomal recessive
Brachyury	Beagle	Simple autosomal dominant with reduced penetrance
Hemimelia	Chihuahua	Unknown
Polydactyly	Great Pyrenees	Autosomal dominant
	Saint Bernard	Autosomal recessive
	Australian Shepherd	X-linked lethal or sex-influenced autosomal

(ii) dysostoses with predominant axial involvement, and (iii) dysostoses with predominant involvement of extremities (Anon., 1983).

Dysostoses with predominantly axial involvement

Hemivertebrae

Hemivertebrae are malformed, wedge-shaped vertebrae (Bailey and Morgan, 1992; Done *et al.*, 1975). Hemivertebrae may be associated with neurological dysfunction which is most commonly seen in screw-tailed breeds like Bulldogs, Pekingese, Pugs and Boston Terriers (Bailey and Morgan, 1992; Done *et al.*, 1975). In German Short-haired Pointers this trait has a simple autosomal recessive mode of inheritance (Kramer *et al.*, 1982).

Spina bifida

Spina bifida is the absence of fusion of the dorsal portion of a vertebra, with (spina bifida aperta) or without (spina bifida occulta) intraspinal dysraphic defects and neurological dysfunction (Bailey and Morgan, 1992). Spina bifida is often associated with faecal and urinary incontinence and may occur with other vertebral malformations like hemivertebrae and block vertebrae (Wilson *et al.*, 1979; Wilson, 1982; Bailey and Morgan, 1992). Bulldogs appear at risk but pedigree analysis did not indicate a heritable mode of transmission (Wilson, 1982).

Axial malformation

The odontoid process (dens) of the axis may be absent, shortened, or angulated abnormally (Bailey and Morgan, 1992). Small and toy breeds are at risk for this malformation and subsequent neurological dysfunction (Beaver *et al.*, 2000).

Table 10.3. Canine osteochondrodysplasias

Breed	Trait	Mode of inheritance
Akita	Achondrogenesis	Unknown
Alaskan Malamute	Chondrodysplasia	Simple autosomal recessive
Beagle	Chondrodysplasia punctata	Unknown
	Multiple epiphyseal dysplasia	Simple autosomal recessive
	Osteogenesis imperfecta	
Bulldog	Osteochondrodysplasia	Unknown
Bull Terrier	Osteochondrodysplasia	Unknown
Cocker Spaniel	Hypochondroplasia	Unknown
Great Pyrenees	Chondrodysplasia	Simple autosomal recessive
Irish Setter	Hypochondroplasia	Simple autosomal recessive
Labrador Retriever	Oculo-skeletal dysplasia	Simple autosomal recessive
Miniature Poodle	Achondroplasia	Simple autosomal recessive
	Epiphyseal chondrodysplasia	Unknown
	Multiple epiphyseal dysplasia	Unknown
Mixed breed dog	Mucopolysaccharidosis VII	Simple autosomal recessive
Norwegian Elkhound	Chondrodysplasia	Simple autosomal recessive
Plott Hound	Mucopolysaccharidosis I	Simple autosomal recessive
Samoyed	Oculo-skeletal dysplasia without haematological abnormalities	Simple autosomal recessive
	Oculo-skeletal dysplasia with haematological abnormalities	Unknown
Scottish Terrier	Achondroplasia	Unknown
	Idiopathic multifocal osteopathy	Unknown
Scottish Deerhound	Pseudoachondroplasia	Simple autosomal recessive

Anury and brachyury

Anury (absence of tail) has a high prevalence in Cairn Terriers, Cocker Spaniels, Doberman Pinschers, Rottweilers and Schipperkes (Pullig, 1953; Fritsch and Ost, 1983; Hall *et al.*, 1987). It appears to be a recessive trait in Cocker Spaniels and a hereditary aetiology in Cairn Terriers has been suggested (Pullig, 1953; Hall *et al.*, 1987). Brachyury (short tail) as a single autosomal dominant trait with reduced penetrance was reported in Beagles (Curtis *et al.*, 1964).

Dysostoses with predominant involvement of the extremities

Hemimelia

Hemimelia is the partial absence of a limb. The most common form of hemimelia in dogs is radial hemimelia (Jezyk, 1985). There are several case reports of canine radial hemimelia, however there is no evidence for a genetic aetiology (Pedersen, 1968; Lewis and Van Sickle, 1970; Ahalt and Bilbrey, 1997). An autosomal recessive form of bilateral pectoral limb hemimelia with involvement of multiple bones in Chihuahuas has been reported (Alonso *et al.*,

1982). Other authors have offered alternative interpretations of the anomalies in this Chihuahua syndrome (Jezyk, 1985; Schultz and Watson, 1995).

Polydactyly

Polydactyly is defined as the presence of one or more extra digits, either on the medial (preaxial polydactyly) or on the lateral (postaxial polydactyly) side of the extremity. An example of preaxial polydactylism is multiple dewclaws. In Great Pyrenees, preaxial polydactyly appears to be an autosomal dominant trait with variable expression (Jezyk, 1985). Preaxial pelvic limb polydactylism in Saint Bernards is an autosomal recessive trait. In this condition, the polydactylism may be associated with palate agenesis, anotia, an incomplete bifid tongue, and an extra thoracic vertebra and rib (Villagomez and Alonso, 1998). A similar syndrome has been described in Australian Shepherds (Freeman *et al.*, 1988; Sponenberg and Bowling, 1985). Male dogs affected with this syndrome express various characteristics including cleft palate, polydactyly, syndactyly, shortened tibia/fibula, brachygnatism and often scoliosis. Females are not as severely affected and lack the cleft palate, brachygnatism and scoliosis. The defect is lethal to males and it has been suggested that the syndrome is inherited as an X-linked lethal or as a sex-influenced autosomal trait.

Osteochondrodysplasias

Osteochondrodysplasias are dysmorphologies with a genetic aetiology, affecting multiple bones and these are summarized in Table 10.3. Osteochondrodysplasias may affect endochondral and/or intramembranous ossification. They develop during the late embryonic period, the fetal period (day 35–birth) and postnatally (Evans, 1979; Noden and de Lahunta, 1985). The human osteochondrodysplasias are subdivided into 32 major groups (Anon., 1983, 1992, 1998).

Achondrogenesis

Akitas affected with this disease are micromelic and short-trunked (Sande *et al.*, 1994). The mode of inheritance, biochemical mechanism, and genetic defect of this condition are unknown.

Achondroplasia

Achondroplasia in man is a disproportionate, short-limbed, rhizomelic (relatively short humerus and femur) dysplasia with a relatively large skull and depression of the nasal bridge (Rimoin, 1975). The trait is due to a mutation in the fibroblastic growth factor-receptor 3 (FGFR3) (Horton, 1997; Webster and Donoghue, 1997). This condition has also been described in Miniature Poodles and Scottish Terriers. In Miniature Poodles, achondroplasia is a simple resessive autosomal trait (Gardner, 1959; Amloff, 1961). However, because of pathological changes in the growth plate, this disease is probably a type of chondrodysplasia rather than achondroplasia (Jezyk, 1985). Biochemical

analysis of growth plate cartilage demonstrated undersulphation of chondroitin sulphate and a primary defect in the sulphation pathway or an increased activity of sulphatase enzymes was suggested (Bingel *et al.*, 1986). An FGFR3 mutation was not reported. There is also a report of achondroplasia in a litter of Scottish Terriers (Mather, 1956). This trait may represent hypochondroplasia because the skulls of the affected pups were normal (Jezyk, 1985). Boston Terriers, Bulldogs, Japanese Spaniels, Pekingese, Pugs and Shi Tzus have phenotypic characteristics of achondroplasia (rhizomelic limb shortening, depressed nasal bridge and shortened maxilla) (Jezyk, 1985). Growth plates from Dachshunds and Beagles have achondroplastic features (Hansen, 1952; Braund *et al.*, 1975). However, an FGFR3 mutation has not been described and German Shepherds, Bulldogs, Dachshunds and Basset Hounds have the same FGFR3 sequence (Martinez *et al.*, 1998).

Chondrodysplasia

Chondrodysplasias are characterized by disproportionate dwarfism and pathological changes in affected growth plates in both axial and appendicular skeleton. Chondrodysplasia in Alaskan Malamutes is associated with biochemical abnormalities in cartilage matrix (Fletch *et al.*, 1973, 1975). Affected growth plates contain type II collagen that is abnormally soluble in neutral salt solutions and contains significantly more proteoglycans with longer proteoglycan monomers, longer chondroitin sulphate side chains with increased amounts of chondroitin-6-sulphate (Bingel *et al.*, 1980, 1985). This dwarfism is a simple autosomal recessive trait with complete penetrance and variable phenotypic expression. Chondrodysplastic Great Pyrenees dogs and Norwegian Elkhounds are disproportionate and short-limbed (Bingel and Sande, 1982, 1994). In both breeds, the condition appears to be a simple autosomal recessive trait, but the biochemical defects and mutations are unknown.

Enchondrodystrophy

Enchondrodystrophy in English Pointers is a dwarfism affecting both bones and joints (Whitbread *et al.*, 1983; Lavelle, 1984). This trait is homozygous recessive. The underlying biochemical and genetic defect is unknown.

Hypochondroplasia

Hypochondroplasia in Cocker Spaniels and Irish Setters is a disproportionate dwarfism (Beachley and Graham, 1973; Hanssen, 1992; Hanssen *et al.*, 1998). In Irish Setters, this condition is transmitted as an autosomal recessive trait, whereas in Cocker Spaniels the mode of inheritance has not been reported. In humans, hypochondroplasia is subclassified in the achondroplasia group, and an FGFR3 mutation has been demonstrated (Webster and Donoghue, 1997). The biochemical and genetic defect in hypochondroplastic Irish Setters and Cocker Spaniels is not known.

Idiopathic multifocal osteopathy

This disease is characterized by multifocal progressive osteolytic lesions in skull, cervical spine, proximal radii, ulnae and femora of Scottish Terriers (Hay *et al.*, 1999). A genetic background is suspected because three of the four reported cases were related via a common male grandparent. The biochemical and genetic defect is unknown.

Mucopolysaccharidosis

The mucopolysaccharidoses (MPS) are a group of hereditary diseases characterized by a deficiency of a lysosomal enzyme required for glycosaminoglycan catabolism. The result of the defect is an accumulation and storage of intermediary metabolites in lysosomes (lysosomal storage disease) (Shull *et al.*, 1982). Two types of MPS in dogs have been described: MPS Type I (MPS-I) and MPS Type VII (MPS-VII). MPS-I was described in Plott Hounds (Shull *et al.*, 1982; Spellacy *et al.*, 1983). Affected pups have retarded growth, progressive lameness and visual difficulty, due to deficient α-L-iduronidase activity. The disease is characterized by a simple autosomal recessive inheritance. Canine MPS-VII has been diagnosed in mixed-breed dogs (Haskins *et al.*, 1984, 1991). Affected pups have retarded growth, a disproportionally large head, and corneal clouding. The biochemical defect is a β-glucuronidase deficiency and inheritance is simple autosomal recessive. α-L-Iduronidase or β-glucuronidase activity in cultured fibroblasts may be used to make a definitive diagnosis.

Multiple cartilaginous exostoses

This disease does not result in dwarfism. Lesions originate from the perichondrium of growth plates and consist of projections of cancellous bone covered with a cartilage cap (Milgram, 1983). Exostoses may cause pain, loss of function and neurological deficits, and may also become neoplastic. In man, multiple cartilagenous exostoses is caused by a mutation of the exostosin gene (Anon., 1998). This disease has been reported in several canine breeds and, although there are familial tendencies, the mode of inheritance is unknown (Gambardella *et al.*, 1975; Sande and Bingel, 1983).

Multiple enchondromatosis

Dogs suffering from this condition develop longitudinally oriented, oval radiolucencies (enchondromata) in long bones, vertebrae, ribs, sternum, and metacarpal and metatarsal bones (Matis *et al.*, 1989). Affected dogs may present with lameness, deformities, and pain on palpation of bones and joints. Enchondromata originate from the central portion of the growth plates (Milgram, 1983). A recessive mode of inheritance has been suggested in Miniature Poodles.

Multiple epiphyseal dysplasia

Affected Miniature Poodles and Beagles have a disproportionate, short-limbed dwarfism, are less active than their phenotypically normal littermates, and have an abnormal posture characterized by marked kyphosis and flexion of

joints with an abnormal gait (shuffle) (Cotchin and Dyce, 1956; Lodge, 1966; Rasmussen, 1971, 1972). In Beagles, the disease has a simple autosomal recessive mode of inheritance. In humans, multiple epiphyeal dysplasia is caused by a mutation in cartilage oligomeric matrix protein (COMP) or in the α-2 chain of Type IX collagen mutation (Briggs *et al.*, 1998; Horton, 1995). The biochemical and genetic defect of canine multiple epiphyseal dysplasia is not known.

Oculo-skeletal dysplasia

Oculo-skeletal dysplasia in Labrador Retrievers is a micromelic, disproportionate dwarfism with vision difficulties (Carrig *et al.*, 1977). Ocular changes are more severe than the skeletal abnormalities and include retinal dysplasia, retinal detachment and cataract formation (Carrig *et al.*, 1988). It has been suggested that the skeletal and ocular abnormalities are the result of a single recessive gene with recessive effects on the skeleton but with incomplete dominant effects on the eye (Carrig *et al.*, 1988).

Two oculo-skeletal dysplasias in Samoyeds have been described. The first is a disproportionate dwarfism, characterized by varus deformity of the elbows, valgus deformity of the carpi and a pelvic girdle that is higher than the thoracic girdle (Meyers *et al.*, 1983). Ocular changes include liquefaction of the vitreous and hyloid artery remnants, cataracts and retinal detachment. This dwarfism may be further classified as a metaphyseal dysplasia with a simple autosomal recessive mode of inheritance. The defect causing this oculo-skeletal dysplasia is not known. Recently, another oculo-skeletal dysplasia in Samoyeds was described (Aroch *et al.*, 1996). This condition is characterized by a marked eosinophilia with immature eosinophils and the absence of Barr bodies. Affected dogs did *not* have liquefaction of the vitreous and hyloid artery remnants. Thus, this oculo-skeletal dysplasia appears to be different from the oculo-skeletal dysplasia without haematological abnormalities.

Osteochondrodysplasia

An osteochondrodysplasia was reported in a litter of Bulldogs and it was suggested that a teratogenic aetiology was more likely than a genetic aetiology (Louw, 1983). The trait was reported recently in Bull Terrier littermates (Watson *et al.*, 1991). The condition may represent multiple enchondromatosis. The mode of inheritance is unknown.

Osteogenesis imperfecta

The underlying defect of osteogenesis imperfecta (OI) is synthesis of an abnormal α-1 or α-2 chain of type I collagen (COL1A1 and COL1A2, respectively). Affected dogs may present as a young pup or later on in life but always with generalized osteopenia and pathological fracture(s). Osteogenesis imperfecta in dogs has been reported in single cases (Beagle, Collie, German Shepherd, Golden Retriever, Great Dane, Standard Poodle) and in littermates (Collies, Standard Poodles, Norwegian Elkhounds) (Calkins *et al.*, 1956; Holmes and Price, 1957; Hoorens and de Sloovere, 1972; Campbell *et al.*, 1997). The mode

of inheritance is unknown. Osteogenesis imperfecta may be diagnosed by analysing type I collagen α chain migration isolated from cultured skin fibroblasts (Campbell *et al.*, 1997).

Pseudoachondroplasia
Pseudoachondroplastic Scottish Deerhound pups have disproportionate short limbs and present with exercise intolerance and retarded growth (Breur *et al.*, 1989, 1992). It appears to be an autosomal recessive trait. It is now known that human pseudoachondroplasia, similarly to multiple epiphyseal dysplasia, is due to a cartilage oligomeric matrix protein (COMP) mutation (Briggs *et al.*, 1998). A similar condition has been reported in Miniature Poodles (Riser *et al.*, 1980). The clinical signs of the affected pups were similar to the clinical signs of affected Scottish Deerhound pups. However, no pathognomonic chondrocytic inclusion bodies were reported. More recently, it was suggested that the underlying biochemical defect is a primary defect in the sulphation pathway or an increased activity of sulphatase enzymes (Bingel *et al.*, 1986).

Short spine syndrome
A short spine syndrome has been described in the Shiba Inu, an old Japanese breed (Suu, 1956; Suu and Ueshima, 1957, 1958; Ueshima, 1961; Hansen, 1968). Although heritable, the mode of inheritance is unknown.

Acquired Bone and Cartilage Diseases

Acquired bone and cartilage diseases are orthopaedic diseases which are not identifiable at birth and whose clinical signs develop during growth or after maturity. Almost all acquired bone and cartilage diseases with a genetic background have a multifactorial aetiology. Most of these diseases have strong gender and/or breed predispositions. Listed breed and gender predispositions are based on epidemiological or genetic studies (Table 10.4).

Fractures

It has been suggested that Italian Greyhounds, Poodles, Papillons, Whippets, Shetland Sheepdogs, Miniature Pinschers and Greyhounds are at increased risk for sustaining fractures (Ljunggren, 1971). These breeds, except Shetland Sheepdogs and Greyhounds, are also at increased risk for radius/ulna fractures but not for tibia/fibula fractures. Radius/ulna fractures in these breeds often occur after minimal trauma and their long legs and gracile long bones may be the cause of the increased risk. Healing of radius/ulna fractures in miniature breeds also can be delayed and inadequate callus formation, refractory healing, non-union and pseudarthrosis are not uncommon (Waters *et al.*, 1993; Larsen *et al.*, 1999). Recently, it was suggested that a decreased vascularization

of the distal radius may be the cause for post-operative complications of radius/ulna fractures in miniature breeds (Welch *et al.*, 1997).

Risk factors for humeral condylar fractures in dogs have been reported in several breeds (Rorvik, 1993) with an increased incidence of medial, lateral, or Y humeral condylar fracture in older spaniels (Piermattei and Flo, 1997). Male Cocker Spaniels are at increased risk and an association between the high prevalence of humeral condyle fractures in Cocker and Brittany Spaniels and incomplete ossification of the humeral condyles was suggested (Marcellin-Little *et al.*, 1994). This condition may have a recessive mode of inheritance.

Developmental orthopaedic diseases

Diseases in this group are often breed-related, have a consistent age of onset, and a consistent clinical course. Most of these diseases are multifactorial or complex, and genetic, nutritional and environmental factors have all been implicated in their aetiology. Developmental orthopaedic diseases (DODs) may affect bones (hypertrophic osteodystrophy, craniomandibular osteopathy, and panosteitis) or joints (hip dysplasia, elbow dysplasia, osteochondrosis, Legg–Calvé–Perthes disease, and patella luxation).

Developmental orthopaedic diseases affecting bones

Craniomandibular osteopathy (CMO)

This is a non-neoplastic proliferative disorder affecting mandible and/or tympanic bullae of young growing dogs. Male and females are equally affected and West Highland White Terriers, Scottish Terriers and Cairn Terriers are at increased risk (LaFond *et al.*, 1998; Munjar *et al.*, 1998). An autosomal recessive mode of inheritance was demonstrated in West Highland White Terriers (Padgett and Mostosky, 1986).

Hypertrophic osteodystrophy (HOD)

HOD is a painful disease of young growing animals affecting the metaphysis of long bones (Piermattei and Flo, 1997). Affected animals may have varying degrees of lameness, pyrexia, depression, weight loss and anorexia. Both genetic and infectious aetiological factors have been suggested (Munjar *et al.*, 1998) and a nutritional component cannot be ruled out. Males are more commonly affected than females and Great Danes, Chesapeake Bay Retrievers, Irish Setters and Weimaraners are most at risk (LaFond *et al.*, 1998; Munjar *et al.*, 1998).

Panosteitis

Panosteitis is a painful condition of long bones characterized by a shifting lameness. The pectoral long bones are more commonly affected than the pelvic long bones (Van Sickle, 1975; Piermattei and Flo, 1997). It has been

suggested that the disease commences with a proliferation of stromal cells in the region of the nutrient foramen, followed by congestion in the vascular sinusoids and endosteal and periosteal bone formation (Van Sickle, 1975). Others have suggested a hormonal-induced inhibition of medullary resorption (Hazewinkel, 1993). Both genetic and non-genetic aetiologies have been

Table 10.4. Breed predilections for selected developmental orthopaedic diseases (DODs)

Trait	Breeds at increased risk
DODs affecting bones	
Cranio-mandibular osteopathy	Cairn Terrier, Scottish Terrier, West Highland White Terrier
Hypertrophic osteodystrophy	Boxer, Chesapeake Bay Retriever, German Shepherd Dog, Golden Retriever, Great Dane, Irish Setter, Labrador Retriever, Weimaraner
Panosteitis	Afghan Hound, Akita, American Cocker Spaniel, American Staffordshire Terrier, Basset Hound, Bearded Collie, Bernese Mountain Dog, Boxer, Bull Terrier, Bulldog, Chesapeake Bay Retriever, Chow Chow, Dalmatian, Doberman Pinscher, English Setter, English Springer Spaniel, Giant Schnauzer, German Shepherd, German Short-haired Pointer, Golden Retriever, Great Dane, Great Pyrenees, Irish Wolfhound, Labrador Retriever, Mastiff, Neapolitan Mastiff, Newfoundland, Rhodesian Ridgeback, Rottweiler, Saint Bernard, Shar Pei, Shih Tzu, Weimaraner, West Highland White Terrier
DODs affecting joints	
Osteochondritis dissecans (OCD) shoulder	Bernese Mountain Dog, Border Collie, Bouvier, Boxer, Bull Mastiff, Chesapeake Bay Retriever, Dalmatian, English Setter, German Short-haired Pointer, German Shepherd, German Wire-haired Pointer, Golden Retriever, Great Dane, Great Pyrenees, Irish Wolfhound, Kuvasz, Labrador Retriever, Mastiff, Munsterland, Newfoundland, Old English Sheepdog, Rottweiler, Saint Bernard, Standard Poodle
OCD elbow	Chow Chow, German Shepherd, Golden Retriever, Great Dane, Labrador Retriever, Newfoundland, Rottweiler
Fragmented coronoid process	Basset Hound, Bernese Mountain Dog, Bouvier, Bull Mastiff, Chow Chow, German Shepherd, Golden Retriever, Gordon Setter, Irish Wolfhound, Labrador Retriever, Mastiff, Newfoundland, Rottweiler, Saint Bernard
Ununited anconeal process	Basset Hound, Bernese Mountain Dog, Chow Chow, English Setter, German Shepherd, Golden Retriever, Labrador Retriever, Mastiff, Newfoundland, Pomeranian, Rottweiler, Saint Bernard, Shar Pei
Hip dysplasia	Airedale, Alaskan Malamute, Bearded Collie, Bernese Mountain Dog, Bloodhound, Border Collie, Bouvier, Briard, Brittany Spaniel, Bulldog, Bull Mastiff, Chesapeake Bay Retriever, Chow Chow, English Springer Spaniel, German Shepherd, German Wire-haired Pointer, Giant Schnauzer, Golden Retriever, Gordon Setter, Great Dane, Great Pyrenees, Keeshond, Kuvasz, Labrador Retriever, Mastiff, Neapolitan Mastiff, Newfoundland, Norwegian Elkhound, Old English Sheepdog, Pointer, Portuguese Water Dog, Rottweiler, Saint Bernard, Samoyed, Tree Walking Coonhound
Legg-Calvé-Perthes disease	Australian Shepherd, Cairn Terrier, Chihuahua, Dachshund, Lhasa Apso, Manchester Terrier, Miniature Pinscher, Pug, Toy Poodle, West Highland White Terrier, Yorkshire Terrier
OCD stifle joint	Boxer, Bulldog, German Shepherd, Golden Retriever, Great Dane, Irish Wolfhound, Labrador Retriever, Mastiff, Rottweiler
Patella luxation (medial and lateral)	Akita, American Cocker Spaniel, Australian Terrier, Basset Hound, Bichon Frise, Boston Terrier, Bulldog, Cairn Terrier, Cavalier King Charles Spaniel, Chihuahua, Chow Chow, Flat-coated Retriever, Great Pyrenees, Japanese Chin, Keeshond, Lhasa Apso, Maltese, Miniature Pinscher, Miniature Poodle, Papillon, Pekinese, Pomeranian, Pug, Shar Pei, Shih Tzu, Silky Terrier, Standard Poodle, Toy Fox Terrier, Toy Poodle, West Highland White Terrier, Wirehaired Fox Terrier, Yorkshire Terrier
OCD talocrural joint	Labrador Retriever, Rottweiler, Bull Mastiff

suggested. Male dogs may be at risk and many breeds are predisposed (see Table 10.4; LaFond *et al.*, 1998). This finding would support the notion of a genetic aetiological component. The heritability of the disease was reported as 0.13 (Lindstedt *et al.*, 1986).

Developmental orthopaedic diseases affecting joints – not including canine hip dysplasia

Osteochondrosis dissecans (OCD) of the shoulder joint
Shoulder osteochondrosis is a local mineralization defect of the articular epiphyseal complex of the proximal humerus (Olsson, 1993). Affected dogs have shoulder pain and lameness. Genetics, rapid growth, nutritional imbalance and trauma have been mentioned as possible causes. Males are more commonly affected than females and many breeds are predisposed (see Table 10.4; Slater, 1991; LaFond *et al.*, 1998). A polygenic mode of inheritance has been suggested.

Elbow dysplasia
Elbow dysplasia includes OCD of the medial humeral condyle (OCD), fragmented medial coronoid process (FCP), ununited anconeal process (UAP) and elbow incongruity. It has been suggested that these diseases are all caused by an asynchronous growth of the radius and ulna and that the proximal ulna in breeds at risk for elbow dysplasia is relatively large (Wind, 1986; Wind and Packard, 1986; Olsson, 1993; Sjostrom *et al.*, 1995). The heritability for osteoarthrosis secondary to elbow dysplasia (OCD and/or FCP) in Labrador Retrievers is 27% (Studdert *et al.*, 1991). A similar study in Rottweilers and Bernese Mountain Dogs demonstrated a heritability between 10 and 45% and between 28 and 40% for OCD and FCP, respectively, for each breed (Grondalen and Lingaas, 1991; Swenson *et al.*, 1997b; Ubbink *et al.*, 1999).

The aetiology of *elbow incongruity* is unknown but genetic and nutritional factors have been implicated. A relatively long radius (radial overgrowth) may precipitate UAP, whereas a relatively long ulna (ulnar overgrowth) is associated with FCP and OCD (Wind, 1986; Olsson, 1993). In Bernese Mountain Dogs, elbow incongruity may be inherited independently from FCP (Ubbink *et al.*, 1999). A heritable form of elbow incongruity secondary to premature closure of the distal ulnar growth plate has been reported in Skye Terriers (Lau, 1977) as an autosomal recessive trait.

Osteochondritis dissecans (OCD) of the medial humeral trochlear ridge is caused by a failure of endochondral ossification of the epicondylar articular-epiphyseal complex. It may be due to ulnar overgrowth and elbow incongruity, placing an abnormal load on the medial humeral trochlear ridge (Wind, 1986; Olsson, 1993). Nutritional imbalance, rapid growth, and genetics have been implicated (Olsson, 1993). Male dogs and several breeds are at increased risk (Table 10.4; LaFond *et al.*, 1998). The condition has a polygenic mode of inheritance.

Dogs with *FCP* present with a swollen elbow and lameness. The lesion may be seen on radiographs but usually the secondary osteoarthritic changes and bony sclerosis are the only visible indirect evidence of the disease. This condition may be bilateral. FCP has been classified as an osteochondrosis with multifactorial aetiology (Olsson, 1993). This condition may be due to ulnar overgrowth with abnormal loading of the medial coronoid process (Wind, 1986; Olsson, 1993). Males are at risk and many breeds are predisposed (Table 10.4). Almost all breeds predisposed to OCD of the medial humeral trochlear ridge also appear to be predisposed to FCP (LaFond *et al.*, 1998). However, several breeds are predisposed to FCP only (see Table 10.4). This may support the notion that OCD of the medial humeral trochlear ridge and FCP are inherited independently (Padgett *et al.*, 1995). The trait has a polygenic mode of inheritance.

Ununited anconeal process is the result of a failure of the anconeal process to unite with the olecranon. Affected dogs have a swollen, painful elbow and lameness. The condition can be bilateral (Olsson, 1993). Many breeds do not have a separate anconeal ossification centre and, thus, do not develop UAP (Piermattei and Flo, 1997). Only selected larger breed dogs do have a separate ossification centre. The development of UAP in dogs with a separate anconeal ossification centre and growth plate may be caused by radial overgrowth and elbow incongruity. This results in increased pressure against the anconeal process and failure of the anconeal process to unite with the ulna (Wind, 1986; Olsson, 1993; Sjostrom *et al.*, 1995). Males are more commonly affected than females and several breeds are predisposed (see Table 10.4; LaFond *et al.*, 1998). A polygenic mode of inheritance is suspected.

Osteochondrosis dissecans (OCD) of other joints

Animals affected with osteochondrosis of the lateral or medial femoral condyle present with a swollen, painful stifle joint and a pelvic limb lameness. Nutritional imbalance, rapid growth, trauma and genetics may be aetiological agents (Olsson, 1993). Males are more frequently affected than females and Labrador Retrievers, German Shepherds and Rottweilers appear to be at highest risk (Slater, 1991; LaFond *et al.*, 1998; Table 10.4). *Talocrural joint OCD* on the medial and lateral trochlear ridge of the talus results in a swollen, painful talocrural joint and affected dogs may present with lameness. The trait can be bilateral and is more common in males. Labrador Retrievers, Rottweiler and Bull Mastiffs are at increased risk (Slater, 1991; LaFond *et al.*, 1998).

Legg–Calvé–Perthes disease (avascular necrosis of the femoral head)

This is a non-inflammatory aseptic necrosis with subsequent deformation of the femoral head and neck resulting in pelvic limb lameness. Ischaemia due to vascular compression and precocious sex hormone activity have been proposed as possible aetiologies (Piermattei and Flo, 1997). Males are more commonly affected and toy breeds and Terriers are predisposed (see Table 10.4; LaFond *et al.*, 1998). In Miniature Poodles and West Highland White Terriers the disease is a simple autosomal recessive trait (Pidduck and Webbon,

1978; Wallin, 1986). In Manchester Terriers, the trait is simple autosomal recessive or multifactorial with a high heritability (Vasseur *et al.*, 1989).

Patella luxation

Patella luxation is common and may occur in lateral or medial direction. Affected dogs may be asymptomatic or present with a lameness. The aetiology is unknown but morphological changes in the hip may precipitate the disease (Putnam, 1968; Piermattei and Flo, 1997). Toy breeds seem to be at particular risk (Table 10.4; Priester, 1972; Hayes, 1994; LaFond *et al.*, 1998). The mode of inheritance is unknown.

Neoplasia

Giant breed (Irish Wolfhound, Saint Bernard and Great Dane) and large breed (i.e. Rottweiler, Irish Setter, Doberman Pinscher) dogs are at increased risk for osteosarcoma whereas breeds of smaller size (i.e. Shetland Sheepdog, Miniature Schnauzer, Miniature Poodle) are at decreased risk (Ru *et al.*, 1998). Patterns of familial aggregation of osteosarcoma for Saint Bernards have been identified (Bech Nielsen *et al.*, 1978). As in humans, a high rate of bone growth may be important in the development of osteosarcoma (Ru *et al.*, 1998).

Acknowledgements

The authors thank Greg Acland, Stuart Bliss, Elaine Ostrander, and George Casella in outcrossed pedigree development, marker heterozygosity testing, and strategies for linkage analysis of CHD. Our thanks go to the Morris Animal Foundation, Ralston Purina, Consolidated Research Grant Program at Cornell, Van Sloan Foundation, 'Gussy' Gruver Foundation, Cornell Veterinary Alumni and National Institutes of Health.

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Cytogenetics and Physical Chromosome Maps

11

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Introduction

A fundamental prerequisite for the development of a comprehensive and effective genome map for an organism is the ability to demonstrate that all chromosomes are represented in such a map. This requirement necessitates

that all chromosomes comprising the karyotype of the organism under investigation can be identified as separate components and thus requires that the organism has been studied at the cytogenetic level. The term 'physical chromosome map' refers to the description of genetic markers that have been located on the chromosomes of an organism, using one of a variety of physical mapping techniques. Depending on the approach used, the location of such markers may either be described by their assignment to a whole chromosome or, more precisely, by regional assignment to a cytogenetically visible chromosome band. This chapter will provide an historical summary of the conventional and molecular cytogenetic work that has been conducted on the domestic dog (*Canis familiaris*), leading to standardized chromosome nomenclature. This will be followed by a brief overview of the current knowledge of nucleolar organizer regions and the distribution of constitutive heterochromatin in the dog karyotype and a summary of the meiotic studies that have been performed. We will summarize the application of molecular cytogenetic approaches to gene mapping studies in the dog and provide an overview of chromosome abnormalities identified by the application of conventional and molecular cytogenetic approaches.

Dog Chromosomes – an Historical Perspective

For many years, cytogenetics of the domestic dog was not intensively explored. This was largely a consequence of the difficulty of chromosome identification, due to the presence of a high diploid chromosome number ($2n = 78$), combined with the similarity in size and banding patterns of many of the smaller autosomes.

The chromosome number of the dog was first determined from studies of meiotic cells by Minouchi (1928) and later confirmed using cultured lymphocytes by Gustavsson (1964). The diploid karyotype is comprised of 38 pairs of acrocentric autosomes, a large sub-metacentric X chromosome and a small metacentric Y chromosome (Fig. 11.1a, b). Conventional Giemsa staining of the chromosomes allows precise identification of only the sex chromosomes, due to their size and morphology, and also chromosome pair number 1, by virtue of its size. Since the remaining autosomes gradually decrease in size, reliable recognition of conventionally stained homologous pairs is an impossible task.

The development of chromosome banding techniques in the early 1970s provided the ability to identify homologous pairs and therefore an opportunity to establish a standard karyotype. Selden *et al.* (1975) presented the first GTG-banded karyotype of the dog, followed by a number of attempts over the subsequent 25 years to produce reliable, complete karyotypes using a variety of conventional banding techniques. Additional GTG banded karyotypes have been presented by Manolache *et al.* (1976), Stone *et al.* (1991a), Graphodatsky *et al.* (1995), Reimann *et al.* (1996) and Graphodatsky *et al.* (2000). R-banded karyotypes have been produced by Howard-Peebles and Pryor (1980), Mayr

et al. (1986), Poulsen *et al.* (1990) and Moreno-Millan *et al.* (1991). There are also reports on karyotypes produced using QFQ banding (Pienkowska and Switonski, 1998) and DAPI banding (Langford *et al.*, 1996; Breen *et al.*, 1999a) of dog chromosomes. Both QFQ and DAPI banding are fluorochrome-based techniques that reveal chromosome banding patterns similar to that of G-banding. There has also been a single report describing the karyotype of the dog prepared by image analysis methodology (Christian *et al.*, 1998).

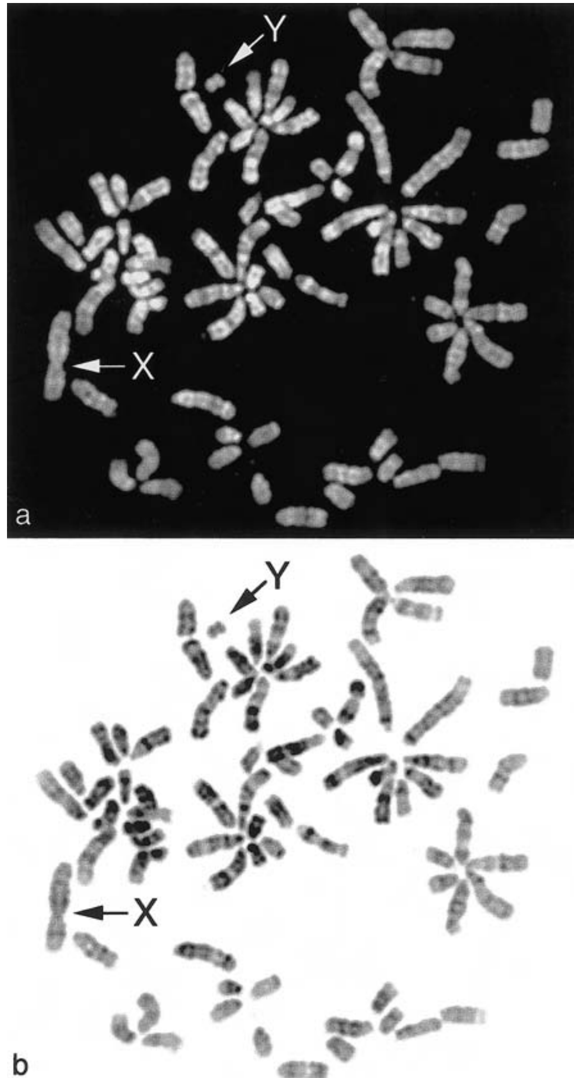

Fig. 11.1. Metaphase chromosomes of the dog. (a) DAPI stained metaphase preparation. (b) inverted DAPI stained metaphase preparation, revealing banding similar to that of conventional GTG banding. The X and Y chromosomes are indicated.

Standardization of the dog karyotype – the classical approach

During the First Domestic Animal Standardization Conference, held at Reading University, UK, in 1976 (Ford *et al.*, 1980), the karyotype of the dog was not discussed, in spite of the report by Selden *et al.* (1975) the previous year. As a consequence, an international standard of the dog karyotype was not established at that time. In 1994, during the 11th European Colloquium on Cytogenetics of Domestic Animals, held in Copenhagen, a committee was established to develop an internationally accepted nomenclature for the dog karyotype (Switonski *et al.*, 1994). The Committee was able to establish only a partial G-banded standard karyotype that included the largest 21 autosomal chromosome pairs and the sex chromosomes (Switonski *et al.*, 1996; Fig. 11.2), numbered according to the system proposed by Selden *et al.* (1975). A further advance towards a description of the entire G-banded dog karyotype was made by Reimann *et al.* (1996) who were able to correctly orientate all the chromosomes by the use of a centromeric repeat probe. These authors were in agreement with the Committee for chromosomes 1–21 and also described an extended nomenclature of the canine karyotype for the remaining 17 chromosomes, based upon the nomenclature of Selden *et al.* (1975) and proposed a revised GTG-banded ideogram at the 460-band level.

Standardization of the dog karyotype – the molecular approach

It was concluded by the Committee for the Standardization of the Dog Karyotype that a complete standard karyotype would require the use of molecular cytogenetic reagents, based upon the application of fluorescence *in situ* hybridization (FISH) techniques, described below. Such reagents included whole chromosome paints (WCP) and single locus probes (SLP). The Committee members independently applied chromosome paints (described by Langford *et al.*, 1996), representing the smallest 17 pairs of autosomes, to metaphase preparations. In May 1997, the Committee met to discuss the findings and concluded that all the chromosomes could be identified with the aid of the painting reagents of Langford *et al.* (1996) and that the identity of each chromosome could be correlated with the nomenclature of Switonski *et al.* (1996) for the larger autosomes (CFA1–21) and with that of Reimann *et al.* (1996) for the smaller autosomes (CFA22–38). These recommendations were presented at the 13th European Colloquium on Cytogenetics of Domestic Animals, Budapest (Breen *et al.*, 1998). The chromosome numbering recommended by the Committee was endorsed by the International Society of Animal Genetics (ISAG) DogMap workshop held in Minneapolis, July 2000 (G. Dolf, personal communication). Although laboratories still use conventional GTG-banding for chromosome identification, an increasing number of laboratories are making use of cooled CCD (chip charge-coupled devices) cameras and sophisticated software for FISH mapping. Consequently, there is a major trend towards the use of fluorochrome-based banding techniques (e.g. DAPI

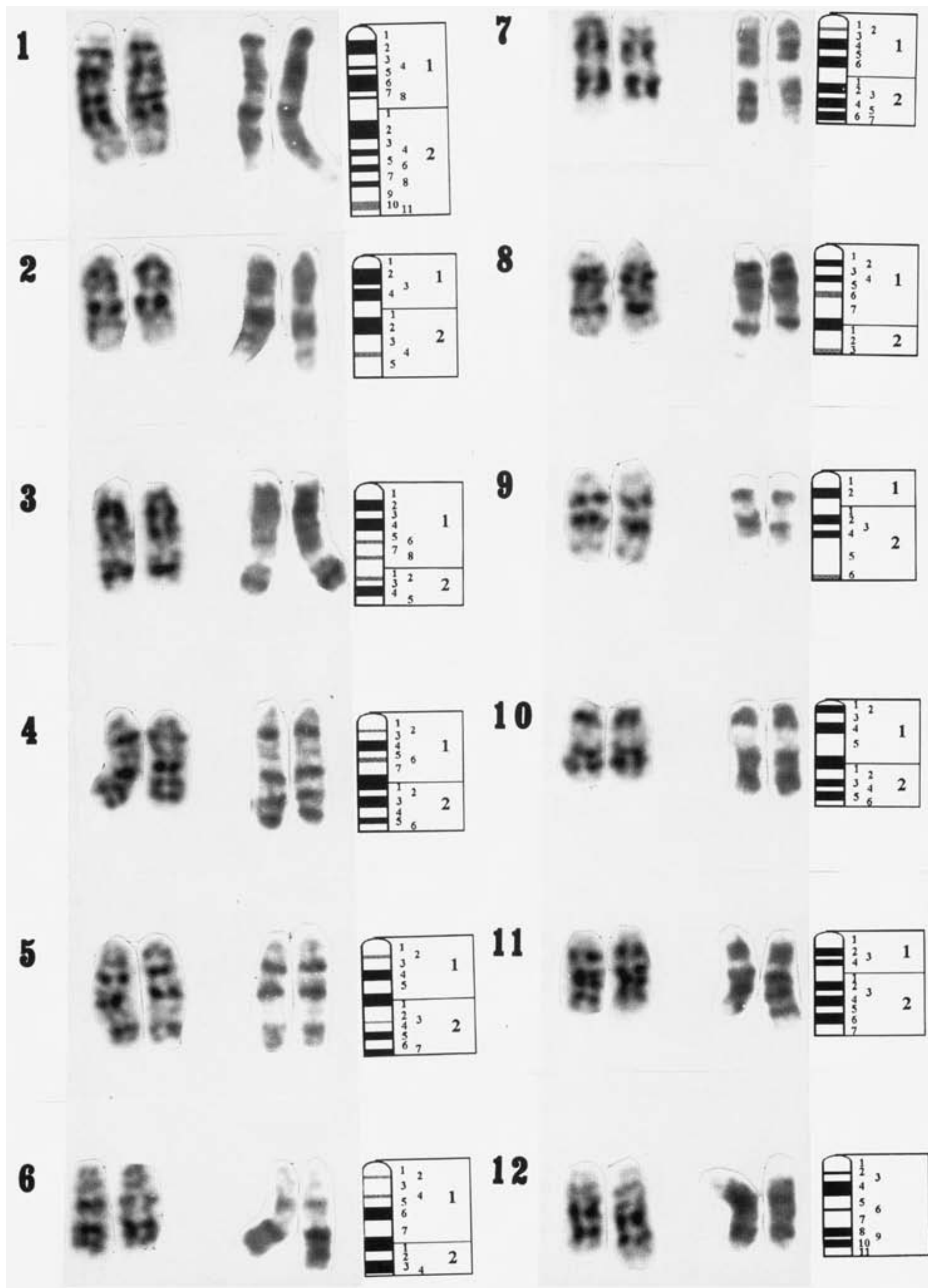

Fig. 11.2. Standard partial G-banded dog karyotype, comprising the 21 largest pairs of autosomes and the sex chromosomes of the 39 total chromosome pairs. The chromosomes were cut out from two representative metaphases. Schematic representation of the distribution of G-bands is shown (Switonski *et al*., 1996).

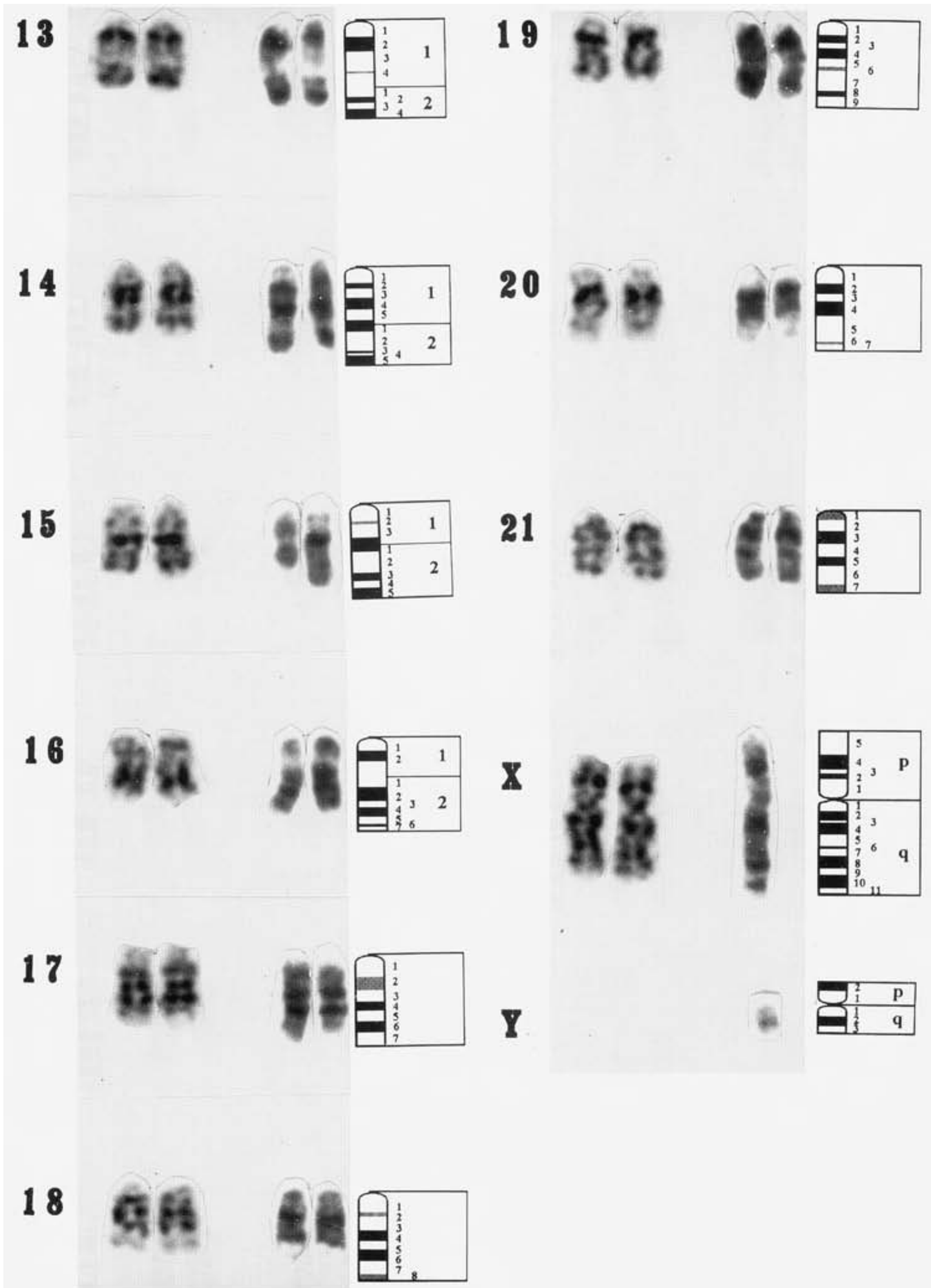

Fig. 11.2. *Continued*.

(DNA binding AT-specific 4′-6-diamidino-2-phenylindole) banding) to facilitate concurrent chromosome identification during FISH analyses in a range of species. Using sequential chromosome painting on single metaphases (using the same paints as were used by the International Committee), Breen *et al.* (1999a), were able to generate a series of DAPI banded karyotypes of the dog in which the numbering of the chromosomes exactly followed that produced by the Committee. In order to facilitate the accurate assignment of FISH mapped loci to all chromosomes comprising the karyotype, Breen *et al.* (1999a) also proposed a 460-band ideogram based on DAPI banding with five grey levels (Fig. 11.3).

Simultaneously, a comprehensive set of chromosome specific single locus FISH probes has been developed by Breen *et al.* (1999b; submitted) which have been made available for the verification of chromosome identity and for the integration of meiotic and radiation hybrid linkage maps. Independently, another study was performed by Yang *et al.* (1999) who applied a different set of dog chromosome-specific painting probes. In this study a comparative chromosome painting approach (dog vs. red fox vs. human chromosomes) was applied in order to indirectly identify the dog chromosomes. The authors attempted to follow the nomenclature of Switonski *et al.* (1996) and Reimann *et al.* (1996) and presented a DAPI banding pattern for the whole karyotype with a black and white ideogram. However, it was indicated by Graphodatsky *et al.* (2000) that there were ten differences in chromosome nomenclature when compared to the paper of Breen *et al.* (1999c) and hence the recommendations of the International Committee.

In conclusion, the standardization of the first 21 autosomes of the dog karyotype was achieved using conventional cytogenetics (Switonski *et al.*, 1996), whilst reliable identification of the remaining 17 pairs of autosomes initially required the use of molecular cytogenetic reagents. There is now an internationally accepted numbering of all 38 autosomes of the dog (Breen *et al.*, 1998, 1999a), endorsed by the ISAG DogMap Workshop. A panel of chromosome-specific single locus probes is now available for each chromosome of the dog to allow verification of chromosome identity where necessary.

Nucleolar Organizer Regions

Nucleolar organizer regions (NORs) consist of genes coding for the 5.8S, 18S and 28S rRNA genes. The NORs can be visualized using silver staining or FISH. When chromosome slides are stained with silver nitrate only active NORs are revealed. Application of FISH facilitates the identification of all NORs (both active and inactive) present in a karyotype. Localization studies of the NORs in the dog karyotype have been performed by several authors, leading to controversy concerning the number of the NORs. The first report, by Pathak *et al.* (1982), showing NORs on the Y chromosome and three pairs of autosomes was finally confirmed by FISH study (Makinen *et al.*, 1997; Pienkowska and Switonski, 1998). The later study demonstrated that NORs are

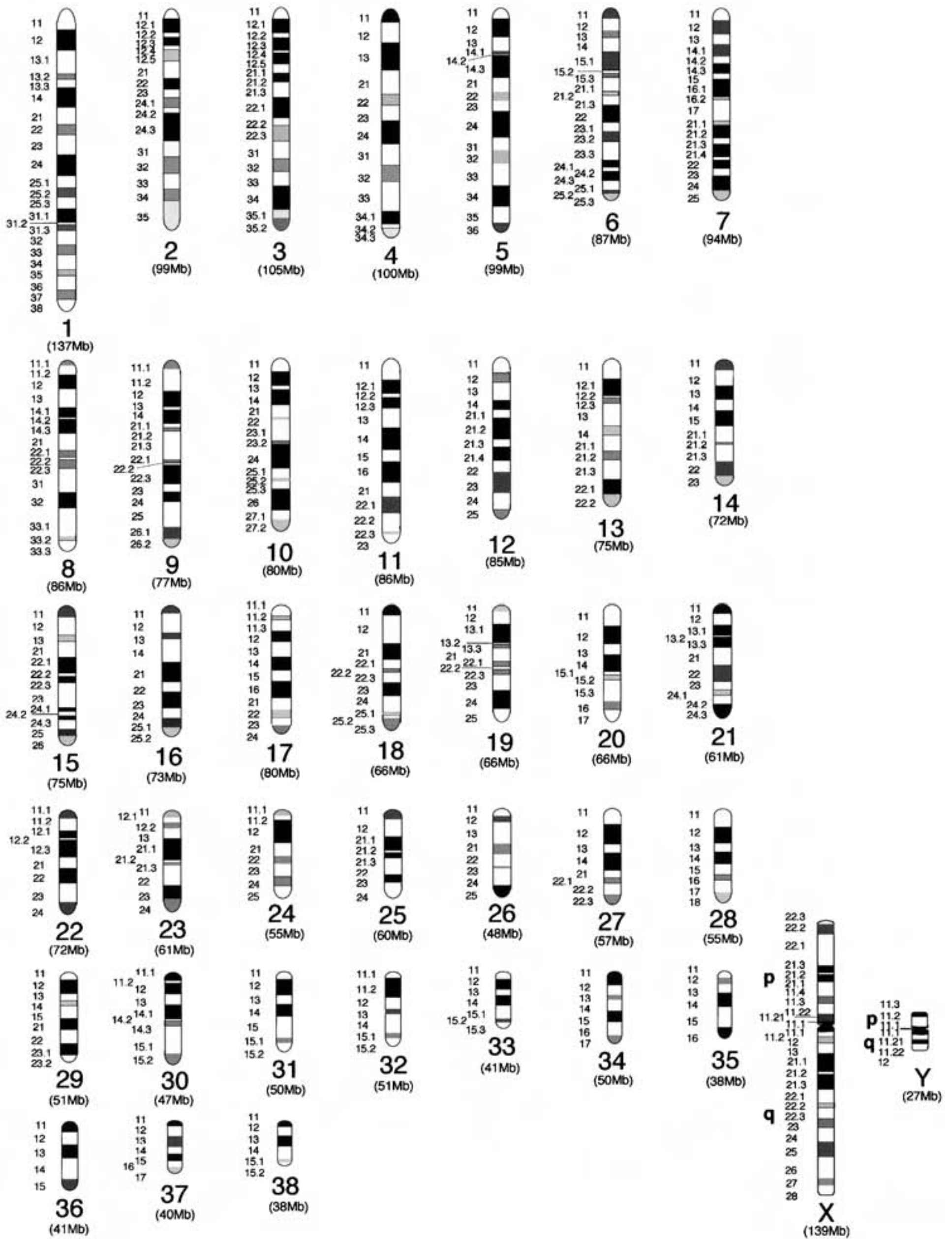

Fig. 11.3. DAPI banded ideogram of the dog at the 460 band resolution, with chromosomes numbered according to the recommendations of the International Committee for the Standardization of the Karyotype of the Dog (Switonski *et al.*, 1996; Breen *et al.*, 1998). The size of each chromosome in megabase pairs is given in parentheses and is based on the data of Langford *et al.* (1996).

located terminally on the long arms of two recognized autosomes (7, 17) and an unidentified small chromosome, which was not included in the standard at that time. Moreover, the NOR resides on the Yqter chromosome. In addition, localization of a gene cluster for 5S rRNA was also observed on chromosome 4q1.4 (Makinen *et al.*, 1997).

Intra- and inter-individual variability in the number of silvered NORs is a well known phenomenon in mammals. In a study carried out on 27 dogs it was shown that an average number of silvered NORs in females was 5.3 and 6.0 in males (Pienkowska and Switonski, 1998). Moreover, inter-individual range of the average number of NORs, namely 4.2–6.0 in females and 4.8–7.0 in males, was also noticed.

Constitutive heterochromatin (C-banding)

The amount of observed constitutive heterochromatin is rather small in the karyotype of the dog (Pathak and Wurster-Hill, 1977; Pathak *et al.*, 1982). There are several autosome pairs showing a darkly stained centromeric area. On the X chromosome the proximal half of the q arm is stained and within this fragment two C-bands can be recognized, one in the centromeric area and another one located interstitially on the q arm. The Y chromosome demonstrates a darkly stained centromeric area and lightly stained q arm.

Meiotic chromosomes

The duration of male meiosis and spermiogenesis, estimated by the use of autoradiography, is approximately 42 days (Ghosal *et al.*, 1983). Meiotic chromosomes are usually visualized by conventional Giemsa staining and/or synaptonemal complex technique. Giemsa staining facilitates the observation of bivalent formation at diakinesis/metaphase I and the study of chromosome segregation at anaphase I, using analysis of chromosome complement at metaphase II (Figs 11.4 and 11.5). Eliasson *et al.* (1968) showed an appearance of autosome and sex bivalents at metaphase I and a chromosome set at metaphase II in the dog spermatocytes. They revealed that among spermatocytes, originating from two males, some cells (*c.* 5%) at metaphase I and metaphase II were polyploid. Similar results (Figs 11.4b and 11.5b) were observed by Switonski (2000; unpublished).

The first report on synaptonemal complexes in the dog spermatocytes was published by Pathak *et al.* (1982). Studies carried out on primary oocytes revealed that these structures occur in postnatal ovaries, which is rather exceptional among mammals (Freixa *et al.*, 1987; Lechniak *et al.*, 1997).

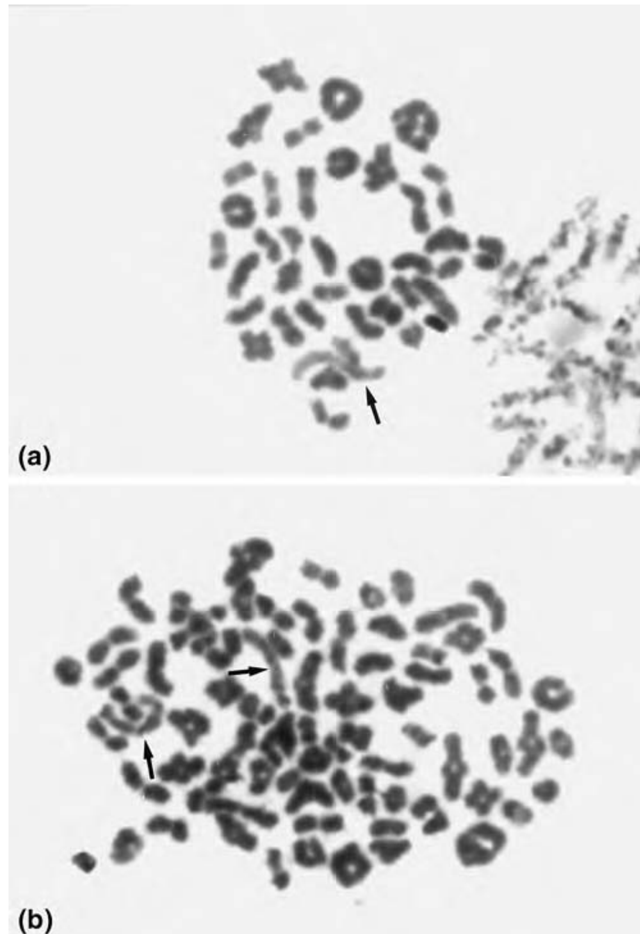

Fig. 11.4. Meiotic chromosomes, conventionally stained with Giemsa, of the male dog: (a) diploid metaphase I with *n* bivalents; (b) tetraploid metaphase I with *2n* bivalents. Sex bivalents are indicated by arrows.

Gene Mapping in Dogs

Over the past decade, gene mapping activity for the domestic dog has shown a dramatic increase. This is largely due to the increasing realization that the dog is a highly suitable model organism for several human diseases. As with other domestic animals, gene mapping studies include the application of somatic cell hybrid analysis, radiation hybrid analysis, meiotic linkage analysis and FISH studies. In this chapter we will restrict the discussion to the way in which FISH has played a key role in the development of the emerging genome map of the dog.

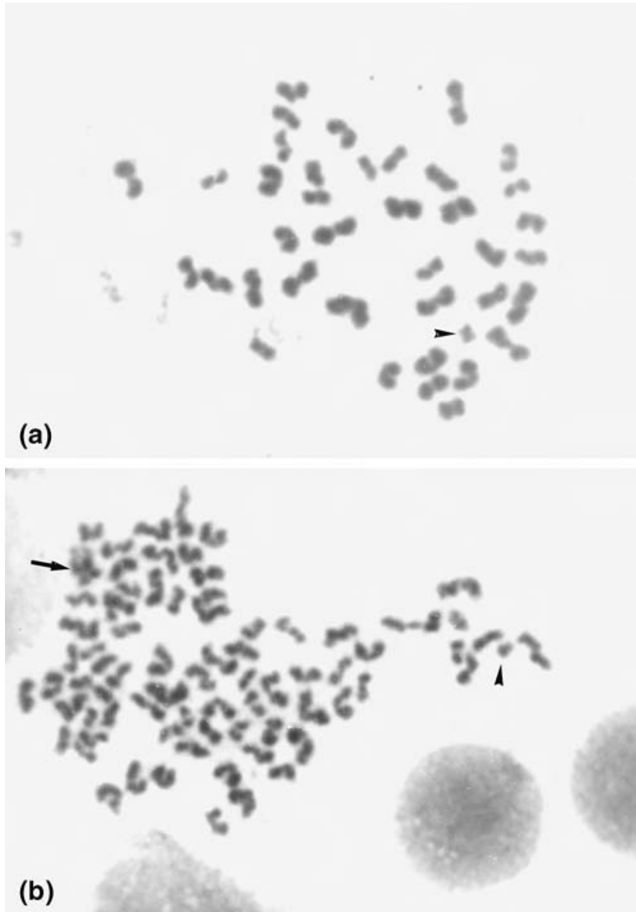

Fig. 11.5. Meiotic chromosomes, conventionally stained with Giemsa, of the male dog: (a) haploid metaphase II, the Y chromosome (arrowhead) is indicated; (b) unreduced, diploid metaphase II. The X (arrow) and presumably Y (arrowhead) chromosomes are indicated.

Fluorescence in situ *hybridization (FISH)*

The development and application of FISH over the past two decades has enabled major progress to be made in the physical mapping of dog genes and anonymous markers. The application of FISH technology, in combination with the increasing accessibility of sophisticated image capture and analysis software, has facilitated significant advances in genome mapping of domestic animals, including the dog. FISH applications in the dog now include chromosome painting, the assignment of single locus probes (SLP) in either single or multi-colours and comparative genomic hybridization (CGH) analysis of dog cancers.

In FISH, the probe DNA is either labelled directly or indirectly. For direct labelling the probe is usually labelled with a fluorochrome conjugated

nucleotide (e.g. fluorescein-isothiocyanate conjugated dUTP (FITC-dUTP), rhodamine conjugated dUTP, Cy3-dUTP, etc.) and, since the probe is itself fluorescent, the probe–target complex is immediately visible following hybridization to the chromosome template. In indirect labelling, the probe is labelled with a hapten (such as biotin-16-dUTP or digoxygenin-11-dUTP), which is not a fluorescent molecule, but has an affinity for fluorochrome-conjugated antibodies, or reporter molecules. For indirectly labelled probes, visualization of the hybridization sites first requires that they be detected with one of a variety of methods that attach fluorochromes to the hapten(s) in the probe–target complex. In most cases the probe DNA is usually a cloned piece of genomic DNA, ranging in size from a few kilobases to hundreds of kilobases. Generally, the size of the resulting FISH signal corresponds to the length of the probe and the amount of probe used. Figure 11.6a (see Frontispiece) illustrates a typical result obtained from the hybridization of a plasmid clone containing a 4 kb genomic DNA insert. The FISH signal resulting from the hybridization of such a small probe/target complex is reflected by the small size of the green spots. Figure 11.6b illustrates the use of dual-colour FISH with probes of two different sizes, hybridized to the same dog chromosome. The red signal results from the hybridization of a bacteriophage clone (insert size of *c.* 15 kb), whilst the much larger green signal results from the hybridization of a bacterial artificial chromosome (BAC) clone with an genomic DNA insert of approximately 150 kb. The development of improved imaging equipment, combined with the available fluorochromes, has allowed the development of multicolour FISH, which allows the simultaneous visualization of a number of probes, as illustrated in Fig. 11.6c.

Few type II (microsatellite) markers have thus far been precisely mapped, by FISH, to dog chromosomes (e.g. Fischer *et al.*, 1996; Dolf *et al.*, 1997; Thomas *et al.*, 1997; Breen *et al.*, 1999b, 2001; Yang *et al.*, 2000). The number of Type I markers (genes) that have been assigned to a chromosome by FISH is increasing, and a summary of those genes is presented in Table 11.1. In earlier reports the probes used for FISH localization were either from bacteriophage or cosmid genomic DNA libraries. However, the development of a BAC genomic DNA library for the dog by Li *et al.* (1999) has seen a trend toward the isolation of larger insert clones for more reliable FISH applications.

Accurate assignment of FISH probes to their chromosome of origin requires a means to identify conclusively each chromosome comprising the karyotype. As described earlier, the similarity in size and banding patterns of many of the chromosomes of the dog renders them notoriously difficult to identify reliably by classical cytogenetics alone. The generation of chromosome specific reagents (Langford *et al.*,. 1996; Breen *et al.*, 1999a,b,c; Yang *et al.*, 1999) has enabled molecular cytogenetics of the dog to be approached with much greater confidence. The application of FISH analysis to cloned DNAs that have also been used in meiotic mapping and radiation hybrid mapping will serve to anchor such maps to the corresponding dog chromosomes. This will ensure that: (i) all chromosomes are represented in the emerging genome maps of the dog and (ii) that the maps extend towards the

Table 11.1. A list of all published genes that have been mapped to dog chromosomes by FISH. The list of loci mapped is ordered through the karyotype from CFA1–38 and then X and Y, according to the nomenclature of the International Committee for the Standardization of the Karyotype of the Dog (Switonski *et al*., 1996; Breen *et al*., 1998, 1999b). Under the heading of 'dog chromosome location', the chromosome location of the gene as reported by the corresponding authors (and band location where applicable) is listed in column 1, the chromosome nomenclature (nom.) used by the reporting authors, where applicable, is shown in column 2, where: 1 = Switonski *et al*. (1996) (GTG-banding for CFA1–21); 2 = Breen *et al*. (1999a) (DAPI-banding for CFA1–38), 3 = Stone *et al*. (GTG-banding for CFA1–38) (1991a). The corresponding chromosome number according to the recommendations of the International Committee for the Standardization of the Karyotype of the Dog (reported in Switonski *et al*., 1996; Breen *et al*., 1998, 1999b) is shown in column 3. Where more than one author has reported a locus, both reports are cited. The human chromosomal assignments are also presented and are based on that reported in the cited paper. Cases where the currently available knowledge of dog–human chromosome synteny (Breen *et al*., 1999c; Yang *et al*., 1999) are not in agreement with the corresponding FISH positions are identified with a '(?)' following the human chromosome location.

Gene symbol	Gene name	Dog chromosomal location			Human chromosomal location	Reference
		Reported[1]	Nom.[2]	ISN[3]		
T	T brachyury (mouse) homologue	1q23	2	1	6q27	Haworth *et al*., 2001a
VIM	Vimentin	2q(12)[a]	1	2	10p13	Werner *et al*., 1999
GOT2	Glutamic-oxaloacetic transaminase 2	2q31–q32	2	2	16q12–q22	Thomas, 2000
NPPA	Atrial natriuretic polypeptide	2q35distal	2	2	1p36	Thomas *et al*., 1999
MYL2	Myosin light chain, regulatory vesicle	3q(21)[a]	1	3	12q23–q24.3 (?)	Werner *et al*., 1999
MSX2	Homeodomain transcription factor	4q23	2	4	5q34–q354	Haworth *et al*., 2001b
ATOX1	Antioxidant protein 1	4q24–q31	2	4	5q32–q33	van der Sluis *et al*., 2001
IGH[e]	Immunoglobulin heavy chain	4qtel.	?	4	14q32 (?)	Dutra *et al*., 1996
CKMM	Creatinine kinase muscle type	4	3	4	19q13.3 (?)	Guevara-Fujita *et al*., 1996
APRT	Adenine phosphoribosyltransferase	3	3	5	16q24	Guevara-Fujita *et al*., 1996
		5q33	2	5		Thomas *et al*., 1999
THY1	Thy-1 cell surface antigen	5q13	2	5	1p32–33	Thomas *et al*., 1999
CDE3	CD3E antigen, epsilon polypeptide (TiT3 complex)	5q13	2	5	11q23	Thomas *et al*., 1999
MMP1	Matrix metalloproteinase 1 (interstitial collagenase)	5q14.3distal	2	5	11q21–q22	Thomas, 2000
TP53	Tumour suppressor protein (p53)	7qdistal[b]	3	5	17p13.1	Guevara-Fujita *et al*., 1996
		5q21	2	5		Thomas *et al*., 1999
GUCY2E	Guanylate cyclase 2E	5q21	2	5	17p13.1	Thomas, 2000
GP1BA	Glycoprotein Ib (platelet), alpha polypeptide	5q21	2	5	17pter–p12	Thomas, 2000

Table 11.1. *Continued*

Gene symbol	Gene name	Dog chromosomal location			Human chromosomal location	Reference
		Reported[1]	Nom.[2]	ISN[3]		
GLUT4/SLC2A4	Solute carrier family 2 (facilitated glucose transporter), member 4	5qprox.	1	5	17p13	Werner *et al*., 1997
		5q21	2	5		Thomas, 2000
VAMP2/SYB2	Vesicle-associated membrane protein 2 (synaptobrevin 2)	5q21	2	5	17pter–p12	Thomas, 2000
MC1R	Melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	5q31–32	2	5	16q24.3	Thomas, 2000
APRT	Adenine phosphoribosyltransferase	5q33prox.	2	5	16q24	Thomas *et al*., 1999
DPEP1	Dipeptidase 1 (renal)	5q33prox.	2	5	16q24	Thomas, 2000
DIO1	Deiodinase, iodothyronine, type I	5q34	2	5	1p33–p32	Thomas *et al*., 1999
TAT	Tyrosine aminotransferase	5q34–q35 prox.	2	5	16q22.1	Thomas *et al*., 1999
HP	Haptoglobin	5q34–q35 prox.	2	5	16q22.1	Thomas, 2000
PMP22	Peripheral myolin protein 22	5qprox.	1	5	17p12–p11.2	Werner *et al*., 1997
ALDOA	Aldolase A, fructose biphosphate	6q14	2	6	16q22–q24	Thomas, 2000
VCAM1	Vascular cell adhesion molecule 1	6q22	2	6	1p32–p31	Thomas, 2000
RPE65	Retinal pigment epithelium-specific protein	6q25	2	6	1p31	Thomas, 2000
TBX19	T-box (like) 19	6q25	2	6	1p	K. Haworth and Y.H. Edwards (personal communication)
PK	Pyruvate kinase	2	3	6	1q21 (?)	Guevara-Fujita *et al*., 1996
GLUT2	Glucose transporter protein-2	5	3	7	3q26 (?)	Guevara-Fujita *et al*., 1996
CSF1R	Colony stimulating factor 1 receptor	5	3	7	5q33 (?)	Guevara-Fujita *et al*., 1996
TG1	Transglutaminase-1	8q11.2–q12	2	8	14q11–q12	Credille *et al*., 2001
BMP4	Bone morphogenic protein 4	8q21	2	8	14q22–q23	Johanson *et al*., 2001
FOS	v-fos FBJ murine osteosarcoma viral oncogene homologue	8q31	2	8	14q24.3	Thomas, 2000
K14	Type I (acidic) keratins	9q21.3	2	9	17q12–q21	Miller *et al*., 1999
P4HB	Procollagen-proline, 2-oxoglutarate 4-dioxygenase	9q(11prox)[a]	1	9	17q25	Werner *et al*., 1997
GALK1	Galactokinase 1	9q(11prox)[a]	1	9	17q23–q25	Werner *et al*., 1997
TK1	Thymidine kinase 1, soluble	9q(11distal)[a]	1	9	17q23.2–qter	Werner *et al*., 1997
HSS	Sperm surface protein	9q11–25	2	9	17q21	van der Sluis *et al*., 2001

GH1	Growth hormone 1	9q(12)[a]	1	9	17q22–q24	Werner *et al*., 1997
BRCA1	Breast cancer, region 1	9q(21distal)[a]	1	9	17q21	Werner *et al*., 1997
THRA1	Thyroid hormone receptor, alpha 1	9q(21distal)[a]	1	9	17q11.2–q12	Werner *et al*., 1997
RARA1	Retinoic acid receptor, alpha	9q23qprox[f]	?	–	17q12 (?)	Park, 1996
		9q(21distal)[a]	1	9	17q12	Werner *et al*., 1997
MPO	Myeloperoxidase	9q(22prox)[a]	1	9	17q21–q23	Werner *et al*., 1997
NF1	Neurofibromin 1	9q(24)[a]	1	9	17q11.2	Werner *et al*., 1997
RXRA	Retinoid X receptor, alpha	9q(25prox.)[a]	1	9	9q34	Werner *et al*., 1998
MDCR[f]	Miller-Dieker	23qtel	?	9	17p13 (?)	Park, 1996
SLC3A1	Solute carrier family 3, member 1	10q(24–q25)[a]	1	10	2pter–q32.2	Werner *et al*., 1999
PROP-1	Prophet of Pit	11q11–q12.1	2	11	5q31–q35	Latinga-van Leeuwen *et al*., 2000b
CTR1	Copper transport 1	11q22.2–q22.5	2	11	9q31–q32	van der Sluis *et al*., 1999
DLA-64/88	Class I MHC	12qtel	?	12	6p21.3	Dutra *et al*., 1996
ALB	Albumin	13q22.2	2	13	4q11–q13	Thomas, 2000
HNRPA2B1	Heterogeneous nucleolar ribonucleoprotein A2/B1	14q21.1	2	4	7p15	Thomas, 2000
DCN	Decorin	15q22–q23	2	15	12q23	Ryder, 2000
IGF1	Insulin-like growth factor 1	15q24	2	15	12q22–q23	Ryder, 2000
TRA1	Tumour rejection antigen (gp96) 1	15q24	2	15	12q24.2–q24.3	Ryder, 2000
PC	Protein C	19q21–22	2	19	?	Leeb *et al*., 1999
PRKCD	Protein kinase C delta	20q(14–q15prox)[a]	1	20	3p	Werner *et al*., 1999
BSN	Basoon	20q15.1–q15.2	2	20	?	Leeb *et al*., 1999
DAG	Dystroglycan	20q15.1–q15.2	2	20	3p21	Leeb *et al*., 2000a
AMT	Aminomethyltransferase	20q15.1–q15.2	2	20	3p21.2–p21.1	Leeb *et al*., 2000b
TCTA	T-cell leukaemia translocation associated gene	20q15.1–q15.2	2	20	3p21	Leeb *et al*., 2000b
ATP7B	P-Type ATPase, copper transporting beta polypeptide (Wilson disease)	22q11	2	22	13q14.2–q21	van der Sluis *et al*., 1999
GLB1[c]	Galactosidase, beta 1	23q(11–q13)[a]	2	23	3p22–p21.33	Werner *et al*., 1999
BMP7	Bone morphogenic protein 7	24q24–25	2	24	20	Johanson *et al*., 2001
IGLC	Immunoglobulin lambda constant region cluster	26q(22–q24)[a]	2	26	22q11.2	Werner *et al*., 1999
K5	Type II (basic) keratins	27q11	2	27	12q12–21	Miller *et al*., 1999
RARG[d]	Retinoic acid receptor gamma	27q(22)	2	27	12q13	Werner *et al*., 1999
PIT-1	Pituitary specific transcription factor	31q11	2	31	3p11 (?)	Latinga-van Leeuwen *et al*., 2000a

Table 11.1. *Continued*

Gene symbol	Gene name	Dog chromosomal location			Human chromosomal location	Reference
		Reported[1]	Nom.[2]	ISN[3]		
PAX3	Paired box homeotic gene 3	37q16–17	2	37	2q35–q37	Leeb *et al*., 2000
MNK	ATPase copper transporting alpha polypeptide (Menkes)	X	?	X	Xq13.2–q13.3	Guevara-Fujita *et al*., 1996
DMD	Duchenne Muscular Dystrophy	Xp21	2	X	Xp21	Schatzberg *et al*., 1999
F VIII	Clotting factor VIII	Xq28	?	X	Xq28	Dutra *et al*., 1996
F IX	Clotting factor IX	Xq26.3–27	?	X	Xq26.3–q27.1	Dutra *et al*., 1996
PGK	Phosphoglycerate kinase	Xq13–q21	?	X	Xq13	Deschenes *et al*., 1994
CHM	Choroidermia	Xq13–q21	?	X	Xq13	Puck *et al*., 1993
SRY	Sex determining region Y	Y	2	Y	Yp11.3	Olivier *et al*., 1999

[a]For these loci, no chromosome band assignment was provided in the cited publication. We have provided a tentative band assignment, in parentheses, based on the chromosome band nomenclature referred to in the original paper.

[b]The mapping of TP53 to the distal end of CFA 5 by Guevara-Fujita *et al*. (1996) does not agree with the human–dog synteny data and may represent the isolation of a clone which did not contain the correct locus. The TP53 clone isolated by Thomas *et al*. (1999) was sequence verified and also maps to the region of CFA5 that shares synteny to HSA17p13, the reported map position of the human TP53 locus.

[c]The FISH of GLB1 was reported by Werner *et al*. (1999) to map to the proximal end of CFA22. However, this was an error in chromosome identification and more recent data (P. Werner personal communication) has demonstrated that the chromosome to which GLB1 mapped was CFA23.

[d]The FISH of RARG was reported by Werner *et al*. (1999) to map to the distal end of CFA17. However, this was an error in chromosome identification and more recent data (P. Werner personal communication) has demonstrated that the chromosome to which RARG mapped was CFA27.

[e]The assignment of IGH to CFA4 by Dutra *et al*. (1996) is not in agreement with human–dog synteny data which suggests that IGH should map to CFA8. The assignment to CFA 8 is also supported by the somatic cell hybrid data of Langston *et al*. (1997) and the radiation hybrid mapping data of Mellersh *et al*. (2000).

[f]In the paper of Park (1996) a number of loci from HSA17 were reported to map to a chromosome identified as CFA23. However, it is now known that HSA17 does not appear to have any conserved synteny with CFA23, only with CFA5 and CFA9. This paper also included the assignment of RARA and MPO to CFA23, both of which have more recently been assigned by Werner *et al*. (1999) to CFA9. This strongly suggests that MDCR actually maps to CFA9 and not to CFA23 as reported by Park (1996).

centromeric and telomeric ends of each chromosome. This has recently been performed and the use of chromosome specific microsatellites and sequence data has allowed each of the 38 dog autosomes to be represented in the integrated radiation hybrid and meiotic map (Breen *et al.*, 2001).

Flow sorting of chromosomes

At the molecular level, the chromosomes within a cell are characterized not only by their size but also by their nucleotide composition. This property is exploited by a technique referred to as bivariate flow sorting using a fluorescence-activated cell sorting system (FACS). In this technique, cultured cells are treated with a spindle inhibitor (e.g. colcemid) to accumulate cells in metaphase. The cells are lysed and the resulting chromosome suspension is stained with two DNA-specific fluorescent dyes that have an affinity for either AT-rich or GC-regions of the DNA. The chromosome suspension is passed as a stream of droplets, each containing a single chromosome, through the path of two lasers. The lasers excite the two fluorochromes, causing each chromosome to emit light that is detected by a pair of photomultiplier tubes. The fluorescent properties of each chromosome are determined by its physical size and also its nucleotide composition. Computer software presents the recorded information as a two-dimensional plot (bivariate karyotype) of the fluorescence emitted from one fluorochrome 'vs.' the fluorescence emission of the second fluorochrome. From this plot the operator is able to direct the hardware to collect many chromosomes of the same emission properties into one tube, isolating them from the rest of the chromosomes comprising the karyotype. In this way the chromosomes comprising the genome of an individual are effectively 'sorted' into their separate groups. Once collected, DNA from the sorted chromosomes may be used to generate chromosome-specific DNA libraries (Collins *et al.*, 1991) or may be amplified by degenerate oligonucleotide primer (DOP) PCR (Telenius *et al.*, 1992). In either case the DNA is suitable for use as a whole chromosome paint (WCP) probe that may be used for a variety of applications including homologous chromosome painting (chromosome identification, chromosome enumeration, identification of translocations etc.) and heterologous chromosome painting (comparative chromosome painting, Zoo-FISH).

Chromosome painting in the dog

In the dog, the first description of a panel of chromosome paint probes was described by Langford *et al.* (1996) who generated 33 discrete bivariate flow peaks, with the paints from eight peaks painting two pairs of autosomes. The generation of eight mixed pools of chromosomes is evidence that many of the dog chromosomes are not only similar in size but also in base pair composition. In their study Langford *et al.* (1996) also co-sorted a mixture of human

and dog chromosomes in order to estimate the physical size (in Mb) of each dog chromosome (Table 11.2). The chromosome paints produced by Langford *et al.* (1996) were first assigned to the largest 21 pairs of autosomes, plus the sex chromosomes, following the nomenclature of the International Committee for the Standardization of the Dog Karyotype (Switonski *et al.*, 1996). Paints were also distributed to members of the Committee to allow a consensus to be reached regarding the assignment of the remaining paints to the smallest 17 pairs of autosomes. In this way, for the first time, international consensus was achieved regarding the numbering of all 38 chromosome of the dog (Breen *et al.*, 1998). An important application of paint probes for the dog has been to conclusively identify the chromosomes to which single locus probes have been assigned. This is done by simultaneous hybridization of both a paint probe and a single locus probe, each labelled with a different fluorochrome. In this way the assignment of both the paint probe and the single locus probe to the same chromosome may be confirmed, as shown in Fig. 11.6d (see Frontispiece).

Three years later, Yang *et al.* (1999) independently produced a second set of dog-paints, some of which also painted more than one pair of chromosomes. However by applying DOP-PCR to the DNA from a single sorted chromosome, 40 paints were obtained with each chromosome represented separately. Although paint probes derived from single chromosomes allow

Table 11.2. Chromosomal assignment of each bivariate flow karyotype peak in the flow karyotype reported by Langford *et al*. (1996). Assignment of peaks A-M, O-R and X and Y are taken from Langford *et al*. (1996). Assignment of peaks N, S-W and Z-ff are taken from the numbering of the chromosomes recommended by the International Committee for the Standardization of the Dog Karyotype (Breen *et al*., 1998). Chromosome sizes taken from Langford *et al*. (1996).

Peak	Number of homologues	Chromosome assignment	Chromosome size (Mb)	Peak	Number of homologues	Chromosome assignment	Chromosome size (Mb)
A	2	1	137	R	2	19	66
B	2	3	105	S	4	21,23	61
C	2	4	100	T	2	25	60
D	2	2	99	U	2	27	57
E	2	5	99	V	4	24,28	55
F	2	7	94	V’	2	28	55
G	2	6	87	W	4	29,32	51
H	4	8,11	86	X	1	X	139
I	2	12	85	Y	1	Y	27
J	4	10,17	80	Z	4	31,34	50
K	2	9	77	aa	2	26	48
L	4	13,15	75	bb	2	30	47
M	2	16	73	cc	4	33,36	41
N	2	22	72	dd	2	37	40
O	2	14	72	ee	2	38	38
P	2	20	66	ff	2	35	38
Q	2	18	66				

chromosome identification, such probes tend to result in a less comprehensive coverage of the chromosome than the conventional amplification of DNA produced from pools of more than 500 sorted chromosomes. Regardless of the origin of the paint probes, the possibility of multicolour chromosome painting is an exciting area for genome mapping in the dog. Figure 11.6e shows the hybridization of just two paint probes to a dog metaphase preparation, where one of the probes is detected in green and the second is detected in red. The extension of this approach by adding additional colours is now possible and currently under development for the dog.

Comparative chromosome painting

Millions of years of evolution have resulted in the genomes of mammals being organized into different chromosome complements. However, all mammals are essentially differential arrangements of the same collection of ancestrally related genes that have retained a remarkable degree of homology. Consequently, large blocks of DNA are conserved between species in the form of evolutionarily conserved chromosome segments (ECCS). This gross synteny can be visualized directly by the use of heterologous chromosome painting, or Zoo-FISH, where chromosome-specific paint probes from one species are hybridized to metaphase spreads of another species (e.g. Scherthan *et al.*, 1994; Rettenberger *et al.*, 1995; Frönicke *et al.*, 1996, 1997; Raudsepp *et al.*, 1996). In this way, data regarding the chromosomal location of genes in a map-rich species, such as the human, may be transferred directly from the human karyotype to the karyotypes of gene-poor species, such as the dog. This approach has now become an integral part in the construction of comparative maps between different mammalian species (reviewed by Weinberg and Stanyon, 1995, 1997; Chowdhary, 1998).

Comparative chromosome painting of the dog and human

Unidirectional chromosome painting involving the dog was first reported by Werner *et al.* (1997), using a human chromosome 17 paint probe to identify corresponding regions on dog chromosomes 5 and 9. The first reciprocal chromosome painting was reported by Thomas *et al.* (1999) who used this approach to identify the conserved segments in the human genome that were shared with dog chromosome 5. A complete reciprocal chromosome painting study using paint probes for the entire karyotypes of human and dog was first reported by Breen *et al.* (1999c), who identified the detailed regions of conserved synteny between these two karyotypes. Figure 11.6f illustrates a typical hybridization of a human paint probe (HSA1) on to seven different dog chromosomes. Figure 11.6g illustrates the hybridization of dog paint probe (CFA26) on to two human chromosomes (HSA12 and HSA22). Later in the same year, Yang *et al.* (1999) also reported a detailed reciprocal study using a

different set of dog chromosome paint probes. The study of Yang *et al.* (1999) made use of the more readily identifiable red fox karyotype as an intermediate between human and dog, and suggested that the red fox karyotype could be used in place of that of the dog for gene mapping studies. Although the study of Yang *et al.* (1999) used a different approach and a different chromosome nomenclature for the dog than that of Breen *et al.* (1999c), both studies are broadly comparable. However, the study of Yang *et al.* (1999) identified a greater number of ECCS and whilst the study of Breen *et al.* (1999c) reported a few gaps in the comparative maps of human and dog, Yang *et al.* (1999) were able to suggest the chromosome homologies that filled these few gaps. Both reports offer a comprehensive analysis of the distribution of ECCS between human and dog which is beyond the scope of this chapter and so the reader is directed to these papers for more detailed information.

Zoo-FISH studies provide valuable information about the gross distribution of conserved segments, but do not offer any indication of the gene order within each conserved segment. Refinement of the boundaries of ECCS and evaluation of the orientation of, and gene order within, such regions, is made possible using comparative FISH mapping and RH-mapping of type-I (gene) markers. This approach was used in the study of Thomas *et al.* (1999) in their study of dog chromosome 5 (CFA5) and forms the basis of the genome-wide study reported by Breen *et al.* (2001).

Chromosome Abnormalities

Identification of chromosome abnormalities in the dog is a difficult task. Among the different types of abnormalities, the presence of aneuploidies and of centric fusions are easy to recognize, although precise identification of the component chromosomes is often difficult. To date, there are relatively more reports on chromosome abnormalities in dog cancer cells than in dogs demonstrating reproductive problems or congenital malformations.

Numerical and structural chromosome abnormalities

Among numerical chromosome abnormalities diagnosed in the dog, the most common are sex chromosome aneuploidies. In a review by Mellink and Bosma (1989), seven cases of XXY trisomy were described, with either mosaic or chimeric XX/XXY status. These dogs were classified as intersexes. Among female dogs, a case of X monosomy (Mayenco-Aguirre *et al.*, 1999) and two cases of X trisomy (Johnston *et al.*, 1985; Switonski *et al.*, 2000) were diagnosed. All these females were investigated by cytogenetic analysis due to their infertility. It should, however, be emphasized that many intersex dogs demonstrate a normal complement of sex chromosomes. McFeely *et al.* (1967) found among four intersexes two with XX and two with XY chromosome sets. Similar results were presented by Chaffaux and Cribiu (1991) who analysed

nine intersexes of which four had female karyotypes (78,XX), four had male karyotypes (78,XY) and only one individual demonstrated chimerism (78,XX/78,XY). Recently, Genero *et al.* (1998) described a phenotypically male dog that presented with normal male behaviour but had malformation of the genitalia. Chromosomal investigation revealed chimerism within the circulating lymphocyte population with 85% of the cells being $2n = 78$,XX and 15% being $2n = 78$,XY. This dog was described as a male pseudohermaphrodite. At present, the analysis of intersexes with a normal sex chromosome set is enhanced by molecular detection of the *SRY* gene and other Y-specific loci (Meyers-Wallen *et al.*, 1995).

Structural chromosome abnormalities have been described rarely in the dog. There are few reports describing the occurrence of Robertsonian translocations (centric fusions). The first cases of centric fusions were found in malformed dogs and dogs suffering from leukaemia (for references, see Larsen *et al.*, 1979). Later, this abnormality was observed in phenotypically normal dogs. Larsen *et al.* (1979) found a translocation designated as rob(13;23), according to chromosome nomenclature proposed by Selden *et al.* (1975). Stone *et al.* (1991b) described a fusion (21;33), following other nomenclature (Stone *et al.*, 1991a). Exceptional results were shown by Mayr *et al.* (1986) who analysed karyotypes of 112 randomly selected dogs, representing 31 breeds. These authors found seven carriers of Robertsonian translocations between chromosome 1 and a small autosome. All the carriers belonged to the poodle breed. In the studies mentioned above (Larsen *et al.*, 1979; Mayr *et al.*, 1986; Stone *et al.*, 1991b) no fertility reduction of the carriers was noticed. Recently, a leucocyte chimerism 78,XX/77,XX,rob(autosome, autosome) was described in a mixed breed bitch with the help of microsatellite polymorphism analysis (Switonski *et al.*, 2000). The incidence of cells carrying the centric fusion was low (*c.* 4%).

Other structural rearrangements (reciprocal translocations, inversions, etc.) were almost exclusively found in cancer cells. An exception is a recently diagnosed case of X–autosome reciprocal translocation in a male to female sex-reversed dog (Schelling *et al.*, 2001). The application of FISH techniques for the identification of aberrant dog chromosomes has been limited by the lack of available resources. However, in a study investigating canine Duchenne muscular dystrophy, Schatzberg *et al.* (1999) used FISH clones containing the DMD locus to identify affected males and carrier females according to whether their respective X chromosomes were deleted for the Xp21 region, known to contain the canine DMD locus (Fig. 11.6h).

Cancer cytogenetics

Over 30,000 karyotypically abnormal human neoplasms are listed in the *Catalogue of Chromosome Aberrations in Cancer* putting similar studies in the dog in its infancy. However, cancer cytogenetics of the dog is a growing area with an increasing number of reports on chromosome abnormalities found in

dog cancer cells. Over a decade ago, Mellink and Bosma (1989) reviewed chromosome analysis of canine neoplasms and indicated that the presence of aneuploidy and additional bi-armed chromosomes, resulting from centric fusions, is a common feature of such cells.

Results published in the last decade confirmed earlier observations that bi-armed chromosomes occur frequently in tumour cells (Mellink *et al.*, 1989; Mayr *et al.*, 1990a, b; Mayr and Kofler, 1991; Nolte *et al.*, 1993; Moreno-Millan *et al.*, 1996; Tap *et al.*, 1998). An interesting suggestion was presented by Reimann *et al.* (1994) who demonstrated, with the use of *in situ* hybridization with a telomere-specific probe, that bi-armed chromosomes in canine mammary tumours are dicentric, due to head-to-head-telomeric fusions of acrocentrics. On the other hand, Tap *et al.* (1998) observed that in mammary carcinoma cell lines some bi-armed chromosomes are actually isochromosomes and some arose by centric fusions. This finding was revealed by application of dog chromosome-specific paint probes.

There are also reports demonstrating other chromosome abnormalities in cancer cells. Bartnitzke *et al.* (1992) found a translocation in mammary tumours that involved chromosome 1. Trisomy of chromosome 1, also in a mammary tumour, was described by Mayr *et al.* (1993). Perhaps the largest studies of aberrations in cancer cells were those reported by Hahn *et al.* (1994) and Reimann *et al.* (1999). Hahn *et al.* (1994) reported on the cytogenetic finding in 61 cases of dog lymphoma and noted that 30% of their cases demonstrated chromosome translocations and 70% demonstrated aneuploidy. Reimann *et al.* (1999) reported the cytogenetic investigation of 270 solid tumours of different histological types. In this study the authors noted a prevalence of involvement of chromosome 2 and the X chromosome, although over 75% of the chromosomes comprising the karyotype had been observed in aberrations.

The application of FISH to studies of dog cancer

Conventional cytogenetics has played an important role in the identification of chromosome aberrations associated with dog cancers. However, the inability to identify many of the smaller autosomes with confidence has potentially led to confusion over the specific chromosomal involvement. A fundamental tool for the classification of many forms of human tumour has recently been provided by molecular cytogenetic evaluation using FISH techniques. Many human tumours are characterized by recurrent genetic abnormalities, including whole chromosome numerical abnormalities (e.g. trisomies and monosomies); partial chromosome numerical abnormalities (e.g. duplications and deletions); and structural abnormalities (e.g. translocations). The FISH techniques widely used for studies of chromosome aberrations associated with cancers include metaphase FISH and comparative genomic hybridization (CGH) (Kallioniemi *et al.*, 1992). Both techniques play an important role in the molecular cytogenetic evaluation of tumours. Metaphase FISH allows a rapid assessment

of the gross numerical and structural characteristics of a cell. This approach looks directly at the aberrations using chromosomes prepared from the tumour cells and therefore requires viable tumour cells as a starting material. Gross numerical aberrations, such as deletions of large chromosomal segments or whole chromosomes, and structural aberrations, such as translocations, are especially evident with this approach. The chromosome aberrations may be conclusively identified by subsequent analysis with chromosome-specific probe DNAs; either chromosome paints or single locus probes. Evaluation of a new tumour with complex aberrations may require the application of an extensive series of such probes. Comparative genomic hybridization allows a detailed and accurate analysis of imbalanced chromosomal material within an entire genome, i.e. the technique is used to examine an entire genome for changes in DNA copy number. Such changes may be either gains, such as amplifications and trisomies, or losses, such as deletions and monosomies, and are assessed using DNA extracted from the tumour as the probe. This approach does not, therefore, depend on the availability of viable tumour cells. Unlike conventional cytogenetic analysis, a previous knowledge of genetic aberrations is not required and all gains and losses may be visualized using 'normal' metaphase spreads. Comparative genomic hybridization does not, however, allow an assessment of chromosomal aberrations that do not involve a gain or loss of DNA, such as balanced translocations. A combination of both metaphase FISH and CGH is a strategy that will maximize the opportunities for the identification of chromosome aberrations.

The development of dog chromosome-specific FISH reagents such as whole chromosome paint probes (Langford *et al.*, 1996; Yang *et al.*, 1999) and single locus probes (Breen *et al.*, 1999b, 2001) will greatly facilitate the conclusive identification of those recurrent chromosome aberrations. These reagents, combined with the development of comparative genomic hybridization for the dog (Dunn *et al.*, 2000) will accelerate the application of FISH technology to the accurate evaluation of chromosome aberrations associated with dog cancers.

Future Prospects

Cytogenetic studies in the domestic dog have benefited from some major breakthroughs and advances in molecular cytogenetic technologies. The development of FISH techniques has seen the increasing application of chromosome paint probes and single locus probes for dog genome mapping and clinical evaluation. The emergence of CGH analysis for the study of dog cancers will undoubtedly drive this important area of research further at an increased rate. The integration of cytogenetic maps with the maps being developed from meiotic linkage analysis and radiation hybrid analysis will serve to enhance the rate at which a workable genome map of the dog is developed. In addition, a detailed comparative map of the dog and human genomes is emerging. All these features in combination will facilitate targeted

identification and cloning of disease-associated genes that, in turn, will provide a mechanism for better diagnosis and will facilitate improvements in health and welfare through selective dog breeding.

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12 Linkage and Radiation Hybrid Mapping in the Canine Genome

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Introduction

The domestic dog is arguably the most diverse species on earth. Selection of variant phenotypes, followed by carefully designed breeding programmes aimed at propagating particular traits has led to the encapsulation of extremes of size, shape, coat colour, body proportion, and behaviour within a single species. But because the breeding practices deemed necessary to maintain these traits involve crossing closely related individuals, dogs of a single breed may have only limited genetic heterogeneity. The use of infrequent founders and small breeding pools to develop major lines within a breed further limit genetic diversity. As a result, dogs of a single breed may share a large amount of genetic material, including mutant alleles of disease genes. In the absence of strong selection against such alleles, inherited diseases have evolved as a significant problem in modern purebred dogs, with autosomal recessive diseases making up a significant percentage of inherited canine diseases.

For dog breeders, therefore, conflicting goals exist. The desire to rigorously maintain physical and behavioural features unique to a given line requires highly restricted breeding programmes using small numbers of dogs that are themselves often related. Unfortunately, development of hybrid vigour by breeding dogs of unrelated breeds to eliminate expression of genetic diseases is not an option. Diseases which appear late in life present a particularly serious problem, as asymptomatic dogs may be bred for several years before the disease is apparent, thus disseminating the disease gene to many members of the next generation. If there is variable expression of the trait, some affected dogs may not be diagnosed, resulting in even more rapid dissemination of unfavourable genes.

Genome-wide maps are the key resource for determining the location of genes of interest in any organism. When DNA from a family carrying a disease of interest is analysed with genetic markers spanning the genome, co-segregation of regions of the genome among affected individuals identifies regions of the genome likely to contain genes of interest. Identification of disease loci is a necessary first step in cloning the disease gene, followed by identification of the deleterious mutation. It is also important if linkage-based genetic testing is to be implemented.

The development of a high-resolution canine map is therefore a necessary step in the improvement of purebred dog health. This chapter reviews the process of developing a canine map, summarizes the current status of the map, and discusses the implications of the map in the development of genetic tests. We review both genetic (meiotic) and radiation hybrid mapping. Finally, we discuss some recent applications of the map.

Genetic Markers

Introduction to polymorphic genetic markers

Maps are composed of linearly ordered *markers*, each of which is assigned a position along the length of the chromosome by either genetic or physical mapping. Historically, markers labelled 'Type I', are small non-polymorphic segments of genes. As we discuss below, Type I markers are useful for building comparative framework maps between the genomes of different mammalian species. 'Type II' markers are short segments of DNA that vary in nucleotide sequence between copies of the same chromosome. Because any given individual has two copies of each chromosome, one inherited from each parent, there will often be variability between the two copies of each marker in an individual. There will always be variability when the population as a whole is considered.

Different copies of the same piece or segment of DNA are referred to as *alleles*. A genetic marker, by definition, must have at least two different alleles. The most useful type of polymorphic markers are those which have a large number of alleles, each of which is found with a high frequency in a random breed population. If the frequency of the most common allele that appears in the population is less than 95%, then the marker is referred to as *polymorphic*. Simple sequence repeats (also called SSRs, microsatellites or SSRPs) meet that criterion (Miesfeld *et al.*, 1981; Hamada and Kakunaga, 1982; Tautz and Renz, 1984; Stallings *et al.*, 1991). Microsatellites are small stretches of DNA, composed of repeating mono-, di-, tri- or tetranucleotide motifs, such as $(CA)_n$, $(GAAA)_n$, or $(CAG)_n$, in which the motif is repeated many times in tandem. At least 50,000 microsatellite repeats are distributed randomly throughout mammalian genomes. A given repeat will typically vary greatly in repeat number within populations, yet with a mutation rate of less than 10^{-4} mutations per chromosome per generation, they are sufficiently stable to be useful for tracking passage of segments of the genome through multiple generations of a family (Weber, 1990).

Alleles of simple sequence repeats are analysed by PCR-based assays developed from the unique DNA sequences which bracket each repeat and which are used to detect the length polymorphisms that characterize the different alleles (Litt and Luty, 1989; Weber and May, 1989). Microsatellite-based markers form the basis of maps of the human, mouse, cow, pig and many others (Ellegren *et al.*, 1993; Fries *et al.*, 1993; Toldo *et al.*, 1993; Bishop *et al.*, 1994). For the canine genome, the distribution of several types of microsatellites has been described (Rothuizen *et al.*, 1994). As in other mammalian genomes, $(CA)_n$ repeats are the most common, occurring about once every 42 kb. A dozen distinct tri- and tetra-nucleotide repeat-based markers have also been reported in the canine genome, with most occurring every 200–400 kb (Francisco *et al.*, 1996).

Informativeness of genetic markers

The first canine microsatellite markers were reported in 1992 and were based exclusively upon $(CA)_n$ repeats (Ostrander *et al.*, 1992). Subsequent reports provide a wealth of markers suitable for mapping the canine genome (Holmes *et al.*, 1993, 1994; Ostrander *et al.*, 1993, 1995). In constructing a map we strive to develop markers which are useful for mapping in at least 70% of the general population. Such markers are said to be highly informative. In dogs this presents something of a challenge since markers which are informative in mixed breed dogs may not be informative in purebred dogs. In addition, markers informative in one breed may not be in another. Ideally, all markers should be tested on a panel of DNA from unrelated purebred dogs prior to placement on the map. However, since there is no uniform set of breeds which have been agreed upon, most laboratories use a panel of unrelated purebred dogs representing a variety of distinct breeds.

For each marker, informativeness is assessed by calculating either the heterozygosity (H) or polymorphic information content (PIC). The heterozygosity (H) is the probability that a random individual is heterozygous for a given polymorphic marker. It is calculated using the equation: $H = 1 - \sum p_i^2$, where p_i is the population frequency of the *i*th allele (Ott, 1991). PIC is a value that gives information about the utility of a given marker in the population. Although originally designed to measure the informativeness of a co-dominant marker segregating in a family with a rare dominant disease (Botstein *et al.*, 1980), it is frequently used as a general assessment of the likelihood that a given marker will be informative in a particular genome scan. Formally, PIC is defined as the probability that one can discern which of two alleles of a given marker was inherited from the parent who is the disease carrier (Ott, 1991).

The task of developing highly informative markers for the dog genome became considerably easier in 1996, when we showed that markers based upon tetranucleotide repeat-based markers, such as $(GAAA)_n$, are unusually polymorphic in the canine genome, having an average PIC value of 0.75 (Francisco *et al.*, 1996). Practically, this means they are polymorphic in over 75% of unrelated dogs and would be likely to be useful in most mapping studies, even those utilizing purebred dogs where lack of genetic heterogeneity is predicted to be a problem. In addition we showed that $(GAAA)_n$ repeats occur very frequently in the canine genome – about 1/130 kb (Francisco *et al.*, 1996). To date, several hundred canine genetic markers based upon microsatellite repeats have been characterized by a variety of researchers, with a growing number of them based upon tetranucleotide repeats. Details of many of the canine microsatellites that have been developed can be found on the following websites: http://www.fhcrc. org/science/dog_genome/dog.html, http://www-recomgen.univ-rennes1.fr/doggy.html, http://ubeclu.unibe.ch/itz/dogmap.html, http://www.msu.edu/~k9genome/microsat.htm and links therein.

Meiotic Linkage Mapping

Principles of linkage map construction

Meiotic linkage maps or *genetic maps* are made up of mapped loci for which the genetic distance between adjacent markers has been determined in centiMorgans (cM). Genetic maps are constructed by tracking the inheritance of alleles and the position of recombination events through multi-generation pedigrees. Because chromosomes undergo frequent recombination during meiosis, markers or genes located far apart on the same chromosome will become disassociated during formation of egg or sperm and as such are said to be unlinked. Markers or genes that are located physically close together on the same chromosome have a low chance of disruption during meiosis and as such they are said to be linked. For a given area of the genome the probability of a genetic recombination event occurring between any given loci, such as two markers or a marker and a disease gene, is directly proportional to the distance between them. The probability of such an event occurring is expressed as a recombination fraction (θ) or, after minor mathematical adjustment for the possibility of double recombinants, as a distance expressed in cM. One per cent recombination is approximately equal to 1cM, which roughly corresponds to a million base pairs in man and a little over two million in the mouse.

The construction of a genetic map takes place in several sequential steps which we have reviewed previously (Mellersh and Ostrander, 1997; Ostrander *et al.*, 2000). Initially, large numbers of markers are isolated. The markers are then placed into linkage groups (markers within the same linkage group are all located on the same chromosome) and finally the order of the markers within each linkage group is determined. The genetic distance between two markers is a measure of the expected number of crossovers occurring on a single chromosome between those two markers during meiosis. The process of counting numbers of recombinant and non-recombinant offspring of a given mating followed by the estimation of map distance and determination of the likelihood of linkage versus non-linkage for a pair of markers is called '*genetic linkage analysis*'. A set of alleles representing several continuous markers that are received by an individual from one parent is called a haplotype. Recombination events can only be detected by observing haplotypes passed from parents to offspring. The larger a family is, the more useful it is for map construction. At a minimum, three-generation families are needed.

Markers that are 'unlinked' segregate independently, with a recombination frequency of 0.5. That is, it is just as likely as it is unlikely that a given pair of alleles will be associated in offspring. However, markers for which there is a consistent deviation from the 1 : 1 ratio of recombinant to non-recombinant offspring are said to be genetically 'linked'. In these cases θ will be less than 0.5. The process of developing a genetic map involves tracking the inheritance of recombination events in haplotypes through multiple generations of a family. In small intervals, where the probability of multiple crossovers is negligible, recombinations may be counted directly as crossovers. Then, the

relationship between the recombination fraction and the distance between two genes (x) is simply $x = \theta$ (Morgan, 1928) and is generally true when $\theta < 0.10$.

When many closely linked markers are available on a chromosome and their order is known, the simplest method of determining map distances between these markers is to estimate the recombination fractions in each interval of adjacent loci. The map distance between more distant markers is the sum of the map distances in the intervals between these loci (Sturtevant, 1913). This method can only be applied accurately when a large number of markers have been mapped to a given region. Recombination fractions cannot be simply calculated over large distances because of the occurrence of multiple crossovers, which decrease the number of recombinant haplotypes observed and, in turn, the genetic distance.

Various mapping functions have been derived to convert a recombination fraction (θ) into a map distance (x). For example, in human genetic mapping, the Kosambi function (Kosambi, 1944) is widely used:

$$x = 1/2 \tan^{-1}(2\theta) = 1/4 \text{ Ln } \frac{1+2\theta}{1-2\theta}$$

For example, it yields a map distance of 0.236 Morgans or 23.6 cM for a value of $\theta = 0.22$.

In estimating how strong the evidence for linkage between two loci, the likelihood for linkage ($\theta < 0.5$) versus the likelihood for random recombination ($\theta = 0.5$) is calculated by first counting the number of recombinant and non-recombinant offspring produced by a given mating. The logarithm of the likelihood ratio (the lod score):

$$Z(\theta) = \log_{10} [L(\theta)/L(0.5)]$$

is then used as the measure of support for linkage. A linkage group is defined as a set of markers in which each marker is linked to at least one other marker with a specified lod score. Historically, linkage has been accepted if a recombination fraction of $\theta < 0.5$ is supported by a lod score of at least 3.0, reflecting an odds ratio of 1000 : 1. Recently, however, the human genetics community has suggested raising the bar to require higher lod scores as evidence of linkage when considering linkage of a disease gene to a marker.

To construct the canine map we used 17 multigeneration families (Mellersh *et al.*, 1997; Neff *et al.*, 1999), which are analogous to the human CEPH (Centre d'Etude du Polymorphisme Humain) reference families used to construct the human map. The canine reference families represent several distinct breeds of dog, including Miniature and Toy Poodles, Norwegian Elkhounds, Irish Setters and several unrelated lines of Beagles. The grandparents of these pedigrees represented distinct crosses derived from the various breeds. The use of a common set of reference families means that many different investigators can contribute to the map simultaneously. In the case of the human map, marker genotypes are contributed to a common repository for a single analysis. In the case of the dog, we have instituted a similar practice. Ralston Purina (St Louis, Missouri) distributes DNA from the

reference families to researchers, who forward genotype data to the Ostrander laboratory in Seattle for incorporation into the map. This approach expedites the process of mapping, minimizes errors that may result from too many groups analysing differentially updated data files and means that at any given time the most up-to-date version of the map is generally available.

Genotyping and data analysis

Placing the markers into linkage groups, and the subsequent determination of the order and spacing of the markers, requires genotyping DNA (that is, determining the alleles) from each family member with every marker for which that family is informative. Genotyping data from each new marker is then analysed together with that from all previous markers in the region to build the map of the chromosome. In the laboratory, different types of genotyping systems can be used. Early versions of the map utilized manual genotyping in which one primer from each marker set was radioactively labelled, DNA from each family member was amplified under a standard set of conditions, and the resulting PCR products resolved on a denaturing sequencing gel and visualized by autoradiography. In most laboratories now contributing to the map, this has largely been superceded by automated genotyping systems based on the ABI, ALF or LICOR automated fluorescent sequencers. Such systems have the advantage that alleles are assigned automatically and not by a person scoring the gels, and thus errors are minimized.

The computations that take the data from raw genotyping form to a finished map are complex. There are various specifically designed computer programs, such as the LINKAGE program package, MAPMAKER and MultiMap, which calculate recombination frequencies and determine the likelihood that two markers are linked and the distance between them.

The canine linkage map was constructed using MultiMap (Matise *et al.*, 1994) which utilizes CRI-MAP (Lander and Green, 1987) to calculate two-point recombination fractions and associated lod scores for all markers. Markers were assigned to linkage groups, using the *find-all-linkage-groups* function of MultiMap. Markers in each group were linked to at least one other marker with a recombination fraction ≤0.4 supported by a lod score of at least 3.0 (equivalent to odds of 1000 : 1 in favour of linkage). In practical terms, this means that each marker was placed in its appropriate linkage group with a high level of statistical support. Meiotic recombination rates are different for males and females, so a sex-averaged framework and comprehensive linkage map are typically displayed for each chromosome. By definition, a framework map is one for which the order and spacing of markers is statistically very well supported. Comprehensive maps are ones in which all markers known to be on a particular chromosome are placed on the map, although the statistical support for order might be very low. Comprehensive maps are generally more dense than framework maps, but might be locally unreliable in terms of marker order. For each linkage group, map construction began with the pair of

markers in each group which had the highest joint PIC value, and for which a recombination fraction of 0.05–0.4 was supported with a lod score of ≥3.0. These were selected automatically by MultiMap. Markers were analysed in decreasing order of informativeness; a marker was only added to the map when it could be localized to a unique interval with a lod score of ≥3.0. Markers that could be positioned with odds of at least 1000 : 1 comprised the framework map. The framework map for each chromosome was considered complete when no further markers fitting the criteria could be added. Markers that could be positioned on the map with a lod score of 0.1 were subsequently added to generate the comprehensive map from each chromosome.

Evolution of the canine linkage map

The first canine linkage groups were described by Lingaas and colleagues in 1997 (Lingaas *et al.*, 1997). Simultaneously in 1997, we published the first meiotic linkage map of the dog (Mellersh *et al.*, 1997). This first-generation map was composed of 150 markers, of which 139 could be assigned to one of 30 linkage groups. Markers were assigned to linkage groups with odds equal or greater to 1000 : 1 (lod scores ≥3.0), and then ordered on the individual chromosomes with lower lod scores. The largest linkage group contained 11 markers and spanned 106.1 cM. The average distance between markers was 14.03 cM and the map was estimated to cover 2073 cM, which we now know to be about 77% of the genome. The sex-averaged linkage map for this work was summarized as a framework map comprising 94 markers that could be ordered with odds greater than 1000 : 1 (lod ≥3.0). In addition, the most likely positions of 45 accessory markers were indicated; these markers could be assigned to linkage groups with a lod ≥3.0 but could not be ordered with odds greater than 1000 : 1.

Addition of markers to the map led to the publication of a second-generation map in 1998. This map comprised 277 markers (Neff *et al.*, 1999), each of which was assigned to linkage groups with odds greater than 100,000 : 1 (≥ lod 5.0). Since maximum likelihood calculations suggested a genome size of about 27 Morgans for the dog, the resulting second-generation map was estimated to cover about 88% of the genome (Neff *et al.*, 1999). This second-generation map included 38 linkage groups and the X.

Because of the difficulty associated with cytogenetic identification of canine chromosomes, in the first- and second-generation maps, assignment of most linkage groups to specific numbered chromosomes was difficult and linkage groups were numbered simply in order of size. During development of a third-generation meiotic linkage map, considerable effort went into assigning linkage groups to specific canine chromosomes (Werner *et al.*, 1999). Two approaches were used to accomplish this. In the first, data from mapped markers made available from a variety of research groups were incorporated into the map. That is, if a given marker had been assigned to, say canine

chromosome one (CFA1), then that marker was analysed on the reference family, assigned to a linkage group, and the linkage group then assigned to the corresponding chromosome (Werner *et al.*, 1999 and references therein). Second, novel cosmids were isolated that contained all or portions of the coding region of the genes of interest. Since microsatellites occur with high frequency in the canine genome, it was relatively easy to identify adjacent microsatellite markers on each cosmid. The gene-associated cosmids were assigned to chromosomes using fluorescent *in situ* hybridization (FISH). The corresponding microsatellite marker was assigned to a linkage group following analysis on the reference family panel. Fifteen linkage groups were thus assigned. This approach relies heavily on identification of FISH mapped chromosomes by conventional cytogenetic banding techniques. While most of the 15 assignments are assumed to be correct, until more definitive technology generated a method for reproducible assignment of chromosomes, at least some of these have to be considered provisional. As we discuss below, such methodology is now at hand, and all linkage groups can now be assigned to their corresponding chromosome (M. Breen *et al.*, unpublished observations).

Assignment of linkage groups to chromosomes

The general method of assigning linkage groups to chromosomes is only successful if technologies exist to unambiguously identify canine chromosomes. Historically this has been problematic. Canine chromosomes are generally small and acrocentric, and as such it has been difficult to develop a reproducible numbering system based on banding patterns for the canine genome, although this issue has been addressed extensively in the literature (Selden *et al.*, 1975; Stone *et al.*, 1991; Reinmann *et al.*, 1996). For some time, only the first 21 canine chromosomes could be reproducibly identified (Switonski *et al.*, 1996).

Improvements in technology have also expedited the naming of canine chromosomes. One exciting development is called D/C R-banding (Christian *et al.*, 1998). In this approach image analysis programs generate chromosome-banding patterns by dividing the image taken with a DAPI excitation filter by the image taken with a chromomycin A3 excitation filter. The end result is a metaphase spread in which the chromosomes possess a banding pattern characteristic of R-banding (Christian *et al.*, 1998). An image analysis program then generates line scans of pixel intensity versus relative position along the length of chromosomes that were banded. Thus, each chromosome in a genome is represented by a characteristic scan profile.

More recently the development of a set of chromosome paints has opened the way for a high resolution DAPI banded karyotype of the dog to be developed (Langford *et al.*, 1996; Breen *et al.*, 1999a). The availability of a second set of paints (Yang *et al.*, 1999) has forced the field to come to agreement on a final nomenclature. Although the chromosomes are not

consistently numbered by size, the nomenclature described initially by Breen and colleagues has now been agreed upon (Third International Meeting on Development of the Canine Genome Map, Seattle, 2000). Regardless of the nomenclature used, the real value of canine whole chromosome paints is the ability to do reciprocal painting, human to dog and vice versa, to determine the regions of the canine and human genomes that correspond. Such information is invaluable in selecting candidate genes to study following identification of initial linkages. There is good agreement between the reciprocal painting studies of Breen and colleagues and Yang and colleagues, which illustrate the relationship between the human and canine genomes (Breen *et al.*, 1999b; Yang *et al.*, 1999). The human–canid map shows extensive rearrangement between the karyotypes of human and dog. Of the 22 human autosomes only the syntenies of three (14, 20 and 21) have been maintained intact in the canid genome; of the 38 canine autosomes, 18 contain material corresponding to more than one human chromosome. It should be noted that fine mapping and small rearrangements cannot be detected by chromosome painting, but will be detected by radiation hybrid mapping of genes derived from regions where small rearrangements are suspected.

Coverage and density

The denser a map is, the more useful it will be for finding loci of interest. The third-generation meiotic linkage map had markers at greater than 10 cM frequency and provided theoretical coverage of nearly all of the canine genome. However, three considerations indicate that significant additional work remains to be done on the canine map if it is to have a high probability of detecting linkage in most pedigrees of interest. The first, which is unique to the dogs, is that while considerable effort has gone into putting markers on the map that are highly polymorphic, markers will be differentially informative in distinct breeds. Mapping will be particularly problematic in breeds that derive from small numbers of founders or in which pedigrees most informative for mapping traits of interest are derived from repeated breeding of related individuals. Second, while the third-generation map placed a marker, on average, every 10 cM, the distribution, as expected, is not random, and holes clearly exist. In addition, estimates of coverage are calculated by assuming that linkage can be detected 10 cM on either side of each marker placed on the map. However, ends of chromosomes are problematic in this calculation. During the early stages of map development it is not obvious which markers are likely to represent the telomeric portion of the chromosome. Finally, even in the most optimal situation, 10 cM density is a minimum that one wishes to conduct a genome screen over. The ideal map has a much greater density of mapped markers available for reducing initial regions of putative linkage and for specific testing of potential candidate genes. Our goal, therefore, was a map of several hundred markers.

Radiation Hybrid Mapping

Principles of radiation hybrid mapping

Parallel with the construction of the canine meiotic linkage map, efforts were ongoing to construct a Whole Genome Radiation Hybrid (WGRH) map of the dog (Priat *et al.*, 1998). In radiation hybrid (RH) mapping, chromosomes from the species of interest (dog) are randomly fragmented by lethal doses of irradiation, and rescued by fusion with non-irradiated hamster cells. Each radiation hybrid clone thus contains a random and unique subset of the canine genome (Fig. 12.1). To construct a map, DNA isolated from approximately 100 independent radiation hybrid clones is amplified with canine-marker specific primers and then scored for the presence or absence of each marker. The principles are the same as for meiotic linkage mapping. Markers or genes which are close together on a chromosome have a lower chance of being separated by the X-ray breakage than markers far apart, and the probability is proportional to the distance between the markers or genes. But in RH mapping, unlike linkage mapping, non-polymorphic markers (such as gene fragments) can be easily placed on the map. This is because the technique relies only on detecting the presence or absence of a specific marker in the DNA from each hybrid cell line, rather than requiring a polymorphism to distinguish between parental chromosomes. Because genes can be easily mapped, RH maps can be used to determine the comparative or syntenic relationship between genomes. The generation of several sets of anchored reference loci referred to as universal mammalian sequence-tagged sites (UMS), comparative anchor-tagged sequences (CATS) and traced orthologous amplified sequence tags (TOASTS), respectively, can be expected to expedite the unravelling of the canine genome (Venta *et al.*, 1996; Lyons *et al.*, 1997; Jiang *et al.*, 1998).

Evolution of the canine radiation hybrid map

Towards that end, a panel of canine–rodent hybrid cell lines was constructed in 1998 by fusing dog fibroblasts irradiated at 5000 rads (50 Grays) with thymidine kinase-deficient hamster cells (Vignaux *et al.*, 1999). The RH panel consists of 126 independent cell lines; the amount of dog genome retained in each clone, calculated as the percentage of markers present, is 20.8%. It has been estimated that the average fragment size is 16.6 Mb, meaning the theoretical limit of mapping resolution of the panel is approximately 0.6 Mb, or 600 kb. The panel appears to contain almost the entire canine genome. To date, of the many hundred markers tested on the panel, only the p53 gene and markers immediately surrounding it on canine chromosome 5, a region syntenic with human chromosome 17p, cannot be detected in the panel, although it was detected in the DNA of the original dog used to make the panel (Priat *et al.*, 1998; Mellersh *et al.*, 2000).

In 1998 this panel was used to develop a first generation WGRH map of the dog that consisted of 400 markers, including 218 genes and 182 microsatellites (Priat *et al.*, 1998). As with meiotic mapping, the relative confidence in assigning markers to RH groups (the RH equivalent of meiotic linkage groups) and determining the order of markers within each group is assessed

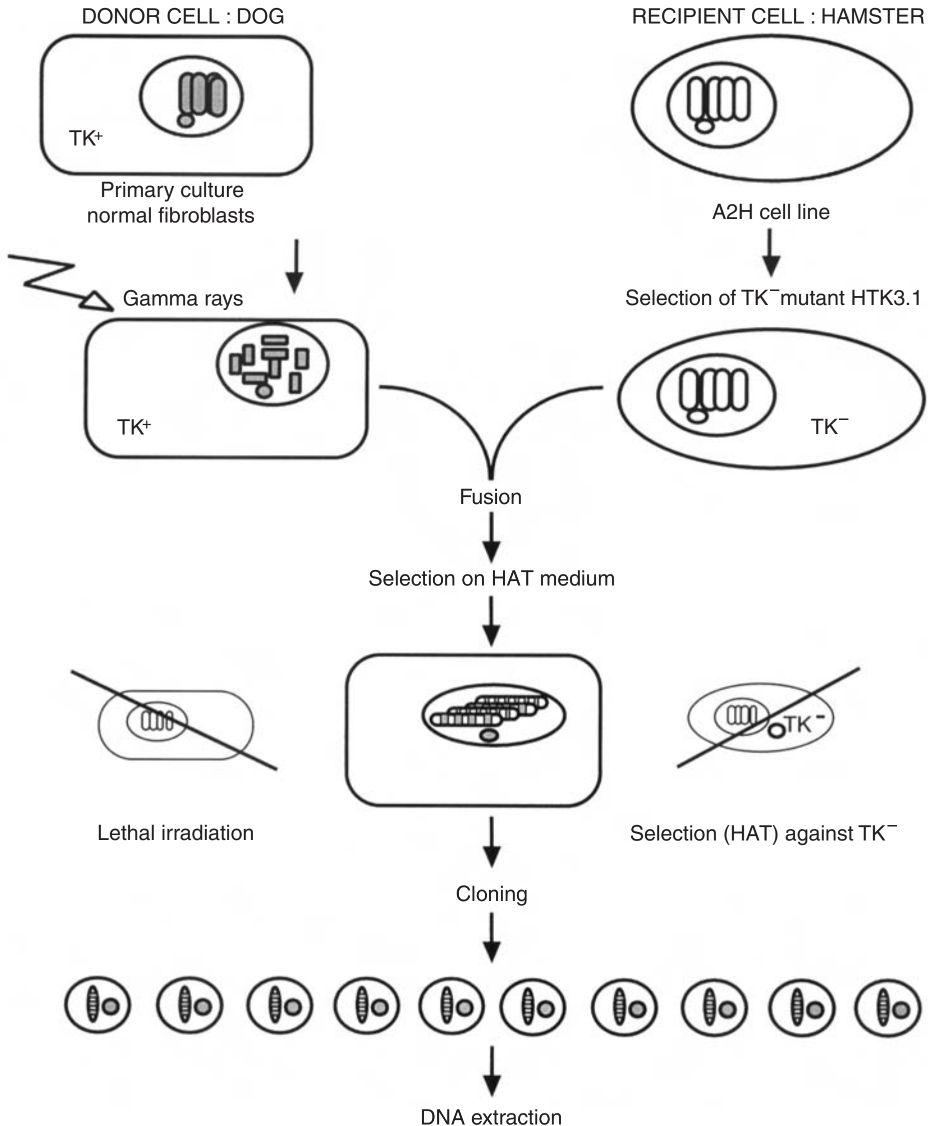

Fig. 12.1. Development of a canine–rodent radiation hybrid panel. Dog fibroblasts initially irradiated at 5000 rad (50 Grays) were fused with thymidine kinase-deficient hamster cells. TK+ clones were then selected following growth in HAT media. Each resultant cell line contains a full complement of rodent chromosomes and some random portion of the canine genome. A total of 126 cell lines were sufficient to provide >99% coverage of the canine genome.

using standard maximum likelihood statistical methods. Initially, a lod score of 6.0 (odds of 1,000,000 : 1) was used to assign markers to RH groups. Three hundred and forty-seven markers were linked to at least one other marker, identifying 57 RH groups. Out of these 57 RH groups, 12 had ten or more markers, with the largest having 21 markers. The average spacing between markers was about 28 cRay, corresponding to a physical distance of about 3.5 Mb. To develop the map with the greatest possible accuracy, a subset of 211 framework markers was initially defined. For 180 of these, the most favourable order of markers was better than any other by a maximum likelihood ratio of at least 1000 : 1; for the remaining 31 the odds were ≥100 : 1.

The first-generation map has since been expanded and now encompasses 600 markers, divided into 77 RH groups, calculated with a lod score of 8.0 (Mellersh *et al.*, 2000). Five hundred and twenty-three markers (87%) were linked to at least one other marker with an average inter-marker distance of 24.3 cR_{5000} and 77 markers (13%) were unlinked to any other marker. RH group sizes ranged from 29 markers (CFA1) to 2, with an average of 7. This iteration of the map also identifies 308 framework markers ordered with odds ≥1000 : 1.

Recently, considerable efforts have focused on adding more markers to the RH map, and as a result, a map of 1500 markers is expected shortly (such a map will be composed of several hundred microsatellite markers and over 400 genes). With a marker placed, on average, every 2 cM and multiple genes localized to each canine chromosome, investigators will have the ability to navigate within small regions of the canine genome as well as to move easily between the canine and human genomes to optimize searches for candidate genes.

Integrating the Linkage and RH Maps

Advantages of an integrated map

Recently, we merged the current linkage and RH maps (Mellersh *et al.*, 2000). In the early stages of genome map development, this process has several advantages. First, due to differences in the distance over which linkage can be detected by RH and linkage data, a low density RH map consists of many more distinct RH groups than a meiotic map does linkage groups, even if the overall density of markers of the two maps is similar. Markers for which there is both linkage and RH data can be used to allocate multiple RH groups to single meiotic linkage groups. As a result, the density of markers per chromosome quickly increases. Second, because it allows genes as well as microsatellites to be positioned on the same chromosomes, when linkage is detected between an anonymous microsatellite and disease phenotype, candidate genes can be selected from corresponding regions of the human map. A list of genes that have been positioned on the canine RH map is described in Table 12.1, including the canine, human and mouse location (where known), the

Table 12.1. Location of RH mapped genes.

Gene symbol	Gene name	Canine location[1]	Human location	Mouse location	GenBank accession number	Forward primer	Reverse primer
ACP5	Acid phosphatase type 5, tartrate-resistant	CFA20	19p13.3–p13.2	9 (6.0)	M98533	ACTTCCCCAGCCCTTACTACCG	GCCGCACAGGTAGGCAGTG
ACTB	Beta actin	UN	7p15–p12	5 (86.0)	M10277	TCGTCACCAACTGGGACGAC	CGGAGTCCATCACGATGCC
ADCY5	Adenylate cyclase 5	CFA29–35	3q13.2–q21	16 (syntenic)	M97886	CAGACGGGGATCCTTTCATTTTC	TTCAGGCAACAGACCAGGAGC
ADRB3	Beta 3 adrenergic receptor	UN	8p12	8 (10.0)	U92468	CGAGGTCTGAAGGATAAGCG	TGTCCACAATCTCGCAGAAG
AGC1	Epidermal growth factor-like domain-1	CFA3	15q26	7 (39.0)	L29487	AGAGTCCCTAGAAACATGCTCACCC	TATGATGTGTCCCTTCATCTGCTGA
ALAS2	Erythroid aminolevulinate synthase	UN	Xp11.21	X (63.0)	U17083	GATAAGCAGAGGTCTGGGAAAGGAA	CTTACCTGGAGATAATGGGGGGAG
ALDOA	Aldolase A	CFA6	16q22–q24	unknown	U03565	TGAGGCAACTGTTCTGGATCCTC	AGCCAAGATCTGATCTCCATGACAG
ALDOC	Aldolase C, fructose biphosphate	CFA9	17q11	11 (44.98)	U03570	GTGGCGTTGTGTGCTGAAAATC	AAAGCCCTCCTAACCCTCTCCC
ANP	Natriuretic peptide precursor A	RH7-b	1p36.3–p36.2	4 (76.5)	X54669	GCCCAGAGAGATGGGGGTG	CGGAAGCTGTTACAGCCCAGTC
ANPEP	Aminopeptidase N	CFA3	15q25–q26	9 (20.0)	Z29522	ATCATCCATAGCAAGAAGCTCAA	GTACTCGCTGCGGTAGAAGC
ANXIIIB	Annexin XIIIb	S8	8q24	unknown	X80209	AGCAGCCTCAAAACGCTTGG	ATTCAGTCTCTACCCACTCTGTGCG
APH	Acylamino acid-releasing enzyme	UN	3p21	unknown	D00524	GGGCTCTATGTGGGGTATGA	TGCCACCTGATTGGTCAGTA
APOH	Apolipoprotein H (beta-2-glycoprotein I)	CFA9	17q23–qter	11 (63.0)	X72933	ATGCATCAGATGTAAAGCCATG	ATTTTTCCACCTTTCTTTATGTTCC
APSP-A	Pulmonary surfactant-associated protein A	UN	10q22	14 (14.0)	L41350	GGTCTTCTCCACCAATGGG	CCATCTGTGTACATCTCCACAC
AR	Androgen receptor	X	Xq11.2–q12	X (36.0)	L11357	CAGCAGCAGCAGCAGGAGAC	TCCAGGCTCTGGAACGCAG
ARGES	Arginine esterase	CFA1	unknown	unknown	M63669	GACCCTGATGAAAGGGAGGATAATG	CCCTGCCCAACATGGTCTTC
ATP1A	H^+,K^+ -ATPase alpha subunit	CFA1	19q13.1	7 (9.8)	M36978	ACCATCATCGGGCGCATT	CATAGGCTACCACGATGGCCAT
ATP4B	H^+,K^+ -ATPase beta subunit	S7	13q34	8 (4.0)	M76486	TCTCAATGTCCCCAGAAACAC	CTCCACTTTCCCTTCATAGGG
BCHE	Butylcholinesterase	RH1-b	3q26.1–q26.2	unknown	M62411	AACACCTAAACCAAAAAACGCCACT	GTCAAATAAGCCCAGGTTCCCTG
BDNF	Brain-derived neurotrophic factor	S2	11p13	2 (62.0)	X16713	CCTTTCGACACAGGGACTTT	GACAGCATTAGCGAGTGGGT
BGT1	Betain-GABA transporter	CFA16	12p13	unknown	D42037	GAGGTGTCCCTAGTCCCACA	TCTCCTCACCCACAAAGTCC

BRCA1	Breast cancer 1, early onset	CFA9	17q21	11 (60.5)	U50709	GAGTCCTAGCCCTTTCACCCATAC	GTGATGTTCCTGAGATGCCTTTG
BRCA2	Breast cancer 2, early onset	S10	13q12.3	5 (89.0)	Z75664	CCATCCTGGCTTTTGTCATT	TGTGGGTCTGTGTCTGCTTC
C5FW	C5FW protein	CFA15	2p23	unknown	X99144	AAGCCTGGAAGATGCCTACA	GTAGCTGCCACTGGTGTTCA
C5R1	Complement c5a receptor	CFA1	19q13	unknown	X65860	CCCCCTCTGCTGATTTTAACCC	TCCCATGCACAATCTCCACCT
CALCA	Calcitonin	S2	11p15.2–p15.1	7 (54.0)	X56994	CGGGGCTGAAACACCTGG	AATGCCAGCTGGAAGAGCAATC
CALCR	Calcitonin receptor	S9	7q21.3	6 (4.5)	M74420	ATGTACCTGAAGGCGGTGAG	CTTCCCAAGCATCTTGTTCG
CALM2	Calmodulin	CFA10	2p21	unknown	D12622	CCATTTTATGAGGCTCGAG0AGGACT	AAGTCCCTCAGAAACCACGGC
CANX	Calnexin	S4	5q35	11 (30.0)	L005594	TGAATGAACTGTAAGCACATCATGC	TGCAGTCAACTCTCAGCCAACC
CAPS	Calcyphosine	CFA20	19p13.3	Unlinked	X14479	TATGCAAGTAGGACAAAGGTTCCGA	CAAGTGGTGGGAAGGGTAGGTG
CD19	Cell differentiation antigen 19	CFA6	16p11	7 (59.0)	AF024717	TTCTGGCTTTGGATGGATTC	TGGTTAGGGTCAGCATCACA
CD28	Cell differentiation antigen 28	S11	2q33	1 (30.1)	L22178	GATTTTGGTGAAGCAGTTGCCC	ATCGCAGTCGAATCCTGTACTTGAG
CD34	Cell differentiation antigen 34	CFA7	1q32	1 (106.6)	U49457	TGAGGCTCAGGGAAAGGCC	GTGTGCAGCAGGAAAGGCTAGAA
CD3E	Cell differentiation antigen 3	CFA5	11q23	9 (26.0)	M55410	TCCAGGTCCTGCTACTCCCAGTAC	CGGTGAGTGGCCATGATGAG
CD4	Cell differentiation antigen 4	CFA16	12p12–pter	6 (57.0)	X68565	TTGAATCTGAAAGAGCAGGCTGC	GTGATAGCCAAGAACTTTGGCCAG
CD44	Cell differentiation antigen 44	CFA18	11p13	2 (56.0)	L28932	TGGAAGAGAAGGTGGACATCTTCC	GGTCACCGGGATGAGGGTC
CD9	Cell differenciation antigen 9	CFA16	12p13	6 (57.0)	U15792	AATGCTTTATTCAATATTGGTATTT	ATGTATCAAATTGTCTTCAACATAA
CGMPPB	cGMP phosphodiesterase beta subunit	UN	4p16.3	5 (57.0)	Z23014	GTGATGTTCCTGAGATGCCTTTG	GTGATGTTCCTGAGATGCCTTTG
CHM	Choroidermia	X	Xq21.1–q21.2	X (96.5)	–	GGGAAGGTGGCAGTAGCAAGG	CAGAGACTAATGAGGGGGCTCC
CHRM3	Muscarinic acetylcholine receptor III	UN	1q41	unknown	X12712	GGTCTGTGACCTGTGGCTCT	AAAGAGATGACCCATGCCAG
CLCN	Chloride channel	UN	unknown	unknown	X65450	TTTACCTGAACCAGTTCTTTTTTTG	GATGCATATAGTGAAAGCAGGAACT
CLN3	Ceroid lipofuscinosis, neuronal 3	CFA6	16p12.1	7 (63.0)	L76281	CAGCTGCCTGTATCTCCGAC	GGGAAGCCAGAGTGTCAAGA
CLPS	Colipase	CFA12	6pter–p21.1	17 (17.1)	M63427	CTGGGCCTCGGGTTACGTC	GACCACCGCAAGGGCAAC
CNCG1	Rod cGMP gated channel alpha subunit	S8	4p14	5 (41.0)	X99913	CTGAGGGTGTGAGGGGAATA	TTGTCAGGGAAGATGGGAAG
COL4A5	Collagen type IV, alpha	UN	Xq22	X (62.0)	U07888	CCCTGGACCAGATGGAATGC	ACCGTGAGCTCTTTTATTTCCTTGG
CfOLF1	Olfactory receptor gene 1	CFA18	11q11	unknown	U53679	GTCTCGGGGCATCTGTGTAT	GATGGCCACAGAAGTCAGGT
CfOLF2	Olfactory receptor gene 2	CFA18	11q11	unknown	U53680	AGAGTGTGCTCCCTGCTGAT	TGCAACAGCAGTTAAGTGGG

Table 12.1. *Continued*

Gene symbol	Gene name	Canine location[1]	Human location	Mouse location	GenBank accession number	Forward primer	Reverse primer
COLF4	Olfactory receptor 4	CFA20	19p13	unknown	U53682	CTTAGCTCCGCTGCTCCC	AGGCTTCCCTCTGAAAAATA
CRTL1	Link protein	CFA3	5q13	unknown	Y00165	GGCGCTACAACCTCAATTTTC	TTGGATGTAAAACAGAAAACATCAT
CRYB	Beta-A3/A1 crystallin	CFA9	17q12	11 (44.71)	U22412	CTCAGGTTGTAGGGTACTGACCCCT	CTCCTCCATGATGGTCACATTCC
CRYGB	Gamma-D-crystallin	UN	2q33–q35	1 (32.0)	U27808	ACTACCAGCAGTGGCTGGGC	GGTAGCTGGTCTTCTGTCCAAACCT
CYP1A1	Cytochrome p450 cholesterol desmolase	CFA30	15q23–q24	9 (31.0)	X13768	GAGCCTGGAAGAAAGACCG	CTCAAAGGCGAAGCGAAAC
CYP3A	Cytochrome P-450, IIIA subfamily	CFA6	7q22	5 (82.0)	X54915	CTATCCCAGAACACCAGAGACCTCA	GTCAGTCACCCCATCCCCC
CZP2	Zona pellucida 2 glycoprotein	UN	unknown	unknown	E07830	GACTCAAAAGGGCACAGGGCT	AACACCATGGTTCTTTTCTTACGCA
DDOST	Oligosaccharyltransferase 48 kDa subunit	CFA2	1p35–p36.1	unknown	M98392	AGGCACTTGGGAGTTCTGCG	AGTGCCGCATCTTCCCTGAG
DGL	Triacylglycerol lipase	CFA26	unknown	unknown	Y13899	ATGCCCAAGCGGTTTACAATG	GGGGGAGCAGCAGGAGGAGAAG
DIO1	Iodothyronine deiodinase, type 1	CFA5	1p32–p33	4 (48.7)	U11762	ACAGGAGGGCTCCTCAAGTCCT	CCCCAGCAGTATCCAGTGGG
DLA-A9	MHC class I DLA-A9/A9 alpha chain	CFA12	6p21	unknown	M32283	AACAACCCTGGCTTGTCTCTGC	TCTCCTGGACAGGAAAGCACATC
DLA-DQA	MHC class II DQA chain	CFA12	unknown	unknown	M74910	GGTTCCTGAGGTGACTGTGTT	CCCCAGTGCTCCACTTTG
DLA-DRA	MHC class II DLA-DRA	CFA12	6p21.3	unknown	L37332	ACCCCTGTGGAACTGGGAGAG	CATCCAAACCCCAGTGCTCC
DLA-DRB1	MHC class II DLA-DR-beta-1	CFA12	6p21	unknown	M29611	CCTTTCCAGCTTCTTCTCAGAGTCC	AAGGTACTGCAGTCAGCACTGGG
DLA79	MHC class Ib	CFA18	6p21	unknown	Z25418	TCTATTCTGGCATTGGGGAC	TGAGTAGCTCCCTCCTTTTCTG
DLA88	MHC class I DLA-88	CFA12	6p21	unknown	U55028	CTGATGTGCTTTCCTGTCCA	CAGCCTCACATCCCAAGACT
DLD	Dihydrolipoamide, NAD^+ oxidoreductase	UN	7q31	12 (15.1)	U19872	CTGGAAAATTTTCTTCAACCCTTAT	TCACCTGGCTTCAAACACAG
DMD	Duchenne muscular dystrophy (dystrophin)	X	Xp21.3–p21.1	X (32.0)	L02114	TGGAGCCGAGCACACAGC	CCAAGAGTAGAGTTCCTTTGCCACA
DTMT	Olfactory receptor	CFA9	17q21	unknown	X64996	AGCCTGAGGAATAAAGACATGAAG	TGGGTAAGAGAAAACATCTGGAATA
EDN	Endothelin	RH5-b	unknown	unknown	X14610	CAGGCTTCTATGTGCTCCTCATGAT	GTTGACCCAGATGATGTCAAGGTG
EDNRB	Endothelin beta receptor	S7	13q22	14 (51.0)	AF026088	CAGCTCCAATTCTCCTTTGC	CCATGCCCAATTCTCTCTTT
EPO	Erythropoietin	CFA6	7q21	5 (80.0)	L13027	AAAGCTGACACTGTACACAGGGGAG	TGCTGAGCAGATCACCCCTG
F7	Coagulation factor VII	S7	13q34	unknown	D21213	CACTGAGAATATGTTCTGTGCCGG	CTGTCAGGTACCATGTGCCCTG
F8C	Factor VIII	X	Xq28	X (30.5)	–	GATCTAGTGCCTGTAAAAAC	CCTGGCTCCCTAGAACCACG

F9	Coagulation factor IX	UN	Xq26.3–q27.1	X (22.0)	M21757	ACATCAACTCCTGCGTCTCATCC	GCCACCAGTACATCCTTCTCCACT
FCER1A	Immunoglobulin E receptor alpha	S17	1q23	1 (94.2)	D16413	AACTGATGCCGCTGCTTAAGAAAC	AAGCCATGACTGCAGACCTTCC
FGA	Fibrinogen A alpha	S6	4q28	unknown	D43756	TCCTGGGGACTACAAGAGCCAG	GTGGAGCTCCCAGGGTTCCT
FGF1	Fibroblast growth factor 1, acidic	CFA2	5q31	18 (19.0)	X60317	CAACATGAAGCCCAAACTCC	GTGCTGGTCGCTCCTGTC
FGG	Fibrinogen, gamma polypeptide	S6	4q28	3 (41.3)	M29510	GAAGCAAAGTGTCAGGAACCTT	ATTTCCAGACCCATCAATTTCA
FN1	Fibronectin 1	S11	2q34	1 (36.1)	U52106	GGACCAGAGATCTTGGATGTTC	CAAGAGATGGTTGTCTGAGAGAGA
FORS	Forssman synthetase	CFA9	9q34	unknown	U66140	CCTTCAACCCTGAGCTTCTG	ACCCTGCATGAAGAACTGCT
FSA	Follistatin	S1	unknown	unknown	M19529	GTCTGTGCCAGTGACAATGC	AAGATCCAGAGTGCTTTACTTCCA
FSHR	Follitropin receptor	CFA10	2p21–p16	unknown	L31966	CCCATCTTTGGCATCAGC	ACACAGTGATGAGGGGCAC
FUCA1	Alpha-l-fucosidase	CFA2	1p35–p34	4 (67.2)	X92675	ACTGTTCCTGTCACCATGGAG	CCCACCACTCTTAGTCAGAAGG
GALK1	Galactokinase	CFA9	17q23	11 (78.0)	–	AATTGAGTTTTTGGGGTGCCTGAG	CAATGAGCTGAGATGGTGTAGTAG
GAPD	Glyceradehyde-3-phosphate dehydrogenase	UN	12p13.31	6 (56.0)	M17851	ATTTTTTGATGTACGTTTTGCTACC	TAAGTGAGTTTTTAGAGCCCCTAGG
GAS	Preprogastrin	CFA9	17q21	11 (60.0)	V01303	CGCCTGCTCTGAAGCTTCTTG	GGCCGAAGTCCATCCATCC
GCSig	DNA sequence with GC signal	CFA9	9	unknown	L07047	CCATGCTTGTTCAGCCGTTG	ATGCTCTTCCCCTTCCCCTG
GGTA1	Alpha-1,3 galactosyl transferase	CFA9	9q33–q34	2 (25.0)	L36535	GAAGAGGTGGCAAGACATCAG	TGGTAATAAAAATCCCCCTGG
GHR	Growth hormone receptor	S1	5p13–p12	15 (4.6)	X54429	CTCACGTCGAGCTGAATCAC	CGGAGACTGGATGATGTGAA
GLUL	Glutamate ammonia ligase	CFA5				CCTTCTTCTCCTGGCCGAC	GGATTCCATGAGACATCCAA
GNRHR	Gonadotropin-releasing hormone receptor	S8	4q21.2	5 (44.0)	L29342	GCTTCTTTCTTGTTGAAACTTCAGA	AGCGGTCCAGGCTAATCAC
GP40	Mucin type membrane protein	RH7-b	unknown	unknown	Z81018	TCCTCTAGGCAGGCATCTGT	GAACGTCGCACTCAACTCAA
GP80	Glycoprotein 80	S10	unknown	unknown	M55251	CCCCTAGAGAGAGCTCTGCATGTC	ACAGGAGTTCTTCCCATGATCAGC
GPCR	Transmembrane receptor	S7	13q32	unknown	L42326	CTCCACACCCCCCACCTG	CCCGTGAAGGAGACTATGAATGATG
GPIB	Glycoprotein Ib	UN	17p12	unknown	U19489	AGGCCTTAAGCACCTTTTCTG	CTCGTCTTGGTGCATCTCTTC
HBB	Beta globin	S2	11p15.5	7 (50.0)	X86791	CAATGCTAAAGTGAAGGCCC	CTGAGCTTAGCAAAGGTGCC
HPD	4-Hydroxyphenyl pyruvic acid dioxygenase	CFA26	12q24	unknown	D13390	CAATCACCAGGTACCAGCAA	GAGGGGGAGAGAGACGAAAG
HSD20B	20 beta hydroxy steroid dehydrogenase	S13	unknown	unknown	M80709	CATGGCATTCTGGACAGGAC	GGAAGACACAAAGAAAGGCG
HSP27	Hsp27-heat-shock protein	CFA6	unknown	unknown	U19368	TGTCCCCTGAGGGCACTCTC	AGTGATGGCTACTGAGGTGAGGATG
HTR1D	5ht1d type serotonin receptor h	CFA2	1p35	4 (66.0)	X14049	CCGGAAAGCCTCCTAGTCTGATTT	CCCAAGACATCAGGATTGACCC
HuEST-D25247	Human EST	CFA3	15q25	unknown	D25247	CGTGCTAAAGGCATCACA	CCAAATGAAGGCAGTGCT

Table 12.1. *Continued*

Gene symbol	Gene name	Canine location[1]	Human location	Mouse location	GenBank accession number	Forward primer	Reverse primer
HuEST-D29070	Human EST	S2	11p15	unknown	D29070	GGTTCCCCAGACTCCAAACA	TGCAGAAGTGCTCGGGC
HuEST-D29206	Caveolin-1	S9	7q31	unknown	D29206	TATTGTGCAGGCTTGTAACC	GCTTTGTGATTCAATCTGTAAAC
HuEST-D29228	Human EST	CFA9	17q12	unknown	D29228	GAATGTCTGAGAGGCTGCT	CACTTTTGTGTCCCAAGGTAA
HUEST-D29618	Human EST	CFA5	unknown	unknown	D29618	CTATTGCTTATTTATTCAGGGTTGT	AGCGAACTTGCATGGTG
HuEST-D59484	Human EST	S12	unknown	unknown	D59484	CCGGGAAGATGTCTATTTTT	TTCAGAGCACTTACATTAGGAAA
HuEST-L03411	Radin blood group	CFA12	6p21	unknown	L03411	GCAGGTCGGATTCATTC	CCATCTTTTCATAGGTGACG
HuEST-L08069	DNAJ protein homolog 2	UN	9	unknown	L08069	GGGTCGCCTAATCATCG	GCCAGCAGTGAGTGTTATTC
HuEST-L08893	–	UN	23	unknown	L08893	TGCGTAAACCCCTTTGC	TACACCCAGTGAACGAGGTC
HuEST-L15189	Mitochondrial stress 70 protein precursor	UN	2	unknown	L15189	ACCTGCTGATGAGTGCAAC	GAACTTCCAGAGCCTTCTCG
HuEST-L33987	Gene from CpG-enriched DNA	CFA1	4p16.3	unknown	L33987	ATCTTCTTGGCGGCAAC	AAACATCGTGTCCCCTG
HuEST-M13150	Human EST	CFA1	6q24–q27	unknown	M13150	TTCGCTATGCCCATGAG	ATCTTTGAAAGCCCTGGTC
HuEST-M14584	Human EST	S9	7p21	unknown	M14584	TGGGCACAGAACTTATGTTG	TTGAGGTAAGCCTACACTTTCC
IAPP	Islet amyloid polypeptide (h) amylin	CFA16	12p12.3–p12.1	6 (62.0)	X59998	TTTAATGCTAATTAAGACCCCATCA	CTTAACTTCACTCTCCGCTGTTTAC
ICAM1	Intercellular adhesion molecule-1	CFA20	19p13.2	9 (7.0)	L31625	ACTGGCAGCGGTGCCATAAT	ACAGGTCAGGCTTAGACGTCCATC
IFNA1	Interferon-alpha subtype 1	S4	9p22	unknown	M28624	ATGGAGAAAGTGCATAAGGGAAAGC	CATCGGGGATCCTGCAGG
IFNA2	Interferon-alpha subtype 2	S4	9p22	unknown	M28625	ATGACAAGCACGCCCATGTAGTT	GGCAAGTGTGGGAACCGC
IFNA3	Interferon-alpha subtype 3	S4	9p22	unknown	M28626	CAAACGCCCAGTGTCAGGG	TGTCTCCTTGTGGCTCTTTCCC
IFNW1	Interferon omega	S4	9p22	unknown	M28627	CAAATACAAAAGAAAAGGCAACAAT	GCTCAGGCTATACCAAGATGATTAA
IGF1	Insulin-like growth factor 1	S6	12q22–q23	10 (48.0)	L08254	AGCCCACAGGGTACGGCTC	CTTCTGAGCCTTGGGCATGTC
IGHA	IgA heavy chain constant region	CFA8	14q32	unknown	L36871	ACCCATCCAGAAGAATCCTGAGC	CCTCACAAACTTTTGTGTGCCAAG
IGHE	IgE heavy chain constant region	CFA8	14q32–q33	unknown	L36872	CCACTGCCACATGGCTGC	CCTCCTTTGTGCCGGGTG
IL10	Interleukin-10	CFA7	1q31–q32	1 (69.9)	L20001	GGTTACCTGGGTTGCCAAG	GCCTCAGCCTGAGGGTCTT
IL1A	Interleukin-1 alpha	RH2-b	2q13–q21	2 (73.0)	M86730	TGCCTGAGACACCCAAAAC	GTTTTCCAGTATCTGAAAGTCAGTG
IL1B	Interleukin-1 beta	RH2-b	2q13–q21	2 (73.0)	Z70047	CTGCTGCCAAGACCTGAAC	CTTCAAAGATGTAGCAAAAGATGC
IL2	Interleukin-2	CFA13/19	4q26–q27	3 (19.2)	U28141	ATTTTTTGATGTAÄCGTTTTGCTACC	TAAGTGAGTTTTTAGAGCCCCTAGG
IL2RG	Interleukin-2 receptor, gamma chain	UN	Xq13.1	X (38.0)	U04361	AGTGAATGGCTCTGCCACGTC	GTGAGCATTGGACCCAGGTTG

IL6	Interleukin-6	S9	7p21–p15	5 (17.0)	U12234	TTCATGGGCATTCCTTTCTC	CGCATAGCATTTTACGTTAACTTTT
IL8	Interleukin-8	S8	4q13–q21	unknown	U10308	GCCAATGAGACTTCAAAAAAATCTA	GTGTGCCTAGACATTGAATAGGG
IVL	Involucrin	S16	1q21	unknown	M34442	AGCAGCCTGCCTTTGTCCC	TCACTGGTGTTCTGGAGGGTCAT
JUNC	Junctional sarcoplasmic reticulum	S3	unknown	unknown	U38414	CTGAAAGCTCTAATACCATCATC	ACATTTATCTGGATACATGATCAA
KCNA4	Potassium channel	S12	11p14	2 (61.0)	U75213	GCTGAAGGAGACAGAGGAGG	CCAGCCCTTGAAGTAGTGGA
KCNA5	Delayed rectifier K^+ channel	CFA16	12p13	unknown	U08596	CAAGGGGTCCTTGTGCAAGG	TCGCCGCAAGTGCACATT
KRT9	Keratin 9	CFA9	17q12–q21	unknown	AF000949	CCCCTGGCCTATGAGAGC	TGTCCCCAAACCACCTT
LALBA	Alpha lactalbumin	CFA16	12q13	unknown	M80520	CAATTTACAAAATGTGAGCTGTCC	GTCACAGGAGATGCCACAGAT
LGALS3	Beta galactosides binding lectin	CFA8	14q21	unknown	L23429	GAAAAATCTCCCGGAAATCAG	AGTCGAAACATGAAGTTTACATGTG
LHB	Luteinizing hormone beta subunit	CFA1	19q13.3	7 (23.0)	Y00518	ATGAGCTGCACTTTGCTTCCATC	TCCTTAGAGGAACAGGAGGCCC
LYZ	Lysozyme	CFA10	12q14	10 (66.0)	L14853	TTTGGCCAAGTGGGAAAGT	GCAGGATATGTGACAGGCATT
MNA	Alpha muscle nicotinic acetylcholine receptor	UN	2q32	2 (44.0)	Z25473	TTGTTTTCCCATCTGTAAAATGG	GCTCCAGTAAAAAAGATTTGATTCA
MUC	Mucin	RH4-b	unknown	unknown	L03387	TGCTCTCTTCCTTCCCACATCC	ACACTGCCGGGAGGAGACAC
MYH7	Myosin heavy chain, cardiac muscle beta	CFA8	14q12	14 (20.0)	L10129	GGTGCTCTACAACCTCAAGGAG	GATGGAGAAGATGTGGGGC
NACL	Na/Cl dependent betaine transporter	CFA16	12p13	unknown	M80403	GAGGCTTCTTGAGGTGTCCCTAGTC	AAGTCCAGAGGTTGGACCAAAGTG
NF1	Neurofibromin 1	CFA9	17q11.2	11 (46.06)	–	TCCCAAGTGACGGTTAGAGC	CCAGTGCCAGGGAGAGTTT
NFKB1A	Eci-6/ikba	CFA8	14q13	unknown	Z21968	GTGTGGGGCTGATGTCAAC	GAACTCTGACTCCGTGTCATAGC
NGFB	Nerve growth factor beta	S16	1p13.1	3 (48.5)	L31898	TGGGATCCACAGTGATGTTG	TCAGCATTCCCTTGACACAG
OCLN	Occludin	CFA2	5q13.1	unknown	U49221	TCTGCAACGTTGTCAGAAGG	ACTCAGTCCTTTAGCACAACAGC
OPSIN	Opsin	CFA20	3q21	unknown	X71380	CCCTACACCTACCCCAGCCAC	GGAGGGGCAGAGATCCCACT
P180RBC	Ribosome receptor	S5	unknown	unknown	X87224	GGGCACTTCTGTCTGAGTCTG	TGTGACAAAGGTTCTTTTACAAACA
P450IIb	Cytochrome p-450 II b	CFA1	19q13.2	7 (6.5)	M92447	TCTTCCTGCCACTGAAAGACCC	GTCAAATCTCCCCTTAATCCCCAG
PAFAH	Plasma paf acetylhydrolase	CFA12	17p13.3	unknown	U34246	TAGGCTATCCCATAAAAGTGATTGA	ATTAGCCTTGTGCCTGTTATACTTG
PCSK2	Prohormone convertase	S5	20p11.2	2 (81.4)	X68603	TGGAGTTTAACCATCTCTTTGG	CCTCCCACACAGTGGAATCT
PDEA2	Cone photo. cGMP-phosphodiesterase alpha sb	S1	10q24	unknown	U52868	CTGCACCGAATGGTACCAC	TGAAAATGATTCTAAGCAAGCAGA
PDEG	Rod photoreceptor phosphodiesterase gamma	UN	17q21.1	11 (75.0)	M36476	AAGCAGCGGCAAACCAGG	CCAGGCCTTCCATTCCAGG
PDYN	Preproenkephalin b	S5	20p12	2 (73.5)	J00650	AGGACCCCAAGGAGCAGGTC	TCTTCCTGAGACCGAGTAACCACC
PFKM	Muscle type phosphofructokinase	CFA16	12q13.3	unknown	U25183	TCTAACCCTCTTTGGAGTGAGGGTC	CAGATGAAGCTGGGGTAGAAGTTGA
PGK1	Phosphoglycerate kinase	Xq13-q21	Xq13.3	X (45.0)	–	AATATTTTTCATGTATAGCACTCC	ATGCTCCCTTCAGTTCTTTCC

Table 12.1. *Continued*

Gene symbol	Gene name	Canine location[1]	Human location	Mouse location	GenBank accession number	Forward primer	Reverse primer
PGR	Progesterone receptor	S2	11q22	9 syntenic	Z66555	ATGAAAGCCAAGCCCTAAGC	GGCAGTGACTTAGACCACTTGAC
PLA2	Phospholipase secretory a2	CFA26	12q23	unknown	M35301	AACAAGGAGCACAAGAACCTGGAC	CTTTATTGGAGGACACAGGGTAGGG
PLN	Phospholamban	CFA1	6q22.1	unknown	M35393	GAAAAGGTCAAGATTCAGAACAAAA	TTTTACAAGCTTTGTTGAGGAATTC
PNLIP	Pancreatic lipase	S14	10q26.1	19 (29.0)	M28151	CTCAAACCAGGCTCTACTCATTCCA	CTCCTCTCCCTTTTGCACGGT
PON2	Paraoxonase 2	S9	7q21.3	6 (1.5)	L48515	TTCTCCAGGGAAGTTCTGTAGC	CGTGGTTAAATTCCCACAAAAT
POR	NADPH p-450 cytochrome oxidoreductase	UN	7q11.2	5 (79.0)	L33893	CCCATGTTCGTGCGCAAGT	CAGCCGTAGTAGAGCAGCGTCTC
PROC	Protein C	CFA13/19	2q13–q14	Unlinked	D43751	TGGACATCAAAGAGGTCCTTATCCA	AGTCACCACGGTCTCCTGGC
PTH	Parathyroid hormone	S2	11p15.3–p15.1	7 (52.5)	U15662	CACTGCTGTAGACAGCATAGGG	CATGGCTAACAATGGATTACATGTA
PTHLH	Parathyroid hormone-like peptide	CFA16	12p12.1–p11.2	6 (73.0)	U15593	AATGCATTGGAATAAAACTGTCTTC	AAAAAATAGAGAAATTCAGCAGCAC
RAB11	Rab11-ras related GTP-binding protein	CFA30	15q	unknown	X56388	CCACTGAAAACAAGCCAAAGGTG	CATTCGGGACAAGTGGATGATTTTA
RAB12	Rab12 related-GTP binding protein	CFA7	unknown	unknown	Z22818	GATGCCTCTGGATATTTTAAGGAAT	AATTAACAGCATCGAACGTGTG
RAB2	Rab2-GTP binding protein	CFA1	unknown	unknown	M35521	ATGAGAAAATCCAAGAAGGAGTCTT	CCTGCTGTCCTCCCTGATT
RAB5C	Rab5c-ras related GTP-binding protein	CFA9	17q12–q21	unknown	Z27110	TAAGTGCCTCTTCACACAGCGC	CAGCTGGTCCATTGGGTCG
RAB7	Rab7 GTP binding protein	CFA20	3p25–p21	unknown	M35522	TCAGCGGAAAGCTGCAGTTG	GTGTCAGGGTGAAGTGTTTGGTAGG
RAB9	Rab9-ras related GTP-binding protein	UN	5q	unknown	X56389	TTCCCTGTGCGATCTAACCAACTTA	GATGTGTCCTTCTCCCACCCC
RAN	Ran/TC4 GTP-binding protein	UN	2q11	unknown	Z11922	CTAACCTGGAGTTTGTCGCCATG	CAGGTCATCATCCTCATCCGG
RARA	Retinoic acid receptor, alpha	CFA9	17q12	11 (57.0)	–	CAGGGCAGGGCGGGTCAT	GAAGATCTGAAGAACTTAGAGGAG
RBP2	Retinol binding protein 2, cellular	S14	10q23–q24	9 (57.0)	M68860	ACCACTGGATCATCGACACGG	CCTGGCCAGGCACAGCTC
RDC1	Rdc1-G protein coupled receptor	S10	3p22	unknown	X14048	AGCGTGCGCGCTTCCTAC	TTACAGAAGTGCAGGCGAGCC
RDC5	Rdc5-G protein coupled receptor	S1	unknown	unknown	X14050	GTACTGTCGCGTGTACATCGTCG	CAGCACAAGATGAACATACCGACC
RDC8	Rdc8-G protein coupled receptor	CFA26	unknown	unknown	X14052	AGAGGAGGGAGCCCTGGCT	GCCCTGCCACATCAGGATG

RDS	Retinal degeneration slow	CFA12	6p21.1–cen	17 (18.84)	U36577	GGTGTGTCCAACCCTGAAGACC	TGGTTACTCTTGCCCAACTTTTTCA
RHO	Rhodopsin	CFA20	3q21	6 (51.5)	Y09004	ATGGTCTCTGGAGCTAGGCA	TTGCTACTGCTGACATTCCG
ROM1	Rom 1	CFA18	11q13	19 (8.0)	X96587	CTCTTTGATCCTCGTCAGCC	TGAGGGTCAGTAGGTCCCTG
RTB	Rod transducin beta	CFA16	unknown	unknown	Z75134	CTGGGACAGCTTCCTCAAAG	ATACTCCCCATTTTCCCCAC
RTG	Rod transducin gamma subunit	S9	7q21.3	unknown	Z75156	GGCAAAAGATGCCAGTGATT	GGAGACCAGCATTCTTTCCA
RYR1	Ryanodin receptor 1, skeletal	CFA1	19q13.1	7 (10.0)	X69465	TGATGGGCATCTTTGGTGAT	TCACAGACTCTGGTAACTTCATCTG
SAA1K	Potential-serum amyloid A like protein	S2	11p15	unknown	M59172	AGGCCGGAGTGGCAAAGAC	TGGCCAGAGATCTCAACGAGTG
SEC61A	Sec61 homologue	CFA20	unknown	unknown	M96629	AGCAGTCGGGCGTTGTGAG	GGGGGGTCTGTTTCCTGGTTAG
SEC61G	Sec61 complex gamma	CFA18	unknown	unknown	L25086	GAGTCTTTCATCTTGGGGATTG	GGAAGACAAAAAAAAACATCAAAGA
SELE	E-selectin	CFA7	1q23–q25	1 (86.0)	L23087	GGAACACCGTACCTCCAGGAACTAG	CGTTGAACTCCTCATGGGGG
SELP	Cell adhesion molecule	CFA7	1q23–q25	1 (86.6)	M88170	CTGCTGGTCTCCTCACTCAAC	ACAAGTATTACCAGAAAACTGCAGC
SLC5A1	Na^+/glucose cotransporter protein	CFA26	22q12	unknown	M34044	TGAAGTGGGAGGTTATGATGC	CTGATCTGTGCACCAGTACCAC
SLC6A6	Na/Cl dependent taurine transporter	CFA20	3p25–p21	6 (38.5)	M95495	GGAGATGCTTCTCTGAGCCTGC	AACCTGCAACTGGAACTGACTCG
SLC8A1	Na^+/Ca^{2+} exchanger	RH3-b	2p23	17 (48.0)	Z49266	CTGGTTACTCCTCAATATGCCAG	AAGTGTTTCCATTTGGCTGTG
SMIT	Na/myo-inositol cotransporter	S13	21q21.2	unknown	M85068	GAAAATACTGGTCTTTGGAAAACAA	TTGAAAATTTACTTTCGTCCATCTC
SMN	Survival of motor neuron	CFA2	5q13	13 (54.0)	U50746	GTGGAAAGTTGGTGACAAATG	CTCCTGAGTATCCTGTTCTAC
SPC18	Signal peptidase complex	CFA3	unknown	unknown	J05466	AAGTATGCCGTCCTCTTCTTGCTG	AAATCCTGTGATTGAGAGTCCCCTC
SPC22/23	Signal peptidase complex 22/23	S12	unknown	unknown	J04067	TATTCCCTGTATGTCCATGATTCTT	ACCCTAAAAGAGAATGTCCACTTCT
SPC25	Signal peptidase complex	S2	unknown	unknown	U12687	GATGCATACGAGCCTGAAATATC	TCTGTTTCCTTCTCTCTCACCC
SRP54	Signal recognition particle 54k	CFA8	14q12	unknown	X16318	TGCTGCTGGCAATATGAAAG	GGAAGTAGGAAGGAAAGAAGATGAG
SRP68	Srp68 sb signal recognition particle	CFA9	unknown	unknown	X53744	CCAGGTCTGCATTGGCCC	ACGACAAAGTTCCGAGCTCCC
SRP72	Signal recognition particle	S8	1q32	unknown	X67813	CTTGGCCTACTTTACCATCCAG	TTCAGACTCTAGTTGAAACCGAAGT
SRY	Sex-determining region	UN	Yp11.3	Y	U15160	GAACGCATTCTTGGTGTGGTCTC	GGCCATTTTTCGGCTTCTGTAAG
SST	Prosomatostatin	RH1-b	3q28	16 (19.0)	L42325	TTGTCCATATAAGACCTCTGATTCC	ACAGTCTTCAATTTCTAATGCAAGG
TBP	TATA box binding protein	CFA12	6q27	17 (8.254)	L47973	CCTTTCTCTTCTGGAGGAAC	CTGCTGGGATGTCGACTG
THRA1	Thyroid hormone receptor, alpha	CFA9	17q11.2–12	11 (57.0)	–	AGCAGCTCCCCTTCACAC	CCCGCCGGTTCCCCCACTAT
TNF	Tumour necrosis factor	CFA12	6p21.3	17 (19.06)	X94932	TACTCCCAGGTCCTCTTCAAGGG	TCAGCGCTGAGTCGATCACC
TP53	Tumour suppressor protein p53	UN	17p13.1	11 (39.0)	U51857	CGGGGGCTACAGATGGGAC	CCTTCCACTCGGATGAGATGCT

Table 12.1. *Continued*

Gene symbol	Gene name	Canine location[1]	Human location	Mouse location	GenBank accession number	Forward primer	Reverse primer
TPCR63	Putative olfactory receptor	CFA18	unknown	unknown	X89661	AGGATACGTTCCTCAGAGGGCC	ATCTAATGAGTGGTTGGTCCCTGGT
TRA1	Glucose regulated protein ppk-98	S6	12q24	10 (49.0)	X90848	TTTGTTGAGCGACTTCTGAAAA	GCAGAGGCTCAAATTCTTTCTC
TRAMP	Tram protein	S3	1p36	unknown	X63678	CCCATCTTTAATTGAGAAGCATTTA	TACTGTGAAGACAACAAAAAAACCA
TSC1	Tuberous sclerosis 1	CFA9	9q34	unknown	–	GTGCACAGGCTACACTTGGGT	GAACCCTGAAAAATTCCACCA
TSHB	Thyrotropin beta	S16	1p13	3 (48.5)	U51644	TCGAAAGGAAAGAGTGTGCTTACT	TTACACTTGCCACACTTACAGCTTA
TSHR	Thyrotropin receptor precursor	UN	14q31	12 (37.0)	X17147	TGATGTTTCATGGGGCAAATTTC	GTGAGAAGCTCAATGTTGACCTGGT
TYRA	Tyrosine aminotransferase	UN	16q22.1–q22.3	8 (55.2)	L47165	CCCGGAGTCACACAGGAG	GTGCCCCAGCAAATGTATTAAT
UOX	Urate oxidase	UN	1p22	3 (75.0)	M27697	CACAGCATAAAGAGGTGGCA	TTCTTGATGGTGTCTGTGGG
VASP	Vasodilator-stimulated phosphoprotein	CFA1	19q13	unknown	Z46388	TCCCTGCCTCAACTCGACTTG	CCAATCCCCAACAGAGGAACC
VCAM1	Vascular cell adhesion molecule 1	CFA6	1p32–31	3 (50.8)	U32086	AATCAAAAGTGTAGCTAATGTTTG	CCTATGGCATAAATTTATTTTACA
VIP21	Vasoactive intestinal peptide 21	S9	7q36	12 syntenic	Z12161	CCGTGTCTATTCCATCTACGTCCAC	ATGTTTCTTTCTGCATGTTGATGCG
VIP36	Vesicular integral-membrane protein	UN	unknown	unknown	X76392	AGGCTGCAGGGCTAGGGAATAG	ATCACTGCTCACATCGTCAGGC
VWF	Von Willebrand factor	CFA16	12p13.3	6 (59.0)	L16903	ACACCTTCAGCGAGGCGC	GGGTTTCCTGTGACCATGTAGACC
WT1	Wilms tumour 1	CFA18	11p13	2 (58.0)	U00687	GGTGCCTGGAAACGTCCG	ACCGGGAGAACTTTCGCTGAC
ZAP47	Zymogen granule membrane associated protein	CFA22	3q23	unknown	D78348	CCTGTGATGAAACTTACTGTGGAC	CTCAGCATTGTTCTCTGTGAGTTTA
ZO-2	Tight junction protein	CFA1	9q13	unknown	L27152	TATAGATGCCTGATAGTCGGCGTGT	AAGACCCAGTTCCACAAACAGAGC
ZP3A	Zona pellucida glycoprotein 3a	CFA6	7q11	5 (79.0)	U05780	CAACCTCTCCTCACACCTCTGTGAT	AAGCAGTACGATGCCTCTTGGC

[1]As described in Mellersh *et al.*, 2000.

GenBank accession number and primer sequences. Linkage and RH data were available for 217 markers, making it a relatively straightforward process to generate a single, integrated map of 724 markers. Examination of 119 pairs of adjacent markers on both maps suggests that 1 cM is equivalent to 6.71 cR (Mellersh *et al.*, 2000).

Properties of the integrated map

In the integrated map, 70 of the 77 RH groups, plus 27 unlinked RH markers, are assigned to a meiotic linkage group (Mellersh *et al.*, 2000). With a single exception, all markers that mapped to the same RH group also mapped to identical linkage groups. There was good agreement between the order of markers assigned by RH mapping and linkage mapping. Specifically, of the 217 markers positioned on both maps, inverted orders are observed for only 18 pairs, and in most cases the two relevant markers are located so close to one another that it is difficult to determine the true order with a high level of statistical support. While the length of the integrated map is virtually the same as that of the third-generation linkage map, the density of markers has essentially doubled. The resulting integrated map spans 1589 cM and provides estimated coverage of over 2600 cM (calculated as described below). The framework markers provide a foundation for developing minimal screening sets of markers to be used when undertaking a genome scan. Examples of the integrated map for two chromosomes are shown in Fig. 12.2. For CFA 6 we note that several human chromosomes are also represented. For CFA8, however, the entire canine chromosome corresponds to a single human chromosome, 14q.

Recently we defined the first canine minimal mapping set of markers (Canine MSS-1), suitable for genome-wide scans (Richman *et al.*, 2001). The 177 markers which make up the set were selected (i) to be highly informative, (ii) to provide as complete coverage as possible of the canine genome, and (iii) were generally markers which could be ordered with a high level of statistical support. The resulting screening set spans all reported meiotic linkage groups and leaves few gaps of ≥20 cM. Importantly, the average PIC value of markers is 0.74. This suggests that most markers will be informative in most pedigrees tested. Coverage estimates suggest 42% of the genome is within 5 cM of at least one marker in the minimal screening set, and 77% of the genome is within 10 cM. While this analysis pays particular attention to the issue of theoretical coverage, the primary reasons any given locus will remain undetected in a genome screen is if markers in the region are uninformative in critical families, or if the pedigrees lack sufficient power to obtain a statistically significant lod score at reasonable values of theta. The former will be a particular problem for pedigrees of dogs derived from breeds with low heterogeneity. Thus we plan to continue to refine the mapping set as the map density increases, substituting more informative markers for less useful ones, and filling gaps where coverage is incomplete. Subsequent iterations of the minimal mapping set will appear on our respective websites.

Integrity of the integrated map

For both linkage and RH mapping, map integrity is a major concern. During development of the meiotic linkage map, efforts were made to minimize genotyping errors (the largest source of potential error) by requiring that each genotype generated on a radioactive gel be scored independently by two individuals. Both scores were entered into a consensus database and disputed genotypes were omitted. In addition, the segregation of each marker in a family is checked for Mendelian segregation using the *prepare* option of the computer program MultiMap (Matise *et al.*, 1994). Finally, some individuals were part of multiple families, perhaps a grandparent in one family and a parent or offspring in another, and the genotypes of these individuals were generated independently, multiple times, thus providing an internal quality control mechanism.

For RH mapping, the largest source of data error comes from 'false negative' results: failure to detect the presence of a particular marker in the

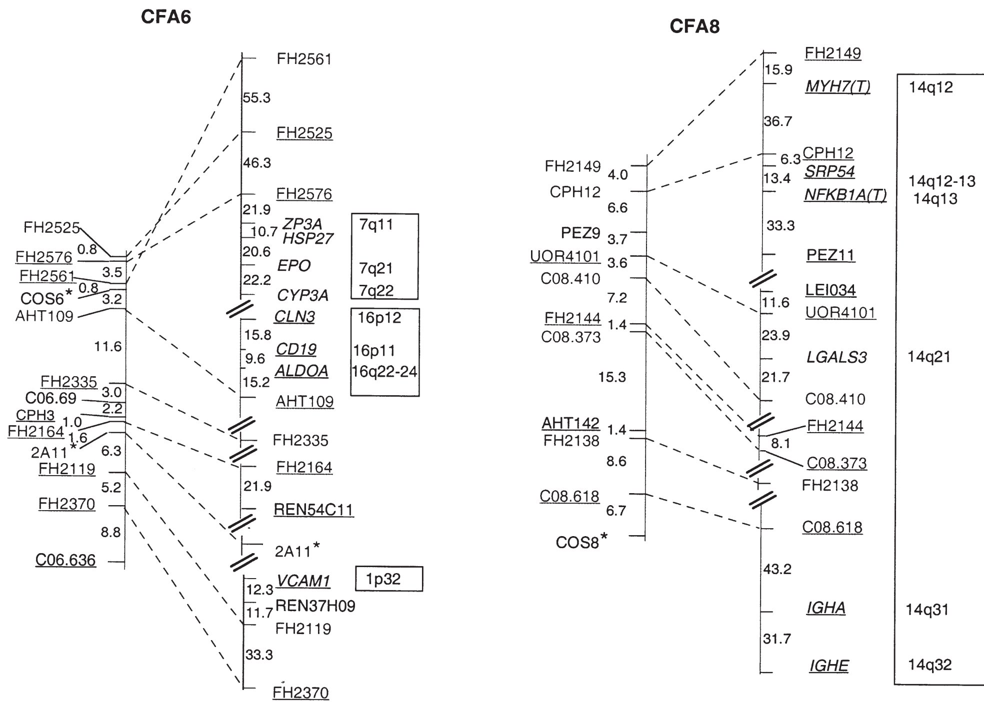

Fig. 12.2. Comparative canine and human maps. The linkage (left) and RH (right) maps for canine chromosomes 6 and 8 are illustrated. Distances between markers are in cM and cRay respectively. Markers mapped on both the canine reference families and the RH panel are indicated by dotted lines. The corresponding parts of the human genome are shown on the right of each map. Canine chromosome 8 corresponds almost entirely to human 14q; by comparison, canine 6 corresponds to regions of several distinct human chromosomes. Asterisks indicate markers used to assign linkage groups to specific chromosomes.

DNA of a radiation hybrid clone or clones even though the marker is in fact present. To overcome this potential source of error, markers are usually typed in duplicate on a panel of radiation hybrids, and consensus data compiled. In addition high lod scores are used to assign markers to RH groups. Whereas, for linkage data, a lod score of 5.0 (odds of 100,000 : 1 in favour of linkage) is typically used to assign markers to linkage groups or chromosomes, a lod score of at least 6.0 and typically 8.0 or higher is used for RH data.

In addition to the above, additional quality control is provided simply by comparing the linkage and RH maps. In the analysis completed thus far, good agreement has been observed.

Use of the Canine Genomic Map

Mapping canine inherited disease genes

There are over 350 reported inherited diseases in dogs (Patterson, 2001). This constitutes the largest number of naturally occurring genetic disorders known in any non-human species. A computerized database of canine genetic diseases summarizes the clinical, pathological and genetic features of these, as well as documenting the 5–10 new disorders reported, on average, each year (Patterson, 2001). Among those in which the mode of inheritance has been defined, over 70% are inherited as autosomal recessive traits, X-linked recessive traits, or traits that are genetically complex and whose prevalence increases with inbreeding.

When mapping disease genes we look for linkage between a marker and a disease state, rather than between two markers, as in map building (Fig. 12.3). The mapping of canine retinal disease genes provides interesting examples of how the syntenic relationship between the human and dog genomes can be explored. For instance, identification of a linkage group flanking the progressive rod cone degeneration locus (*prcd*) localizes the gene to the end of CFA9 in a region that includes the genes *TK1*, *GALK1*, *MYL4* and *RARA* (Acland *et al.*, 1998). The conservation between this region of CFA9 and distal human chromosome 17q, as shown previously (Werner *et al.*, 1997), establishes the potential locus homology of *prcd* in the dog with RP17, a human RP locus for which no gene has yet been identified, in part because the number of informative families is limited.

Using canine pedigrees to study complex diseases

The mapping and even cloning of disease genes shared by humans and dogs is likely to benefit both species. The comparative genetics of humans and dogs is most likely to be useful when considering complex disorders to which multiple genes contribute, sometimes simultaneously. Identification of genes for cancer, epilepsy, neurological and psychiatric disorders have all proven

problematic for human geneticists. While there are clearly strong genetic components for each, the contribution of multiple genes, the presence of phenocopies, and the inability to stratify the often bewildering array of affected families into homogeneous genetic groups makes analysis extremely difficult.

Many of these difficulties can and will be overcome through analysis of canine families with the same disorder. Catastrophic events in the last 100 years, such as the World Wars, have produced severe bottlenecks in many breeds, at times reducing the effective breeding stock to only a few dogs. In addition, for some breeds, diversity has been further reduced by the use of 'popular sires'. These dogs have features that make them particularly popular as stud dogs and as such they may produce dozens of litters in their lifetime. In many cases, therefore, purebred dogs of today represent a limited genetic pool, characterized by disease predispositions that derive from one or a small number of recent genetic founders.

Two strategies exist to exploit this favourable population structure. In the first, a single large family or group of families derived from the same lineage is analysed. The fact that many canine lineages, and sometimes even whole breeds, derive from a limited number of founders provides a means to overcome locus heterogeneity problems. In the case of epilepsy or deafness, for instance, there are likely to be many susceptibility genes in the gene pool. But analysis of a small subset of related families from within a single breed would reduce the statistical complexity associated with mapping such a

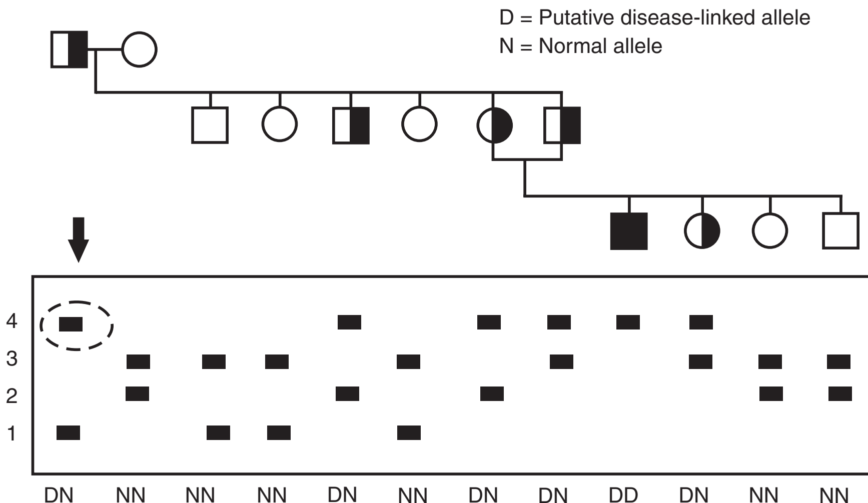

Fig. 12.3. Schematic of a single marker linked to a putative disease locus. Pedigree appears at the top of the figure and a schematic of a single marker analysed on a denaturing gel appears in the bottom portion of the figure. 1. Males are represented by squares and females by circles. Affected individuals appear as fully coloured circles or squares. Heterozygotes appear as partially filled circles or squares. The circled allele, originally inherited from the maternal grandfather, segregates with the disease in all individuals shown.

complex trait enormously. We used this approach, recently, to map a gene for susceptibility to kidney cancer in German Shepherds (Jónasdóttir *et al.*, 2000). Using a small group of six families all derived from a single common founder, we were able to localize a gene responsible for a complicated syndrome termed canine hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis (RCND) to a small region of canine chromosome 5. The most closely linked marker maps to a region of CFA5 syntenic with portions of human chromosomes 1p and 17p. Interestingly, these two regions of the genome contain a number of loci which are suitable candidates, including the p53 gene. Linkage and mutation screening, however, eliminate p53 as the causative gene. It is likely that RCND is caused by a gene which has been identified in humans, but to which a cancer susceptibility phenotype has not yet been ascribed. The final identification of this gene, therefore, is likely to benefit both human and canine health.

A second approach for mapping diseases of interest to humans and dogs which makes use of the natural history of dogs is that based upon linkage disequilibrium (LD). Using data provided by the American Kennel Club Pure-bred Dog Registry Database, we and collaborators (Ostrander and Kruglyak, 2001) have conducted simulation studies to determine the extent over which LD can be expected in breeds such as the Rottweiler, which originated from a small group of founders, remained small for many generations, but which has expanded in popularity enormously during the last decade. Taking into account breed history, registration patterns and litter size, we estimate that LD will extend by as much as 5–10 cM around a disease mutation in particularly structured breeds. This means that genome-wide screens of a 5–10 cM density on unrelated dogs of the same breed who share a common disease phenotype may be suitable for identification of disease loci. This approach is particularly attractive for the study of diseases that are difficult to study because they are frequently lethal or affect fertility. In such families it is often relatively easy to obtain samples from affected individuals, but virtually impossible to collect three-generation families suitable for tracking the Mendelian inheritance of chromosomal regions through multiple generations of a family. This strategy opens the door to more economical mapping of disease genes and allows geneticists to map genes of interest without breeding additional animals, simply by taking advantage of naturally occurring pet populations.

Conclusion

Most scientists working in mammalian biology occasionally find themselves at uncomfortable crossroads, where they must choose between studying a disease which is common in humans, but for which there are few suitable animal models, or conversely, a disease which is common and interesting in animals but which is not a suitable model for any relevant human disease. Those of us working in canine genetics are fortunate; we are seldom forced to make such choices. Canine biology and disease patterns mirror the human

condition with uncanny similarity. Virtually anything we learn about predisposition to disease from canine studies is likely to benefit human genetics, and much of what is known about human disease predisposition has the potential to lead to dramatic improvements in canine health.

The much anticipated availability of the complete human and mouse genome sequence in the year 2000 heralds the opening of a new chapter in comparative mammalian genetics. The availability of dense and integrated human linkage and radiation hybrid maps provides us with the means to map loci of interest to a few cM. In addition, it provides us with the resources to determine the corresponding regions of the human and mouse genomes. The biggest challenge at present for canine geneticists is to determine how best to access and manipulate the human sequence for the joint benefit of canine and human health. It is no longer appropriate to simply think of each mammalian genome and each mammalian genome project as separate and distinct. As more and more canine disease genes are mapped, we can turn to the human map for selection of appropriate candidates for mutation analysis. Those that are indeed disease associated can be tested against DNA from humans displaying similar phenotypic disease profiles. Clearly, the greatest strides in human and canine health in the coming decade will come from those who think about the collective 'comparative genome' and who are willing to take advantage of all this unique resource has to offer.

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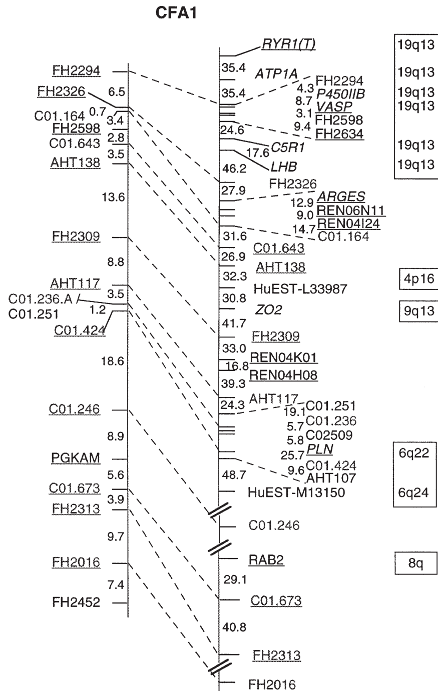
Appendix

Figures from Mellersh *et al.* (2000) *Mammalian Genome* 11, 120–130.

Comprehensive linkage maps are presented by vertical bars on the left, and the corresponding RH maps are shown on the right. The 217 markers positioned on both maps are joined by dotted lines. Gene-based markers are indicated in italics, chromosome-specific markers are designated by an asterisk and markers corresponding to TOASTs (Jiang *et al.*, 1998) are indicated by the suffix (T). Framework markers ordered with statistical support higher than 1000 : 1 are underlined. When two or more RH groups were associated with a single chromosome, the orientation of the RH groups with respect to each other and to the chromosome was established by comparison with markers mapped by linkage analysis and by analysis of two-point breakage probability between markers in the different RH groups. CFA nomenclature is used for linkage groups and their associated RH groups that have been assigned to specific chromosomes; anonymous groups are termed Syntenic Group 1 (S1), Syntenic Group 2 (S2) etc., in order of decreasing size. Distances between markers on the linkage map and the RH map are measured in centiMorgans (cM) and centiRays$_{5000}$ (cR$_{5000}$), respectively, and were calculated using the Kosambi and the RH Haldane mapping functions. Known chromosomal locations are reported for human orthologous genes. Primer sequences are available at http://www-recomgen.univ-rennes1.fr/doggy.html and http://www.fhcrc.org/science/dog_genome/dog.html.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C01.251 1.2
C01.424
18.6
C01.246
8.9
PGKAM
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C01.673
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FH2016
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35.4
ATP1A
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35.4
8.7 VASP
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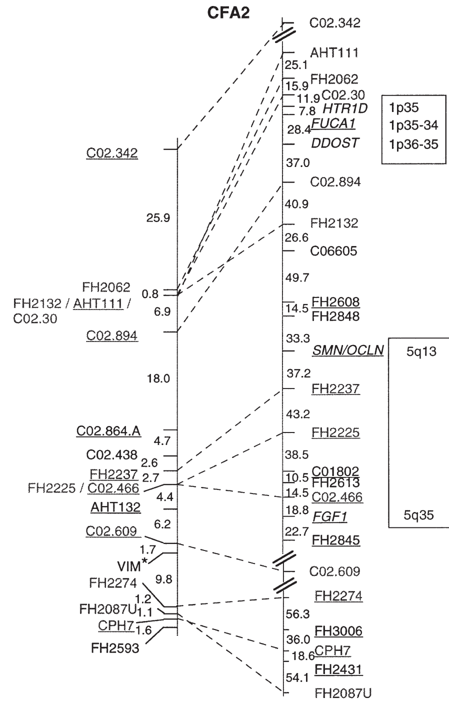
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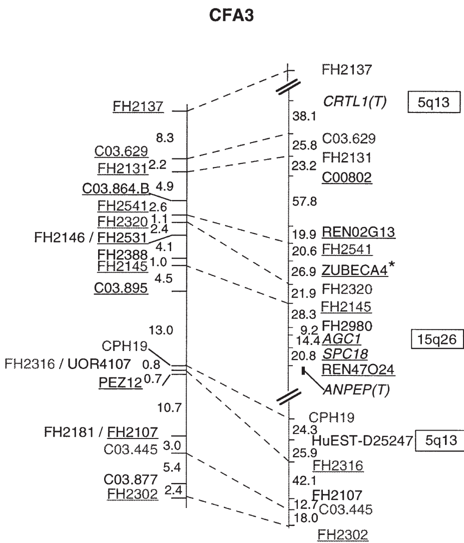
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10.7
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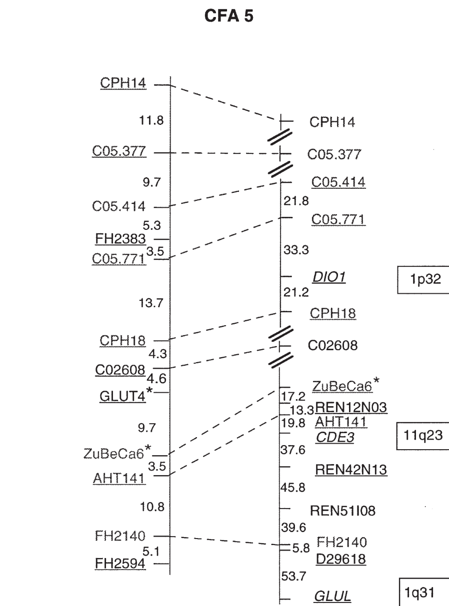
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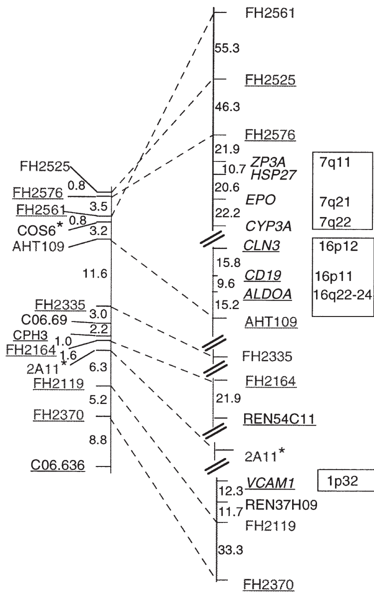
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1.0
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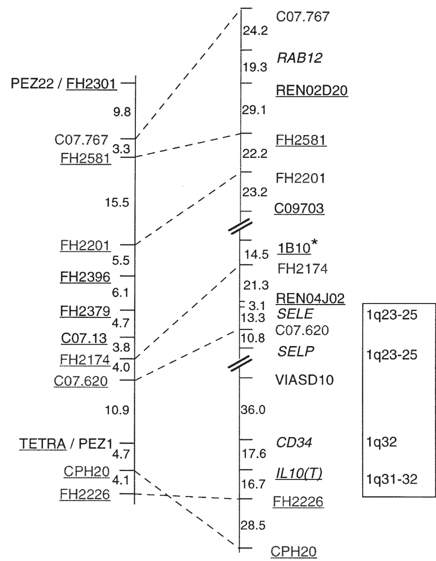
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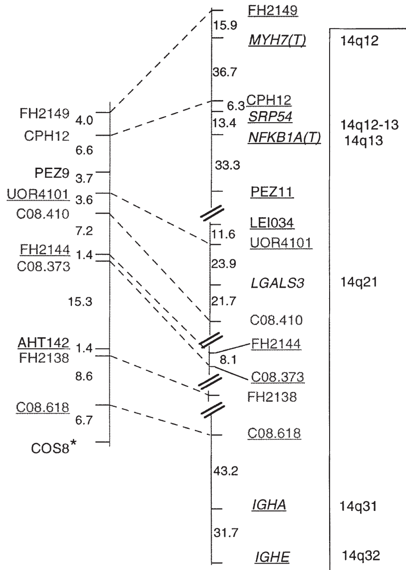
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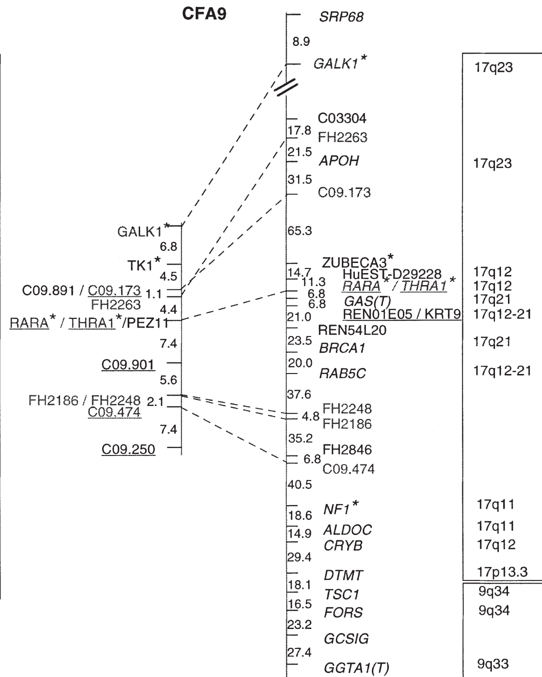
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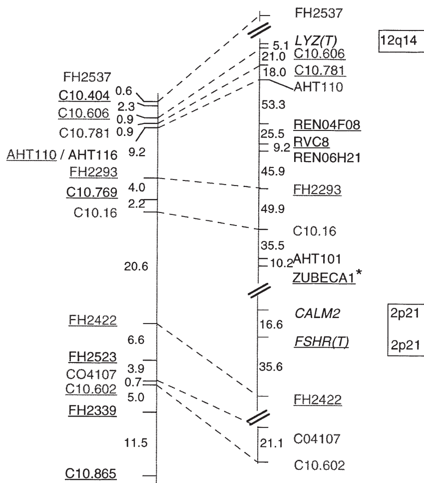
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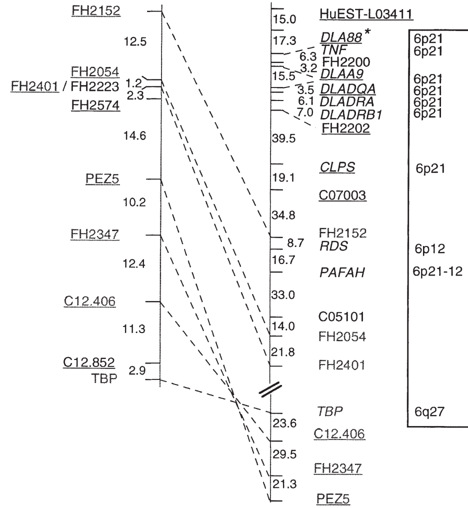
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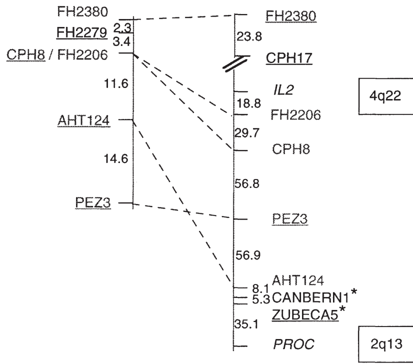
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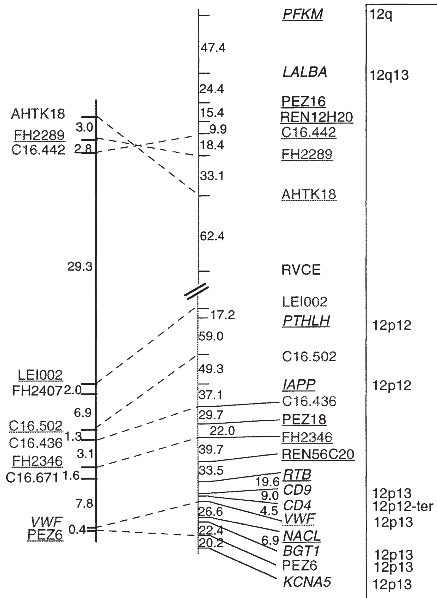
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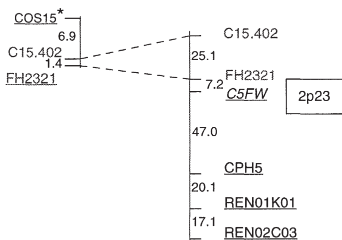
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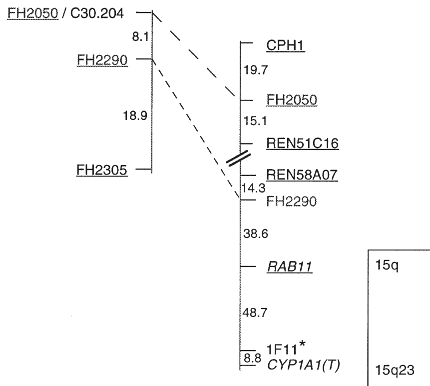
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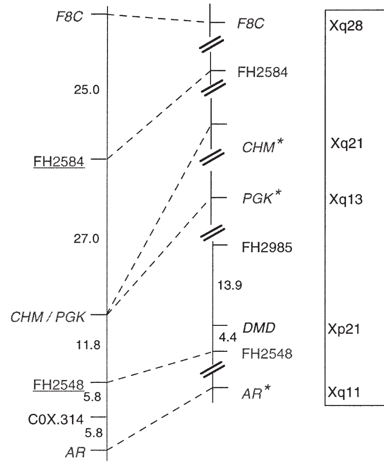
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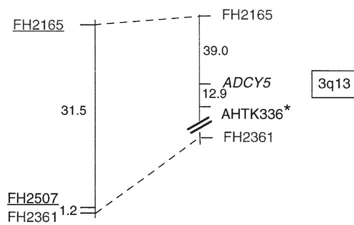
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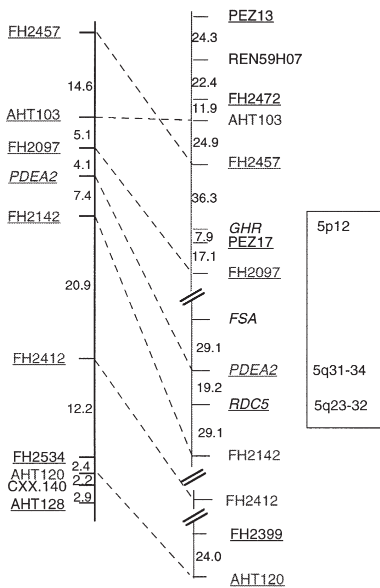
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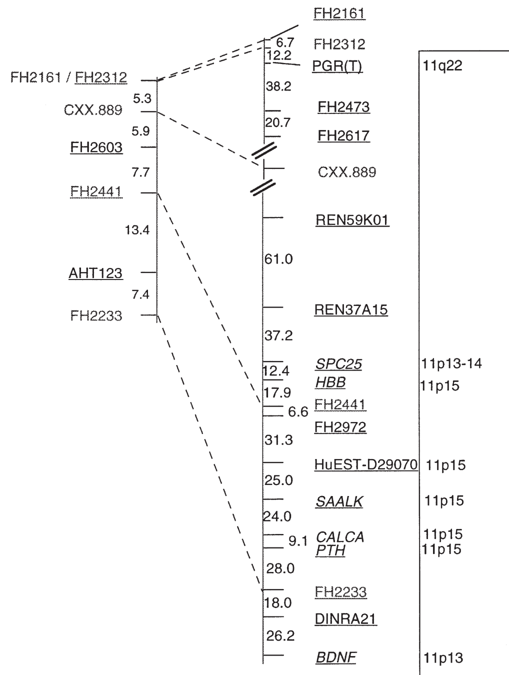
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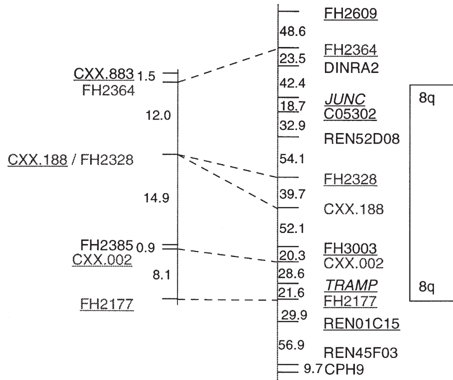

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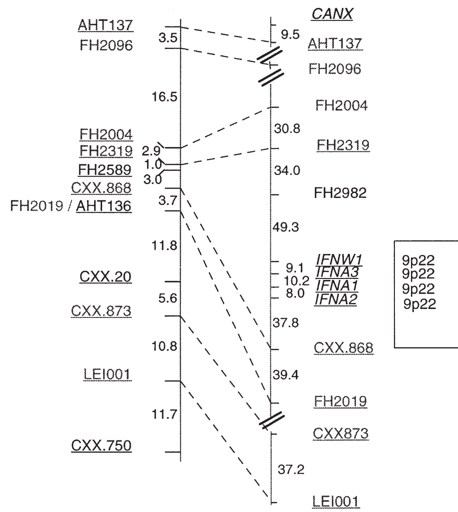

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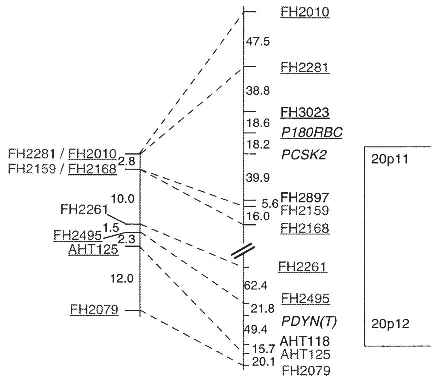

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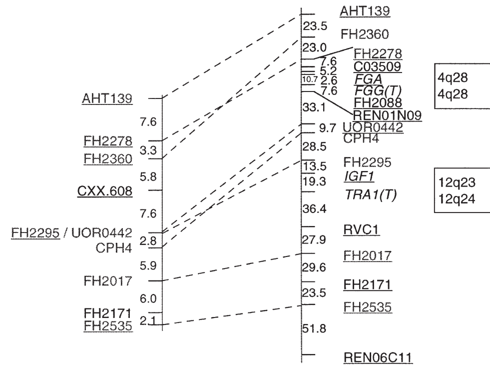

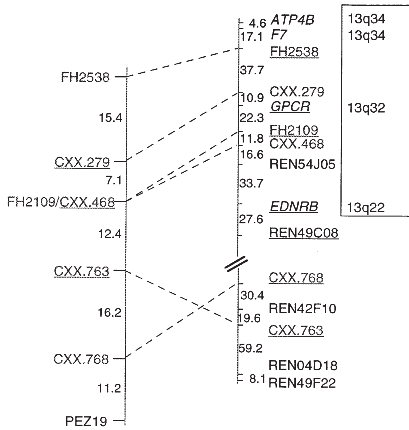

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17.1 F7
FH2538
37.7
CXX.279
10.9 GPCR
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FH2109
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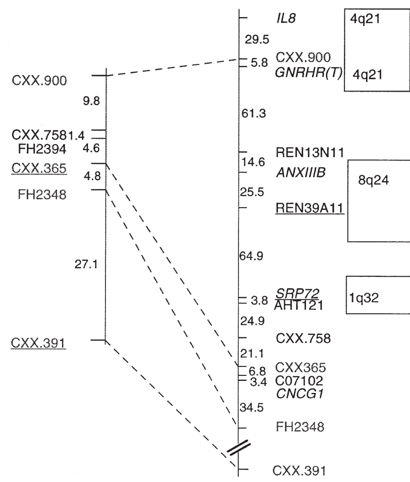

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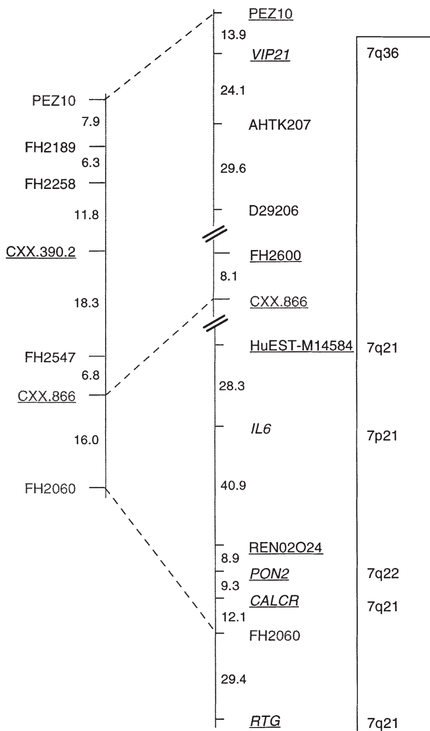

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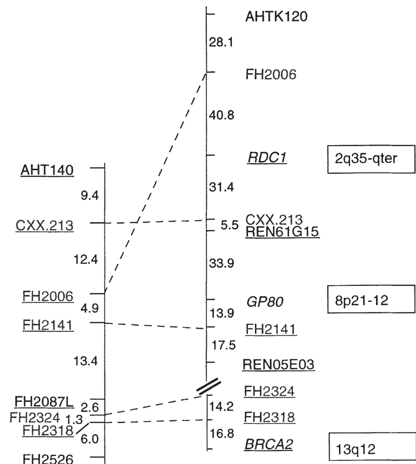

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FH2324 1.3
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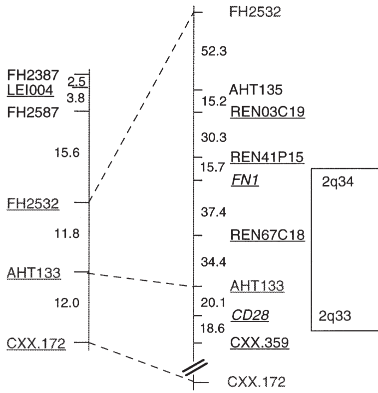
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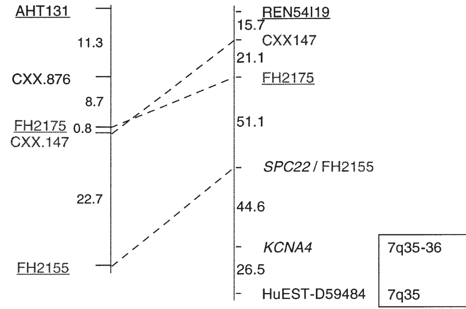
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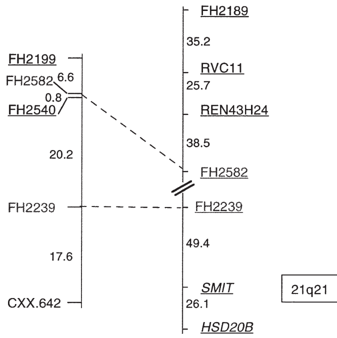
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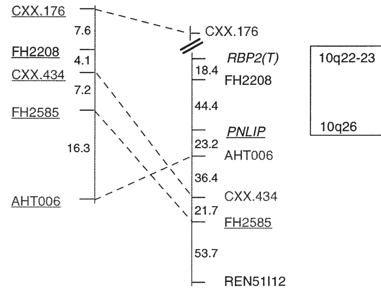
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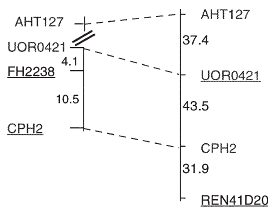
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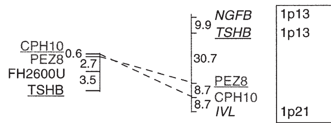
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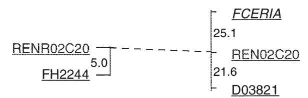
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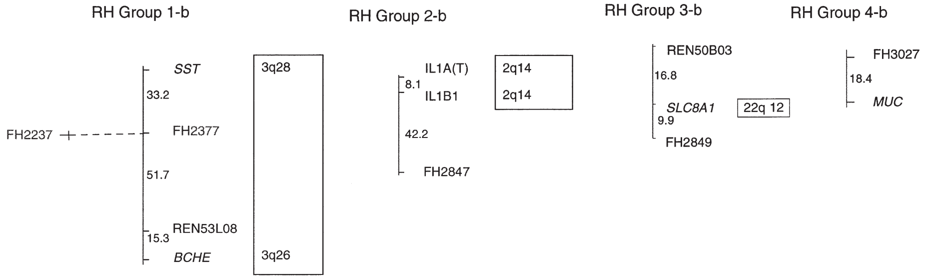
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8.1
IL1B1
42.2
FH2847
2q14
2q14
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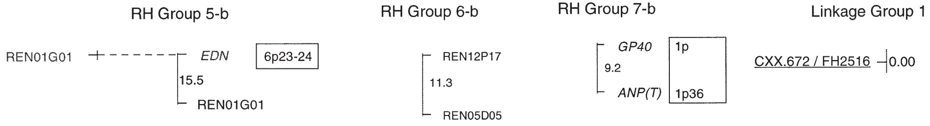
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RH Group 7-b
GP40
9.2
ANP(T)
1p
1p36
Linkage Group 1
CXX.672 / FH2516
0.00

Genetics of Behaviour 13

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Introduction

The dog was the first species to be domesticated by man some 10,000 years ago though an association with humans may go back 40,000 years (Clutton-Brock, 1984). Although there has been debate as to the origins of the domestic dog the review by Clutton-Brock (1995) suggests that the wolf is the principal, if not sole, progenitor of the dog. Mitochondrial DNA control region

sequences of wolves and 67 breeds of dogs suggest that dogs and wolves diverged over 100,000 years ago and that there were four separate occasions when animals which became dogs became distinct from wolves (Vilà *et al.*, 1997, 1999). For this reason dogs are more genetically distinct from one another than if there had been just one instance of separation. From these origins the dog has been diversified into numerous breeds and innumerable shapes. Some of these breeds originated some 3000–4000 years ago (Harcourt, 1974), but others trace back to the Middle Ages and some modern breeds trace, in their present form, only to the end of the 19th century. Because of the wolf relationship considerable attention has been given to the similarities and differences between wolves and domestic dogs but it is inevitable that, after 10,000 years of domestication and living in close proximity to humans, major differences will have evolved. The dog, like the wolf, still rests for 12 hours of every 24, but the human has to some degree determined when and where the dog will rest.

Wolf Behaviour

Although wolves are ancestral to dogs, there are many differences between their behaviour and that of domestic dogs. Some of these differences are due to the fact that domestic dogs are provisioned and usually restrained, so that dogs do not spend long hours hunting or travelling in search of food sources. Wolves dig dens, but dogs rarely do. Vocal communications have also changed; wolves howl more than most breeds of dogs, but they seldom bark. Barking has been selected for in dogs.

Wolves are better at problem solving than dogs. For example, Frank and Frank (1985) found that wolves could learn to manipulate bowls from a box much more quickly than Malamutes, but were worse at passive inhibition (stay training) or active inhibition (leash training) when compared with Malamutes (Frank and Frank, 1982). Furthermore, wolves are very difficult to house-break and are often very destructive. Wolves kept as pets can be very dangerous.

Wolves breed only once a year, and in most cases only the dominant female has a litter (Asa *et al.,* 1985). Most dogs breed twice a year; the Basenji being an exception. Male wolves help to provision the pups. Wolves live in stable packs of up to 15 animals with a dominant pair, but feral dogs live in unstable groups. Wolves travel 120 km per day around territories that vary with the pack size from 40 to 200 hectares (Mech, 1966).

When wolves fight with pack members, they inhibit their aggression if the victim gives submissive and fearful signals such as rolling over, exposing the groin area, avoiding eye contact, tucking the tail, etc. If wolves fight with another pack, the victim will be bitten whether or not he shows submissive signs (Schenkel, 1947).

The effect of human selection on canid behaviour is well exemplified by the study of Goodwin *et al.* (1997). They identified 15 signals in wolves during social interactions and then observed social interactions among dogs

interacting in same-breed groups. Some breeds used very few signals. These authors then plotted the number of signals against physical similarity to wolves. Some breeds such as the French Bulldog and Cavalier King Charles Spaniel neither looked nor behaved like wolves, whereas Alaskan Malamutes looked and acted like wolves. Golden Retrievers behaved more like wolves than their appearance would indicate, but German Shepherd dogs (GSD) behaved less like wolves.

The signals that appear earliest in lupine development are the only ones retained by the non-wolf-like breeds, such as the Cavalier King Charles Spaniel, the Norfolk Terrier, Shetland Sheepdog and French Bulldog (Goodwin *et al.*, 1997).

Major Changes in Behaviour During Domestication

A long-term study (> 40 generations) at the Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirisk, revealed that foxes selected for tameness not only became docile and friendlier towards people, but they also developed curly tails, pendant ears and white spotting (Belyaev, 1979; Trut, 1999; Chapter 2). Piebald spotting is due to the *star* gene and is incompletely penetrant (Belyaev *et al.*, 1981).

On the one hand, the appearance of mutants such as curly tails and pendant ears may not be due to selection for tameness but just coincidental findings in a population undergoing inbreeding due to small effective population size. On the other hand, deliberate selection for tameness in foxes also led to white spotting and selection for spotting in Cocker Spaniels led to less aggression in these Cocker Spaniels (Podberscek and Serpell, 1996) and this is associated with less aggressive breeds such as the Cavalier King Charles Spaniel and Beagle.

Coppinger and Schneider (1995) have ranked breeds of dogs according to their degree of neotenization. Heelers such as Corgis and Huskies are the closest to the adult wild canids. Next, are the headers and stalkers such as the Collie, then the object players such as the hounds, retrievers and poodles (all dependent-eared dogs) and finally those breeds which act adolescent as adults, the Saint Bernard, Komondor, Maremma and Great Pyrenees. Domestication of the dog is not simply a process of neotenization, but breeds selected for certain purposes such as lap dogs (Chihuahua, Pomeranian, Cavalier King Charles Spaniel, French Bulldog) appear and act puppy-like, whereas working dogs and terriers are much less neotenized in appearance and behaviour.

As with other domestic species, there is a relative shortage of scientific data on canine behaviour. Faure (1994) gave some reasons for this which are as valid for the dog as they are for the farm livestock. Faure was referring to in his paper: (i) behaviour is difficult to measure and behavioural traits are often of long duration, making enough data difficult to obtain; (ii) measures of behavioural traits are often not normally distributed, making genetic parameters difficult to estimate; and (iii) the importance of environmental

influences was often overestimated in the past. It can be added that the dog lives in closer communication with humans and is subject to many more varied environmental influences than farm livestock in addition to living in a variety of 'pack' sizes ranging from one upwards. Housing conditions can also influence behaviour (Hetts *et al.*, 1992) as can the interactions between mother and offspring around weaning time (Wilsson, 1984/1985).

According to Mackenzie *et al.* (1986), behavioural work on the dog began around the start of the 20th century, but much of the work was directed towards Mendelian type explanations. Thus, Humphrey and Warner (1934) suggested that gunshyness in the GSD was controlled by a simple gene series with two alleles while Thorne (1944) attributed extreme nervousness in a crossbred population to a single dominant allele.

The Ontogeny of Behaviour and Neotenization

In essence, canine development can be divided into four stages or sensitive periods: the neonatal period, the transition period, the socialization period and the juvenile period. Because some long-term influences on behavioural development may occur *in utero* (Joffe, 1969) an additional prenatal period may be included. The subject has been reviewed by Serpell and Jagoe (1995) who conclude that prenatal effects are unlikely in the dog. In the neonatal period (0–14 days), puppies are in a fairly altricial state; they cannot see, hear or bear weight on their limbs. They can locomote by pulling themselves forward with their forelimbs. They find the mammary gland by tactile cues – warmth, hairlessness and resiliency, and then find the nipple as a protuberance. The neonatal period extends until the puppies open their eyes around day 10. They can learn simple associations, but they are unlikely to be of a lasting nature (Scott and Nagy, 1980). It is, however, thought that daily handling can have long-term effects upon subsequent behaviour (Fox, 1978) and it is known that wolf cubs handled from birth or 6 days were more reliable and friendly than those handled from 15 days or later (Fox, 1971; Zimen, 1987).

The transition period starts from around 2 weeks of age, by which time the eyes are open, and ends about a week later with the opening of the ear canals. The pup's sensory world is now more complete. In this period puppies begin to urinate and defecate without maternal stimulus and they crawl, commence play fighting with siblings and start an interest in solid food. This period corresponds to the wolf cub leaving its nest but, according to comparative studies (Frank and Frank, 1982, 1985; Zimen, 1987), wolves start the transition period slightly ahead of puppies and complete it sooner. According to Scott and Fuller (1965), learning ability in the pup does not reach adult levels prior to an age of 4–5 weeks.

The socialization period was identified by Freedman and his colleagues (Freedman *et al.*, 1961) who found that if a bitch and her litter were completely isolated behind a solid fence for 14 weeks, the puppies were essentially feral and were frightened of people. The socialization period runs through from 3–4

weeks to 14 weeks of age but does not appear to end suddenly with the upper boundary being far from clear-cut. These periods were originally termed critical periods, but now are known as sensitive periods because dogs can be socialized before or after 4–14 weeks, albeit with greater difficulty.

Few puppies have absolutely no socialization. Even the puppy born in a kennel still encounters a human because the bitch is fed and the kennel cleaned. Nevertheless, it is important to expose puppies to people during the second and third months of their lives. Ideally, the people should be those with whom the puppy will spend the rest of its life. For example, a puppy that has not seen a child will not make the ideal pet for a family with small children. Socialization is not only to people, but also to conspecifics. During this period, play among puppies commences and allows puppies to practise adult behaviours and learn which behaviours are acceptable to other dogs.

Work with the Seeing Eye (Guide Dogs for the Blind) by Pfaffenberger *et al.* (1976) led to practical recommendations that socialization between owner and pup should be achieved between 6 and 8 weeks of age. They also suggested that puppies should face their adult conditions of housing at this time. However, Slabbert and Rasa (1993), following work with GSD puppies, came to the conclusion that puppies were best left with their mothers for 12 weeks. This is contrary to the normal rehousing conditions faced by most puppies and does not appear to have been adopted on any real scale by breeders.

The juvenile period follows the socialization period. Puppies may be more fearful and neophobic following the socialization period. Although dogs are believed to reach social maturity between 18 months and 2 years, puberty, which usually occurs between 6 and 9 months, marks the end of the juvenile period because the dog is physically mature. See Fig. 13.1 for more details on development in dogs.

Coat Colour and Behaviour

The relationship of coat colour to behaviour is intriguing. The wild type coat colour for many mammals is agouti – bands of brown (eumelanin) and yellow pigment (phaeomelanin). The precursor molecule for both melanin and the neurotransmitter dopamine is DOPA so that an increase or decrease in synthesis of one will affect the other. When the responses to stress of agouti and non-agouti mice are compared, the non-agouti mice are slower to recover and to reproduce (Hayssen, 1998). Experiments on yellow deer mice indicated that they were tamer and less aggressive than agouti deer mice (Hayssen, 1997).

Hemmer (1990) has shown that coat colour in domestic livestock can be associated with temperament but coat colour *per se* may not be the explanation in this case. Non-white sheep are more likely to lamb out of season than white ones (Dyrmundsson and Adalsteinsson, 1980).

In order to determine whether coat colour was related to aggressive behaviour, the incidence of each coat colour – black, yellow and chocolate – among all the Labrador Retrievers that were patients at The Veterinary Medical

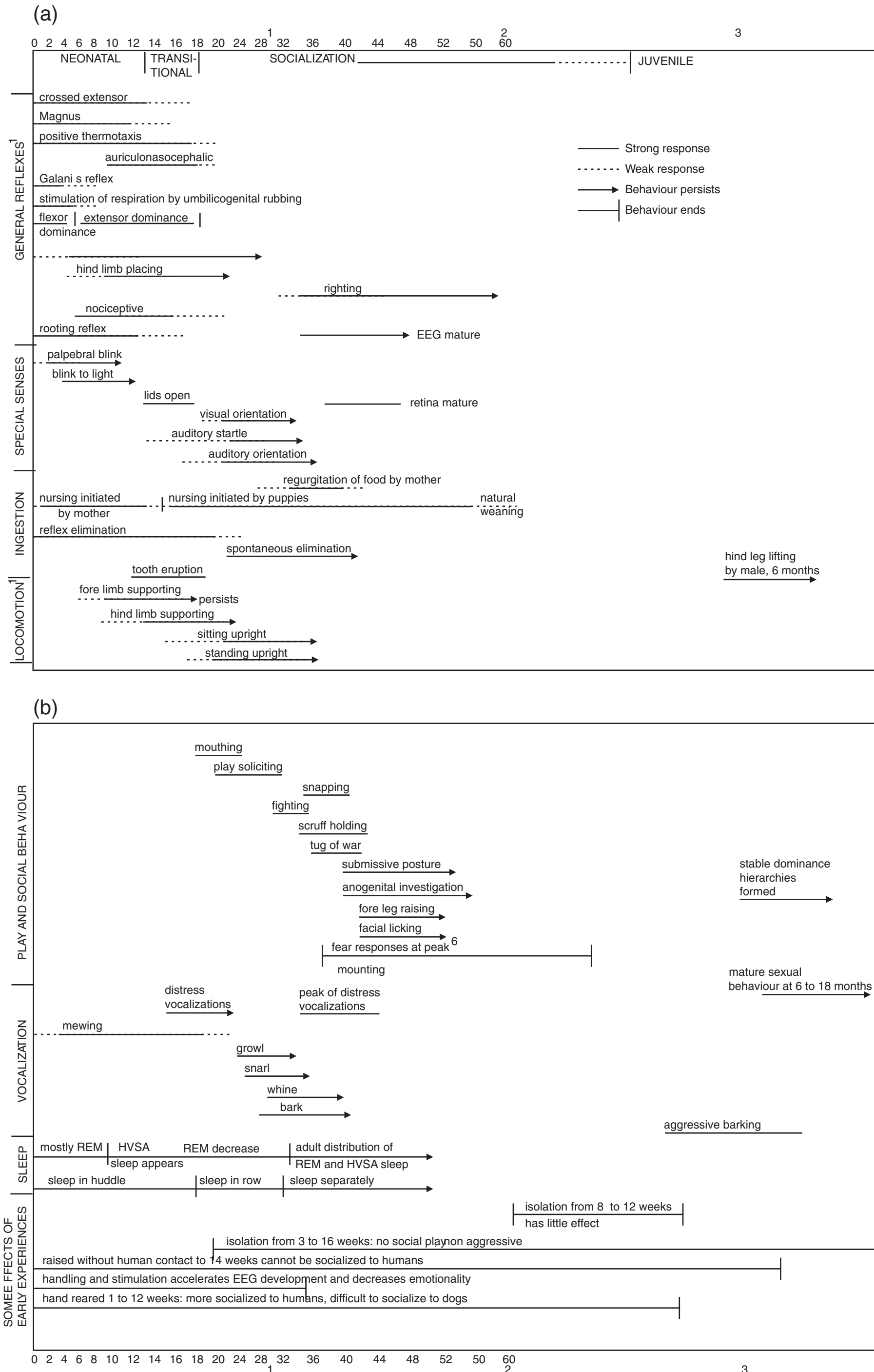

Fig. 13.1. The development of the dog (Houpt, 1998; with permission from Iowa State University Press).

Teaching Hospital of Cornell University was compared to the incidence of the three coat colours among Labradors presented to the Animal Behavior Clinic for aggression. Chocolate Labradors were less likely to be presented for behaviour problems than their numbers in the hospital population would have predicted. Black Labradors (the dominant colour in this breed) were presented for aggression in proportion to their numbers whereas yellow Labradors tended to be over-represented for aggression (Fig. 13.2).

The effect of breeding foxes for tameness has revealed that piebald foxes are tamer than their solid colour counterparts (Trut, 1999). Podberscek and Serpell (1996) found that solid colour Cocker Spaniels were more likely to be aggressive than white spotted ones (particolours). Between the solid colours, red Cocker Spaniels were more likely to be aggressive than black ones. It is known that bloodlines of reds, blacks and particolours tend to be distinct (Lloyd Carey, 1992) and what may be being observed is an inherited trait that arose in some red line and has remained predominant in reds because of largely separate breeding policies.

Genetics and Behavioural Selection of Working Dogs

Dogs have not only developed over a large range of body sizes with, for example, a Chihuahua being a tiny portion of the body weight of a St Bernard,

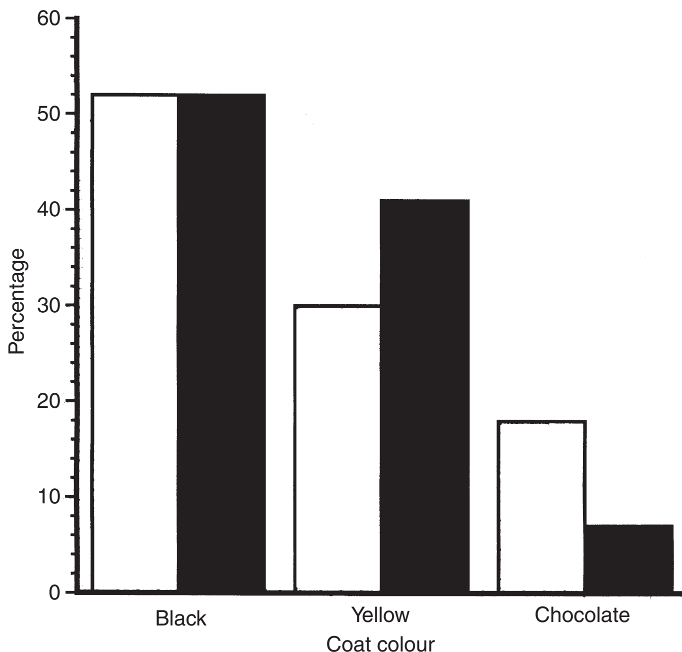

Fig. 13.2. A comparison of the proportion of Labrador Retrievers presented for medical problems (□) with those presented for aggression (■).

but they have also developed to undertake a variety of different purposes. Without herding breeds such as the Border Collie or the Kelpie, the handling of European, Australasian and American sheep on a large scale would be well nigh impossible. Similarly the non-deployment of livestock protection dogs such as the Maremma, Great Pyrenees or Anatolian Shepherd dog would make sheep losses to predators unacceptable in parts of North America. In addition to these tasks, dogs serve as drug/explosive detection animals, as general purpose police dogs, as gundogs, sled dogs, trackers and hunting dogs, as eyes for those who cannot see and ears for the deaf, as well as just companions to those whose lives might be less full without them.

This plethora of activities for which dogs are employed may be taken to mean that humans have selected for these features over a period of time. It would however be erroneous to suggest that humans have been selecting for a specific feature over many millennia. As Coppinger and Schneider (1995) have suggested, most traits may be a fortuitous accident. Many of the distinguishing features of some dog breeds such as hanging ears, curly tails, long coats, hanging jowls and various coat colours have no real selectional advantage (Bemis, 1984). They may have arisen by chance and been retained for their distinguishing nature or by the desire to retain the dog in a juvenile state (Frank and Frank, 1982).

Guide dogs for the blind

Guide dogs are a product of the early 20th century, primarily using GSD and more recently greater use of Labrador and Golden Retrievers and their crosses. Initially, dogs were selected from gift dogs with a high failure rate (Scott and Bielfelt, 1976; Goddard and Beilharz, 1982) but more recently guide dog organizations have undertaken their own breeding programmes with greater success. American studies (Bartlett, 1976; Scott and Bielfelt, 1976) tended to show low heritabilities for most traits even though based, in Bartlett's case, on over 1800 animals. In most instances heritabilities did not differ significantly from zero. The later Australian work based on 394 Labrador Retrievers (Goddard and Beilharz, 1982) also showed low values but not for fear, the major cause of guide-dog failure, or for overall success (Table 13.1). Both these had moderate heritabilities which, although specific both to the population under study and the time that the work was undertaken, could explain why guide dog breeding kennels now have higher rates of success.

In addition to moderate heritabilities for fear or 'nervousness' strong positive correlations were observed with 'sound shyness' and negative correlations with 'willingness' (see Table 13.2). Most breeders believe that nervous animals give rise to nervous animals in increased proportions and, though empirical, this would seem to be borne out by the Australian work (see Goddard and Beilharz, 1983, 1984, 1985, 1986). It also substantiates the work on Pointers selected for fearfulness (Brown *et al.*, 1978). In many breeds, breeding programmes are dictated by the show ring. Thus conformational features rank

Table 13.1. Heritability estimates for guide dog traits

	Sire		Dam		Combined	
Trait	h^2	SE	h^2	SE	h^2	SE
Success	0.46	0.19	0.42	0.18	0.44	0.13
Fear	0.67	0.22	0.25	0.15	0.46	0.13
Dog distraction	–0.04	0.08	0.23	0.14	0.09	0.08
Excitability	0	0.09	0.17	0.13	0.09	0.08

Goddard and Beilharz (1982).

Table 13.2. Genetic correlations (below diagonal) and heritabilities (diagonal) in Labrador Retrievers

Trait	N	S	C	W	D	SS	BS
Nervousness (N)	**0.58**						
Suspicion (S)	0.53	**0.1**					
Concentration (C)	–0.01	–0.31	**0.28**				
Willingness (W)	–0.57	–0.2	0.67	**0.22**			
Dog distraction (D)	0.11	0.63	–0.5	–0.41	**0.08**		
Sound-shy (SS)	0.89	0.47	0.33	–0.78	0.28	**0.14**	
Body sensitivity (BS)	0.72	0.51	–0.3	–0.74	–0.21	0.59	**0.33**

Goddard and Beilharz (1983).

high, yet the majority of puppies end up in pet homes far removed from the show ring. Attention to behavioural traits and character are crucial in breeding puppies going to the pet market. According to Mugford and Gupta (1983) undesirable character traits seen on the bench were not apparent in the confines of the show ring. This work seems to fail to appreciate that a dog may be protective on a bench yet not in the ring. Nevertheless it is true that many judges fail to recognize or appreciate character failings and the sort of dog being exhibited could reflect the judges' discriminatory ability. Some effective character test for show dogs would thus seem desirable.

Hunting dogs

Although hunting was believed to be a major use of the dog in its first associations with humans (Clutton-Brock, 1995) there are those who question this (Manwell and Baker, 1984). Nevertheless men have used dogs for hunting at least from some 3000 years BC (sight hounds) and in more recent centuries as pack animals pursuing fox or deer and as gundogs in a variety of roles. A German study on German Wire-haired Pointers (Geiger, 1972) looked at four major traits and found that, although maternal heritabilities were high, sire values did not differ from zero (Table 13.3). More recently Scandinavian workers have sought to examine the genetics of hunting traits in English

Setters and Finnish Spitz (Vangen and Klemetsdal, 1988). The studies were based on 5285 English Setter tests and 4864 Finnish Spitz tests. Heritability studies were higher in English Setters than Finnish Spitz but none were substantial (see Tables 13.4 and 13.5). It was pointed out that some traits did not show a normal distribution but breeding values were calculated from some of the Finnish Spitz traits. It was concluded by the researchers that the 'scoring' of hunting traits was probably the reason for low heritabilities and that better scoring techniques could lead to greater progress in hunting dogs.

Schmutz and Schmutz (1998) calculated heritabilities on seven hunting traits in five breeds using tests devised by the North American Versatile Hunting Dog Association (NAVHDA). Values are shown in Table 13.6 and

Table 13.3. Heritability estimates in German Wire-haired Pointers

Trait	Sire	Dam
Hare tracking	0.03	0.46
Nose	0.01	0.39
Obedience	0.01	0.19
Seek	0	0.41

Geiger (1972).

Table 13.4. Genetic (above diagonal) and phenotypic (below diagonal) correlations and heritabilities (diagonal) for English Setters

Trait	HE	SS	FW	CO	SI
Hunting eagerness (HE)	**0.22**	0.79	0.72	0.33	0.72
Style and speed (SS)	0.94	**0.18**	0.68	0.31	0.67
Field work (FW)	0.97	0.92	**0.18**	0.44	0.74
Cooperation (CO)	0.41	0.43	0.52	**0.09**	0.72
Selection index (SI)	0.8	0.74	0.64	0.61	**0.17**

Vangen and Klemetsdal (1988).

Table 13.5. Genetic (above diagonal) and phenotypic (below diagonal) correlations and heritabilities (diagonal) for Finnish Spitz

Trait	TS	SA	FB	MK	BK	HB	FB	TI
Total score (TS)	**0.11**	0.48	0.51	0.57	0.48	0.66	0.60	0.72
Searching ability (SA)	0.61	**0.07**	0.22	0.30	0.35	0.22	0.48	0.43
Finding birds (FB)	0.94	0.79	**0.11**	0.13	0.10	0.17	0.16	0.28
Marking (MK)	0.77	0.97	1.00	**0.04**	0.48	0.35	0.33	0.47
Barking (BK)	0.46	–0.77	1.00	1.00	**0.02**	0.30	0.31	0.42
Holding birds (HB)	0.77	–0.01	0.31	0.55	–0.38	**0.18**	0.22	0.47
Following birds (FB)	0.59	1.00	0.55	0.37	–0.26	0.03	**0.10**	0.50
Total impression (TI)	0.83	–0.05	0.50	0.50	–0.14	1.00	0.13	**0.09**

Vangen and Klemetsdal (1988).

Table 13.6. Heritabilities of hunting traits using the NAVHDA test and midparent–offspring regression

	German SH Pointer		German WH Pointer		Griffon		Large Munsterlander		Poodle Pointer		Overall	
Trait tested	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE
Number of dogs	80		99		75		86		144			
Nose use	0.35	0.11*	0.32	0.12	0.33	0.08*	0.19	0.12	0.19	0.07*	0.02	0.04
Search	0.48	0.07*	0.31	0.10*	0.18	0.12	0.19	0.11	0.12	0.08	0.19	0.05*
Water retrieve	0.13	0.01	0.32	0.28*	0.30	0.10	0.24	0.16	0.31	0.09*	0.17	0.07*
Pointing	0.25	0.22	0.13	0.22	0.13	0.09	0.31	0.11*	0.10	0.15	0.18	0.08*
Tracking	0.48	0.09*	0.14	0.12	0.13	0.08	0.80	0.10	0.17	0.06	0.07	0.06
Desire to work	0.31	0.12*	0.14	0.10	0.2	0.10	0.22	0.11	0.05	0.08	0.10	0.05
Cooperation	0.22	0.12	0.34	0.11*	0.08	0.09	0.25	0.11	0.09	0.07	0.13	0.04*
Weighted total score	0.34	0.13*	0.27	0.14	0.22	0.10	0.33	0.13*	0.08	0.08	0.09	1.13

$*P < 0.05$.
After Schmutz and Schmutz (1998).

were significant only in those marked with an asterisk. The authors suggested that low values may indicate an advanced state of development in these breeds and thus reduced variation. Repeatabilities of NAVHDA tests were low and non-significant but dogs taking second tests were those which had failed originally and thus could have undergone extra training to seek to improve.

Police and armed service work

Dogs were used in the First World War (1914–1918) by most participants, with the British relying on Airedales and the Germans on GSD. Since that time the GSD has been the principal breed used by police and armed forces around the world. In more recent times gundogs such as English Springer Spaniels, Cocker Spaniels and Labrador Retrievers have been used in drug and explosive detection work, not through any lack of ability of the GSD but because of its tendency, when seeking drugs, to have part of its attention on the protection role, which gundogs do not have.

Heritability studies on Swedish army dogs (GSD) were undertaken by Reuterwall and Ryman (1973) based on over 900 animals bred in the training centre at Solleftea, puppy walked in private homes and then temperament tested at 18 months. Heritability estimates were derived from their data by Willis (1976) and are shown in Table 13.7. Values were disappointingly low but, as Mackenzie *et al.* (1986) suggested, the scoring system was complex and 18 month assessments may not be truly reflecting inherited differences. This may be equally true of the study of Schutzhund testing by Pfleiderer-Hogner (1979). Schutzhund testing comes in three grades termed SchH I, SchH II and SchH III with the higher numbers indicating more advanced work. Each test involves tracking, obedience, man work (protection) and character (courage). Thousands of tests are undertaken on GSDs in Germany each year and

Table 13.7. Heritabilities (half sib) of mental traits in German Shepherd dogs

	Paternal half-sib values	
Trait	Males	Females
Number of animals	488	438
Affability	0.17	0.09
Disposition for self defence	–0.11	0.26**
Disposition for self defence and defence of handler	0.04	0.16
Fighting disposition	0.16*	0.21*
Courage	0.05	0.13
Ability to meet sudden strong auditory disturbance	–0.04	0.15
Disposition for forgetting unpleasant incidents	0.10	0.17
Adaptiveness to different situations	0.00	0.04

$^*P < 0.05$, $^{**}P < 0.01$.
Willis (1976) after Reuterwall and Ryman (1973).

show dogs must obtain specific SchH grades to be exhibited as adults. Pfleiderer-Hogner analysed 2046 tests on 1291 GSDs from 37 sires with all testees being born in 1973. Her heritability figures did not differ from zero whether derived from sire/dam or combined components. She did, however, obtain a phenotypic correlation between Man Work and Courage of 0.76 ($P < 0.001$). It is difficult to believe that Schutzhund testing has no genetic value and one has to conclude that either the traits are under non-additive control or that, as with hunting dogs, the flaws in testing are serious enough to recommend new designs of testing which, 21 years later, has not happened. The latter explanation is more likely.

Tracking is an important feature for all police and service dogs. Pioneering work by Kalmus (1955) showed that dogs could distinguish between the body odours of different individuals, even of the same family and twins. King *et al.* (1964) found that dogs were beginning to fail to detect human odour after 7–14 days when samples were kept outside but could detect samples kept indoors for up to 6 weeks. Dogs joining a track at some mid-point are expected to go in the forward direction of the track but Mackenzie and Schultz (1987) showed that this was not the case. Even within a tracking breed, dogs vary in their ability for, and interest in, tracking but the genetic basis – if any – is as yet unclear.

An important feature of a working dog would be a good standard of hip conformation or an absence of hip dysplasia. In a study of 575 GSD army dogs, Mackenzie *et al.* (1985) graded hip dysplasia and temperament each on a scale of 1–9 with higher numbers being better. They found a heritability of 0.26 for dysplasia and 0.51 for temperament with a negative genetic correlation of –0.33. This suggests that better hips were associated with poorer temperaments, which is difficult to explain when a positive correlation might have been expected. This does, however, suggest that physical and mental traits may be linked.

Herding dogs

Herding breeds such as the Border Collie and Kelpie herd livestock, usually sheep, from one location to another. That they can do so relies as much upon the sheep being selected to flock combined with their flight behaviour as it does on the dog's instinctive ability to chase and nip. Some breeds like the GSD were used, in addition, to patrol the edge of an unfenced field acting in the role of a mobile fence to keep sheep on one side. Failure to select for sheep working potential means that most modern GSD have no sheep working instinct although a few lines exist based on HGH (a sheep working qualification) animals. Most breeds herd silently with their head down but some breeds like the New Zealand Huntaway work with head erect and are vocal. It has long been assumed that the herding dog is acting in a similar manner to its wolf ancestor stalking prey but, whereas the wolf stalk ends with a kill, the Collie stalk ends in moving or holding the sheep.

As Burns (1969) has pointed out, a Border Collie should understand and respond to at least eight commands to work effectively. Some dogs show 'eye' which means that they lower their head, half crouch and stare fixedly at the sheep. Other dogs may 'clap' that is they drop to the ground and may crawl towards the sheep or may get up and rush at them. Breeders select for such traits, but the mode of inheritance is unclear. Faced with African sheep, an ability to bark and move at the sheep was found to be necessary (Burns, 1969). It is astonishing to realize that the mode of inheritance of sheep work is little understood despite the value of the sheep industry world-wide and the absolute necessity to use dogs to handle sheep effectively. Jasper Rine and his colleagues (McCaig, 1996) are, as described below, attempting to find the genes for these traits in Border Collies and hybrids.

Livestock protection dogs

Livestock protection or guarding dogs trace back many centuries in their use for protecting sheep flocks. Most breeds doing this task today tend to be white in colour, whereas herding breeds are not. Breeds such as the Pyrenean Mountain Dog, Maremma, Komondor and Anatolian Shepherd are the modern-day descendants. Such dogs have no herding instinct but develop instinctive protectionist traits against predators (Linhart *et al.*, 1979; Coppinger *et al.*, 1983, 1985, 1988; Green, 1990). In the USA, the predators would be the coyote, the cougar, dogs and perhaps bears and re-introduced wolves. The role of the guarding dog is not to engage the predator in a fight but to frighten it away by the implied threat of a fight (Black, 1987).

It does appear that the livestock guarding breeds have a basic instinct. Breed differences in effectiveness were not significant for Great Pyrenees, Komondor, Askbash, Anatolians, Maremmas and hybrids (Green and Woodruff, 1983a,b,c, 1987; Green, 1989). All were aggressive towards predators, other dogs and occasionally people. It was seen that dogs reared with livestock by 8 weeks of age were more successful than those placed with livestock at an older age (Green and Woodruff, 1988). It is known that behavioural differences between herding and guarding breeds exist (Coppinger *et al.*, 1987), with the former showing a high level of partial predator behaviour and the latter a high level of agonistic play. According to Coppinger and his colleagues (1987) the two types of livestock dog are neotenic polymorphs unconsciously selected by differential retardation of ontogeny.

Genetics and Behavioural Selection of Pet Dogs

Breed differences

Scott and Fuller (1965) at Jackson Laboratory in Bar Harbor, Maine, performed the most comprehensive study of genetic differences in canine behaviour.

They used five breeds: Cocker Spaniels, Beagles, Shetland Sheepdogs, Basenjis and Wire-haired Fox Terriers. They performed a battery of tests to determine genetic differences in behaviour. Cocker Spaniels were the easiest to teach to sit quietly. The different breeds acted differently in response to leash training. Cocker Spaniels and Beagles were easiest to leash train and Basenjis were the worst; they baulked, fought the leash and strayed out of position. Shetland Sheepdogs fought the leash whereas Beagles were vocal (Fig. 13.3). Basenjis gave the best performance in reward training and Shetland Sheepdogs the worst, but the authors noted that the Shetland Sheepdogs were not as food motivated. Basenjis were best and Shetland Sheepdogs worst on a motor skill test in which the dogs had to climb a ramp and walk along a narrow elevated platform. Later, Scott and Fuller tested problem solving. The dogs had to learn to pass around a barrier to reach a dish of food instead of travelling directly to the dish. The next test involved pulling a dish from under a barricade. There were no particular breed differences in those two tasks. Beagles were best at learning a six choice point maze. A trailing test was administered in which fish was used to entice the dogs to follow a specific route. There were no breed differences. Cocker Spaniels were most successful and Shetland Sheepdogs the

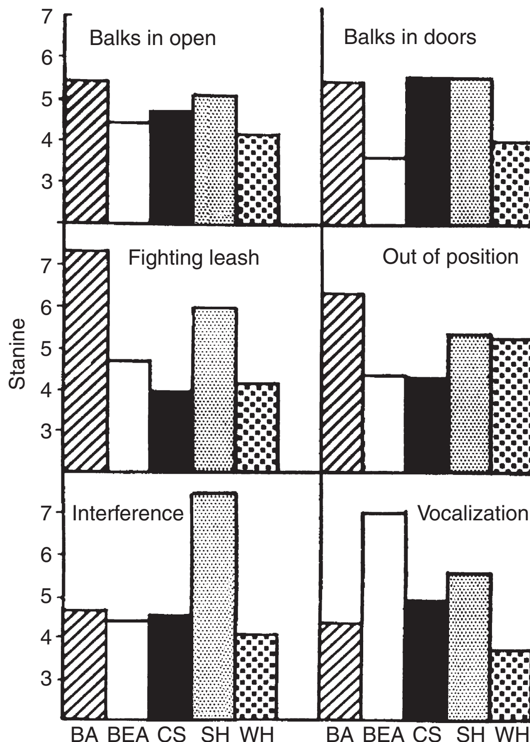

Fig. 13.3. Errors made by five breeds of dogs during leash training: BA = Basenji, BEA = Beagle, CS = Cocker Spaniel, SA = Shetland Sheep Dog, WH = Wire-haired Fox Terrier (with permission from University of Chicago Press).

worst in a problem solving test in which the dogs had to climb a ramp and walk across a narrow plank and to find a food reward. When testing puppies for complete dominance within litters of puppies, Scott and Fuller found that Wire-haired Fox Terriers, Shetland Sheepdogs and Basenjis showed complete dominance by 1 year of age, but Cocker Spaniels and Beagles did not. Dominance was determined by control of a bone.

Hart and Hart (1985) looked at 13 traits thought to be of importance to dog owners and then ranked 56 breeds on the opinions of 48 obedience judges and 48 veterinarians. Each person ranked only seven breeds. The authors then used analyses of variance to determine the magnitude of the *F*-test as a means of distinguishing between breeds. The results are summarized in Table 13.8 and indicate that features such as excitability and excessive barking were reliable means of distinguishing between breeds, whereas the ease with which a breed was house-trained was not. Thus, the Rottweiler ranked in the lowest decile for excitability but the highest for watchdog barking, whereas the Bloodhound ranked in the lowest decile on both traits and the GSD intermediate on excitability and the highest on watchdog barking. Allocating genetic parameters to such traits has not yet been done. Judges and veterinarians could be biased and limited in their knowledge of some breeds but in this instance concentrated on seven breeds each which they were obviously familiar with. The test may have flaws but the results seem feasible to those practically involved with dogs.

Hart and Hart (1985) found that cluster analysis revealed that three factors: reactivity, aggression and trainability, could explain most of the 13 traits. Not all aggression fell into the aggression factor; snapping at children clustered with reactivity. The breeds believed to be most likely to snap at children were:

Table 13.8. Behavioural traits ranked in decreasing order in differentiating between dog breeds

Trait	*F* Ratio
Excitability	9.6
General activity	9.5
Snapping at children	7.2
Excessive barking	6.9
Playfulness	6.7
Obedience training	6.6
Watchdog barking	5.1
Aggression to dogs	5.0
Dominance over owner	4.3
Territorial defence	4.1
Affection demand	3.6
Destructiveness	2.6
House training ease	1.8

Hart (1995).

Scottish Terrier, Miniature Schnauzer, West Highland White Terrier, Chow Chow, Yorkshire Terrier and Pomeranian. In a similar study performed in the UK, Bradshaw *et al.* (1996) ranked dogs according to cluster analysis using factors for aggression, reactivity and immaturity. The latter include traits such as playfulness and destructiveness. Dogs could rank high, average or low for each factor, giving eight categories. Breeds that ranked high in aggression, average reactivity and low immaturity were Rottweilers, GSD, Doberman Pinschers and Bull Terriers. Those which ranked low in aggression, high reactivity and low immaturity were King Charles Spaniels and Shetland Sheepdogs.

Intelligence and trainability

Coren (1994) surveyed 208 obedience judges from the USA and Canada. He concluded that for obedience and working intelligence, the top five breeds were Border Collie, Poodle, GSD, Golden Retriever and Doberman Pinscher (see Table 13.9). Coren admits that to do a scientifically valid test would require 25 people working for 3–5 years and cost US$14,000,000. Coren also performed a series of intelligence tests on many breeds of dogs. The tests he performed on a small number of dogs of each breed involved long- and short-term memory (finding food under one of several cups), problem solving using barriers, response to own or another name, reaction to a rearrangement of the furniture, etc. The highest overall (both memory and problem solving) were Doberman Pinschers, GSDs, Norwegian Elkhounds, Poodles, Pulis and Shetland Sheepdogs. His ranking for adaptive intelligence is shown in Table 13.11. Perhaps the most interesting observation on canine learning is that dogs in a companionship relationship with humans had decreased performance on problem solving compared with those that had a working relationship with humans (Topál *et al.*, 1997).

Table 13.9. Ranking of dogs for obedience and working intelligence

Rank	Breed
1	Border Collie
2	Poodle
3	German Shepherd
4	Golden Retriever
5	Doberman Pinscher
6	Shetland Sheepdog
7	Labrador Retriever
8	Papillon
9	Rottweiler
10	Australian Cattle Dog

Coren (1994).

Aggression

Because the dog lives in close association with humans, its behaviour is of great importance to the well-being of both. Although various behavioural traits are important to owners the main problem with dogs that affects this relationship is aggression towards humans (Mugford, 1995). Sudden unprovoked aggression against owners and their families was observed in Bernese Mountain Dogs in the Netherlands (Van Der Velden *et al*., 1976). Data on 404 animals were obtained, with aggression being graded into five categories of severity. Willis (1998) has tabulated these data, and it can be seen (Table 13.10) that increasing aggression grade of parents led to a definite increase in grade of aggression of progeny, which is indicative of a polygenic trait.

There are two types of canine aggression, desirable and undesirable, from a human standpoint. Several breeds of dogs have been selected for aggression, particularly aggression to strangers. Rottweilers, Doberman Pinschers, German Shepherds and Akitas are examples of breeds selected for that behaviour,

Table 13.10. Highest ranking dogs for adaptive intelligence

Highest overall intelligence	Good in learning and memory	Good in problem solving
Doberman Pinscher	Beagle	Australian Terrier
German Shepherd dog	Belgian Malinois	Basenji
Norwegian Elkhound	Belgian Sheepdog	Cairn Terrier
Poodle	Belgian Tervuren	Chihuahua
Puli	Bernese Mountain Dog	Fox Terrier
Shetland Sheepdog	Border Collie	Kerry Blue Terrier
	Bouvier des Flandres	Malamute
	Golden Retriever	Samoyed
	Labrador Retriever	Schipperke
	Welsh Corgi	Schnauzer
		Siberian Husky
		West Highland White Terrier

Breeds listed in alphabetical order. From Coren (1994).

Table 13.11. Aggression in Bernese Mountain dogs

Parents (at least one)	Number of progeny	Progeny grades 1	2	3	4	5	Mean grade
Grade 1	131	58.8	5.3	28.2	2.3	5.3	1.9
Grade 2	63	41.3	9.5	36.5	6.3	6.3	2.27
Grade 3	136	36.8	4.4	36.8	4.4	17.6	2.62
Grade 4	41	19.5	4.9	51.2	9.8	14.6	2.95
Grade 5	114	25.4	0.9	31.6	6.1	36.0	3.62
Total	485	39.2	4.5	34.4	4.9	16.9	2.56

After Van Der Velden *et al*. (1976).

and Hart and Hart (1985) found that those breeds were in the top decile for territorial defence. Many trainers believe that dogs are aggressive because their owners spoil them. According to Voith *et al.* (1992) dogs whose owners reacted anthropomorphically were no more likely to have behaviour problems.

No matter how desirable a dog is for protection, nobody wants a dog that bites him or her. The Hart and Hart (1985) study indicated that Fox Terriers, Siberian Huskies, Afghans, Miniature Schnauzers, Chow Chows and Scottish Terriers were most likely to be dominant over the owner. Dogs that attack other dogs can also be a problem. Scottish Terriers, Miniature Schnauzers, West Highland White Terriers, Chow Chows and Fox Terriers were believed to be the breeds most prone to this problem. Some breeds have been selected deliberately for fighting purposes, e.g. the American Pit Bull Terriers and increasingly steps are being taken by governments to control the breeding of such dogs. Politicians have to be careful that the right breeds are targeted.

Aggression may not be a unitary phenomenon, but there is considerable disagreement in how to subdivide it. Overall (1997) has 13 subcategories – maternal, play, fear, pain, territorial, protective, interdog, redirected, food-related, possessive, predatory, dominance and idiopathic aggression. Askew (1996) has 11: dominance, possessive, protective, predatory, fear, intermale, interfemale, pain-elicited, punishment-elicited, maternal and redirected aggression. Landsberg *et al.* (1997) listed the types of aggression as dominance, possessive, fear, territorial, predatory, pain, interdog, redirected, maternal and intraspecies, idiopathic and learned. Houpt (1998) has fewer categories: territorial, fear (including fear of pain), predatory, maternal, interdog and owner-directed (dominance).

Borchelt (1983) defined aggression in the dog in eight ways. These were fear-elicited (22), dominance aggression (20), possessiveness (17), protectiveness (17), predation (1), punishment-elicited (7), pain-elicited (2) and intraspecific aggression (12). The figures in brackets refer to the percentage of cases in the examination of 245 cases of aggressive behaviour. Breed differences were reported, with breeds like the Doberman Pinscher, Toy Poodle, Lhasa Apso and English Springer most likely to show dominance aggression. In contrast, working breeds, notably the GSD, showed more protective aggression and fear-elicited aggression was most likely in GSD and commonly seen in Cocker Spaniels (Borchelt, 1983). Similar findings were seen by Beaver (1983, 1993). In a smaller series, Line and Voith (1986) found that mixed breeds, Cocker Spaniels, English Springer Spaniels, Lhasa Apsos, Miniature Poodles and Brittany Spaniels were most likely to be presented for dominance aggression. The pure breeds of dogs most likely to be presented to five referral practices for aggression are shown in Table 13.12. Springer Spaniels, Wheaten Terriers, Doberman Pinschers, Old English Sheepdogs and Dalmatians appeared out of proportion to their breed registration (Landsberg, 1991). Notice that Springer Spaniels are included in the list from several clinic studies despite the fact that this is not a numerous breed in America.

It is difficult to decide which populations to use as a control for the behaviour cases. The national kennel club registration numbers, which

Landsberg (1991) used, or the dogs presented for behaviour problems can be compared with dogs presented for medical problems, but the breeds may vary in number geographically. Table 13.13 compares kennel club registration with breed incidence of aggression. The hospital population is more likely to reflect the breeds in the surrounding area, but, depending on the specialist in a referral hospital, some breeds may be over-represented. For example, dogs with progressive retinal atrophy are more likely to be presented if there are board-certified ophthalmologists on the staff.

Table 13.12. Breeds of dogs most frequently presented because of aggression

New York City[a]	Texas[b]	Toronto[c]	Kansas City[c]	Ithaca, NY[c]
German Shepherd	Cocker Spaniel	Cocker Spaniel	Cocker Spaniel	Springer Spaniel
Cocker Spaniel	German Shepherd	Springer Spaniel	Poodle	Cocker Spaniel
Poodle (all)	Springer Spaniel	Golden Retriever	Irish Wolfhound	German Shepherd
Lhasa Apso	Schnauzer (all)	Lhasa Apso	Golden Retriever	Golden Retriever
Doberman Pinscher	Dalmatian	Wheaten Terrier	German Shepherd	Lhasa Apso
Springer Spaniel	Basset Hound	Schnauzer	English Bulldog	Doberman Pinscher
	Old English Sheepdog	Bull Terrier	Dachshund	Labrador Retriever
	Poodle (all)	German Shepherd	Chow Chow	Poodle (all)
	Wire-haired Fox Terrier			

[a]Borchelt (1983), [b]Beaver (1983), and [c]Landsberg (1991).

Table 13.13. Breed incidence of behaviour problems in comparison to breed registration

Behaviour problems	CKC Registration 1989	AKC Registration 1989
Springer Spaniel	German Shepherd	Cocker Spaniel
German Shepherd	Labrador Retriever	Labrador Retriever
Cocker Spaniel	Poodle	Poodle
Golden Retriever	Shetland Sheepdog	Golden Retriever
Labrador Retriever	Golden Retriever	German Shepherd
Lhasa Apso	Cocker Spaniel	Rottweiler
Doberman Pinscher	Shih Tzu	Chow Chow
Poodle	Schnauzer	Dachshund
Dachshund	Lhasa Apso	Schnauzer
Schnauzer	Yorkshire Terrier	Beagle
Shetland Sheepdog	Rottweiler	Shetland Sheepdog
Wheaten Terrier	Pomeranian	Yorkshire Terrier
Old English Sheepdog	Siberian Husky	Shih Tzu
Yorkshire Terrier	Bichon Frise	Pomeranian
Beagle		Lhasa Apso
Dalmatian		

CKC = Canadian Kennel Club, AKC = American Kennel Club. All breeds listed in order of frequency.
Landsberg (1991).

In a study from the Animal Behaviour Clinic, College of Veterinary Medicine, Cornell University, the most frequently represented breeds presented for aggression toward strangers and for separation anxiety were compared to the breeds most often presented to the Veterinary Medical Teaching Hospital over the same time period (Takeuchi *et al.*, 2001; Table 13.14). Only individual breeds with ten or more dogs in at least one of the behaviours were evaluated. English Springer Spaniels were significantly more common among dogs with aggression to owners (10.7%) than among those with aggression to strangers (0%) ($P = 0.002$). Among dogs with aggression to strangers, 12% were Labrador Retrievers as compared to none observed among dogs with aggression to owners ($P < 0.0001$). English Springer Spaniels and Rottweilers (but not German Shepherds) had significantly ($P < 0.005$) greater representation among dogs with aggression to owners as compared to the hospital population. This is the first study to reveal that Rottweilers are presented more frequently than expected for dominance aggression. There was a significantly ($P = 0.02$) higher proportion of GSDs (but not Labrador Retrievers) among dogs with aggression to strangers as compared to the proportion observed in the hospital population. Other breeds were not evaluated, in light of the small numbers.

It is interesting that all four studies indicated that English Springer Spaniels are frequently presented for dominance aggression (Borchelt, 1983; Landsberg, 1991; Line and Voith, 1986; Takeuchi *et al.*, 2001). The changes in neurotransmitter levels in English Springer Spaniels indicate that a genetic cause, even a single gene, might be responsible. There does appear to be decreased levels of serotonin and dopamine metabolites in the cerebrospinal fluid of English Springer Spaniels euthanized for aggression (Reisner *et al.*, 1996). A survey of Springer Spaniel owners indicated that over 20% of English Springer Spaniels have bitten people (Reisner, 1996).

When all behaviour problems were combined, Wright and Neselrote (1987) found that sporting breeds were presented more often, followed by mixed breeds, working dogs, terriers, non-sporting, hounds and toys. The specific breeds of dogs most likely to be presented were Cocker Spaniels, GSDs, English Springer Spaniels, Doberman Pinschers, Golden Retrievers and Dachshunds.

Table 13.14. A comparison of breeds presented for behavioural problems with those presented for medical problems

Aggression to owner	Aggression to strangers	Separation anxiety	Hospital
English Springer Spaniel	Labrador Retriever	Labrador Retriever	Labrador Retriever
Rottweiler	German Shepherd	Dalmatian	Golden Retriever
German Shepherd	Dalmatian	Cocker Spaniel	German Shepherd
		Beagle	Cocker Spaniel
			Rottweiler

Takeuchi *et al*. (2001).

Abnormal behaviour

A long term study on Pointers selected for human aversion in comparison with a line selected for normal behaviour has been an interesting study on the genetics of behaviour. The nervous line was hypervigilant with a strong avoidance of humans and a low flight threshold. The nervous Pointers were much slower to learn operant conditioning (pressing a lever for a food reward), but were better at classical conditioning (pairing a sound with shock). They froze at loud sounds and would not approach a person (Dykman *et al.*, 1969). Phenothiazine-derivative tranquillizers were not effective in overcoming the fearful responses (Angel *et al.*, 1974). The nervous line showed a high sensitivity to the mange mite but few biochemical differences (DeLuca *et al.*, 1974). Inbreeding levels reached values in excess of 40% in the timid strain but inbreeding had minimal effect and it was concluded that human aversion was largely caused by additive genetic effects, suggesting a high heritability (Brown *et al.*, 1978).

Obsessive compulsive behaviour

Among the most interesting behavioural abnormalities are stereotypic or compulsive behaviours (Luescher, 1998). These are repetitive non-functional behaviours. They include circling, which is seen in Bull Terriers (Dodman *et al.*, 1996) and in a slightly different presentation in German Shepherds (Hewson *et al.*, 1998), digging as if for vermin by Rottweilers, anal checking by Schnauzers and flank sucking by Doberman Pinschers. The breed predispositions indicate a hereditary basis.

Sex differences in behaviour

The reaction of dogs to humans is very much influenced by the sex of both the dog and the human. Lore and Eisenberg (1986) found that in a kennel situation female dogs showed little reluctance to approach humans of either gender but male dogs were much less likely to make body contact with unfamiliar men. Wells and Hepper (1999) showed that the gender of both human and dog had an effect upon response that the dog displayed in some traits such as barking and eye orientation. This could have a bearing upon kennel staff, with females being more widely acceptable than males as regards relationship with the dog.

Hart and Hart (1985) found that obedience judges and veterinarians felt that male and female dogs differed in ten of 13 traits. Females were easier to house-break and obedience train. Females demanded more affection. Males were more playful and active, but were more likely to be aggressive to other dogs, to snap at children and to be dominant over the owner. Male dogs were also more likely to exhibit territorial defence.

According to Borchelt (1983), males were more aggressive than females and entire males more so than neutered males, while the reverse situation applied in females. Males were more likely to be presented for dominance and territorial aggression (Landsberg, 1991). Wright and Neselrote (1987) found that males outnumbered females for all behaviour problems, but the difference was not significant for destructive behaviour. Intact males and neutered females were most likely to be presented for behaviour problems. There were more intact than neutered males in the catchment population, but more neutered than intact females.

Takeuchi *et al.* (2000) found that male dogs significantly outnumbered females in cases of destructive behaviour. In contrast, 117 dogs (61 males (52%), 56 females (48%)) with separation anxiety were examined at the Behaviour Clinic of the Veterinary Medical Teaching Hospital at the University of California during the same period. In the study from New York and in previous studies by Borchelt and Voith from Pennsylvania (Voith and Borchelt, 1996) and by Podberscek *et al.* (1999) from the UK, significantly more male dogs than females were examined because of separation problems, whereas Wright and Neselrote (1987) from Georgia found no sex difference. There may be an interaction between sex and climate, particularly if male dogs that display destructive behaviour indoors can be kept outdoors in warmer areas.

Candidate genes for canine misbehaviour

In the case of aggression, there are many candidate genes: monoamine oxidase A in humans (Brunner *et al.*, 1993) and mice (Cases *et al.*, 1995), serotonin 5-HT1B receptor in mice (Saudou *et al.*, 1994) and alpha-calmodulin-dependent protein kinase II (CaMKII) in mice. This enzyme is required for activation of tryptophan hydroxylase which is the rate limiting step in serotonin synthesis (New *et al.*, 1998). In humans, a genetic mutation in the gene for tryptophan hydroxylase is associated with aggression (Manuck *et al.*, 1999). Transgenic mice over-expressing transforming growth factor alpha (TGF α) are also aggressive, but the relation of this to neurotransmitter or brain lesions is unknown. Finally, nitric oxide is involved in neural events throughout the body and, as a result, affects aggression. Knockout mice, lacking the neural form of nitric oxide synthetase, are aggressive (Nelson *et al.*, 1995). Mutation of any of these genes could be responsible for aggression in dogs.

The first identification of a gene that appears to be related to canine behaviour was that by Niimi *et al.* (1999), who compared the D4 dopamine receptor gene, *D4DR*, of Golden Retrievers and Shiba Inu. More allelic variation was found for Shiba than for Golden Retrievers, but that may reflect the larger gene pool of the native Japanese breed than of the imported Golden Retrievers. The Shiba is more reactive and aggressive but less trainable and playful than the Golden Retriever according to the ratings of Hart and Hart (1985) and Tanabe *et al.* (1999); they have more of the long-D allele than the

Golden Retriever. Humans with longer repeats in the *D4DR* gene have higher scores in personality traits for novelty seeking.

Summary

The changes in behaviour with domestication have, in general, followed changes in anatomy such as dropped ears, curly tail and spotted coats. Classical canine behavioural genetics indicates low heritability of such traits as hunting. Working dog behaviour has been better characterized than that of pet dog behaviour. The effects of sex and breed on behaviour and behaviour problems have been quantified, but single gene causes of abnormal behaviour have not been identified. The rapid advances in genomics, however, encourage us to expect that dogs will be screened for the gene alleles associated with aggression, anxiety and compulsive behaviour in the next decade, just as they are screened now for alleles associated with retinal pathology.

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14 Biology of Reproduction and Modern Reproductive Technology

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Introduction

Wild canids have only one oestrus cycle per year and are seasonal, whereas the domestic dog has one or two, sometimes even three, oestrous cycles per year and no obvious seasonality, although recent data indicates that fertility may be lower during the warm season (C. Linde-Forsberg and K. Andersson, Uppsala, 2000, unpublished data). Some breeds of dogs still show the original pattern of seasonal reproduction, notably the Basenji, and the vast majority of Basenji pups are born during the winter (see also Chapter 2). The long oestrous cycle, which is peculiar to the canid species, and the great variation in its length between individual bitches, the combined prooestrus and oestrus period varying from as little as 7 days to 27 days or more, makes it difficult to decide on which days mating or artificial insemination (AI) should be performed, and poor timing of the mating is a common cause of failure to conceive.

The dog has been used in medical research as a model for humans, but the lack of appreciation of the differences, especially in reproductive pattern and hormonal effects and sensitivity, has led to some classic misconceptions for instance regarding the tumorigenic effects of progestagens on mammary glands. In contrast, the dog has proved to be a very useful model in studies on human prostatic functions. The domestic dog is also increasingly being used as a model in research aiming at the preservation of the many species of wild canids that are threatened by extinction; projects popularly referred to as 'The Frozen Zoo'. During the last 10–15 years our knowledge of canine reproduction has made major progress. This chapter aims at summarizing the basic reproductive physiology of the dog, including the latest discoveries within this field, and also to give an update on the applications of new reproductive technology in this species.

Reproductive Endocrinology

The reproductive events, both in the male and the female dog, are orchestrated from the hypothalamus which, in response to some as yet partly unknown stimuli, produces and releases the gonadotrophin-releasing hormone (GnRH), which, in turn, influences the pituitary gland to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotrophic hormones induce ovarian follicular development and ovulation in the bitch and testicular development, androgen production and spermatogenesis in the male. The hypothalamic–pituitary–gonadal axis is regulated via intricate feedback mechanisms (Fig. 14.1a,b) whereby the gonadal hormones, having reached a certain concentration via negative feedback, downregulate further release of GnRH, and thus FSH and LH.

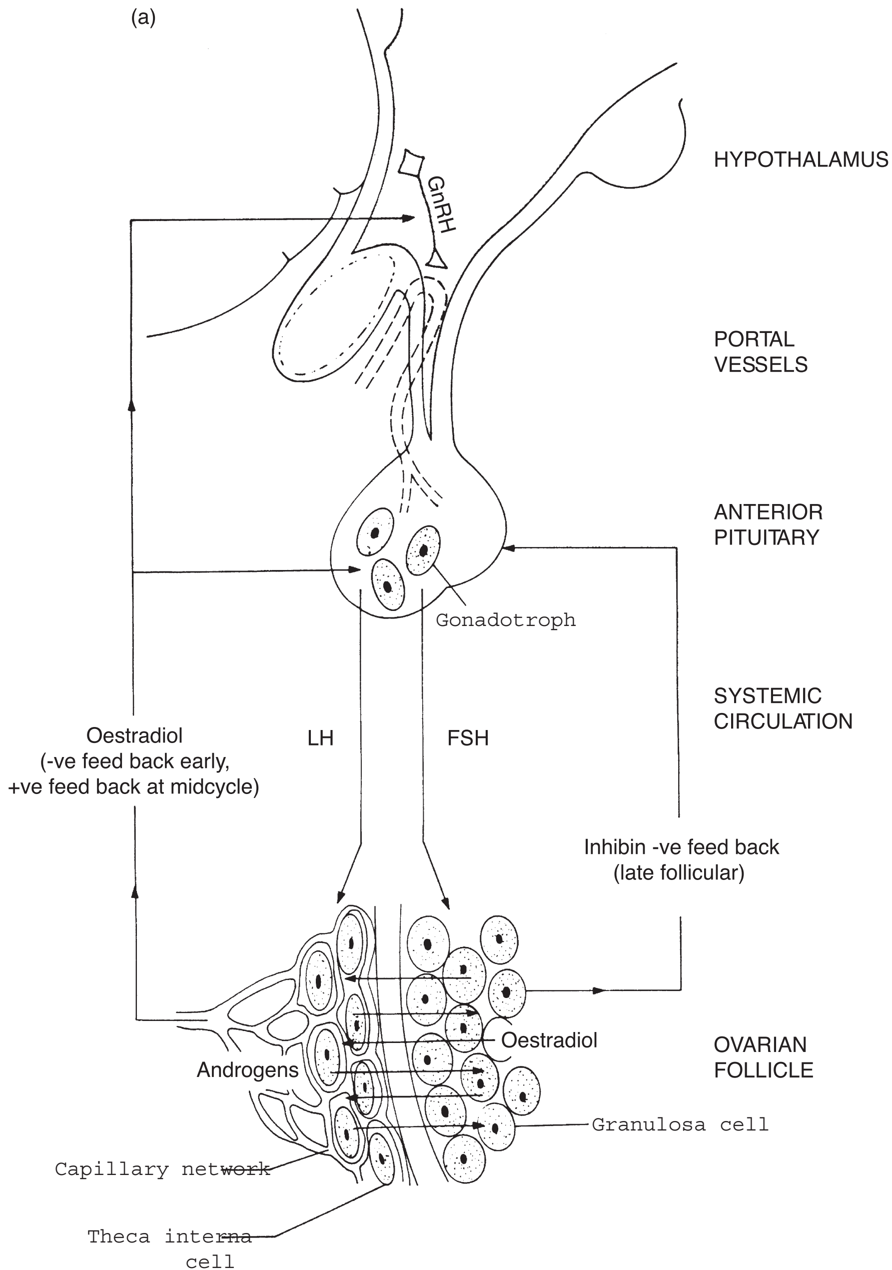

Fig. 14.1. (a) Summary of hypothalamic–pituitary–ovarian interactions during the follicular phase of the cycle.

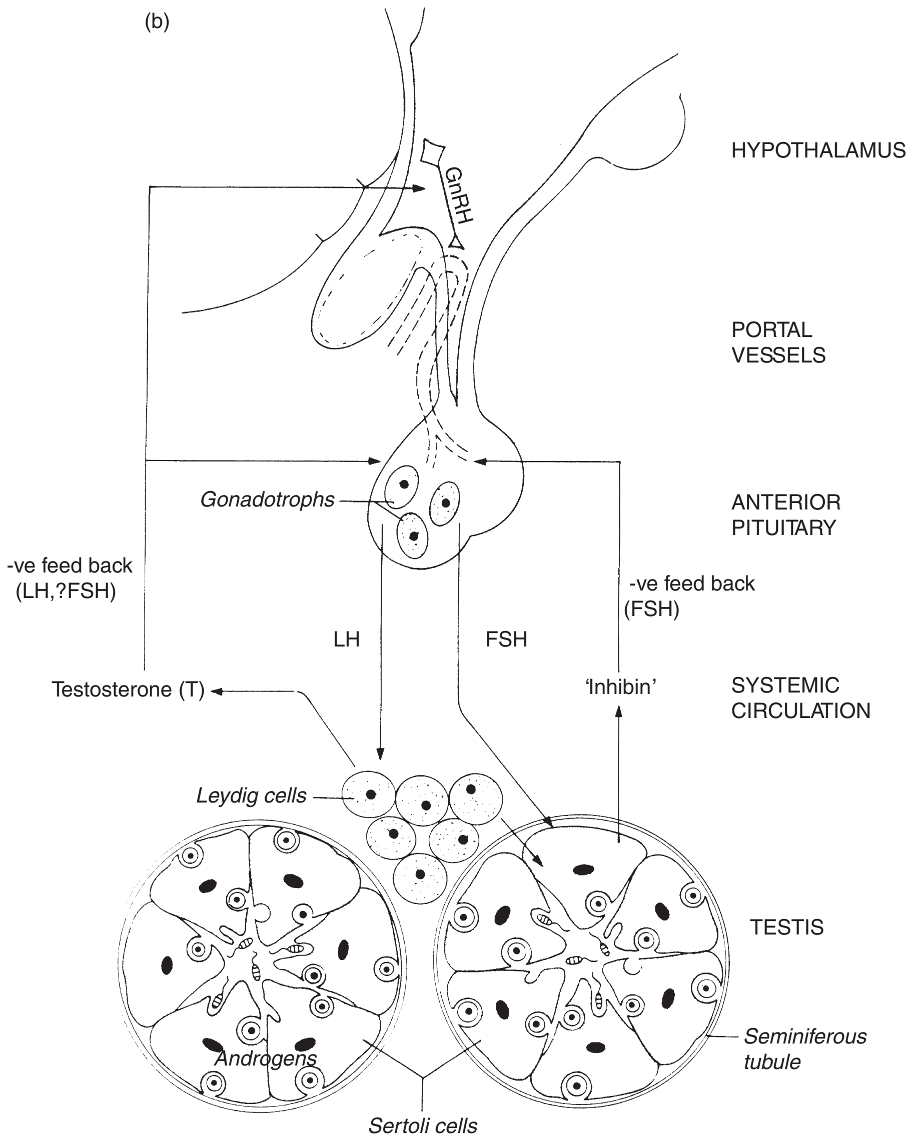

Fig. 14.1. (b) Summary of hypothalamic–pituitary–testis interactions in the male. (From Johnson and Everitt, 1990.)

Reproduction in the Male

The reproductive organs of the male dog consist of the testicles with epididymides and the vas deferens, the prostate gland, the urethra and the penis. The testicle contains the seminiferous tubules, producing spermatozoa, and the interstitium with Leydig cells which produce steroids, particularly testosterone, in the sexually mature individual. The epididymis consists of a

single long duct in which the spermatozoa during passage undergo maturational changes and obtain the capacity for motility. The distal part of the epididymis, the cauda, is the storage site for the matured spermatozoa. Prostatic fluid constitutes the major portion of the ejaculate, and contains several enzymes, cholesterol and lactate. The penis consists of a pelvic part and the glans penis, which is some 5–15 cm long, depending on the size of the dog. The glans penis has two cavernous parts, the bulbus glandis and the pars longa glandis, which fill with blood during sexual arousal, creating an erection (Fig. 14.2). The dog also has a penile bone, located dorsally of the urethra, which enables coital intromission of the non-erect penis.

Testicular descent

During fetal development the bipotential primordial germ cells migrate to the gonadal ridge, located caudal to the kidneys, where they differentiate into ovaries or testes. In the males, a gubernaculum testis, a mesenchymal structure, will develop in the caudal pole of the fetal testis and extend via the inguinal canal towards the scrotum. Structural changes in this gubernaculum are essential in the process of testicular descent which takes place in two phases. During the first phase, the extra-abdominal part of the gubernaculum increases in length and volume and extends past the inguinal canal, dilating

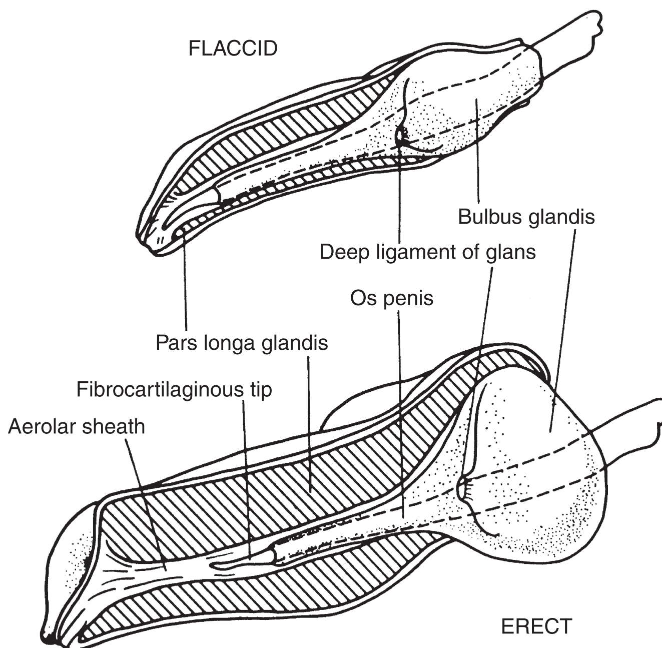

Fig. 14.2. Schematic representation of the changing relationship of the glans and os penis during erection in the dog. (From Grandage, 1972.)

this and creating the processus vaginalis, and incorporating the intra-abdominal part. During the second phase the gubernaculum is transformed into a fibrous structure enabling the testis to descend into the scrotum. At the time of birth the testes are located halfway between the kidney and the deep inguinal ring, at day 3–4 after birth they pass through the inguinal canal and they reach their final position in the scrotum at day 35–50 after birth (Baumans, 1982; Johnston and Archibald, 1991).

Puberty, sexual maturity and senescence

Puberty in the male dog usually occurs at between 6 and 12 months of age (Harrop, 1960). It is thought to depend on size, the larger breeds developing more slowly than smaller breeds, and it is not unusual that males of the giant breeds are 18 months or more of age before they can be used for breeding. Age at puberty is likely also to be influenced by genetic as well as environmental factors, such as nutrition. Attainment of puberty in the male dog is not obvious as in the bitch experiencing her first oestrus, but is a rather protracted process involving not only the display of sexual behaviour but also the beginning of sperm production and maturation during epididymal transit, as well as storage of the mature spermatozoa in the tail of the epididymis. The ejaculates from young dogs contain high percentages of abnormal spermatozoa (Taha *et al.*, 1981). Andersen and Wooten (1959) found that male dogs usually become sexually mature 2–3 months after they have reached adult body weight. Takeishi *et al.* (1980a) reported that Beagles reached puberty at 6 months, but optimal sperm production was first seen at 15–16 months of age. Little information is available on the effects of ageing on sexual activity in and fertility of the male dog. There is anecdotal data that 14-year-old dogs have sired litters. However, clinical data indicate that it is unusual for male dogs over 11 years to have good fertility, and attempts to freeze semen from dogs over 9 years old often yield disappointing results, even though the dogs may still have good fertility by natural mating (C. Linde-Forsberg, unpublished data).

Spermatogenesis/spermiogenesis

The production of spermatozoa is a continuously ongoing process throughout the fertile life of the male dog. The tubuli seminiferi in the testicles are lined by the spermatogonia, and the supporting and nurturing sertoli cells. In the sexually mature dog the spermatogonia undergo a series of mitoses resulting in primary spermatocytes which in turn divide by meiosis to become haploid round spermatids. The process of differentiation of the round spermatids into spermatozoa is called spermiogenesis. It includes the condensation of the DNA in the nucleus of the spermatid and the formation of the compact sperm head. The acrosome formation takes place and the arrangement of the mitocondria

into the sperm midpiece, as well as the growth of microfilaments into the sperm tail. The duration of the cycle of the seminiferous epithelium is 13.9 days in the dog. During epididymal transit, which takes around 15 days, the spermatozoa mature and a residual cytoplasmic droplet moves from a proximal to a distal position along the midpiece, and the spermatozoa acquire capacity for motility. The entire process of spermatogenesis, from spermatogonium to mature spermatozoa, takes 62 days (Davies, 1982; Amann, 1986).

Mating

Surprisingly little research has been done on mating behaviour in the dog (e.g. Beach and LeBoeuf, 1967; Hart, 1967; LeBoeuf, 1967; Beach, 1968, 1969; Fuller and Fox, 1969; Beach and Merari, 1970; Daniels, 1983; Ghosh *et al.*, 1984). From these studies it is apparent that most female dogs in oestrus demonstrate clear-cut mating preferences, which tend to persist from one breeding season to the next. Bitches also differ in their degree of attractiveness to the males. The sexual selectivity is influenced by social experience and familiarity, and individuals that are accepted as playing partners are often not the same ones as those accepted for mating. Pups reared from weaning to sexual maturity in isolation show deficient copulatory behaviour. Canids are basically monogamous, which is still the case in many wild canids.This can, for instance, be seen in the wolf packs, in which in most instances only the alpha couple mate, and live in a life-long relationship. The sexual promiscuity rather than pair-bond mating seen among dogs is considered to be a domestication phenomenon (Kretchmer and Fox, 1975).

The mating procedure in the canid species is different from in all other species studied in that it includes a copulatory tie (Fig. 14.3) which usually lasts for between 5 and 20 min, and although it may seem quite irrational, this apparently must serve a purpose as it has remained despite apparent drawbacks, such as vulnerability to attacks during the act.

The ejaculate

The dog ejaculates in three distinct fractions. The first fraction is emitted during courting and mounting of the female, and consists of from 0.5–7 ml of clear, prostatic fluid. The second, sperm-rich, fraction is emitted after intromission and begins before accomplishment of the copulatory tie and continues for a couple of minutes. The volume is from 0.5 to 3 ml and it contains the major portion of the spermatozoa. Its colour is whitish with an intensity that varies depending on the sperm concentration. The third fraction, again, consists of prostatic fluid. It is emitted during the major part of the tie, and its volume can be up to 30–40 ml in the larger breeds. The accomplishment of the tie is not necessary for the attainment of pregnancy, but is considered to increase the chances of conception.

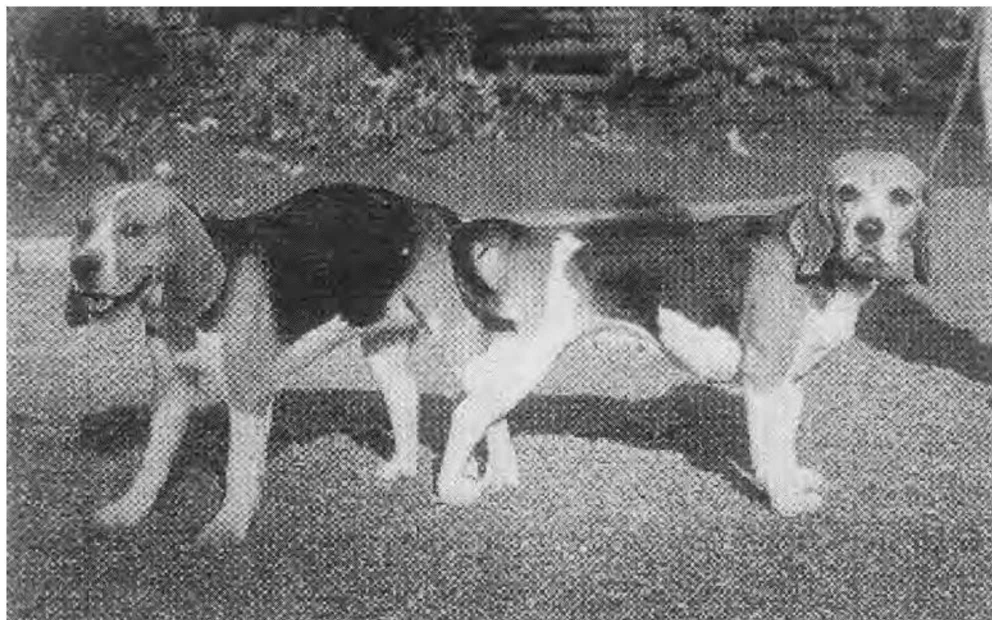

Fig. 14.3. The copulatory tie.

The ejaculate contains between 100 and 5000×10^6 spermatozoa, depending on the size of the dog. The percentage of abnormal spermatozoa should not exceed 20–40% and motility should be at least 70% (Feldman and Nelson, 1987; Oettlé, 1993). It has been suggested that a higher number of spermatozoa may compensate to some extent for a higher percentage of abnormal spermatozoa (Linde-Forsberg and Forsberg, 1989). Mickelsen *et al.* (1993) also found that in dogs the total number of normal, motile spermatozoa was more important than the percentage.

Sperm capacitation and the acrosome reaction

Spermatozoa must go through a process of capacitation to be able to undergo the acrosome reaction, thereby acquiring the capacity to fuse with and fertilize an ovum. The process of capacitation is poorly understood at the molecular level. Capacitated spermatozoa display a hyperactivated pattern of motility, which is characterized mainly by an accentuated curvilinear line velocity and lateral head displacement. Capacitation time varies between species, and has been found to be 3–7 hours for dog spermatozoa when studied in different culture media *in vitro* (Mahi and Yanagimachi, 1976, 1978; Tsutsui, 1989; Yamada *et al.*, 1992, 1993; Kawakami *et al.*, 1993; Guérin *et al.*, 1999). Rota *et al.* (1999) found that the preservation of dog spermatozoa by extending and chilling, or freezing and thawing, significantly shortened the time for

capacitation-like changes from 4 hours in fresh semen to 2 hours in the preserved samples.

The acrosome reaction is necessary for a spermatozoon to acquire its fertilizing capacity. It is believed to be triggered by an intracellular rise of Ca^{2+}. During the acrosome reaction the apical and pre-equatorial domains of the sperm plasma membrane fuse with the outer acrosomal membrane (e.g. Wassarman, 1990) leading to a release of the acrosomal contents, including hydrolytic enzymes which are necessary for the spermatozoon to be able to penetrate the zona pellucida of the oocyte and accomplish fertilization.

Daily sperm production

The daily sperm production has been found to be $12–17 \times 10^6$ spermatozoa per gram testis parenchyma (Davies, 1982; Olar *et al.*, 1983). The volume of the testicular parenchyma, the total number of spermatozoa and the ejaculate volume show a distinct correlation with body weight (Günzel-Apel *et al.*, 1994) and daily sperm production, therefore, normally varies with the size of the dog. It is generally considered that mature, healthy dogs can accomplish matings every second day without a decrease in ejaculate volume or number of spermatozoa (Boucher *et al.*, 1958).

Reproduction in the Female

The genital organs of the bitch consist of the two ovaries which contain the oocytes, and the tubular genital ducts, i.e. the oviducts, the bi-cornuate uterus with a short uterine body, the cervix, the vagina, the vestibulum and the vulva (Fig. 14.4). The vestibulum is quite large in this species, to be able to accommodate the bulbus glandis of the male during the copulatory tie. In the female all the oocytes are present already from birth, unlike in the male in which the spermatozoa are produced by the testicles throughout the dog's fertile life.

Puberty, sexual maturity and senescence

Puberty in the bitch appears, in most breeds, not to depend on day length, an exception being the Basenji which usually cycles only in the autumn. Puberty seems to be related to size and weight, in that it occurs when the bitch has reached around 85% of the adult weight and, consequently, bitches of the smaller breeds in general have their first oestrus at an earlier age than those of the larger breeds. Under the influence of the sexual hormones the growth plates of the long bones close, and little further growth will take place after this time. Most bitches, then, reach puberty at between 6 and 15 months of age, but some, especially of the large breeds, not until at 18–20 months of age. During the pubertal oestrus, circulating hormone levels are often low and

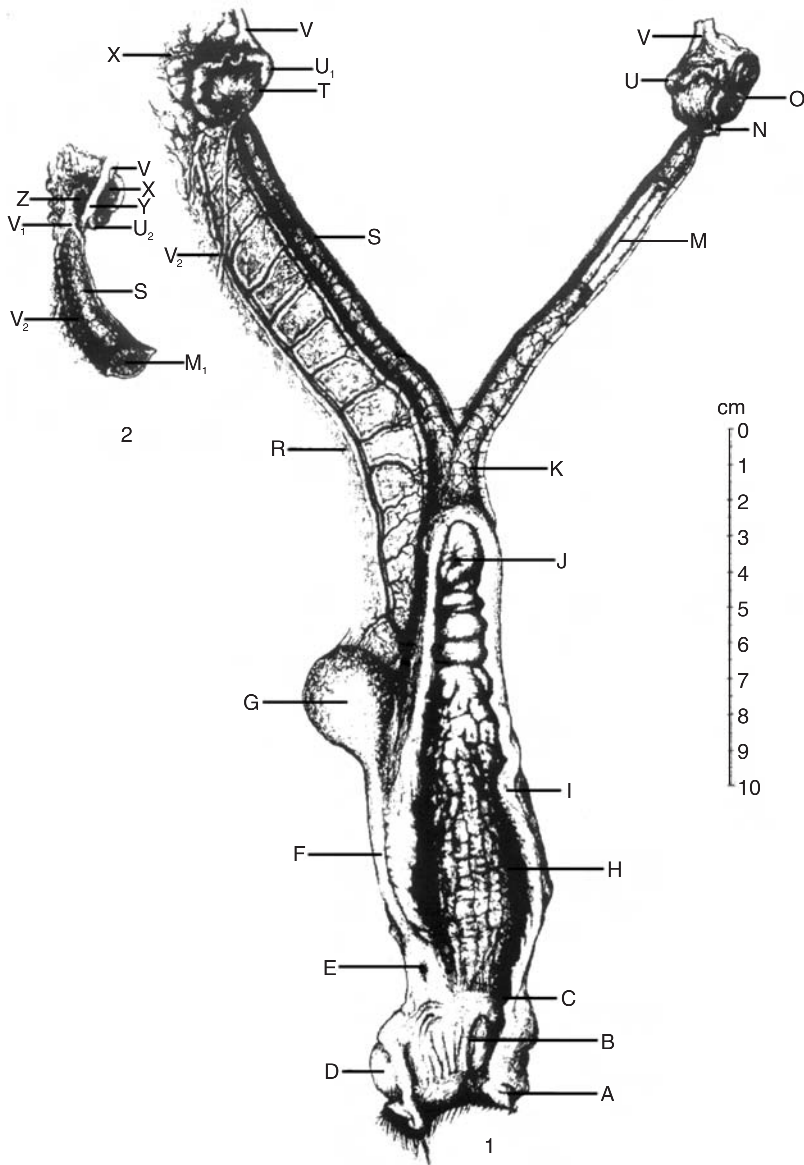

Fig. 14.4. The genital organs of the bitch. (From Andersen and Simpson, 1973.) A, vulva; B, vestibulum; C, cingulum; E, urethral orifice; F, urethra; G, urinary bladder; J, external os of cervix; K, body of uterus; M and S, uterine horns; U, oviduct.

fluctuating, causing absence of or incomplete ovulation and the bitch may not show standing oestrus (Chakraborty *et al.*, 1980; Wildt *et al.*, 1981). Few studies exist describing whelping rates after natural mating, but it appears to be around 85% in German Shepherd, Golden Retriever and Labrador Retriever guide dogs (England and Allen, 1989) and 90% in research colony Beagles

(Daurio *et al.*, 1987), although in the experience of ordinary dog breeders it is probably considerably lower because of breed differences in fertility and varying levels of skill among the breeders. Litter size is breed-related, and it increases until 3 years, and decreases after 7 years of age (see Christiansen, 1984). Senescence is considered not to occur in the dog. Bitches cycle and, if mated, may become pregnant all their life, even though their fertility decreases with age. Sometimes the periods between oestrus cycles may increase in the older bitch.

The oestrous cycle

The oestrus cycle of the bitch is classically divided into four stages: prooesterus, oestrus, metoestrus and anoestrus (Heape, 1900). Some prefer the terminology dioestrus instead of metoestrus for the luteal period. Prooestrus is considered to begin on the day when a vaginal haemorrhage can first be seen from the turgid vulva. Prooestrus lasts on average for 9 days, but can be as short as 3 days or as long as 27 days. The beginning of prooestrus is gradual and a precise first day is often difficult to assess with certainty. The bitch is inviting the male, but is not ready to mate. The vulval turgidity and the haemorrhage subside towards the end of prooestrus. In oestrus, by definition, the bitch allows mating, usually for a period of 9 days, but some only for 2 or 3 days and some for as long as 21 days. In metoestrus the bitch rejects the male again. The progesterone-stimulated uterine epithelium desquamates as the progesterone concentration subsides over 2–3 months. The endometrial repair process is completed after 4.5–5 months (corresponding to the human menstruation period). Anoestrus lasts for between 1 and 9 months, depending on whether the bitch has one, two, or three cycles per year. The interval between two oestrus periods is usually around 2 months longer after a pregnant cycle (Linde-Forsberg and Wallén, 1992). Breed differences in cycle length have been described, but they are controversial and difficult to discriminate from familial and individual variations (see Willis, 1989).

The bitch is a spontaneous ovulator, i.e. mating is not necessary for release of LH and subsequent ovulation. With the great individual variation in length of prooestrus, and the uncertainty about which exact day it starts, it is obvious that it is not possible to determine the fertile days of the bitch's cycle accurately if the timing is based on the days from onset of prooestrus. Some bitches may ovulate as early as day 3–4, and others as late as day 26 or 27 from the beginning of prooestrus. The only consistent relationship is the time from the LH peak until the onset of ovulation, ovulation in most bitches beginning 24–72 h after the LH peak (Concannon *et al.*, 1975, 1977a; Wildt *et al.*, 1978; Lindsay and Jeffcoate, 1993). All ova are not released simultaneously, but ovulation may take from 24 to 96 h. Boyd *et al.* (1993), using ultrasound, noticed that the ova were released from one ovary at a time. The whole process took 36 h. Unlike most other mammals, the dog ovulates primary oocytes that are at the beginning of the first meiotic division at metaphase

I (MI) and the germinal vesicle break down (GVBD) takes place shortly thereafter. *In vivo*, canine oocytes mature in the oviducts and there is a multilayered and tight cumulus mass around the oocyte, which is seen to expand as the oocyte matures, a process that takes 2–5 days to complete (Holst and Phemister, 1971; Mahi and Yanagimachi, 1976; Tsutsui, 1989; Yamada *et al.*, 1992, 1993).The oviductal transit takes 5–10 days (Andersen and Simpson, 1973; Tsutsui, 1989). Fertilization occurs during this passage, in the distal part of the oviduct. Mature canine ova may remain alive and fertilizable for 2–4.5 days (Concannon *et al.*, 1989; Tsutsui, 1989). Canine spermatozoa can fuse with immature oocytes (Mahi and Yanagimachi, 1976). Recent data indicate that the interval from fertilization to the eight-cell stage is 5 days if the bitch is inseminated before the oocytes have matured and only 3 days when insemination takes place after maturation, whereas 16-cell embryos were observed at day 11 after the LH surge with either early or late insemination (Concannon *et al.*, 2000). Thus, apparently, embryonic cleavage between 2 and 16 cells occurs more rapidly following fertilization of more mature oocytes. Canine spermatozoa have been reported to survive in the uterus of the female for at least 4–6, and in one case 11 days, after a single mating (Doak *et al.*, 1967). Theoretically, therefore, the bitch could conceive after one mating from about 1 or 2 days before until about 7 or 8 days after the LH peak, a period referred to as the 'fertile period' (Fig. 14.5). Available data suggest that the most fertile days are from 2 to 5 days after ovulation, i.e. from 4 to 7 days after the LH peak when the oocytes have all been released and have matured and are ready to be fertilized, a period referred to as the 'fertilization period' (Fig. 14.5).

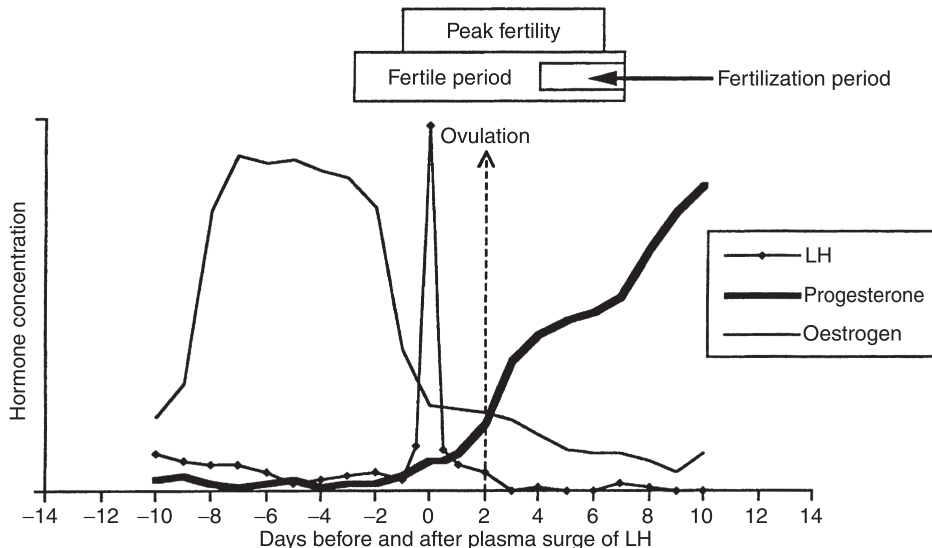

Fig. 14.5. Schematic representation of the changes in plasma progesterone, oestrogen and luteinizing hormone (LH) in relation to ovulation, and the fertile and fertilization periods of the bitch. (From England and Pacey, 1998.)

The most widespread indirect method of studying the oestrus cycle of the bitch is by way of vaginal exfoliative cytology. The vaginal epithelial cells respond to the rising oestradiol levels during prooestrus in a regular manner. The cell layers of the vaginal mucous membrane increase from 2–3 to 20–30 during oestradiol stimulation. The cells change during prooestrus from small parabasals, with a high nucleus : cytoplasm ratio, over the larger intermediary cells, which still have a large nucleus, to the fully cornified superficial cells, which are usually irregular in shape and sometimes have folded borders and contain either a small pycnotic nucleus or are anuclear. The vaginal epithelial cells respond to the increase in oestradiol in the peripheral plasma with a lag-time of 3–6 days (Linde and Karlsson, 1984). Maximal cornification can be seen in some bitches for up to 14 days, appearing during late prooestrus or early oestrus and remaining unchanged during the period of the abrupt fall in oestradiol and rise in progesterone preceding ovulation, and throughout oestrus. In metoestrus there is a quick shift from merely superficial cells to intermediary cells and parabasals. Very characteristic of metoestrus is the appearance of a large number of polymorphonuclear leucocytes. Because the changes in the vaginal cells are caused by oestradiol, while no effect has been reported to be caused by the rise in progesterone following ovulation, this method cannot be used to determine whether, or when, the bitch actually ovulates. Vaginal cytology thus is not an exact enough method for timing the bitch for artificial insemination. The technique is, however, useful in that a smear will show whether the bitch is still in prooestrus or already in metoestrus.

Measurements of peripheral plasma LH levels may be the most exact method for predicting ovulation in the bitch. LH assays are available, but because the LH peak only lasts for 1–2 days in the bitch, blood samples would have to be taken daily or every second day during prooestrus, which makes the method impractical and expensive. The method that best combines the practical and economic aspects with the requirement of exactness is measurement of the peripheral plasma progesterone concentration. The level of progesterone is basal (< 0.5 nmol l^{-1}) until the end of prooestrus, when the follicles change from producing oestradiol to producing progesterone shortly before the LH-peak. The bitch is unique with this long preovulatory progesterone production. When the LH peaks, the progesterone level is usually between 6 and 9 nmol l^{-1}. Ovulation occurs 1–2 days later at a progesterone level of between 12 and 24 nmol l^{-1}. Progesterone then rapidly rises to a maximum of around 150 nmol l^{-1} in about a week's time, then slowly decreases during the ensuing 2–3 months (Fig. 14.5). Because canine ova are released as primary oocytes and need 2–5 days to mature, the optimal time for mating or AI would be 2–5 days after ovulation, during the fertilization period, when the progesterone level is between 30 and 80 nmol l^{-1}. It should, however, be remembered that plasma levels of progesterone fluctuate considerably during the day, by up to 20–40%, but not in a regular diurnal fashion (Linde-Forsberg, 1994). Thus, even though the values obtained by a validated RIA or EIA assay are very exact, they should be interpreted with this daily variation borne in mind.

Sperm transport within the female genitalia, and fertilization

The male dog deposits the spermatozoa in the bitch's cranial vagina, but because of the copulatory tie and the large volume of the third fraction of the ejaculate, the spermatozoa are forced through the cervical canal into the uterine lumen and further through the uterotubal junction into the oviducts where fertilization ultimately takes place. Of the several hundred million spermatozoa that are deposited at mating, maybe only 1000 will finally reach the oviducts. Active contractions of the vagina and uterus contribute to this transport and spermatozoa are found in the oviducts only minutes after being deposited in the bitch's genital tract (Tsutsui *et al.*, 1988). The main sperm storage site in the bitch is considered to be the crypts of the uterine glands (Doak *et al.*, 1967; England and Pacey, 1998). Temporary attachment of the spermatozoa to the oviductal epithelium is thought to be an integral part of the capacitation process, ensuring the slow release over time of a sufficient population of spermatozoa during the long fertilization period of the bitch (England and Pacey, 1998). When the spermatozoon has succeeded first in attaching to and then in penetrating through the zona pellucida of the ovum into the perivitelline space, it elicits a zona blockage that prevents polyspermy, the equatorial segment of the sperm head binds to the plasma membrane of the oocyte and the two cells fuse.

Pseudopregnancy

As many as 40% of non-pregnant bitches experience a condition during the luteal phase called pseudopregnancy, a syndrome which to varying degrees mimicks the signs of pregnancy, including behavioural changes and/or mammary gland enlargement and milk production. Pseudopregnancy is believed to have been an evolutionary advantage in the wild dog, because it made it possible for other females in the group to produce milk and take over the nursing of the pups if something should happen to the mother. The cause of pseudopregnancy is considered to involve increased prolactin secretion and/or increased sensitivity of various tissues including the mammary gland to prolactin. Prolactin is necessary for luteal function during pregnancy in the dog, but is also secreted during non-pregnant luteal periods, although to a lesser degree. It can be seen to rise in response to an abrupt decline in progesterone. Prolactin concentrations in clinical cases of pseudopregnancy have not been adequately studied, but it was recently found that an important rise in prolactin levels was the only difference between animals developing pseudopregnancy and those that did not (Gobello *et al.*, 2000).

Pregnancy and parturition

Pregnancy in the dog is dependent on the ovaries for progesterone production during the entire 9-week period (Sokolowski, 1971). The major luteotrophic

hormones in the bitch are LH and prolactin (Concannon *et al.*, 1989). There is no apparent difference in progesterone level during non-pregnant and pregnant cycles. A haemodilution occurs from around day 25, progressing throughout pregnancy, causing a normochromic and normocytic anaemia and haematocrit values as low as 29–35% (Concannon *et al.*, 1977b).

Apparent gestation length in the bitch averages 63 days, with a variation of from 56 to 72 days if calculated from the day of the first mating to parturition. This surprisingly large variation in the comparatively short canine pregnancy is due to the long behavioural oestrus period of the bitch. Actual gestation length determined endocrinologically is much more constant, parturition occurring 65 ± 1 days from the preovulatory LH peak, i.e. 63 ± 1 days from the day of ovulation. Gestation length in the dog has been reported to be shorter for larger litter sizes, but this remains equivocal. Breed differences in gestation length, although not well documented, have been postulated (Okkens *et al.*, 1993) and Okkens *et al.* (2000) studying 113 bitches from six breeds found a mean gestation length for West Highland White Terriers of 62.8 ± 1.2 days, which was significantly longer than in German Shepherds with 60.4 ± 1.7 days, Labrador Retrievers with 60.9 ± 1.5 days and Dobermanns with 61.4 ± 1.0 days. They also found a negative correlation between mean length of gestation and litter size and concluded that breed was the main factor influencing the length of gestation and that this might be ascribed to breed-related differences in litter size. In contrast, Linde-Forsberg *et al.* (1999) studying fertility data from 327 frozen-semen AIs found no influence of either breed or litter size on gestation length.

The litter size in dogs varies with breed, or size, ranging from as few as one pup in the miniature breeds to more than 15 in some of the giant breeds. It is smaller in the young bitch, increases up to 3 to 4 years of age, to decrease again as the bitch gets older. A litter size of only one or two pups predisposes to dystocia because of insufficient uterine stimulation and large pup size, 'the single-pup syndrome' (Darvelid and Linde-Forsberg, 1994). This can be seen in dog breeds of all sizes. Breeders of the miniature breeds tend to accept small litters, but should be encouraged to breed for litter sizes of at least three or four pups to avoid these complications.

Based on a number of surveys, puppy losses up to weaning age appear to range between 10% and 30% and average around 12% (Linde-Forsberg and Forsberg, 1989, 1993). More than 65% of puppy mortality occurs at parturition and during the first week of life; few puppies die after 3 weeks of age. The possible genetic background of fetal and neonatal deaths has not been investigated in the dog.

Stress produced by the reduction of the nutritional supply by the placenta to the fetus stimulates the fetal hypothalamic–pituitary–adrenal axis, this results in the release of adrenocorticosteroid hormone and is thought to be the trigger for parturition. An increase in fetal and maternal cortisol is believed to stimulate the release of prostaglandin $F_{2\alpha}$, which is luteolytic, from the feto-placental tissue, resulting in a decline in plasma progesterone concentration. Increased levels of cortisol and of prostaglandin $F_{2\alpha}$-metabolite

have been measured in the prepartum bitch (see Concannon, 1998). Withdrawal of the progesterone blockade of pregnancy is a prerequisite for the normal course of canine parturition; bitches given long acting progesterone during pregnancy fail to deliver. In correlation with the gradual decrease in plasma progesterone concentration during the last 7 days before whelping there is a progressive qualitative change in uterine electrical activity, and a significant increase in uterine activity occurs during the last 24 h before parturition with the final fall in plasma progesterone concentration. In the dog, oestrogens have not been seen to increase before parturition as they do in many other species. Oestrogens sensitize the myometrium to oxytocin, which in turn initiates strong contractions in the uterus when not under the influence of progesterone. Sensory receptors within the cervix and vagina are stimulated by the distension created by the fetus and the fluid-filled fetal membranes. This afferent stimulation is conveyed to the hypothalamus and results in release of oxytocin. Afferents also participate in a spinal reflex arch with efferent stimulation of the abdominal musculature to produce abdominal straining. Relaxin, which is pregnancy-specific, causes the pelvic soft tissues and genital tract to relax, which facilitates fetal passage. In the pregnant bitch this hormone is produced by the placenta, and it rises gradually over the last two-thirds of pregnancy. Prolactin, the hormone responsible for lactation, begins to increase 3–4 weeks following ovulation and surges dramatically with the abrupt decline in serum progesterone just before parturition.

The final abrupt decrease in progesterone concentration 8–24 h before parturition causes a drop in rectal temperature. This drop in rectal temperature is individual but also seems to depend on body size to some extent. Thus, in miniature-breed bitches it can fall to 35°C, in medium-sized bitches to around 36°C, whereas it seldom falls below 37°C in bitches of the giant breeds (Linde-Forsberg and Eneroth, 1998, 2000). This difference is probably an effect of the surface area : body volume ratio. Several days before parturition, the bitch may become restless, seeks seclusion or is excessively attentive, and may refuse all food. The bitch may show nesting behaviour 12–24 h before parturition concomitant with the increasing frequency and force of uterine contractions and shivering in an attempt to increase the body temperature.

In primiparous bitches, lactation may be established less than 24 h before parturition, while after several pregnancies, colostrum can be detected as early as 1 week prepartum.

Parturition is divided into three stages, with the last two stages being repeated for each puppy delivered. The duration of the first stage is normally between 6 and 12 h. Vaginal relaxation and dilation of the cervix occur during this stage. Intermittent uterine contractions, with no signs of abdominal straining, are present. The bitch appears uncomfortable, and the restless behaviour becomes more intense. Panting, tearing up and rearranging of bedding, shivering and occasionally vomiting may be seen. The unapparent uterine contractions increase both in frequency and intensity towards the end of the first stage. During pregnancy the orientation of the fetuses within the uterus is 50% heading caudally and 50% cranially, but this changes during first-stage labour

as the fetus rotates on its long axis extending its head, neck and limbs to attain the normal birth position, resulting in 60% of pups being born in anterior and 40% in posterior presentation (van der Weyden *et al.*, 1981, 1989).

The duration of the second stage is usually 3–12 h, in rare cases up to 24 h. At the onset of second-stage labour, the rectal temperature rises and quickly returns to normal or slightly above normal. The first fetus engages in the pelvic inlet, and the subsequent intense, expulsive uterine contractions are accompanied by abdominal straining. On entering the birth canal the allantochorionic membrane may rupture and a discharge of some clear fluid may be noted. Covered by the amniotic membrane, the first fetus is usually delivered within 4 h after onset of second-stage labour in the dog. In normal labour the bitch may show weak and infrequent straining for up to 2 h and at the most 4 h before giving birth to the first fetus. If the bitch is showing strong, frequent straining without producing a pup this indicates the presence of some obstruction and she should not be left for more than 20–30 min before seeking veterinary advice.

The third stage of parturition, expulsion of the placenta and shortening of the uterine horns, usually follows within 15 min of the delivery of each fetus. Two or three fetuses may, however, be born before the passage of their placentas occurs. Lochia, i.e. the post-partum discharge of fetal fluids and placental remains, will be seen for up to 3 weeks or more, being most profuse during the first week. Uterine involution is normally completed after 12–15 weeks in the bitch (Al-Bassam *et al.*, 1981).

The total incidence of canine difficult births, dystocia, has not been reported, but probably averages below 5% in most breeds. In some breeds, however, it approaches 100%. Many of the achondroplasic breeds, such as the Bulldog breeds, Boston Terriers and Scottish Terriers, have whelping problems. In Boxers 21%, in French Bulldogs 43% and in Boston Terriers 62% of bitches needed caesarean section (C. Linde-Forsberg, unpublished data; Eneroth *et al.*, 1999). Uterine inertia is the most common cause for dystocia (Darvelid and Linde-Forsberg, 1994), and some breeds seem to be more prone to developing this disorder, for instance the Boxer with 28% dystocia, and the Smooth-haired Dachshund with around 10% of bitches needing veterinary assistance at whelping (C. Linde-Forsberg, unpublished data). In the Boston Terrier and Scottish Terrier breeds there is also a significant flattening and narrowing of the pelvis (Eneroth *et al.*, 1999) causing obstructive dystocia. There is a strong tendency in Boston Terriers for a hereditary influence on pelvic shape from both the mother and the father (Eneroth *et al.*, 2000).

Assisted Reproductive Technologies

The first scientific publication on the use of reproductive biotechnology in a mammal is by Abbé Lazzarro Spallanzani in 1784, in which he describes how he performed artificial insemination of a bitch. Despite this promising start, little further happened within this field in the dog until some 170 years later.

However, the number of studies on fresh, chilled and frozen–thawed dog semen, although initially greatly lagging behind in comparison to the interest shown in the preservation of semen from farm animals, is nowadaws growing exponentially by the year. However, biotechnology in the form of *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and embryo transfer (ET) has not been extensively studied in this species, and attempts at cloning are just beginning.

Artificial insemination

Although the first artificial insemination in the dog was performed more than 200 years ago, it was not until the late 1950s that interest began to focus on this field of research. Harrop (1960) described the first successful AI using chilled extended semen. The first litter by frozen–thawed dog semen was reported by Seager in 1969. Since then, interest in canine artificial insemination has grown exponentially. With the advent of AI technology, breeders not only have the potential to use dogs from all over the world, but can also save deep-frozen semen from valuable dogs to be used in later generations. In many countries, the Kennel Clubs, in one way or another, controls the use of artificial insemination and restricts to approved agencies the right to perform AI and to freeze, store and release dog semen. The main concern of the Kennel Clubs is that the identity of the semen doses is strictly controlled. New knowledge is constantly accumulating, and techniques for semen preparation, oestrus detection, and insemination are being improved.

The keys to obtaining good results are proper timing of the insemination, the use of high quality semen, and good semen handling and inseminaton techniques. AI in the dog can be performed by depositing the semen either in the cranial vagina or in the uterus. Recent field data shows that intrauterine deposition of the semen significantly improves whelping rates and litter sizes using fresh, and chilled extended, as well as frozen semen (Linde-Forsberg, 2000; Table 14.1). Linde-Forsberg and Forsberg (1989) reported whelping rates of 83.8% with fresh and 69.3% with frozen semen under optimal conditions. In 2211 AIs performed under varying conditions, however, pregnancy rates were 48.5% for fresh, 47.0% for chilled extended and 51.8% for frozen–thawed semen (C. Linde-Forsberg, unpublished data). Interestingly, the whelping rate in 170 bitches that were both artificially inseminated and mated was 83.5%, indicating that some AIs may have been performed at the wrong time during oestrus, usually too early (Linde-Forsberg, 2000).

Information on the relationship between semen quality and fertility is very sparse in the dog. The recommended total number of spermatozoa per breeding unit is $150–200 \times 10^6$ (Andersen, 1975, 1980). Good pregnancy results have, however, been achieved with as few as 20×10^6 fresh spermatozoa deposited surgically at the tip of the uterine horn (Tsutsui *et al.*, 1989a) and with two doses of $30–35 \times 10^6$ normal, frozen–thawed spermatozoa deposited into the uterus through the cervix with the aid of an endoscope (Wilson, 1993). By applying intrauterine instead of vaginal deposition of semen, around 10

Table 14.1. Whelping rate and litter size after vaginal or intrauterine artificial insemination (AI) using fresh, chilled and frozen–thawed semen (n = 2041) (Linde-Forsberg, 2000)

	Whelping rate (%)		Litter size	
Semen type	Vaginal AI	Intrauterine AI	Vaginal AI	Intrauterine AI
Fresh	47.8	65.2	5.8 ± 2.8	6.5 ± 2.5
Chilled	45.1	65.6	5.8 ± 3.0	6.4 ± 3.2
Frozen–thawed	34.6	52.0	4.7 ± 2.6	5.0 ± 3.2

times fewer spermatozoa are required (Tsutsui *et al.*, 1989a; Linde-Forsberg *et al.*, 1999). Breed differences in fertility have been described (Linde-Forsberg and Forsberg, 1989, 1993). Semen quality may also vary betwen breeds and was found to be generally poor in Irish Wolfhounds (Dahlbom *et al.*, 1995, 1997).

The vast majority of canine inseminations are performed with fresh semen. It is easy to handle, and the semen can be deposited in the cranial vagina, which is technically quite easy, although results are better after intrauterine deposition (Linde-Forsberg, 2000). One reason why pregnancy rates tend to be low following AI using fresh semen is probably that many of those are performed in dogs that have a problem and for some reason will not mate naturally. Some Kennel Clubs have set up ethical rules for when AI is acceptable and when it is not.

Semen to be stored or shipped should always be extended and chilled. The extender helps to protect the spermatozoal membranes from damage caused by changes in temperature and shaking during transport, while also providing energy and stabilizing the pH and osmotic pressure. Furthermore, chilling lowers the metabolic rate, thereby increasing sperm longevity. Spermatozoa in an extender may survive cooling to 4°C for several days (Rota *et al.*, 1995). Chilled, extended semen is both easier and cheaper to handle and to ship than frozen semen. A disadvantage with chilled, extended semen is that everything has to be arranged on the day most suitable for the bitch.

Dog semen is frozen either in straws (usually 0.5 ml but occasionally 0.25 ml) or in pellets. The pellets have some disadvantages in that they are more difficult to identify, and they can become contaminated by spermatozoa and infectious agents from other pellets; consequently, freezing in straws is preferred by most semen agencies. Extenders used for freezing dog semen usually contain glycerol as cryoprotectant. Rapid thawing at 70°C for 8 s has been shown to be significantly better than at 37°C for 15 s (Rota *et al.*, 1998; Peña and Linde-Forsberg, 2000). Advantages of frozen semen include the fact that it can be shipped at a time convenient for all parties and that many doses can be sent in a single shipment to be used when desired. The semen banking option also may prove to be of exceptional value in dog breeding. Deposition of frozen semen in the cranial vagina generally results in a poor pregnancy rate (Linde-Forsberg, 1991, 1995, 2000; Linde-Forsberg *et al.*, 1999) although there are some reports of good success (Seager, 1969; Nöthling and Volkmann,

1993). Furthermore, individual dogs differ markedly in terms of how well their semen freezes. Normal fertility at natural mating is no guarantee that the semen will still be viable after freezing and thawing. Litter size was estimated to be 23.3% and 30.5% smaller, respectively, in bitches inseminated with frozen compared with fresh semen (Linde-Forsberg and Forsberg, 1989, 1993).

Methods for AI in bitches include vaginal deposition of semen, transcervical intrauterine deposition, surgical intrauterine deposition, and intrauterine insemination by laparoscopy. For vaginal inseminations, a 20–45 × 0.5 cm disposable plastic catheter can be used. The correct placing of the tip of the catheter, close to the cervix, should always be checked by abdominal palpation.

For transcervical intrauterine inseminations, the Scandinavian catheter, a 20–50 cm long steel catheter with a 0.5–1 mm diameter tip and an outer protecting nylon sheath, is used (Andersen, 1975; Linde-Forsberg, 2001). The AI is performed with the bitch standing in the normal position. The cervix is fixed between the inseminator's fingers by abdominal palpation and the catheter is introduced through the cervix. No sedation is needed, most bitches in oestrus freely accept this type of handling. Transcervical intrauterine insemination can also be done with the aid of an endoscope, for instance a rigid cysto-urethroscope, and a urinary or angiographic catheter (Wilson, 1993). Sedation of the bitches is not necessary.

Intrauterine AI is also performed surgically with the bitch under general anaesthesia and in dorsal recumbency (Hutchison, 1993). Whether it is ethically acceptable to resort to surgery to achieve pregnancies is, however, a matter of debate. The anaesthetic risks and the risks for infection and other problems associated with surgery and the limited number of surgical AI that can be performed on a given bitch are obvious disadvantages. The method is also costly and time-consuming. Abdominal laparoscopy is a well established technique in human gynaecology and should offer a more acceptable alternative to full surgery for AI in the dog (Wildt, 1986).

In vitro *maturation (IVM) and* in vitro *fertilization (IVF)*

When attempts are made to mature canine oocytes in artificial media *in vitro* only high quality oocytes, with at least two layers of cumulus cells, with a dark and homogeneous ooplasm, and an intact zona pellucida should be selected. The size of the oocyte seems of importance as significantly more large ones (> 112 μm) than smaller ones (36.2% vs. 19%) mature to metaphase I, anaphase-telophase I (A-TI) or metaphase II (MII) (Theiss, 1997). Various culture media have been used: TCM 199, Hams F-10 with BSA or homologous serum, and Kreb's–Ringer lactate. There are conflicting results concerning the beneficial effects of the addition of hormones such as FSH, LH, oestradiol or progesterone to the culture media on the maturation rate (Yamada *et al.*, 1992, 1993; Nickson *et al.*, 1993; Hewitt and England, 1999). It has been suggested

that the granulosa cells may contain an FSH-dependent pathway that is capable of controlling oocyte maturation (Kalab *et al.*, 1997). Even when all details are optimized and the canine oocytes are successfully stimulated to resume meiosis the maturation rate *in vitro* is considerably lower (from 0 to 58%; Mahi and Yanagimachi, 1976; Robertson *et al.*, 1992; Nickson *et al.*, 1993; Yamada *et al.*, 1993; Hewitt and England, 1997, 1998, 1999; Theiss, 1997; Bolamba *et al.*, 1998) than that observed in oocytes from other species.

A peculiarity of canine spermatozoa is that they will not fuse with zona-free hamster eggs (Yanagimachi, 1988). No sperm penetration of the zona pellucida has been observed in IVF studies of canine oocytes that have been *in vitro* matured for less than 24 h, whereas in those matured for 48–72 hours spermatozoa were seen to penetrate the zona within 1 h. Metcalfe *et al.* (2000) found that IVM and IVF are possible also using oocytes recovered from naturally cycling bitches and that embryos could be produced with only 24 h oocyte maturation time and that induction of sperm capacitation is not necessary prior to IVF when the spermatozoa are added to cumulus–oocyte complexes. They, however, conclude that the conditions for IVM and IVF in the dog are still suboptimal (Metcalfe *et al.*, 2000).

Embryo transfer

Only a few studies have described the transfer of non-frozen *in vivo* derived canine embryos, and fewer still were successful resulting in the birth of live young (Kinney *et al.*, 1979; Kraemer *et al.*, 1979; Takeishi *et al.*, 1980b; Tsutsui *et al.*, 1989b, 2000; Kim, 1994). The embryos were obtained from the donors by uterine flushing through surgery. Success rates were from 100% (one bitch), 40% (two out of five bitches) and 12.5% (one out of eight bitches), with litter sizes of 2, 1 and 2, and 1 pup respectively. Tsutsui *et al.* (2000) reported about 50% success rate (four bitches out of eight) by transfer of embryos obtained by tubal flushing after salpingectomy 3–7 days after ovulation, when the embryos were transplated into the lower oviduct in recipient bitches, while none of the seven bitches that were transplanted into the upper oviduct became pregnant. One of the major problems with ET in dogs is the need for synchronization of donor and recipient, which is difficult because of the peculiarities of the reproductive pattern in this species.

Cloning

Cloning is a way of creating identical individuals. It is done by using either a cell from an embryo (blastomere), an embryonal stem cell from a fetus, or a somatic cell from an adult individual, and transferring them into oocytes from which the endogenous genetic material (i.e. the nucleus containing the chromosomes) has been removed (Fig. 14.6). The egg cell and the embryo cell are stimulated to fuse by an electric impulse. The cytoplasm of the oocyte

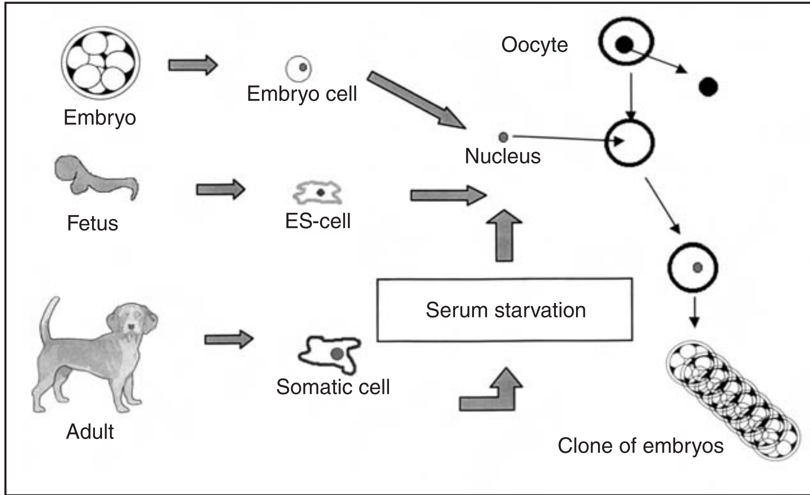

Fig. 14.6. Schematic representation of the ways to create a clone of embryos.

changes the programming of the embryo cell so that it can develop into a new embryo, with the identical genetic set-up as the original embryo. This procedure can be repeated again and again, thus creating unlimited numbers of clones. The embryo death rate is, however, high. Embryonal stem cells are lines of undifferentiated cells that can be grown in cell cultures *in vitro* and can be manipulated before being transplanted. Such cell lines have been created in some of the laboratory animals. The well-known sheep, Dolly, born in 1996, was the first clone created using a somatic cell (Wilmut *et al.*, 1997). A cell from the mammary gland was used. The cell was cultured *in vitro* during 'serum starvation' to enter a resting stage. After transplantation to the anucleated oocyte and an electric impulse, it behaved like an undifferentiated embryonal cell and developed into an embryo. From the 277 embryos created in this way and transferred into recipient sheep, one lamb, Dolly, was born.

Cloning using somatic cells from adult animals has, so far, been successfully performed in sheep, cattle and mice. An American group of researchers are working on a project to clone dogs, financed by an eccentric dog owner to create a copy of his beloved pet 'Missy'. From the name of this dog the project has become known worldwide as the 'Missyplicity Project' and it can be followed through its own homepage on the Internet (www.Missyplicity.com). The process is, however, more complicated in the dog, depending on the peculiarities of the reproductive pattern of this species, compared to that of most of the other species studied, and because many of the basic mechanisms controlling reproduction are as yet unknown. Some of the techniques involved, such as superovulation, oestrus synchronization, embryo transfer and *in vitro* production of embryos are not yet developed in the dog. The issue of oestrous synchronization may prove particularly difficult in this species,

because of the protracted cycle, and the great individual variation in oestrus periodicity and length. The need for a large number of metaphase II oocytes of high enough quality is also a challenge.

The American group has used *in vivo* matured dog oocytes as recipients. Of 109 such oocytes, 63 were enucleated and 43 of those successfully fused with cells from adult dogs, resulting in the production of ten cleaved embryos, but no pups (Westhusin *et al.*, 2000). In another study by the same group, bovine oocytes were used as recipients for the adult canine cells, and 43% developed to the 8–16 cell stage *in vitro*. Forty-seven of the cloned embryos were transferred into four recipient bitches, resulting in one single conceptus which, however, was lost after 20 days of gestation (Westhusin *et al.*, 2000). The interest in cloning companion animals will no doubt be great, but a lot more research remains before this can become a routine procedure in dogs.

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Developmental Genetics

15

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Introduction

Recent progress in mammalian developmental genetics has been significant. However, the vast majority of information has been generated through the use of the mouse model. The dog was not significantly involved in these investigations. Some domestic animals were studied more intensively. Surprisingly even detailed embryological description of dog development is not available. Therefore, it may appear premature to write a chapter entirely devoted to developmental genetics of the dog. Nevertheless a high level of similarity in mammalian development and homology of genetic systems involved in

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regulation of the major processes seems to be sufficient justification for inclusion of this chapter into the book. It should allow collecting all available data and placing them on a general background of mammalian development. Hopefully this approach could provide a useful basis for future research to dog genetics.

Developmental Stages of the Dog Embryo

Gamete maturation and fertilization, which comprise the first crucial steps in each new developmental cycle in mammals, have been considered in Chapter 14. The major embryological steps and their genetic determination are discussed here. Table 15.1 summarizes the essential events and timing of early embryonic and fetal development in the dog. The exact time of fertilization in bitches is difficult to establish, while the surge of luteinizing hormone (LH-peak) in plasma immediately preceding ovulation is well characterized (Concannon *et al.*, 1989; England and Yeager, 1993). The interval between LH-peak and ovulation is about 2 days (Concannon *et al.*, 1989), fertilization follows within 1–2 days, or sometimes even longer (Badinand *et al.*, 1993; M.J. Harvey, personal communication, 2000). In order to reconstruct the timetable of different embryological events in the dog, we compared numerous publications and generally agreed that the interval between LH-peak and fertilization is roughly about 3–4 days. This reconstruction may not necessarily be absolutely correct, but gives some information, which can more easily be compared with other species. Three periods during the dog's prenatal development can be distinguished: (i) preimplanation, (ii) organogenesis, (iii) fetal growth and final differentiation, similar to what is observed in other mammals.

The ovum period covers the first 12 days after fertilization. It is characterized by several crucial events including cleavage, morula formation and compaction, blastocyst development, gastrulation, and the start of extra-embryonic mesoderm migration (Concannon *et al.*, 1989; Renton *et al.* 1991; Stabenfedt and Shille 1977; Yamada *et al.*, 1993). After compaction of the morula at about day 4, the conceptus enters the uterus between days 7 and 8 of development and coincidentally begins to undergo blastocyst formation. Tight intercellular junctions develop and this provides a condition for accumulation of fluid within the central cavity (the blastocoele). The majority of external cells of the blastocyst called trophoblasts are concerned with development of extra-embryonic tissues and placenta. A group of cells, located at a pole, form the embryoblast or inner cell mass (ICM). Later during gastrulation, the ICM differentiates into the three primary germ layers of the embryo: ectoderm, mesoderm and endoderm. The zona pellucida, so common for mammals at this stage, is an essential feature of the early canine conceptus. The presence of lectins and other carbohydrates in the dog's oocytes and zona pellucida itself was studied recently (Parillo and Verini-Supplizi, 1999). Hatching from zona pellucida indicates a preparation for implantation and thus entering the next stage of development.

Table 15.1. Essential events and timing of prenatal development in the dog

Stage of development	Days after fertilization	Cells/stage
Ovum period	0–12	
Two cell	1–2	2
Eight cell	2.5–3	8
Genome activation	~3	8
Morula compaction	4–5	~16
Blastocyst formation	~6	32–64
Entry into the uterus	7–8	~64+
Free migration between uterine horns	8–10	1–2 mm
Hatching from zona pellucida	~12	2–3 mm
Embryonic period	13–34	
Implantation begins	14–15	—‖—
Primitive streak (gastrulation begins)	15–16	~4 mm
First somite pair	~16	~4–6 mm
Notochord	16–17	somites
Neural groove	17–18	—‖—
Head fold	~19	—‖—
Closing of neural tube	19–20	—‖—
Vascularized yolk sac	20–21	—‖—
Beating heart	20–22	—‖—
Visible limb buds	24–25	vertebrae
Optic and otic vesicles visible	25–26	—‖—
Well developed tail and elongating limbs	32–34	—‖—
Fetal period	35–65	
Testicular differentiation begins	35–36	Growing fetus
Pituitary gland developed	38	—‖—
Eyelids close, lids fused, claws on digits	40	—‖—
Ossification recognizable	~42	—‖—
Mammae begin to grow	~42	—‖—
Hair coat developed	55	—‖—
Birth	62–64	(considerable variation)

Note:
It is generally agreed that the interval between LH-peak and fertilization is roughly 3–4 days. Relevant comments can be found in the section 'Developmental Stages of the Dog Embryo'.
Compiled from: Barrau *et al*. (1975), Concannon *et al*. (1989), England and Yeager (1993), Harvey *et al*. (1993), Meyers-Wallen *et al*. (1994), Renton *et al*. (1991), Sasaski and Nishioka (1998), Stabenfeldt and Shille (1977), Valtonen and Jalkanen (1993), Yamada *et al*. (1993).

During the embryonic period (days 13–34) a number of crucial events happen. These include implantation and development of placenta, final differentiation of ectoderm, endoderm and mesoderm, and the major organogenesis events. The zonary placenta, in which the chorionic villi are restricted to an equatorial band, is typical for the dog (Steven, 1975). The cross-section of uterus in the area of zonary placenta is shown in Fig. 15.1. The placenta in the dog, as in most Carnivora, belongs to the endotheliochorial type (Steven, 1975).

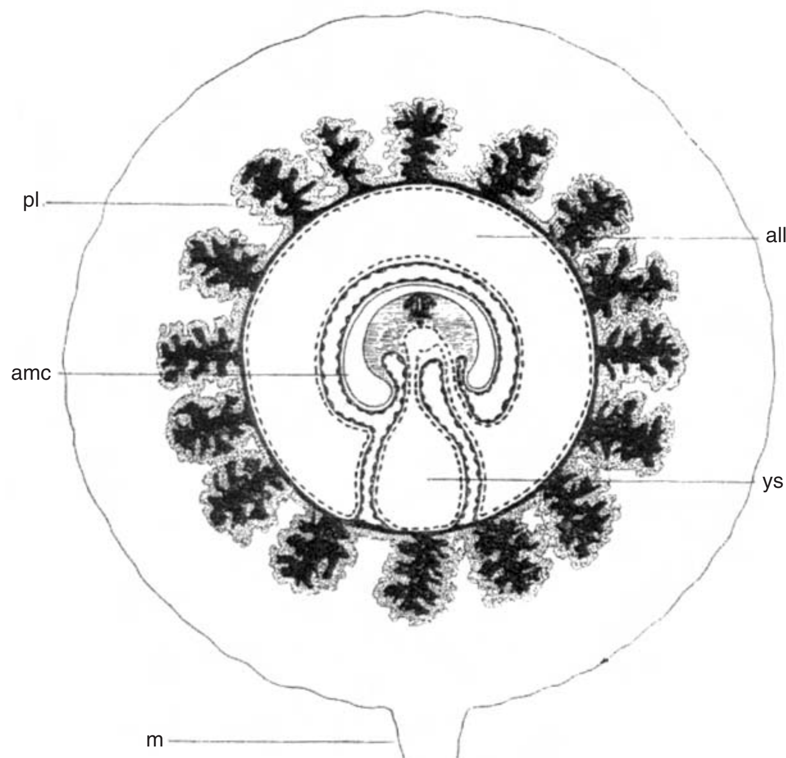


Fig. 15.1. Cross-section of the dog uterus in the area of zonary placenta at 20–22 days after fertilization. all, allantois, amc, amniotic cavity, m, mesometrium, pl, zonary placenta, ys, yolk sack. The yolk sack is small compared to the allantois and of no importance in the later stages of development, though it is essential at first. The embryo itself is shown as a black body surrounded by amniotic cavity. (Reprinted from Jenkinson, 1925.)

During this period, the embryo develops its entire structures, major organs and tissues. The shape of the embryo changes dramatically at this time. Essential morphogenetic events such as head, vertebrae and appendage formation, development of the nerve system and blood circulation and all other major internal organs occur during this time. By day 34 of gestation, the embryo has developed easily recognizable taxonomic features.

The last and longest fetal period takes a month, during which the canine fetus undergoes extensive growth and final development. Numerous morphological changes, although definite, are not radical. These gradual changes shape the fetus, its major structures and function towards the requirements of postnatal life. Sexual differentiation occurs during this period.

A comparison of developmental events and gene regulation during the dog embryogenesis with that of other domestic mammals can be fruitful (Cockett, 1997; Pomp and Geisert, 1998; Ruvinsky and Spicer, 1999; Ruvinsky and Stewart, 2000).

Genetic Control of Preimplantation Development

Expression of maternal genes and embryonic genome activation

In the dog, as in other mammals, the embryonic genome seems to be inactive until after the 4-cell stage, at approximately 2 days of development. Harvey *et al.* (1993), have shown that the marked change from maternally produced to embryonically controlled protein synthesis probably takes place at or soon after the 4-cell stage. The total RNA content in the zygote and in early blastomeres in mammals is commonly much higher than in somatic cells. The oocyte and the following early stages of development are able to synthesize polypeptides in the absence of active transcription. Experiments with a specific inhibitor of RNA polymerase II (α-amanitin) show that cleavage and likely polypeptide synthesis are not significantly affected until the 4–8 cell bovine embryo, addition at later stages completely inhibits further embryonic development (Barnes and First, 1991; Liu and Foote, 1997).

Figure 15.2 shows the poly-A$^+$ RNA profile during early preimplantation development in the mouse embryo. Soon after fertilization the amount of embryonic transcripts start to increase and somewhere in the middle between fertilization and morula it exceeds the level of maternal transcripts. A similar picture is expected for dog development. The major anticipated difference between species would be in the time scale.

Recent data indicate that the leptin and STAT3 proteins play critical roles in early mammalian development. Both may be involved in the determination of the animal pole of the mammalian oocyte and in the differentiation of the trophoblast and inner cell mass (Antczak and Van Blerkom, 1997). A potential role of these proteins in early development is indicated at the morula stage

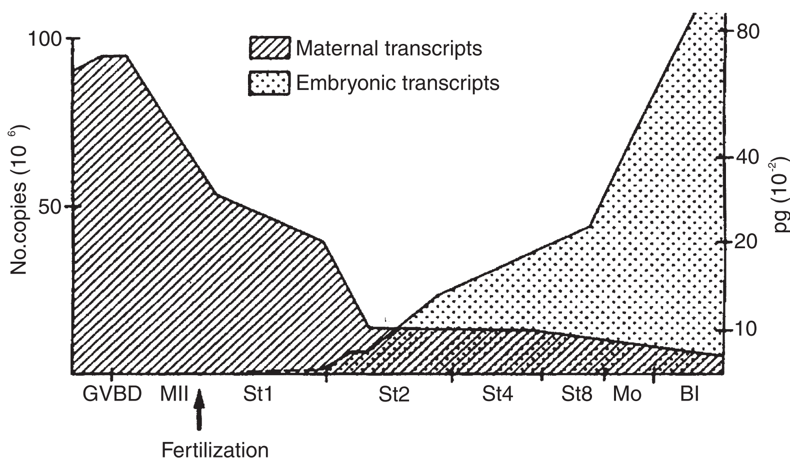


Fig. 15.2. Evolution of polyA$^+$ RNA during the early preimplantation development of the mouse embryo (from Ménézo and Renard, 1993, with permission of Ellipses). MII, second meiotic metaphase; Mo, morula; Bl, blastula.

where the 'inner' cells consist of blastomeres that contain little, if any, leptin/STAT3 while 'outer' cells contain both leptin/STAT3-rich and -poor cells (Antczak and Van Blerkom, 1997). It was also shown in the mouse that tropomyosin, an actin-binding cytoskeletal protein, becomes associated with both the blastomere cortex after fertilization and the cleavage furrow during cytokinesis. The interphase cortical association is uniform until the 8-cell stage, when tropomyosin becomes associated with the developing apical pole and is excluded from the basolateral cortex. Thus, the early mouse conceptus contains a unique and specific set of tropomyosins, which responds to polarizing signals (Clayton and Johnson, 1998). Several more regulatory proteins have also been described, including Bax, Bcl-x, TGF beta 2, VEGF, EGF-R, whose distribution among fragmenting blastomeres during the earliest stages may affect development (Antczak and Van Blerkom, 1999).

Increasing cell polarity was described at the 8-cell stage in both the mouse and rat (Gueth-Hallonet and Maro, 1992). In cattle, cell polarization has occurred in some blastomeres at the 8- to 16-cell stage; however, distinct polarity is not evident until after the 16-cell stage, with approximately 40% polar cells per embryo (Koyama *et al.*, 1994). It is suggested that the changes in the cell surface and nuclear position within a cell are the first manifestations of cell polarity (Suzuki *et al.*, 1999).

It is well known that in *Drosophila melanogaster* and *Caenorhabditis elegans* gradients of morphogens in the zygote and early embryo are crucial for establishing positional information (St Johnston and Nüsslein-Volhard, 1992; Nüsslein-Volhard, 1996). These gradients are essentially products of expression of maternal genes. In what degree similar gradients and elements of the cytoskeleton are important during the earliest stage of mammalian development remains to be seen, but it seems unlikely that these factors are not important in specifying major polarities (Holliday, 1990). Cell fate, controlled by positional information seems to be at least partially reversible and provides the developing embryo with a certain degree of flexibility.

Embryonic gene expression

Later, blastocyst formation creates two different cell lineages: non-polarized inner mass cells and polarized trophectoderm or outer cells with prominent microvilli. Louvet *et al.* (1996) showed that this process in mice is accompanied by specific redistribution of the actin-associated protein ezrin, which is proposed to play a role in the formation of microvillous structures that are crucial for normal implantation. Before morula compaction, ezrin is located around the cell cortex. However, after blastocyst formation, it segregates to the outer trophectoderm cells which have microvilli. Two phosphorylated forms of ezrin are present from the ovum period throughout preimplantation development, but they gradually decrease in amount. A third non-tyrosine-phosphorylated isoform appears at the 8-cell stage and increases to the blastocyst stage (Louvet *et al.*, 1996). Several other actin-associated proteins

(α-fodrin, vinculin and E-cadherin), which are involved in cytokeratin bundles, are not observed until the early blastocyst both in the mouse and the pig (Reima *et al.*, 1993). E-cadherin cell adhesion function is essential for the establishment and maintenance of epithelial cell morphology during embryogenesis and adulthood. Mouse embryos homozygous for a targeted mutation of the gene show severe abnormalities before implantation, because dissociation of adhesive cells of the morula has occurred shortly after compaction and their morphological polarization is then destroyed. Maternal E-cadherin is able to initiate compaction, but cannot maintain the process (Riethmacher *et al.*, 1995). Significant defects in the cell-junctional and cytoskeletal organization were found in E-cahedrin null mouse embryos and the trophectoderm layer failed to differentiate (Oshugi *et al.*, 1997).

Although the rate of embryonic development is not the same in different mammals, there is a close correlation between the developmental stage and cytoskeletal organization in the studied species. In the expanded bovine blastocyst, for instance, the distribution of several cytoskeletal and cytoskeleton-related proteins appeared similar (Shehu *et al.*, 1996). Extracellular fibronectin was first detected in the early blastocyst before differentiation of the primitive endoderm, and at this stage was localized at the interface between the trophectoderm and extraembryonic endoderm (Shehu *et al.*, 1996). Cingulin, the tight junction peripheral membrane protein, also contributes to morphological differentiation in early mouse development and it is likely that other mammals may have the same gene. Its synthesis is tissue-specific in blastocysts, and is up-regulated in the trophectoderm, and down-regulated in the inner cell mass (Javed *et al.*, 1993).

It is commonly accepted that proto-oncogenes are involved in numerous processes of embryonic development and that they encode a series of nuclear transcription factors, intracellular signal transducers, growth factors and growth factor receptors. For example, activation of the c-fos and c-jun proto-oncogenes in the sheep conceptus occurs during the period of rapid growth and elongation (Wu, 1996) and a similar pattern probably occurs in canine embryos. These proto-oncogenes are involved in the regulation of gene expression, cell proliferation and differentiation.

Information on the expression of housekeeping genes during canine embryonic development is sparse but it is likely to be similar to that observed in bovine embryos. The mRNA levels for various genes studied in bovine embryos remain constant or decrease slightly from the mature oocyte to the 6–8 cell or morula stage and greatly increase in blastocysts. These changes in gene expression were significant, ranging from 2–6-fold to 110–118-fold (Bilodeau-Goeseels and Schultz, 1997). However, caution is required when extrapolating from one species to another, particularly if the species belong to different orders.

Many efforts have been made to try and ascertain the relative importance of internal versus external factors in controlling the development of pre-implantation embryos. Most experiments with rodents show that it is unlikely that preimplantation development is significantly dependent on external

factors. Furthermore, none of the known endogenously produced factors and their receptors is essential until the blastocyst stage (Stewart and Cullinan, 1997). However, later during development, the importance of growth factors rises sharply.

It was found that some of the regulatory substances secreted by the uterus can act as growth factors. Together with a number of growth factors and their receptors produced by the embryo itself, they create the medium essential for development. A detailed review of these regulators of mammalian embryonic development can be found elsewhere (Schultz and Heyner, 1993).

Trophoblast gene expression

Differentiation of trophoblast cells, the first and perhaps the most radical event during mammalian embryonic development, provides an embryonic component for future fetal–maternal interface during implantation and placentation. A detailed description of current knowledge about the genetic control of trophoblast development and implantation is presented in a special issue of *Developmental Genetics* (Schultz and Edwards, 1997). Clearly, many features of these processes are common to the majority of eutherian mammals and applicable to the dog. More than 44 loci with different functions implicated in preimplantation or peri-implantation events have been identified so far (Rinkenberger *et al*., 1997).

A basic helix–loop–helix (bHLH) transcription factor gene, *Hxt*, is expressed in early trophoblast and in differentiated giant cells of mouse embryos (Cross *et al*., 1995). The negative HLH regulator, *Id-1*, inhibited rat trophoblast (Rcho-1) stem cell differentiation and placental lactogen-I transcription. These data demonstrate a role for HLH factors in regulating trophoblast development, at least in mice, and indicate a positive role for *Hxt* in promoting the formation of trophoblast giant cells. A separate gene, *Hed*, encodes a related protein that is expressed in maternal decidium surrounding the implantation site (Cross *et al*., 1995). Gene *Mash*-2, homologous to *Drosophila achaete/scute* complex of loci, also determines transcription factor. Its expression begins during preimplantation development, but is restricted to the trophoblast lineage after the blastocyst stage (Nakayama *et al*., 1997). This murine locus belongs to the quite rare category of imprinted genes (Guillemot *et al*., 1995). Mouse embryos which inherit a mutant allele from the mother and normal from the father die after implantation. The cause of death is a lack of placental spongiotrophoblasts (McLaughlin *et al*., 1996). Although gametic imprinting has not been observed in the dog so far, there are good reasons to expect that it will. Further information concerning the gametic imprinting is published elsewhere (Ruvinsky, 1999). Several more genes are currently under investigation in mammals to determine their role in trophoblast development and implantation/placentation events (Schultz and Edwards, 1997).

Genetic determination of integrin trafficking, which regulates adhesion to fibronectin during differentiation of the mouse peri-implantation blastocyst,

has been studied by Schultz *et al.* (1997). The regulation of several metalloproteinase and corresponding genes may also shed additional light on the process of implantation and further trophoblast development (Bass *et al.*, 1997; Das *et al.*, 1997).

Implantation and Maternal Recognition of Pregnancy

Implantation is a result of mutual embryo–maternal interactions, which start prior to physical attachment of the embryo to the uterine wall. It is known that after 4–5 days of free blastocyst migration in the bitch uterine horns, the zona pellucida is shed (Table 15.1). This is so called blastocyst 'hatching', which is a critical step in the preparation of the conceptus for implantation. At this stage in other species, the conceptus releases an antiluteolitic substance (trophoblastin), which supports the activity of the corpora lutea (CL) and thus leads to high levels of progesterone production (Cockett, 1997). Whether the same process is typical in the dog remains unclear. The dog CL seems to have an internally programmed life span (Meyer, 1994). It is known, however, that progesterone levels reach a peak around the time of implantation (Concannon *et al.*, 1989). Treatment of pregnant bitches with natural prostaglandin F2 alpha in low doses before implantation resulted in the arrest of luteal progesteron production and prevention of nidation (Lange *et al.*, 1997). Unfortunately, there is no clear picture of the exact timing and specific features of maternal recognition of pregnancy in the bitch due to a lack of information. However, a description of the morphological changes prior and soon after implantation is available.

It was observed that on day 13 after the end of oestrus, knobs of trophoblast syncytium formed and became wedged between cells of the uterine luminal epithelium in the dog (Barrau *et al.*, 1975). It can be tentatively suggested that maternal recognition of pregnancy occurs at least at this stage, but perhaps even earlier. The process of invasion continued and after day 26 the syncytial trophoblast covering the tips of the villi degenerated. Finally haematomas were formed by focal necrosis of fetal and endometrial tissue at the poles of the implantation sites. Large pools of extravasated blood accumulated and red cells were phagocytozed by trophoblastic cells (Barrau *et al.*, 1975). This process makes former implantation and placentation sites visible even a few weeks after parturition. A detailed description of the placentation process in the bitch can be found elsewhere (Grether *et al.*, 1998).

A study of proteins secreted by the endometrium and the conceptus around the time of implantation in the dog revealed several specific proteins, one of which, canine Conceptus Protein 7 (cCP7) was found to be secreted prior to implantation, but only by blastocysts (Thatcher *et al.*, 1994). Polypeptide relaxin, a member of the insulin-like superfamily and a hormone of pregnancy in mammals, plays an important role in the dog. It is first detected soon after implantation, reaching peak levels at 6–8 weeks of gestation (Steinetz *et al.*, 1987). Relaxin is considered as a marker of pregnancy in

the dog (Steinetz *et al.*, 1989). Detailed investigation of canine preprorelaxin provided a clear idea concerning the structure of the gene and the protein. It was also found that the syncytiotrophoblast is the source of relaxin in the canine placenta (Klonisch *et al.*, 1999). Interestingly, the concentration of acute-phase reactant protein fibrinogen increases soon after implantation in dogs at or just before the increase in relaxin, approximately starting from day 22–23 of gestation (Concannon *et al.*, 1996). The serum C-reactive protein (CRP) concentration also increases significantly at about the same time, reaching peak activity after 30 days of gestation (Eckersall *et al.*, 1993).

In other species, many proteins, steroids and other substances are known to be involved in maternal recognition of pregnancy, implantation and placentation (reviewed in Cockett, 1997; Pomp and Geisert, 1998; Ruvinsky and Spicer, 1999; Ruvinsky and Stewart, 2000). Hopefully further studies will also discover the molecular basis of these processes in the bitch.

Genes Involved in the Control of Morphogenesis

Gastrulation and notochord formation

Gastrulation in the dog starts around day 14 of development (Table 15.1). Lack of any clear-cut embryological description of the process in the dog prevents an opportunity to discuss this in detail. Cell proliferation and rearrangement in the germinal disc are the main events during gastrulation in eutherian embryos. The visible indication that gastrulation has begun is formation of the primitive streak. In mammals 'this process begins with the production and proliferation of mesodermal progenitor cells at the proximal (allantoic) end of the primitive streak; this position marks the future caudal end of the fetus. As ectodermal cells migrate through the primitive streak, they move both laterally and distally towards the future cranial end of the embryo, extending the primitive streak towards the distal lip' (Wilkins, 1993).

While genetic mechanisms that are responsible for gastrulation in mammals are still mainly unknown, new data are appearing (Viebahn, 1999). The next step, establishing anterior–posterior orientation, has recently become a subject of intensive investigations (Beddington and Robertson, 1998). Two genes, the homeobox gene *goosecoid* (*gsc*) and the winged-helix gene *Hepatic Nuclear Factor-3beta* (*HNF-3*β) are co-expressed in all three germ layers in the anterior primitive streak and at the rostral end of mouse embryos during gastrulation (Filosa *et al.,* 1997). *Fgf-4,* a member of the fibroblast growth factor gene family which shows expression in the primitive streak and a sequential expression in developmental pathways such as mesoderm formation and myogenesis, is believed to play a role in specific epithelial–mesenchymal interactions (Niswander and Martin, 1992).

The recently discovered murine *Axin* gene seems to be a crucial regulator in embryonic axis formation in vertebrates. This gene inhibits the Wnt/ Wingless signalling pathway, involving several polypeptides and enzymes (Zeng

et al., 1997). This pathway plays an important role not only in embryonic development but also in tumorigenesis. Interaction of Axin protein with glycogen synthase kinase-3beta is required for beta-catenin down-regulation. Beta-catenin and Axin are positive and negative effectors of the Wnt signalling pathway, respectively (Nakamura *et al.*, 1998). The whole transduction pathway related to *Axin* is beginning to emerge (Fagotto *et al.*, 1999).

The next step in development is the so called 'head process', which gives rise to the notochord. The notochord is a rod-shaped structure which extends along the embryo and represents the initial axial skeleton, playing an important role in induction of the neural plate, chondrogenesis, and somite formation (Gomercic *et al.*, 1991). Development of the notochord in the canine embryo has not been studied. The only available data show that notochordal cells may persist into adulthood in some canine breeds, thus preventing intervertebral disc degeneration (Aguiar *et al.*, 1999). Clearly, activation of nuclear genes responsible for basic morphogenetic rearrangements is a requisite for notochord formation and development.

The *T* gene, which is required for extension of the posterior axis and for several other essential steps in mammalian development, plays an essential role in differentiation of the notochord and the formation of mesoderm during posterior development (Clements *et al.*, 1996). The T protein is located in the cell nuclei and acts as a tissue-specific transcription factor (Kispert *et al.*, 1995). Cloning and sequencing of the *T* gene led to the discovery of the *T-box* gene family, which is characterized by a conserved sequence, called T-box (Bollag *et al.*, 1994). This ancient family of transcription factors, which underwent duplication around 400 million years ago, is common to all vertebrates (Ruvinsky *et al.*, 2000). There are indications that several murine T-box genes are essential in different mesodermal sub-populations and one is essential in early endoderm during gastrulation (Papaioannou, 1997). Involvement of the T-box genes *Tbx2–Tbx5* in vertebrate limb specification and development has been shown recently (Gibson-Brown *et al.*, 1998; Schwabe *et al.*, 1998). Formation of the notochord leads to several key ontogenetic events including induction of the neural tube and development of the gut, heart and brain. A putative morphogen secreted by the floor plate and notochord, Sonic hedgehog (Shh), specifies the fate of multiple cell types in the ventral aspect of the vertebrate nervous system. Shh in turn induces expression of the oncogene *Gli-1*, which affects later development of dorsal midbrain and hindbrain (Hynes *et al.*, 1997).

HOX genes and development of axial identity

The homeotic genes, which encode transcription factors, were first described in *Drosophila* as the primary determinants of segment identity. They all contain a similar 180-bp DNA sequence motif named the homeobox. Comparative analysis of the *Drosophila* homeotic gene complex called *HOM-C* and the mammalian homeobox genes called the *HOX* complex, demonstrates

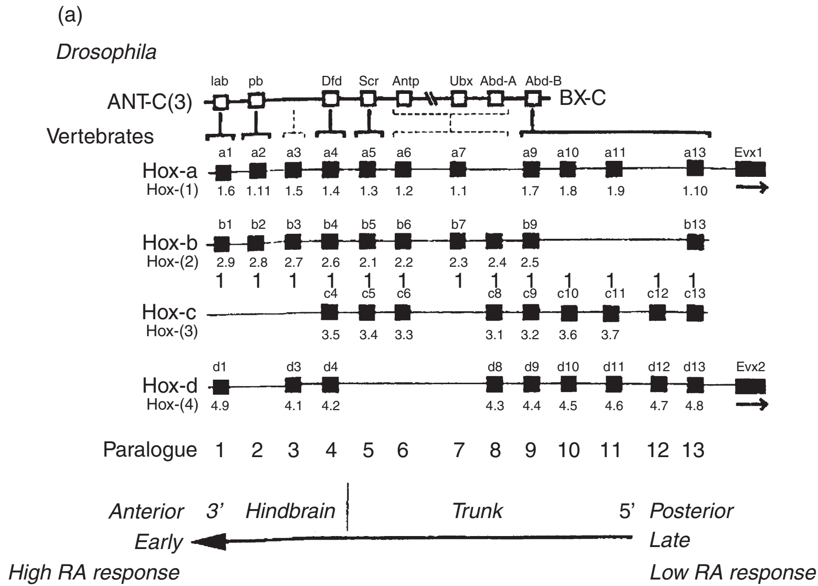
an excellent example of evolutionary conservation. The *HOX* gene family determines a set of transcription factors crucial for development of axial identity in a wide range of animal species (Maconochie *et al.*, 1996). Figure 15.3 shows the striking similarity and colinearity found in the molecular anatomy of the insect and mammalian (vertebrate) *HOX* complexes. The main difference is the number of complexes per genome. In insects there is only one, while mammals and some other vertebrates have four paralogous sets of genes. It is most likely that *HOX* complex in the dog has a structure very similar to mouse and other mammalian species that have been studied.

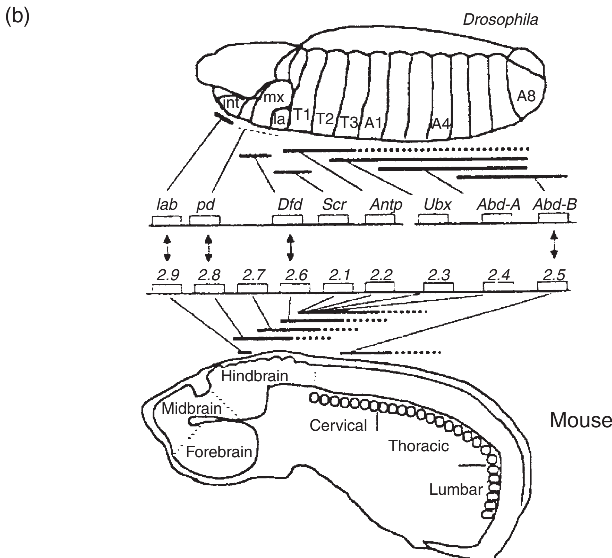
The *HOX* genes are expressed in segmental fashion in the developing somites and central nervous system and each *HOX* gene acts from a particular anterior limit in a posterior direction. The anterior and posterior limits are different for different murine *Hox* genes (Fig. 15.3). The genes located at the 3′ end have the most anterior limit of activity. Transcription of the genes, however, moves in the usual 5′ to 3′ direction. The genes located at the 3′ end are expressed earlier while genes located at the 5′ end are expressed later. The process of segmentation moves along the anterior–posterior axis but there are differences in development of segmentation between the hindbrain and the trunk (Maconochie *et al.*, 1996). Thus, the vertebrate body is, at least partially, a result of interactions of *HOX* genes that provide cells with the essential positional and functional information. Signals from the *HOX* genes force embryonic cells to migrate to the appropriate destination and generate certain structures. Retinoids can affect the expression of *HOX* genes and there is a 5′ to 3′ gradient in responsiveness of *HOX* genes to retinoids (Marshall *et al.*, 1996). It was found that a bitch endometrium secretes a family of proteins (cP6), some of which bind retinol and are likely to be involved in early development, supplying retinol or derivates to the embryo (Buhi *et al.*, 1995). Embryonic retinoic acid synthesis is essential for post-implantation development in mammals (Niederreither *et al.*, 1999)

It is well known that the neural crest plays a key role as the major source for numerous cell lineages, including sensory neurons, glial cells, melanocytes, some bone and cartilage cells, thyroid cells and smooth muscle. There has been considerable progress during the past few years in identifying genes controlling development of the neural crest and associated cell migration (Anderson, 1997). Several growth factors affect the developmental fate of

Fig. 15.3. (a) Alignment of the four mouse *Hox* complexes with that of *HOM-C* from *Drosophila*. The vertical shaded boxes indicate related genes. The 13 paralogous groups are noted at the bottom of alignment. The colinear properties of the *Hox* complexes with respect to timing of expression, anteroposterior level, and retinoic acid (RA) response are also noted at the bottom (from Maconochie *et al*. (1996) with the author's permission). (b) Summary of *HOM-C* and *Hox-2* expression patterns. The upper part of the figure is a diagram of a 10 h *Drosophila* embryo with projections of expression patterns of different genes from *HOM-C* complex to particular body segments. The lower part of the figure is a diagram of a 12-day mouse embryo with projections of expression patterns of different genes from the *Hox-2* complex to particular body segments (from McGinnis and Krumlauf (1992) with the author's permission).

neural crest cells: glial growth factor (GGF), transforming growth factor (TGF-β) which promotes smooth muscle differentiation and a bone morphogenic protein (BMP2/4) involved in bone morphogenesis. Transcription factors are also important in neural crest lineage determination, including the basic helix– loop–helix (bHLH) transcription factors Mash1 and Mash2 (Anderson, 1997).





Organogenesis: T-box, PAX and other genes

Some of the *T-box* genes are involved in limb morphogenesis and specification of forelimb/hindlimb identity. It has been shown that murine *Tbx5* and *Tbx4* expression is primarily restricted to the developing fore- and hindlimb buds, respectively. These two genes appear to have been divergently selected in vertebrate evolution to play a role in the differential specification of fore- (pectoral) versus hind- (pelvic) limb identity (Gibson-Brown *et al.*, 1998). Mutations in the human *TBX3* gene cause the ulnar-mammary syndrome characterized by posterior limb deficiencies or duplications, mammary gland dysfunction and genital abnormalities. It has been suggested that *TBX3* and *TBX5* evolved from a common ancestral gene and each has acquired specific, yet complementary, roles in patterning the mammalian upper limb (Bamshad *et al.*, 1997). *Tbx4* and *Tbx5* are essential regulators of limb outgrowth whose roles seem to be tightly linked to the activity of three signalling proteins that are required for limb outgrowth and patterning: fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and Wnt (Rodriguez-Esteban *et al.*, 1999) Full sequence analysis of the *T* gene from a number of different dog breeds identified several polymorphisms and a unique missense mutation in a bob-tailed dog and its bob-tailed descendants (Haworth *et al.*, 2001). This mutation is situated in a highly conserved region of the *T*-box domain and alters the ability of the T protein to bind to its consensus DNA target.

PAX genes are another family of developmental genes coding nuclear transcription factors. They contain the paired domain, a conserved amino acid motif with DNA binding activity. *PAX* genes are the key regulators of development in organs and structures such as the kidney, eye, ear, nose, limb muscles, vertebral column and brain. Vertebrate *PAX* genes are involved in pattern formation possibly by determining the time and place of organ initiation or morphogenesis (Dahl *et al.*, 1997). Murine *Pax-1*, for instance, is a mediator of notochord signals during the dorsoventral specification of vertebrae (Koseki *et al.*, 1993). The *Pax-3* gene may mediate activation of *MyoD* and *Myf-5*, the myogenic regulatory factors, in response to muscle-inducing signals from either axial tissues or overlying ectoderm and may act as a regulator of somitic myogenesis (Maroto *et al.*, 1997). Mutations in the *Pax-2* gene show that the gene is involved in eye formation, causing renal coloboma syndrome. Mutations in the *Pax-6* gene result in eye malformation, known as *Aniridia* in humans and some other mammals and *Small eye* syndrome in mice (Dahl *et al.*, 1997). Several other genes such as *Bmp-4*, *Msx-1* and *Msx-2*, which encode bone morphogenetic proteins and are expressed before and after neural tube closure, interact with *Pax-2* and *Pax-3* (Monsoro-Burq *et al.*, 1996). Recent results show that *Lbx1h* gene is a key regulator of muscle precursor cell migration and is required for acquisition of dorsal identities of forelimb muscles (Schafer and Braun, 1999).

Firulli and Olson (1997) have reviewed recent progress on genetic mechanisms of muscle development in mammals. Skeletal, cardiac and smooth muscle cells express overlapping sets of muscle-specific genes, although some

genes are only expressed in one particular muscle type. So called genetic modules or independent *cis*-regulatory regions are required to direct the complete developmental pattern of expression of individual muscle-specific genes within each muscle type and the temporospacial specificity of these regulatory modules is established by unique combinations of transcription factors (Firulli and Olson, 1997). A gene encoding an actin-modulating protein, gelsolin, has been described in several mammalian species.

It is well established that mitogens inhibit differentiation of skeletal muscle cells, but the insulin-like growth factors (IGFs), acting through a single receptor, stimulate both proliferation and differentiation of myoblasts. For example, an inhibitor of mitogen-activated protein (MAP) kinase inhibits IGF-stimulated proliferation of L6A1 myoblasts and associated events, such as phosphorylation of the MAP kinases and elevation of c-fos mRNA and cyclin D protein. This inhibitor caused a dramatic enhancement of differentiation, evident both at a morphological and biochemical level. In sharp contrast, an inhibitor of phosphatidylinositol 3-kinase and p70 S6 kinase completely abolished IGF stimulation of L6A1 differentiation. These data demonstrate that the MAP kinase pathway plays a primary role in the mitogenic response and is inhibitory to the myogenic response in L6A1 myoblasts, while activation of the phosphatidylinositol 3-kinase/p70(S6k) pathway is essential for IGF-stimulated differentiation. Thus, it appears that signalling from the IGF-1 receptor utilizes two distinct pathways leading either to proliferation or differentiation of muscle cells (Coolican *et al.*, 1997). Morphological changes and electrical/mechanical activity of the canine proximal colon during ontogeny has been documented (Ward and Torihashi, 1995; Ward, 1996).

A canine *CIC*-1 (skeletal muscle voltage-dependent chloride channel) mutation causes autosomal recessive myotonia congenita in Miniature Schnauzers. The mutation results in replacement of a threonine residue in the D5 transmembrane segment with methionine. This developmental abnormality provides a new and potentially valuable animal model of an inherited skeletal muscle disease (Rhodes *et al.*, 1999). Canine X-linked muscular dystrophy is homologous to human Duchenne muscular dystrophy and proves the existence of one more genes, which is known to be involved in muscle development in dogs (Lanfossi *et al.*, 1999)

Investigation of ontogeny of the granulocyte/macrophage cell pool shows it is established in the yolk sac after 23 days of gestation, with a maximum on 36–37 days (Nortdurft *et al.*, 1984). Detailed description of the pituitary gland development in the dog has been recently made by Sasaki and Nishioka (1998). It becomes recognizable from 25 days of gestation and by 38 days the morphology of the organ looks the same as in adult dogs. It can be expected that *PAX6* (like in humans) participates in early organogenesis of the pituitary gland (Terzic and Saraga-Babic, 1999).

More information is becoming available concerning the development of brain and nerve system. Data suggest that PB-cadherin-mediated cell-cell adhesion has a functional role in pattern formation and morphogenesis of neural tissues, including the embryonic brain and spinal cord (Kitajima *et al.*,

1999). It has also been found that at about the same time two genes *Otx2*, expressed in the forebrain and midbrain, and *Gbx2*, expressed in the anterior hindbrain, play an essential and interactive role in positioning of the mid/hindbrain junction. This junction acts as an organizer, directing development of midbrain and anterior hindbrain (Millet *et al.*, 1999).

Investigation of early morphogenesis of the canine eye shows that histologically defined structures can be identified as early as 23–24 days after fertilization (Boeve *et al.*, 1989). It is likely that during early dog development, as in humans, *PAX6* participates in organogenesis of the eye (Terzic and Saraga-Babic, 1999). It has also been shown that *Tbx5* is involved in mammalian eye morphogenesis and is a topographic determinant of the visual projections between retina and tectum (Koshiba-Takeuchi *et al.*, 2000).

Developmental effects of coat colour mutations

A great number of colour variations in dogs are caused by mutations at several loci (Chapter 3). Some of these mutations lead to developmental abnormalities and eventually to disorders. This includes the colour dilution alopecia (absence of hair) in diluted (mostly 'blue') pigmentation with its subtype of Black Hair Follicular Dysplasia (BHFD) in black pigmentation (Laukner, 1998). The potentially homologous human locus was recently cloned and mapped on chromosome 8p21–22 (Cichon *et al.*, 1998).

Another defect linked to extreme piebalds is deafness. Dalmatian dogs are the best known example, where the classical association with blue eyes was also found (Cattanach, 1999). The overall rate of deafness in the tested Dalmatians was 18.4%, of which 13.1% were unilaterally deaf and 5.3% were bilaterally deaf. The overall deafness in females (21.1%) was significantly higher ($P = 0.014$) than that in males (15.5%) (Wood and Lakhani, 1998). As in other mammals, the mutant allele apparently affects migration of cells from the neural crest causing pleiotropic effects (Cattanach, 1999; Holme and Steel, 1999). Significant genetic variation existing beyond the contribution of a few loci was revealed. Eye colour, colour patch, sex and the hearing status of the parents were all significant contributions to deafness (Famula *et al.*, 1996). Selection seems to be able to reduce the harmful effect of this mutation. Undesirable coat colours, which are connected with defects, are the extreme dapple of the merle-syndrome and the 'grey' Collie with cyclic haematopoiesis (Laukner, 1998).

It seems likely that in the Australian Shepherd dog, microphthalmia with coloboma is related to white hair coat (Gelatt *et al.*, 1981). A similar observation was made in Dachshunds, where homozygotes for the merle allele demonstrated a high level of microphthalmia and microcornea, microcoria, corectopia, cataracts and colobomas, rudimentary lenses and ectasia of episcleral vessels. Heterozygous merles with less than 50% white coat colour (*Mm*) have been reported to lack tapetum lucidum, have depigmented retina and have papillary anomaly and ectosia of episcleral vessels (Dausch *et al.*,

1978). Molecular and developmental causes of this disorder are not yet known in the dog, but potential homology with Waardenburg Syndrome type 2 (WS2) in humans and mice may be suspected. This syndrome is caused by mutations in the *MITF* (microphthalmia-associated transcription factor) gene, which encodes a basic helix–loop–helix–leucine zipper (bHLHZip) factor and regulates expression of tyrosinase and other melanocytic genes during differentiation. It has been shown that Ser298, which locates downstream of the bHLHZip and was previously found to be mutant in individuals with WS2, plays an important role in MITF function. These findings suggest that the Ser298 is important for MITF function and is phosphorylated probably by glycogen synthase kinase 3 beta (Takeda *et al.*, 2000). It is possible that the developmental abnormalities result from interactions of mutant proteins in a cascade that involves genes for PAX3, MITF, human MyoD, MYF5, c-MET, c-KIT, tyrosinase, TRP-1, human QNR-71, SOX10, EDNRB and EDN3 (Tachibana, 1999).

Neoteny

The enormous variability and morphogenic plasticity of the dog still remains a fundamental question. Specific type of selection during domestication is certainly the cornerstone of any explanation of this exciting phenomenon (see Chapter 2). It was observed long ago that adult dogs show numerous traits typical of wolf puppies. Since then the idea that neoteny was involved in the spectacular developmental changes in dogs has been discussed. Coppinger and Schneider (1995) provided the latest and most comprehensive discussion of this issue. Clearly retardation of some developmental processes and acceleration of others seems to be the essence of dramatic differences between the dog breeds. This can hardly be achieved without relevant tuning of gene activities during development. In turn this tuning is likely to be a result of changes in the activity of a few highly influential genes involved in production of key hormones and other biologically active substances (Belyaev, 1979; Trut, 1999; Chapter 2). While the dog, unlike laboratory animals, is not an easy model for developmental genetics, one may suggest that the contrasting dog breeds may provide a unique opportunity for further investigations in the field.

Sex Determination

The major steps in gonad differentiation

Normal sexual development in mammals requires a series of steps, which occurs under genetic control. Three major steps are usually recognized: initial sex determination during fertilization, gonadal sex and phenotypic sex. Recent data show the differential expression of genes located on the X chromosome (*G6PD* and *HPRT*) in the early bovine embryos (7–8 days) prior to gonadal

differentiation and thus indicate that preimplantation blastocysts have sexually dimorphic gene activity (Gutierrez-Adan *et al.*, 2000).

The earliest stages of gonadal development in mammals occur at a similar stage in XX and XY embryos. Primordial germ cells, which differentiate relatively late in mammals, migrate into the gonads of either presumptive sex indiscriminately and may function even across a species barrier (McLaren, 1998, 1999). To be functional, a gonad needs both germ cells and somatic cells. Assuming that gonadal development in the dog does not strongly deviate from that of the mouse and other mammals, one may expect that a few dozen germ cells, originating from the proximal region of the embryonic ectoderm start their journey inside the embryo along with the invaginating hindgut. A recent study in mice showed that expression of *Bmp4* (bone morphogenetic protein 4 gene) in the trophectoderm layer which is in closest contact with the epiblast is responsible for the differentiation of both the primordial germ cells and the allantois (Lawson *et al.*, 1999).

Due to ongoing proliferation, a significant number of germ cells reach the genital ridge, which consists of a thin layer of mesenchymal cells located between the coelomic epithelium and the mesonephros. Two genes, *Sf1* and *Wt1*, are particularly important in the development of the murine genital ridge (McLaren, 1998). Eventually, four different cell lines comprise the genital ridge: primordial germ cells, somatic steroidogenic cells, supporting cells and connective tissue. The fate of each lineage depends on the sexual determination of the embryo in which they develop and their structure, function and pattern of genetic activity is quite different in testes and ovaries.

It has long been known that sex determination in mammals depends on the presence or absence of the Y chromosome. Embryos without a Y chromosome develop as females and those with a Y chromosome develop as males. The breakthrough in molecular understanding of sex determination and differentiation in the mouse and human (Goodfellow and Lovell-Badge, 1993) paved the way for studies in other mammals. Usually gonadal sexual differentiation starts later during embryonic development and morphological differences in XY embryos appear prior to those in XX embryos. In the dog, testicular differentiation has been observed at 35–36 days of gestation (Meyers-Wallen *et al.*, 1991, 1994; Table 15.1).

Testicular development is a key element in establishing mammalian sex. The chromosomal constitution determines the migration of cells into gonads and the final differentiation into a testis or an ovary (Hunter, 1995). Testicular development in mammals is triggered by a gene on the Y chromosome encoding the testis determining factor (*TDF*), or sex determining region of the Y chromosome (*SRY* or *Tdy*). In genetic males, this factor induces differentiation of Sertoli cells (reviewed by McLaren, 1991) and secretion of anti-Müllerian hormone (AMH or MIS). AMH, which belongs to the transforming growth factor β family, causes regression of Müllerian ducts, promotes development of Wolffian ducts and the differentiation of Leydig cells which secrete the male steroid hormone, testosterone (Behringer, 1995). Testosterone binds to androgen receptors, which in turn act as transcription factors.

In canine embryos, secretion of AMH by Sertoli cells and regression of Müllerian ducts begins immediately after the start of testis differentiation, i.e. on 35–36 days of gestation (Meyers-Wallen *et al.*, 1991, 1994). A whole chain of developmental events follow, and the phenotype typical for males arises. In females, Müllerian ducts develop, no Leydig cells form in the gonad, no testosterone is produced and gonad development steadily moves towards the female phenotype. The female developmental programme is therefore usually considered as the 'default', while the male programme requires switching on of the *SRY* gene followed by a cascade of activation of autosomal genes. However, the latest finding of the signalling protein Wnt-4, which is crucial for female sexual development, may affect this perception (Vainio *et al.*, 1999).

SRY gene and sex differentiation

The testis determining role of the *SRY* gene in mammals is widely accepted after the experiments performed in the early 1990s (reviewed by Goodfellow and Lovell-Badge, 1993). Available data also suggest that the cell-autonomous activity of the murine *Tdy* (*Sry*) gene in Sertoli cell precursors results in differentiation of Sertoli cells (Burgoyne *et al.*, 1988). The complete coding sequence of cDNA (1578 bp) for the canine *SRY* gene has recently been determined (Meyers-Wallen *et al.*, 1999).

After demonstration that SRY is a transcription factor (Ramkissoon and Goodfellow, 1996; Greenfield, 1998), several autosomal genes acting downstream of *SRY* were shown to be involved in the mammalian sex differentiation pathway. This set of genes includes the *SRY*-related high-mobility group box (*SOX*) autosomal gene family, which display properties of both classical transcription factors and architectural components of chromatin (Pevny and Lovell-Badge, 1997). *Sox9* has an essential function in sex determination in mice as well as in other mammals and is critical for Sertoli cell differentiation (Morais da Silva *et al.*, 1996). The human *DAX-1* gene and its mouse homologue are X chromosome located and encode an unusual member of the nuclear hormone receptor superfamily. Mutations in this gene cause adrenal hypoplasia (Greenfield, 1998). The autosomal *SF-1* gene produces another nuclear receptor, steroidogenic factor 1. Mutations in this gene may cause gonadal and adrenal agenesis and other disorders and act at the same time (Greenfield, 1998).

Anomalies in sexual differentiation in the dog

As proposed by Lyon (1961) and widely accepted now, one of the X chromosomes in mammalian females undergoes inactivation during early embryonic development. Numerous investigations have shed light on different aspects of X chromosome behaviour, including preferential inactivation of the paternal X chromosome in the trophoblast, random inactivation in the inner

cell mass, and molecular mechanisms of inactivation (Goto and Monk, 1998). The dog is not an exception from the common rule, although direct evidence is still missing. Thus, females of eutherian mammals are natural mosaics, having a different active X chromosome in different groups of somatic cells.

Detailed description of numerous defects of sexual development in dogs has been given by Meyers-Wallen (1993, 1999), who recognizes anomalies of chromosomal, gonadal and phenotypic sex. Three major disorders: XXY, XO and XXX, which are based on variations of the normal chromosome set, are described in the dog. Underdeveloped genitalia and sterility associated with lack of one X chromosome (XO karyotype) observed in the bitch (Meyers-Wallen, 1993) appears to be similar to the human Turner syndrome. XXY dogs resemble Klinefelter's syndrome in humans. Chimeras carrying cells with chromosome sets, like XX/XY and XX/XXY demonstrate deviations from normal development (Meyers-Wallen, 1999). This includes true hermaphrodites having both ovarian and testicular tissue.

Gonadal sex abnormalities refer to the situations when chromosomal and gonadal sex are contradictory. Such animals are called sex-reversed. In several breeds such as the American Cocker Spaniel, the English Cocker Spaniel, Chinese Pug, Kerry Blue Terrier, Weimaraner and German Short-haired Pointer, animals with 78,XX chromosome constitution were described who developed varying amounts of testicular tissue (Meyers-Wallen, 1993). *SRY* gene, as mentioned above, is crucial for development of the testis, thus directing sexual development towards the male phenotype. In humans and the mouse, translocation of the *SRY* (*Sry*) region from the Y to X chromosome is responsible for reversal XX males (Cattanach *et al.*, 1982). It seems that this is not necessarily always the case in the dog. It has been demonstrated, for instance, that SRY high-mobility group (HMG) box was absent in genomic DNA of XX sex-reversed American Cocker Spaniel dogs and Short-haired Pointer dogs (Meyers-Wallen *et al.*, 1995a,b). A possibility was discussed that a mutant autosomal gene may cause activation of the testis differentiation cascade in the absence of *SRY* (Meyers-Wallen *et al.*, 1995a,b).

In a number of situations, chromosal and gonadal sex agree, but the internal and external genitalia are ambiguous or even alternative. These cases are categorized as abnormalities of phenotypic sex and belong to two groups: male or female pseudohermaphrodites (Meyers-Wallen, 1993, 1999). In such abnormal embryos, with XY constitution and gonads developing into testes, two groups of events can be recognized. One of them is failure of Müllerian duct regression which diverts embryos from the typical male developmental pathway, allowing female sexual structures such as oviducts, uterus and vagina to appear. Another is failure of androgen-dependent masculinization, when Müllerian ducts regress but structures dependent upon androgens (i.e. testosterone) do not respond and masculization does not occur. There are obviously different genetic and developmental grounds for this phenomenon. One of them seems to be similar to a syndrome known in humans as testicular feminization, which is caused by an X-linked mutation responsible for defects in the androgen receptor.

Female pseudohermaphrodites with XX constitution developing ovaries have also been reported. Such females may be a result of androgen or progesterone administration during development (Meyers-Wallen and Patterson, 1989).

The most common disorder of sexual development in dogs is cryptorchidism, accounting for about 13% of males presented to small animal clinics (Dunn *et al.*, 1968, cited by Meyers-Wallen, 1993). It seems that there is more than one reason for cryptorchidism. In some cases sex-limited autosomal recessive inheritance can be an explanation. However, it may not exclude other options. In foxes, for instance, cryptorchidism was one of the pleiotropic effects in males homozygous for an autosomal dominant mutation *Star* causing black and white spotting. Other known pleiotropic effects in these foxes include heterochromia, deafness and the pathological condition of vestibular apparatus causing abnormal head shaking behaviour (Belyaev *et al.*, 1981).

These numerous abnormal outcomes of sexual differentiation highlight the multiplicity of genes involved and complexity of developmental interactions.

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16 Pedigree Analysis, Genotype Testing and Genetic Counselling

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Introduction

Since domestication, *Canis familiaris* has undergone tremendous diversification into different breeds, so that today we recognize over 400 breeds throughout the world (Padgett, 1998). Individual members of a breed are clearly identifiable as a member of that breed and distinguishable from

other breeds. Presumably, man first domesticated the wolf to provide help with hunting and guarding. When later other species were domesticated, such as cattle and sheep, herding breeds would have been developed. These three functions, hunting, guarding and herding, must have predominated throughout the vast majority of time since domestication.

The major expansion in the number of different breeds is very recent and has certainly only occurred during the last few centuries. The impetus for this expansion has been man's desire to produce breeds that have characteristics other than those that permit dogs to perform a particular function. Since the 19th century there has been a tremendous growth in dog shows and that has greatly increased the breeding of dogs exclusively for exhibition and competition. Even the breeds that were traditionally developed for a particular function are now also bred for exhibition and competition. The end of the 19th century saw the development of kennel clubs throughout the world designed to control the registration of pedigree breeds and their exhibition at dog shows. Today, the vast majority of purebred dog production throughout the world is controlled by these kennel clubs and new litters of puppies will only be added to their registries if both parents are already registered on the breed database.

Origin of mutations

The canine genome has accumulated many mutations during the time since domestication. Some of these would already have been present in the wolf populations that were involved in domestication, others have occurred *de novo*. Not all of these have necessarily been deleterious. Some have been neutral and therefore have had no consequence for the dog. Some have been exploited by breeders over the centuries to produce the great diversification in breed types. Others have been more detrimental and have resulted in inherited disease in the various dog breeds. There are now over 370 different diseases that have been shown to be the result of the inheritance of mutant genes, or in which inheritance is believed to play a major role (see Chapter 9). These diseases have been made more imperative in the minds of breeders because as the veterinary field has conquered infectious and parasitic disorders, health issues now more frequently involve disorders that have a genetic basis.

The breeding practices of linebreeding and inbreeding developed over the centuries, initially to establish new breeds and then to maintain breed-specific characteristics, have not caused any of the disease gene mutations, but they have certainly increased their frequency once introduced into a breed. These defects are not intentionally added to the dog's DNA by dog breeders but rather represent the concentration of mutations that accompanied the development of dog breeds (Ott, 1996). In 1965, Scott and Fuller investigated the inheritance of behaviour, and in doing so uncovered other ramifications of current dog breeding practices. They stated, 'In our experiments we began with what were considered good breeding stocks, with a fair number of

champions in their ancestry. When we bred these animals to their close relatives for even one or two generations, we uncovered serious defects in every breed.' They concluded that 'all the evidence is in favour of the conclusion that injurious recessive mutations have accumulated in the dog and become extremely common' and 'current dog breeding practices can be described as the ideal system for the spread and preservation of injurious recessive genes' (Scott and Fuller, 1965). Although their report was written in the mid-1960s, the breeding practices of dog owners have remained largely unchanged since their work was published.

However, the very same breeding practices that have been used to generate and maintain the various breeds have enabled, and will continue to allow, breeders to construct breeding programmes designed to eliminate disease alleles from a breed population. The key is for breeders to identify the stock that is likely to pass on mutant alleles to future generations. Armed with this information, they will be able to use their skills as breeders to select against them. Ultimately, the new DNA technologies will provide breeders with the opportunity to better understand the genes carried by their animals. However, even in the absence of specific DNA tests for disease alleles, there are steps that breeders can take to minimize their spread to future generations.

Breeder involvement

There are several components to be considered when a disorder is found to be recurrent in a breed: accurate diagnosis of the disease, evaluation of the severity of the disease, determination of whether therapy exists to ameliorate the disease, consideration of personal opinions on the responsibilities of being a 'breeder' of defects, detection of the mode of inheritance of the disease, and assignment of a genetic risk to the disorder (Fisher, 1982; Dodds, 1995). Accurate diagnosis is essential to establish if the disorder has a genetic basis and therefore can be selected against. In some instances, the severity of the disorder may not be great enough in the minds of breeders to warrant a concerted selection programme against the disorder even if a genetic basis exists (Hall and Wallace, 1996). In these cases, little effort may be expended if the disorder is thought not to impact on the well-being of the dog. This may be the case too if readily available therapeutics minimize the impact of the disorder on the dog (Dodds, 1995). All of these issues will influence the relative weight a breeder will place on trying to eliminate that particular disorder from a line of dogs, especially if the 'breeder culture' is such that breeders routinely castigate other breeders for either inadvertently producing a defective puppy or for openly discussing the genetic short-comings of the breed (Fowler *et al.*, 2000). Should the breeders agree that the trait is worthy of consideration in their breeding programmes, the breeding community's involvement is crucial to obtain the necessary information and data needed to make informed genetic suggestions.

Pedigree Analysis and Modes of Inheritance

Knowledge of just how a particular disease is inherited is absolutely essential to the control of genetic disease. The first indication that a condition might be inherited occurs when a higher than expected incidence of the condition is noted within a breed, a line or a distinct family of dogs, provided that all possible environmental factors have been considered. Once this alert has been sounded, decisive evidence for or against a genetic causation can be collected by analysing the patterns of occurrence of affected individuals within a closely related family. The characteristics of the major forms of inherited disease (autosomal recessive, autosomal dominant, sex-linked recessive and polygenic) are presented in Table 16.1, and pedigrees are analysed to see if the observed pattern of appearance of affected individuals fits one of these.

Of the 370 inherited diseases that have thus far been reported in the dog, 203 have been assigned a particular mode of inheritance (Patterson, 2000). A survey of the publications relating to these show that often insufficient numbers of dogs have been studied to allow a statistical evaluation of the results and their interpretation. Thus patterns of appearance of affected individuals have been shown to be consistent with a particular mode of inheritance, but these conclusions have not been supported by statistical analyses. Cursory evaluations of pedigree data, without statistical support, may lead to false conclusions on the mode of inheritance, resulting in improper breeding recommendations.

Where sufficient pedigree information is available, for example the study of the inheritance of lethal acrodermatitis in Bull Terriers (Jezyk *et al.*, 1986), the inheritance of multifocal retinal dysplasia (MRD) in the Golden Retriever (Long and Crispin, 1999) and the inheritance of epilepsy in the Keeshond (Hall and Wallace, 1996), several statistical evaluations have been used. In all three cases the condition has been shown to result from a single autosomal recessive mutation. Generally, two different statistical approaches have been used: the Singles Method of segregation analysis (Li and Mantel, 1968; Davie, 1979; Nicholas, 1982, 1987) and the *a priori* method (Emery, 1976). In the former, data are analysed to see how well they fit the expected segregation pattern of three normal offspring to one affected for a condition caused by an autosomal recessive mutation. In the latter, the number of affected offspring observed in sibships is compared with the expected number under the hypothesis of autosomal recessive inheritance.

Simple segregation analysis is very straightforward provided that specific matings can be arranged. For example, if a condition is thought to result from a single autosomal recessive mutation, specifically planned matings between obligate carriers will be expected to produce 25% affected offspring, a segregation frequency of 0.25. However, it is not generally the case that specific matings are set up to test a hypothesis of inheritance. Rather, data are obtained by analysing existing pedigrees where one or more individuals are affected with the condition. This immediately introduces a bias into the data because there could be carrier/carrier matings in the pedigree that have gone

Table 16.1. General characteristics of the main modes of inheritance in canine disease

Autosomal recessive
- The mutant gene responsible is on one of the 38 pairs of autosomes.
- To be affected dogs must be homozygous for the mutant gene.
- The condition tends to skip a generation until such time as two heterozygous carriers are mated and produce affected offspring.
- Each parent of an affected offspring must be a heterozygous carrier.
- A carrier bred to a carrier will, on average, produce 25% affected, 50% carrier and 25% normal offspring.
- Male and female offspring are equally affected.

Autosomal dominant
- The mutant gene is located on one of the 38 pairs of autosomes.
- The mutant gene is generally present in the heterozygous state and is less common in the homozygous state.
- At least one parent of an affected offspring must have the condition (unless the condition shows partial penetrance or there is a new mutation).
- Generally there is no skipping of generations.
- Assuming that an affected dam or sire is heterozygous, on average 50% of its offspring will be affected.
- Males and females will be equally affected.

Sex-linked recessive
- Approximately 50% of the male offspring of a carrier female will be affected.
- By the same token, 50% of a carrier dam's female offspring will be carriers.
- There is a characteristic pattern of transmission: clinically normal females produce affected sons who in turn produce clinically normal carrier daughters.
- Clinically affected males pass the mutant gene on to all of their daughters, but none of their sons.
- If both parents are affected, all offspring will be affected.
- An affected male will often have affected relatives on his dam's side, but hardly ever on his sire's side.

Polygenic
- Both dam and sire must contribute one or more genes to affected offspring, but this contribution need not necessarily be equal.
- There are no predictable ratios in pedigrees because we do not know the number of genes involved.
- Both sexes are affected, but not necessarily in equal numbers.
- The condition will often appear erratic in pedigrees where there are affected dogs.

unnoticed simply because, by chance, no affected offspring have been produced. This means that although all affected offspring of carrier/carrier crosses will be scored, not all of the normal offspring will be scored. Thus, even if the condition does result from an autosomal recessive mutation, the segregation ratio will be greater than 0.25.

The Singles Method was derived to provide an efficient and unbiased estimate of segregation frequency. The only requirement for this method of statistical analysis is that all members of each reported family are included in the analysis. This method of analysis can be used in its simplest form when one of the two following criteria can be met: (i) all families with affected offspring are included in the data, or (ii) a random sample of families with affected offspring has been included in the data. Often, the investigator may be unsure that one or other of these criteria hold and, in such a case, the best that

can be achieved is for the investigator to compute an upper and lower estimate of the segregation frequency.

Although segregation analysis is simple in principle, in practice difficulties can be encountered. A common observation is that most of the data will fit an expected segregation frequency, but within the data-set there are observations that are not compatible with the general theory. These anomalies are usually explained by misdiagnosis of phenotype or the occurrence of phenocopies (a phenotype caused by some sort of environmental influence that mimics the phenotype caused by the gene mutation).

The recent investigation into the inheritance of multifocal retinal dysplasia (MRD) in the Golden Retriever (Long and Crispin, 1999) serves as a good example of the statistical methodology available for pedigree analysis. MRD had already been reported to be inherited as a simple autosomal recessive condition in the American Cocker Spaniel (MacMillan and Lipton, 1978) and the English Springer Spaniel (Schmidt *et al.*, 1979) and this investigation sought to confirm a similar mode of inheritance in the Golden Retriever. The report contains litter information from 27 different litters, containing a total of 202 offspring. Of these, 56 were affected with MRD (28 males and 28 females) and 146 were clinically unaffected. In addition, affected offspring had clearly been produced in litters where both parents were clinically normal. The equal numbers of affected males and females rules out a sex-linked mode of inheritance and the fact that affected puppies had been produced from clinically normal parents tends to rule out a dominant mode of transmission. The data are therefore highly suggestive of an autosomal recessive mode of inheritance.

If MRD is due to an autosomal recessive gene, the segregation frequency of affected individuals (p_0) would be 0.25. The probability that the segregation pattern in the litter screening data presented is consistent with an autosomal recessive mode of inheritance was calculated using the Singles Method (Nicholas, 1987; Davie, 1979). In the final analysis there was no statistical difference between the expected and observed segregation frequency allowing the authors to conclude that MRD in the Golden Retriever results from an autosomal recessive mutation.

Breeding Programmes to Address Inherited Disease

Pedigree information can be invaluable for determining the genetic status of a dog for a given inherited condition, provided that the mode of inheritance is known, correct parentage can be relied upon and that accurate clinical diagnoses are available for dogs in the pedigree. Once pedigrees have been studied and evaluated and a specific disorder's transmission assessed, breeders can predict the likelihood that puppies produced by a specific breeding will exhibit a particular trait or disorder. This information can then be built into breeding programmes in order to avoid producing affected offspring and, hopefully, to reduce the frequency of mutant genes within the breed's gene pool. Certainly most breeders would agree that it is desirable to eliminate genetic anomalies through selective breeding.

Autosomal dominant diseases

For a condition that is known to result from a single autosomal dominant mutation, the solution is relatively straightforward because all animals carrying the mutation will be clinically affected (Fig. 16.1). It is simply a question of identifying affected individuals by clinical examination and selecting against them when developing breeding programmes. For example, von Willebrand's disease in Doberman Pinschers is inherited as an autosomal dominant condition (Riehl *et al.*, 2000). Unfortunately, even with autosomal dominant conditions there can be problems. For example, the age of onset of the condition can be crucial. Early onset conditions that result from an autosomal dominant mutation will be diagnosed in affected animals early in life, before sexual maturity, and these can be selected against. However, if the condition is late onset, affected individuals may not be diagnosed until after they have been used for breeding. Additionally, if the age of onset is late in life an affected dog may have been used for breeding and died before the age that it would have developed the condition and revealed it as having a dominant mutation. Another complication that could arise is if the dominant mutation shows less than 100% penetrance. If the condition demonstrates incomplete penetrance, some animals carrying the dominant mutation will not become clinically affected and therefore will go unnoticed.

These aspects of a dominant mutation will certainly complicate efforts to reduce the frequency of the mutant gene by selecting against animals

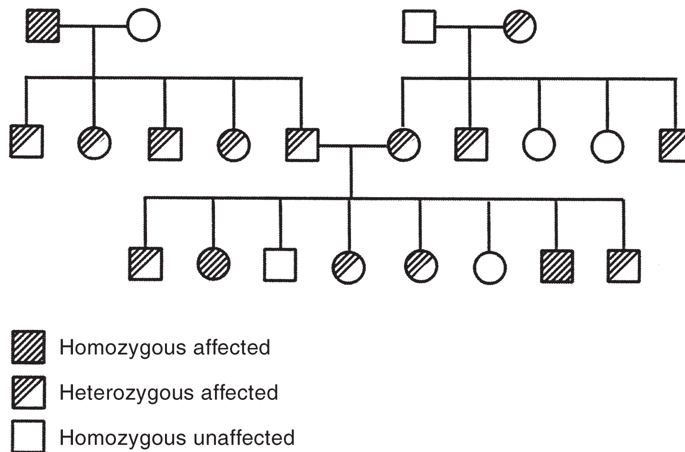

Fig. 16.1. Idealized pedigree for inheritance of an autosomal dominant disorder. Segregation of an autosomal dominant disorder. Provided that there is full penetrance, individuals with one mutant allele will be clinically affected. Dominant conditions may express themselves differently if they are homozygous affected (two copies of the dominant, mutant allele) rather than heterozygous affected (only one dominant mutant allele and one normal allele). This differential effect is illustrated in the condition known as Ehlers–Danlos syndrome in English Springer Spaniels where the homozygous dominant allele seems to be lethal.

that carry it. However, given sufficient clinical screening within a breed, particularly those dogs that form the nucleus of a breed's breeding stock, it should be relatively easy to reduce the frequency of a dominant gene within a breed, provided that the condition that it causes has a relatively early age of onset. The identification of the genes involved in these diseases and the development of DNA-based tests for the mutation will greatly advance breeders' ability to select against the disease alleles. Additionally, early DNA screening of animals will identify those that carry mutations that cause late onset conditions or are not fully penetrant.

Polygenic diseases

Reduction in the incidence of a polygenic condition is certainly possible by selecting to breed from those animals that do not exhibit the condition and removing those more severely affected from the breeding programme. The more heritable the trait and the more rigorous this selection the more likely it will be that the incidence of the disease will fall. Attempts to address the problem of hip dysplasia (discussed in detail in Chapter 10) in a number of breeds worldwide demonstrate what can be achieved. Many countries and kennel clubs have established hip screening programmes based on the radiographic evaluation of the phenotype. Radiographs of both hips of an animal are assessed and either a grade or score is given for each hip. For example, in the UK each hip is assessed on the basis of nine radiographic features of the hip joint (Gibbs, 1997; Willis, 1997; Dennis, 1998). Each of these nine features for each joint is scored by a panel of expert scrutineers and the dog is then given an overall hip score. The lower the hip score, the more normal the dog's hips. Similar schemes, although with different evaluation systems, operate in other countries of the world. The benefit of such schemes is that they can be used to select animals for breeding, for example only breeding from tested animals with low scores or, as in the UK, animals that have scores lower than the breed mean score. Control programmes based on such selection have shown variable results (Corley, 1992; Fluckiger *et al.*, 1995; Willis, 1997). However, generally, the results of these studies confirm the benefits of breeding from tested animals with low scores for the reduction of the extent of hip dysplasia.

The best progress was found in Sweden where disease prevalence decreased during the period of selective breeding in all breeds investigated (Swenson *et al.*, 1997). This study encompassed 83,229 dogs from seven different breeds registered by the Swedish Kennel Club. Since 1984 it has been mandatory in Sweden that the hip score is known and recorded for both the dam and sire if their progeny are to be registered by the Swedish Kennel Club. This measure has led to a dramatic shift to use, as breeding stock, dogs that have had their hips evaluated and have low scores. Prior to 1984 individual breed clubs operated their own scheme for selecting breeding stock. The data presented in Fig. 16.2 represent the prevalence, by year of birth, of hip dysplasia during the period of investigation (1976–1988).

While utilizing selection schemes based on phenotypes has been productive as noted above, additional progress in minimizing the incidence of disorders with a polygenic or complex mode of inheritance may lie in the application of quantitative trait locus (QTL) mapping. Even complex traits appear to be governed by a restricted suite of loci exerting significant impact on expression of that trait (Georges, 1997) and these can be identified. A more thorough discussion of applying QTL methodology appears in the following chapter. It is important to note here though, that to detect QTLs, divergently selected populations must be bred to permit segregation of the phenotypic trait of interest (Fisler and Warden, 1997). In the case of dogs, that would require cross-breeding distinct breeds, thereby producing mixed breed offspring.

Autosomal recessive diseases

Genetic counselling is less straightforward for conditions caused by an autosomal recessive mutation. Identifying affected dogs and selecting against them will certainly reduce the number of affected puppies born but there will be a less significant reduction in the number of carriers. These carriers will act as a silent reservoir of the recessive mutant allele. The only way to make significant inroads into autosomal recessive conditions is to develop ways of identifying carriers so that they can be taken account of when breeding programmes are developed. The vast majority of canine genetic disorders tend to be either recessive or polygenic in nature (Willis, 2000). Examples of autosomal recessive disorders include phosphofructokinase deficiency and

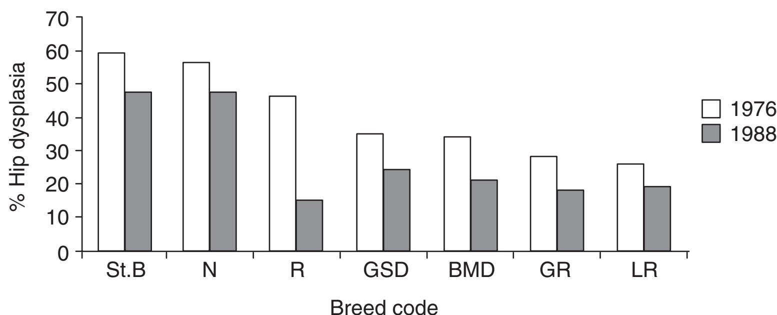

Fig. 16.2. Prevalence, by year of birth, of hip dysplasia (all grades) in seven breeds in Sweden. The overall prevalence of hip dysplasia in 1976 and then 1988 for seven breeds registered with the Swedish Kennel Club. During this period it became mandatory for the hip score to be recorded for both dam and sire before their progeny were registered, a measure that led to a dramatic shift toward the use, as breeding stock, of dogs that have had their hips evaluated and have low scores. In all breeds analysed there was a significant reduction in the prevalence of hip dysplasia in 1988 compared to 1976. [Data taken from Swenson *et al*., 1997.] Breed Code: St.B, St Bernard; N, Newfoundland; R, Rottweiler; GSD, German Shepherd Dog; BMD, Bernese Mountain Dog; GR, Golden Retriever; LR, Labrador Retriever

fucosidosis in Springer Spaniels, narcolepsy in Doberman Pinschers, and canine leucocyte adhesion deficiency in Irish Setters (Patterson, 2000).

Pedigree analysis

Traditional pedigree analysis will identify the existence of carriers of recessive alleles (Fig. 16.3). When two clinically normal parents produce one or more offspring affected by a condition known to result from an autosomal recessive condition, both are identified as obligate carriers. However, it is certainly possible that carriers will go undetected by merely analysing pedigree information. Carrier bitches may go undetected because during their limited breeding life they may not have been mated to a carrier dog, or if they have, they may, by chance, not have produced an affected offspring, which is certainly possible in breeds that have small litter sizes. Carrier dogs are less likely to go undetected because dogs usually have a much greater mating potential. However, even carrier dogs may go undetected, particularly if the frequency of carrier bitches is low in the population. Late onset conditions that result from autosomal recessive mutations pose exactly the same problems as mentioned above. Carriers may have been used extensively for breeding before their carrier status becomes known. Again, an increased understanding of the genes involved and the development of DNA tests for the mutations will greatly facilitate the breeders' attempts to determine the genotype of individual animals before they are used in a breeding programme.

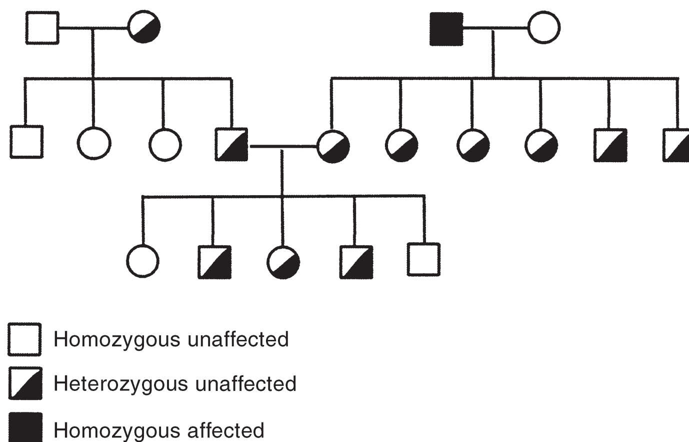

Fig. 16.3. Idealized pedigree for inheritance of an autosomal recessive disorder. Idealized pedigree in which an autosomal recessive disorder is segregating; clinically affected individuals have two copies of the mutant allele. The key to controlling such disorders through selective breeding is to identify the heterozygous carriers that are clinically unaffected (heterozygous unaffected). Traditional pedigree analysis and, increasingly, DNA testing will help breeders to identify carrier animals and then take note of this when designing breeding programmes.

Test matings

In the past, breeders of livestock have resorted to test matings in order to verify the genetic status of a particular animal when that animal potentially carries a recessive allele. Test matings rely on mating the animal under test to a mate with a known genotype. The ideal way to test-mate is to breed the dog in question to a known affected animal. If the test dog is homozygous normal no affected progeny will be produced, if it is a carrier then, on average, half of the progeny will be affected. Test matings rely on producing sufficient progeny to ensure that the results can be reliably interpreted. If the test mating involves a known affected, then if six normal offspring are produced the test dog has approximately 98.5% chance of being genetically clear. The mate with the known genotype could also be a carrier; and in this case 15 normal progeny will need to be produced in order that the test animal has a 98.5% chance of being homozygous normal. Although it is possible to test-mate both males and females, it is really only feasible to undertake test matings for males because females may not produce sufficient offspring in one litter, necessitating mating the female several times, which spreads the test mating over a long period of time. The other thing to note about the outcome of test matings is that there can still be doubt about the outcome. So, although there is a 98.5% chance that the test animal is genetically clear when six normal offspring have been produced from a mating to an affected mate, there is still a 1.5% chance that the tested animal is a carrier. Thus out of every 200 dogs tested in this way, three carriers will go undetected and slip through the net as genetically clear.

Performing test breedings may provide useful information on the mode of inheritance of a particular trait, but intentionally breeding dogs with the objective of producing disease is not considered ethically responsible. In a scientific venue, test breeding dogs may have merit, but there is still the issue of placing puppies known or suspected of being afflicted with a particular disorder. Furthermore, many breed clubs have codes of ethics that specifically preclude the provision of dogs for scientific research, except under limited circumstances. Pedigree analysis generally relies upon the unintentional 'test breedings' done in the dog community; that is, breedings done without the knowledge of the genotypes of the broodstock. Puppies generated from these breedings have provided extremely valuable information on the parental genotypes. Analyses of these pedigrees and the epidemiological data of the puppies, coupled with deductive reasoning, allow owners and scientists to infer the genotype of a given dog.

Biochemical genotyping

Carriers of recessive disease alleles are generally phenotypically normal and clinical examination cannot distinguish carriers from normals. However, 'genotyping' tests of genetic disorders have been developed by characterizing the relative abundance or activity of a particular protein known to be associated with a disorder (Dodds, 1995). In other words, if a defective protein product was detected, you could conclude that the gene had been mutated (van Oost, 1998). For example, in English Springer Spaniels, a deficiency in the

activity of a particular enzyme known as muscle type phosphofructokinase is related to the disease symptoms of acute haemolytic anaemia and jaundice that accompanies exercise (Patterson, 2000). Affected homozygotes have only 8–24% of the normal activity values for this enzyme while heterozygote carriers have 40–60% of normal activity (Vora *et al.*, 1985; Giger *et al.*, 1986). While affected individuals can be definitively diagnosed by this enzymatic assay, the results cannot unequivocally determine heterozygote carriers, making this tool less than ideal in breeding programmes. Additionally, some disorders affect systems that lack a ready protein-based test. Alternatively, obtaining the tissue to be tested may be traumatic to the dog's health, for example, disorders affecting the retina (Patterson, 2000). The lack of reliable and/or available protein-based tests has served as the impetus for developing DNA-based genotyping methods.

Genetic Counselling Based on Predictive Models

There have been few breed-wide genetic counselling schemes based on predicting the probable genotype of an individual dog from knowledge of its pedigree. However, Hall and Wallace (1996) used exactly this approach to address the problem of epilepsy in the Keeshond, a condition that results from an autosomal recessive mutation. They were able to analyse pedigrees of individual dogs sent in by their owners and calculate the probabilities of a mating producing epileptic or carrier dogs, and the inbreeding coefficients of the progeny of such matings. Based on the outcome of these calculations they have been able to advise the breeder whether or not to proceed with the mating. Since the start of the counselling scheme in 1988, the mean probability that a proposed mating would result in carriers has declined significantly, consistent with a decline in the frequency of the mutation for epilepsy in the Keeshond.

DNA Technology and the Identification of Mutant Genes Responsible for Inherited Disease

The advent of molecular biological techniques has enabled the identification of genes causal in the expression of disorders (Sack, 1997). These can then be cloned and used as the basis for genetic tests. The development of DNA-based tests permits the classification of individual dogs as carriers for recessive genes or can identify those puppies that will be afflicted with late onset disorders as adults (van Oost, 1998). This information can then be used judiciously by breeders to propagate the desired traits of certain dogs while minimizing the spread of known disorders in their breed (Dodds, 1995; Ubbink *et al.*, 1998a).

DNA tests for disease gene mutations

The first gene demonstrated to be causal for a canine genetic disorder was reported in 1989. The mutation was a single base pair substitution in the canine clotting factor IX gene and caused haemophilia B in Cairn Terriers (Evans *et al.*, 1989) when present in two copies. Carriers of the defective allele could be identified with a genetic test designed to detect that substitution. In the intervening years, many additional genes have now been cloned and characterized as causing canine disorders (Patterson, 2000). Genetic tests, based on the DNA sequence data of these cloned genes, have been or are being developed (Fowler *et al.*, 2000).

The development of these tests and the identification of the causal genes are based upon the verification of the mode of inheritance by pedigree analysis to determine that the disorder is in fact, genetically based (Famula *et al.*, 2000). DNA is collected from affected and unaffected dogs of a particular breed, ideally representative of particular lines or families within the breed (Ubbink *et al.*, 1998b), and then it is evaluated for a unique association between a particular DNA profile and the disorder's phenotype. The screening utilizes molecular markers derived from the emerging canine linkage map (discussed in Chapter 6 and in Mellersh and Ostrander, 1997) or candidate genes which are genes characterized as causing similar disorders in humans or other species (Sargan, 1995).

Genotyping using DNA tests

Genotype testing is based either on mutations within the causal gene itself or on linkage to a particular gene. Mutation-based tests, sometimes called 'direct tests', are preferred because the genotyping is 100% accurate (*Today's Breeder*, 2000). However, DNA linkage-based tests, sometimes called 'marker tests', also provide valuable information (Petersen-Jones, 1998). Both of these tests utilize the polymerase chain reaction (PCR) in which the DNA of an individual is duplicated billions of times in a laboratory test tube. Rather than copying an animal's entire DNA, a particular region of DNA is targeted to be amplified which creates substantial quantities of that region of DNA that can then be analysed (Sack, 1997). This PCR amplification process has the advantage of permitting very small quantities of a dog's DNA to be expanded into useful quantities. It is through this process that the small amount of DNA collected from the cells sloughed off on the inside of a dog's cheek or isolated from a modest volume of blood can be used in genotyping, because every cell in the body contains *all* of the animal's DNA (van Oost, 1998). The region targeted for amplification is either the gene that causes the disorder (mutation-based tests), or a region of DNA adjacent to the gene that causes the disorder (linkage-based tests) (Mellersh and Ostrander, 1997).

Mutation-based DNA tests

As mentioned above, mutation-based tests provide accurate genotyping of homozygous affected, heterozygous carriers, or homozygous normal animals. Examples of such tests commercially available are listed in Table 16.2. In these tests, the DNA representing the particular gene is amplified by PCR and run on a thin gel matrix in a laboratory. The amplified DNA migrates through the gel matrix differentially and exhibits discrete patterns when visualized: the normal gene is distinctly different from the mutant gene (Fig. 16.4). The amplified DNA migrates as a function of its sequence so DNA copies of the exact same sequence accumulate into bands within the gel matrix with each band

Table 16.2. Available canine mutation-based DNA tests

Disorder	Applicable breed(s)	Testing laboratory
Canine leucocyte adhesion deficiency (CLAD)	Irish Setters	AHT; OptiGen
Congenital stationary night blindness (CNSB)	Briards	Optigen; AHT; GeneSearch
Cystinuria	Newfoundlands	PennGen
Fucosidosis	English Springer Spaniels	PennGen; AHT; Cambridge
Mucopolysaccharidosis type VII	German Shepherd dogs	PennGen
Myotonia congenita	Miniature Schnauzers	PennGen
Narcolepsy	Labrador Retrievers, Doberman Pinschers, Dachshunds, Poodles	Stanford
Phosphofructokinase deficiency (PFK)	American Cocker Spaniels, English Springer Spaniels	GeneSearch; OptiGen; PennGen
Pyruvate kinase deficiency (PK)	Basenjis, Dachshunds, West Highland White Terriers	PennGen; VetGen
Rod–cone dysplasia (*rcd-1*)	Irish Setters	GeneSearch; AHT; OptiGen; VetGen
Rod–cone dysplasia (*rcd-3*)	Cardigan Welsh Corgis	Cambridge; GeneSearch
Severe combined immunodeficiency (SCID)	Bassett Hounds, Pembroke Welsh Corgis	PennGen
von Willebrand's disease	Doberman Pinschers, Shetland Sheepdogs, Poodles, Scottish Terriers, Pembroke Welsh Corgis, Manchester Terriers	GeneSearch; VetGen; AHT

Optigen, Cornell Business & Technology Park, Ithaca, New York, USA.
PennGen Laboratories, 3850 Spruce Street, Philadelphia, PA 19104–6010, USA.
GeneSearch, LLC, 11014 Schuylkill Rd., Rockville, MD 20852, USA.
Center for Narcolepsy, Department of Psychiatry, Stanford University School of Medicine, California, USA.
VetGen, 3728 Plaza Drive, Suite One Ann Arbor, Michigan, USA.
Cambridge DNA Testing, Centre for Veterinary Science, University of Cambridge, Madingley Road, Cambridge CB3 03S, UK.
AHT: Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK.

representing an allelic form of a gene. In the case of an autosomal recessive disorder, a homozygous dog genetically free from the mutation will have two copies of the normal gene while the affected dog will have two copies of the mutant version of the gene. Because the amplified products from the homozygous normal dog are identical, a single band image would be seen. The same would be true for the homozygous affected dog, but the band image would be distinctly different from that of the genetically clear dog. A heterozygous carrier has both a normal and a mutant gene copy, therefore, the amplified DNA from this dog will show two discrete bands, one band representing the normal gene and a separate band representing the mutant gene. Thus, the DNA from carriers is readily distinguishable from the DNA of genetically normal or genetically affected individuals (van Oost, 1998; Wilkie, 1999).

Interpretation of mutation-based genotyping tests is relatively straightforward. An individual has zero, one, or two copies of the mutant gene. Such tests can be done on the DNA of puppies as early as it is safe to collect the small amount of tissue or blood necessary to complete the test. Breeding a carrier to a genetically normal dog would produce, on average, 50% carrier puppies and 50% genetically normal puppies. With the ability to genetically test using a mutation-based test, puppies will be immediately identifiable as being genetically free from possessing a mutant allele or carrying the mutation. A breeder will not have to rely upon statistical odds that half the puppies will be genetically normal; the breeder will know unequivocally. It is important to note, though, that the application of statistical odds still holds true. On average, such a breeding will produce 50% carriers and 50% genetically normal (Mellersh and Ostrander, 1997). However, segregation of the gene copies during the formation of reproductive cells and fertilization is an independent event, so within any given litter, the percentages will vary considerably from

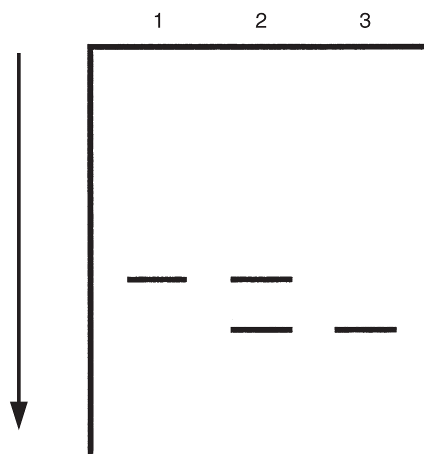

Fig. 16.4. Image of PCR-based genotyping products. Image of PCR-based genotyping products run on a polyacrylamide gel for homozygous normal (lane 1), heterozygous carrier (lane 2), and homozygous mutant (lane 3) DNA samples. Arrow denotes direction of DNA migration.

the overall average. The advantage of employing genotype testing is that the breeder will know precisely the genotypes, for the disorder under consideration, of the puppies produced (Holmes, 1998). This is important to know because deleterious versions of genes can spread rapidly throughout a dog breed when popular sires or genetic founders carry mutations (*Today's Breeder*, 2000)

Armed with such information, the breeder can make informed and educated breeding decisions. Educated decisions include recognizing that no single trait defines a breed and exerting selection pressure designed solely to eliminate a particular trait, without consideration for other attributes that a particular dog possesses, is not prudent (Binns *et al.*, 1996). The advocacy of eliminating all dogs that are carriers from the breeding programme will result in eliminating many other superior qualities (Dodds, 1995; Holmes, 1998). It is imperative to maintain the breed's attributes while selecting against the genetic disorder. By applying genetic testing, the breeder can judiciously use those sires or dams that may carry, or even be affected with, a particular disorder in their breeding schemes. Ideally, if the carrier possesses quality attributes, breeding a dog that lacks any mutant allele to the carrier will result in offspring including superior individuals that do not carry the mutant allele and which can be used as broodstock in subsequent generations. In some cases, if the mutation is prevalent within the breed and the sire or dam was an affected individual, it will take two generations to eliminate the mutant gene from the broodstock (Binns *et al.*, 1996).

Linkage-based DNA tests

Mutation-based genotype tests, while offering many advantages, are laborious to develop (van Oost, 1998) and tend to be breed-specific. Even if a particular disorder affects many breeds, it is likely that each breed will require the development of its own unique mutation-based test as seen in the different mutations causing von Willebrand disease in Dutch Kooiker dogs and West Highland Terriers and Dobermans (Slappendel *et al.*, 1998). In contrast, linkage-based tests are less likely to be breed-specific but are not as precise in their diagnosis (Curtis *et al.*, 1991; Patterson, 2000).

Linkage-based tests require considered interpretation of their results. That is because, just as the name implies, the DNA test is linked to the disorder but does not detect the genetic mutation causing it (Holmes, 1998; Ubbink *et al.*, 1998a). In these tests, a region of DNA physically adjacent to the gene causing the disorder is the target of the PCR amplification (Curtis *et al.*, 1991). Just as in the mutation-based genotyping tests, there are distinct banding patterns that represent the different genotypes derived from the amplification of the DNA. However, the genotypes obtained from these tests are associated with the disease phenotype but are not themselves the disease genotypes (Holmes, 1998). The closer the linkage of the target DNA to the causal gene, the greater the utility of such a linkage for detecting carrier animals (Ubbink *et al.*, 1998a).

Linkage-based tests are developed by screening the DNA of animals with genetic markers that span the entire genome (Curtis *et al.*, 1991; Wilkie, 1999; Ostrander *et al.*, 2000). These markers are anonymous markers in that they do not represent a particular gene, but can detect inherent polymorphism or genetic variation between individuals within a breed. Their utility lies in their widespread and unique distribution across the genome. If a marker is physically close enough to the gene that causes the disorder such that the marker and the disease gene are never separated during recombination, then that marker can be utilized to detect individuals possessing the mutant allele. Because the marker does not detect the disease gene itself, there is some error due to recombination occurring between the marker and disease gene during the process of forming the reproductive cells (Slappendel *et al.*, 1998). The degree of linkage, reflective of the distance between the disease gene and the marker, is associated with the risk of disease inheritance of the disorder when the particular marker is present (Wilkie, 1999). In some instances this can be calculated if the genetic distance between the causal gene and the marker region is known. If the distance is great enough, false positives and false negatives will be detected, that is, animals with one or two mutant alleles at that locus will be detected as genetically clear or vice versa. The advantage to linkage-based tests is that the exact gene involved need not be known in order to utilize their linkage with the disease gene (Wilkie, 1999). Commercially available linkage-based tests are: copper toxicosis for Bedlington Terriers; progressive rod-cone degeneration (PRCD) for Portuguese Water Dogs, English Cocker Spaniels, Chesapeake Bay Retrievers and Labrador Retrievers; and renal dysplasia for Lhasa Apsos, Shih Tzus, and Soft-coated Wheaten Terriers.

It should also be noted at this point in time that the DNA tests utilized to verify parentage rely upon anonymous markers (Chapter 7; Sack, 1997). These markers were selected for their inherent polymorphism and reliability in being transmitted from one generation to another, not as indicators of particular genes (van Haeringen, 1998). As such, parentage genotypes do not inform the owner of the presence or absence of disease alleles; instead they can be thought of as a permanent microchip number that was bestowed by the dog's parents.

Genetic Counselling Based on DNA Tests

As previously mentioned, there are in excess of 370 documented canine genetic disorders. This number is expected to grow by 5–10 new diseases annually (Ostrander *et al.*, 2000; Patterson, 2000) as additional disorders are characterized as having a genetic basis or new, spontaneous mutations arise. While dominantly inherited diseases are readily selected against by breeders, by far the vast majority of genetic disorders are inherited either as autosomal recessive, X-linked recessives, or are polygenic. Unfortunately, while genotyping exists for some disorders, many hundreds lack any type of genetic tool to aid breeders.

Genetic counselling, guided by the results of DNA tests or the prevalence of the disorder in the breed, will assume a greater role in making complex breeding decisions (Fowler *et al.*, 2000). Again, it cannot be emphasized enough that breeding should be done for the whole dog, not just based on one aspect, even if that aspect causes severe health consequences. The Portuguese Water Dog exemplifies this. Gangliosidosis (GM1) was prevalent in Portuguese Water Dogs when a blood protein-based test for GM1 became available to breeders in the mid-1980s. Carriers of GM1 were accurately diagnosed and eliminated from the breeding programmes. While the incidence of GM1 dramatically declined in Portuguese Water Dogs, the prevalence of progressive retinal atrophy (PRA) increased; the lines of dogs free from GM1 were carriers for PRA (Woodsen, 1999). The breeders had traded one disease for another simply because one disorder had an available test. While the great majority of genetic diseases do not have an available test, implementation of the results for diseases with a test must be done judiciously. The role of the genetic counsellor is to provide explanations of test results, quantify the risks associated with a particular breeding, and enumerate the possible outcomes from such a breeding (Fig. 16.5). The counsellor should not pass judgement but merely supply accurate information (Fowler *et al.*, 2000).

The potential role of kennel clubs

The kennel clubs around the world could have a role to play in encouraging the establishment of breed control schemes to eliminate disease alleles from a breed gene pool, particularly since in most countries they register a very significant number of the purebred dogs produced. The Kennel Club in the UK has recently collaborated with the Irish Setter breed clubs to introduce a breed control scheme to reduce the incidence of CLAD in the breed. The scheme is based on the availability of a gene mutation-based test (direct test) that affords 100% reliability in genotyping individual animals with respect to CLAD. The scheme recommends that all Irish Setters are genotyped before they are used for breeding. This might involve direct DNA testing or it may be inferred from parental genotypes, for example a dog that has both its parents DNA tested clear of CLAD will be hereditarily clear. Carrier/carrier matings are to be avoided, but carriers can be mated to genetically clear dogs; the resultant offspring are required to be DNA-tested to identify the carrier and clear offspring. The progeny of carriers mated to clears will be registered by the Kennel Club for a period of 5 years. However, thereafter the Kennel Club will only register Irish Setters that are either hereditarily clear of CLAD or are shown to be clear by direct DNA testing. Irish Setters that are carriers of CLAD will not be registered after this 5-year period has elapsed. In this way it is hoped that the mutant allele causing CLAD will be gradually removed from the Irish Setter population without compromising the overall gene pool of the breed. That spectacularly good Irish Setter bitch that just happens to be a carrier can still be bred from, the breed will benefit from all of her qualities, but only her

genetically clear offspring will be used to continue the pedigree. This scheme will serve as a future model for the treatment of inherited disease in the UK once a mutation-based test becomes available for a breed-specific inherited condition. The only major variable is likely to be the window of time before

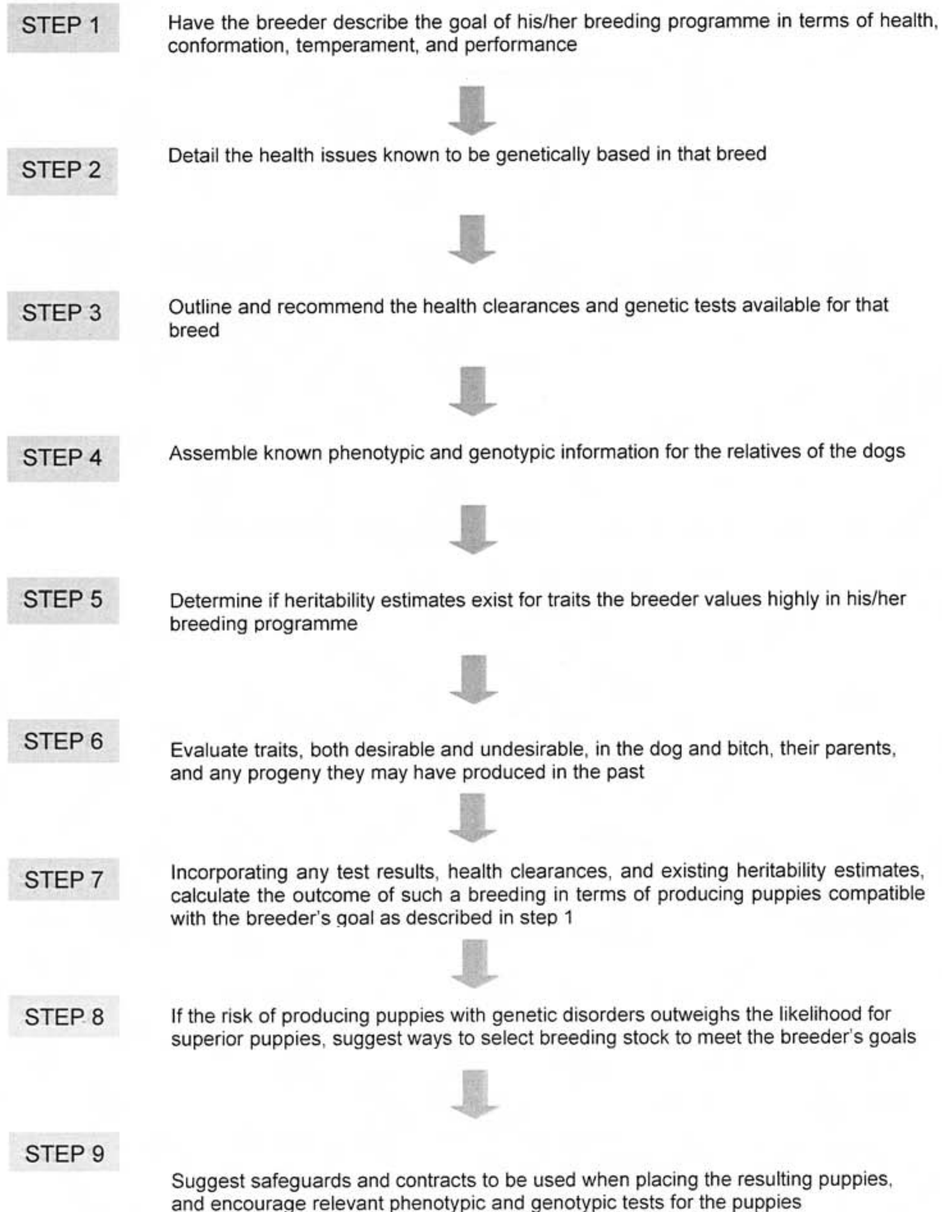

Fig. 16.5. Steps in genetic counselling to follow once a breeder suggests the breeding of a dog and bitch of a given breed.

registration restrictions are made and during which carriers can be mated to clears. In the case of the Irish Setters, the breed clubs involved decided that a 5-year period would be sufficient; for some conditions and breeds it may be decided that a longer period of time will be required.

Many kennel clubs and dog registries currently encourage phenotypic health clearances, such as ophthalmic examinations and hip and elbow evaluations, prior to breeding. As more genetic tests become available, more kennel clubs are likely to take the stance that the Kennel Club of the United Kingdom has taken with respect to requiring the CLAD tests for Irish Setter registration. Additionally, kennel clubs, by offering limited registrations, allow breeders the luxury of time (Willis, 2000). Often, phenotypes of disease, structure, or behaviour require time to be expressed. Breedings in which the potential of producing superior puppies also carries the risk of producing disease need to have the puppies placed in homes under limited registration; that is, registration that requires the breeder's signature to become fully registered. This allows the breeder to evaluate the outcome of the breeding without the resulting puppies being bred prematurely or bred at all if considered to be of inferior quality.

Breeding for Specific Functions

Although the emphasis of this chapter has been on genetic health disorders, pedigree analyses and breeding decisions encompass more than just disease. While genetic disorders command interest due to their widespread nature as well as the emotional and economic impact, concerted breeding programmes include pedigree analyses for additional traits such as behavioural attributes, structural characteristics, and sensorial capabilities. Dogs selected/used in service capacities must be free of a suite of behaviours that includes shyness, fear biting, noise sensitivity and distractability, in order to successfully meet the needs of their human partners' disabilities (Wilsson and Sundgren, 1997). Dogs utilized in search and rescue operations or for bomb detection need exceptional scenting abilities, known to be governed by olfactory receptor genes found in all dog breeds (Chapter 7; Issel-Tarver and Rine, 1996). At this time, only moderate effort has been applied to developing the necessary extensive databases to evaluate the heritability of some of these traits, but the time and cost inputs of training dogs to such tasks are too great to allow inferior genetics to result in failures due to character flaws (Willis, 1995). Early efforts sought to identify simple Mendelian inheritance patterns to account for complex physiological traits, such as noise and physical touch sensitivity. While this approach is now perceived to be overly simplistic, that is selection as if the traits were Mendelian, it proved to be successful in the production of guide dogs for the blind (Humphrey and Warner, 1934).

Guide dog breeding efforts

The continued evaluation of genetic inputs into traits that may influence production of guide dogs was reported by Goddard and Beilharz (1982), Bartlett (1976), and Scott and Bielfelt (1976) and was summarized by Willis (1995). These investigators estimated heritabilities of traits favourable to the successful graduation rate of guide dogs as well as traits thought to negatively affect graduation. Large heritability estimates denote that direct selective breeding programmes can produce genetic change over generations (Famula and Oberbauer, 1998). In the majority of the traits investigated, the heritability estimates did not differ significantly from zero, possibly reflecting either long-term selection ongoing in the breeding programmes or variability in assessing the traits investigated (Willis, 1995). The same was true for heritability estimates of traits associated with police or guard dog work (Willis, 1995). More encouraging heritability estimates were obtained for hunting characteristics (Willis, 1995); additionally, fear and nervousness were highly heritable (Murphree and Dykman, 1965; Murphree *et al.*, 1977; Goddard and Beilharz, 1985).

Institutes are continuing to explore the heritabilities of traits influential on successful graduation of guide and service dogs with greater efforts to minimize the variability in trait assessment. Such institutes as Guiding Eyes and Canine Companions for Independence are establishing large databases of behavioural traits that can be correlated with graduation rate and placement; these correlations will then be used in breeding decisions. Although heritability estimates vary, reports suggest that selective breeding should prove fruitful for improving service dog graduation rates (Humphrey and Warner, 1934; Willis, 1995, Wilsson and Sundgren, 1997).

Breeding for the overall dog

The aforementioned research focuses on traits that govern utilitarian traits. However, the same principles apply to any trait of interest to breeders be it conformational, reproductive or temperamental. Seemingly complex traits that control structural or behavioural attributes may be selected for if the traits have sufficient genetic input, as determined by heritability estimates. Unfortunately, the scientific knowledge base on the inheritance of many of these traits is virtually non-existent. However, once heritabilities for the desired traits are ascertained, and the heritabilities are large enough to assure that selection for these traits will produce phenotypic improvement, breeders should be encouraged to incorporate sire and dam selection schemes designed to maximize the genetic progress (Famula *et al.*, 2000). Breed clubs can initiate

the collection of phenotypic traits along with pedigrees to allow heritabilities to be assigned to the various traits, as outlined in Chapter 17. Sires and dams can then be ranked as to their predisposition to pass on particular traits, and breeders can utilize that information to select prepotent sires and dams for the traits they deem to be particularly important, while weighing other attributes of the sires and dams.

Minimizing the spread of defects requires the concerted effort of the breeders involved to identify the defect, characterize whether the defect has a genetic basis and, if so, determine the mode of inheritance (McNeil and Ponce de Leon, 1992; Ubbink *et al.*, 1998b). It is at that point that breeders can then make some informed decisions on the likelihood of producing an animal with the defect in its pedigree. The breeders can also generate interest in the creation of a DNA-based test to assist in their breeding selections. Given that the majority of genetic defects are either polygenic or inherited as an autosomal recessive and lack a genetic diagnostic test (Patterson, 2000; Ostrander *et al.*, 2000), the breeders must be counselled as to the wisdom of breeding suspected carriers (Ubbink *et al.*, 1998b). The likelihood and probability estimates of breeding carriers were discussed earlier in this chapter (and also in Ubbink *et al.*, 1998b). The primary difference in breeding suspected carriers or affecteds, as compared to those of known genotypes, is that the breeder must rely more heavily upon the estimated likelihood of producing an affected animal. The issues become more complex when polygenic traits are considered. When making breeding decisions to minimize the incidence of polygenic conditions, the breeder must consider the 'breadth of the pedigree' and carefully evaluate the phenotypes of not just the sire and dam of the potential mate, but of all the relatives of the breeding pair (Padgett, 1998).

Summary

Implementing selective breeding practices based either on the genetic marker/mutation tests available or upon breeding values that represent the likelihood that a particular sire or dam will pass on a particular trait (Hall and Wallace, 1996), will result in the improvement of dogs. Until such time as molecular diagnostic tools become available to aid in selection of broodstock, heritability estimates for each trait to rank prospective parents can be used, provided the breed clubs have collected the appropriate data. The role of genetic counselling remains in interpreting molecular test results or statistical rankings, judiciously applying the results to mate selection, and weighing the overall attributes of a given mate against the faults that may be produced if that animal is bred. Analysis of pedigrees to determine the heritability of particular traits and the development of genetic tests will greatly influence the manner in which dogs are bred in the future, hopefully to produce healthier and more sound animals.

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Genetics of Quantitative Traits and Improvement of Dog Breeds

17

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Introduction

'Like begets like', one of the principal axioms of the acknowledged pioneer of animal breeding, Robert Bakewell (1725–1795) of Dishley Manor (Pawson, 1957), is the bedrock of quantitative genetics. Combined with Bakewell's second axiom, 'breed the best to the best', we see that the integration of quantitative genetics and animal improvement is guided by two very simple principles, whether we discuss livestock or the domestic dog. Breed improvement, or the process of genetic change, is a function of genetic variation, intensity of selection and the accuracy of identifying superior genotypes (Van Vleck, 1993). Of the three, animal breeders concentrate on the accuracy of identifying superior genotypes. The present chapter will examine the tools available to dog breeders that can enhance the accuracy of selection decisions. The number of papers that have examined quantitative genetics in the dog has been increasing over the past 20 years. However, the great majority of these investigations have done little more than estimate the heritability of a few traits in a handful of breeds. Understanding the quantitative traits of the dog is more

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than estimating a few parameters (Kempthorne, 1997; Lynch and Walsh, 1998). Much more remains to be accomplished. In particular, we must examine how the explosion of knowledge in the canine genome can be used to breed better, healthier dogs.

Traits of Interest

Unlike breeders of livestock, dogs do not have production traits; objectively measured quantitative characters which breeders hope to increase through selection (Patterson, 2000). Nevertheless, there are traits which dog breeders hope to modify. The traits that dominate our interest can be broadly described under the headings of reproduction, conformation, temperament and disease. These characters are typically polygenic, where the variation we observe is a function of environmental contributions as well as the action of many individual loci distributed throughout the dog genome.

The statistic most commonly used to quantify genetic variation is heritability, the ratio of genetic variation to phenotypic variation (Falconer and Mackay, 1996). Most of the scientific literature in dog quantitative genetics is the publication of heritability estimates (see Willis, 1989, 1992). Yet, the job of animal improvement does not stop with the publication of this statistic, because heritability is a means to an end and not an end in itself. Heritability is used to predict the response to selection and as an adjunct to the prediction of genetic merit (Kennedy and Sorensen, 1988; Kempthorne, 1997). Moreover, the heritability of a character in one breed is no indication of the heritability of that character in a second or third breed (Bulmer, 1985). This caution aside, Willis (1992) presented a useful generalization of heritability values for a variety of traits in a cross-section of breeds.

Unfortunately, no large data sets of reproductive data in dogs have been presented that can lead to useful estimates of the heritability of reproductive traits. Willis (1989, 1992), drawing upon allied research in livestock, prudently supposes that the heritability of fertility, litter size, semen quality and early postnatal survival is low, with values that are likely to range between 0.10 and 0.20. The implication of these low values is that selection to improve reproductive performance is likely to be slow. Other than selection against reproductive disease states such as inherited cryptorchidism (Brandsch, 1964; Sittmann, 1976), or a variety of other inherited reproductive disorders (see Meyers-Wallen, 1993), breeders are more likely to concentrate their selection decisions on other inherited characters.

Although adequate data exists only sparingly, the heritability of body conformation traits suggests a moderate to high heritability, with values ranging between 0.35 and 0.65 across breeds. Table 17.1 presents the heritabilities, genetic and phenotypic correlations among hip, elbow, hock and shoulder measurements in a study of 3500 Labrador Retrievers collected by the Institute for Genetic Disease Control (unpublished data). Such results demonstrate that, not only are structural traits heritable, but that they are typically positively

Table 17.1. Estimates of heritability (diagonal), phenotypic (above diagonal) and genetic (below diagonal) correlations for joint measurements in Labrador Retrievers.

Trait	Hips	Elbows	Hocks	Shoulders
Hips	0.65	0.22	0.06	0.07
Elbows	0.55	0.48	0.06	0.08
Hocks	0.21	0.00	0.18	0.10
Shoulders	0.41	0.43	0.12	0.15

genetically correlated to one another. Early studies in German Shepherd dogs from the Swedish Army suggest a heritability for 60-day weight of approximately 0.4 (Reuterwall and Ryman, 1973; Hedhammar *et al.*, 1979). Other stature traits in German Shepherd dogs suggest that measures of body size are moderate to high in heritability, with values of 0.81 for chest width or 0.54 for height at the withers (Verryn and Geerthsen, 1987). The interpretation of these high values is that a large percentage of the observed variation in body conformation is genetic variation, suggesting that selection could change body size in only a few generations of directional selection. However, dog breeders are interested in stabilizing selection (see Hartl, 1988) when thinking of conformation, hoping to eliminate dogs at the extremes of size or structure (Willis, 1989; Craige, 1997).

Behaviour traits, among the most important characters of interest to dog breeders, are also the most difficult to evaluate genetically (Willis, 1989). Clearly, behaviour patterns are inherited, most visibly illustrated by the range of breed differences in temperament, nervousness and any of a variety of behavioural characteristics (see, for example, Scott and Fuller, 1965; Burns and Fraser, 1966; Hart and Hart, 1985; Willis, 1989). But behaviour is also highly dependent upon acquired and environmental influences, so that the interaction of heredity and the environment can never be far from one's mind. Add to this the subjective nature in evaluating the phenotype of behaviour traits, and one can readily see the dilemma for the quantitative geneticist who hopes to separate nature from nurture.

Depending upon the character to be studied, and the breed to be investigated, the heritability (h) of behavioural traits can vary from low to high, from 0.10 to 0.60. Probably the most reliable genetic work on behaviour has been done through breeding organizations with an emphasis on service performance. At the Royal Guide Dogs for the Blind Association of Australia, Goddard and Beilharz (1982, 1983) were able to estimate the heritability of a variety of behavioural traits in Labrador Retrievers: nervousness ($h = 0.58$), suspicion ($h = 0.10$), nose distraction ($h = 0.0$) and successful graduation from the training programme ($h = 0.44$). Working with German Shepherd dogs bred by the Division of Bio-Sensor Research of the US Army, Mackenzie *et al.* (1985) estimated the heritability of temperament (as measured by the dog's ability to chase and attack a decoy) as 0.51. In more recent work at the Swedish Dog Training Centre, Wilsson and Sundgren (1997) found a range of heritability

estimates in German Shepherd dogs and Labrador Retrievers from a low of 0.05 for prey drive in Retrievers to a high of 0.37 for affability (i.e. friendliness) in German Shepherds.

The reason for citing these few reports is not to present a comprehensive review of the genetics of behaviour (that can be found in Chapter 13). The intent of this brief outline is to demonstrate that behaviour can be modified by selection. Yet, as the titles given for these behavioural traits suggest, we also see the difficulty in drawing universal conclusions about the inheritance of behaviour traits. For example, how does one quantify 'affability' (a trait evaluated by Wilsson and Sundgren, 1997) in a repeatable manner? While most of us can distinguish a friendly dog from an unfriendly one, how can this be quantified reliably and consistently. This remains one of the important challenges for dog breeders in the coming years, the effort to better quantify temperament traits for use in selection programmes.

Finally, breed organizations hope to reduce the incidence of disease through breeding. The American Kennel Club has established the Canine Health Foundation (AKC-CHF) with this purpose in mind, surveying breed clubs on the diseases they hope to eliminate through selection and breeding (Wilkie, 1999). At the top of the list are the diseases of hip dysplasia and epilepsy (Wilkie, 1999). The heritability of hip dysplasia has been variously estimated in the range of 0.26 (Mackenzie *et al.*, 1985) to 0.35 (Leighton, 1997) in German Shepherd dogs. Although investigators now universally agree that hip dysplasia is a polygenic trait (Willis, 1989; Todhunter *et al.*, 1999), the environmental effect of food consumption also plays a role in the prevalence of this disease (Kealy *et al.*, 1992, 1997). As for epilepsy, Famula *et al.* (1997) recently estimated the heritability of this disease in Belgian Tervuren at 0.71. Further investigation suggests that a single locus with a large effect on epilepsy is segregating in this population at a frequency of 0.14 (Famula and Oberbauer, 2000a). Kathmann *et al.* (1999) conclude that epilepsy in the Bernese Mountain Dog is polygenic, yet provide no estimate of genetic variation.

Of course there are a great many other health disorders of dogs that have a polygenic component, ranging from inherited cardiac arrhythmias (Moise, 1999), the vertebral disease spondylosis deformans in boxers (with a heritability of 0.4; Langeland and Lingaas, 1995), calcification of intervertebral discs in the Dachshund (with a heritability of 0.22; Stigen and Christensen, 1993), primary angle-closure glaucoma in Samoyeds (with a heritability of 0.58; Ekesten and Torrang, 1995) or histiocytosis in Bernese Mountain Dogs (with a heritability of 0.30; Padgett *et al.*, 1995). Chapter 9 presents a more complete discussion of the genetics of disease in dogs.

In the context of breed improvement, the emphasis on disease as a component of selection decisions is increasing. A recent examination of the effects of selection for improved hips in German Shepherd dogs of the Finnish Kennel Club (Leppanen *et al.*, 2000) showed no improvement over the years 1985 to 1997. This analysis stands in contrast to the reduction of hip dysplasia in German Shepherd dogs and Labrador Retrievers observed in a closed colony trained as guide dogs for the blind (Leighton, 1997). Table 17.2

Table 17.2. Percentage normal hearing, unilaterally deaf and bilaterally deaf Dalmatians by year of hearing test (brainstem auditory-evoked response) at the University of California, Veterinary Medicine Teaching Hospital, from 1984 to 1998

	Percentage		
Year of test	Normal hearing	Unilaterally deaf	Bilaterally deaf
1984	70	30	0
1985	64	27	9
1986	75	20	5
1987	74	17	9
1988	65	24	9
1989	67	25	8
1990	79	14	7
1991	76	15	9
1992	74	19	7
1993	71	18	11
1994	80	16	4
1995	86	10	4
1996	74	19	7
1997	64	27	9
1998	82	9	9

demonstrates a similar absence of observable genetic improvement in the reduction of deafness in Dalmatians from 1984 to 1998 (unpublished data). Apparently, although breeders may be interested in reducing the incidence of disease through breeding, they may lack the resolve to turn their interest into action, a point also suggested by Leppanen *et al.* (2000).

This summary of traits, though by no means complete, illustrates that breeders do not suffer from a lack of selection objectives. Whether behaviour, conformation or disease, breeders have sufficient raw material to manifest genetic change. The future will show whether breeders have the resolve to establish the genetic change that the parameters listed above suggest can materialize.

Genetic Improvement in Quantitative Traits

Interestingly, the use of statistical decision aids is a new and exciting topic in dog breeding (Canine Health Foundation), although the techniques and tools available to breeders are not new, having been used by livestock breeders for generations (Henderson, 1977, 1984, 1988; Searle *et al.*, 1992; Mrode, 1996). Yet, dogs are not livestock, and the problems and interests of dog breeders are quite different from those that interest agriculturally oriented animal breeders. In essence, dog breeders hope to create dogs of a uniform standard of performance across several traits (Frankling, 1974), avoiding the extremes of phenotype that are typically the goal of livestock breeders (Patterson, 2000).

As evidence of this growing interest to apply statistical tools to breed improvement, the American Kennel Club is developing a database of health information (Canine Health Foundation) and other private businesses (e.g. Institute for Genetic Disease Control) and breed clubs are considering the same. Assuming such data can and will be assembled, what use will such information provide for dog breeders? It is unlikely that individual breeders, those with only one or two dogs (the majority of the dog owning population), will be able to take advantage of these computerized databases and the associated statistical decision aids such data can provide (Leppanen *et al.*, 2000; Patterson, 2000). However, large breeding organizations such as Canine Companions for Independence (Santa Rosa, California) or Guiding Eyes for the Blind (Patterson, New York) can, and no doubt will benefit from the use of statistical tools. Armed with objective, quantifiable traits, pedigree information and statistical advice, these organizations will be able to select better animals efficiently and effectively (Famula and Oberbauer, 1998), in a manner that will mirror that of livestock geneticists (e.g. Ollivier, 1998).

Quantitative methods for animal improvement

Most dog breeding is based on individual selection, using observations from the animals in question and deciding which is best on a subjective scale (Hutt, 1979; Willis, 1989; Robinson, 1990; Patterson, 2000). Many breeders attempt to incorporate family information and knowledge of the performance of relatives as well, but do so on ‘intuition’ (Robinson, 1990; Craige, 1997). Ideally this incorporation is done through formal statistical procedures for weighting pedigree information (Van Vleck, 1993) or other mating strategies outlined in texts of animal breeding (e.g. Bourdon, 2000). However, much of dog breeding is done on subjective opinion and a sense of what constitutes an ideal dog (Craige, 1997). The weakness of such a strategy is the accuracy of identifying superior genotypes, one of the three components of genetic progress.

Of course, genetic progress is still possible without elaborate statistical techniques. Figure 17.1 presents a plot of average breeding values computed via the mixed model techniques discussed by Henderson (1984) for the joint measurements discussed in Table 17.1 for the population of Labrador Retrievers collected by the Institute for Genetic Disease (unpublished data). This process, plotting estimated breeding values against year of birth, is typically how livestock geneticists evaluate genetic trends, or the response to selection (e.g. Nizmani and Berger, 1996). Although the analysis of this data was done with mixed model methods, the selection decisions were not based on these calculations. Breeders could only make use of the phenotypes of individuals and their relatives with only ‘intuition’ of superiority as a guide. Nevertheless, breeders were able to show a dramatic change in hip and elbow measurements between 1991 and 1998, with virtually no change in hock and shoulder measurements. So change is possible, only likely to be slower than that possible with more advanced statistical technology.

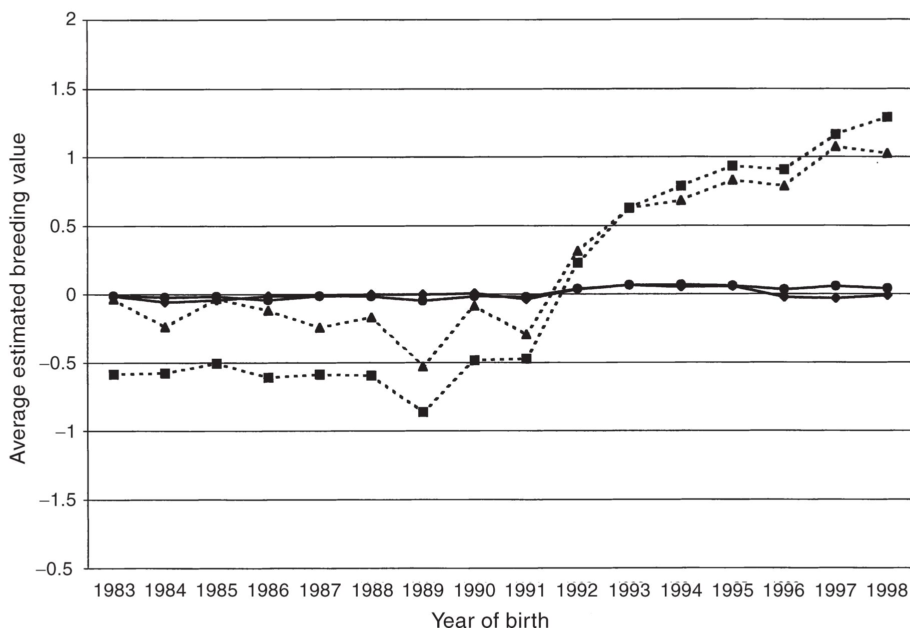

Fig. 17.1. Average estimated breeding values for joint measurements on hips (■), elbows (▲), hocks (♦) and shoulders (●) in Labrador Retrievers by year of birth.

As databases accumulate objective data, we should anticipate the application of mixed linear model techniques to dog improvement (suggested by Leppanen and Saloniemi, 1999; Leppanen *et al.*, 2000). The advantage of a mixed model approach is the broad applicability to a variety of genetic models (e.g. maternal effects, repeated measures) and the simultaneous ability to correct for non-genetic factors (Henderson, 1984). These techniques have been extended to evaluate and rank animals for traits that are categorical rather than continuous (Gianola and Foulley, 1983; Harville and Mee, 1984), a form of data quite typical for disease (e.g. deafness in Dalmatians by Famula *et al.*, 1996) or behavioural traits (e.g. hunting behaviours evaluated by Schmutz and Schmutz, 1998). Mixed model techniques are also useful in analysing several traits simultaneously (Henderson, 1984, 1988). Yet, as Patterson (2000) and Leppanen *et al.* (2000) suggest, with the exception of large, institutional breeders of service dogs, most breeders are unlikely to use statistical decision aids and mixed models. How then, can breeders improve the accuracy of selection decisions without turning to computers, databases and statistical algorithms?

Breed Improvement and Genomics

The past 10 years has brought an explosion of genetic information including genome maps (Mellersh *et al.*, 1997, 2000; Langston *et al.*, 1999), DNA markers (Rothuizen *et al.*, 1999; van de Sluis *et al.*, 1999) and a host of new acronyms in

molecular biology (Lewin, 1999). The future will see the integration of the pedigree based selection tools of mixed linear models with the expanding canine genetic map through marker-assisted selection (Hospital and Charcosset, 1997; Spelman and Bovenhuis, 1998). Many dog breeders can already benefit from the direct use of DNA information (e.g. the von Willebrand's disease mutation, Venta *et al.* (2000) see Chapter 16) for simply inherited Mendelian traits. The integration of map information into selection decisions for quantitative traits is more problematic.

Currently there are no concrete examples of the identification of quantitative trait loci (QTL) and the application of this information to dog breeding and improvement. Before this process can begin, researchers have several obstacles to overcome. The first step is to establish that the trait of interest (often a polygenic disease trait) is inherited, along with an evaluation of the impact the environment may have on expression of the trait. With inheritance firmly established, investigators must next evaluate the role that single genes play in the expression of the trait. Armed with this basic information, investigators can take the final step to identify the specific genes that govern expression of the quantitative trait. Though no example illustrating all three phases of such work can be given for dogs at present, our own work in reducing the prevalence of epilepsy in the Belgian Tervuren can serve as a model for the direction of quantitative genetics research in dogs.

Establishment of inheritance

As discussed earlier, estimation of heritability is not an end in itself (Lynch and Walsh, 1998). Knowledge of genetic variation is required to evaluate the potential success of breeding programmes (de Andrade *et al.*, 1999), as well as to serve as an adjunct to the prediction of breeding values (Kennedy and Sorensen, 1988). Not to be ignored, the process of estimating heritability (Searle *et al.*, 1992) also compels investigators to examine the environmental influences on quantitative traits. An example of where investigators are still sorting out fixed non-genetic contributions to genetic disease is in the influence of gender on hearing loss in Dalmatians (Wood and Lakhani, 1997, 1998).

Regardless of the problem, the methodology for estimating heritability and genetic correlations is well understood and software is widely available (Van Vleck, 1993; Lynch and Walsh, 1998). The advantage of mixed model methods is their ability to incorporate all known relationships among animals, to accommodate inbreeding and fixed non-genetic effects, to evaluate potential environmental covariances and to do all this in multiple trait settings (Kennedy and Sorensen, 1988; Kennedy *et al.*, 1992). In addition, these strategies of parameter estimation can be applied to discrete phenotypes and threshold models (Famula *et al.*, 1996, 1997).

Complex segregation analysis and major genes

Finding those loci (i.e. QTL) that are known to contribute to quantitative traits of interest could lead to efficient, workable selection aids (Xie and Xu, 1998). Genes large enough to detect, and thus large enough to facilitate significant genetic change when the subject of a selection decision, are referred to as major genes (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Though no formal definition is necessary, a major gene is usually defined as a locus where the difference between homozygotes exceeds one phenotypic standard deviation (Morton and MacLean, 1974). Methods to uncover major genes extend from the detection of a departure from a normal distribution of phenotypes (Hammond and James, 1970), to tests based on family variances (Fain, 1978), pedigree analysis (Karlin *et al.*, 1979; Famula, 1986) and mixture models (Elston, 1984; Hoeschele, 1988). However, such methods have not been used on data from dogs.

Complex segregation analysis (CSA), developed for work in human genetics (Elston and Stewart, 1971; Morton and MacLean, 1974), is the ideal method for the detection of major genes in dogs, although also the most computationally difficult. The intent of this statistical tool is to search for the hidden patterns of Mendelian inheritance in a polygenic trait. For complex quantitative traits (e.g. behaviour) or polygenic disease traits (e.g. hip dysplasia, epilepsy), we can not directly observe the Mendelian segregation of individual loci. Complex segregation analysis is the integration of Mendelian transmission genetics, allele frequency and penetrance with the patterns of covariance among relatives expected in polygenic models of inheritance (Bonney, 1986; Bonney *et al.*, 1989).

A detailed discussion of CSA can be found in a variety of sources (e.g. Lynch and Walsh, 1998; Sham, 1998). Jarvik (1998) has recently reviewed the use and limitations of complex segregation analysis, asserting that prudence dictates the use of CSA prior to a molecular search for QTL. Although CSA has seen limited use thus far in dog genetics, Famula *et al.* (2000a) and Famula *et al.* (2000b) are two recent applications of this technique to major gene detection in dogs. The purpose in both investigations has been to provide sufficient statistical evidence for QTL prior to proceeding with more expensive and time-consuming linkage studies.

Elston *et al.* (1975) outline criteria that must be satisfied before acceptance of the major gene model. The principal concern in the interpretation of the results of CSA is the risk of false positives (Demenais and Bonney, 1989; Jarvik, 1998). Skewness or kurtosis in the distribution of phenotypes can be misinterpreted as evidence of a major locus (MacLean *et al.*, 1975), as can other departures from normality (Go *et al.*, 1978; Eaves, 1984; Morton, 1984; Tiret *et al.*, 1993). Population sampling is also an issue in CSA. Many studies of disease are structured around the discovery of diseased animals, called probands. This ascertainment bias, the bias of how animals and their relatives

are selected for study, must be accommodated to ensure appropriate interpretation of CSA and avoid the risk of a false positive declaration of major gene inheritance (Elston and Sobel, 1979; Lalouel and Morton, 1981; Greenberg, 1986; Vieland and Hodge, 1995).

Although the statistical power of this method for dichotomous traits can be disappointing (Greenberg *et al.*, 1998), quantitative and polychotomous traits can benefit from dissection with these tools. Investigators are urged to apply these techniques to problems in dog genetics prior to the search for QTL via linkage analysis (Jarvik, 1998) through a variety of commercially available software packages (e.g. SAGE, 1997).

Complex linkage analysis

If we assume that dog breeders are unlikely to apply the statistical methods traditionally employed in other species (Leppanen *et al.*, 2000; Patterson, 2000), then the long-term hope for improvement will have to rely upon the identification of specific loci as an adjunct to making selection decisions. Assuming complex segregation analysis suggests a major gene is segregating, our attention then turns to locating this gene amongst the thousands present in the genome of the dog.

One strategy of locating QTL is based on the knowledge of candidate genes. Investigators have already identified important biochemical pathways and the genes that control their expression (e.g. Veldhoen *et al.*, 1999). It remains a relatively simple statistical process to correlate a dog's candidate genotype to a phenotype of interest. The statistics are simple (e.g. analysis of variance) and the results easy to interpret (Kennedy *et al.*, 1992). Several AKC-CHF supported investigators (Wilkie, 1999) are using this approach to finding QTL associated with epilepsy and canine cancer.

The limitation to this process is candidate genes. We can only investigate loci that have been previously identified, are polymorphic and which we suspect are involved in the expression of a phenotype. For many of the disease and behavioural traits that interest dog breeders we have, as yet, no knowledge of where to begin this search. In epilepsy, for example, there are hundreds of loci that are responsible for a properly functioning nervous system. Yet, beyond a handful of possible candidates borrowed from the human genetic map (Greenberg *et al.*, 2000), dozens more surely remain unknown.

A more powerful, though more complicated, strategy is to search for loci of which we are not already aware. In this approach we use anonymous sections of DNA, commonly small repeated sequences (e.g. microsatellites). These non-coding, highly polymorphic sections of each dog chromosome are easily identified and of known map location (Mellersh *et al.*, 2000). Although microsatellites do not translate into important physiological information, they

are physically located near potential QTL. To make use of this information, samples of tissue (e.g. blood, buccal swab) are taken from dogs within a breed, the DNA extracted and genotyped at each of potentially hundreds of marker locations across the genome. The hope is to link a particular marker to a trait of interest.

Strategies of linkage analysis for complex traits vary in their degree of statistical complexity. A recent review of the assumptions of several methods (Elston, 1998) outlines the applicability of these techniques to various forms of pedigree data (Lander and Schork, 1994; Lander and Kruglyak, 1995; Kruglyak *et al.*, 1996; Morton, 1998). As part of his review of methodology, Ott (1999) includes a discussion of the software available for analysing pedigree and phenotypic data. Chase *et al.* (1999) discuss methods traditionally used in plant genetics, where inbred lines form the basic unit of investigation, and their application to breeds of dog of small population size such as the Portuguese Water Dog. The rationale behind each of these strategies is that the variation observed between the phenotypes of siblings can be related to the probability of identity by descent for the QTL influencing the trait in question (Liu, 1998).

As with complex segregation analysis, these methods have not yet seen wide use in dogs. In our continuing investigation of epilepsy in the Belgian Tervuren, we have made use of sib pair analysis (SAGE, 1997) to identify several promising markers associated with a putative QTL for epilepsy (unpublished data). Having screened 88 tetra-, tri- and di-nucleotide microsatellite markers, we have found 11 markers with a significant association to seizures. Of these, two markers fall to the same chromosome assignment (unpublished data). However, debate continues on the appropriate criterion for the declaration of a ‘significant’ result (Lander and Kruglyak, 1995; Witte *et al.*, 1996; Morton, 1998). As one might expect, violations of model assumptions can decrease the power of linkage detection (Elston, 1998; Jarvik, 1998) and lead to erroneous conclusions. As the literature on QTL in dogs expands in the coming years, this concern may dissipate, but the first efforts in this area are likely to be fraught with as many errors as useful discoveries.

Summary

Naturally, this brief discussion only introduces the basic concepts for the future of quantitative genetics and breeding in dogs. The key to this discussion has been increasing the accuracy of making selection decisions. This search for increased accuracy begins with the collection of reliable phenotypic data and pedigrees. The future will increasingly make use of marker information and the identification of QTL influencing traits of importance. What remains is for dog breeders to maintain the resolve necessary to turn research results into wise breeding decisions.

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18 The Canine Model in Medical Genetics

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Introduction

Difficulties in the study of diseases in humans can be overcome by using animal models in which experimentation is possible that will give results relevant to human biology (Galibert *et al.*, 1998). The mouse is a popular experimental model for human genetic diseases because of the large amount of genetic information available and the ease of their maintenance. However, the dog has several advantages over the mouse as model of human disease and recent progress in molecular biology and in genomics in the dog makes it a choice model in medical genetics research. Some of these advantages are: (i) its size, which is similar to humans in some breeds; (ii) the dog is evolutionarily more closely related to human than is the mouse; and (iii) veterinary medicine in dog diseases has reached high standards, while comparatively little is known about treatment of diseases in rodents.

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In addition to the above, perhaps the main interest of the canine model for studying human genetics lies in human interference with canine reproduction, that has culminated in the current breeding structure of the dog populations. Although the exact date the first representatives of *Canis lupus* were domesticated is far from agreed (Vilà *et al.*, 1997), it was certainly before the Neolithic revolution that occurred some 10,000 years ago, as the dog already shared the life of our ancestors when hunting and gathering progressively gave way to agriculture and the domestication of farm animals (Clutton-Brock, 1995). Subsequently, breeding practices aimed at adapting the physical characteristics or the aptitudes of the dog were initiated as new needs arose. The long-term selection pressure imposed by the desires of the breeders or the purchasers resulted in the high number of extant breeds – a commonly accepted estimate is 350 – and their extreme diversity. More importantly, many breeds were initiated from only a few progenitors. Each breed is genetically isolated, and the widespread use of a few animals for mating within a breed followed by crossing to related animals has led to the accumulation of several genetic diseases in most breeds. So in parallel with the fantastic diversification shown by the various breeds, most present-day canine breeds have specific genetic defects – for example Dalmatians have deafness, Yorkshire Terriers have hydrocephalus and Collies have a series of ocular defects (see Chapter 5). Several different genetic defects can be associated with a single breed. Some genetic diseases with similar characteristics can be found in a number of different breeds. Many of these are likely to have arisen independently and may be due to different defects in the same gene or defects in different genes giving similar phenotypes.

The aim of this chapter is not to catalogue the many genetic diseases in dogs with human counterparts, which will increase in number as the genetic diseases affecting the two species are identified. Rather, it is to underline the interest of the dog as a model for human genetic diseases and to define the conditions in which the canine model can be exploited optimally. The importance of the dog model in human genetics can be illustrated at four different levels: (i) identification of disease genes, (ii) studies of conservation of synteny, (iii) analysis of protein function, and (iv) treatment of disease.

Identification of Disease Genes in Humans

Human genetic diseases can be a consequence of a range of different genetic causes from monogenic (single-gene) to complex diseases involving several genes and influenced by environmental factors. The interest in the dog model for human diseases can best be viewed by dividing the diseases into categories, as its application varies according to the type of genetic disease.

Categories of human genetic diseases

Single-gene diseases with complete or nearly complete penetrance
Monogenic diseases result from alterations in a single gene. For a few, the corresponding disease genes were identified long before the present tools of genomic analysis became available through information about the defective protein and its function or from some chromosomal rearrangement which indicated that the gene was in a specific region of the genome. During the last decade, new tools and strategies have been developed and used to isolate the genes involved in about 800 diseases and localize these genes in another 500.

Complex aspects of genetic disease determinism
The data accumulated during the last decade have revealed a greater complexity than was initially realized. In particular, there is often no simple correspondence between genotype and phenotype, as illustrated below.

Intralocus (or allelic) heterogeneity A set of diseases with a variety of symptoms can be ascribed to alterations in a single gene. Each different alteration may result in different symptoms. The site of the mutation can also play a part in disease severity and age at onset. For instance, over 400 different alterations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene have been reported that cause autosomal recessive disease [Online Mendelian Inheritance in Man; OMIM 219700]. The phenotypes of homozygotes range from cystic fibrosis, with death at an early age from respiratory distress, to azoospermia from congenital bilateral aplasia of vas deferens (CBAVD) [OMIM 277180]. Age of onset of symptoms may be affected by the mutation that is present but it may also depend upon other factors. Similarly, mutations in the *RET* gene [OMIM 164761] mapped to hum-10q11.2 are associated with the disorders multiple endocrine neoplasia [OMIM 171400, 162300), Hirschsprung disease (HSCR; aganglionic megacolon) [OMIM 142623] and medullary thyroid carcinoma (MTC) [OMIM 55240].

Interlocus (or genetic) heterogeneity The same symptoms can result from defects in different genes (non-allelic diseases); for example, osteogenesis imperfecta [OMIM 166200] can result from a defect in the collagen genes *COL1A1* on hum-17q21.31–q22 or *COL1A2* on 7q22.1. In addition to causing several clinical forms of osteogenesis imperfecta, other defects in these genes are responsible for other collagen diseases, e.g. Ehlers–Danlos syndrome, type VII [OMIM 130060]. Indeed, the family of *Collagen* genes includes over 20 genes on at least 12 different chromosomes. Available evidence suggests that defects in any of those will lead to genetic disease. Another example of genetic heterogeneity is the disease caused by defects in a receptor which is indistinguishable from disease caused by defects in the receptor's ligand. Defects in either will result in the same clinical manifestations, e.g. c-kit and its ligand which are referred to as the c-kit receptor/ligand axis (Abkowitz *et al.*, 1991).

Phenotypic heterogeneity A particular disease-causing allele may not always produce the same symptoms when present. The same mutation in a gene – for instance an abnormal allele segregating within the same family – can give different clinical manifestations. This can be due to modifying genes, to parental imprinting (the expression of the disease gene depends on its paternal versus maternal origin), or to environmental interactions. The phenotypic heterogeneity in phenylketonuria (PKU) has been reviewed at length by Scriver and Waters (1999).

Incomplete penetrance and polygenic or multifactorial diseases
Even in monogenic diseases, genotype and phenotype are not always in accordance (see Scriver and Waters, 1999, for a review devoted to phenylketonuria). The phenotype is conditional on other factors such as interaction with other genes (i.e. the genetic background) and the physical environment. Diseases of late onset, e.g. Wilson disease or polycystic kidney disease, show incomplete penetrance, with the penetrance level increasing as age increases. Some diseases may require exposure to a pathogen before symptoms develop, e.g. multiple sclerosis, type 1 diabetes (IDDM). In diabetes type 2 (NIDDM) [OMIM 125853], over ten predisposing loci have been reported, with some loci being more commonly associated with disease in a given human population, e.g. loci on 2q in Mexicans, or 12q in Finns. The contribution of each locus cannot easily be determined and it is not even possible to tell whether a given locus is dominant or recessive. In addition to diabetes, there are many common diseases that are multistage processes. These so-called polygenic, or multifactorial diseases – because several loci are thought to be involved, together with the environment (food, chemicals, pathogen exposure etc) – also include atherosclerosis, hypertension [OMIM 145500], some forms of mental illness, arthritis and cancer.

A major difficulty of the study of human genetic diseases is the respective role of each of the sometimes numerous loci involved and of the genetic background and of the environment. The latter may be so important that it can inhibit the expression of the disease phenotype (incomplete penetrance), or modulate or enhance the phenotype (phenotypic heterogeneity). Hypertension is an example of a disease phenotype that can result from monogenic disease or from polygenic disease, the latter being the more common form. Intralocus heterogeneity also exists at some loci that contribute to hypertension, e.g. several alleles of the *Angiotensinogen* gene.

The dog model for the various types of human genetic diseases

Monogenic disease with full penetrance
In the case of monogenic disease with full penetrance, the major interest of the dog model is in finding the genes responsible for rare or orphan diseases. The location of a disease gene through gene mapping requires large pedigrees. In human populations, such large pedigrees for rare diseases are not available in

countries where adequate medical follow-up is customary. Data from several small pedigrees can be combined for mapping but this solution is often not advisable because of the possibility of genetic heterogeneity.

An illustration of the role of the dog model in investigating a rare genetic disease is provided by narcolepsy (reviewed by Kadotani *et al.*, 1998). Narcolepsy affects 0.6% of the population. In humans, it is a sleep disorder characterized by excess daytime drowsiness and low alertness, disturbed nocturnal sleep, and pathological manifestations of rapid eye movement sleep, including sleep-onset rapid eye movement periods, cataplexy, sleep paralysis and hypnagogic hallucinations. Although genetic factors have been suspected, the paucity of multicase families tended to hinder further genetic studies in human. A canine condition with a similar phenotype was first observed in the dachshund (Knecht *et al.*, 1973) and subsequently in many other dog breeds. It was inherited as an autosomal recessive disorder with full penetrance (Foutz *et al.*, 1979; Baker *et al.*, 1982). Colonies of narcoleptic dogs have been established from affected litters of Doberman Pinschers and Labrador Retrievers (Baker and Dement, 1985). Lin *et al.* (1999) mapped the gene to canine chromosome 12, in a region bearing orthologous synteny with human chromosome 6p21, which greatly facilitated the development of a BAC contig across the region and the identification of the gene encoding the hypocretin type 2 receptor (HCRTR2) [OMIM 602393] as a plausible candidate. By genomic sequencing of the *Hcrtr2* gene of the narcoleptic Doberman, they found that in the Hcrtr2 transcripts from narcoleptic Doberman Pinschers, exon 4 was spliced out, while it was exon 6 in narcoleptic Labrador Retrievers. The disrupted gene creates a truncated transcript and a defective receptor.

These results culminated in the report of hypocretin deficiency in human narcoleptics: hypocretin was undetectable in seven out of nine people with narcolepsy, indicating abnormal hypocretin transmission (Nishino *et al.*, 2000). Narcolepsy is a good example of the impact that a dog model can have on human genetic studies.

The dog model in the study of incomplete penetrance or multifactorial diseases

The dog model can help simplify complex diseases such as cases of incomplete penetrance or multifactorial diseases. In animals the genetic background and environment can be manipulated to give some control over penetrance or the many factors involved in multifactorial diseases.

In medical genetics, it is sometimes difficult to distinguish between a recessive disease with complete penetrance, or a dominant disease with low penetrance. The causes of incomplete penetrance are difficult to determine and are usually not known. Many genes that modify the expression of other genes have been described in model organisms such as *Drosophila*, maize and yeast. In humans, the existence of genetic interactions leading to incomplete penetrance can only be suspected. However, dog could constitute an excellent model to study factors contributing to incomplete penetrance. The same defect experimentally introduced into several canine breeds may show different levels of penetrance. The level of penetrance can also be enhanced or reduced

by selection. Crosses between high and low penetrance lines (homozygous for the disease genotype) can then be used to map genetic factors modifying the expression of the disease. Such a drastic change in penetrance may be difficult to obtain, because it requires that the number of suppressors is low – ideally only one – and they are not linked to the mutated gene. Nevertheless, as the genetic background varies from breed to breed, the chances of obtaining such a combination are reasonably high provided the parents in the crosses are chosen with care, as will be specified below. More importantly, such experiments will have to make use of a monogenic disease described both in dog and human and having a reasonably low penetrance, to increase the chances of detecting penetrance value modifications. Severity of expression can result from the position of the gene alteration causing the disease, but also from interaction with other genes. Analysis of severity of expression of multifactorial diseases is amenable to quite similar approaches.

Optimal use of the dog model in medical genetic studies

There are limitations inherent in the use of human pedigrees to study disease. Pedigrees segregating for the disease are needed to localize a disease gene, both in cases of simple inheritance, e.g. a monogenic disease with full penetrance, and in more complex inheritance. In humans, the required pedigrees cannot be generated, they must be located among the existing population. This task of collecting pedigrees can be extremely difficult. A major drawback is that in developed countries families are small, while in developing countries pedigrees are often poorly informative owing to inadequate medical records. The collection of unrelated affected pedigrees introduces another possible problem which is heterogeneity of the disease. As discussed above, defects in several different genes can lead to the same symptoms. In practice, the best solution in humans is to resort to studies in isolated populations or ethnically homogeneous groups (e.g. Ashkenazim, Parsees, Icelanders). Due to founder effects, the disease in different families within a group is likely to result from the same defect that has been inherited from a common ancestor and is therefore identical at the molecular level.

Dog pedigrees as a substitute Dog breeds are resources of natural isolated populations with high levels of inbreeding. Within a breed a disease is likely to be homogeneous but similar diseases in different breeds could result from defects in different genes and are therefore a good source for identification of several genes involved in similar functions or pathways, the defects in which can lead to similar phenotypes. An example is the neuronal ceroid lipofuscinoses (NCL), referred to in humans as Batten's disease when it was thought to be a single disease. Defects in eight different genes have now been postulated to cause human NCL (Mole *et al.*, 1999). Four of the genes have been characterized. NCL occurs in English Setters and the disease gene has been located and is likely to be homologous to one of the uncloned human genes (Lingaas *et al.*, 1998).

NCL also occurs in Border Collies and mapping data suggests that the disease gene is unlikely to be the same as for the English Setters (F. Lingaas and A.N. Wilton, unpublished data). The identification of these genes could provide two missing pieces to the puzzle of Batten's disease in humans.

The advantages of dog pedigrees One advantage of dog pedigrees over human pedigrees is the large number of close relatives that are usually available. The use of inbreeding also tends to expose any recessive defects that are present. One problem lies in the identification of genetic diseases in animals. The reluctance of some breeders to readily disclose information, because of fear that it may tarnish the image of their kennel, results in many missed opportunities for finding more dog models of human diseases.

Another advantage of studying diseases in dogs is that the same gene may be altered in different breeds, which amounts to having a given gene in a different genetic background. This allows a study of the variation in symptoms that may arise due to genetic interactions. As mentioned above, it also provides a model system for investigating aspects of penetrance. This may entail breeding special stocks and making crosses between lines, which is only possible in animal models. The ability to manipulate matings in animal models has several advantages. It allows an increase in the number of informative individuals in a pedigree for mapping disease genes so that statistical significance can be reached and it allows outcrossing to increase the amount of heterozygosity at marker loci needed for gene mapping. Until recently, the usual approach was to use any pedigree that was available. However, because: (i) many purebred pedigrees have undergone complex crosses, resulting in intricate genealogies that tend to confuse the computer programs used for calculating linkages and (ii) owing to the high degree of inbreeding, the heterozygosity of the markers positioned on extant maps may have been lost; it is often advisable to construct a specific pedigree for the purpose with the markers heterozygous in the parents. The chances that the parents have different alleles at a majority of marker loci are of course highest if they come from unrelated breeding stocks. The parents could be chosen from two different breeds, but this raises possible difficulties of another nature, as such crosses will change the genetic background and this may affect the disease phenotype.

Jónasdóttir *et al.* (2000) illustrated the power of this strategy and of the dog model. They constructed a dedicated pedigree to study a naturally occurring hereditary renal cancer syndrome (cystadenocarcinoma and nodular dermatofibrosis, RCND) in dogs. In this paradigmatic study, the dog was deliberately selected as a model owing to the rarity of informative high-risk cancer families in humans. The pedigree was obtained by crossing an affected German Shepherd sire with six unaffected females (one German Shepherd and five English Setters). These crosses yielded 67 F_2 offspring from nine litters. Linkage mapping data eliminated several likely candidate genes to suggest that RCND is caused by a previously unidentified tumour suppressor located on CFA5.

Synteny Studies

Significant advances in comparative genomics of human and dog have recently been made. With a view to establishing a map of the canine genome, a dog/hamster radiation hybrid panel has been constructed (Vignaux *et al.*, 1999). This panel was employed to map polymorphic microsatellite markers to be used in linkage studies and, additionally, gene markers in order to define zones of conserved synteny in humans, mice and other species (Priat *et al.*, 1998). This map was merged with the genetic linkage map into an integrated map including over 600 markers (Mellersh *et al.*, 2000). Although marker density will have to be increased, this map can already be used to localize genes involved in genetic diseases (see Chapter 12) and has already identified a number of conserved syntenic (homosyntenic) groups. Reciprocal chromosome painting has revealed about 70 homosyntenic regions in human and dog (Breen *et al.*, 1999; Yang *et al.*, 1999; Chapter 11). This will greatly facilitate the identification of true homologues of human genes in the dog genome.

Most of the human genome sequence is already available. As sequence homology of genes between human and canine is generally high, this will undoubtedly promote the dog model by facilitating cross comparisons between genomes. For example, Wilson's disease [OMIM 277900] is a late-onset autosomal recessive disorder causing copper accumulation predominantly in the liver and the brain, which results in liver dysfunction and progressive dementia. The mutated locus (*WD*) has been assigned to hum-chr-13q14–q21, where it is closely linked to the *Esterase D* and *Retinoblastoma* genes. A similar condition in dog, canine copper toxicosis [Online Mendelian Inheritance in Animals; OMIA 001071] is designated as Wilson disease in dog. The designation copper toxicosis has been introduced as an across-species synonym. Ceruplasmin is deficient in both species, and it is considered a model of human Wilson disease. However, the *Copper Toxicosis* (*CT*) locus is not linked to either *Esterase D* or *Retinoblastoma* in the dog and the latter two are not even linked with one another (Yuzbasiyan-Gurkan *et al.*, 1993). Subsequently, the canine disease gene has been mapped to can-chr-10, in a region orthosyntenic with hum-chr-2p13–p16 and devoid of any positional candidate genes. This suggests that *CT* might not be the exact counterpart (orthologue) in dog of *WD* (van de Sluis *et al.*, 1999). Synteny studies are a prerequisite to any serious study of trans-species genetics: if a canine disease is considered as a potential model for a human disease, it needs to be confirmed that the genes are orthologous before it can be developed as a model for trials of therapeutics or other treatments.

Analysis of Protein Function

The physiology and pathophysiology and even the classification and symptomatology of canine diseases are better documented than in other species – including apes – since dogs as pets are commonly in frequent

contact with veterinarians. An example in point is the contribution that investigations of narcolepsy in dog have brought to the study of this condition in human. In their studies summarized above, Lin *et al.* (1999) speculated that transcript truncation resulting from the aberrant splicing in gene *Hcrtr2* impairs the proper membrane localization or transduction functions of the receptor. Physiological and pharmacological studies suggested a close similarity between canarc-1 phenotype in the Doberman and human narcolepsy (Nishino and Mignot, 1997). In humans, de Lecea *et al.* (1998) described a novel peptide system, hypocretins, so designated on the analogy of the gut hormone secretin and to recall their hypothalamic origin. At about the same time, Sakurai *et al.* (1998) identified peptides that bind to two related receptors. They named these peptide ligands orexins (appetizers) because they stimulate food intake. The peptides described by the de Lecea and the Sakurai groups are identical (Chemelli *et al.*, 1999; Lin *et al.*, 1999; Siegel, 1999, for a review). Chemelli *et al.* (1999) studied *Hcrt* knockout mice, whose symptoms differ from both those observed in narcoleptic humans and dogs, which have a defective Hcrt2 receptor. Finally, the finding that Hcrt2 is undetectable in human cases of narcolepsy is in favour of a similar pathophysiology in narcoleptic humans and dogs.

Thus, concomitant – rather than parallel – investigations in dogs and humans have led to the discovery of a novel peptide family that will serve as a clue, to say the least, in future studies of a human disease.

Treatment

Amelioration of the symptoms of genetic diseases requires restoring or enhancing the defective function or blocking the unwanted expression of a gene left out of control, which may be achieved by administering a drug. Restoration of function can be achieved by enzyme replacement from an exogenous source. It can also be achieved by gene or cellular therapy, which involves replacing the defective gene in a fraction of the somatic cells so that a sufficient amount of the gene product can perform the function of the defective product. In both cases, the dog makes a better model for trialling treatments than the mouse. In fact, the discrepancy in size between human and mouse creates large differences in responses to treatment.

In pharmacology, dosage determination is facilitated by a comparable weight range and reactivity/responsivity is easy to evaluate, follow up and compare in dogs. A particularly active area of research in medical treatment in the dog model is gene therapy. Leaving aside germ line gene therapy, which is for the moment – and with reason – banned from human applications, somatic gene therapy experiments have much to gain from the canine model. While cloned genes can be efficiently transferred into somatic cells and be expressed *in vitro*, adequate expression of the gene product of interest once the cells have been returned to the patient is very difficult to obtain, despite some successful endeavours. Many studies of the conditions that regulate expression

are needed, and preliminary studies in dog can provide the data. For example, Hcrt has been reported to interfere with appetite, and might also interact with the immune system or the control of anterior pituitary hormones, and can therefore disturb a large number of brain systems. The development of specific Hcrt agonists for Hcrt2, the receptor mutated in narcoleptic dogs, might reduce these problems (Siegel, 1999).

Dogs have been used as models for the treatment of both haemophilia A (factor VIII deficiency) and haemophilia B (factor IX deficiency). They were used to trial enzyme replacement therapies using plasma-derived enzymes and enzymes produced by recombinant DNA methods (Brinkhous *et al.*, 1996). They have also been used to trial gene therapy methods of treatment which has led to establishing clinical trials in humans (Connelly *et al.*, 1996; Snyder *et al.*, 1999). Factor IX gene replacement was also trialled simultaneously in mouse. The dose required in mice for long-term correction of the defect had to be greatly increased for the larger dog model. A dose that was one tenth per body weight that given to mice resulted in only 1% of normal factor IX levels in dog. This shows the necessity of a large animal model close to humans such as the dog for use in preclinical trials.

Other enzyme defects have been the target of therapy trials in dogs. Krabbe's disease (globoid cell leukodystrophy; GLD) [OMIM 245200] is a storage disease due to a deficiency in galactosylceramide β-galactosidase, an enzyme that breaks down sugar moieties. West Highland White Terriers have a naturally occurring form of Krabbe's disease (Victoria *et al.* 1996). Wenger *et al.* (1999) showed successful transduction of cultured skin fibroblasts from a West Highland White Terrier with GLD and normal canine bone marrow, using a retroviral vector containing the human GALC cDNA.

Despite concerns about the appropriateness of such experiments on ethical grounds that could restrict the use of this resource, clearly the dog is an appropriate model for drug, cellular and/or gene therapy, as illustrated by the examples shown in Table 18.1.

Conclusions

The title of this chapter is slightly misleading in that it conveys the impression that only human genetics can benefit from the dog model. In reality, the title could be reversed: dog genetics also has much to learn from human genetics. All in all, genetic studies in dogs and humans would be better viewed as assisting each other. This approach has been convincingly set out by Patterson *et al.* (1988), who pointed out the reciprocal benefits that can be derived from studies of congenital heart defects or inborn errors of metabolism in humans and in animals. Admittedly, the advances in the study of human genetic diseases in humans in the past 20 years have no parallel in the dog. For humans large numbers of disease genes have been located, cloned, sequenced, and for some the 3-D structure of the encoded protein has been determined in the normal and the diseased individual. Now knowledge

Table 18.1. Examples of genetic diseases in canine breeds in use as models for human diseases

Disease	Dog breed	Use	Reference
Haemophilia A	German Shepherd	Gene therapy	Connelly *et al*., 1996
Haemophilia B	Labrador Retriever	Transfusion therapy	Brooks *et al*., 1997
		Gene therapy	Monahan *et al*., 1998
Muscular dystrophy	Golden Retriever	Gene therapy	Howell *et al*., 1997, 1998; Howell, 1999
		Antisense therapy	
Mucopolysaccharidosis I α	Labrador Retriever	Bone marrow transplant	Gompf *et al*., 1990; Lutzko *et al*., 1999;
L-iduronidase deficiency		Enzyme replacement therapy	Kakkis *et al*., 1996
Mucopolysaccharidosis II Hunter syndrome		Potential	Wilkerson *et al*., 1998
Mucopolysaccharidosis VII Hunter syndrome		Disease identification	Ray *et al*., 1998
X-linked severe combined immune deficiency		Disease identification	Deschenes *et al*., 1994
Globoid cell leukodystrophy Krabbe disease		Potential	Wenger *et al*., 1999
Factor VII deficiency Sly syndrome	Beagle	Evaluate treatment	Ferguson *et al*., 1991
			Haskins *et al*., 1991
Fucosidosis	English Springer Spaniel	Potential	Occhiodoro and Anson, 1996
Muscle phosphofructokinase deficiency		Potential therapeutics	Smith *et al*., 1996
Erythrocyte pyruvate kinase deficency	West Highland White Terrier	Potential	Skelly *et al*., 1999
Sex reversal – Sry-negative	American Cocker Spaniel	Sex determination	Meyers-Wallen *et al*., 1999
Myotonia congenita, skeletal muscle voltage-dependent chloride channel defect	Miniature Schnauzer	Potential	Rhodes *et al*., 1999
Retinitis pigmentosa RP17	Several	Potential	Acland *et al*., 1998
X-linked retinitis pigmentosa RP3	Several	Potential	Zeiss *et al*., 2000
Leucocyte adhesion deficiency (integrin β2)	Irish Setter	Potential clinical treatments	Kijas *et al*., 1999
Narcolepsy	Doberman Pinscher Labrador Retriever	Potential	Faraco *et al*., 1999

may well start to flow in the dog–human direction. For this to happen, it is necessary: (i) to increase our knowledge of the canine genome; (ii) to construct pedigrees dedicated to a specific problems and (iii) to envisage the possibility of generating dog models for human disease either by screening dog populations for rare variants in heterozygous form that can be bred into affected colonies or through the development of knockout technology in puppies if the exact canine counterpart of a given genetic disease cannot be found. The sequencing of the dog genome will make the comparisons between human disease genes and dog disease genes a much simpler task. The rate of sequencing of genomes is now so fast that it should not be too many years before this is a reality. Once the genomes of economically important domesticated animals and plants have been sequenced, the medically important human disease model, man's best friend the dog, should be high on the agenda.

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19 Dog Genetic Data and Forensic Evidence

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Introduction

DNA analysis of human material was first used in a forensic investigation in 1986 and since then has been established as a powerful method for the identification of individuals. It is probably the most important single advancement in the field of forensic science in the last century. It gives a considerable increase of the discrimination power compared to the blood-grouping systems previously used, and the application of the PCR method means that very small amounts of sample, down to single-cell amounts, can be analysed. Forensic DNA analysis is now routinely used in most developed countries to analyse almost every type of biological material originating from humans.

Biological material from animals, as well as material from humans, is frequently found at crime scenes. The most likely type of biological material to be found, originating from a furred animal, is hairs. Shed human hairs are one of the most commonly secured biological evidence materials at crime scenes, and hairs from dogs and other animals are also frequently found. Indeed, a dog owner is more likely to leave hairs from his dog, directly from the dog or indirectly via clothes and equipment, than from himself at a scene of crime.

However, the potential of this source of evidence has so far not been fully exploited. Morphological methods can usually be used to distinguish between species but can never give a positive identification of an individual, and can only rarely be used to exclude an individual or a breed. The main reason for this is the large variation that exists not only within species and breeds but also between hairs from the same individual (Moore, 1988; Seta *et al.*, 1988). Therefore the application of DNA analysis to these samples represents a great improvement to the forensic investigations.

Normally, in forensic investigations of biological material from humans, microsatellite loci situated in the nuclear DNA are analysed. The PCR-based analysis of nuclear DNA has been used successfully not only for blood and semen samples but also for hairs that have been pulled from the skin and for samples containing epithelial cells, such as used stamps and cigarette butts (Hochmeister *et al.*, 1991; Allen *et al.*, 1994). There are a wealth of canine microsatellites reported which could be used in forensic analyses (e.g. Ostrander *et al.*, 1993; Mellersh *et al.*, 1997), and the use of microsatellite analysis of dog DNA has also been reported in one case (Muller *et al.*, 1999). However, some materials, for example shed hairs and hair shafts and very old and severely decomposed materials contain DNA that is so degraded that analysis of nuclear DNA mostly fails. For these materials, the remaining possibility is to analyse mitochondrial (mt) DNA. The fact that the most common forensic material originating from dogs is shed hairs implies that mtDNA analysis is the method of choice for forensic dog DNA analyses in most situations.

Analysis of Mitochondrial DNA

There are about 100 mitochondria per cell and 10 copies of mtDNA per mitochondrion, and therefore about 1000 copies of mtDNA per cell (Nass, 1969; Bogenhagen and Clayton, 1974). This implies that there is a much greater chance of finding an intact copy of mtDNA than a copy of nuclear DNA in a sample containing very small amounts of DNA or severely degraded DNA, and the chance of a useful result is therefore higher using mtDNA analysis. The mitochondrial DNA molecule is approximately 16,500 bp in dogs and other mammals (Wolstenholme, 1992; Kim *et al.*, 1998). Most of the molecule is coding for proteins or RNAs and in these parts the sequence variation between individuals is small. However, there is also a region of about 1200 bp that is non-coding but contains most of the sites that are involved in the regulation of replication and transcription. This region is called the control region (CR) and shows a large degree of sequence variation between individuals (Fig. 19.1). The CR is therefore the region most commonly used in forensic analyses.

Forensic study of the CR is performed by DNA sequence analysis, either directly by Sanger sequencing (Hopgood *et al.*, 1992; Sullivan *et al.*, 1992; Allen *et al.*, 1998) or indirectly by mini-sequencing (Tully *et al.*, 1996). In humans, there are two regions of approximately 400 bp showing especially

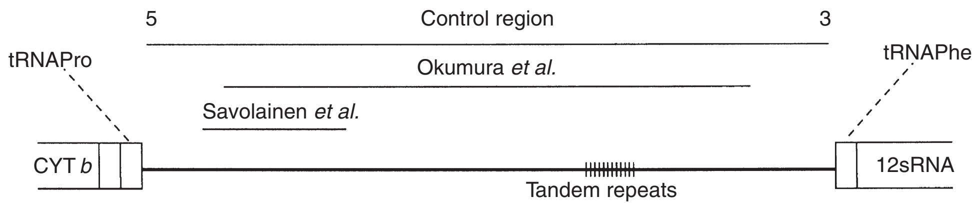

Fig. 19.1. Schematic diagram of the mitochondrial control region. The surrounding coding regions, the tandem repeat region and the segments analysed in Savolainen *et al*. (1997) and Okumura *et al*. (1996) are indicated.

high sequence divergence between individuals, the so-called hypervariable regions one and two (HVR1 and HVR2). In dogs only one part, corresponding to HVR1 in humans, shows this especially high sequence variation, while the part corresponding to HVR2 has a sequence variation comparable to that of the rest of the CR (Okumura *et al*., 1996). However, in 'HVR2' in dogs there is a highly variable array of tandem repeats. This region and its application to forensic analysis is discussed below under the heading New Technologies. The greatest disadvantage of analysing mtDNA compared with nuclear DNA is the limited discrimination power that can be obtained. Because of the relatively small size of the mtDNA genome and the maternal inheritance, many individuals have identical mtDNA sequences. This implies that if an mtDNA analysis is used in court for the inclusion of an individual, it can only be used as circumstantial evidence. However, in combination with other material it may form a very important piece of evidence.

DNA Analysis of Hairs

Hairs can be divided into two principal parts: the hair root, which is the site of biological synthesis and organization, and the shaft (permanent hair), which consists of dead, mostly fibre-shaped, keratinized cells (Chatt and Katz, 1988). Mammalian hair follicles go through three phases of a growth cycle: (i) the anagen phase which is the active growth phase for the hair, (ii) the catagen phase in which the viable part of the root, the bulb, goes through apoptosis, and finally (iii) the telogen phase in which the dead hair rests until it is shed, normally pushed out by a new hair growing from underneath (Chatt and Katz, 1988; Abell, 1994). It is the telogen hairs that are spontaneously shed and they are therefore the most commonly found hairs at crime scenes. The average human individual sheds 50–100 hairs during daily activity (Abell, 1994). The telogen hair consists entirely of dead cells, and the DNA molecules are therefore degraded to a great extent. The possibility of analysing nuclear DNA from human hairs in different developmental stages was studied by Linch *et al.* (1998). Hairs were gently removed from the skulls of human individuals and DNA was extracted and analysed. It was found that telogen hairs with follicular

tissue, and also anagen/catagen hairs with bulb only (no follicular tissue) were easily typed by nuclear DNA amplification. However, telogen hairs with no adhering follicular tissue did not give a nuclear DNA amplification product. In a study on shed monkey hairs (Vigilant, 1999), mtDNA could be amplified from 85% of the hairs, while a nuclear microsatellite could be amplified from 28% of the samples. Furthermore, in a forensic case study of the analysis of shed human hairs from caps worn during robberies, mtDNA could be amplified from 23 of 24 hairs, while a nuclear locus could be amplified from only two of the 24 hairs (Allen *et al.*, 1998). In conclusion, mtDNA analysis is, for the majority of shed hairs, the only possible method for genetic analysis.

Population Genetic Studies

In order to find out the forensic informativeness of a genetic region, a population study must be performed, in which the frequencies of the different sequence types and the total discrimination power is calculated. The domestic dog originates from the wolf (Wayne, 1993) and the earliest archaeological remains of domestic dog date from 10,000–15,000 years ago (Olsen, 1985). The rate of sequence divergence of CR in humans has been estimated to be about 10% per million years (Pesole *et al.*, 1992; Tamura and Nei, 1993). Assuming the same rate of sequence evolution for dog mtDNA (Pesole *et al.*, 1999), the sequence divergence rate would correspond to about one mutation every 12,000 years in the CR. Thus, only a few mutations would have occurred since the time of domestication and, in the present-day dog population, almost all sequence variation within this region would originate from variations within the wolf population. If the domestication were an isolated event the sequence variation of the domestic-dog CR would therefore be very limited. Fossil records suggest, however, a multiple domestication (Olsen, 1985), and a date for the time of domestication up to 100,000 years ago has been indicated by mtDNA studies (Vilà *et al.*, 1997). Some variation among dogs would therefore be anticipated. Furthermore, because of the different geographical origins of the breeds, some correlation between dog breeds and sequence variants would be anticipated. Another possible source of genetic variation in dogs is hybridization between domestic dogs and wolves. However, only sporadic examples of this are known, and interbreeding between sympatric wolf and feral-dog populations was not apparent in two Italian studies (Randi *et al.*, 1993).

Genetic variation

So far, two major studies of mtDNA sequence variation in dog populations have been performed, one Swedish (Savolainen *et al.*, 1997) and one Japanese (Okumura *et al.*, 1996). The two studies give a concordant picture of the mtDNA sequence variation in the domestic dog population. In the Swedish

study 102 dogs representing 52 different breeds were studied. The individuals were chosen so that several different breeds were represented, but more samples were from the most common breeds, in order to achieve an approximation of the frequencies of different sequence variants in the total population. A 257 bp segment containing the most variable part of the CR,'HVR1', was analysed (Fig. 19.1). Among the 102 dogs, 19 sequence variants were found (Table 19.1). The frequencies of the sequence variants varied between 20.6% and less than 1% (one individual per sequence variant) (Table 19.2). To obtain a measure of the discrimination power of the region, the random match probability, defined as the sum of the squares of the sequence-variant frequencies, was calculated. It was found to be 0.12, which implies that the probability of two randomly selected individuals in the population having identical sequence variants is 0.12 or, conversely, that on average 88 out of 100 randomly selected individuals can be excluded by this analysis. These figures can be compared to the results of a study of HVR1 of 100 British white Caucasian humans, which gave a random match probability of 0.03 (Piercy *et al.*, 1993).

In the Japanese study, 94 dogs were analysed, 73 of ancient Japanese breeds and 21 of non-Japanese breeds. Nine hundred and seventy bp were studied (Fig. 19.1). In the region overlapping with the Swedish study, 22 sequence types could be defined, showing a similar amount of genetic diversity as in the Swedish population. Most of the sequence types were identical to those found in the Swedish study. In the 650 bp segment on the 5′ side of the repetitive region, 35 sequence variants could be defined, and in the whole 970 bp fragment 38 sequence types were found. Similarly, preliminary results from an extended study of the Swedish population, analysing 609 bp of the 5′ side of the CR, show a decrease of the random match probability to about 0.06. This shows that by the analysis of the most variable part of the CR, which can be made from one PCR fragment, most of the genetic information in the CR is obtained, but that considerably more information is obtained if a larger part of the 5′-part of the CR is analysed. If shed hairs are analysed, this should be done from three separate amplifications covering about 200 bp each, because of the degraded state of the DNA in hair shafts (see Methodology section).

Breed-sequence correlations

In the Swedish study no general correlation between dog breed and sequence variant was found (Table 19.2). However, for some breeds, one sequence variant was more frequent. Among the various Retriever breeds, 9 out of 15 dogs (60%) were of the same variant (No. 4) and among the Labrador Retrievers, 5 out of 6 (83%) were of variant No. 4. In the total population there were 15% Retrievers while 43% of the individuals of variant No. 4 were Retrievers. Of the German Shepherd dogs, 4 out of 7 (57%) were of variant No. 5. Importantly, one breed-specific variant was found. Sequence variant 8 was only found in four dogs belonging to two closely related northern Scandinavian breeds, Jämthund and Norwegian Elkhound. In further analyses of 300 Swedish dogs,

Table 19.1. List of the 23 polymorphic nucleotide positions of the 19 sequence variants found among the 102 dogs and two wolves studied by Savolainen *et al*. (1997). The nucleotides are numbered according to the sequence of one individual used as a reference, and only differences from this sequence are shown for the various sequence variants. One additional nucleotide, found in sequence variant No. 10 but not in the reference sequence, is numbered with a decimal number. The corresponding deletion in the reference sequence is indicated by –. Sequence variant No. 13, marked *, contained an insertion of a 67 bp tandem repeat (nucleotides 101–167). A 24th polymorphic nucleotide position (No. 162), indicated by bracketed figures, was found in a wolf-sequence, variant W2

Variant	Polymorphic nucleotide positions																							
	5	22	30.1	53	78	96	103	123	155	(162)	165	181	182	190	195	197	202	204	206	209	213	220	222	223
1	G	T	–	T	C	C	C	A	A	T	C	T	T	T	T	G	C	G	T	A	A	T	G	A
2	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
3	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·
4	·	·	·	C	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·
5	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	T	·	·	·	·
6	A	·	·	C	·	T	·	·	·	·	T	·	C	·	·	A	T	·	·	G	G	·	A	·
7	A	·	·	C	T	T	·	·	·	·	·	C	·	·	·	A	·	·	·	G	·	C	·	·
8	A	·	·	C	·	·	·	·	·	·	·	·	·	·	C	A	T	A	C	G	G	·	·	·
9	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·
10	A	·	C	C	·	T	·	·	·	·	·	·	C	·	·	A	T	·	·	G	G	·	A	·
11	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·
12	·	·	·	C	·	·	·	·	G	·	·	·	·	·	·	A	·	·	·	T	·	·	·	·
13*	·	·	·	C	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·
14	·	·	·	C	·	·	·	G	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·
15	·	C	·	C	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	T	·	·	·	·
16	·	·	·	C	·	·	·	·	·	·		·	·	·	C	A	·	·	·	T	·	·	·	·
17	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	T	·	·	·	G
18	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·
19	A	·	·	C	T	T	T	·	·	·	·	C	·	·	·	A	·	·	·	G	·	C	·	·
W2	A	·	·	C	·	T	·	·	·	C	T	·	·	·	·	A	·	·	·	·	·	·	A	·
	5	22	30.1	53	78	96	103	123	155	(162)	165	181	182	190	195	197	202	204	206	209	213	220	222	223

Table 19.2. List of the 102 dogs examined by Savolainen *et al*. (1997) and their distribution among the sequence variants. The breeds, the number of individuals of each breed and the sequence-variant frequencies are shown. Two wolves are listed but not included in the frequency calculations

Variant	Breed	Number	Frequency (%)
	Border Collie	2	
	Irish Setter	2	
	Chow-Chow	1	
1	Collie	1	9.8
	Leonberger	1	
	Norwegian Buhund	1	
	Samoyed	1	
	Crossbreed	1	
	Chow-Chow	1	
2	Tibetan Terrier	1	2.9
	Chinese Crested	1	
	Rottweiler	2	
	English Setter	2	
	Norwegian Elkhound	2	
	Border Terrier	2	
	Papillon	1	
3	Whippet	1	15.7
	Springer Spaniel	1	
	Poodle	1	
	Icelandic Sheepdog	1	
	Fox Terrier	1	
	Japanese Spitz	1	
	Siberian Husky	1	
	Labrador Retriever	5	
	Golden Retriever	2	
	Flat-coated Retriever	2	
	Norfolk Terrier	2	
	Papillon	1	
	Newfoundland	1	
4	Alaskan Husky	1	20.6
	Giant Schnauzer	1	
	Boxer	1	
	Leonberger	1	
	Kuvasz	1	
	Schipperke	1	
	German Shepherd	1	
	Crossbreed	1	

Continued

Table 19.2. *Continued*

Variant	Breed	Number	Frequency (%)
	German Shepherd	4	
	English Setter	2	
	Dachshund, Wire-haired	2	
	Samoyed	1	
5	Border Collie	1	14.7
	Wachtelhund	1	
	Keeshond	1	
	Hamiltonstövare	1	
	Crossbreed	1	
	Old English Sheepdog	1	
	Afghan Hound	3	
	Golden Retriever	2	
	Groenendael	1	
6	Irish Water Spaniel	1	8.8
	Otter Hound	1	
	Tibetan Spaniel	1	
(W1)	Russian Wolf	(1)	
	Giant Schnauzer	2	
	Alaskan Husky	1	
7	German Shepherd	1	6.9
	Airedale Terrier	1	
	Jämthund	1	
	West Highland White Terrier	1	
	Jämthund	2	
8	Norwegian Elkhound	1	3.9
	Crossbreed (Elkhound grandmother)	1	
	Irish Setter	1	
9	St Bernhard	1	3.9
	Greyhound	1	
	Tibetan Terrier	1	
10	Flat-coated Retriever	1	2.0
	Dachshund Wire-haired	1	
11	Irish Wolfhound	2	2.9
	Pyrenean Mastiff	1	
12	Labrador Retriever	1	1.0
13	Chesapeake Bay Retriever	1	1.0
14	Fox Terrier, Wire Haired	1	1.0
15	Golden Retriever	1	1.0
16	Norwegian Lundehund	1	1.0

Table 19.2. *Continued*

Variant	Breed	Number	Frequency (%)
17	Cavalier King Charles Spaniel	1	1.0
18	Siberian Husky	1	1.0
19	German Shepherd	1	1.0
(W2)	Estonian Wolf	(1)	

sequence variant 8 was only found in these and some other North Scandinavian breeds, in 14 of 30 individuals belonging to this group of breeds. Furthermore, when a larger part of the CR is studied a clearer pattern is obtained, also concerning some other breeds. In the extended Swedish study, dogs of a few breeds could be distinguished. For example, a single mutation distinguishes half of the German Shepherds from all other breeds studied, and another mutation partitions one common mtDNA type into two types, one of which was only found in Afghan Hounds and Salukis. Obviously, by the analysis of a larger part of the CR, the breed of a dog can be predicted in many cases.

Methodology

The principal methodology for the analysis of DNA from dog hairs involves three steps. First the hair is enzymatically digested, mostly by the use of proteinase K, by one of several methods, depending on the quality of the hair (Higuchi *et al.*, 1988; Allen *et al.*, 1998; Savolainen and Lundeberg, 1999). Next, the genetic region to be analysed is amplified by PCR (Savolainen *et al.*, 1997; Savolainen and Lundeberg, 1999) and finally the DNA polymorphism is analysed by DNA sequencing, in the case of mtDNA analysis, or by fragment length analysis, in the case of microsatellite analysis. The amplification of long PCR products is usually not successful for shed hairs. This is in accordance with observations that mtDNA is fragmented to a small average size, around 200 bp, under degrading conditions (Pääbo, 1989). In amplifications of shed chimpanzee hairs, a 300 bp segment of mtDNA was successfully amplified from 80% of the samples, while a 500 bp segment could be amplified from only 15% of the samples (Vigilant, 1999). In a forensic case study of dog hairs (Savolainen and Lundeberg, 1999), DNA amplification was initially performed on a segment spanning 348 bp, but since some of the evidence materials failed to yield amplification products a 148 bp segment was tried out instead, which resulted in a higher success rate. Therefore, if a region much larger than 200 bp is studied, it should be amplified in several short fragments.

In forensic DNA analysis using the PCR method considerable precautions are necessary. The crucial advantage of the PCR method for the forensic scientist is that it allows the amplification of minute amounts of DNA. However, herein lies also a great risk of contamination. Even a few molecules of DNA coming from another source than the disputed material, that find their

way into the samples, will be amplified and if the number of contaminating molecules is larger than the number of molecules from the sample it will produce a false result. It is therefore important that precautions are taken to avoid contamination when forensic materials are handled. This is exemplified for the handling of ancient and archaeological materials in Handt *et al.* (1994) but the same rules are applicable in forensic analyses. A few obvious rules to follow are: (i) the place where DNA is extracted and the PCR amplifications are set up should be strictly physically isolated from the rest of the laboratory; (ii) dedicated clothing and instruments should be used and surfaces and equipment should be cleaned with 5% sodium hypochlorite; (iii) care and cleanliness should be observed at all times; (iv) multiple negative controls should be performed in parallel with each sample through all steps of the analysis. Three additional important rules for forensic analyses are to keep the evidence materials and reference materials strictly physically separated, to perform the DNA extractions and PCR amplification set ups of evidence materials and reference materials in separate parts of the laboratory, and to complete the analysis of the disputed material before starting the analysis of the reference material.

An important question that has been raised in the last few years is that of the incidence and amount of mtDNA heteroplasmy, that is, the presence of more than one type of mtDNA in an individual. Early studies showed a very low level of point mutation heteroplasmy in humans and mtDNA has therefore been regarded as homoplasmic in practice. However, a few more recent studies of human mtDNA have found heteroplasmy in some individuals (Howell *et al.*, 1996; Parsons *et al.*, 1997; Bendall *et al.*, 1997). Cases of triplasmy (the presence of three molecule types) have also been reported (Howell *et al.*, 1996). Furthermore, two studies of hair samples from heteroplasmic individuals have shown different amounts of mtDNA types when hairs, blood and buccal samples are compared (Bendall *et al.*, 1997; Wilson *et al.*, 1997). Blood samples and buccal swabs gave similar amounts of mtDNA types, but hairs showed varying amounts (between 38% and more than 99% in proportion) of two mtDNA types between hairs and compared to the other tissue types. The fact that the hairs show a much larger variation of heteroplasmy compared to the blood samples may be explained by the fact that each hair stems from a small number of stem cells in the hair follicle, while peripheral blood consists of lymphocytes produced from very large numbers of haematopoietic stem cells (Bendall *et al.*, 1997). Still, point mutation heteroplasmy is a rare phenomenon in humans (Huhne *et al.*, 1999). In our own work on dog hairs, we have performed more than 100 comparisons between mtDNA sequences from pairs of hairs, but only found one instance where two hairs have shown different majority nucleotides at a heteroplasmic position. Even though the finding of different levels of heteroplasmy is a rare phenomenon, it is of significance for the forensic applications of mtDNA analysis and must be considered in the determination of whether two samples originate from the same source. If there is a difference in one or two nucleotide positions between two samples, one should consider the possibility that they come from the same source but differ because of heteroplasmy.

However, two samples from a heteroplasmic individual showing different homoplasmic types would be very exceptional. In most cases at least one of the samples would show heteroplasmy. Nevertheless, until more is known about the incidence of heteroplasmy and the mechanisms behind the segregation of mtDNA molecules between cells, this is a matter for the forensic scientist to take into account.

Case Examples

The use of analysis of dog DNA in forensic cases has so far been described in three publications. In Savolainen and Lundeberg (1999) mtDNA analysis was used in six forensic cases. One 148 bp fragment was amplified in a semi-nested fashion and 79 bp were sequenced. In one case, a female was found murdered and dog hairs were found on the body. Shortly after, another female was found murdered and several dog hairs were recovered from objects connected with the crime. Because of the similarities between the two cases and because dog hairs with similar morphology were found in connection with the bodies it was hypothesized that the crimes had been performed by the same individual. The mtDNA analysis showed that the evidence material was of sequence variant 8 (Table 19.2) in both cases. As this is a relatively rare sequence variant, it is probable that the hairs came from the same dog. Reference hairs were collected from dogs associated with seven suspects, and the mtDNA from the reference materials was shown to be of different sequence variants compared with the evidence material. It could therefore be ruled out that the reference materials originated from the same source as the evidence materials. Importantly, the mtDNA variant found in the evidence materials was of variant 8, which has only been found among northern Scandinavian Spits dogs. It is therefore probable that the evidence materials originated from a dog belonging to this group of breeds.

In another case a female was reported missing and was believed to have been murdered. The female's body has not been found, but eight hairs believed to belong to the dog of the female's family were found in the car of a suspect. These hairs were collected as evidence material, while hairs plucked from the dog were used as reference material. The mtDNA of the reference material was of variant 5, while that of the hairs found as evidence was of variant 6 and variant 3. It was therefore concluded that the evidence material and the reference material originated from different dogs.

In a third case a medieval Bible was stolen from a public exhibition but later retrieved. Two hairs believed to derive from dog were found in the cover of the Bible. Reference material was obtained from dogs in two apartments where suspects had resided. mtDNA was isolated from one of the hairs found in the Bible and was shown to be of variant 3. The mtDNA from reference material 1 was also of sequence variant 3 while that from reference material 2 was of sequence variant 4. The analysis could therefore exclude the latter from the investigation, whereas it could not be excluded that reference material 1

could originate from the same dog as the evidence material. Sequence variant 3 was found in about 15% of the dogs analysed by Savolainen *et al.* (1997).

These case examples show that the analysis of 79 bp from one short amplification product may have a great value for the exclusion of suspects, and that the breed of a dog may sometimes be predicted, but that for the inclusion of suspects it mostly has a low value. However, by analysing the complete 5′-part of the CR, in three separate amplifications, the random match probability can be decreased from 0.14 to 0.06, increasing considerably both the chances for exclusions and the value of inclusions.

In two studies by Muller *et al.* (1999) and Schneider *et al.* (1999), dogs could be excluded from having caused traffic accidents by the analysis of hairs left on the vehicles and comparison with blood tests of the suspected animals. In Muller *et al.* (1999) microsatellite loci were successfully analysed from hairs collected from the tyre of the vehicle, which exemplifies that the analysis of nuclear DNA often works well for hairs removed by force.

New Technologies

In order to investigate the possibility of obtaining a higher discrimination power for the analysis of dog mtDNA, a highly variable tandem repeat array, situated in the control region of dog mtDNA (Fig. 19.1), was studied by Savolainen *et al.* (2000b). Tandem repeat arrays are found in the mtDNA of a large number of animals but not in humans. In dogs, the region is composed of an array of 10-bp repeats that are tandemly repeated, usually between 20 and 40 times. Among dogs there are two types of these 10-bp repeats, differing in one position. The different repeat types form a sequence and, therefore, there are two types of variation in this region, variation of length and variation of the sequence of repeat types. Because of the high mutation rate it also shows heteroplasmy, i.e. more than one type of DNA can be found in one individual (Savolainen *et al.*, 2000a). Therefore it is necessary to clone the PCR product to enable sequence analysis. Initially, it was shown that the heteroplasmic fragment length pattern could not be used for analysis from hairs, as different hairs from one and the same individual show different patterns. However, identical repeat sequences were found in the different hairs, and sequence analysis can therefore be used for identifications. In order to obtain an estimation of the genetic variation between individuals, several individuals having the same non-repetitive mtDNA sequence variant were studied: eight individuals having sequence variant 5 (found in 21% of the dogs in Savolainen *et al.* (1997)), five individuals having variant 6 (9%), and two individuals having variant 8 (4%). Seven of the eight dogs having sequence variant 5 were German Shepherds. When the DNA types were compared between individuals, no two identical array types were found. Thus, the tandem repeat region displays a large amount of variation between individuals. A very conservative estimate of the amount of genetic variation of the region, applied to the data base presented in Savolainen *et al.* (1997) indicates that at

least 48 different repeat sequence types should be found among the 102 dogs, giving a random match probability of 0.025 for this genetic marker. The fact that all seven German Shepherds of sequence variant 5 could be distinguished by the analysis and that the breeding of German Shepherds started from a small group of animals in 1899 indicate that dogs of different breeds having the same sequence variant can always be distinguished by sequence analysis of this region and that the random match probability is even lower than 0.025. In conclusion, this study shows that the analysis of repeat type sequence in dogs adds a substantial amount of discrimination power to forensic mtDNA analysis of domestic dogs. The drawbacks of this analysis method are that the PCR product must be cloned and that several clones, probably at least 20, must be sequenced from each sample in order to enable an evaluation of the genetic variation within the individual. Both these facts suggest that the analysis method is more laborious than normal DNA sequence analysis. However, due to its seemingly very high discrimination power, it could have a great value in investigations of serious crimes.

Conclusions

Dog hairs are a very common source of evidence often found at scenes of crime. The morphological analyses mostly used up to now are of limited value for the inclusion or exclusion of individuals, and analysis of DNA therefore implies a great improvement to forensic investigations. As most hairs found at a crime scene are spontaneously shed hairs, from which amplification of nuclear DNA generally fails, mtDNA analysis is the method of choice in most cases. The use of mtDNA analysis of dog hairs has so far been described in a limited number of forensic investigations. However, with the establishment of databases for the mtDNA sequence variation in dogs and the continuous reduction in time and cost for DNA analysis, the forensic DNA analysis of dog hairs will be routinely used in the near future.

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Standardized Genetic Nomenclature for the Dog

Locus

Locus name
Choice of name

- The name in English should be as brief as possible, but more than a single letter. The name may indicate, for example, a morphological character, a disease character or a biochemical property.
- As far as possible the locus name should reflect interspecies homology.
- All Greek symbols should be spelt out in roman letters and placed after the name, e.g. *Haemoglobin Beta.*

Printing the name

- The locus name should be in roman letters or a combination of roman letters and arabic numerals. The locus name should be printed in italics. The initial letter of the locus name should be a capital roman letter.
- Both nouns and adjectives in locus names should begin with capital roman letters.

Locus symbol
Choice of symbol

- For newly reported loci, unmapped DNA segments and proteins which have no known homologues or official names or symbols, special care should be exercised in selecting an appropriate symbol to avoid duplication and confusion with existing nomenclature. Every effort should be made to ensure that the symbols selected conform with those in current use for homologous loci.
- The locus symbol should consist of as few roman letters as possible, or a combination of roman letters and arabic numerals.
- The initial character should always be a capital roman letter which, if possible, should be the initial letter of the name of the locus.
- For loci other than those for coat colour and visible traits, upper case roman letters only, or upper case letters combined with Arabic numerals, should be used.
- If the locus name is of two or more words and the initial letters are used in the locus symbol, then these letters should be in capitals.
- All characters in a locus symbol should be written on the same line; no superscripts or subscripts should be used, nor should roman numerals or Greek letters.
- Where appropriate, the symbol should indicate the biochemical property or designate a particular nucleotide segment.
- The rules of mammalian interspecies homology already used in the choice of the name of the locus should be applied to the choice of the symbol.
- The designation of prefixes denoting mammalian species of origin, when being used to distinguish between the species homologues of a locus (e.g. *CFA* for the dog and *HSA* for humans), should follow the recommendations of the Human Genome Nomenclature Committee.

Printing the symbol
The locus symbol should be in italics; e.g. the symbol of the *Agouti* locus: *A*.

Allele

Allele name

Choice of name

- The name should be as brief as possible, but should convey the variation associated with the allele. If not given names, alleles should be given symbols as described in the 'Allele symbol' section below.
- If a newly described allele is similar to one which is already named, it should be named according to the breed, geographic location or population of origin. The names of new alleles at a recognized locus should conform to the nomenclature established for that locus.
- Should a new allele be identified later as being the same as an allele already named, the name invoking breed, geographic location or population of origin should be abandoned.

Printing the name

The allele name should be in italics. A lower-case initial letter of the allele name is preferred. This does not apply when a symbol is used instead of an allele name; for example, an allele at the *Haemoglobin Beta* locus: *B*.

Allele symbol

Choice of symbol

- The allele symbol should be as brief as possible, consisting of roman letters and/or arabic numerals.
- As far as possible, the allele symbol should be an abbreviation of the allele name, and should start with the same letter. In the loci detected by biochemical, serological or nucleotide methods, the allele name and symbol may be identical.
- Greek letters and roman numerals should not be used.
- The symbol + can be used alone for identification of the standard allele ('wild type') for alleles having visible effects. Neither + nor – symbols should be used in alleles detected by biochemical, serological or nucleotide methods. Null alleles should be designated by the number zero.
- The initial letter of the symbol of the top dominant allele should be a capital letter. When there are codominant alleles only, they should each have a capital initial letter. The initial letter of all other alleles should be lower-case.

Printing the symbol

- The allele symbol should always be written with the locus symbol. It may be written as a superscript following the locus symbol or it may be written following an asterisk on the same line as the locus symbol. The allele symbol should be printed immediately adjacent to the locus symbol, i.e. with no gaps.
- The allele symbol should be in italics.

Genotype terminology

- The genotype of an individual should be shown by printing the relevant locus and allele symbols for the two homologous chromosomes concerned, separated by a solidus.
- Unlinked loci should be separated by semicolons.
- Linked or syntenic loci should be separated by a space and listed in alphabetical order when gene order and/or phase are not known.
- For X-linked loci, the hemizygous case should be designated by /X following the locus and allele symbols.
- Y-linked loci should be designated by /Y following the locus and allele symbols.

Phenotype terminology

The phenotype symbol should be in the same characters as are the locus and allele symbols. The difference is that the characters should not be in italics, and should be written with a space between locus characters and allele characters instead of with an asterisk. Square brackets [ ] may also be used.

Recommendation

The guidelines for gene nomenclature for mapped loci and other genetic systems recommend that, if an unnamed, newly mapped locus is identified, contact be made with Dr Sue Povey, University College, London, Medical Research Council (MRC) Biochemical Genetics Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK. Phone: +44-207-380 7410; Fax: +44-207-387 3496; e-mail: mpovey@hgmp.mrc.ac.uk, before the locus is named to ensure that the symbol proposed for the dog has not already been adopted for the human.

Acknowledgement

The Editors are grateful to Mr C.H.S. Dolling, President of COGNOSAG, for allowing the use of his previous publications in this series of books in order to compile Standardized Genetic Nomenclature for the Dog.

List of Kennel Clubs of the World

Kennel clubs and organizations

Country	Name of club	Address	Contact numbers
Argentina	Federacion Cinologica Argentia	Moreno 1325, C1091 ABA, Buenos Aires, Argentina	Tel: +54-11-4383-0031/4381 0465/ 4381 3945/4381 3945/ 4384 7714/4384 7732 Fax: +54-11-4384 7785 Internet: http://www.fca.org.ar E-mail: fca@fca.org.ar
Australia	Australian National Kennel Council	PO Box 1005, St Mary's, NSW 2760, Australia	Tel: +61-2-9834 4040 Fax: +61-2-9834 6038 Internet: http://www.ankc.aust.com E-mail: dogsaust@ozemail.com.au
Austria	Osterreichischer Kynologenverband	Johann Teufelgasse 8, A–1230 Vienna, Austria	Tel: +43-1-888 70 92–888 70 93 Fax: +43-1-889 26 21 Internet: http://www.oekv.at
Bahrain	Kennel Club of Bahrain	PO Box 28555, Rufa State of Bahrain (Arabian Gulf)	
Barbados	Barbados Kennel Club	PO Box 344, Bridgetown, Barbados, West Indies	Tel: +1-246-417 0607 Fax: +1-246-425 4207 E-mail: klaurie@sunbeach.net
Belarus	Belorussian Cynological Union (BCU)	Revolutzionnaja Str., 13, PO Box 532, 220050 Minksk, Belarus	Tel/Fax: +375-17-289 30 92
Belgium	Union Royale Cynologique Saint-Hubert	Avenue A. Giraud 98, B–1030 Brussels, Belgium	Tel: +32-2-245 48 40 Fax: +32-2-245 87 90
Bermuda	The Bermuda Kennel Club	PO Box HM 1455, Hamilton, Bermuda HM FX	
Bolivia	Kennel Club Boliviano	Edif. Alborada, piso 13, Of 1301, C/Mercado esq. Loayza, Casilla 5978, La Paz, Bolivia	Tel/Fax: +591-2-339 177 Fax: +591-2-358 272
Brazil	Confederacao Brasileira de Conofilia CBKC (Brazil Kennel Club)	Rua Newton Prado, 74 Sao Cristovao, Rio de Janeiro, RJ Brazil-Cep 20930440	Tel: +55-21-580 0812 Fax: +55-21-580 8178 E-mail: cbkc@uninet.com.br
Bulgaria	Federation Cynologique Bulgare	Pres de l'Union des Casseurs et des Pecheurs de Bulgarie, 31–33 Boulevard Vitocha, BG–1000 SOFIA, Bulgaria	Tel: +359-2-87 68 07, 80 25 19 Fax: +359-2-80 36 33 88 33 83
Canada	The Canadian Kennel Club	Commerce Park, 89 Skyway Avenue, Suite 100, Etobicoke, Ontario, M9W 6RA Canada	Tel: +1-416-675–5511 Fax: +1-416-675–6506
Chile	Kennel Club de Chile	Casilla 269T, Santiago, Chile	Tel: +56-2-235 8589/235 3338 Fax: +56-2-235 8589

Country	Name of club	Address	Contact numbers
Colombia	Asociacion Club Canino Colombiano	Calle 121 A 52–23, Apartado Postal 102268, Bogota D.C., Bolivia	Tel: +57-1-22 64 707/22 66 437 Fax: +57-1-22 64 195 E-mail: canino@impsat.net.co
Costa Rica	Asociacion Canofila Costarricense	Apartado 593, 1002 Paseo de los Estudiantes, San Jose, Costa Rica	Tel: +506-253–0809 Fax: +506-253–0809 E-mail: canoficr@sol.racsa.co.cr
Croatia	Hrvatski Kinoloski Savez	Ilica 61, HR 10000 Zagreb, Croatia	Tel: +385-1-48 46 124 Fax: +385-1-48 46 124
Cuba	Federacion Cinologica de Cuba	Apartado 6135, La Habana 6, 12300 Havana, Cuba	Tel: +53-7-815 037/7819 815 Fax: +53-7-326 164 E-mail: rosefcc@mixmail.com Rosefcc@lettera.net
Cyprus	Cyprus Kennel Club	44 Vas. Voulgaroctonou Street, Flat 3–4, CY–Nicosia, Cyprus	Tel:+ 357-2-667 309 667 102 679 202 672 803 677 488 Fax: +357-2-669 407 E-mail: cy.kenne.club@cytanet.com.cy
Czech Republic	Ceskomoravská Kynologická Unie	U Pergamenky, CZ-170 00, Prague, A 73, Czech Republic	Tel: +420-2-66710829 Fax: +420-2-66712827 E-mail: cmku@cmku.cz Internet: http://www.cmku.cz/
Denmark	Dansk Kennelklub	Parkvej 1, DK–2680, Solrod Strand, Denmark	Tel: +45-56-1881 00 Fax: +45-56-1881 91
East Africa	East Africa Kennel Club	Jamhuri Park, PO Box 24792, Karen, Nairobi, Kenya	Tel: +254-2-566067 Fax: +254-2-566067
Ecuador	Asociacion Ecuatoriana de Registros Caninos	Apartado Postal n 17–08–8548, Calle Azuay n 269 y Avenida Republica, Quito, Ecuador	Tel: +593-2-247 170 Fax: +593-2-229 551
El Salvador	Asociacion Canofila Salvadorena	Prolongacion Juan Pablo II, n 18, Residencial Escalon, San Salvador, El Salvador	Telefax: +503-262 2560 Tel: +503-262 1820
Estonia	Eesti Kennellitt	Siili 2–100, EE0034–Tallinn, Estoria	Tel: +372-654 2601 Fax: +372-654 2448 Internet: http://www.online.ee/~ekl
Finland	Suomen Kennellitto – Finska Kennelklubben	Kamreerintie 8, FIN–02770, Espoo, Finland	Tel: +358-9-887300 Fax: +358-9-88730331 Internet: http://www.kennelliitto.fi
France	Societe Centrale Canine	155 avenue Jean Jaures, F93535, Paris-Aubervilliers, Cedex, France	Tel: +33-1-49 37 54 00 Fax: +33-1-49 37 01 20
Georgia	Federation Cynologique de Georgie	Rue Leselidze 22, 380005 Tbilisi, Georgia	Tel: +995-32-88 32 98 96 41 Fax: +995-32-88 32 98 60 42

Country	Name of club	Address	Contact numbers
Germany	Verband fur das Deutsche Hundewesen	Westfalendamm 174, D-44141, Dortmund, Germany	Tel: +49-231-56 50 00 Fax: +49-231-59 24 40 Internet: http://www.vdh.de E-mail: info@vdh.de
Gibraltar	Gibraltar Kennel Club	PO Box 493, 19/23 Naval Hospital Road, Gibraltar	Tel: +350-40826 Fax: +350-41791
Greece	Kennel Club of Greece	Koimisseos Theotokou & Eirinis, GR–14565 Agios Stefanos, Greece	Tel: +30-1-81 45 165 Fax: +30-1-81 45 167
Guatemala	Asociacion Guatemalteca de Criadores de Perros	16 calle–32, zona 10, Ciudad, Guatemala	Tel: +502-368 20 91 Fax: +502-333 59 82
Guernsey	Guernsey Dog Club	Cashinua, Portinfer Vale, Guernsey	Tel: +44-01481-55767
Holland	De Raad van Beheer op Kynologisch gebied in Nederland	Postbus 75901, 1070 AX, Amsterdam, The Netherlands	Tel: +31-20-6644 471 Fax: +31-20-6710 846
Honduras	Asociacion Canofila de Honduras	Prolongacion Juan Pablo II, no. 18, Residencial Escalon. San Salvador, Honduras	Telefax: +503-262 2560 Tel: +503-262 1820
Hong Kong	Hong Kong Kennel Club	3rd Floor, 28b Stanley Street. Hong Kong	Tel: +852-25 23 39 44 Fax: +852-2869 4464/2521 8747 E-mail: hkkcltd@netvigator.com
Hungary	Magyar Ebtenyesztok Orszagos Egysulete	Tetenyi ut 128/b-130, H–1116 Budapest, Hungary	Tel: +36-1-208 2304-3-1-0-6 Fax: +36-1-208 2307
Iceland	Hundareaktarfelag Islands	Icelandic Kennel Club, Sioumula 15, IS–108 Reykjavik, Iceland	Tel: +354-588 52 55 Fax: +354-588 52 69
India	Kennel Club of India	PO Box 481, 9 Balar Kalvi Nilayam Avenue, off Ritherdon Rd, Purasawalkam, Madras 600007, India	Tel: +91-44-53 222 82 Fax: +91-44-53 222 82 E-mail: kenclub@md3.vsnl.net.in
Indonesia	Perkumpulan Kynologi Indonesia (Perkin Pusat) The All Indonesia Kennel Club	Kpmpleks Roxy Mas Blok D III no. 28, 3rd Floor, J1 K.H. Hasyim Ashari, Jakarta 10150, Indonesia	Tel: +62-21–6306905 Fax: +62–21-6306904
Ireland	The Irish Kennel Club	Fottrell House, Harold's Cross Bridge, Dublin 6, Eire	Tel: +353-1-453 3300 Fax: +353-1-453 3237
Israel	Israel Kennel Club	PO Box 10555, Ramat Gan 52005, Israel	Tel: +972-3-672 71 74 Fax: +972-3-672 71 73
Italy	Ents Nazionale della Cinofilia Italiana	Viale Corsica 20. 1-20137 Milan, Italy	Tel: +39-2-700 20 31/700 20 334 Fax: +39-2-700 20 364
Jamaica	The Jamaica Kennel Club Ltd	73 Lady Musgrave Road, Kingston 10, Jamaica, West Indies	

Country	Name of club	Address	Contact numbers
Japan	Japan Kennel Club	1–5 Kanda, Suda-cho, Chiyoda-ku, Tokyo 101–8552, Japan	Tel: +81-3-32 511 651 Fax: +81-3-32 511 659 KDD no TOKINBTH J 29 400 Internet: http://www.jkc.or.jp.
Jersey	Jersey Dog Club	La Maison Du Verger, La Rue de Mount Pellier, Trinity, Jersey JE3 5JL	Tel: +44-1534-863492 Fax:+44-1534-863824
Kazakhstan	Union of Cynologists of Kazakhstan	Gagarin Avenue 73, Almaty 480 009, Kazakhstan	Tel/Fax: +7-3272-42 66 01
Latvia	Latvijas Kinologiska Federacija	Daugavgrivas 6, LV–1007 Riga, Latvia	Tel: +371-7 614819 Fax: +371-7 614819
Lithuania	Lietuvos Kinologu Dragija	Paupio G. 13, LT–2007 Vilnius, Lithuania	Tel: +370-2-62 18 52 Fax: +370-2-62 18 52
Luxembourg	Union Cynologique Saint Hubert	Du Grand Duche de Luxembourg, Boite Postale 69, L–4901 Bascharage, Luxembourgh	Tel: +352-50 28 66 Fax: +352-50 54 14
Macedonia	Kennel Association of the Republic of Macedonia	Kosta Novakovic 4–8, PO Box 728, 91000 Skopje, Macedonia	Tel: +389-91-165 333 Fax: +389-91-165 333
Malaysia	Malaysian Kennel Association	No. 8 Jalan Tun Mohd Faud Dua, Taman Tun Dr Ismail, 60000, Kuala Lumpur, Malaysia	Tel: +60-3-719 2027/717 4839 Fax: +60-3-718 2312
Malta	The Malta Kennel Club	54 Rue d'Argens, Msida MSD 05, Malta	Tel: +356-34 35 24
Mexico	Federacion Canofila Mexicana	Apartado Postal 22 535, C.P. 14001 Mexico D.F., Mexico	Tel: +52-5-573 81 82/573 81 46 Fax: 593-5-513 14 39
Moldova	Uniunea Chinologica Din Moldova	Armeana str. 30, ap. 103 B, Chisinau, Moldova	Tel: +373-2-270383 Fax: +373-2-271 897
Monaco	Societe Canine de Monaco	Palais de Congres, Avenue d'Ostende 12, MC–98000, Monte Carlo, Monaco	Tel: +377-935 05514 Fax: +377-933 05503
Morocco	Societe Centrale Canine Marocaine	Boite Postale 15941, Casablanca Principal, Morocco	
Nepal	Nepal Kennel Club	GPO Box 9875 E.P.C. 4079, Kathmandu, Nepal	
New Zealand	New Zealand Kennel Club	Prosser Street, Eldson, Private Bag 50903, Porirua, New Zealand	Tel: +64-4-237 4489 Fax: +64-4-237 0721 Internet: http://www.nzkc.org.nz E-mail: nzkc@nzkc.org.nz
Nicaragua	Asociacion Canina Nicaraguense	Hotel Intercontinental, 2 Cs al Sur y l c. Abajo, Managua, Nicaragua	Tel: +505-2-278 0633/278 3970 Fax: +505-2-222 3492/277 1004 E-mail: ancla@ibw.com.ni
Norway	Noesk Kennelklub	Postbox 163, Bryn, 0611 Oslo, Norway	Tel: +47-22 65 60 00 Fax: +47-22 72 04 74

Country	Name of club	Address	Contact numbers
Pakistan	The Kennel Club of Pakistan	4 Church Road, Old Anarkali, Lahore 54000, Pakistan	Tel: +92-42-427 323 883 Fax: +92-42-427 239 972 E-mail: crkmr@paknet4.ptc.pk
Philippines	Philippines Canine Club	PO Box 649, Greenhills 1502, Philippines	Fax: +63-2-721 7152
Poland	Zwiazek Kynologiczny w Polsce	Ul. Nowy-Swiat 35, PL–00 029 Varsovie, Poland	Tel: +48-22-8 26 05 74 Fax: +48-22-8 26 46 54
Portugal	Clube Portuges de Caniculturea	Rua Frei Carlos 7, P–1600–095 Lisbone, Portugal	Tel: +351-21 799 47 90 Fax: +351-21 799 47 99 E-mail: cpc@mail.telepac.pt Internet: http://www.cpc.pt
Romania	Asociata Chinologica din Romania	Str. Popa Tatu 61, Sector 1, RO-70771 Bucharest, Romania	Tel/Fax: +40-1-314 3763 Internet: http://www.netsoft.ro/mcane E-mail: achr@logicnet.ro
Russia	Russian Kynological Federation	BP 64, 125 015 Moscow, Russia	Tel/Fax: +7-095-285 81 24
Republic of San Marino	Kennel Club San Marino	Via F. Fiori 27, RSM–47895 Domagnano, Republic of San Marino	Tel/Fax: +378-901842 904465
Scotland	The Scottish Kennel Club	3 Brunswick Place, Edinburgh EH7 5HP, UK	Tel: +44-131-5572877 Fax: +44-131-5566784
Singapore	Singapore Kennel Club	Suite 12–02, 12th Floor, Bukit Timah Shopping Centre, 170 Upper Bukit Timah Road, Singapore 2158	Tel: +65-469 48 21 Fax: +65-469 91 18
Slovakia	Slovenska Kynologicka Jednota	Stefanikova 10, SK–811 05 Bratislava, Slovakia	Tel/Fax: +1421-7-5249 2298 E-mail: kynologia@skj.sk Internet: http://www.skj.sk
Slovenia	Kinoloska Zveza Slovenije	Ilirska 27, SLO–1000 Ljubljana, Slovenia	Tel: +386-61-32 0 9 49 Fax: +386-61-31 54 74 E-mail: kinoloska.zveza-slo@siol.net Web site: http://www.kinoloska-zveza.si
South Africa	Kennel Union of South Africa	6th Floor, Bree Castle, 68 Bree Street, Cape Town 8001, South Africa; PO Box 2659, Cape Town 8000, South Africa	Tel: +27-21-2390 27/8 Fax: +27-21-2358 76 E-mail: kusa@africa.com
South Korea	Korean Pet Animal Protection Association	275–5 Ulchi-ro 5-Ga, Dongshin Building no. 203, Jung-Su, Seoul, S. Korea	Tel: +82-2-2278 0661/2 Fax: +82-2-2277 4073
Spain	Real Sociedad Canina de Espana	Agasca, 16 Bajo derecha, E–28001 Madrid, Spain	Tel: +34-91-4264960 Fax: +34-91-4351113 or 4352895 E-mail: administracion@rsce.es Web: http://www.rsce.es
Sri Lanka	The Kennel Association of Sri Lanka	19 Race Course Avenue, Colombo 7, Sri Lanka	Tel: +94-95831

Country	Name of club	Address	Contact numbers
Sweden	Svenska Kennelklubben	Rinkebysvangen 70, S-163 85 Spanga, Sweden	Tel: +46-8-795 30 00 Fax: +46-8-795 30 40 Internet: http://www.skk.se
Switzerland	Schweizerische Kynologische Gesellschaft	Langgasstrasse 8, Postfach 8217, CH-3001 Bern, Switzerland	Tel: +41-31-301 5819 Fax:+ 41-31-306 62 60 Internet: http://www.hundeweg.org http://www.chienweb.org http://www.dogweb.org E-mail: skg-scs@bluewin.ch
Taiwan	Kennel Club of the Republic of China	148 Cheng Hsing Road, Kaohsiung, Taiwan, Republic of China	Tel: +886-7-3892957/3892964/ 3893006 Fax: +886-7-3893060
Thailand	Kennel Club of Thailand	9/338 SOI KM, 25 Phaholyothin Road, Sai May – Bangkok 10220, Thailand	Tel/Fax: +66-2-990 3618 990 3619
Trinidad & Tobago	The Trinidad and Tobago Kennel Club	PO Box 737, Port of Spain, Trinidad, West Indies	
Ukraine	Ukrainian Kennel Union	Rognedinskaya Str. 3, 252 004, Kiev, Ukraine, PO Box no. 8, Kiev 252023	Tel: +380-44-227 01 43 Tel/Fax: +380-44-225 31 24
United Kingdom	The Kennel Club	1–5 Clarges Street, Piccadilly, London W1J 8AB, UK	Tel. +44-870-6066750 Email: info@the-kennel-club.org.uk www.the-kennel-club.org.uk
United States of America	American Kennel Club	4th Floor, Madison Avenue, New York, NY 10016, USA	Tel: +1-212-698 8200 Fax: +1-212-696 8329
Uruguay	Kennel Club Uruguayo	Avenue Uruguay 864, 11100 Montevideo, Uruguay	Tel: +598-2-902 0484 Fax: +598-2-902 6278
Yugoslavia	Jugoslovensky Kinolosky Savez	Federation Cynologique Yugoslave, Alekse Nenadovica 19–23, YU–1100 Beograd, Yugoslavia	Tel/Fax: +381-11-437 652 E-mail: jks@yubc.net
Zambia	Kennel Association of Zambia	PO Box 21262/ 21064, Kitwe, Zambia	Tel: +260-2-212423/221070 Fax: +260-2-214242
Zimbabwe	Zimbabwe Kennel Club	PO Box BE61, Belvedere, Zimbabwe	Tel: +263-4727 854 Fax: +263-4757 854

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Federation Cynologique Internationale (F.C.I.)
13 Place Albert 1er
6530 Thuin
Belgium

World Kennel Club
PO Box 60771
Oklahoma City, OK-73146
+1-(405) 745 9520
http://www.worldkennelclub.com/

List of Relevant Databases

Dog breeds

The biggest dogs related search engine: http://www.dogomania.com/kennels.shtml
Dog breeds: http://www.petnet.com.au/dogs/dogbreedindex.html
Dog breeds: http://www.digitaldog.com/breeds.html
Dog breeds http://www.cofc.edu/~huntc/breedpage.html#Top
Dog Breeds http://www.dogbiz.com/dogbiz-main-menu.htm
http://www.doginfomat.com/breedsintro.htm#books
Dog breeds : http://www.akc.org/breeds/index.cfm
Dog breeds: http://www.ankc.aust.com/groups.htm
AcmePet http://acmepet.petsmart.com/canine/genetic/article/primer.html

Dog genomic and cDNA libraries:

http://www.atcg.com/index.htm.

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