Genetic evaluation of guide dogs in the UK

By

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ABSTRACT

Guide Dogs is the largest assistance dogs charity providing mobility support for people who are blind or partially sighted. Approximately 70% of dogs bred become working guide dogs, and this value is high compared with other guide dog schools. However, it is still desirable to increase further the proportion of dogs that are bred which successfully become guide dogs. This study aimed to complete a thorough genetic evaluation of Guide Dogs' programme and colony to investigate the potential for using quantitative genetic tools in service of this aim.

Firstly, reasons for withdrawal of dogs from Guide Dogs' programme between 1995 and 2012 were analysed, and a survey of selection aims was undertaken among the seven individuals involved with selecting breeding stock, to ascertain which health and behavioural traits were of most importance. Health and behavioural traits were approximately equally weighted in the survey, but behavioural reasons accounted for 68% of withdrawals of dogs from Guide Dogs' programme. A key finding of the survey was that selection aims were breed-specific.

Genetic evaluation of health and behavioural traits recorded by Guide Dogs was then undertaken. Historical health records were interrogated and cases of disease conditions were collated. Heritability analyses were conducted and genetic correlations between disease conditions were investigated. Atopic dermatitis, cranial cruciate ligament (CCL) disease, diabetes mellitus, distichiasis, elbow dysplasia, entropion, hip dysplasia, laryngeal paralysis, multifocal retinal dysplasia, panosteitis, patellar luxation and seizures were all found to be heritable in Labrador Retrievers. High, positive genetic correlations were found in this breed between hip and elbow dysplasia, hip and elbow dysplasia and panosteitis, and elbow dysplasia and seizures. Heritability estimates were reported for atopic dermatitis, congenital

ichthyosis, entropion, Horner's syndrome and panosteitis in Golden Retrievers. In German Shepherd Dogs atopic dermatitis, hip dysplasia, panosteitis and sebaceous cysts were found to be heritable. Most heritability estimates were small or moderate in magnitude. Selective breeding strategies that identify those animals with low genetic risk, such as the use of estimated breeding values (EBVs), could be used to reduce the incidence of these conditions.

Crossbreeding parameters were investigated in Labrador Retrievers, Golden Retrievers and crosses between the two breeds for atopic dermatitis, CCL disease, elbow dysplasia, entropion, hip dysplasia, Horner's syndrome, panosteitis and seizures. Heterosis appeared to slightly reduce the likelihood of developing elbow dysplasia, Horner's syndrome and seizures. Recombination loss appeared to slightly increase the likelihood of developing either hip or elbow dysplasia. Increasing Labrador fraction was associated with a greater probability of developing elbow dysplasia but a lower probability of developing Horner's syndrome. These results suggest that there are small benefits of heterosis for the first generation (F1) cross but that these may be lost when the F1 is crossed back to a purebred dog.

Current Guide Dogs practice was to "measure" behavioural traits using two different behavioural assessments. Results from these assessments were also subjected to genetic evaluation. Firstly genetic and environmental parameters relating to scores in the Canine Assessment Summary (CAS) were estimated in purebred Labrador Retrievers and Golden Retrievers and then in crossbreed models between the two breeds and their crosses. Although many of the models measured heritability estimates which were not detectably larger than zero, and the assessor effect was considerably larger than the heritability estimate in most cases, the heritability estimates suggest that there is substantial genetic variation in many of the traits being measured by CAS. Therefore EBVs for these traits could be used, with scores at the first CAS assessment in advanced training looking particularly suitable. Bivariate models in Labrador Retrievers identified three negative genetic correlations

between behavioural traits: between confidence and distraction, eagerness and interaction with people and calmness and eagerness. These could be problematic as selection for one of these traits could lead to a worsening in the other.

Most of the crossbreeding parameter estimates were not detectably larger than zero. However, seven CAS elements had small to moderate heterosis estimates, six of which were negative i.e. beneficial. Four CAS elements had small to moderate estimates of recombination loss, three of which were positive i.e. detrimental. This suggests that, as with the disease conditions, there may be benefits in behavioural terms in the F1 but these may be at least partially lost in the backcrosses. Repeatability models were also undertaken for two CAS elements, calmness and eagerness. For both traits estimates of permanent environmental effects were larger than heritability estimates suggesting that they are more important than genetic influences on these traits.

Finally heritability and crossbreeding parameters were estimated for scores in the Puppy Profiling Assessment (PPA). Nine of the 11 PPA components had heritability estimates which were detectably larger than zero, five of which were moderate in magnitude, indicating that performance in these tests had an inherited element. Crossbreeding parameter estimates for PPA components were mostly not detectably larger than zero however; the PPA dataset may have lacked the power to detect crossbreeding effects due to its relatively small size.

This study provides a platform for the implementation of quantitative genetic techniques to improve the accuracy of Guide Dogs' selection decisions. Many of the findings of the study will also be of interest to the wider dog breeding community.

PUBLICATIONS ARISING FROM THIS THESIS

Conference presentations

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DEDICATION

This thesis is dedicated to the memory of my big sister and my Mum:

Geraldine Ruth Evans

18.10.1974 – 14.02.1982

and

Glynis April Evans

19.04.1948 – 10.04.2014

I hope they would both have been proud of me.

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COMMON ABBREVIATIONS

AT	Advanced training
BBH	Brood bitch holder
BBS	Brood bitch supervisor
BLUP	Best linear unbiased prediction
BV	Breeding value
C-BARQ	Canine behavioural assessment and research questionnaire
CAS	Canine assessment summary
CAT	Character assessment tracker
CCL	Cranial cruciate ligament
CDRM	Chronic degenerative radiculomyelopathy
CRM	Customer relationship management
DCWA	Dog care and welfare advisor
DMA	Dog mentality assessment
DNA	Deoxyribose nucleic acid
EBV	Estimated breeding value
EPI	Exocrine pancreatic insufficiency
ET	Early training
FCI	Fédération Cynologique Internationale
GD	Guide Dogs
GDB	Guide Dogs for the Blind Inc., California
GDI	Guide Dogs Interactive
GDI-R	Guide Dogs Interactive Replacement
GDMI	Guide dog mobility instructor

GDO	Guide dog owner
GDT	Guide dog trainer
GEB	Guiding Eyes for the Blind, New York
GEBV	Genomic breeding value
GR	Golden Retriever
GRD	Geographic retinal dysplasia
GS	Genomic selection
GSD	German Shepherd Dog
GWAS	Genome-wide association study
IBD	Identical-by-descent
KC	The Kennel Club
LR	Labrador Retriever
LRT	Likelihood ratio test
MRD	Multifocal retinal dysplasia
mtDNA	Mitochondrial DNA
MTM	Mobility team manager
NBC	National breeding centre
OA	Osteoarthritis
OCD	Osteochondritis dissecans
PBS	Prospective breeding stock
PIM	Pre-intake meeting
PPA	Puppy profiling assessment
PTS	Puppy training supervisor
PW	Puppy walker or puppy walking
QTL	Quantitative trait loci
REML	Restricted maximum likelihood

s.e.	Standard error
SGDT	Senior guide dog trainer
SNP	Single nucleotide polymorphism
SQL	Structured query language
TSE	The Seeing Eye, Inc., New Jersey
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America

1. INTRODUCTION

Dogs and humans have a unique relationship, and the relationship between a guide dog and its visually-impaired owner is perhaps the ultimate example of this. Traditionally, Guide Dogs (GD) and other guide dog breeding organisations internationally have relied on phenotypic data when making selection decisions. For health-related traits, the advent of DNA tests for some single gene disorders has allowed the incorporation of genetic information into the decision-making process. However there are a number of heritable diseases affecting breeds used as guide dogs, including hip and elbow dysplasia and atopic dermatitis, which are not single gene disorders and whose inheritance pattern is complex. Many behavioural traits have also been shown to be heritable, but again their inheritance pattern is complex.

Current figures from GD estimate that the full lifetime cost of a guide dog from birth to retirement is £50,000 (Guide Dogs, 2014). The majority of costs which make up this sum are incurred during the breeding and training stages, and thus any dog which is withdrawn from the training programme at any stage represents a significant financial loss. More importantly a dog lost to the training programme cannot simply be replaced with another dog purchased from outside the organisation, rather a replacement dog will have to be bred, leaving the visually impaired person without the mobility support they need. GD has a very high success rate in terms of the proportion of dogs bred which become guide dogs when compared with the rates of other guide dog breeding organisations internationally, at approximately 70%. However, it is still highly desirable to increase further the proportion of dogs bred by Guide Dogs which become guide dogs. This thesis represents a thorough genetic evaluation of Guide Dogs programme and colony to investigate the potential for using quantitative genetic tools to aid the selection of breeding stock in the service of this aim.

Firstly, in Chapter 2, there is a review of relevant literature. Subsequently, in Chapter 3, a brief history of the use of guide dogs and their breeding is given, followed by a discussion of current practices at Guide Dogs. In Chapter 4 the efforts which were undertaken to prepare Guide Dogs pedigree file for use in subsequent analyses are described.

In Chapter 5, two pieces of work which were undertaken in order to ascertain which health and behavioural traits were of most importance to Guide Dogs are described. Then Guide Dogs' historical health records were used to estimate genetic and environmental parameters of disease conditions in the three purebred dog breeds used in the largest numbers (Chapter 6) and using crossbreed models (Chapter 7). Behavioural traits measured by Guide Dogs throughout the dogs' early life until qualification are subjected to genetic analyses in two purebred dog breeds (Chapter 8) and using crossbreed models (Chapter 9). Finally, in Chapter 10, crossbreed models are used to estimate genetic and environmental parameters of a standardized behavioural test applied to potential guide dog puppies at approximately six weeks of age.

Chapter 11 then brings together results and conclusions from the preceding chapters in a discussion of the findings of this project. Future work suggested by these findings is also discussed.

2. LITERATURE REVIEW

The following is a review of scientific literature around the subject of guide dog breeding. The history and process of guide dog breeding itself will be considered in Chapter 3. The literature review begins with an exploration of canine genetics and the emergence and diversity of modern dog breeds. Sections 2.4 and 2.5 examine the genetics of two groups of traits which are of fundamental importance in guide dog breeding: canine health and inherited diseases and canine behaviour. Finally quantitative genetic concepts and how they might be applied to guide dog breeding are explained.

2.1. Canine genetics and dog breed formation

Different "types" of dog have existed for thousands of years. By Roman times most of the main "types" were well-defined, with hunting dogs, guard dogs, sheep dogs and lap dogs being common (Clutton-Brock, 1995). Ancient humans selected on specific behavioural traits such as aggression and obedience to shape dogs into "types" which could undertake various tasks such as herding, guarding or simply companionship (Careau et al, 2010). Most, if not all, of the physical traits of the traits subjected to selection in early times had direct utility and functional benefits (McGreevy & Nicholas, 1999). Reproductive isolation between them, formalising "breeds", was cemented with the advent of breed clubs and written breed standards in the mid-1800s and the formation of the Kennel Club (KC) in 1873 (Parker et al, 2004). Since that time more than 100 countries around the world have adopted the KC system for the registration of pedigree dogs (Collins et al, 2011). A breed has been defined as an intraspecies group that has relatively uniform physical characteristics developed under controlled condition by man (Irion et al, 2003). Each dog breed is a closed population with breed membership requiring both parents to be registered members of the same breed (Parker et al, 2007). However, all dog breeds are inter-related and stem

from one or more common founding populations with the relationships among established breeds not always being evident (Parker et al, 2007).

Cluster analysis based on microsatellite markers and single nucleotide polymorphisms (SNPs) found at least four different breed groupings: an ancient Asian, African and Arctic group including breeds such as the Chow Chow, Akita, Basenji and Siberian Husky; a Mastiff-type group including the Mastiff, Bulldog and Boxer and other larger breeds that are reported to have had ancient Mastiff-type ancestors such as the Rottweiler and Bernese Mountain Dog; a herding dog group including the Border Collie, Rough Collie, Belgian Tervueren and breeds which are either progenitors or descendants of herding types such as the Irish Wolfhound and Saint Bernard; and the final "hunting" cluster contained breeds of relatively recent European origin – scent hounds, terriers, spaniels, pointers and retrievers (Parker et al, 2004). Subsequent research largely supported these clusters but identified a new cluster, termed the "mountain" cluster, which contained some dogs which had previously fallen into other clusters such as the Bernese Mountain Dog and German Shepherd Dog which had previously been in the Mastiff cluster (now refined as the Mastiff-Terrier cluster), the Saint Bernard which had previously been in the herding dog cluster (now the Herding-Sighthound cluster) and the Clumber, Field and English Cocker Spaniels, previously in the Hunting cluster (Parker et al, 2007). Subsequently, a genome-wide survey of more than 48,000 SNPs in dogs and wolves was able to show distinct genetic clusters largely corresponding to groupings based on phenotype or function, including spaniels, retrievers and small terriers (vonHoldt et al, 2010). The phylogenetic relationships which have been found between breeds are summarized in Figure 2.1.

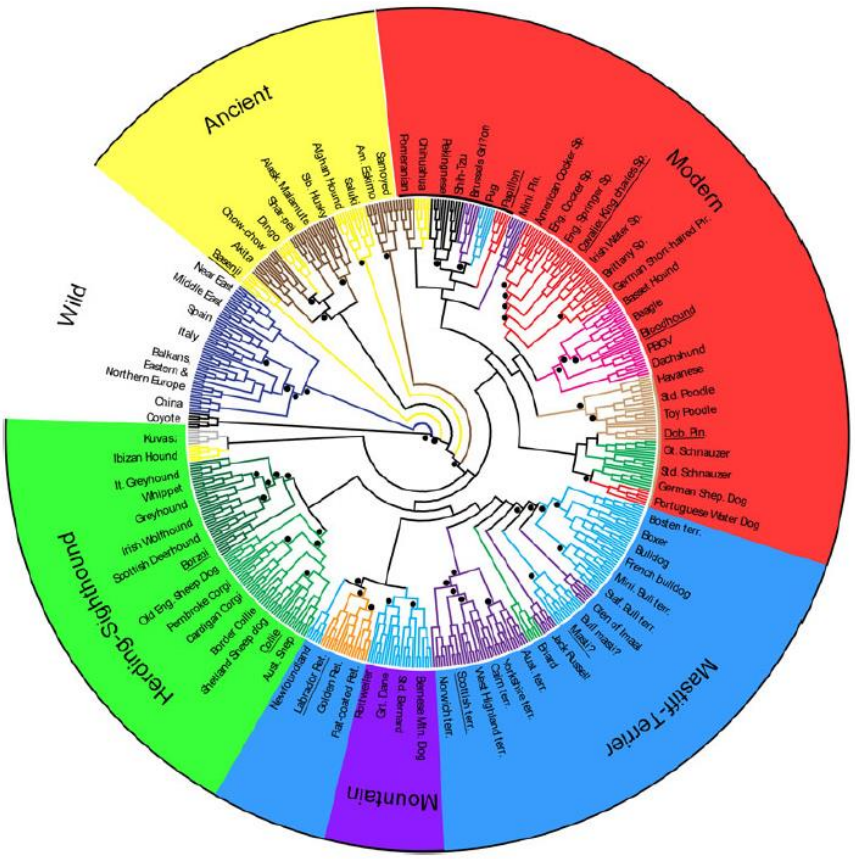

Figure 2-1 Breed clusters based on SNP markers. The inner circle shows a neighbour-joining tree built by comparing 10-SNP haplotypes which grouped 80 dog breeds into approximately 10 breed clusters (VonHoldt et al, 2010). The coloured bars encircling the tree show comparable clusters from a microsatellite analysis of 130 breeds (Parker et al, 2007). Figure taken from Parker, 2012.

2.1.1. Labrador Retriever

The exact origin and early history of the Labrador Retriever is unclear but it is thought that its ancestors, black dogs known as St. John's Dogs or Lesser Newfoundlands, came to the UK on cod boats from Newfoundland and that at a later date pointers were crossed with these dogs to create the breed as we know it (Eley, 1921). The earliest kennels breeding Labrador Retrievers were established around 1850 in Scotland, with the first major English kennel being set up in Netherby in 1860 and from here the breed spread far and wide (Eley,

1921). The Labrador Retriever has been the most popular breed in the UK, as shown by KC registration statistics, for at least 50 years (Farrell, C., KC, personal communication, 2013).

There are multiple, distinct lines bred for showing, field and obedience trials, scent detection and as service dogs of various kinds – its long popularity and selection for lines with particular behaviours support greater haplotype diversity than seen within some other breeds (Sutter et al, 2004). The morphology of the show and working types is becoming increasingly divergent over time, and this will likely result in the establishment of distinct genetic clusters within the breed (Björnerfeldt et al, 2008).

2.1.2. Golden Retriever

Lord Tweedmouth is regarded as having begun the creation of the breed by crossing a yellow wavy coated retriever with a Tweed Water Spaniel, both now extinct breeds (Pounds-Longhurst, 2003). The resultant puppies were bred and outcrosses were performed with Labrador Retrievers, Irish Setters, Bloodhounds and probably others (Ostrander & Kruglyak, 2000). A study found that 84% of chromosomes from Golden Retrievers and Labrador Retrievers (20 unrelated individuals of each breed) carried shared haplotypes (Sutter et al, 2004), reflecting the relatively close relationship between the two breeds. The breed was recognised by the KC in 1913 (Pounds-Longhurst, 2003), and has consistently been in the top 10 most popular breeds in the UK based on KC registration statistics.

2.1.3. German Shepherd Dog

Willis (1991) stated that before 1899 there were various types of German sheepdogs, but with the founding of the Verein fur Deutsche Schaferhunde in 1899 the modern German Shepherd Dog began to develop. Some German Shepherd Dogs had been imported to the UK before the First World War, but

the breed became properly established in the UK after the end in hostilities. The breed's name was initially changed to Alsatian (relating to the border region of Alsace-Lorraine between France and Germany) to avoid negative connotations associated with its Germanic origins. The first Challenge Certificates (certificates given by a judge stating that in his or her opinion the dog is worthy of becoming a Show Champion) were awarded by the KC in 1920 and after this the breed flourished rapidly becoming one of the top ten breeds in terms of KC registration numbers and it has remained one of the most popular breeds in the UK. The German Shepherd Dog falls into the Mastiff-type cluster, and it was argued that their history as a military and police dog alongside breeds such as the Boxer shaped the genetic history of the breed (Parker et al, 2004).

2.2. Canine health and inherited diseases

As scientific progress reduced the frequency of once common infectious, parasitic and nutritional disorders the importance of inherited diseases has grown (Patterson et al, 1989). There is no real evidence of an increase in the frequency of genetic diseases, but as the frequencies of diseases with environmental causes have decreased this has resulted in a relative increase in those diseases in which genetics plays a major role (Patterson, 2000).

Over 1000 inherited conditions have been reported in dogs making the dog the species with the second highest number of reported hereditary disorders after humans in whom approximately 5000 genetic disorders have been described (Mellersh, 2008). This partly reflects the fact that dogs receive the highest level of medical care and investigation at an individual level of any species other than humans (Patterson, 2000). However, the emergence of inherited diseases is an inevitable byproduct of maintaining purebred lines (Meyers-Wallen, 2003).

It is generally the case today that there are more breeding females than males within a breed, with the existence of a small number of "popular sires" which

may produce a very large number of litters in their lifetime (Ostrander & Kruglyak, 2000). A phenotypic trait found in one male can be passed on to a larger number of offspring per generation than if that trait was found in a female, so strong selection on males allows a faster fixation of the phenotype in a new breed (Sundqvist et al, 2006). Shariflou et al (2011) highlighted the fact that the impact of a popular sire will only be felt if his progeny go onto have progeny of their own. It has been shown that the popular sire effect leads to a dissemination of genetic disorders unlike the practices of line breeding and close breeding, but all three practices increase the average inbreeding coefficient within a breed which may have negative effects on fitness (Leroy & Baumung, 2010). Inbreeding also increases the probability of homozygosity of recessive genes whether they encode desirable traits or undesirable traits (Meyers-Wallen, 2003). It has been suggested that the average number of deleterious recessive alleles carried by an individual dog could be as high 20 (McGreevy & Nicholas, 1999).

Inherited conditions can be divided into three main types: those involving gross alterations to large regions of one or more chromosomes, single gene defects and complex traits (Mellersh, 2008). Most biological traits and common diseases are complex traits, meaning that they are influenced by numerous genes and environmental factors (Andersson & Georges, 2004). Complex traits present greater challenges for breeding management than single-locus Mendelian disorders (Wilson & Wade, 2012). The identification of genes that cause single-locus traits is straightforward, but dissecting the genetic basis of complex traits presents a significant analytical challenge (Andersson & Georges, 2004).

It is important to remember that although the frequency of genetic disorders can be greatly reduced by genetic testing and appropriate breeding programmes, they will never be entirely eliminated. The spontaneous occurrence of new mutations mean that over the long term new genetic disorders will arise and those previously known may reemerge (Patterson, 2000).

2.2.1. Chromosomal abnormalities

This category of genetic conditions refers to diseases in which there are recognisable abnormalities in the number or structure of chromosomes. Most are rare, occur as chance events and are not inherited as they are lethal in utero or in the neonatal period (Patterson et al, 1989). Historical studies of chromosomal abnormalities in the dog are few because their identification using conventional cytogenetics is very difficult in this species (Breen & Thomas, 2012). The most frequently reported type is constitutional numerical chromosome abnormalities affecting the sex chromosomes and even these are rare. Monosomy and trisomy of the X chromosome have been reported (e.g. Smith et al, 1989; O'Connor et al, 2011) as have Klinefelter syndrome (XXY) and XX/XXY (e.g. Reimann-Berg et al, 2008; Chaffaux & Cribiu, 1991).

2.2.2. Single gene defects

Single gene diseases are the result of mutations that cause the loss of function of a biologically important gene (Mellersh & Sargan, 2011). The first identification of a disease-causing mutation in a canine gene, along with development of a DNA test for diagnosis and detection of carriers, was reported in 1989 when a single missense mutation (a point mutation) in the gene for canine clotting Factor IX was shown to be the cause of haemophilia B in the Cairn Terrier (Evans et al, 1989). Identification of the mutations responsible for single gene disorders has become very much quicker since the publication of the first full dog genome in 2004 (Mellersh, 2008). DNA from affected and unaffected dogs is compared to locate and identify the mutation responsible for the condition under investigation.

Since 1989 approximately 100 different canine mutations have been identified and DNA tests are available in more than 120 breeds of dog (Mellersh, 2013). Many single gene diseases have a recessive mode of inheritance. If a

mutation is recessive dogs with no or a single copy of the mutation will remain clinically free of the disease although heterozygous carriers will pass on the mutation to half of their offspring (Mellersh & Sargan, 2011). Dogs which are homozygous for the mutation will almost certainly develop the disease even if they are clinically unaffected at the time of testing.

DNA tests are available for several single gene disorders of the Labrador Retriever, Golden Retriever and German Shepherd Dog and those which Guide Dogs use are discussed briefly in Chapter 3.

2.2.3. Complex traits

For complex traits, defining the same phenotype across different breeds may be difficult as identical phenotypes may have different genetic components (Karlsson & Lindblad-Toh, 2008). This is illustrated by coat colour in which the genetics underlying black coat colour in Labrador Retrievers are different to the genetics underlying black coat colour in German Shepherd Dogs despite the two being phenotypically identical. In the Labrador Retriever the tyrosinase-related protein 1 (*TYRP1*) gene on CFA11 has been found to determine black coat colour which is inherited as a dominant trait (Schmutz et al, 2002). Solid black coat colouration in the German Shepherd Dogs on the other hand is inherited as a recessive trait which has been found to be due to a recessive allele *a* at the agouti signal peptide (*ASIP*) gene on CFA24 (Kerns et al, 2004).

A recent study of the prevalence of disorders recorded in dogs attending primary-care veterinary practices in central and southeast England, using electronic patient records, concluded that the most prevalent disorders within the 3,884 dogs were complex disorders (O'Neill et al, 2014a) The five most prevalent disorders found in that study were otitis externa, periodontal disease, anal sac impaction, nail disorders and osteoarthritis. Interestingly only one of these disorders, otitis externa, had a higher prevalence in purebred dogs than crossbred dogs (O'Neill et al, 2014a).

A study of patient records at an American veterinary teaching hospital looked specifically at the prevalence of 24 inherited conditions over a 15 year period, amassing data on 90,004 dogs (Bellumori et al, 2013). The five most prevalent conditions found in that study were intervertebral disc disease, cataracts, patellar luxation, hip dysplasia and hypothyroidism. There was no difference in the prevalence of 13 inherited disorders (including hip dysplasia) between purebred dogs and mixed-breed dogs, and the authors presented three alternative hypotheses as to why this might be: causal mutations may have arisen multiple times; a common distant ancestor may have carried the mutations, perhaps because the traits which made dogs suitable for domestication were linked to alleles for these conditions; or selection for desirable morphological traits may be linked to the presence of deleterious alleles (Bellumori et al, 2013). This final hypothesis appears to be given credence by a study which found a direct correlation between inherited disorders in purebred dogs and the morphological characteristics required in breed standards (Asher et al, 2009).

Asher et al (2009) found that all of the top 50 most popular breeds of dog in the UK, as measured by KC registration statistics, were predisposed to at least one inherited disorder linked to breed standards with 84 disorders being directly or indirectly associated with conformation. A related study estimated that there are at least 312 inherited disorders not related to breed standards in these 50 breeds of dog which are inherited and may have emerged as result of inbreeding and restricted gene pools (Summers et al, 2010).

The Labrador Retriever, Golden Retriever and German Shepherd Dog were sixth, joint third and first respectively when ranked in these recent reviews of inherited disease in pedigree dogs, coming sixth, joint third and first respectively (Asher et al, 2009; Summers et al, 2010). Two factors may slightly confound these findings: firstly, these breeds are among the most popular in terms of KC registrations in the UK and are also very numerous across the Western world; secondly, as these breeds are amongst the most commonly used as service dogs across the world they may attract more

funding for research which may mean that more conditions have been investigated in these breeds (Collins et al, 2011). Complex conformation-linked diseases to which the Labrador Retriever, Golden Retriever and German Shepherd Dog were described as predisposed included hip dysplasia, panosteitis and entropion (Asher et al, 2009). In this category, the two Retrievers were also considered predisposed to elbow dysplasia. Complex diseases to which all three breeds were considered predisposed but which were not linked to the breed standards included atopic dermatitis, cataracts, hypothyroidism and idiopathic epilepsy (Summers et al, 2010).

When a more complete understanding of the genes underlying complex diseases is achieved it may be possible to identify mutations in key genes which could lead to the development of DNA tests (Patterson, 2000). However, few of the sequence mutations underlying complex traits have been found in dogs (Karlsson & Lindblad-Toh, 2008) and currently no DNA tests for complex diseases are available. Thus breeding strategies to reduce the incidence of complex diseases must currently rely on quantitative genetic techniques.

2.3. Canine behavioural genetics

The success of any breeding programme involving behavioural traits in dogs depends on an understanding of the genetics of those traits (Mackenzie & Houpt, 1986). The genetic influence on behaviour appears to involve multiple genes and nongenetic sources of variance are at least as important as genetic factors (Plomin, 1990). Nevertheless, behavioural differences between dog breeds have a genetic basis (Saetre et al, 2006). However, behavioural traits can only be incorporated in breeding programmes if they can be measured as accurately and objectively as possible, and if there is significant genetic variation (Ruefenacht et al, 2002).

Attempts to study dog behaviour, and to investigate the inheritance of canine behavioural traits, have generally fallen into four main categories: applied

stimulus tests, observer ratings, surveys of expert opinion and surveys of owner-reported behaviour (Jones & Gosling, 2005). A comprehensive review of studies into canine behavioural genetics is beyond the scope of this literature review, but the results of some key studies from all four categories are discussed below. Not surprisingly a large amount of study into behavioural genetics has been focused in the area of working and service dog breeding. The term "working dog" usually refers to police or military dogs.

2.3.1. Canine behavioural testing

Whitney (1929) first drew attention to differences in behaviour between certain breeds of dog. Among characteristic behaviours considered, Whitney discussed the reticence of the Bloodhound to bite humans and the lower level of inhibition of the German Shepherd Dog regarding the same activity. The Labrador Retriever, Golden Retriever and German Shepherd Dog are among the breeds which have most often been the subject of behavioural tests (Diederich & Giffroy, 2006), and this reflects both their worldwide popularity but also their widespread use as working and assistance dogs.

Behaviour testing of dogs has been used since at least 1934 as an aid for selection of service dogs for various types of work and for breeding (Humphrey, 1934). Jack Humphrey described the work with German Shepherd Dogs over 10 years at Fortunate Fields in Switzerland which had led to the formation of the first guide dog school in the USA, The Seeing Eye, Inc. (TSE). They defined a list of behavioural characteristics which they scored, mostly on a 1 to 5 scale. The names of the characteristics they measured in this way were body sensitivity, ear sensitivity, nose ability (described as willingness to trail), intelligence, willingness, energy, self-right (described as the dog exhibiting that it feels a right to be right where it is), confidence, sharpness (defined as the willingness to bite a human) and fighting instinct (defined as the desire to fight with other dogs). Based on the scores they allocated to each of these characteristics they could identify for which line of

work the dog was most suited, with a dog suitable for guiding work possessing high confidence, medium ear sensitivity and low nose ability (Humphrey, 1934).

2.3.1.1. *Scott & Fuller*

The extensive work by Scott and Fuller (1965), begun in 1945 and lasting 13 years, at Jackson Laboratory in Maine, USA, is often regarded as the most important and influential work on canine behavioural genetics (Mackenzie et al, 1986). The objective was to compare different breeds of dog in the same environmental conditions so that any behavioural differences between breeds would be attributable to genetics rather than genetics plus environment. They investigated differences in temperament between five breeds: Basenjis, American Cocker Spaniels, Beagles, Shetland Sheepdogs and Wire-haired Fox Terriers, and crosses between the breeds, using a battery of behavioural tests and found strong and statistically significant effects of breed on many of the traits measured. They also found variation in behaviour within each individual breed. Scott and Fuller (1965) thus became the first researchers to estimate the heritability of canine behavioural traits, as the proportion of total variance due to breed differences, with estimates ranging from 0.42 for “playful fighting” at 13-15 weeks of age to 0.79 for “running time long barrier” (the time taken to find a way past a long barrier).

Subsequently, Scott, Fuller and Bielfelt (1976) worked with Guide Dogs for the Blind, Inc. (GDB) in San Rafael, California to improve their behavioural testing procedures and breeding programme. One of their recommendations was that experimental crosses between the three breeds which GDB had found to be successful guide dogs, the German Shepherd Dog, Labrador Retriever and Golden Retriever, should be carried to evaluate the possible use of first-generation (F1) crosses. They hypothesized that such crosses should at the minimum show a marked reduction in puppy mortality and a significant improvement in physical health due to heterosis (Scott et al, 1976).

2.3.1.2. *Goddard & Beilharz*

Goddard & Beilharz (1974-1986) at the University of Melbourne were the first to report attempts to use quantitative genetics in a guide dog breeding programme in a research project beginning in 1973. They worked with the Royal Guide Dogs for the Blind Association in Australia analysing data already collected by the association on its dogs, mainly Labrador Retrievers but also a few Golden Retrievers, to define essential traits and to estimate their heritabilities and correlations. They also started a cross-breeding programme with Labrador Retrievers, Kelpies, German Shepherd Dogs and Boxers to estimate the degree of heterosis (Goddard & Beilharz, 1974).

Using data relating to 1031 dogs (929 Labrador Retrievers and 102 other breeds, mostly Golden Retrievers) born between 1963 and 1975, Goddard and Beilharz (1982) first used least-squares analysis to estimate the heritabilities of three behavioural reasons for dogs to be withdrawn from the guide dog program. These were fearfulness, dog distraction and excitability. Heritability estimates were 0.46±0.13 for fearfulness, 0.09±0.08 for dog distraction and 0.09±0.08 for excitability (Goddard & Beilharz, 1982).

Goddard and Beilharz (1983) then moved onto scores of a behavioural assessment used by guide dog trainers. Dogs returned to the training centre from their puppy walkers at 12-18 months of age and were assessed for approximately three weeks during which time the trainers rated the dogs on 17 different scales, each on a 0-5 scale with 0.5-point intervals, which they believed measured traits important in guide dogs. These traits were: nervousness, suspicion, sound shy (fear of loud noises), anxiety, aggression, nervous aggression, concentration, distraction, dog distraction, cat distraction, nose distraction, willingness, hearing sensitivity, body sensitivity, temperamental stability (overall suitable temperament), initiative (decision-making ability e.g. in traffic or negotiating obstacles) and excitability. Estimation of heritabilities and genetic correlations did not include all traits as some were excluded due to missing values. Heritability estimates for those

eight traits for which they were calculated ranged from 0.08 for distraction to 0.58 for nervousness, and there were no unfavourable correlations between traits (Goddard & Beilharz 1983).

In 1985, results of the cross-breeding experiment were reported. Goddard & Beilharz (1985b) performed a diallel cross using the four breeds of dog mentioned above: the Labrador Retriever, German Shepherd Dog, Boxer and Kelpie. A diallel cross is a mating scheme used by animal (and plant) breeders and geneticists to investigate the genetics underlying quantitative traits in which each line is crossed with every other line (Falconer & Mackay, 1996). They recorded 38 measures of fearfulness in the dogs at between 12 and 18 months of age, and found that the Labrador Retrievers were the least fearful and the German Shepherd Dogs were the most fearful of the four breeds. They also found no evidence of heterosis for fearfulness (Goddard & Beilharz, 1985).

The final report of this research project related to a puppy test on 102 puppies (a maximum of four puppies per litter) produced from the diallel cross detailed above (Goddard & Beilharz, 1986). The puppies underwent a battery of tests from four weeks until six months of age and were scored on a variety of scales which sought to measure fearfulness, activity and learning ability. As with the adult dogs, the Labrador Retrievers puppies were the least fearful and the German Shepherd Dog puppies were the most fearful. They found that consistent individual differences in fearfulness were detectable at approximately eight weeks of age, but that the ability to predict adult fearfulness increased with age (Goddard & Beilharz, 1986).

2.3.1.3. *Swedish Dog Training Centre behavioural test data*

Wilsson & Sundgren (1997a, 1997b, 1998) and then van der Waaij et al (2008) reported a series of studies relating to a canine behavioural test that has been in use at the Swedish Dog Training Centre. In the test the dog was exposed to seven novel situations and a trained tester allocated scores on a numerical

scale for ten traits: courage (ability to overcome fear), sharpness (tendency to react with aggression), defence-drive (tendency to defend itself or its handler), prey-drive (willingness to engage in competitive games e.g. tug of war), nerve stability (appropriateness of the dog's reaction to a situation), reaction to gunfire, temperament or energy level, hardness (lack of lasting effect of a pleasant or frightening experience), ability to cooperate and affability (willingness to make contact with people). The first study involved the scores of 1310 German Shepherd Dogs and 797 Labrador Retrievers aged between 15 and 20 months which underwent the test between 1983 and 1991. German Shepherd Dogs were found to score significantly higher for sharpness and defence-drive while Labrador Retrievers scored significantly higher for courage, nerve stability, hardness, reaction to gun fire, ability to cooperate and affability; the authors concluded that these differences made Labrador Retrievers more suitable as guide dogs and German Shepherd Dogs more suitable as police or protection dogs (Wilsson & Sundgren, 1997a). However, some of the German Shepherd Dogs successfully became guide dogs and these dogs scored higher for ability to cooperate, courage and nerve stability and lower for sharpness, defence-drive and prey-drive compared to the breed average suggesting that there was variation in these traits within the breed. The authors concluded that this subjective evaluation of complex behavioural parameters could be used as a tool for selection of dogs appropriate for a variety of working roles.

Subsequently these results were used in genetic analyses (Wilsson & Sundgren, 1997b). Heritability, estimated from intraclass correlation between sibs within groups of full and half sibs and based on the combined sire and dam variance components, ranged from 0.13±0.05 for sharpness to 0.37±0.08 for affability in the German Shepherd Dog and from 0.05±0.07 for prey-drive to 0.35±.0.09 for ability to cooperate in the Labrador Retriever. The differences in heritability between breeds were mostly small and not statistically significant except for affability and prey drive (Wilsson & Sundgren, 1997b).

The next piece of work reported by those authors was a comparison of results of a behavioural test for eight week old puppies with later results in behavioural test used above between 15 and 20 months of age (Wilsson & Sundgren, 1998). The dataset comprised 554 German Shepherd Dogs which underwent both tests. The puppy test consisted of 10 elements named yelp, shriek, contact I, fetch, retrieve, large ball, tug of war, activity, contact II and objects visited, some of which involved observing the puppy's reaction to a novel stimulus and others were counts of behaviours observed. Heritabilities were estimated using the same methodology as their previous study, and ranged from 0.20±0.08 for retrieve to 0.53±0.13 for activity with all but two elements (contact I and contact II) having moderate to high heritability estimates (Wilsson & Sundgren, 1998). However they found no correlation between results of the puppy test and later results in the adult behaviour test and concluded that adult behaviour could not be predicted at eight weeks of age (Wilsson & Sundgren, 1998).

Finally, van der Waaij et al (2008) analysed the adult behavioural test results of a larger dataset using more robust quantitative genetic techniques. Test results were available for 2757 German Shepherd Dogs and 1813 Labrador Retrievers which had undergone the test between 1980 and 2003. Heritability estimates ranged from 0.14±0.03 for hardness to 0.38±0.06 for affability in the German Shepherd Dog and from 0.13±0.05 for sharpness to 0.56±0.08 for gun shyness in the Labrador Retriever. All the heritability estimates were smaller than those estimated by Wilsson & Sundgren (1997b) which may have been related to the different estimation methods; those presented in the later study should be more accurate (van der Waaij et al, 2008).

2.3.1.4. *Dog Mentality Assessment*

Svartberg and Forkman (2002) described another standardised canine behavioural test in Sweden called the Dog Mentality Assessment (DMA) used for dogs between 12 and 24 months of age. This was an applied stimulus test in which each dog was exposed to 10 novel situations and their reactions were scored by trained observers. It was developed by the Swedish Working Dog Association as a breeding tool for working dogs but has since become widely used in many different breeds. They performed factor analysis on the DMA test results of 1175 dogs, representing 47 breeds, and found five primary factors which they named playfulness, curiosity/fearlessness, chase-proneness, sociability and aggressiveness. They also found a broad personality dimension, shyness-boldness, which related to all the narrower primary factors except aggressiveness (Svartberg & Forkman, 2002).

A subsequent study by Svartberg (2002) used DMA data from 2655 German Shepherd Dogs and Belgian Tervuerens aged between 12 and 18 months and related them to performance data from working dog trials. The performance data consisted of scores for obedience and function (either tracking, searching, messenger dog or handler protection). They found that a high score on the shyness-boldness axis, which relates to sociability towards strangers, playfulness, interest in chasing, exploration and fearlessness correlated with high success in working dog trials (Svartberg, 2002).

Up to 2005 no heritability estimates had been published for components of, or dimensions identified from, the DMA but this was addressed by Strandberg et al (2005). They extracted DMA data for 5959 German Shepherd Dogs which had been tested between 1989 and 2001. Heritability estimates for four of the personality traits previously identified by Svartberg & Forkman (2002), playfulness, curiosity/fearlessness, chase-proneness and aggressiveness, were 0.22, 0.24, 0.10 and 0.15 respectively (standard errors were not reported). The broader personality dimension of shyness-boldness

was found to have a heritability of 0.27 and a moderate, positive genetic correlation with aggression of 0.37 (Strandberg, 2005).

Svartberg (2006) later compared the results of the DMA, described above, for 13,097 dogs of 31 breeds. An interesting result was that the Golden Retriever clustered with the German Shepherd Dog and the Labrador Retriever was in a different group to both breeds, which differed to the studies of expert opinion detailed below. The most striking difference between the two Retrievers was that on average the Labrador Retriever was found to be very bold whereas the Golden Retriever was rather fearful (Svartberg, 2006). However Svartberg also reported that there was considerable variation in scores between members of the same breed.

2.3.2. C-BARQ

Serpell and Hsu (2001) described the development and validation of a novel questionnaire method for assessing behaviour and temperament of prospective guide dogs which, after modification, subsequently became known as the Canine Behavioral Assessment and Research Questionnaire (C-BARQ©). The C-BARQ at this stage was a simple 40-item questionnaire about dog behaviour and temperament which used a series of five-point semantic differential-type rating scales. The initial study involved 1097 one year old prospective guide dogs at TSE where volunteer puppy-raisers used the C-BARQ to provide a quantitative assessment of their dog's typical response to a variety of environmental events and stimuli. The scores for the 40 items were subjected to factor analysis which identified eight common factors: dog-directed fear/aggression, stranger-directed fear/aggression, owner-directed aggression, non-social fear, energy level, chasing, trainability and attachment. These eight factors were then validated against TSE's own criteria for rejecting dogs for behavioural reasons confirming the construct validity of the puppy raiser's questionnaire assessments of their dogs. The authors concluded that

this behavioural questionnaire provided a useful and accurate means of predicting the suitability of dogs for guiding work (Serpell & Hsu, 2001).

Hsu and Serpell (2003) subsequently described the development and validation of the C-BARQ, which now consisted of 152 questions, for assessing behaviour and temperament traits in pet dogs. Some 2054 pet owners scored their dogs for 152 items some on five-point frequency scales and some on semantic differential-type rating scales. The scores for 68 of these items were subjected to factor analysis which identified 11 common factors: dog-directed fear/aggression, stranger-directed fear, stranger-directed aggression, owner-directed fear, non-social fear, trainability, attachment or attention-seeking behaviour, separation-related behaviour, chasing, excitability and touch sensitivity. The factors which differed between this study and Serpell & Hsu (2001) were in areas of behaviour which had not been covered by one of them due to differences in the questions. The authors concluded that the findings of the two studies suggested that the questionnaire factors and the behavioural and temperament traits they represent were stable and consistent across different populations of dogs (Hsu & Serpell, 2003).

Serpell & Hsu (2005) looked specifically at the C-BARQ factor of trainability from a 101 element questionnaire, a factor which is consisted of eight questionnaire items scored from 0-4, in 1563 dogs of 11 breeds. The Golden Retriever was second only to the Labrador Retriever in terms of mean trainability score, with mean scores of 3.14 and 3.24 respectively. The breed with the lowest mean trainability score of the 11 breeds in the study was the Basset Hound with a mean score of 2.06. Labrador Retrievers which had been bred for field work tended to obtain significantly higher i.e. better scores for trainability than those which had been bred for showing. The authors suggested that selection for more specialised and interactive working skills may have heightened the trainability of some breeds compared with others (Serpell & Hsu, 2005).

Svartberg (2005) compared results of the DMA with subsequent results 1-2 year later of the (122 question) C-BARQ for 697 dogs of 16 breeds and found that playfulness, curiosity/fearlessness and sociability results in the DMA were associated with corresponding behaviour in the dogs' home environment as assessed by the dogs' owners in the C-BARQ.

Liinamo et al (2007) were the first to undertake genetic analysis of any C-BARQ traits. The authors used 27 C-BARQ questions relating to stranger-, owner- and dog-directed aggression and sought to identify if any of the questions or factors exhibited significant genetic variation in a population of 325 Golden Retrievers in The Netherlands. Heritability estimates for the individual C-BARQ questions ranged from zero to one, but heritability estimates for the four C-BARQ factors of stranger-directed aggression, owner-directed aggression, dog-directed aggression and familiar dog-directed aggression were all high ranging from 0.43 for dog-directed aggression to 0.87 for stranger-directed aggression (Liinamo et al, 2007). Unfortunately standard errors were not obtained for most traits, and the sample size was very small, so these results must be viewed with some caution.

Schiefelbein (2012) sought to estimate genetic parameters for each of the 101 questions and 12 factors measured by the C-BARQ among 6 month old and 12 month old prospective guide dogs at TSE. Questionnaire responses were obtained for 989 and 1187 Labrador Retrievers, 608 and 692 Golden Retrievers and 966 and 1348 German Shepherd Dogs between 2002 and 2010. Many of the factors and most of the questions had low heritability estimates of less than 0.1 but the estimates of heritability for the factor "trainability" at 12 months of age were moderate to high at 0.46±0.07 in the Labrador Retriever, 0.47±0.20 in the German Shepherd Dog and 0.20±0.08 in the Golden Retriever. Three other factors were found to have moderate heritabilities: familiar dog-directed aggression/fear in the Golden Retriever at six months (0.27±0.12), chasing in the Golden Retriever at six months (0.22±0.10) and non-social fear in the Golden Retriever at 12 months (0.27±0.09). The author suggested that the genetic variation in scores in

these C-BARQ factors could be exploited in selection to improve these traits (Schiefelbein, 2012).

Significant differences in owner-reported behaviour using the C-BARQ questionnaire have been found between Labrador Retrievers of different coat colours in the UK (Lofgren et al, 2014). Chocolate Labrador Retrievers showed lower noise fear, trainability and fetching behaviour and higher unusual behaviour, agitation when ignored and excitability compared to black and yellow dogs. The authors postulated that this could perhaps be due to genetic associations between the mutated TYRP1 genes responsible for chocolate coat colour and behaviour or that the demand for chocolate dogs may have led the gene pool of dogs carrying the mutant alleles to have become slightly separated from that of the other colours. Yellow Labrador Retrievers also showed more separation anxiety than black dogs. However, it should be noted that differences in personality were more commonly associated with working status of the dog than with coat colour (Lofgren et al, 2014).

Differences in problem behaviours between Labrador Retrievers of different coat colours have also been reported by Houpt and Willis (2001), who found that yellow Labrador Retrievers were more likely to be referred to a veterinary teaching hospital in America due to aggressive behaviour than black or chocolate coloured Labrador Retrievers. Takeuchi and Houpt (2003) postulated that the link between coat colour and aggression could be a direct metabolic one as both dopamine, a neurotransmitter, and melanin have DOPA as a precursor so genes that code for DOPA and its products could be involved in the aggressive behaviour associated with coat colour.

2.3.3. Expert opinion of breed behavioural differences

Hart and Hart (1985) in the USA ranked 56 breeds of dogs on 13 behavioural traits and then performed factor analysis which found three factors, which they named reactivity, aggression and trainability, accounted for 81% of the variance in the 13 behavioural traits. They then performed cluster analysis of dog breeds on the basis of similarity in scores for each of the three factors. On this basis Labrador Retrievers and Golden Retrievers were in the same cluster characterized by low aggression, high trainability and low reactivity. German Shepherd Dogs on the other hand clustered with Rottweilers, Dobermann Pinschers and Akitas, in a cluster characterized by very high aggression, very high trainability and very low reactivity (Hart & Hart, 1985).

A subsequent study in the UK ranked 49 breeds of dogs on the same 13 behavioural traits to see if nominally identical breeds were behaviourally distinct populations in the USA and UK (Bradshaw et al, 1996). The three factors founds in that study were labelled aggressivity, reactivity and immaturity and between them accounted for nearly 88% of the variance in the 13 behavioural traits. Cluster analysis of dog breeds on the basis of scores for each of the three factors again saw the Labrador Retriever and Golden Retriever in the same cluster characterized by low aggressivity, average reactivity and high immaturity. German Shepherd Dogs shared a similar cluster to in Hart and Hart (1985), although the Akita was replaced by the Bull Terrier, and the cluster was characterized by high aggressivity, average reactivity and low immaturity (Bradshaw et al, 1996). The major difference between this study and the earlier American study was in the three factors which accounted for the majority of the variance. In Hart and Hart (1985), trainability was one of the major factors while immaturity was not, but this was reversed in the British study and the authors hypothesised that this could reflect a different emphasis in dog use in the two countries with working potential being valued more highly in America and their role as companions being more important in the UK (Bradshaw et al, 1996).

The methodology of Hart and Hart (1985) was repeated again in Japan, the same three factors were found to account for the majority of the variance as in the USA and breeds largely fell into the same clusters; the Labrador Retriever and Golden Retriever still clustered together and the German Shepherd Dog was in a distinct cluster with breeds including the Dobermann Pinscher and Akita (Takeuchi & Mori, 2006). Thus it seems that behavioural characteristics of breeds are largely consistent across countries and continents even if the populations may be relatively genetically isolated from each other.

2.3.4. Molecular genetics of canine behaviour

In 2001, Houpt and Willis predicted that the rapid advances in genomics made it likely that DNA tests for aggression, anxiety and compulsive behaviour in dogs would become available within the next decade. This prediction has not come to fruition to date. Candidate gene and genome-wide association study (GWAS) approaches have been used in attempts to identify the genes controlling these and other behavioural traits with little success (Spady & Ostrander, 2008).

Candidate gene studies involve choosing genes which may be involved in the trait of choice on the basis of the known function of the proteins which the genes encode, using a case-control design in which dogs are matched so that for every dog which displays the trait in question there is one which doesn't (Overall et al, 2014). For example, van den Berg et al (2008) used the candidate gene approach with four genes of the canine serotonergic system, as alterations in serotonin metabolism in the brain had been described in aggressive dogs previously, in aggressive and non-aggressive Golden Retrievers. They concluded that none of the four genes played a major role in aggression in the breed (van den Berg et al, 2008).

The GWAS approach has become increasingly popular and investigates the association between common genetic variation and a particular phenotype,

for example a behavioural trait. It is likely that, using the GWAS approach, several novel loci associated with behavioural traits in dogs will be discovered (Overall et al, 2014). However, as most behavioural traits probably involve several genes and environmental effects this does not necessarily mean that DNA tests will become available for problem behaviours in all breeds. It is more likely that potential DNA tests may at most be able to offer information on the risk of development of separation anxiety, for example (Overall et al, 2014). Therefore, as with complex diseases, breeding strategies involving behavioural traits must currently rely on quantitative genetic techniques.

2.4. Quantitative genetic concepts and their application

The basic premise of quantitative genetics is that, if the relationships between individuals in a population are known, useful inferences about the inheritance of traits for which phenotypic data are available can be made without explicit knowledge of the genetic loci involved (Wilson et al, 2010). The ideal data set on which to use quantitative genetic techniques is one comprising data on a large number of individuals in a well-connected pedigree (Wilson et al, 2010).

2.4.1. Heritability

Heritability is a fundamental concept in animal breeding. It is a descriptive statistic that provides an estimate of the extent to which observed variability between individuals is due to genetic variability (Plomin, 1990). The broad sense heritability (H^2) of a trait is defined as the ratio of genetic variance to the total variance of the phenotype;

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

where σ denotes variance, and g and p denote genetic and phenotypic environmental components respectively (Falconer & Mackay, 1996). Total

phenotypic variance is defined as the variation in a trait after accounting for that which is attributable to known fixed effects such as sex or age. It is composed of both genetic and environmental components:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where subscripts *e* denotes the environmental component (Falconer & Mackay, 1996). Genotypic variance can be subdivided into the additive genetic effects, dominance and epistatic genetic effects i.e. the effects of alleles at an individual locus and interactions between genes at different loci. The environmental variance can also be subdivided into all variation not due to genetic influences, such as the environmental variance common to specified groups such as siblings or litters, as well as individual stochastic error variance and measurement error. In this way the equation above can be expanded as follows:

$$\sigma_p^2 = \sigma_a^2 + \sigma_d^2 + \sigma_i^2 + \sigma_f^2 + \sigma_e^2$$

where subscripts *a*, *d*, *i*, *f* and *e* refer to additive genetic, dominance and epistatic genetic effects, fixed environmental effects and residual error (combining individual stochastic errors and measurement error) respectively (Falconer & Mackay, 1996). Only genes rather than genotypes are transmitted from parents to offspring, thus the effects of dominance and epistasis are not inherited. Therefore narrow sense heritability, defined as the additive genetic variance as a proportion of total phenotypic variance, rather than broad sense heritability is used in breeding programmes, as it is this that determines the degree of resemblance between relatives not total genetic variance. Narrow sense heritability, h^2, can be written as (Falconer & Mackay, 1996):

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

Because the heritability estimate depends on the value of all the components of variance, a change in the magnitude of any one of these will affect it.

Therefore in theory heritability is a feature of a specific population at a specific time and should not be extrapolated to other scenarios. However estimates of heritability for various traits have been found to be remarkably similar at different times and across different populations (Visscher et al, 2008). Therefore heritability becomes a very useful parameter allowing comparison of the relative genetic contribution to variation between different traits or diseases.

As a mathematical measure, heritability is always positive and ranges from zero to one. Traits with heritabilities below 0.2 are considered lowly heritable, those with heritabilities between 0.2 and 0.4 are considered moderately heritable and traits with heritabilities greater than 0.4 are considered highly heritable (Bourdon, 2000). When the heritability is high the phenotype will be a good predictor of the underlying genotype and relatives will tend to resemble each other in that trait. Low heritability implies that only a small proportion of the total phenotypic variation between individuals for the traits in question is determined by additive genetic effects, with environmental effects playing a greater role, and there will be little resemblance between relatives.

The accuracy of a heritability estimate depends on bias and on its sampling error, which is a function of the sample size and pedigree structure. Hundreds of observations are needed to obtain a standard error of less than 0.1, and thousands of observations are needed to obtain very precise estimates (Visscher et al, 2008).

The numerator in the equation defining narrow sense heritability is also known as the breeding value (BV). The BV of an individual is the sum of the average effects of that individual's genes which give rise to the mean genotypic value of their offspring (Falconer & Mackay, 1996). BVs are not directly measurable but they can be predicted using performance data to produce estimated breeding values (EBVs). The EBV of an individual related to a particular trait can be judged by the mean value of its progeny or siblings

for that trait (Nicholas, 2010). EBVs have been used extensively in livestock industries to rank breeding individuals to ensure maximal response to selection since the 1950s (Flint & Woolliams, 2008). More recently there has been growing interest in their use in dogs. TSE in the USA have been using EBVs as the basis of their selection decisions since 1995 (Leighton, 1997) and some other international guide dog organisations have followed suit (e.g. Russenberger & Havlena, GEB, 2013; Bullis, GDB, personal communication, 2014). Since March 2014, EBVs for hip and elbow dysplasia have been freely available for all Labrador Retrievers, Golden Retrievers and GSDs (and twelve other breeds) registered with the UK Kennel Club, through a section of the KC website called "Mate Select" (The Kennel Club, 2014). The EBV of all individuals in a pedigree, regardless of whether they have reproduced or whether they have phenotypic information, can be obtained using modern statistical techniques.

The benefits of EBVs when making selection decisions have been summarized by Lewis et al (2010): (i) EBVs are more accurate than using the phenotype as a measure of genetic merit, since they use all the available information, including relatives, not just the phenotype of the individual; (ii) the EBV for an individual, unlike its phenotypic score, will increase in accuracy over time as further information on relatives becomes available, e.g. from offspring and siblings; (iii) EBVs can provide predictors for those animals that do not have their own phenotypic record or scores, but have relatives that are scored, hence increasing selection intensity and progress; (iv) the EBV of an individual is available from the moment of birth for selection (although newborn littermates will have identical EBVs); and (v) EBVs will have been corrected for fixed effects, for example sex and age, which bias phenotype as a predictor of genetic merit.

Traditionally, heritability was estimated using simple, balanced studies of regression of offspring on parents, correlation of full or half sibs and twin studies (Visscher et al, 2008). These methods have been superseded by linear mixed models which can incorporate information from all individuals within a

pedigree and which are more robust with unbalanced data. In particular the "animal model" has become the model of choice in animal breeding and evolutionary genetics. As well as making use of phenotypic information from all types of relationships within complex, unbalanced pedigrees the animal model is flexible enough to cope with missing data and non-genetic influence on phenotype can also be explored (Wilson et al, 2010).

2.4.2. Genetic correlations

Genetic correlations occur when traits are related either positively or negatively through their genes, due either to pleiotropy or linkage of genes. Pleiotropy is the phenomenon whereby a gene influences the expression of more than one trait. A positive genetic correlation suggests that improvement in one trait will also lead to improvement in the other trait with which it is genetically correlated. A negative genetic correlation on the other hand suggests that improvement in one trait will lead to a reduction in the correlated trait.

2.4.3. Selection indices

As many traits may influence an animal's value as breeding stock, a method of combining EBVs for different traits was needed. The selection index represents such a tool. The use of selection indices in animal breeding was first described in a seminal paper in 1943, regarding a herd of swine in Iowa (Hazel, 1943). The total genetic improvement which can be brought about by selecting a group of animals is the sum of the genetic gains made for the multiple traits of importance, in the case of swine economic importance. The gain made for each trait is weighted by the relative (economic) value of that trait. Selection for an index which weights each trait appropriately is more efficient than selection for one trait at a time (Hazel, 1943). When constructing a selection index genetic correlations between traits must be taken into consideration.

2.4.4. Managing a breeding programme

A prerequisite for a well-defined breeding programme is an integrated database which contains all information on each individual animal, including pedigree, reproduction data, health information and behavioural test results (Lindhé & Philipsson, 1998). The organizational requirements of a breeding programme are illustrated in Figure 2.1.

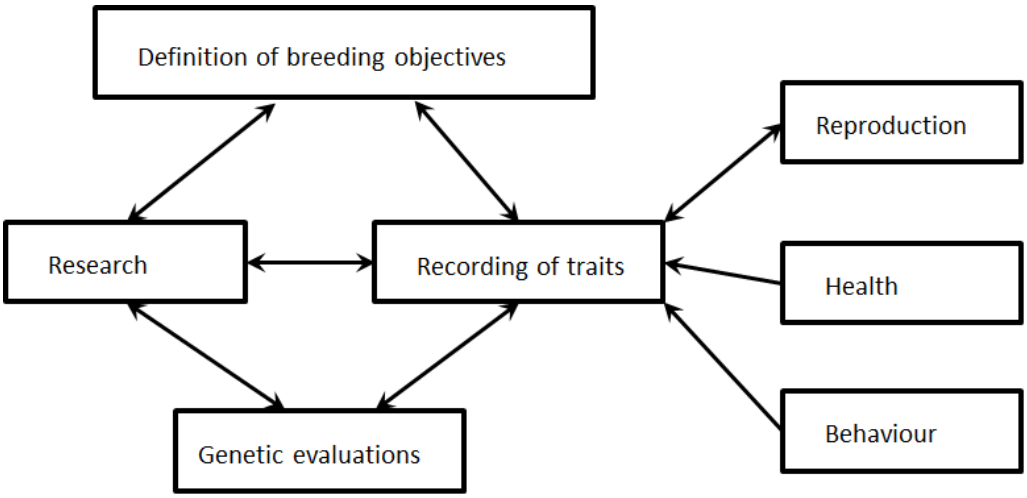

Figure 2-2 Interactive parts of a breeding programme, adapted from Lindhé & Philipsson (1998).

A breeder of animals or plants controls most of the evolutionary forces acting on a population, by defining the population size, the mating system and the intensity and direction of artificial selection (Lynch and Walsh, 1998). An ideal breeding programme should consider all traits of importance (for dogs that would include health, behaviour and appearance) whilst also taking population structure and genetic variation into account (Hedhammar et al, 2011). Many of the challenges in dog breeding relate to breeding within small, closed populations (McGreevy & Nicholas, 1999). Therefore within a breed-specific breeding programme, monitoring genetic progress and restricting the rate of inbreeding must go hand in hand (Hedhammar et al, 2011). Simulation studies have suggested that the rate of inbreeding should be lower when a broadly defined breeding goal is used rather than a limited

focus on one or two traits (Sørensen et al, 1999). Which traits are included in a breeding goal depends predominantly on their genetic aspects, particularly heritabilities but also genetic correlations, and their relative importance (Kanis et al, 2005).

2.5. Aims and objectives

This study was conducted with the following aims:

- Identify breeding priorities at Guide Dogs
- Assess the presence/absence and magnitude of heritability for disease conditions in the three purebred dog breeds used in the largest numbers by Guide Dogs
- Examine whether genetic correlations exist between disease conditions
- Quantify crossbreeding parameters for disease conditions
- Assess the presence/absence and magnitude of heritability for behavioural traits measured by Guide Dogs during puppy walking and training in the two purebred dog breeds used in the largest numbers
- Examine whether genetic correlations exist between behavioural traits measured by Guide Dogs during puppy walking and training
- Quantify crossbreeding parameters for behavioural traits measured by Guide Dogs during puppy walking and training
- Estimate permanent environmental effects for behavioural traits measured by Guide Dogs during puppy walking and training
- Assess the presence/absence and magnitude of heritability for components of a puppy test used at Guide Dogs
- Quantify crossbreeding parameters for components of a puppy test used at Guide Dogs

3. HISTORY OF THE GUIDE DOG MOVEMENT AND CURRENT PRACTICES AT GUIDE DOGS

3.1. History of the guide dog movement

The first methodical training of dogs to act as guides for blind people began in Germany in 1916 in response to the needs of blinded German war veterans (Turner, 1989). Schools were established in Oldenburg, Württemberg and later Potsdam and Munich (Putnam, 1979). Mrs. Dorothy Harrison Eustis had established a dog breeding and training facility called Fortunate Fields in Vevey, Switzerland in 1923, providing German Shepherd Dogs for the Swiss army, police and customs service (International Guide Dog Federation, 2014). She visited the guide dog training school in Potsdam in 1925 and 1926 and was inspired to set up L'Oeil qui Voit in 1928 and shortly afterwards the first guide dog school in the United States of America (USA) which shares its name, but in translated form. The Seeing Eye, Inc. (TSE) in Morristown, New Jersey, USA, was founded in 1929 and is the oldest existing guide dog school in the world (Putnam, 1979).

The guide dog movement in the United Kingdom (UK) began a short time later in 1931, led by Muriel Crook and Rosamund Bond, and The Guide Dogs for the Blind Association (now known as Guide Dogs (GD)) was incorporated in 1934. Its training methods were instituted by Captain Nikolai Liakhoff, a graduate of L'Oeil qui Voit (Hartwell, 1942). Originally, German Shepherd Dogs were used almost exclusively, and were donated or bought for a few pounds (Lane, 1981). A breeding programme for guide dogs was founded in 1959 by the late Derek Freeman, with the purchase of a German Shepherd Dog called Reiner who became GD's first brood bitch in 1960 (Guide Dogs for the Blind Association, 2011). The number of puppies being bred by GD grew steadily,

and in 1970 Tollgate House near Leamington Spa became the headquarters of GD's breeding programme (Lane, 1981).

3.2. Current practices at Guide Dogs

GD opened a new, purpose-built National Breeding Centre (NBC) adjacent to Tollgate House in 2011, which provides capacity to breed up to 1500 puppies a year. Currently the number bred per year is approximately 1350. There are approximately 80 stud dogs and 275 brood bitches. As of 2013 there are more than 4,700 guide dog owners (GDOs) in the UK, and GD are currently responsible for approximately 7,800 dogs including breeding stock and retired animals.

3.2.1. Breed usage

Derek Freeman, who began GD's breeding programme, wrote "the following breeds have been found to be the most satisfactory as working guide dogs in Britain and other parts of the world: Labradors, German Shepherd Dogs, Golden Retrievers, occasionally Curly Coat and Flat Coat Retrievers, and some Collies. However, our greatest success has been with the Labrador crossed with the Golden Retriever and the Labrador crossed with the Curly Coat Retriever" (Freeman, 1991). GD have experimented with using at least 21 different pure breeds including those listed previously and many different crosses between these breeds, but after a review in 2009 it was decided that the only breeds and crosses that would continue to be bred were:

1. Labrador Retriever
2. Golden Retriever
3. German Shepherd Dog
4. Border Collie

5. Curly Coated Retriever
6. Flat Coated Retriever
7. Standard Poodle
8. Labrador Retriever cross Golden Retriever
9. Flat Coated Retriever cross Golden Retriever
10. Curly Coated Retriever cross Labrador Retriever
11. Border Collie cross Golden Retriever
12. Standard Poodle cross Labrador
13. German Shepherd Dog cross Golden Retriever

A subsequent review in 2013 led to a decision to phase out use of Border Collies, Flat Coated Retrievers and their crosses with Golden Retrievers. The numbers of Curly Coated Retrievers and their crosses, Standard Poodles and their crosses and German Shepherd Dog cross Golden Retrievers are also relatively low. For these reasons it was decided only to include pure Labrador Retrievers, Golden Retrievers and German Shepherd Dogs and crosses between Labrador Retrievers and Golden Retrievers for evaluation in this thesis.

3.2.2. Stages of a guide dog's life

A schematic diagram illustrates the potential paths of a puppy entering GD's colony, either by birth for dogs bred by GD or for puppies bought in from outside breeders (Figure 3.1), with a training pathway and a breeding pathway. Health or behavioural issues can lead to dogs being withdrawn at any stage and this is explored further in Chapter 5.

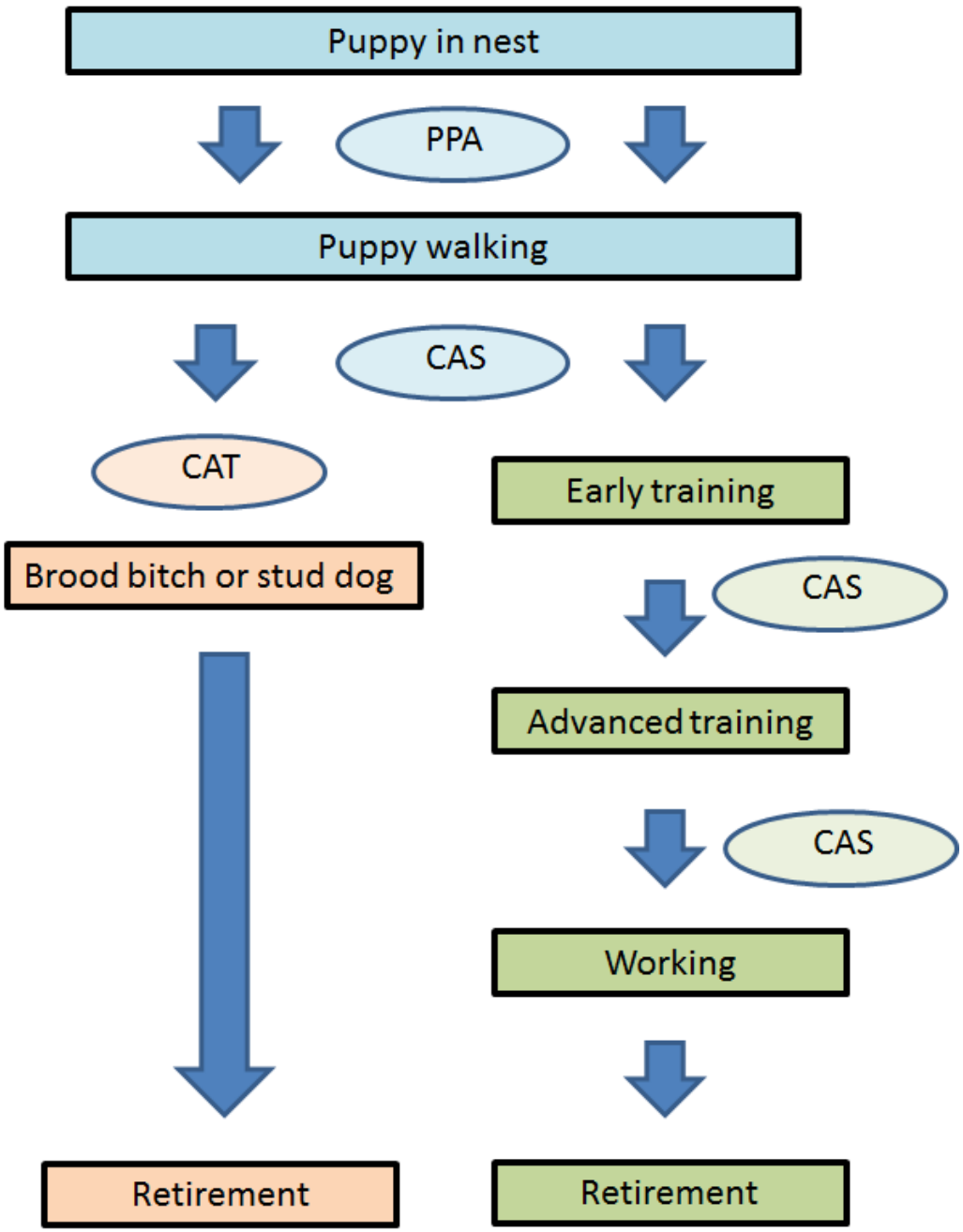

Figure 3.1 Schematic diagram showing potential pathways of a puppy entering GD's colony.

The terms in each box represent the “dog stage” names used by GD. Stages where behavioural tests are undertaken are illustrated. PPA= Puppy profiling assessment, CAS = Canine assessment summary, CAT = Character assessment tracker.

3.2.2.1. *Puppy in nest*

The majority of GD's brood bitches whelp in the home of their Brood Bitch Holder (BBH) and the puppies remain there from birth until entering the NBC at six to seven weeks of age. However, a small number of GD's brood bitches whelp at the NBC. Of these, some of the bitches and their puppies will remain in the NBC for the entire "puppy in nest" period, while others return to their BBH home for part of this time. At six to seven weeks of age the puppies will undergo the Puppy Profiling Assessment (PPA), health checks and vaccinations and remain in the NBC for approximately a week before going to live with volunteer puppy handlers, known as "Puppy Walkers" (PWs). Outside bred puppies will be brought into the NBC to undergo the PPA etc. at a slightly older age of around eight to nine weeks but from then on the pathway is the same for both GD-bred and outside bred puppies.

3.2.2.2. *Puppy walking*

Puppies live in the homes of volunteer puppy PWs, under the supervision of a Puppy Training Supervisor (PTS). Puppies remain with their puppy walker until 12-14 months of age, undergoing basic training and gaining exposure to as many different situations, environments and people as possible. They also undergo an observational behavioural assessment, called the Canine Assessment Summary (CAS) at the start of puppy walking and at five, eight and 12 months of age. Those dogs which have not been selected to be breeding stock will be neutered during the puppy walking stage. There is a pre-intake meeting to discuss the health and training of the dog before it progresses into training.

3.2.2.3. *Early training*

Dogs typically enter this stage at between 12 and 14 months of age. Most of them will stay in kennels during this period, but a few which fail to settle in a kennel environment will be boarded with volunteers. This stage is when they will start to learn the tasks and skills that a guide dog needs to be able to perform, and when they learn to work in a harness. They also learn left and right commands and to sit or stand at kerbs until told to move on by their instructor. In addition, they have to master a concept termed "right shoulder work", which involves considering the space needed for their handler when avoiding obstacles. Guide Dog Trainers (GDTs) usually train five dogs at a time, and usually these five dogs are at different stages of training.

GD has four training schools, at Leamington Spa, Atherton, Redbridge and Forfar. In general dogs remain in early training for approximately 16-18 weeks, until they are approved by a Senior Guide Dog Trainer (SGDT) to start advanced training. At this point there is a process known as "bidding" or "pick-up". Mobility team managers (MTMs) from the surrounding areas come to view any dogs which are ready for advanced training. The MTMs each have a list of the potential GDOs waiting for dogs in their area and will look for dogs which they think will suit these best. The MTMs bid for the dogs they want, and the dogs are allocated in order of priority with potential GDOs that have been waiting for longest or those who have had a dog previously and are awaiting a replacement having the highest priority.

3.2.2.4. *Advanced training*

Dogs enter advanced training at approximately 18 months of age. They spend 10 weeks being trained by a Guide Dog Mobility Instructor (GDMI) building on what was learnt in early training. Final matching takes place at this stage. The GDMI will visit the potential GDO with the dog and evaluation usually includes

a walk with the dog in harness to evaluate suitability of the match. Finally there is four to six weeks of what is known as “on-class training” working with the GDMI and their new GDO. The GDMI trains both the GDO and the dog to work together and when the GDMI and MTM feel that the pair is safe to work together they are formally certified as “qualified” and the dog goes to live with its new GDO.

3.2.2.5. *Qualified / working*

Once the guide dog and its GDO have qualified the GDMI will visit the partnership to ensure that everything is going well and to address any concerns the GDO may have. Visits from the GDMI are frequent at first, with at least four visits within the first three weeks, then six, 16 and 24 weeks after the last visit. Thereafter routine visits occur every second year with a telephone call on the year in between. This pattern continues until the dog is eight years old at which point visits are made every six months to ensure that the dog is coping with the demands of its working life as it ages. The average working life of a guide dog (after qualification) is five to six years.

3.2.2.6. *Retired*

Once it is felt that a guide dog is no longer able to carry on working it is retired. The GDO is usually offered the chance to keep the dog on as a pet, but if this is not possible the dog will be rehomed. The GDO can recommend friends or family that would like to have the dog, and then the puppy walker or any boarders the dog has stayed with are asked if they would like them. If none of these options are available there is a waiting list of people wishing to adopt a retired guide dog.

3.2.3. Management of potential breeding stock

Potential breeding animals of the future are identified at the planned mating stage and the resultant offspring are then monitored throughout their early life. Dogs making suitable progress return to the NBC after puppy walking at approximately 12 months of age and undergo a Character Assessment Tracker (CAT) which assesses three main areas of temperament – compliance, environmental awareness and willingness. During the CAT, dogs are exposed to a series of imposed visual and aural stimuli in a controlled environment and their responses are recorded (N.B. the CAS and CAT differ substantially by the fact that the latter measures responses to imposed stimuli whilst the former records general traits seen in the course of normal life). It is not a pass or fail test, rather the result of the CAT (a score from one to seven with values in the middle being desirable) is used as part of the decision-making process.

Approximately 5% of puppies are outside-bred. Currently these are mostly Standard Poodles and Curly-coated Retrievers (the vast majority of Labrador Retrievers, Golden Retrievers and German Shepherds are GD-bred). In addition, GD stud dogs are hired out to selected breeders and payment is one puppy from the litter that is produced – these are mostly Labrador Retrievers or Golden Retrievers. Outside-bred and half outside-bred puppies come to the NBC at approximately eight weeks of age and then follow the same pathways as GD-bred puppies except that all are flagged as potential breeding stock.

From 12 to 18 months accepted breeding stock live at brood stock holders' houses. The dogs are visited at 16 months by the Breeding Selection Supervisor for an assessment. Consideration of health history and training progress of littermates is carried out, as well as examination of the individual's health history. At 18 months they are considered at the breed review meeting and a decision is made as to whether they will remain in the breeding programme and be used for breeding or not.

3.2.3.1. *Breed review meeting*

A breed review meeting is held once a month, at the NBC. All breeding stock is reviewed at least annually following the assessment undertaken at 18 months of age.

Attendees:

Head of Breeding Programme

Breeding Stock Manager

Breeding Selection Supervisor

Chief Veterinary Officer

Canine Services Advisor*

Dog Care and Welfare Manager, Breeding Centre*

* these people collate information on individual dogs but do not actually make the decision.

The decision for any animal can be one of five outcomes:

- Clear to breed without any restrictions
- NB – No breeding.
- A1 alert – "be aware" e.g. there is a likely tendency to have a health or temperamental problem in this line. Review date given.
- A2 alert – more prescriptive. An issue has been identified and breeding decisions must be managed appropriately. For example, if the dog is a carrier of a particular condition with a genetic test it can only be mated to dogs which are clear of that condition.

- A3 alert – stop, pending further information from, for example, siblings or ancestors.

3.2.3.2. *Management of GD brood bitches*

Bitches begin to be used for breeding from 19 months of age onwards - they may be mated as soon as they have a season after the breed review meeting assuming they are fit and well. They are generally mated every other season except if they have a particularly long inter-season interval. However a consecutive-season mating is now usual, generally after a bitch's second litter, following research outcomes (data not shown). A maximum of five litters per bitch is permitted, with brood bitches retiring by 8 years of age. GD has special dispensation from the Kennel Club (KC) to register five litters from the same bitch on written application (as of 2012 the KC will only register four litters per bitch from other breeders).

The majority of bitches whelp at the BBH's home whilst potentially complicated cases and a small number of normal cases (for staff training purposes) whelp at the NBC. Complicated cases include a small or large litter or where the BBH may have limited ability. Approximately 15% whelp at the NBC. Brood bitch supervisors are on call 24 hours a day during the whelping period.

3.2.3.3. *Management of GD stud dogs*

Semen examination forms part of the assessment procedure for stud dogs. A new stud dog will be used to sire three litters then will be put "on hold" for a time until the progress of the puppies from these litters has been assessed. Generally stud dogs are used to produce five more litters and then held again to allow assessment of these further offspring. Then he will be allowed to sire

further litters. There is currently no set limit on the number of litters a dog may sire.

3.2.4. Genetic testing

Five DNA tests are currently used routinely by GD. Prospective breeding stock which are offspring of parents which are classed as either affected or carriers for a particular condition or whose status is unknown are tested at approximately 12 months of age before being accepted into the breeding programme. The DNA tests used are: for three forms of Progressive Retinal Atrophy (PRA) which all prospective breeding stock Labrador Retrievers, Golden Retrievers and crosses of these breeds must have; the superoxide dismutase 1 (*SOD1*) mutation for canine degenerative myelopathy in German Shepherd Dogs and Golden Retrievers but not currently their crosses; and for congenital ichthyosis in Golden Retrievers.

3.2.5. Guide Dogs Interactive (GDI)

GDI is a portal to a structured query language (SQL) relational database which can be accessed via a browser over GD's internal network. The database contains all the information, including pedigree, health records and behavioural test results, relating to all of GD's dogs. It came into use in 2004 and has enabled staff both to enter and access information more quickly than the previous 15-year-old database systems (Lamb, 2004). The data held in this SQL database will be used in subsequent analyses.

4. PREPARATION OF THE PEDIGREE FILE FOR ANIMAL MODEL RESTRICTED MAXIMUM LIKELIHOOD (REML) AND BEST LINEAR UNBIASED PREDICTION (BLUP) ANALYSIS

4.1. Introduction

Guide Dogs (GD) have maintained pedigree records since commencing their breeding programme in 1959. Initially this was done as paper records for each dog but over time it has become one electronic record and is now a table in Guide Dogs Interactive (GDI).

Correct pedigree information is vitally important in a successful breeding program, especially when the animal model is being used as in this scenario an incorrectly identified sire of an individual will affect the EBV not only of that individual but also the EBV of all of its relatives (Visscher et al 2002). Indeed, genetic evaluations of individuals by best linear unbiased predictors (BLUP) methods assume that all genetic relationships among individuals included in the analysis are correct (Israel & Weller, 2000; Parlato & Van Vleck, 2012). Phenotypes from as many animals as possible, including those with incomplete pedigrees, are critical for accurate genetic evaluations but the utility of such phenotypes is decreased considerably by unknown relationships to other animals in the population (Cassell et al, 2003). Incomplete pedigrees also results in underestimation of the rate of inbreeding (Woolliams & Mäntysaari, 1995).

A pedigree file to be used in heritability analyses should have one entry per individual and should accurately reflect all familial relationships. It became apparent that this was not the case with the raw pedigree file obtained from GD – many dogs had multiple duplicate entries and many dogs were listed with no known parents when in fact their parents were in the pedigree. Both

of these things mean that the degree of relationship between many individuals in the pedigree would appear considerably lower than it actually ws, which would artificially lower the estimated heritability of any disease condition or behavioural trait and make it impossible to have any confidence in the EBVs produced. A great deal of work was required to clean and recode the original pedigree file held in the main Guide Dogs database, and this is described in this chapter. This process was essential to ensure the accuracy of heritability and EBV calculations for both disease conditions and behavioural traits.

4.2. Materials and methods

4.2.1. Description of dataset

GD provided a copy of their main database which contained data up to 2nd February 2012. The pedigree file contained therein was exported from SQLServer® to Access® (both Microsoft) for scrutiny. The raw pedigree file contained 49189 records (supposedly 49189 individual dogs) of 59 different breeds and crosses, a full list of all 59 breeds and crosses is in Appendix 1.

The pedigree file contained rudimentary details of all individuals purchased or bred by GD and their known ancestors – when a dog is purchased by GD as much of their pedigree information as possible is added to the master pedigree file. This is actually a source of many of the errors which have arisen in the file as dogs have been added when they were already in the pedigree file. The information for each dog in the pedigree file were ID number (a unique identifier), GD ID number (another unique identifier which not all dogs in the pedigree file have; it links to health and behavioural records), KC name, KC ID number, sire ID number, dam ID number, breed code, sex and litter ID number. KC name and ID number were blank for 30941 dogs, partly reflecting the fact that many dogs bred by GD are not registered with the KC, but also

that these data are not consistently entered for KC-registered dogs in the pedigree. Breed code is also inconsistently entered, particularly when whole pedigrees of purchased dogs are entered. Some 11219 dogs had the code 59 which indicates “unknown breed – migration purposes only”.

Microsoft Access® allows moderate data manipulation through directed use of tables and queries, but some of the more complex data manipulation required the writing of simple specific programmes. Such programmes were written using MATLAB ® (The Mathworks, Inc.). It will be indicated in the subsequent editing steps where such programmes were used.

4.2.2. Data validation

The pedigree was edited with two main aims: firstly, the removal of duplicated records to create a pedigree in which each dog had only one record which linked to all its relatives; secondly, the addition of new records for dogs which were the relatives of dogs in the pedigree if this revealed cryptic relationships. Table 4.1 shows the number of dogs in the dataset before and after each round of validation. The final dataset contained 53283 dogs. This number is larger than the original raw dataset due to the addition of new records mentioned above.

4.2.2.1. *Removal of duplicated records*

The first stage of establishing the extent of the problems with the pedigree and attempting to rectify them was to run a simple query in Access® to identify duplicates in the raw pedigree file. The resultant duplicates file, which only contained exact duplicates, contained 6777 records. This included duplicates that appeared exactly the same to the program (such as Abben Brecan and Abben Brecan), but did not find those which appeared different due to the inclusion of honorifics or the presence of spelling mistakes or

abbreviations (for example, Sh.Ch.Va. Italian Sieger Natz v Arminius and Natz von Arminius both describe the same dog but are not recognised as such by this simple query).

The duplicates file was used as a starting point for identifying more duplicates in the raw pedigree file – the kennel affix part of each dog name appearing in the duplicate file was used as a search term against the raw pedigree file in Access® and this identified many additional duplicated individuals. A new file was created for each individual dog with more than one entry in the raw pedigree file, collating all of its identifying numbers, allocating one name (minus honorifics and spelt correctly) and assigning the correct breed code. Some 1855 files were created in this way.

Programs were then created in MATLAB® to bring together all the duplicates for each dog into one identity per dog, to flag up if as a result of this any dog appeared to have multiple sires or dams (which were then corrected) or if any dog appeared as both a sire and dam (which was then corrected). At each stage, additional duplicates were identified and collated.

4.2.2.2. *Addition of ancestors*

When individuals appeared to be founders, i.e. with no parents listed, online pedigree databases including www.pedigreedatabase.com, www.k9data.com, www.huntinglabpedigree.com and www.universal-dog.eu were searched to see if parents could be identified. If no online pedigree databases yielded information then the KC pedigree files held by a colleague in the Kennel Club Genetics Centre at the Animal Health Trust were interrogated. If it was found that the parents of these supposed founders were actually in the pedigree file or were offspring or siblings of dogs in the pedigree file then this information was added. An additional file was created in Excel® (Microsoft) to contain this information. This step was undertaken both for dogs with duplicated records and for those with only a single record and yielded a file containing sire and

dam information for 8356 dogs, some of which were new dogs whose addition to the pedigree file revealed cryptic relationships.

A final MATLAB® program to bring together all of the newly gathered information and create a final master pedigree file was created. Then a relatively simple recoding protocol was applied to create a pedigree file ready for use in heritability analyses in ASReml with sire and dam ID numbers being lower than that of their offspring.

Table 4.1 Number of dogs retained, removed and added at each editing step described in the materials and methods. The percentage expressed is with reference to the number of dogs in the raw dataset.

Editing step	Number remaining	Number lost or added	Percentage remaining
Raw data set	49189	-	-
Removal of duplicates	44806	4383	91%
Addition of ancestors	53283	8477	108%

4.2.3. Estimation of inbreeding coefficients

The coefficient of inbreeding, generally symbolised by *F*, is a measure of genetic diversity first defined by Wright (1922), and represents the probability that the two alleles at any locus in an individual are identical by descent (IBD). It refers to an individual and reflects the degree of relationship between that individual's parents (Falconer & Mackay, 1996). An individual whose parents are completely unrelated will have an inbreeding coefficient of F = 0. Individual inbreeding coefficients were calculated using the algorithm of Meuwissen and Luo (1992) and all available pedigree information. This algorithm enables the calculation of Wright's inbreeding coefficient in large populations quickly and only requires a small amount of computer memory (Meuwissen & Luo, 1992). A MATLAB ® program was used to calculate

inbreeding coefficients using this algorithm for all 53283 individuals in the amended pedigree.

4.3. Results

The final pedigree file contained 53283 dogs, of which 20219 were Labrador Retrievers, 8226 were Golden Retrievers and 6290 were German Shepherd Dogs. There were 6041 unique sires and 12137 unique dams. The mean number of offspring per sire was 7.24, with a minimum of 1 and a maximum of 620. The mean number of offspring per dam was 3.87, with a minimum of 1 and a maximum of 61. The mean inbreeding coefficient was 0.05 (standard deviation 0.06).

4.4. Conclusion

A considerable amount of time and effort was spent on preparation of the pedigree file for use in REML and BLUP analysis. The resultant pedigree file can now be used in subsequent analyses.

5. SELECTION AIMS AT GUIDE DOGS AND REASONS FOR WITHDRAWAL OF DOGS FROM THE GUIDE DOG PROGRAMME.

5.1. Introduction

The first step in designing or evaluating a breeding programme is to define its objectives clearly, and breeding objectives must lead to selection criteria which relate to predicted genetic change (Goddard, 1998). To accomplish selection whole animals must be selected, even if they possess some undesirable traits along with their more highly prized ones (Leighton, 1997).

Lane (1981) stated that a guide dog should stand not less than 48.5cm to the shoulder, must be physically fit and capable of walking at least 10 miles a day at a normal pace, must be reasonably bold, free from suspicion and with no aggressive tendencies and above all it must be intelligent and willing to please. However, as there is substantial variation among guide dogs owners (GDOs) there is no single perfect "type" of guide dog but rather there must also be variation among the dogs available for matching with prospective GDOs (Schiefelbein, 2012). A guide dog which will be a perfect match for a small, frail, elderly person living in a rural location is unlikely to fit the needs of a younger, fitter person based in a city with a demanding schedule.

Knol et al (1988) gave a general description of what makes a good guide dog, comprising physical characteristics, skills and behaviour. They stated that a guide dog must be healthy and of appropriate size, build and coat type; must be able to perform necessary tasks without a previous command, on command and to refuse a command if it is unsafe; and must be obedient and eager to perform tasks although not being easily excited and remaining calm. It can be seen that there will be some variation among dogs with respect to these characteristics such as "appropriate size and build" as this will vary for

different GDOs. Guide dogs need to be heavy enough to guide their GDOs safely and must also be the appropriate height for the owner to reach the harness handle comfortably, but dogs which are too heavy or tall may be difficult for GDOs to handle comfortably (Helmink et al, 2003).

Between 2003 and 2007 the success rate at Guide Dogs (GD), defined as the proportion of dogs which successfully complete their training, was 70% which is high compared to other guide dog organisations (GD, unpublished data). However, it is important for GD not just that the vast majority of dogs they breed or otherwise obtain successfully qualify as guide dogs but also that they are able to work for a number of years after qualification. Currently the average working life of a guide dog is five to six years (Guide Dogs for the Blind Association, 2014). Working dogs of any kind that have to be withdrawn from service prematurely represent not only the loss of valuable assets in their own right but also the loss of resources used to purchase or breed them, to train them and care for them (Evans et al 2007). In addition any premature retirement of a guide dog will cause significant emotional distress to the guide dog owner (GDO) (Olson et al, 2004).

Selection for many traits simultaneously is possible by means of a selection index, as discussed in Chapter 2. The relative importance of different traits, their heritabilities and genetic correlations between all traits must be considered. In livestock production the relative importance of different traits is judged in terms of economics. Each trait is given an economic weight which reflects its importance for production. However the weighting of traits may also include ethical values and animal welfare aspects (Lindhé & Philipsson, 1998). Non-economic weightings are more difficult to quantify and are more subjective (Kanis et al, 2005). Although it may be possible to quantify some economic impact of traits measured by GD, for example if the presence or absence of a characteristic necessitates withdrawal of a dog from GD's programme, it is likely that the importance of many traits will be largely non-economic. It is important to remember that selection priorities, the resultant selection index and measurements of progress are not, and should not be, set

in stone but rather should be reviewed regularly in light of the latest data (Collins et al, 2011).

In this chapter two avenues used to explore breeding priorities at GD are described. Firstly, the reasons why dogs were withdrawn from GD's programme were examined to establish the major reasons dogs either failed to qualify as guide dogs or were prematurely retired as working guide dogs. Secondly a short survey of the key members of staff involved in the decision-making process around which dogs to use as breeding stock is described.

5.2. Materials and methods

5.2.1. Description of withdrawal dataset

GD provided a spreadsheet of all dogs withdrawn between 1995 and 2012. Information for each dog included breed, sex, which stage the dog was at prior to withdrawal, primary withdrawal reasons and any additional notes. The full dataset consisted of 8432 dogs of 43 breeds and crosses (see Appendix 3). GD categorise dogs that are withdrawn from work before the age of 9 years as withdrawals rather than retirement, but this has only been rigorously applied in the last few years so some dogs that stopped work before the age of 9 years in the early part of the period under consideration may have been missed as they may have been recorded as retirements.

5.2.2. Data validation

5.2.2.1. *Breed*

The data were edited based on breed. For the reasons given in Chapter 1, German Shepherd Dogs (GSDs), Golden Retrievers (GRs), Labrador Retrievers (LRs), and Golden Retrievers crossed with Labrador Retrievers were included

in subsequent analyses (Table 5.2), as there were insufficient numbers of individuals of other breeds and crosses for quantitative genetic analysis. Breeds which were removed included Australian Shepherds, Bernese Mountain Dogs, Border Collies, Chesapeake Bay Retrievers, Curly Coated Retrievers, Flat Coated Retrievers, Irish Water Spaniels, Italian Spinones, Leonbergers, Standard Poodles, Tervuerens and Weimaraners and crosses involving these breeds. This excluded 540 dogs as shown in Table 5.1. The final data set analysed contained 7892 dogs.

Table 5.1 Number of dogs retained and removed at each editing step described in the materials and methods. The percentage expressed is with reference to the number of dogs in the raw dataset.

Editing step	Number remaining	Number lost	Percentage remaining
Raw data set	8432	-	-
Breed	7892	540	94%

Table 5.2 Number of records available for the breeds and crosses included in subsequent analyses.

Breed	Number of dogs
German Shepherd Dog (GSD)	627
Golden Retriever (GR)	1121
GR x (GR x LR)	29
Golden Retriever x LR	2403
Labrador Retriever (LR)	3303
LR x (LR x GR)	409
Total	7892

5.2.2.2. *Recoding of reasons for withdrawal*

Vague reason for withdrawal codes in the raw dataset included death/died, euthanasia, unknown reason for withdrawal, withdrawn for health and "not due to health or temperament of dog – DO NOT USE IN GDI". The notes of any dogs with these withdrawal codes were checked and where possible a more specific code was allocated. New codes of "GDO-related" and "no match found" were created and allocated to some of these dogs. Disease conditions necessitating withdrawal were grouped by organ system. Specific disease conditions affecting less than three individuals were then grouped together into categories such as "other disorder of skin".

5.2.3. Survey of selection aims

At the Breed Review Meeting on 14th April 2011, a short survey form was handed out to the seven regular attendees of these meetings. These people are those most closely involved with deciding which young stock will become breeding stock. They are the Breeding Centre Manager, Dog Care and Welfare Manager (Breeding Centre), Chief Veterinary Officer, Breeding Stock Manager, Breeding Selection Supervisor, National Dog Health Screening Co-ordinator (now called the Puppy Selection and Screening Supervisor and no longer an attendee of the meeting) and Canine Services Advisor. A copy of the blank survey form is included in Appendix 2.

The survey was designed to elucidate clearly the desired traits for breeding stock and how important each of these selected traits were considered when selecting new breeding stock. The survey form was designed following a review of the literature regarding desirable characteristics of guide dogs. A draft version of the form was created and then amended after input from a former manager of GD's breeding programme and members of the

Epidemiology of Guide Dog Behaviour research group at the University of Nottingham.

Each respondent was asked to allocate 100 points in any way that they wished (so long as the total did not exceed 100) to a list of traits, such that the number of points allocated to each trait reflected how important a selection criterion that trait is to the respondent. They were asked to repeat this procedure for each of the four breeds/crosses which are maintained in numbers by GD – Labrador Retriever, Golden Retriever, Labrador Retriever cross Golden Retriever and German Shepherd Dog (GSD).

The list of traits was separated into three categories – appearance, temperament and health. Criteria listed under appearance were breed conformation and movement, height, weight, coat length and cosmetic appearance. The criteria relating to temperament were compliance, environmental awareness, willingness, confidence and lack of aggressive behaviour. Under health, the listed criteria were acceptable hip score, acceptable elbow score, acceptable shoulder "score" and clear eye examination. There was also space for respondents to add any other criteria they felt had been omitted, in each of the three categories and at the end. The survey was conducted anonymously, although respondents were told that they could put their name on their form if they wished. Postage-paid addressed envelopes were provided for return of completed forms.

5.3. Results

5.3.1. Reasons for withdrawal

The stage at which dogs were withdrawn from GD's programme is shown in Table 5.3. The majority of withdrawals took place during early training (3675 of 7892 dogs, 47%) with the smallest proportion occurring after qualification (939 of 7892 dogs, 12%). Categories of withdrawal reasons are shown in

Table 5.4. Most dogs were withdrawn for behavioural reasons (5327 of 7892 dogs, 68%) with health reasons accounting for 29% (2257 of 7892 dogs) of dogs withdrawn between 1995 and 2012. The proportions of dogs withdrawn for health, behaviour and other reasons were approximately equal between the different breeds and crosses, as shown in Table 5.5.

Table 5.3 Stage at which the dog was withdrawn from GD's programme for 7892 dogs which were withdrawn between 1995 and 2012.

Withdrawal stage	Number	Percentage
Puppy walking	1679	21.27
Early training	3675	46.57
Advanced training	1599	20.26
Qualified	939	11.90
Total	7892	100

Table 5.4 Categories of withdrawal reasons for 7892 dogs which were withdrawn between 1995 and 2012.

Withdrawal category	Number	Percentage
Health	2257	28.60
Behaviour	5327	67.50
GDO-related	180	2.28
No match found	4	0.05
Other	6	0.07
Unknown	118	1.50
Total	7892	100

Table 5.5 Number and percentage of dogs of each breed and cross in the dataset which were withdrawn for health, behavioural or other reasons. The percentage is shown in brackets.

Breed	Health	Behaviour	Other	Total
German Shepherd Dog	205 (32.7)	409 (65.2)	13 (2.1)	627
Golden Retriever	325 (29.0)	761 (67.9)	35 (3.1)	1121
GR x (GR x LR)	11 (37.9)	16 (55.2)	2 (6.9)	29
GR x LR	633 (26.3)	1656 (68.9)	114 (4.7)	2403
Labrador Retriever	993 (30.1)	2185 (66.2)	125 (3.8)	3303
LR x (LR x GR)	90 (22.0)	300 (73.3)	19 (4.6)	409
Total	2257 (28.6)	5327 (67.5)	308 (3.9)	7892

5.3.1.1. *Health reasons for withdrawal*

The stage at which dogs were withdrawn for health reasons is shown in Table 5.6. Compared with withdrawals for all reasons (Table 5.3) a greater proportion of dogs were withdrawn during puppy walking (781 of 2257 dogs, representing 35%) for health reasons. However early training was still the stage during which most withdrawals took place, with 40% (894 of 2257 dogs) of dogs which were withdrawn for health between 1995 and 2012 being withdrawn during early training. Reasons for withdrawal for health by body system affected are shown in Table 5.7. Reasons for withdrawal for health by body system affected for each of the different breeds and crosses considered and a full list of all disease conditions with 3 or more affected individuals necessitating withdrawal from GD's programme are in Appendix 4.

Table 5.6 Stage at which the dog was withdrawn from GD's programme for 2257 dogs which were withdrawn for health reasons between 1995 and 2012.

Withdrawal stage	Number	Percentage
Puppy walking	781	34.6
Early training	894	39.6
Advanced training	343	15.2
Qualified	239	10.6
Total	2257	100

Table 5.7 Reasons for withdrawal for health by body system affected for the 2257 dogs withdrawn for health reasons between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Musculoskeletal	975	43.20
Dermatological	474	21.00
Ophthalmological	182	8.06
Neurological	120	5.32
Gastrointestinal	82	3.63
Cancer	57	2.53
Cardiovascular	55	2.44
Death (cause unspecified)	47	2.08
Urological	44	1.95
Trauma or accidental death	43	1.91
Other	34	1.51
General health deterioration	33	1.46
Hepatic	23	1.02
Aural	21	0.93
Physical appearance	21	0.93
Autoimmune	17	0.75
Endocrinological	15	0.66
Respiratory	14	0.62
Total	2257	100

Musculoskeletal conditions were by far the biggest group of conditions necessitating withdrawal of dogs from GD's programme, accounting for 43% (975 of 2257 dogs) of all withdrawals for health between 1995 and 2012. Within this category, hip dysplasia and elbow dysplasia were the top two diagnoses, accounting for 70% of withdrawals due to musculoskeletal disorders between them as shown in Table 5.8.

The next largest group of conditions was dermatological, accounting for 21% (474 of 2257 dogs) of withdrawals for health between 1995 and 2012. Atopic dermatitis or allergic skin disease accounted for 86% of withdrawals due to dermatological conditions, as shown in Table 5.9. The third largest group of conditions necessitating withdrawal from GD's programme was ophthalmological conditions, accounting for 8% (182 of 2257 dogs), with cataract comprising 46% (84 of 182 dogs) of ophthalmological conditions as shown in Table 5.10.

Neurological conditions were the fourth largest group of conditions necessitating withdrawal from GD's programme accounting for 5% (120 of 2257 dogs) of withdrawals for health reasons between 1995 and 2012, with epilepsy or seizures comprising 80% (96 of 120 dogs) of withdrawals due to neurological conditions as shown in Table 5.11. Thus the top five diagnoses necessitating withdrawal from GD's programme between 1995 and 2012 were atopic dermatitis or allergic skin disease, hip dysplasia, elbow dysplasia, epilepsy or seizures and cataract.

Table 5.8 Disease conditions given as reasons for withdrawal for 975 dogs withdrawn due to a musculoskeletal condition between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Hip dysplasia	353	36.21
Elbow dysplasia	328	33.64
Other forelimb lameness	55	5.64
Other musculoskeletal disorder	44	4.51
Arthritis	38	3.90
Osteochondritis dissecans	32	3.28
Cruciate ligament disease	27	2.77
Patellar luxation	23	2.36
Limb deformity	15	1.54
Disorder of spine	14	1.44
Other hindlimb lameness	14	1.44
Poor conformation	12	1.23
Congenital spinal abnormalities	5	0.51
Panosteitis	5	0.51
Amputation	4	0.41
Short radius syndrome	3	0.31
Total	975	100

Table 5.9 Disease conditions given as reasons for withdrawal for 474 dogs withdrawn due to a dermatological condition between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Atopic dermatitis or allergic skin disease	407	85.86
Other disorder of skin	46	9.70
Discoid lupus erythematosus	12	2.54
Congenital ichthyosis	9	1.90
Total	474	100

Table 5.10 Disease conditions given as reasons for withdrawal for 182 dogs withdrawn due to an ophthalmological condition between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Cataract	84	46.15
Other ophthalmological condition	42	23.08
Geographic retinal dysplasia	27	14.84
Multifocal retinal dysplasia	12	6.58
Hypoplasia of optic nerve	4	2.20
Retinal degeneration	4	2.20
Generalised progressive retinal atrophy	3	1.65
Epiphora	3	1.65
Retinopathy	3	1.65
Total	182	100

Table 5.11 Disease conditions given as reasons for withdrawal for 120 dogs withdrawn due to a neurological condition between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Epilepsy or seizures	96	80.00
Other neurological condition	13	10.83
Meningitis	7	5.83
Congenital abnormalities of brain	4	3.34
Total	120	100

5.3.1.2. *Behavioural reasons for withdrawal*

The stage at which dogs were withdrawn for behavioural reasons is shown in Table 5.12. The behavioural withdrawal reasons given all related to traits measured in GD's Canine Assessment Summary (CAS) except for social behaviour and post-qualification habits. Early training was the stage during which most withdrawals took place, with 52% (2775 of 5327 dogs) of dogs which were withdrawn for behavioural reasons between 1995 and 2012 being withdrawn during early training. Behavioural reasons necessitating withdrawal of dogs from GD's programme between 1995 and 2012 are shown in Table 5.13. Behavioural reasons necessitating withdrawal of dogs from GD's programme for the six breeds and crosses under consideration and a full list of all specific behavioural reasons necessitating withdrawal from GD's programme, showing for example what a dog was distracted by, are in Appendix 5. Distraction accounted for the majority of withdrawals for behavioural reasons, accounting for 22% (1150 of 5327 dogs) of such withdrawals. High suspicion, low attentiveness, low stress resilience and aggression towards people all accounted for more than 10% of withdrawals from GD's programme for behavioural reasons between 1995 and 2012.

Table 5.12 Stage at which the dog was withdrawn from GD's programme for 5327 dogs which were withdrawn for behavioural reasons between 1995 and 2012.

Withdrawal stage	Number	Percentage
Puppy walking	895	16.8
Early training	2775	52.1
Advanced training	1231	23.1
Qualified	426	8.0
Total	5327	100

Table 5.13 Reasons for withdrawal for the 5327 dogs withdrawn for behavioural reasons between 1995 and 2012 in descending order.

Withdrawal reason	Number	Percentage
Distraction – high	1150	21.61
Suspicion – high	832	15.62
Attentiveness – low	696	13.07
Stress resilience - low	610	11.45
Aggression towards people	562	10.55
Confidence – low	464	8.71
Social behaviour - unacceptable	356	6.68
Willingness - low	340	6.38
Aggression towards animals	191	3.59
Body sensitivity - high	84	1.58
Unacceptable post-qualification habits	42	0.79
Total	5327	100

5.3.2. Survey of selection aims

All seven completed forms were returned. Only one respondent had put their name on their form. Data were entered into an Excel® (Microsoft) spreadsheet for analysis. The mean and standard deviation of the score were calculated for each trait in each breed. The mean score allocated to each selection trait (including those added by some respondents) for each of the four breeds and crosses are shown in Table 5.14. In the appearance section, no respondents had added any criteria, but one respondent had noted that selection for breed conformation and movement may actually involve breeding away from the Kennel Club (KC) breed standard, particularly in the case of the GSD. Under temperament, one respondent had added the criterion adaptability. In the health section, five respondents had added at least one criterion which they felt had been omitted. Two respondents added freedom from atopy, freedom from exocrine pancreatic insufficiency (EPI) and freedom from canine degenerative myelopathy (also known as canine degenerative radiculomyelopathy, CDRM). One respondent added freedom from EPI and freedom from CDRM and one respondent added freedom from skin disease. One respondent added general health, skin, freedom from CDRM and clear genetic tests for Progressive Retinal Atrophy (PRA) – however, it was decided that absence of the mutations causing two forms of PRA had been considered to form part of a clear eye examination by other respondents, and so the points allocated to this additional criterion by this respondent were added to those for clear eye examination. Freedom from atopy, freedom from skin disease and skin were combined into a single criterion “freedom from atopy”. One respondent added a note next to “clear heart examination”, saying that not all breeds have a heart examination but if one was done then only animals with a clear examination would be used for breeding.

Box-and-whisker plots of the distribution of scores for selection traits for the four breeds and crosses are shown in Figures 5.1-5.4. A box-and-whisker plot comprises a box which represents the interquartile range (enclosing the central 50% of the data) and is bounded by the upper and lower quartiles. The median is marked by a solid line in the box. The whiskers are lines extending from the box to the 2.5th percentile and the 97.5th percentile; outliers are indicated by hollow circles. The box-and-whisker plot of selection aims for Labrador Retrievers (Figure 5.1) and that of selection aims for Labrador x Golden Retrievers (Figure 5.2) was almost identical. There were marked differences however between these two and those for Golden Retrievers (Figure 5.3) and German Shepherd Dogs (Figure 5.4). Freedom from EPI and CDM were both allocated points by the majority of respondents in the GSD, but not in any other breed. Breed conformation and movement tended to be allocated more points in the GSD compared with the other breeds and crosses which fits with the comment of one respondent noted above. A clear heart examination was given the most points by the majority of respondents in the Golden Retriever, closely followed by the Labrador cross Golden Retriever. The criterion of adaptability which had been added by one respondent only applied to the Labrador Retriever and Labrador cross Golden Retriever. Lack of aggressive behaviour appeared to be the most important behavioural trait for selection in all four breeds and crosses.

Table 5.14 Mean score allocated to the different selection criteria for the four breeds and crosses by seven respondents.

Criteria	LR	LR x GR	GR	GSD
Appearance traits				
Conformation and movement	4.9	3.0	4.8	5.1
Height	1.9	1.1	1.9	2.0
Weight	2.1	1.4	2.1	2.1
Coat length	1.4	1.1	1.9	1.7
Cosmetic appearance	2.9	2.1	2.9	3.6
Behavioural traits				
Compliance	7.6	9.0	7.5	8.0
Environmental awareness	6.8	8.1	6.7	6.9
Willingness	7.7	9.0	7.3	7.3
Confidence	8.6	9.1	9.5	8.7
Lack of aggressive behaviour	10.6	12.0	10.5	10.9
Adaptability	0.4	0.7	0	0
Health traits				
Acceptable hip score	8.0	7.0	7.6	7.9
Acceptable elbow score	9.0	8.1	8.8	8.7
Acceptable shoulder “score”	7.3	6.3	6.9	6.9
Clear eye examination	10.8	10.0	9.5	9.4
Clear heart examination	5.4	7.1	8.5	4.3
Freedom from atopy	4.0	3.9	4.1	1.1
Freedom from EPI	0	0	0	1.6
Freedom from CDRM	0	0	0	3.1
General health	0.7	0.7	0.7	0.7
Total	100	100	100	100

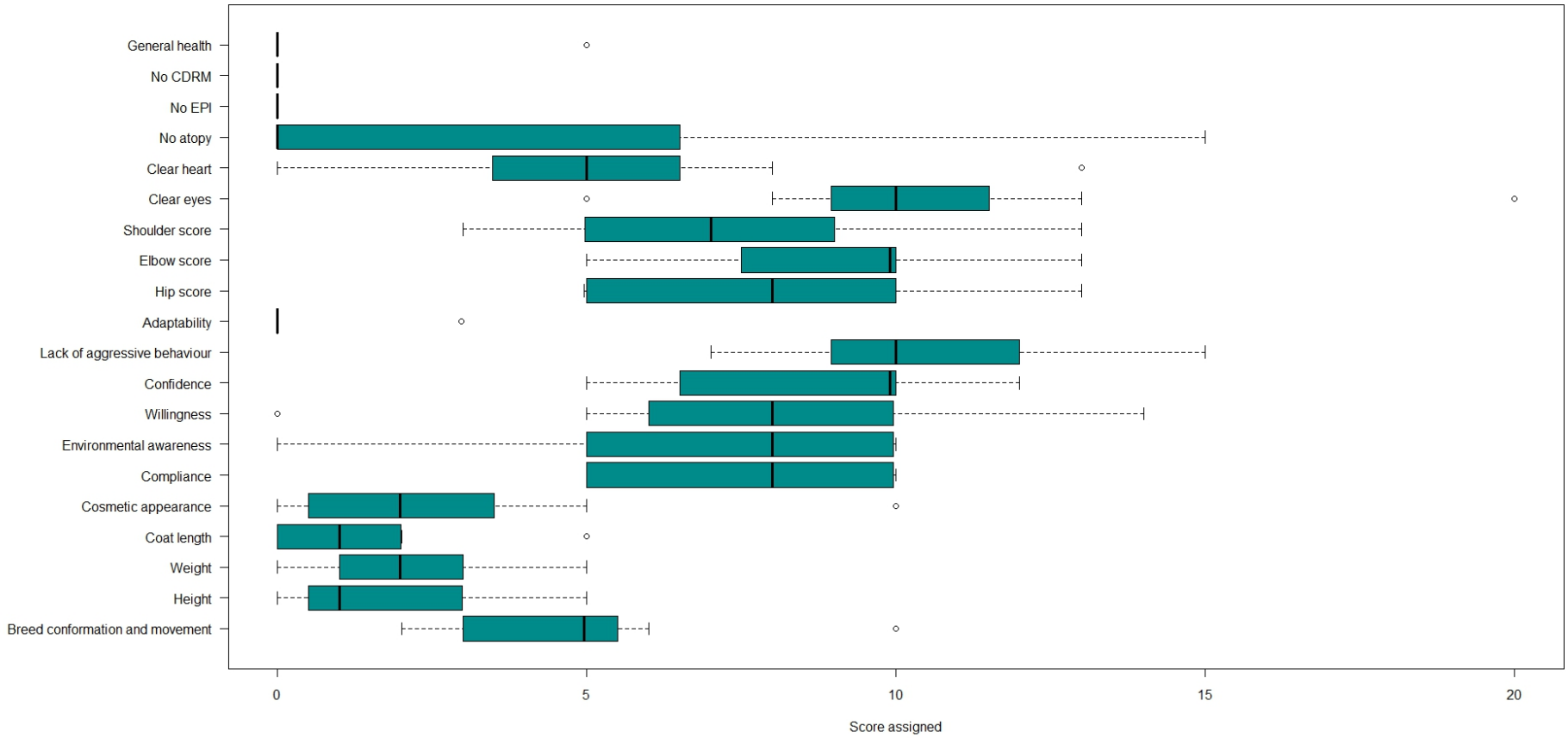

Table 5.15 Box-and-whisker plot showing the points allocated to different selection aims by 7 respondents for Labrador Retrievers.

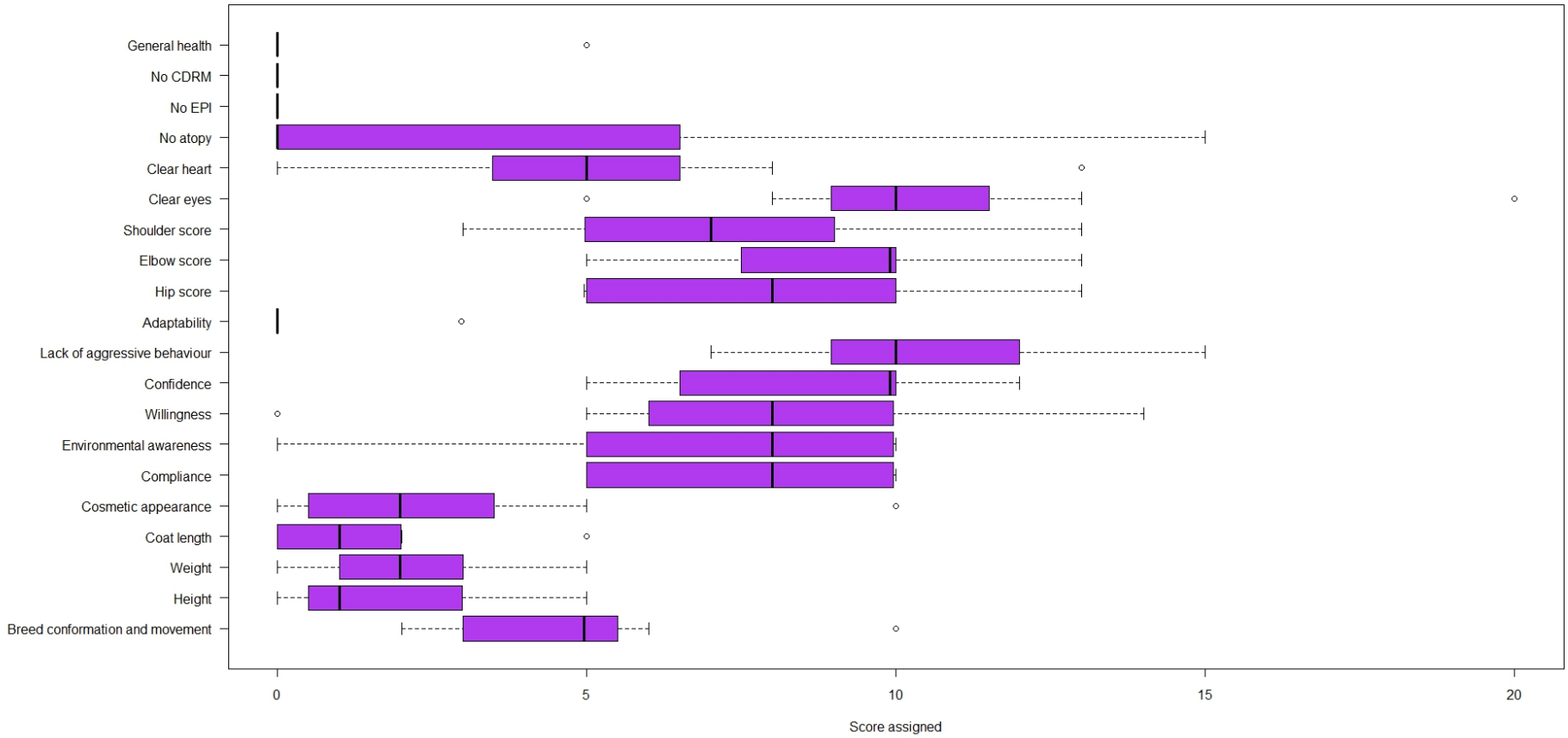

Table 5.16 Box-and-whisker plot showing the points allocated to different selection aims by 7 respondents for Labrador x Golden Retriever.

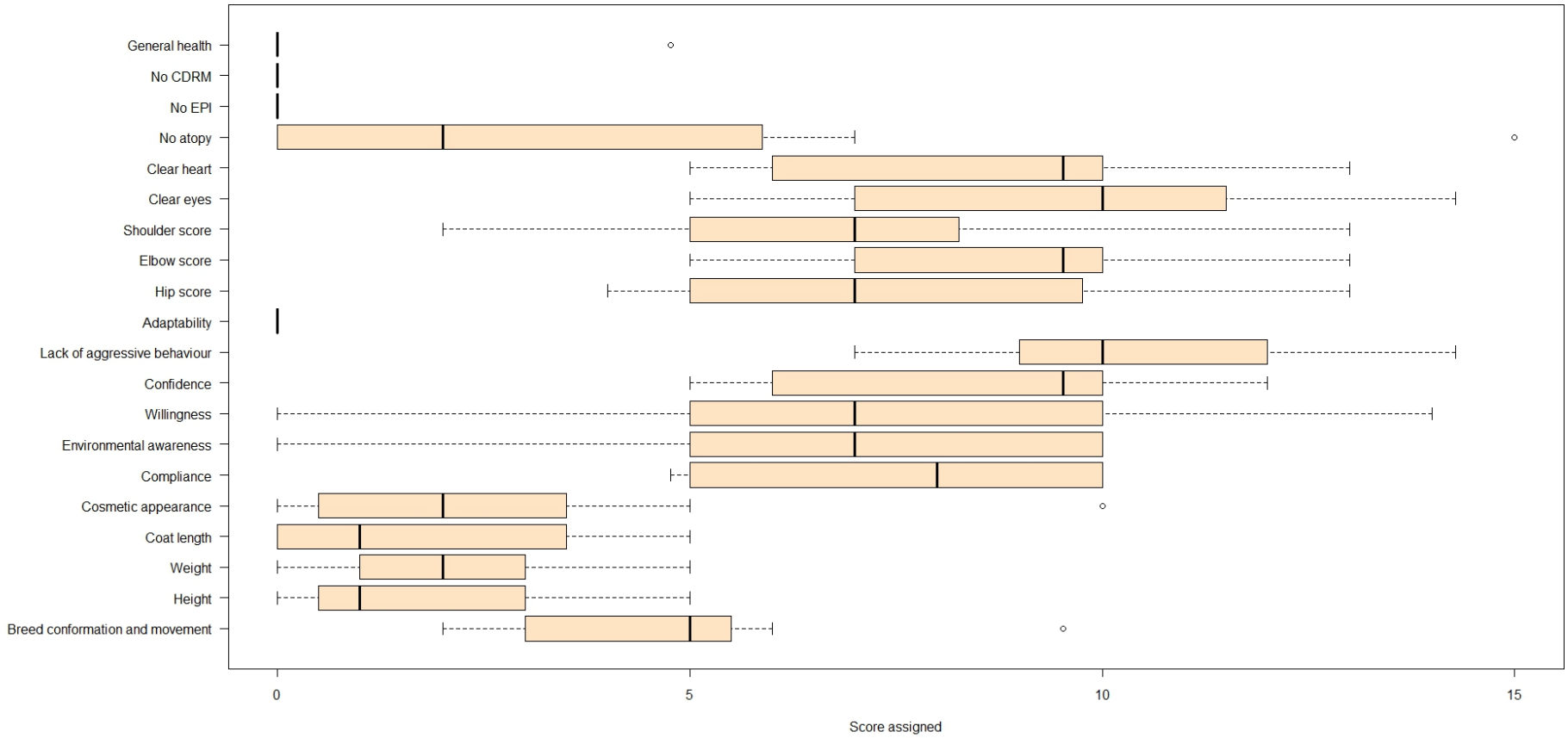

Table 5.17 Box-and-whisker plot showing the points allocated to different selection aims by 7 respondents for Golden Retrievers.

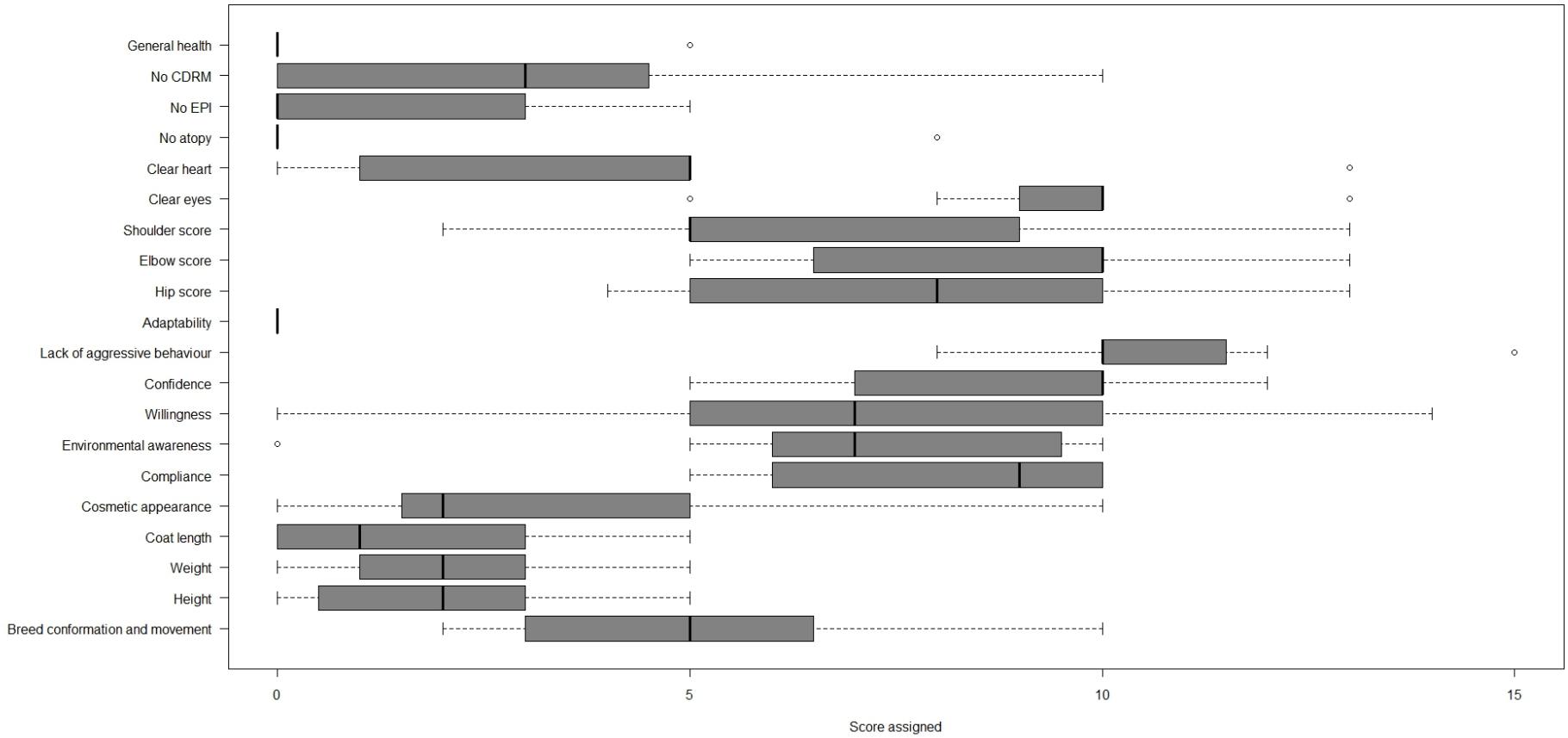

Table 5.18 Box-and-whisker plot showing the points allocated to different selection aims by 7 respondents for German Shepherd Dogs.

5.4. Discussion

Two methods were used to explore breeding priorities at GD; examination of reasons for withdrawal of dogs from GD's programme and a survey of selection aims. It was hoped that the results of these two analyses might indicate which traits should be included in a selection index, subject to suitable results of genetic analyses, and also possibly what the relative weightings of the different traits might be.

Although theoretically a selection index can contain any number of traits, if the number of traits in a selection index is not limited problems may arise with calculating index weights and monitoring of the selection process may be difficult (Lindhé, 1999). Lindhé & Philipsson (1998) reported details of a selection index in use in dairy cows in Sweden which consisted of 11 traits with individual heritabilities ranging from 0.02 to 0.25. The selection index in use in dairy cattle in Denmark comprises 13 traits (Sørensen et al, 1999), while that used in Canada is made up of nine traits (Van Doormaal et al, 2001). The selection index used at Guiding Eyes for the Blind (GEB) in New Jersey, USA, consists of eight traits (Russenberger & Havlena, 2013). These examples give an indication of the number of traits that could reasonably be included in a selection index for GD.

5.4.1. Reasons for withdrawal

Behavioural reasons accounted for 68% (5327 of 7892 dogs) of dogs withdrawn from GD's programme between 1995 and 2012, with health reasons accounting for 29% (2257 of 7892 dogs). Specific behavioural reasons (high distraction, high suspicion, low attentiveness, low stress resilience, aggression towards people, low confidence, unacceptable social behaviour and low willingness) accounted for eight of the 10 most frequent specific reasons for withdrawal, with atopic dermatitis or allergic skin disease and hip

dysplasia being the only two individual health conditions in the 10 most frequent specific withdrawal reasons. Reasons for withdrawal of dogs from guide dog training or work have been reported for several international guide dog organisations and are summarised below.

The most common reasons for Labrador Retrievers born between 1963 and 1975 failing to qualify as guide dogs at the Royal Guide Dogs for the Blind Association of Australia were fearfulness, dog distraction, excitability, various physical or health conditions and hip dysplasia (Goddard & Beilharz, 1982). When grouped together behavioural reasons accounted for 77% of dogs rejected from training at this Australian guide dog organisation. Similarly nearly 70% of dogs withdrawn from guide dog training at the Japan Guide Dog Association between 2003 and 2005 were withdrawn due to behavioural problems (Arata et al, 2010). These figures are both close to that for the proportion of GD's dogs withdrawn due to behavioural reasons at 68%.

It was reported that only 43% (313 of 735 dogs) of puppies bred by Guide Dogs for the Blind Inc. (GDB) in California were successfully trained as guide dogs in the year 2000, with approximately half of the dogs which were withdrawn being withdrawn due to health conditions and half due to behavioural issues (Olson et al, 2004). Cataract, hip dysplasia, elbow dysplasia, allergies and cancer were reported to be the top health disorders leading to withdrawal of dogs from guide dogs training at GDB. This list is similar to the list of conditions necessitating most withdrawals from GD's programme although epilepsy or seizures account for more withdrawals than cataracts or cancer.

In 2006 it was reported that 53% of dogs entering training at The Seeing Eye (TSE) were successful as guide dogs (Ennik et al, 2006). Of 323 dogs withdrawn from guide dog training for behavioural reasons at TSE 18% were rejected due to suspicion of people, 17% due to lack of confidence, 14% due to distraction and 12% due to aggression towards other dogs (Serpell & Hsu, 2001). It can be difficult to compare behavioural traits between different

organisations as behavioural traits are often poorly defined and may actually be different traits despite the same names being used to describe them (Mackenzie et al, 1985). However it seems that the most frequent behavioural reasons for withdrawal from guide dog training at TSE are similar to those necessitating withdrawal from GD's programme.

Evans et al (2007) reported that 38% (93 of 245 dogs) of military working dogs (GSDs and Belgian Malinois) were withdrawn from service due to behavioural problems such as having low drive or being overly aggressive, with the remaining 62% (152 of 245 dogs) being withdrawn for a variety of health reasons of which spinal cord disease and arthritis were the most common diagnoses.

5.4.2. Survey of selection aims

Selection aims at GD have never been reviewed in this way before which makes these findings particularly interesting and useful. Well researched definitions of breeding objectives and selection may never be accepted and implemented if those definitions do not take into account the perceptions and wishes of the actual breeders for whom they are intended (Dekkers & Gibson, 1998).

It is apparent that selection aims and breeding objectives differ between the different breeds and crosses. Therefore selection indices would need to be breed-specific. Traits relating to health and behaviour were approximately evenly weighted across all breeds and crosses, with traits relating to appearance appearing to be judged as less important. Some of the traits used as selection criteria can only be judged subjectively – particularly "cosmetic appearance" and "breed conformation and movement". If a trait is not accurately measured and recorded it will not be possible to use this trait as a basis for selection. Several traits which appear to be selection criteria based on the results of the survey of selection aims are not systematically measured

or recorded. This may represent a failing of the questionnaire used in the survey – perhaps only traits which are directly measured should have been included as potential selection criteria. Alternatively it could be suggested that GD need to consider including measures of these traits in their programme.

5.4.2.1. *Appearance traits*

Height and coat length were both allocated points by four of seven respondents and weight by six of seven respondents for all four breeds and crosses. However, weight is the only one of these three criteria which is measured and recorded. If height and coat length are genuinely important criteria, although it seems unlikely that coat length varies significantly in Labrador Retrievers unlike in the other breeds/crosses, it would be useful to institute some form of measurement and recording of data on these traits. Work at TSE has shown that mature height and weight is heritable (Helmink et al, 2001). They studied 2334 German Shepherd Dogs and 2028 Labrador Retrievers and the heritability of mature weight was estimated as 0.57 ± 0.07 for GSDs and 0.44±0.07 for Labrador Retrievers, while heritability estimates for mature height were 0.35 ±0.08 for GSDs and 0.46 ± 0.08 for Labrador Retrievers. These moderate-to-high heritability estimates suggest that improvement in the proportion of dogs of each breed attaining the desired height and weight could be increased by selecting for these traits (Helmink et al, 2001).

5.4.2.2. *Temperament traits*

Lack of aggressive behaviour appeared to be the most important behavioural trait for selection in all four breeds and crosses, with mean scores allocated ranging from 10.5 in the Golden Retriever to 12.0 in the Labrador Retriever cross Golden Retriever. The mean scores allocated to willingness and

confidence were between 7.3 and 9.5 for all four breeds and crosses. Aggression towards animals or people, willingness and confidence are all measured in CAS and genetic parameters relating to CAS elements are described in Chapters 8 and 9. Confidence is also measured in the Puppy Profiling Assessment (PPA) and genetic parameters relating to PPA components are described in Chapter 10.

Compliance and environmental awareness received mean scores between 6.7 and 9.0 in all breeds and crosses but these traits are not measured in any behavioural test used at GD. The trait of adaptability which was added by one respondent is also not measured at GD. Traits which are not measured and recorded cannot be used as a basis for selection so again perhaps GD need to consider measuring these traits.

5.4.2.3. *Health traits*

"Clear eye examination" was allocated points in all breeds and crosses by all seven respondents, with the mean being approximately 10 points for all breeds and crosses. Genetic parameters relating to some ophthalmological conditions are explored in Chapters 6 and 7.

All prospective breeding stock has screening radiographs taken of their hips, elbows and shoulders. However, the numbers of dogs tested is quite low and the scores received are not systematically recorded, rendering it impossible to use quantitative genetic techniques to analyse genetic parameters of these scores. However, cases of hip and elbow dysplasia are recorded and genetic parameters relating to these disease conditions are explored in Chapters 6 and 7. Cases of shoulder OCD are also recorded but the number of confirmed cases was too low for genetic parameters to be estimated for this condition.

With respect to "clear heart examinations", only Golden Retrievers and Golden Retriever crosses routinely receive a heart examination (assumed to be a cardiac ultrasound) before being accepted as breeding stock.

Nevertheless, all but one respondent allocated points to “clear heart examination” for German Shepherd Dogs and all respondents allocated points to this criterion for Labrador Retrievers. It could be argued, therefore, that perhaps all prospective breeding stock should undergo cardiac examination before acceptance as breeding stock. Alternatively, if cardiac examination is not to be performed routinely in German Shepherd Dogs and Labrador Retrievers, “clear heart examination” cannot be considered a selection aim in these breeds.

Freedom from atopy was added to the survey form by several respondents and the mean score allocated ranged from 1.1 for the GSD to 4.1 in the Golden Retriever. Genetic parameters relating to atopic dermatitis in GD’s dogs are explored in Chapters 6 and 7.

Freedom from two disease conditions, EPI and CDRM, were added to the survey form by several respondents for the German Shepherd Dogs. Between 1995 and 2012 nine dogs were withdrawn due to EPI, eight of these were GSDs and one was a Labrador Retriever cross Golden Retriever. No dogs were withdrawn due to CDRM but the condition tends to occur in older dogs which may explain why it does not seem to have caused any premature withdrawals from GD’s programme. Although both conditions are causes for concern, their apparent prevalences in GD’s GSD population are very low which makes the use of quantitative genetic techniques for their analysis and management difficult. Genetic parameter estimation could not be successfully undertaken for either condition, as described in Chapter 6.

5.5. Conclusion

Not surprisingly there are considerable similarities between the lists of behavioural and health traits considered selection aims and those which are high on the list of traits necessitating withdrawal from GD’s programme. Aggression towards people, low confidence and low willingness, atopic

dermatitis, hip dysplasia and elbow dysplasia are high in both categories and thus estimated breeding values (EBVs) for these traits, combined into a selection index, may be particularly useful. The possibility of producing EBVs for these and other traits is explored in later chapters.

6. USE OF HISTORICAL HEALTH RECORDS FOR GENETIC EVALUATION OF HEALTH TRAITS IN THREE PUREBRED DOG BREEDS.

6.1. Introduction

Approximately 30% of dogs withdrawn from the training and breeding programme at Guide Dogs (GD) are withdrawn for health reasons. Even those health conditions which do not necessitate withdrawal of a dog from the programme can have a significant impact on the individual dog's welfare and also on the mobility of the guide dog owner (GDO) or their need to provide ongoing care for the dog. For these reasons a principal objective of the GD breeding programme, and indeed any breeding programme, must be to reduce the number of health cases which arise for as many disease conditions as possible. In many breeding programmes including that of GD this is attempted by monitoring the prevalence of health conditions.

GD implements a preventative health programme of regular vaccinations against infectious diseases and regular ecto- and endo-parisiticidal treatment to prevent parasitic diseases. Each time a GD dog is seen by a veterinary surgeon the details of the consultation, any procedures carried out, medication given and if any test results are awaited are recorded and entered into Guide Dogs Interactive (GDI) by a support worker as a health note. This information is then checked and coded appropriately by a Dog Care and Welfare Advisor (DCWA). A previous coding system using only 200 codes was replaced in 2009 by the current coding system which includes approximately 2100 different codes. Codes may indicate a clinical sign e.g. polyuria, a diagnosis e.g. chondrosarcoma or a procedure e.g. routine worming. The coding system is not error-proof, with misclassification sometimes occurring,

although the accuracy of the coding has improved substantially since 2009 (Adams, W., GD, personal communication 2011).

In the past decade three major reports into pedigree dog health concluded that, although pedigree breeding practices did compromise welfare in dogs, a lack of accurate prevalence data regarding inherited conditions in dogs made effective reforms difficult (Rooney & Sargan, 2008; APGAW, 2009; Bateson, 2010). Three projects have since been developed to address this lack of data using primary-care practice electronic patient records: VetCompass (Veterinary companion animal surveillance system) at the Royal Veterinary College, SAVSNET (Small animal veterinary surveillance network) at the University of Liverpool which utilises data from veterinary diagnostic laboratories in addition to veterinary practices, and work at the CEVM (Centre for Evidence-based Veterinary Medicine) at the University of Nottingham (O'Neill et al, 2014b).

During the same period efforts were made to attempt to quantify the welfare impact of different diseases on individual dogs and dog breeds. The Generic Illness Severity Index for Dogs (GISID) described by Asher et al (2009) was developed with the intention of allowing the severity of different disease conditions, based on their intensity and duration, to be compared. This index can then be multiplied by the prevalence in a particular breed to give the Welfare Index (WI) as described by Collins et al (2010) which allows different disease conditions to be compared across different breeds, and into a Breed-Disorder Welfare Impact Score (BDWIS) by taking account the duration of the disorder as a proportion of the dog's life (Collins et al, 2011).

When deciding which health traits should be selected upon as part of a breeding programme, it should be ensured that disease conditions upon which selection is based are both of clinical relevance for the dogs' health and well-being and also are heritable (Hedhammar et al, 2011). Efforts to establish the prevalence of disease conditions, and to compare their severity, as detailed above can provide evidence about their clinical relevance to dogs'

health. None of the above projects address the matter of whether and to what extent the diseases in question are heritable. One of the limitations of projects such as VetCompass and SAVSNET is that the data collected cannot currently be linked to any pedigree information. GD's database therefore presents a unique resource as it contains phenotypic information, related to both disease conditions and behavioural traits, for a substantial number of dogs linked by a large pedigree. Although the breeds used by GD are not representative of the full spectrum of pedigree dog breeds, the three breeds used in the largest numbers are all consistently in the top 10 breeds in terms of registration numbers at the UK Kennel Club (KC) with the Labrador Retriever having been the most popular breed by this measure for at least fifty years (Farrell, C., KC, personal communication, 2013). Therefore heritability and genetic correlation estimates for disease conditions for GD's dogs will be of interest and utility to the wider dog breeding community, particularly for those conditions for which estimates have not previously been reported.

Welsh et al (2013 & 2014) described the use of historical health records for genetic analyses of musculoskeletal conditions in Thoroughbred racehorses and reported small to moderate heritability estimates for conditions including osteoarthritis, tendon injury and fracture. Historical health records have also been used to estimate genetic parameters for incidence of recorded clinical lameness in dairy cattle, although the heritability estimates were very low (Chawala et al, 2013).

EBVs, primarily for production traits, have been used for many years in livestock production to improve selection decisions with great success. For example, average milk production per lactation in Holstein cows in the USA nearly doubled between 1960 and 2000 with more than half of this increase due to improved genetics (Dekkers & Hospital, 2002). In Sweden, the time taken to increase the average milk production per lactation in dairy cows by 1000 kg has been reduced from 45 years for the first interval to eight years for the latest interval reported by improved selection (Lindhé & Philipsson, 1998).

EBVs for health (and behavioural) traits have been used to aid selection decisions at The Seeing Eye (TSE) since 1995 (Leighton, 1997). The use of EBVs by TSE has been credited with virtually eliminating hip dysplasia, as diagnosed by hip-extended radiographs, in their Labrador Retrievers and German Shepherd Dogs (Smith, 2013). Guiding Eyes for the Blind (GEB) in New Jersey and Guide Dogs for the Blind (GDB) in California followed suit in using EBVs for health and behavioural traits and have reported success at reducing the incidence of several disease conditions (Russenberger & Havlena, GEB, 2013; Bullis, GDB, personal communication, 2014). The benefits of using EBVs were described in Chapter 2.

The characteristics of GD's database, as described above, should enable EBVs to be developed and used to aid selection decisions in the future. The precursor to estimating breeding values is to estimate heritabilities of the different health traits. EBVs can then be produced for the more heritable traits. In this chapter the estimation of health trait heritabilities and genetic correlations is described for three purebred GD lines.

6.2. Materials and methods

6.2.1. Description of dataset

GD provided a copy of their main database which contained data up to 2nd February 2012. The full health data set (including all data held in the database up to 2nd February 2012) consisted of 36992 dogs of 56 breeds and crosses (see Appendix 6).

6.2.2. Data validation

The health data were edited based on two inclusion criteria (date of birth and breed) as explained below. Table 6.1 shows the number of dogs in the dataset before and after each round of validation. The final dataset analysed contained 19540 dogs.

6.2.2.1. *Date of birth*

For the purpose of analysis only dogs which could potentially have a complete health record from birth to death or current date were included. A complete health record was defined as at least one health note per year of life. This meant that only dogs with a date of birth of 01/01/1995 or later were considered, as when GD changed its data recording system to GDI only health records from 01/01/1995 onwards were imported onto the system. Using inclusion criteria as described above to include only dogs born on or after 1st January 1995 resulted in a total of 21263 dogs of 38 breeds and crosses (Table 6.1).

6.2.2.2. *Breed*

For the reasons given in Chapter 1, German Shepherd Dogs (GSDs), Golden Retrievers (GRs), Labrador Retrievers (LRs), and Golden Retrievers crossed with Labrador Retrievers were included in subsequent analyses (Table 6.2), as there were insufficient numbers of individuals of other breeds and crosses. Breeds which were removed included Australian Shepherds, Bernese Mountain Dogs, Border Collies, Boxers, Chesapeake Bay Retrievers, Curly Coated Retrievers, Flat Coated Retrievers, Irish Water Spaniels, Italian Spinones, Leonbergers, Standard Poodles, Tervuerens and Weimaraners and crosses involving these breeds. This excluded 1723 dogs (Table 6.1), 424 of which had no health records as they were “unknown breed - for migration

purposes only" i.e. they were given a database entry for purposes of the pedigree but had no health records.

Table 6.1 Number of dogs retained and removed at each editing step described in the materials and methods. The percentage expressed is with reference to the number of dogs in the raw dataset.

Editing step	Number remaining	Number lost	Percentage remaining
Raw data set	36992	-	-
Date of birth 01/01/95 or later	21263	15729	58%
Breed	19540	1723	53%

Table 6.2 Number of dogs of each of the breeds and crosses with health records available for use in subsequent analyses.

Breed	Number of dogs
German Shepherd Dog	1162
Golden Retriever	2424
Golden Retriever x (Golden Retriever x Labrador)	43
Golden Retriever x Labrador	7376
Labrador	7451
Labrador x (Labrador x Golden Retriever)	1084
Total	19540

6.2.3. Case identification

The list of all potential health codes was examined by a veterinary surgeon and codes were categorised as relating to potentially inherited conditions, traumatic injury, infectious disease or routine procedures. The frequency of health notes with each health code in the dataset was determined, excluding codes relating to traumatic injury, infectious disease or routine procedures. The number of health notes with each code is not the same as the number of

dogs with that condition as, particularly for chronic conditions requiring frequent veterinary attention, a dog may have the same code attached to multiple health notes. Any conditions with a substantial number of health notes with that code were investigated further, alongside any conditions for which GD monitor the affected number of offspring of breeding stock. For each condition a set of cases in dogs of the three chosen breeds and their crosses born between 1st January 1995 and 2nd February 2012 was generated. For most conditions multiple health codes were investigated to compile all possible cases of a condition, health codes investigated for each condition are listed in section 6.2.3.1. In addition to the health code and associated notes, information such as dog identification number, breed, sex, date of birth was also captured. Further investigation involved veterinary interpretation of the attached notes of each individual dog, and if necessary of additional notes in the dog's health records in GDI, to determine whether each dog could be considered as a genuine case of the condition or not. If the dog could be considered as a case of the condition one record for that dog was saved. If the dog could not be considered a case of that condition all its records were deleted from that dataset. This represented a huge volume of work and had to be undertaken by a veterinary surgeon in order to accurately interpret all of the relevant information. Conditions which were subsequently found to have affected fewer than 30 individuals in the three breeds are not presented here.

6.2.3.1. *Case definitions*

To ensure reliability of case classification the following definitions of specific disease conditions were used:

Atopic dermatitis – diagnosis confirmed by a veterinary dermatologist. Codes searched: atopic dermatitis, suspected atopic dermatitis, allergic skin disease, allergic contact dermatitis, flea allergy.

Chronic degenerative radiculomyelopathy (CDRM) – diagnosis by veterinary surgeon based on presenting clinical signs. Code searched: chronic degenerative radiculomyelopathy.

Congenital ichthyosis (primary seborrhoea) – diagnosis confirmed by a veterinary dermatologist. Codes searched: congenital ichthyosis, primary seborrhoea, seborrhoea.

Cranial cruciate ligament (CCL) disease – diagnosis by veterinary surgeon based on presenting clinical signs. Codes searched: rupture of cruciate ligaments, ligament rupture, disorder of ligament, radiography of stifle, disorder of stifle.

Diabetes mellitus – diagnosis confirmed by blood biochemical analysis. Codes searched: diabetes mellitus, disorder of endocrine system.

Distichiasis – diagnosis by veterinary surgeon. Codes searched: ectopic cilia of eyelid, distichiasis, trichiasis.

Elbow dysplasia/elbow osteoarthritis (OA) – either diagnosis of elbow dysplasia confirmed radiographically or radiographic or symptomatic diagnosis of elbow osteoarthritis. Codes searched: ununited anconeal process, medial coronoid process disease, fragmented coronoid process, canine elbow dysplasia, osteochondritis dissecans of the elbow, disorder of elbow, osteoarthritis of elbow, osteoarthritis, chronic osteoarthritis, arthritis, degenerative joint disease, forelimb lameness, abnormal forelimb gait, radiography of elbow, radiography of elbow 2 views, radiography of foreleg.

Entropion – diagnosis by veterinary surgeon. Codes searched: entropion, lower eyelid entropion, upper eyelid entropion, entropion-ectropion combination.

Geographic retinal dysplasia (GRD) – diagnosis by a veterinary ophthalmologist. Codes searched: retinal dysplasia, geographic retinal dysplasia, multifocal retinal dysplasia.

Hip dysplasia/hip OA – either diagnosis of hip dysplasia confirmed radiographically or radiographic or symptomatic diagnosis of hip osteoarthritis. Codes searched: hip dysplasia, bilateral dysplastic hip, unilateral dysplastic hip, osteoarthritis of hip, osteoarthritis, chronic osteoarthritis, arthritis, degenerative joint disease, disorder of hip, hindlimb lameness, abnormal hindlimb gait, radiography of hindleg, radiography of hip, radiography of hip bilateral.

Histiocytoma – diagnosis by veterinary surgeon based on characteristic appearance or histopathological examination. Codes searched: cutaneous histiocytoma, benign fibrous histiocytoma of skin, histiocytoma, neoplasm of skin, benign neoplasm of skin

Horner's syndrome – diagnosis by veterinary surgeon. Codes searched: Horner's syndrome, Horner's syndrome pupil.

Hypothyroidism – diagnosis confirmed by thyroid hormone and/or thyroid stimulating hormone (TSH) levels or positive response to thyroid supplementation therapy. Codes searched: disorder of thyroid gland, hypothyroidism, hyperthyroidism.

Juvenile cellulitis (head gland disease) – diagnosis by veterinary surgeon. Code searched: head gland disease.

Laryngeal paralysis – diagnosis by veterinary surgeon. Codes searched: paralysis of larynx, laryngeal spasm, laryngismus stridulus, laryngitis, disorder of larynx.

Lymphoma – diagnosis confirmed by histopathological analysis. Codes searched: lymphoma, lymphosarcoma.

Mast cell tumour – diagnosis confirmed by histopathological analysis. Codes searched: mast cell tumour, neoplasm of skin, malignant neoplasm of skin.

Multifocal retinal dysplasia (MRD) – diagnosis by veterinary ophthalmologist. Codes searched: retinal dysplasia, multifocal retinal dysplasia, geographic retinal dysplasia.

Pancreatitis – diagnosis by veterinary surgeon. Codes searched: pancreatitis, acute pancreatitis, acute haemorrhagic pancreatitis, chronic pancreatitis, recurrent pancreatitis, disorder of pancreas.

Panosteitis – diagnosis by veterinary surgeon. Codes searched: panosteitis, forelimb lameness, radiography of foreleg, hindlimb lameness, radiography of hindleg.

Patellar luxation – diagnosis by veterinary surgeon, cases occurring due to trauma discarded. Codes searched: subluxation of the patella, disorder of stifle, hindlimb lameness, abnormal hindlimb gait, radiography of hindleg, radiography of stifle.

Posterior polar subcapsular cataract (PPSC) – diagnosis by veterinary ophthalmologist. Codes searched: cataract, bilateral cataracts, posterior polar subcapsular cataract, posterior polar capsular cataract, posterior cortical cataract.

Renal failure – diagnosis by veterinary surgeon. Codes searched: renal failure, kidney disease.

Sebaceous cyst – diagnosis by veterinary surgeon based on characteristic appearance or histopathological examination. Code searched: sebaceous cyst.

Seizures – history of two or more seizures in the absence of obvious precipitating causes (such as brain pathology), requiring maintenance therapy with anti-epileptic medication. Codes searched: seizure, grand mal seizure, petit-mal seizure, epilepsy, epileptic seizure, idiopathic generalised epilepsy, status epilepticus, disorder of brain, disorder of central nervous system.

Shoulder OCD - diagnosis made by veterinary surgeon based on radiographs and/or arthroscopic examination. Codes searched: osteochondritis dissecans of the shoulder, osteochondritis dissecans, juvenile osteochondritis dissecans, disorder of shoulder, forelimb lameness, abnormal forelimb gait, radiography of shoulder, radiography of foreleg.

Spondylosis – diagnosis made by veterinary surgeon based on radiographs or clinical signs. Codes searched: spondylosis, cervical spondylosis, thoracic spondylosis, lumbar spondylosis, disorder of spine, disorder of cervical spine, disorder of thoracic spine, disorder of lumbar spine, radiography of cervical spine, radiography of spine, x-ray of cervicothoracic junction, radiography of thoracic spine, x-ray of thoracolumbar spine, diagnostic radiography of lumbar spine, x-ray of lumbosacral spine, pain in spine, pain in cervical spine, pain in thoracic spine, pain in lumbar spine.

Umbilical hernia – diagnosis by veterinary surgeon. Code searched: umbilical hernia.

6.2.3.2. *Selection of "non-cases"*

Dogs which could be categorised as "non-cases" for each condition first had to fit the inclusion criteria for cases, that is to say they had to be of the same breed(s) as the cases and to have been born since 1st January 1995. In addition, they had to have a complete health record, which was defined as consisting of at least one health record entry per calendar year with no more than a one year gap between consecutive entries. The ages between which the health record had to be complete are shown in Appendix 7. They varied by condition and were chosen based on the literature and on the minimum and maximum age at diagnosis of the cases. Ideally, particularly for those conditions which tend to develop later in life, a "non-case" would have a complete health record from birth to death but attempting to use such strict criteria reduced the number of dogs which could be used in the analyses too severely.

6.2.4. Statistical analysis

The statistical analysis of the data had the objective of fitting univariate and bivariate mixed linear models, using ASReml version 3.0 (Gilmour et al, 2009), to estimate genetic and environmental parameters associated with the disease conditions in the Labrador Retriever, Golden Retriever and German Shepherd Dog. ASReml is a commercially available software package which uses Restricted Maximum Likelihood (REML) methodology to estimate variance components and to produce best linear unbiased predictors (BLUP) of breeding values. The concepts of heritability and genetic correlations were outlined in Chapter 2.

The validated dataset of cases (reduced to include only one record per dog) and non-cases for each disease condition was separated into individual breed datasets. A binary profile for each dog for that condition was created, whereby a dog was coded "1" as a case it if had been diagnosed with that condition and "0" if it had never received such a diagnosis.

The pedigree file used in all analyses was described in Chapter 4.

6.2.4.1. *Univariate linear models*

Each disease condition was analysed using REML univariate animal models. The general form of the linear model was as follows:

$$\mathbf{Y = Xb + Za + Wc + e}$$

where **Y** is the vector of observations; **X**, **W** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \sigma^2_a\mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \sigma^2_c\mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \sigma^2_e\mathbf{I})$; and where **I** denotes an identity matrix of the appropriate size, **A** is the numerator

relationship matrix, and σ^2 is a scalar denoting variance. The subscripts *a*, *c* and *e* denote additive genetic, litter and residual variances respectively. The fixed effects included in the model were gender, year of birth, whether the dog was bred by GD or not, inbreeding coefficient and colour (for LRs only). The random effects fitted were a litter effect and individual animal effect. Mathematically, the heritability is the ratio of additive genetic variance to phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$.

In order to determine whether the heritability estimates were significantly different from zero, Likelihood Ratio Tests (LRTs) were performed between the univariate animal models and null models in which the random effect for the individual had been omitted. The significance of other estimated effects was determined using approximate t-tests, with the number of degrees of freedom corresponding to the number of records from which the estimate could be determined.

Estimation of the heritability of a disease on the underlying continuous liability scale can be made using the binary scale heritability estimate and the prevalence of the disease by the following equation (Dempster & Lerner, 1950):

$$h_c^2 = h_{01}^2 \left(\frac{1-p}{i^2 p} \right)$$

where subscripts *c* and 01 signify heritability estimates on the continuous liability and binary scale, respectively; *p* is the prevalence of the condition in the data; and *i* is the mean liability of individuals with the condition at prevalence p, in SD units, from the population mean, assuming normally distributed liability. Mean liabilities were obtained using Appendix Table A of Falconer & Mackay (1996). Estimates of heritability on the continuous liability scale were approximated for all conditions found to have a statistically significant heritability estimate on the binary scale.

6.2.4.2. *Bivariate linear models*

Bivariate linear models were only attempted when there were at least 100 cases of each condition in the pair. No such models were attempted for the GSD breed as hip dysplasia was the only condition in this breed with more than 100 affected individuals. The bivariate linear models fitted to estimate the genetic correlations between pairs of disease conditions were of the following form:

$$\mathbf{Y = Xb + Za + Wc + e}$$

where **Y** is the vector of observations; **X**, **W** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \Sigma_a \otimes \mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \Sigma_c \otimes \mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \Sigma_e \otimes \mathbf{I})$; **I** is an identity matrix of the appropriate size, **A** is the additive genetic relationship matrix. The subscripts *a*, *c* and *e* denote additive genetic, litter and residual (co)variances respectively. The variance terms such as σ^2_a used in the univariate models were replaced by the appropriate bivariate covariance matrices (Σ) for the traits using the Kronecker product e.g. $\Sigma_a \otimes \mathbf{A}$. Thus Σ_a represents the additive genetic covariances of the two conditions in the base population. The fixed effects included in the model were the same as for the univariate models, namely: gender, year of birth, whether the dog was bred by GD or not, inbreeding coefficient and colour (for LRs only). The random effects fitted were a litter effect and individual animal effect.

In order to determine whether the genetic correlation estimates were significantly different from zero, LRTs were performed between the unconstrained bivariate animal models and null models in which the genetic correlation was constrained to 0.00001.

6.3. Results

The number of confirmed cases of each condition investigated is shown in Table 6.3. The case numbers shown in Table 6.3 include individuals of all three pure breeds and crosses between Labrador Retrievers and Golden Retrievers. When the datasets containing all affected dogs of the chosen breeds and crosses were split by breed there were insufficient case numbers per breed for some conditions to be investigated in any breed. Conditions for which this was the case were GRD, lymphoma and shoulder OCD.

Year of birth exclusion criteria were subsequently applied (which were disease-specific) to avoid the situation in which dogs born in certain years could only be cases due to the inclusion criteria used for the selection of "non-cases". The case and non-case numbers shown in subsequent numbers reflect these exclusion criteria and are therefore smaller than those shown in Table 6.3.

Table 6.3 Number of cases of 28 disease conditions identified from health records of 19450 GD dogs. LR = Labrador Retriever, GR = Golden Retriever, GSD = German Shepherd Dog.

Condition	LR	GR	GSD	All breeds
Atopic dermatitis	352	116	76	981
CDRM	8	5	22	39
Congenital ichthyosis	0	150	0	154
CCL disease	83	23	0	195
Diabetes mellitus	22	1	0	33
Distichiasis	28	25	0	103
Elbow dysplasia/elbow OA	491	60	48	774
Entropion	74	23	7	180
GRD	10	9	3	56
Hip dysplasia/hip OA	394	145	124	1076
Histiocytoma	120	35	19	367
Horner's syndrome	14	55	1	127
Hypothyroidism	24	23	6	101
Juvenile cellulitis	19	13	2	64
Laryngeal paralysis	52	6	0	99
Lymphoma	14	15	7	53
Mast cell tumour	46	30	1	156
MRD	30	20	4	106
Pancreatitis	28	14	4	76
Panosteitis	286	20	91	498
Patellar luxation	32	5	0	47
PPSC	31	50	2	149
Renal failure	34	13	2	73
Sebaceous cyst	81	39	60	293
Seizures	108	16	6	199
Shoulder OCD	9	11	10	52
Spondylosis	46	21	13	124
Umbilical hernia	22	3	84	129

The diagnoses of elbow dysplasia/elbow osteoarthritis and hip dysplasia/hip osteoarthritis were divided into cases that had been confirmed as dysplasia by radiography (Category 1), cases that had been confirmed as osteoarthritis of the joint by radiography but for which underlying dysplasia had not been explicitly diagnosed (Category 2) and cases which had been diagnosed as dysplasia and/or osteoarthritis of the joint on the basis of clinical signs without radiological confirmation (Category 3). The breakdown of cases for the three breeds on this basis, after exclusion criteria of year of birth had been applied which for these conditions removed any dogs born outside of the range of 1995 to 2004, is shown in Table 6.4.

Table 6.4 Case numbers of the subcategories of elbow dysplasia/elbow osteoarthritis and hip dysplasia/hip osteoarthritis by breed. LR = Labrador Retriever, GR = Golden Retriever, GSD = German Shepherd Dog.

Category	LR	GR	GSD
Elbow dysplasia (Category 1)	258	19	24
Elbow osteoarthritis (Category 2)	78	11	2
ED/OA clinical signs only (Category 3)	70	18	3
Hip dysplasia (Category 1)	209	84	85
Hip osteoarthritis (Category 2)	46	19	11
HD/OA clinical signs only (Category 3)	89	24	8

In subsequent heritability analyses, hip and elbow dysplasia comprised only Category 1 cases. Looser case definitions, described as “hip dysplasia or osteoarthritis” and “elbow dysplasia or osteoarthritis”, consisted of the dogs in Category 1 as well as the additional Category 2 and 3 cases.

6.3.1. Labrador Retriever models

6.3.1.1. *Labrador Retriever univariate models*

The results of univariate disease models run for the Labrador Retriever are shown in Table 6.5.

Table 6.5 Number of cases and non-cases and heritability estimates for the 21 disease conditions investigated in Labrador Retrievers.

Disease	Cases	Non-cases	h^2	s.e.†	*p* value
Atopic dermatitis	341	2558	0.0941	0.0346	<0.01
CCL disease	69	1392	0.3147	0.0897	<0.01
Diabetes mellitus	22	1406	0.0816	0.0447	<0.05
Distichiasis	28	2796	0.0628	0.0286	<0.05
Elbow dysplasia	258	1373	0.2637	0.0712	<0.01
Elbow dysplasia or OA	406	1273	0.1618	0.0587	<0.01
Entropion	74	2770	0.1118	0.0388	<0.01
Hip dysplasia	209	1374	0.0851	0.0559	NS
Hip dysplasia or OA	344	1287	0.1257	0.0636	<0.05
Histiocytoma	73	1392	0	0	NS
Hypothyroidism	22	1402	0	0	NS
Juvenile cellulitis	19	2808	0.0013	0.0168	NS
Laryngeal paralysis	52	1550	0.1077	0.0403	<0.01
Mast cell tumour	41	1387	0	0	NS
MRD	26	2427	0.0897	0.0352	<0.01
Pancreatitis	25	1571	0.018	0.0242	NS
Panosteitis	277	2688	0.1804	0.0447	<0.01
Patellar luxation	29	1412	0.1357	0.0486	<0.01
PPSC	31	1400	0.0354	0.0283	NS
Renal failure	34	1401	0	0	NS
Sebaceous cyst	69	1375	0	0	NS
Seizures	108	1371	0.1195	0.0549	<0.01

†standard error

Heritability estimates were not significantly different from zero for nine (hip dysplasia, histiocytoma, hypothyroidism, juvenile cellulitis, mast cell tumour,

pancreatitis, PPSC, renal failure and sebaceous cyst) of the 22 conditions investigated in the Labrador Retriever. In most cases this was probably due to the low number of confirmed cases of the disease condition being considered, ranging from 19 for juvenile cellulitis to 209 for hip dysplasia. The heritability estimates for 11 conditions were low, with estimates ranging from 0.06 (s.e. 0.03, $p<0.05$) for distichiasis to 0.18 (s.e. 0.04, $p<0.01$) for panosteitis. Two conditions had moderate heritability estimates. These were cranial cruciate ligament disease with heritability estimate 0.31 (s.e. 0.09, $p<0.01$) and elbow dysplasia diagnosed radiologically with a heritability estimate of 0.26 (s.e. 0.07, $p<0.01$).

Litter was a very small but significant effect for patellar luxation, PPSC, renal failure and sebaceous cyst with variance component estimates ranging from 2.4×10^{-9} (s.e. 4.4×10^{-10}) for patellar luxation to 5.2×10^{-9} (s.e. 6.4×10^{-10}) for sebaceous cyst. Coat colour was a significant fixed effect for three conditions. For atopic dermatitis the estimated effect for chocolate colour (relative to yellow) was 0.18 (s.e. 0.08). For diabetes mellitus and juvenile cellulitis black colour relative to yellow was a significant effect with effects of -0.02 (s.e. 0.007) and 0.01 (s.e. 0.003) respectively. For diabetes mellitus this reflects the fact that only two of the 22 cases were black, the rest being yellow, and in the case of juvenile cellulitis all the cases were black dogs.

Whether or not a dog was bred by GD was a significant fixed effect for cranial cruciate ligament disease and hip dysplasia or osteoarthritis (Categories 1-3). In both cases being bred by GD had a small protective effect, with estimated regression coefficients of -0.10 (s.e. 0.03) for cranial cruciate ligament disease and -0.09 (s.e. 0.04) for hip dysplasia or osteoarthritis.

Sex was a significant fixed effect for seven conditions. For elbow dysplasia (Category 1 cases) the estimated effect for female was -0.10 (s.e. 0.02) and for elbow dysplasia or osteoarthritis (Categories 1-3) the estimated effect for male was 0.10 (s.e. 0.02), both implying that females are at lower risk of developing elbow dysplasia. There were small, significant, positive effects of

being male for Category 1 hip dysplasia (0.08, s.e. 0.02), Category 1-3 hip dysplasia or osteoarthritis (0.09, s.e. 0.02) and panosteitis (0.06, s.e. 0.01). Small, negative effects of being male were estimated for hypothyroidism (-0.01, s.e. 0.007) and renal failure (-0.02, s.e. 0.008).

Heritability estimates on the continuous liability scale are shown in Table 6.6. As expected for all conditions the heritability estimates increased compared to the estimates from the univariate linear models. Those for CCL disease, MRD and patellar luxation exceeded the theoretical maximum.

Table 6.6 Apparent prevalence and heritability estimates on the continuous liability scale for the 13 conditions for which heritability estimates in univariate linear models were significantly larger than zero in Labrador Retrievers.

Disease	Prevalence	h^2
Atopic dermatitis	0.12	0.24
CCL disease	0.05	1.38
Diabetes mellitus	0.02	0.67
Distichiasis	0.01	0.84
Elbow dysplasia	0.16	0.59
Elbow dysplasia or OA	0.24	0.49
Entropion	0.03	0.69
Hip dysplasia or OA	0.21	0.26
Laryngeal paralysis	0.03	0.69
MRD	0.01	1.25
Panosteitis	0.09	0.56
Patellar luxation	0.02	1.17
Seizures	0.07	0.43

6.3.1.2. *Labrador Retriever bivariate models*

Bivariate models were only attempted between disease conditions for which more than 100 confirmed cases had been found. Those which produced genetic correlation estimates which were significantly larger than zero are shown in Table 6.7. The heritability estimates from the bivariate analyses were consistent with those estimated using univariate models so are not shown. All the genetic correlation estimates were positive and strikingly large.

Table 6.7 Statistically significant genetic correlations between pairs of disease conditions (each with more than 100 cases) in Labrador Retrievers.

Disease conditions	r_G*	s.e.†	*p* value
ED§ – ED or arthritis (Categories 1-3)	0.9782	0.0222	<0.01
ED – hip dysplasia or arthritis (Categories 1-3)	0.8815	0.0974	<0.01
ED - panosteitis	0.6939	0.1172	<0.01
ED - seizures	0.9345	0.0658	<0.01
ED or arthritis – hip dysplasia or arthritis	0.7448	0.1699	<0.05
ED or arthritis - panosteitis	0.4819	0.1951	<0.05
ED or arthritis - seizures	0.6781	0.2156	<0.05
Hip dysplasia or arthritis - panosteitis	0.6128	0.1983	<0.05

*Genetic correlation †standard error § elbow dysplasia (category 1)

6.3.2. Golden Retriever models

6.3.2.1. *Golden Retriever univariate models*

The results of univariate disease models run for the Golden Retriever are shown in Table 6.8. Significant heritability estimates were measured for five of the 14 conditions investigated in the Golden Retriever. Those conditions for which heritability estimated were not significantly different from zero were cranial cruciate ligament disease, elbow dysplasia (Category 1), elbow

dysplasia or osteoarthritis (Categories 1-3), hip dysplasia (Category 1), hip dysplasia or osteoarthritis (Categories 1-3), hypothyroidism, mast cell tumour, PPSC and spondylosis. For all these conditions the size of the datasets, which ranged from 449 dogs with phenotypes for cranial cruciate ligament disease and hypothyroidism to 510 dogs with phenotypes for spondylosis, was not sufficient to allow heritability greater than zero to be detected. Horner's syndrome had a low heritability estimate of 0.09 (s.e. 0.07, p<0.01), this may also have been affected by the relatively small size of the dataset with only 523 dogs with phenotypes.

Table 6.8 Number of cases and non-cases and heritability estimates for the 14 disease conditions investigated in Golden Retrievers.

Disease	Cases	Non-cases	h^2	s.e.†	*p* value
Atopic dermatitis	116	854	0.3106	0.0859	<0.01
Congenital ichthyosis	116	767	0.4067	0.107	<0.01
CCL disease	22	427	0.1851	0.1395	NS
Elbow dysplasia	19	440	0	0	NS
Elbow dysplasia or OA	48	419	0.1216	0.0916	NS
Entropion	23	924	0.7386	0.0832	<0.01
Hip dysplasia	84	358	0	0	NS
Hip dysplasia or OA	127	381	0.0256	0.0649	NS
Horner's syndrome	55	468	0.0935	0.0663	<0.01
Hypothyroidism	23	426	0.0367	0.0717	NS
Mast cell tumour	24	429	0	0	NS
Panosteitis	20	932	0.5317	0.1305	<0.05
PPSC	30	430	0.12	0.08	NS
Spondylosis	21	489	0.0504	0.0906	NS

†standard error

The datasets for the remaining four conditions were larger, with the datasets all including more than 880 individuals with defined phenotypes. Atopic dermatitis and congenital ichthyosis both appeared to be moderately heritable, with heritability estimates of 0.31 (s.e. 0.09, p<0.01) and 0.41 (s.e.

0.11, p<0.01) respectively. Entropion and panosteitis both had high heritability estimates, of 0.74 (s.e. 0.08, p<0.01) for entropion and 0.53 (s.e. 0.13, p<0.015) for panosteitis. However both of these conditions had very low case numbers (23 cases of entropion and 20 cases of panosteitis) so these results should be viewed with some caution.

There were significant litter effects for elbow dysplasia, Horner's syndrome, mast cell tumour and PPSC but the effects were all very small, with litter variance component estimates ranging from to $1.5x10^{-9}$ (s.e. $4.2x10^{-10}$) for Horner's syndrome to 0.008 (s.e. 0.003) for mast cell tumour. Sex was a small but significant effect for atopic dermatitis and mast cell tumour, with the effect of being female being -0.05 (s.e. 0.02) for atopic dermatitis and 0.05 (s.e. 0.02) for mast cell tumour.

Heritability estimates on the continuous liability scale are shown in Table 6.9. As with the Labrador Retriever the heritability estimates were considerably larger than those estimated in the linear models, with estimates for congenital ichthyosis, entropion and panosteitis exceeding the theoretical maximum.

Table 6.9 Apparent prevalence and heritability estimates on the continuous liability scale for the five conditions for which heritability estimates in univariate linear models were significantly larger than zero in Golden Retrievers.

Disease	Prevalence	h^2
Atopic dermatitis	0.12	0.82
Congenital ichthyosis	0.13	1.04
Entropion	0.02	6.19
Horner's syndrome	0.11	0.25
Panosteitis	0.02	4.43

6.3.2.2. *Golden Retriever bivariate models*

Bivariate models were attempted between atopic dermatitis and congenital ichthyosis, atopic dermatitis and hip dysplasia or osteoarthritis and congenital ichthyosis and hip dysplasia or osteoarthritis. None of the genetic correlation estimates was statistically significant.

6.3.3. German Shepherd Dog models

6.3.3.1. *German Shepherd Dog univariate models*

The results of univariate disease models for the GSD are shown in Table 6.10. Heritability estimates which were not significantly different from zero were estimated for five (CDRM, elbow dysplasia, elbow dysplasia or osteoarthritis, hip dysplasia or osteoarthritis and umbilical hernia) of the nine diseases investigated in the GSD, probably due to the small size of the datasets. The four conditions for which heritability estimates were significantly larger than zero all appeared to be moderately heritable. Atopic dermatitis had a heritability estimate of 0.36 (s.e. 0.15, $p<0.05$), hip dysplasia had a heritability estimate of 0.34 (s.e. 0.17, $p<0.05$), panosteitis had a heritability estimate of 0.30 (s.e. 0.13, $p<0.01$) and sebaceous cysts had a heritability estimate of 0.29 (s.e. 0.13, $p<0.01$). None of the fixed effects were significant and there was no significant litter effect in any of the GSD models.

Table 6.10 Number of cases and non-cases and heritability estimates for the 9 disease conditions investigated in German Shepherd Dogs.

Disease	Cases	Non-cases	h^2	s.e.†	*p* value
Atopic dermatitis	75	350	0.3643	0.1458	<0.05
CDRM	22	177	0	0	NS
Elbow dysplasia	24	168	0	0	NS
Elbow dysplasia or OA	29	164	0.0758	0.1754	NS
Hip dysplasia	85	149	0.3351	0.1724	p<0.05
Hip dysplasia or OA	104	138	0.0376	0.1562	NS
Panosteitis	91	365	0.2950	0.1275	<0.01
Sebaceous cyst	55	143	0.2855	0.1656	<0.01
Umbilical hernia	79	542	0.0999	0.0936	NS

†standard error

Heritability estimates on the continuous liability scale are shown in Table 6.11. The heritability estimates were all larger than the estimates from linear models.

Table 6.11 Apparent prevalence and heritability estimates on the continuous liability scale for the four conditions for which heritability estimates in univariate linear models were significantly larger than zero in German Shepherd Dogs.

Disease	Prevalence	h^2
Atopic dermatitis	0.18	0.77
Hip dysplasia	0.36	0.56
Panosteitis	0.20	0.61
Sebaceous cyst	0.28	0.52

6.4. Discussion

Univariate and bivariate linear mixed models were used to estimate heritabilities of, and genetic correlations between, disease conditions in GD's Labrador Retrievers, Golden Retrievers and German Shepherd Dogs. Although the number of individuals with a defined phenotype (case or non-case) for most conditions was relatively small, many of the models produced estimates which were detectably larger than zero. For several of the health traits studied this represents the first attempt to quantify the genetic contribution to development of the disease in the dog. For those conditions previously analysed elsewhere the results reported here offer interesting comparisons and help to shed light on whether the genetic contribution to development of the diseases differs between breeds, countries or management systems.

A major limitation of these analyses was the small size of the datasets – although the pedigree is large, as discussed in Chapter 3, the number of individuals of defined phenotype for any condition was relatively low even for the largest single breed population, the Labrador Retriever. Datasets comprising observations on several thousand individuals are necessary to have the requisite power to quantify genetic variation (Bishop & Woolliams, 2010). The datasets for the crossbreed models described in subsequent Chapter 6 are all much larger and it will be seen that the estimates produced had lower standard errors and were all statistically significant, which was not the case in this chapter.

The success of any breeding programme relies on the data available relating to the breeding stock under consideration and an incomplete database limits its success (Lang et al, 1998). It has been shown that incomplete recording and inaccurate diagnosis of a condition both result in underestimation of the heritability of the condition (Bishop & Woolliams, 2010). TSE and GEB send annual health surveys to all owners of dogs bred by them which have been removed from their programmes or which have retired (Leighton, TSE &

Russenberger, GEB, personal communication 2014). Such a system, if implemented by GD, would enable valuable health information to be collected on dogs they have bred which otherwise have to be discounted from any analyses as their disease status for any condition is unknown. This would increase the size of the dataset for any condition being investigated, and probably also decrease the apparent prevalence, thus increasing their power for quantifying genetic variation.

6.4.1. Univariate linear models

The results of the univariate linear models are discussed by body system affected and in the order that disease affecting that body system caused withdrawal of dogs from GD's programme, as described in Chapter 5.

6.4.1.1. *Musculoskeletal conditions*

Musculoskeletal conditions were ranked first in terms of withdrawals for health reasons of dogs from GD's programme, accounting for 43% (975 of 2257 dogs) of dogs withdrawn for health between 1995 and 2012. Hip dysplasia was the single biggest diagnosis within that category, accounting for 15.6% (353 of 2257) of withdrawals for health in that period. This is not particularly surprising as it is the most common orthopaedic disease of large breed dogs (Smith et al, 2001). It is a multifactorial disorder with a polygenic genetic predisposition, involving many genes, being modulated by many non-genetic factors (Hedhammar, 1979; Leighton, 1997; Wood, 2002; Krontveit, 2012).

The severity of hip dysplasia is a continuous variable but it is difficult to measure, hip scores on the other hand are ordinal (Wood et al, 2002). There are various hip scoring schemes worldwide, with most using the hip-extended radiographic projection (Verhoeven et al, 2012). The scoring system used in the UK is the British Veterinary Association/Kennel Club (BVA/KC) Hip Scheme,

established in 1984, under which each hip is scored from 0 to 53 giving a total hip score of 0-106 with 0 being the best score and 106 being the worst.

A strong association between hip status at radiographic hip screening and subsequent requirement for hip dysplasia-related veterinary care and mortality has been shown, and this was more marked for the GSD than for the Labrador Retriever and Golden Retriever (Malm et al, 2010). Published heritability estimates based on hip score from hip-extended radiographs vary from 0.34-0.60 in the Labrador Retriever from 0.34 to 0.47 in the Golden Retriever and from 0.10-0.60 in the GSD (e.g. Hedhammar, 1979; Leighton, 1997; Swenson et al, 1997b; Leppänen et al, 2000; Wood et al, 2002; Hamann et al, 2003; Lewis et al, 2010; Wilson et al, 2012; Lewis et al, 2013).

Distraction methods of hip screening such as the Pennsylvania Hip Improvement Program (PennHIP) and Dorsolateral Subluxation (DLS) system measure passive hip joint laxity (Verhoeven et al, 2012). These measures are reported to be more heritable than hip scores based on hip-extended radiography, with heritability estimates of 0.50 for GSDs and 0.60 for Labrador Retrievers being reported for the PennHIP score (Leighton et al, 1994). Unfortunately such distraction methods are not possible in the UK due to radiation safety regulations, so they are not considered further here.

As hip scores were poorly and inconsistently recorded in GDI, and also as only prospective breeding stock are hip scored, it was not possible to estimate the heritability of hip score in GD's dogs. Instead, as with the other disease conditions investigated, hip dysplasia was treated as a binary trait with dogs being categorized as either affected or unaffected. The heritability estimates for hip dysplasia in the Labrador Retriever and Golden Retriever were not statistically significant, but in the GSD the heritability estimate was 0.34 (s.e. 0.17, $p<0.05$). Hip dysplasia or osteoarthritis (categories 1-3) including cases which had been diagnosed based on clinical signs without radiological confirmation in the Labrador Retriever had a heritability estimate of 0.13 (s.e. 0.06, $p<0.05$).

It has been estimated that fewer than 5% of pet dogs which have radiographic evidence of hip osteoarthritis secondary to hip dysplasia have clinical abnormalities such as lameness and altered gait (Smith et al, 2001). This suggests that many dogs which would have been diagnosed with hip dysplasia had they undergone hip screening radiography have been missed and may have been classified as non-cases, even under the looser case definition of hip dysplasia or osteoarthritis, which may have adversely affected the heritability estimates.

Elbow dysplasia was ranked second in terms of orthopaedic conditions leading to withdrawal of dogs from GD's programme, accounting for 14.5% (328 of 2257 dogs) of dogs withdrawn for health reasons between 1995 and 2012. Both hip and elbow dysplasia are considered quantitative genetic traits which are also influenced by environmental factors (Guthrie & Pidduck, 1990; Padgett et al, 1995; Swenson et al, 1997b; Janutta et al, 2006; Malm et al 2008). The term "elbow dysplasia" covers four different conditions: ununited anconeal process (UAP), fragmented medial coronoid process (FCP), osteochondrosis (OC) or osteochondritis dissecans (OCD) and incongruity of the elbow joint, all of which often result in osteoarthritis of the joint (Hazewinkel, 2007). This grouping of syndromes may result in underestimates of heritability (Bishop & Woolliams, 2010; Lewis et al, 2013). The results of a study in GSDs suggested that UAP and OCD are genetically distinct from FCP and incongruity of the elbow joint in this breed (Janutta et al, 2006). A study in Labrador Retrievers also suggested that OCD and FCP were inherited independently (Padgett et al, 1995).

A higher prevalence of elbow dysplasia has been reported in male dogs than in females (Guthrie & Pidduck, 1990; Janutta et al, 2006; Malm et al, 2007) and the data examined here concurred with that finding, with 62% of cases (479 of 774 dogs) occurring in male dogs. Sex as a significant fixed effect in the elbow dysplasia models in Labrador Retrievers is consistent with these reports.

Elbow scoring in the UK under the BVA/KC Elbow Scheme follows the International Elbow Working Group (IEWG) protocol with extended and flexed lateral view radiographs of the elbows being scored from 0 to 3 for each elbow, 0 being the best score and 3 being the worst (Woolliams et al, 2011). Unlike for hip scoring, the scores are not summed but the worst score of left or right elbow is considered the official elbow score. This scheme was initiated in 1998, much later than the hip scheme, and the participation rate is much lower than for the hip scheme (Woolliams et al, 2011).

Heritability estimates of the BVA/KC elbow score of 0.19±0.03 for the Labrador Retriever, 0.30±0.05 for the Golden Retriever and 0.18±0.06 for the GSD were reported by Lewis et al, 2013. As with hip scores, only prospective breeding stock undergo elbow scoring, elbow scores were recorded poorly and inconsistently in GDI and there was an additional problem in that, prior to 2004, the elbow score field in the database could not be left blank so a 0 in that field was the default. It was thus not possible to estimate the heritability of the BVA/KC elbow score for GD's dogs, but instead elbow dysplasia was treated as a binary trait with dogs classified as either affected or unaffected. A heritability estimate of 0.26 (s.e. 0.07, $p<0.01$) was measured for Category 1 elbow dysplasia in the Labrador Retriever, and for the looser case definition of elbow dysplasia or osteoarthritis (Categories 1-3) the heritability estimate was 0.16 (s.e. 0.07, $p<0.01$). The datasets for the Golden Retriever and GSD were insufficiently larger to detect a heritability significantly different from zero for elbow dysplasia (Category 1) and elbow dysplasia or osteoarthritis (Categories 1-3). Although specific codes do exist within GDI for UAP, FCP and OCD of the elbow, these were not used extensively and the number of individuals with any of these codes was too low for heritability analysis to be attempted.

Since March 2014, EBVs for hip and elbow dysplasia have been freely available for all Labrador Retrievers, Golden Retrievers and GSDs (and some other breeds) registered with the UK Kennel Club, through a section of the KC website called "Mate Select" (The Kennel Club, 2014). These use data from the BVA/KC Hip and Elbow Schemes, together with the KC's extensive

pedigree files, to estimate EBVs for hip and elbow score. As GD's purebred breeding stock are KC registered they will have EBVs for hip and elbow score which can be accessed through Mate Select. The accuracy of their EBVs could be improved by submitting GD's hip and elbow scores to the BVA/KC Schemes. The accuracy of EBVs is the correlation between the estimate and the true breeding value and is a measure of how much information has been used to calculate the EBV. EBVs of individuals which have been scored themselves and have several relatives with scores will have higher accuracies than those of individuals which have not been scored and with few relatives with scores.

Labrador Retrievers (and Golden Retrievers) have been described as predisposed to developing cranial cruciate ligament disease, a multifactorial condition in which progressive ligament deterioration often results in CCL rupture and subsequent instability of the femorotibial joint leading to osteoarthritis (Duval et al, 1999; Guthrie et al, 2012). The heritability estimate for CCL disease in the Labrador was moderate at 0.31 (s.e. 0.09, $p<0.01$). This is similar to the heritability estimate of 0.27 reported for CCL rupture in the Newfoundland (Wilke et al, 2006). The moderate heritability estimate might make CCL disease a good candidate for the use of EBVs to reduce its prevalence. The lack of cases of CCL in GD GSDs concurs with the findings of Duval et al (1999) that GSDs are at decreased risk of CCL rupture compared to many other breeds.

Panosteitis is a self-limiting developmental inflammatory condition of unknown aetiology that affects the long bones of large-breed dogs, typically occurring between 5-18 months of age (Demko & McLaughlin, 2005). The Labrador Retriever, Golden Retriever and GSD have been found to be at increased risk of developing the condition (LaFond et al, 2002; Demko & McLaughlin, 2005). No heritability estimates for panosteitis were found in the literature, so those presented here for the three breeds investigated are of particular interest.

In the Labrador Retriever, panosteitis had a heritability estimate of 0.18 (s.e. 0.04, $p<0.01$) which was the lowest seen in the three breeds considered. The condition was found to be highly heritable in the Golden Retriever, with a heritability estimate of 0.53 (s.e. 0.13, $p<0.05$), and moderately heritable in the GSD with a heritability estimate of 0.30 (s.e. 0.13, $p<0.01$). Although the condition is usually self-limiting GD dogs which are severely affected are held back in puppy walking with associated cost implications and enter training and therefore work at a later age. The worst affected individuals may be permanently withdrawn; between 1995 and 2012 0.2% (5 of 2257 dogs) of dogs withdrawn for health was withdrawn due to panosteitis. EBVs for the condition therefore may be of value, particularly in the Golden Retriever and GSD.

Patellar luxation is another developmental orthopaedic condition to which Labrador Retrievers have been described as predisposed (Gibbons et al, 2006; Bound et al, 2009). A heritability estimate of 0.17 ± 0.03 has been reported in the Flat Coated Retriever (Lavrijsen et al, 2013). This is similar to the heritability estimate from GD Labrador Retrievers of 0.14 (s.e. 0.04, $p<0.01$).

6.4.1.2. *Dermatological conditions*

Atopic dermatitis and allergic skin disease were the diagnoses accounting for the most withdrawals for health of dogs from GD's programme, with 18.0% (407 of 2257 dogs) of dogs withdrawn for health reasons between 1995 and 2012 as discussed in Chapter 5. Atopic dermatitis, also known as atopy, is a complex multifactorial disease involving flare factors, a poor skin barrier, allergic sensitisation and cutaneous inflammation (Nuttall et al, 2013). Clinical signs usually start to develop between six months and three years of age (Favrot et al, 2010). It is a chronic condition with most affected individuals requiring life-long treatment (Nuttall et al, 2014).

Labrador Retrievers, Golden Retrievers and GSDs have all been described as predisposed to developing atopic dermatitis (Favrot et al, 2010; Jaeger et al,

2010). In a previous study of GD's Labrador Retrievers and Golden Retrievers (born prior to 1992) a heritability estimate of 0.47 ± 0.17 for the condition was measured (Shaw et al, 2004). Heritability estimates in this study were 0.09 (s.e. 0.03, $p<0.01$) for the Labrador Retriever, 0.31 (s.e. 0.09, $p<0.01$) for the Golden Retriever and 0.36 (s.e. 0.15, $p<0.05$) for the GSD. It is unclear why the heritability estimate is so low for the Labrador Retriever compared to the previous estimate and to the other two breeds, although as heritability estimates are population specific they would not necessarily be the same in different breeds or in the same breed at different times. Also, the estimate by Shaw et al (2004) was not measured using an animal model or REML methodology, but instead the heritability was estimated from the regression of the offspring atopic dermatitis score on the midparent atopic dermatitis score. Their study involved 429 dogs born prior to 1992 and used a three point scoring scale for the condition. Thus the heritability estimate from Shaw et al (2004) would not be expected to be the same as those estimated here.

GEB use a five-point scoring system for diagnoses presumed to relate to underlying allergy with the highest score of five for normal skin and ear and worst score of zero for conditions including atopic dermatitis and chronic otitis externa. Russenberger & Havlena (2013) estimated the heritability of a "skin allergy/otitis score" as 0.25 ± 0.025 in Labrador Retrievers. Importantly, using EBVs for this score reduced the incidence of moderate and severe cases (conditions graded 1 and 0) from 18% in 2002 to 7% in 2009 (Russenberger & Havlena, 2013). GD could consider using a similar scoring system for diagnoses presumed to relate to underlying allergy in their dogs.

Congenital ichthyosis in the Golden Retriever is characterised by scaling (not affecting the head or extremities) which can be mild to severe with whitish scales initially progressing to greyish or blackish scales and hyperpigmentation and roughness of the ventral skin (Guaguere et al, 2009). These signs are sometimes detected as early as 3-6 weeks of age in affected puppies, but diagnosis is usually made before 3 years of age (Guaguere et al, 2009). A mutation in the *PNPLA1* gene has been found to be responsible for congenital

ichthyosis in the Golden Retriever, and the gene has an autosomal recessive mode of inheritance (Grall et al, 2012).

GD now uses the DNA test which is commercially available for this mutation on all prospective Golden Retriever breeding stock. The mutation is described as perfectly segregating with the disease (Grall et al, 2012). Although the heritability estimate for congenital ichthyosis for GD's Golden Retriever was high at 0.41 (s.e. 0.11, $p<0.01$) which was not as high as might be expected if the disease were truly a single gene disorder, it should be remembered that this heritability estimate is on the binary scale. The maximum heritability estimate of a trait on the binary scale is approximately 0.64 even if it is fully additive on the liability scale (Lynch & Walsh, 1998). The heritability estimate for congenital ichthyosis on the liability scale, approximated using Dempster & Lerner (1950), was 1.04 and although this exceeds the theoretical maximum value of heritability this suggests that the heritability of underlying liability to the condition is very high. As discussed further in 6.4.2, approximations using Dempster & Lerner (1950) are dependent on the apparent prevalence of the condition in the population. All the cases of congenital ichthyosis were diagnosed by veterinary dermatologists so it is unlikely that any dogs categorised as cases actually did not have the condition. However it is possible that some dogs which have been categorised as non-cases may actually have been cases, particularly if they were only mildly affected. This would artificially decrease the apparent prevalence of the condition in the population and could explain why the heritability estimate on the continuous liability scale is greater than one.

Sebaceous cysts are benign, cystic skin masses filled with sebum which can affect any species and are relatively common in the dog. Although they are benign they are unsightly, unpleasant if they rupture and can become infected requiring antibiotic treatment and sometimes necessitating surgical removal. No heritability estimate for sebaceous cysts could be found in the literature. The moderate heritability estimate of 0.29 (s.e. 0.17, $p<0.01$) in the GSD is

therefore particularly interesting especially as no references were found which referred to tendency to develop sebaceous cysts as inherited.

6.4.1.3. *Ophthalmological conditions*

Distichiasis is a condition involving the growth of eyelashes in abnormal locations on the eyelid margin which may cause ocular irritation. It is considered likely to be an inherited condition due to the high incidence in some breeds (Genetics Committee of the American College of Veterinary Ophthalmologists, 2009). The only published heritability estimate found in the literature for the condition in dogs was 0.043 ± 0.028 for Tibetan Terriers in Germany, although the authors of this study found that distichiasis was underreported by veterinary ophthalmologists and that the heritability was therefore underestimated (Ketteritzsch et al, 2004). The heritability estimate calculated for GD's Labrador Retrievers of 0.06 (s.e. 0.03, $p<0.01$) is similar to the estimate for Tibetan Terriers. This estimate may be lower than in reality as the terms distichiasis, trichiasis and ectopic cilia of eyelid appeared to be used interchangeably by DCWAs when entering health notes into GDI and the three are not technically exactly the same condition.

Entropion is the turning inwards of one or both eyelids which may cause corneal irritation. It is considered to be an inherited condition in many breeds of dog including the Labrador Retriever and Golden Retriever (Genetics Committee of the American College of Veterinary Ophthalmologists, 2009). No heritability estimate for the condition could be found in the literature for any breed of dog. The heritability estimates for the Labrador Retriever and Golden Retriever were markedly different, at 0.11 (s.e. 0.04, $p<0.01$) and 0.74 (s.e. 0.08, $p<0.01$) respectively. Heritability estimates are population specific, so it is not unexpected that they could be so different in two different breeds. The considerably higher heritability estimate in the Golden Retriever suggests that the response to selection against this condition might be more rapid than in the Labrador Retriever.

Hereditary retinal dysplasia, of which MRD is one form, is the most common type of retinal dysplasia reported in dogs (Crispin et al, 1999). It has been suggested that MRD in the Golden Retriever has a simple recessive mode of inheritance (Long & Crispin, 1999). This was also considered to be the case in the Labrador Retriever (Crispin et al, 2008). The apparent higher prevalence of MRD in Labradoodles compared to Labrador Retrievers despite the fact that the condition is not seen in poodles has cast this mode of inheritance into doubt (Oliver & Gould, 2012). The heritability estimate for MRD in GD's Labrador Retrievers was low at only 0.09 (s.e. 0.04, $p<0.01$), but there were only 26 confirmed cases.

A statistically significant heritability estimate for PPSC was not measured in either Labrador Retrievers or Golden Retrievers, probably due to the small number of confirmed cases in each breed (49 and 50 respectively). There were only two confirmed cases in the GSD so heritability analysis was not even attempted. PPSC is considered a hereditary cataract (Oliver & Gould, 2012). GDB have been using EBVs for cataracts as a single diagnosis rather than subdivided into more precise categories and have reduced their incidence (Bullis, GDB, 2014, personal communication). As cataracts are the fifth biggest reason for withdrawal for health, accounting for 3.7% (84 of 2257) of all withdrawals for health reasons between 1995 and 2012, GD could consider a similar approach.

6.4.1.4. *Neurological conditions*

Horner's syndrome is characterised by unilateral miosis (constriction of the pupil), with ptosis (drooping of the upper eyelid), prolapse of the third eyelid and apparent enophthalmos frequently being present. It occurs due to lesions resulting in interruption of the oculo-sympathetic path at any point between its origin in the brain (although lesions here are the least common cause) and termination in the orbit (Van Den Broek, 1987). Common causes include cranial thoracic neoplasia and otitis media (Boydell, 2000). In

approximately half of dogs with Horner's syndrome which underwent clinical investigations a specific cause could not be determined and these cases are described as idiopathic (Van Den Broek, 1987; Morgan & Zanotti, 1989; Kern et al, 1989). A high incidence of idiopathic Horner's syndrome has been reported in the Golden Retriever, particularly among males (Boydell, 1995; Boydell, 2000; Simpson et al, 2013).

No attempt was made to remove cases of Horner's syndrome in GD dogs for which a definitive underlying cause had been established; nevertheless the number of cases in the Golden Retriever was striking representing 43% (55 of 127 dogs) of all confirmed cases between 1995 and 2012. The heritability estimate in this breed was low at 0.09 (s.e. 0.07, $p<0.01$), this might be increased by removing cases which had a diagnosed cause and were not idiopathic.

Acquired laryngeal paralysis typically occurs in old, large-breed dogs. The Labrador Retriever has been reported to be the most commonly affected breed (Jeffery et al, 2006; MacPhail & Monnet, 2001; Snelling & Edwards, 2003), but none of these studies appeared to take into consideration the breed's popularity. Labrador Retrievers were found to be at increased risk, and GSDs at decreased risk, of being found to have undiagnosed laryngeal paralysis when undergoing general anaesthesia compared to a control population of other breeds (Broome et al, 2000). Evidence is accumulating suggesting that acquired laryngeal paralysis in older dogs is often a sign of a generalised peripheral neuropathy (Jeffery et al, 2006; Stanley et al, 2010; Thieman et al, 2010).

No heritability estimate for acquired laryngeal paralysis in dogs could be found in the literature, so the heritability estimate of 0.11 (s.e. 0.04, $p<0.01$) in GD's Labrador Retrievers is particularly interesting. As age at onset can be quite old, some dogs which were included as "non-cases" may have subsequently developed the condition which may have affected the ability to accurately estimate the heritability. The median age at diagnosis has been

reported as 10.5-11 years old (MacPhail & Monnet, 2001; Snelling & Edwards, 2003) which is older than the age (8 years) by which GD's dogs had to remain clear of the disease to be classed as a "non-case". The late age of onset and low number of cases suggest that there would be little value in attempting to select against the condition by using EBVs. There were no confirmed cases of laryngeal paralysis in GSDs which may support the finding of Broome et al (2000) that they may be at decreased risk of laryngeal paralysis.

Epilepsy or seizures accounted for 4.3% (96 of 2257 dogs) of withdrawals for health reasons between 1995 and 2012. Idiopathic epilepsy is a diagnosis of exclusion which can only be made once other causes of recurrent seizure activity have been ruled out (Ekenstedt et al, 2012). As this is a time-consuming, laborious and expensive process, and may well not affect the treatment regime of choice, this had not been done for many of GD's dogs which had suffered recurrent seizures. This is why a looser case definition of seizures was used. Epilepsy in Boxers, with a similar case definition to that used for GD's dogs, has been estimated to have a heritability of 0.36 (Nielen et al, 2001). Idiopathic epilepsy in the Labrador Retriever and Golden Retriever has been proposed to be polygenic, recessive and neither sex-linked nor sex-modified (Jaggy et al, 1998). GEB use a 3-point scoring system for epilepsy with a score of 0 for dogs aged 3 years or older which have had no seizures, a score of 1 for dogs which have had 1 seizure and a score of 2 which have had more than 1 seizure, and have estimated the heritability of epilepsy based on this scoring system as 0.54 in Labrador Retrievers (Russenberger & Havlena, 2013). The use of EBVs for epilepsy at GEB has reduced the incidence of the condition from 7% in 1999 to 3% in 2007. The case numbers for GD's Golden Retrievers and GSDs were too low (at 19 and 6 respectively) for heritability estimates to be measured in these breeds. In the Labrador Retriever a low heritability estimate of 0.12 (s.e. 0.05, $p<0.01$) was calculated.

6.4.1.5. *Endocrinological conditions*

In both humans and dogs diabetes mellitus is a multifactorial disease with both genetic and environmental factors (Davison et al, 2004). Canine diabetes mellitus is not as well understood as the condition in humans, as there are several potential pathological mechanisms leading to hyperglycaemia, although it has been suggested that the underlying cause of the hyperglycaemia can be categorised as due to absolute or relative insulin deficiency (Catchpole et al, 2008). Insulin deficiency diabetes, in which there is an absolute deficiency of insulin, is usually diagnosed in dogs aged between five and 12 years of age and is considered the most common type of diabetes in the UK dog population (Davison et al, 2004).

The heritability of diabetes mellitus in GD Labrador Retrievers was estimated at 0.08 (s.e. 0.04, $p<0.05$). Cases of diabetes mellitus in the GD population were diagnosed on the basis of persistent hyperglycaemia on blood biochemical analysis and no attempt was made to separate cases into more specific categories of the disease. This may have lowered the heritability estimate. The low number of confirmed cases (22 dogs) is also not ideal for a heritability study and will have an impact on the accuracy of the heritability estimate. However, no heritability estimate for diabetes mellitus in any breed of dog was found in the literature, so there is nothing to compare this against.

The Golden Retriever and GSD have been described as “diabetes-resistant” (Catchpole et al, 2008) and were also found to be at decreased odds of developing diabetes mellitus in a study of dogs attending first opinion veterinary practices in the UK (Mattin et al, 2014). GD’s data appear to concur with this as there were no confirmed cases of diabetes mellitus in the Golden Retriever and only one in the GSD between 1995 and 2012.

6.4.2. Heritability estimates on the underlying liability scale

Heritability estimates made using binary data such as these are an underestimate of the heritability on the underlying liability scale, which is assumed to be continuously distributed, since the binary present/absent phenotype is a less precise indicator of disease susceptibility than a phenotype measurable on a more graduated scale (Gianola, 1982).

Heritability estimates on the continuous liability scale were approximated using Dempster & Lerner (1950) for all conditions for which the heritability estimate was detectably larger than zero on the binary scale. In all cases the heritability estimate increased markedly, but in several cases the estimate was > 1, which is greater than the theoretical maximum value of heritability. This can happen with ad hoc corrections (e.g. Lewis et al, 2011a). Dempster & Lerner (1950) approximations are dependent on the prevalence of each condition in the population under investigation. The prevalence estimates used in their calculations here were based on the number of confirmed cases and the number of dogs which could be classed as non-cases. It is likely that these prevalence estimates are quite inaccurate. Low prevalence estimates, particularly when combined with high heritability estimates on the binary scale, tend to produce very large heritability approximations on the liability scale. Heritability approximations on the liability scale tend to be significantly overestimated when the heritability is high and the prevalence is either very high or very low as epistatic genetic variance on the observed scale is transformed to the liability scale as additive genetic variance (Tenesa & Haley, 2013). However, these approximations suggest that the heritabilities of the underlying liabilities to these diseases (cranial cruciate ligament disease and MRD in Labrador Retrievers and congenital ichthyosis, entropion and panosteitis in Golden Retrievers) are all very high.

6.4.3. Bivariate models

The estimation of genetic correlations between traits requires substantially larger sample sizes than are necessary in univariate analysis (Lynch & Walsh, 1998). The datasets used in this chapter were often at the limit of what is viable even in univariate analysis, so it is not surprising that many of the bivariate models attempted either would not converge or produced non-significant estimates of genetic correlations between traits. For example, none of the three bivariate models attempted in the Golden Retriever produced genetic correlation estimates which were significantly larger than zero. A simulation study in horses found that additive genetic correlations between binary traits produced using REML methodology were almost always overestimated (Stock et al, 2007), therefore some caution should be applied when considering the results discussed here.

The genetic correlation between elbow dysplasia and the looser case definition of elbow dysplasia or osteoarthritis in Labrador Retrievers was 0.98 (s.e. 0.02, $p<0.01$) suggesting that they are genetically the same trait. The genetic correlations between elbow dysplasia and the loose case definition of hip dysplasia or osteoarthritis, and the loose case definitions of elbow dysplasia or osteoarthritis and hip dysplasia or osteoarthritis were both high and positive at 0.88 (s.e. 0.10, $p<0.01$) and 0.74 (s.e. 0.17, $p<0.05$) respectively. Positive genetic correlations imply that selecting for one trait should produce concurrent improvements in the second trait. A moderate, positive genetic correlation of 0.40-0.42 between hip and elbow score in the BVA/KC Hip and Elbow Schemes has been estimated (Lewis et al, 2011b).

High, positive genetic correlation estimates were found for elbow dysplasia and panosteitis, the loose case definition of elbow dysplasia or osteoarthritis and panosteitis and hip dysplasia or osteoarthritis and panosteitis, at 0.69 (s.e. 0.12, $p<0.01$), 0.48 (s.e. 0.20, $p<0.05$) and 0.61 (s.e. 0.61, $p<0.05$) respectively. No genetic correlation estimates between hip or elbow

dysplasia and panosteitis were found in the literature. All three conditions are developmental orthopaedic conditions in which rapid growth is thought to play a role and to which Labrador Retrievers are considered predisposed so it is probably not surprising to find high, positive genetic correlations between them. It seems quite plausible that a proportion of the genes conferring risk of developing panosteitis should also confer risk of developing hip and elbow dysplasia, but this could perhaps be an area of future research.

The high, positive genetic correlations between elbow dysplasia and seizures and the looser case definition of elbow dysplasia or osteoarthritis and seizures in Labrador Retrievers, at 0.93 (s.e. 0.07, $p<0.01$) and 0.68 (s.e. 0.22, $p<0.05$) cannot be explained and may warrant further investigation. Although unexpected these findings suggest that risk genes are shared by these conditions, or are in linkage with each other, such that genetic susceptibility to one of these conditions tends to occur concurrently with genetic susceptibility to the other condition.

6.5. Conclusion

The results of these analyses suggest that EBVs produced from univariate linear models of some diseases as binary traits could be used for selection in GD's Labrador Retrievers, Golden Retrievers and Germans Shepherd Dogs. In the Labrador Retriever, diseases for which EBVs could potentially be used are cranial cruciate ligament disease, panosteitis, patellar luxation, entropion and seizures. In the Golden Retriever, panosteitis and atopic dermatitis look like suitable candidates for EBV use. In these two breeds, crossbreed models will be investigated for these and other diseases in Chapter 6. In the German Shepherd Dog, panosteitis, atopic dermatitis and sebaceous cysts could potentially be selected against using EBVs from these models.

Heritability estimates have been measured for the first time for panosteitis, sebaceous cysts, entropion, multifocal retinal dysplasia, Horner's syndrome, laryngeal paralysis and diabetes mellitus. In addition heritability estimates for

cranial cruciate ligament disease, patellar luxation and distichiasis for the Labrador Retriever have been measured, having only previously been reported for the Newfoundland, Flat Coated Retriever and Tibetan Terrier respectively. These results will be of interest to the wider dog breeding community and herald the potential to select against these conditions.

High, positive genetic correlations have been estimated between clinical cases of hip and elbow dysplasia, in contrast to the previously reported moderate genetic correlation between hip and elbow scores. The high, positive genetic correlations between hip and elbow dysplasia and panosteitis have not been reported before. Most unexpectedly, and potentially warranting further research, high positive genetic correlations were found between elbow dysplasia (both Category 1 and Categories 1-3) and seizures.

7. USE OF HISTORICAL HEALTH RECORDS FOR GENETIC EVALUATION OF HEALTH TRAITS IN CROSSBREED MODELS.

7.1. Introduction

The Golden Retriever (GR) crossed with the Labrador Retriever (LR) has been the most successful of all the breeds and crosses Guide Dogs (GD) have used for guiding blind and partially sighted people, allegedly as a result of combining the best attributes of both breeds (Freeman, 1991). Between 2003 and 2007, 79% of Golden Retriever cross Labrador Retrievers successfully qualified as guide dogs, compared with 63% of purebred Labrador Retrievers and Golden Retrievers and 61% of purebred German Shepherd Dogs (GSDs) (GD, unpublished data). Currently, 60% of GD's working guide dogs are Golden Retriever crosses (mostly crossed with Labrador Retrievers, but a smaller number of Golden Retriever cross GSDs are also used), 24% are purebred Labrador Retrievers and 10% are purebred Golden Retrievers, with the remaining 6% being GSDs and other breeds or crosses (Guide Dogs for the Blind Association, 2013).

It has been suggested that first-generation (F1) hybrids have a far lower chance of being affected by the disorders that commonly afflict the parental breeds (McGreevy & Nicholas, 1999). The genetic health of F1 dogs is expected to be substantially higher than the pure parental breeds, due to the positive benefits of hybrid vigour (McGreevy & Nicholas, 1999). However, a survey of Labradoodles in the UK found the incidence of multifocal retinal dysplasia (MRD) was higher in this crossbreed than the purebred Labrador Retriever, despite MRD not being seen in the Poodle (Oliver & Gould, 2012). Unfortunately Labradoodles in that study were a heterogeneous group comprised of F1 crosses between Labrador Retrievers and Poodles, offspring

of two Labradoodles and offspring of Labradoodles crossed back to either a Labrador Retrievers or Poodle. Therefore, as will be shown later, some of the Labradoodles will have had two Labrador Retriever alleles at some loci. It would have been interesting to see what the prevalence of MRD was in the different categories of Labradoodle.

In a study of patient records at an American veterinary teaching hospital there was no difference in the prevalence of 13 inherited disorders (including hip dysplasia) between purebred dogs and mixed-breed dogs, and interestingly mixed-breed dogs were found to have increased risk of ruptured cranial cruciate ligament (Bellumori et al, 2013). The authors hypothesised that the increased risk of CCL rupture could be caused by multiple musculoskeletal alleles from different physical conformations which together reduce the resilience of the ligament (Bellumori et al, 2013). This could be an illustration of the possible disadvantages of crossbreeding, when advantageous gene combinations present in the pure breeds are lost through recombination in the crossbreed.

A study of mortality data among owned dogs attending first opinion veterinary practices in central and southeast England showed that longevity in crossbred dogs exceeded that in purebred dogs by 1.2 years (O'Neill et al, 2013). The authors stated that this supported the concept of hybrid vigour in dogs. However, the "crossbred" group included dogs of unknown mixed breeds and F1 crosses between specified breeds. They did however acknowledge that, although median age at death for the crossbreed group was 13.1 years, 10 purebred breeds of dog had a higher median age at death than this figure (O'Neill et al, 2013). These results corroborated the findings of a Danish questionnaire survey of dog mortality which found that mixed-breed dogs had a higher median age of death (11.0 years) than purebred dogs in general, but that several breeds of dog exceeded this age (Proschowsky et al, 2003).

As GD use a large number of F1 crosses between Labrador Retrievers and Golden Retrievers (and a smaller number of backcrosses to one of the parental breeds), and of both pure breeds, crossbreed models are appropriate for the genetic analysis of disease (and later behavioural) data. GD's dataset, linked as it is to a large pedigree containing purebred and crossbred dogs, also enables the quantification of genetic parameters such as heterosis and recombination loss in dogs for the first time.

Inbreeding depression is the reduction of the mean phenotypic value in the population shown by traits connected with "fitness" such as reproductive capacity or physiological efficiency (Falconer & Mackay, 1996). Inbreeding depression is caused by increased homozygosity of individuals which can lower fitness in two distinct ways: increased homozygosity for recessive detrimental mutations and increased homozygosity for alleles at loci for which the heterozygous state is advantageous (Charlesworth & Willis, 2009). The second mechanism is termed overdominance.

The opposite of inbreeding depression is hybrid vigour or heterosis (Falconer & Mackay, 1996). When two inbred lines are crossed, F1 hybrids show an increase in the mean phenotypic value in the traits that previously suffered a reduction due to inbreeding or, more simply, the fitness lost due to inbreeding is generally restored by crossing. Often this improvement in performance exceeds the mid-parent breed mean and even both parental breed means too.

The genetic basis of crossbreeding effects can be divided into two major components, additive and non-additive effects. The additive component for any trait in crossbred individuals is that which is due to the simple averaging of merit in the parental breeds (Kinghorn, 1987). The non-additive component is heterosis, and it explains why crossbred individuals often perform better than the average of their parent breeds.

Although heterosis is frequently observed in F1 crosses much of its effect is lost in the subsequent F2 generation and in some cases this loss of fitness is

greater than would be seen if it were just due to the loss of heterosis. If the F2 progeny are significantly less fit than the original parental breeds this is due to the phenomenon of recombination loss (Lynch and Walsh, 1998).

These phenomena can be explained most easily using simple diagrams, after Kinghorn (1987). These simple diagrams depict a number of gene pairs, using letters to describe breed of origin (L for Labrador Retriever and G for Golden Retriever), and two rows to describe from which parent each alleles originates. For simplicity it is assumed that the two breeds are totally inbred, that is to say that they are homozygous for alternative alleles at all loci.

Purebred LR	Alleles from sire:	L L L L L L L L L
	Alleles from dam:	L L L L L L L L L
Purebred GR	Alleles from sire:	G G G G G G G G G
	Alleles from dam:	G G G G G G G G G

Heterosis and recombination loss expression in the purebred individuals is 0%, because in all cases both alleles are derived from the same breed.

F1 cross LR x GR	Alleles from sire:	L L L L L L L L L
	Alleles from dam:	G G G G G G G G G

In the F1 cross all gene pairs involve one allele from each parent breed and so heterosis expression is 100%. Recombination loss is still 0% in the F1.

F2 cross (LR x GR) x (LR x GR)	Alleles from sire:	L L G G L L G G L L
	Alleles from dam:	L G L G L G L G L G

This diagram reflects one possible re-pairing of alleles due to meiotic recombination. In the F2 cross, on average only half of the gene pairs are

expected to involve a difference in breed of origin so heterosis expression is 50%. Recombination loss is seen in this situation. Breed-specific epistatic effects on any given trait are maintained in the F1 with an entire chromosome from both parental breeds but these are broken during recombination of the gametes of the F1 generation.

Backcross LR x (LR x GR)	Alleles from sire:	L L L L L L L L L L
	Alleles from dam:	L G L G L G L G L G

In the backcross, half of the gene pairs involve a difference in breed of origin and in the other half both alleles come from the Labrador Retriever. Heterosis expression is thus 50%. Recombination loss is less in backcrosses than F2 crosses, as at least one allele from the breed of the purebred parent is present in all gene pairs.

Crossbreeding parameters have been quantified in many other species. Examples can be found in the literature on crossbreeding relating to production traits in pigs, cattle, sheep, goats, mink, rabbits, rats, mice, chickens, turkeys, ducks and quail, among others. For example, studies in pigs have shown significant heterotic effects on number of piglets born alive and heterotic and recombination loss effects on litter birth weight (Baas et al, 1992). In cattle, significant heterosis and recombination loss effects were found for milk yield, calving interval, days open and days dry (Ahmad et al, 2001). Similarly in rabbits significant positive heterosis was detected for milk yield of the doe, litter size and weight of the pups at 21 days (Lukefahr et al, 1983).

There are relatively few published studies investigating crossbreeding effects on health traits. Most focus on the effects of inbreeding and heterosis on resistance to infectious or parasitic diseases in livestock (e.g. Li et al, 2001; Hielscher et al, 2006; Murray et al, 2013). Inbreeding depression has been linked to reduced cell-mediated immunity in sparrows (Reid et al, 2003) and this may at least partially explain heterotic effects on resistance to infectious

and parasitic diseases. Fewer studies are found relating to crossbreeding effects on non-infectious disease. One study into recorded clinical lameness in dairy cattle in New Zealand reported a small heterotic effect reducing the incidence of this broad category of conditions (Chawala, 2011).

In this chapter, crossbreeding parameters will be estimated for 10 disease conditions in GD's dogs. In addition the utility of crossbreed models for producing EBVs for these traits, compared to using single breed models, is evaluated.

7.2. Materials and methods

Data acquisition and validation, case identification, cases definitions and selection of "non-cases" were as described in Chapter 6.

7.2.1. Estimated crossbreed parameter calculation

The expected heterosis and recombination loss for each individual was calculated from the proportion of Labrador Retriever and Golden Retriever of each animal's sire and dam, after Van der Werf and de Boer (1989). The non-additive effects of heterosis and recombination loss originate either through dominance effects, from interactions between Labrador Retriever and Golden Retriever alleles within loci, or epistatic effects from interactions between loci. Using this method heterosis was calculated as $h = ½ [(P_S (1-P_D)) + (P_D (1-P_S))]$, and recombination loss as $r = ½ [(P_S (1-P_S)) + (P_D (1-P_D))]$, where P_S and P_D are the proportion of Labrador Retriever in the sire and the dam respectively, and thus the probability of inheriting a Labrador Retriever allele from the sire or dam. The first equation gives the probability that the two alleles inherited from the parents at any one locus originate from different breeds and represents dominance effects as well as half of the additive effect that is confounded with dominance. The second equation gives the probability that

any two loci inherited from the same parent originate from different breeds and represents the sum of dominance and epistatic effects.

The proportion of Labrador Retriever and estimates of heterosis and recombination loss for the different breeds and crosses in the dataset are shown in Table 7.1.

Table 7.1 Estimates of heterosis (h) and recombination loss (r loss) for the different breeds and crosses in the dataset. Estimates for the F2 (GR x LR) x (GR x LR) are shown for interest, but there were none of this cross in the dataset.

Breed or cross	Lab*	h	r loss
Golden Retriever	0	0	0
Labrador Retriever	1	0	0
Golden Retriever x Labrador Retriever	0.5	0.5	0
Golden Retriever x (GR x LR)	0.25	0.25	0.125
Labrador Retriever x (GR x LR)	0.75	0.25	0.125
(GR x LR) x (GR x LR)	0.5	0.25	0.25

* Labrador Retriever fraction

7.2.2. Statistical analyses

Statistical analysis of the data had the objective of fitting a univariate linear mixed model using ASReml version 3.0 (Gilmour et al, 2009) to each disease condition to estimate the heritability and independent regression coefficients of estimated Labrador Retriever fraction, heterosis and recombination loss. In addition univariate binomial mixed models were fitted for each disease condition to compare their use for heritability estimation to the linear models. The significance of estimated effects and regression coefficients from zero was determined using approximate t-tests, with the number of degrees of freedom corresponding to the number of records from which the estimates can be determined.

The pedigree file used in all analyses was described in Chapter 4.

7.2.2.1. *Univariate linear mixed models*

The general form of the univariate linear mixed model fitted for each disease condition was as follows:

$$\mathbf{Y = Xb + Za + Wc + e}$$

where **Y** is the vector of observations; **X**, **W** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \sigma^2_a\mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \sigma^2_c\mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \sigma^2_e\mathbf{I})$; and where **I** denotes an identity matrix of the appropriate size, **A** is the numerator relationship matrix, and σ^2 is a scalar denoting variance. The subscripts *a*, *c* and *e* denote additive genetic, litter and residual variances respectively. The fixed effects included in the model were sex, year of birth, whether the dog was bred by GD or not and inbreeding coefficient. Labrador Retriever fraction, heterosis and recombination loss were included as covariates. The random effects fitted were a litter effect and individual animal effect. Mathematically, the heritability is the ratio of additive genetic variance to phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$.

In order to determine whether the heritability estimates were significantly different from zero Likelihood Ratio Tests (LRTs) were performed between the univariate animal models and null models in which the random effect for the individual had been omitted.

Estimates of heritability on the continuous liability scale were approximated for all conditions using the following equation (Dempster & Lerner, 1950):

$$h_c^2 = h_{01}^2 \left(\frac{1-p}{i^2 p} \right)$$

where subscripts *c* and 01 signify heritability estimates on the continuous liability and binary scale, respectively; *p* is the prevalence of the condition in the data; and *i* is the mean liability of individuals with the condition at prevalence p, in SD units, from the population mean, assuming normally distributed liability. Mean liabilities were obtained using Appendix Table A of Falconer & Mackay (1996).

7.2.2.2. *Univariate binomial mixed models*

Binomial mixed models fitted for each disease condition had the same general form, fixed effects and covariates and random effects as the linear mixed models. Unlike the linear models, binomial models link the response variable to an underlying linear predictor via a logit link function which is based on the logistic distribution. This is more flexible when the response variable is categorical or binary, as is the case in these disease models (De Risio et al, 2011).

7.3. Results

The total dataset for crossbreed models consisted of 18004 dogs. Table 7.2 shows the number of dogs of each breed and cross in the dataset and their relative proportions.

Table 7.2 Numbers of each breed and cross in the dataset. The breed of the sire is listed first.

Breed or cross	Number	Proportion
Golden Retriever	2345	0.130
Labrador Retriever	7264	0.400
Golden Retriever x Labrador Retriever	5801	0.320
Labrador Retriever x Golden Retriever	1470	0.080
Golden Retriever x (GR x LR)	42	0.002
Labrador Retriever x (GR x LR)	968	0.054
Labrador Retriever x (LR x GR)	114	0.006

Diseases for which there were more than 100 confirmed cases, and for which a statistically significant heritability estimate had been measured in at least one of the pure breeds, were investigated further. The breakdown of cases of each condition by breed and cross is shown in Table 7.3. The number of confirmed cases, and the number of dogs classed as “non-cases”, for each condition investigated is shown in Table 7.4.

Table 7.3 Cases of each condition in the breeds and crosses under investigation.

Condition	LR	GR	F1	BC	Total
Atopic dermatitis	341	112	353	46	852
CCL disease	69	22	67	1	159
Elbow dysplasia	258	19	40	1	318
Elbow dysplasia/elbow OA	406	48	131	3	588
Entropion	74	23	63	8	168
Hip dysplasia	209	84	201	4	498
Hip dysplasia/hip OA	127	344	336	7	814
Horner’s syndrome	12	55	54	0	121
Panosteitis	277	20	70	15	382
Seizures	108	16	50	0	174

Table 7.4 Cases and non-cases for each condition under investigation using crossbreed models, and apparent prevalences.

Condition	Cases	Non-cases	Total	Prevalence
Atopic dermatitis	852	6804	7656	0.11
CCL disease	159	3350	3509	0.05
Elbow dysplasia	318	3369	3687	0.09
Elbow dysplasia/elbow OA	588	3188	3776	0.16
Entropion	168	7334	7502	0.02
Hip dysplasia	498	3287	3785	0.13
Hip dysplasia/hip OA	814	3056	3870	0.21
Horner's syndrome	121	3348	3469	0.03
Panosteitis	382	7249	7631	0.05
Seizures	174	3355	3529	0.05

7.3.1. Linear models

The regression coefficient estimates of heterosis, recombination loss and Labrador fraction for the 10 conditions investigated are shown in Table 7.5.

Table 7.5 Crossbreeding parameter estimates for the 10 conditions investigated. Estimates are shown followed by their standard errors in brackets to two decimal places.

Disease	h*	r loss†	Lab§
Atopic dermatitis	-0.04 (0.04)	-0.01 (0.15)	-0.01 (0.04)
CCL disease	0.02 (0.04)	0.85 (0.76)	-0.01 (0.04)
Elbow dysplasia	-0.07 (0.05)	0.38 (0.98)	0.18 (0.05)
Elbow dysplasia/OA	-0.13 (0.06)	2.30 (1.17)	0.24 (0.06)
Entropion	0.02 (0.02)	0.03 (0.07)	-0.01 (0.02)
Hip dysplasia	-0.05 (0.05)	2.48 (0.99)	0.07 (0.06)
Hip dysplasia/OA	-0.00 (0.07)	3.87 (1.07)	0.07 (0.07)
Horner's syndrome	-0.08 (0.03)	-0.51 (0.73)	-0.11 (0.03)
Panosteitis	-0.03 (0.03)	-0.07 (0.11)	0.03 (0.03)
Seizures	-0.12 (0.04)	-0.44 (0.87)	0.05 (0.04)

* heterosis † recombination loss § Labrador fraction

Estimates of heterotic effect were negative and significantly larger than zero (using an approximate t-test) for elbow dysplasia or osteoarthritis (Categories 1-3), Horner's syndrome and seizures, at -0.13 (s.e. 0.06), -0.08 (s.e. 0.03) and -0.12 (s.e. 0.04) respectively. This suggests that heterosis reduced the likelihood of developing any of these three conditions, although the effect was quite small as the maximum heterosis probability for any individual is 50% meaning that values should be halved to obtain estimates for F1s. For the other seven conditions investigated, estimates of heterotic effect were small and positive for cranial cruciate ligament disease and entropion and small and negative for the remaining five conditions but none of these estimates were detectably greater than zero.

Estimates of the effect of recombination loss were positive and significantly larger than zero (using an approximate t-test) for elbow dysplasia or osteoarthritis (Categories 1-3), hip dysplasia (Category 1) and hip dysplasia or osteoarthritis (Categories 1-3), at 2.30 (s.e. 1.17), 2.48 (s.e. 0.99) and 3.87 (s.e. 1.07) respectively. Recombination loss thus increases the chance of

individuals developing these conditions, although as the maximum recombination loss probability in this dataset was 12.5% (in the backcrosses) the effects were smaller than they initially appeared at 0.29, 0.31 and 0.48 for the three conditions because the regression coefficient shown was scaled to a recombination loss value of 1. Positive estimates of the effect of recombination loss were also found for cranial cruciate ligament disease, elbow dysplasia (Category 1) and entropion with their values ranging from 0.03 (s.e. 0.07) for entropion to 0.85 (s.e. 0.76) for cranial cruciate ligament disease but they were not significantly larger than zero. Negative values for the effect of recombination loss were estimated for atopic dermatitis, Horner's syndrome, panosteitis and seizures with their magnitude ranging from -0.01 (s.e. 0.15) for atopic dermatitis to -0.51 (s.e. 0.73) for Horner's syndrome but none of them were detectably larger than zero.

Estimates of the effect of Labrador Retriever fraction were positive and detectably greater than zero (using an approximate t-test) for elbow dysplasia (Category 1) and the looser case definition of elbow dysplasia or osteoarthritis (Categories 1-3), at 0.18 (s.e. 0.05) and 0.24 (s.e. 0.06) respectively, meaning that increasing Labrador Retriever fraction was associated with a greater probability of having the conditions. The estimate of the effect of Labrador Retriever was negative and significantly larger than zero for Horner's syndrome, at -0.11 (s.e. 0.03), translating as increasing Labrador Retriever fraction decreasing the probability of being diagnosed with Horner's syndrome. Estimates of the effect of Labrador Retriever fraction for the remaining seven conditions were positive for four conditions (hip dysplasia (Category 1) and hip dysplasia or osteoarthritis (Categories 1-3), panosteitis and seizures) and negative for three conditions (atopic dermatitis, cranial cruciate ligament disease and entropion) but not detectably greater than zero.

Heritability estimates for the 10 conditions from these crossbreed univariate linear models, and those estimated in the single breed univariate linear models for the Labrador Retriever and Golden Retriever which were discussed in Chapter 6, are shown in Table 7.6. All the heritability estimates from the crossbreed univariate linear models were significantly greater than zero at $p < 0.01$.

Table 7.6 Comparison of heritability estimates from the crossbreed models and those estimated in single breed models for the Labrador Retriever and Golden Retriever. Estimates are shown followed by their standard errors in brackets to two decimal places.

Disease	Crossbreed h^2	LR h^2	GR h^2
Atopic dermatitis	0.12 (0.02)	0.09 (0.03)	0.31 (0.09)
CCL disease	0.18 (0.04)	0.31 (0.09)	0.19 (0.14)*
Elbow dysplasia	0.20 (0.04)	0.26 (0.07)	0 (0)*
Elbow dysplasia/OA	0.13 (0.03)	0.16 (0.06)	0.12 (0.09)*
Entropion	0.06 (0.02)	0.11 (0.04)	0.73 (0.08)
Hip dysplasia	0.16 (0.04)	0.09 (0.05)*	0 (0)*
Hip dysplasia/OA	0.20 (0.04)	0.13 (0.06)	0.03 (0.06)*
Horner's syndrome	0.09 (0.03)	NA†	0.09 (0.07)
Panosteitis	0.16 (0.03)	0.18 (0.04)	0.53 (0.13)
Seizures	0.10 (0.04)	0.12 (0.05)	NA†

* not significantly different from zero

† analysis not run in the purebred due to insufficient number of cases

The estimates in the crossbreed models were broadly similar to those estimated in the single breed models with a few exceptions. The heritability estimates for entropion and panosteitis in the crossbreed models, at 0.06 (s.e. 0.02) and 0.16 (s.e. 0.03), were much lower than those estimated in the Golden Retriever models which were 0.73 (s.e. 0.08) and 0.53 (s.e. 0.13) respectively.

Litter had a small but significant variance component estimate for all conditions except entropion, with estimates ranging from 0.02 (s.e. 0.01) for atopic dermatitis to 0.14 (s.e. 0.02) for seizures. Sex was a very small but significant fixed effect for atopic dermatitis, cranial cruciate ligament disease, elbow dysplasia (Category 1) and elbow dysplasia or osteoarthritis (Categories 1-3), hip dysplasia (Category 1) and hip dysplasia or osteoarthritis (Categories 1-3), panosteitis and seizures, with estimated effects of being male ranging from 0.01 (s.e. 0.007) for seizures to 0.06 (s.e. 0.01) for elbow dysplasia (Category 1) and elbow dysplasia or osteoarthritis (Categories 1-3). Whether or not a dog was externally bred was a significant but very small fixed effect for cranial cruciate ligament disease, elbow dysplasia (Category 1) and elbow dysplasia or osteoarthritis (Categories 1-3), with the effect for being externally bred of 0.05 (s.e. 0.02), 0.07 (s.e. 0.002) and 0.06 (s.e. 0.03) respectively. None of the other fixed effects were significant.

Heritability estimates on the continuous liability scale are shown in Table 7.7. As expected for all conditions the heritability estimates increased compared to the estimates from the univariate linear models.

Table 7.7 Apparent prevalences and heritability estimates on the continuous liability scale.

Disease	Prevalence	h^2
Atopic dermatitis	0.11	0.33
CCL disease	0.05	0.80
Elbow dysplasia	0.09	0.62
Elbow dysplasia or OA	0.16	0.30
Entropion	0.02	0.50
Hip dysplasia	0.13	0.40
Hip dysplasia or OA	0.21	0.40
Horner's syndrome	0.03	0.57
Panosteitis	0.05	0.71
Seizures	0.05	0.45

7.3.2. Binomial models

The results of the binomial models are presented in Table 7.8. Although the heritability estimates in the binomial models were similar to those estimated in the linear models none of the results were statistically significant.

Table 7.8 Heritability estimates from binomial models of 10 disease conditions.

Condition	h^2	s.e.
Atopic dermatitis	0.1557	0.1422
CCL disease	0.2704	0.3514
Elbow dysplasia	0.1904	0.2771
Elbow dysplasia/elbow OA	0.1134	0.1617
Entropion	0.0956	0.3597
Hip dysplasia	0.1158	0.1898
Hip dysplasia/hip OA	0.1074	0.1487
Horner's syndrome	0.2401	0.3935
Panosteitis	0.2304	0.2190
Seizures	0.1410	0.3343

7.4. Discussion

Univariate linear mixed models were successfully used to estimate heritabilities and crossbreeding parameters for 10 disease conditions in GD's Labrador Retrievers, Golden Retrievers and crosses between these two breeds. This represents the first attempt to quantify the crossbreeding parameters of heterosis, recombination loss and breed effects in the dog. In addition all 10 models measured heritability estimates which were detectably larger than zero ($p<0.01$). These simple crossbreed models assume that the additive genetic variance of the traits under investigation is broadly the same in the parent breeds and crossbred groups which is justifiable for two closely

related breeds with minimal inbreeding and when the traits are determined by many loci (Van der Werf & de Boer, 1989).

The datasets available for the crossbreed models were all much larger than those used in Chapter 6. This was reflected in the more moderate heritability estimates and smaller standard errors found in the present chapter. In addition, when the heritabilities were transformed to the underlying liability scale using Dempster & Lerner (1950) although all the estimates increased they remained lower than the theoretical maximum of one. Although all of the heritability estimates on the binary scale were low they were moderate to high when transformed to the underlying liability scale. This suggests that the underlying liabilities to these diseases are high.

The estimates in the crossbreed models were broadly similar to those estimated in the single breed models with a few exceptions. The heritability estimates for entropion and panosteitis in the crossbreed models, at 0.06 (s.e. 0.02) and 0.16 (s.e. 0.03), were much lower than those estimated in the Golden Retriever models which were 0.73 (s.e. 0.08) and 0.53 (s.e. 0.13) respectively. However the numbers of confirmed cases of these two conditions in the Golden Retriever were very low with just 23 cases of entropion and 20 cases of panosteitis. In such situations it could be that cases occur within just a handful of families artificially inflating the heritability estimates. More confidence can be placed in the crossbreed heritability estimates which were based on 168 and 382 confirmed cases respectively of the two conditions.

The level of heterosis exhibited in crossbred animals is determined by the degree of genetic difference between the parent breeds (Simms et al, 1990). The Labrador Retriever and Golden Retriever, although clearly distinct breeds with characteristic physical and behavioural traits, are more closely related than some other breeds. As discussed in Chapter 2, the Labrador Retriever and Golden Retriever were both in the “hunting” cluster of breeds based on microsatellite markers and SNPs (Parker et al 2004). The Labrador Retriever

was used in outcrosses in the early stages of breed formation of the Golden Retriever, and Sutter et al (2004) found that 84% of chromosomes from Golden Retrievers and Labrador Retrievers (20 unrelated individuals of each breed) carried shared haplotypes. Larger estimates of the effects of heterosis and recombination loss might thus be seen between crosses of more distantly related breeds. GD are using increasing numbers of Golden Retriever cross GSD and it would be interesting to attempt to quantify crossbreeding effects between these two breeds which are in different genetic clusters (Parker et al, 2007). Unfortunately the numbers were insufficient to allow such analyses.

Lewis et al (2010) found a small but significant detrimental effect of increasing inbreeding coefficient on BVA/KC hip score in Labrador Retrievers. Thus, as heterosis is the opposite of inbreeding depression (Falconer & Mackay, 1996), a small positive heterotic effect on hip phenotype might be expected. In the binary disease model of hip dysplasia this would manifest as a small negative heterotic effect, and this was seen for hip dysplasia (Category 1) and the looser case definition of hip dysplasia or osteoarthritis (Categories 1-3) but in both cases the effect was very small and not detectably larger than zero. In contrast no significant effect of inbreeding coefficient on BVA/KC elbow score was found (Lewis et al, 2011b). A small but significant negative heterotic effect in the binary disease model of elbow dysplasia or osteoarthritis (Categories 1-3) was however estimated in the present study, but the small, negative heterotic effect for the stricter case definition of elbow dysplasia (Category 1) was not significantly larger than zero.

Purebred dogs were not found to be at increased odds of idiopathic epilepsy compared with crossbred dogs although specific individual breeds, including the Labrador Retriever and Golden Retriever, were at increased odds of the disease (Kearsley-Fleet et al, 2013). The average inbreeding coefficient was not significantly different in Labrador Retrievers diagnosed with idiopathic epilepsy compared to those which were clear of the disease (Jaggy et al, 1998). The small but significant negative heterosis estimate of -0.12 (s.e. 0.04) found in the univariate linear model of seizures suggests that heterosis

lessens the likelihood of having seizures, although as explained in the results section as the maximum heterosis probability is 50%, seen in the F1 crosses, the effect is smaller than it initially appears.

The negative estimate of heterotic effect for Horner's syndrome was small but detectably larger than zero at 0.08 (s.e. 0.03), implying that heterosis reduced the likelihood of developing this condition. There was also a small but significant negative effect of Labrador fraction of -0.11 (s.e. 0.03) for Horner's syndrome, implying that increasing Labrador Retriever fraction decreases the probability of developing Horner's syndrome. Golden Retrievers have been described as being predisposed to the idiopathic form of the condition but the condition was also frequently seen in Golden Retriever x Labrador Retrievers (Boydell, 1995; Boydell, 2000). The number of confirmed cases in GD's Labrador Retrievers (12) between 1995 and 2012 is markedly lower than that for Golden Retrievers (55) and F1 (54), especially when the much greater number of Labrador Retrievers than Golden Retrievers in the dataset is considered. When the overall population size at GD is considered the prevalence of Horner's syndrome is also lower in the F1 than in the purebred Golden Retriever.

That the estimates of heterotic effect were small and not significantly different from zero for the other six conditions suggests that there is little dominance between alleles of genes that influence these traits and/or that the allele frequencies are similar in the Labrador Retriever and Golden Retriever. Given the relatively close relationship between the two breeds it is quite likely that they would have similar allele frequencies at some loci at least.

Moderate positive estimates of the effect of recombination loss were found for elbow dysplasia or osteoarthritis (Categories 1-3) and for both hip dysplasia (Category 1) and hip dysplasia or osteoarthritis (Categories 1-3) of 0.29, 0.31 and 0.48 respectively (given that the maximum recombination loss probability in this dataset was 12.5%). These estimates imply that

recombination loss increases the chance of individuals being diagnosed with these conditions. This suggests that there may be breed-specific epistatic effects which are protective against developing hip and elbow dysplasia. Therefore, in terms of hip and elbow dysplasia, it could be argued that GD should not use any of their F1 crosses for breeding but should only breed from the purebred parent lines.

Binomial models were not attempted for the within-breed disease models due to the small size of the datasets and even the much larger crossbreed datasets presented in this chapter were too small for the models to produce heritability estimates which were detectably larger than zero. The results of binomial models such as these are known to suffer from biases. For binary data with small group sizes estimation bias, usually towards zero, can be over 50% (Rodriguez & Goldman, 1995). These authors reported that, although the random effects (such as heritability) tended to be underestimated the standard errors tended to be accurate. This could explain why none of the heritability estimates from the binomial models were significantly larger than zero as the heritability estimates are being underestimated while the standard errors are not.

Studies have shown that combining data on purebred and crossbred individuals improves the reliability of genetic evaluations, particularly when both purebred and crossbred performance is of interest and when substantial crossbred information is available (Lutaaya et al, 2002; Ibáñez-Escriche et al, 2011). The benefit of using crossbred information has been shown to be highest for traits with low heritability as such traits benefit more from information on siblings (Bijma & van Arendonk, 1998). Although substantial genetic improvement can result from crossbreeding it does not accumulate over time and long term trends in genetic improvement come from selection within breeds (Kinghorn, 1987).

7.5. Conclusion

The results of these analyses suggest that EBVs produced from univariate linear crossbreed models could be used for selection in GD's Labrador Retrievers and Golden Retrievers to produce both purebred and crossbred litters. As discussed in Chapter 6, selection against hip and elbow dysplasia in the purebred lines may be best achieved by using the EBVs available through Mate Select. These conditions had the highest heritability estimates in these crossbreed models; selection against these two diseases could thus perhaps be undertaken in the F1 using EBVs produced from these models. Atopic dermatitis, CCL disease, panosteitis and seizures have heritability estimates between 0.10 and 0.20 suggesting that EBVs for these diseases would enable more efficient selection against these conditions than selecting based on phenotype or even phenotype of close relatives. Information on many more individuals is available when crossbreed models are used compared to single breed models and this should lead to more accurate heritability estimates and EBVs.

Crossbreeding parameters for 10 health traits have been quantified for the first time in the dog. Most of the effects were not detectably larger than zero, but a few disease conditions showed evidence of heterosis, recombination and/or breed effects. Such effects may be larger when the breeds being crossed are less closely related. These results will be of interest to the wider dog breeding community.

8. GENETIC ANALYSIS OF A BEHAVIOURAL SCORING SYSTEM USED BY GUIDE DOGS IN TWO PUREBRED DOG BREEDS

8.1. Introduction

Behaviour testing of dogs has been used since at least 1934 as an aid for selection of service dogs for various types of work and for breeding (Humphrey, 1934). Since this time many service dog organisations have created their own bespoke behaviour assessment protocols. Guide Dogs (GD) use three different behavioural scoring systems to assess their dogs during puppy walking and training to determine skills needing further work and to evaluate potential for suitability for guiding work. The first of these is an applied stimulus test used just before puppies go into their puppy walking homes, the puppy profiling assessment (PPA), and this is explored in Chapter 10. A second applied stimulus test is used with prospective breeding stock (PBS) at the end of puppy walking; this is the character assessment test (CAT). The CAT dataset was too small for quantitative genetic analyses at this time as only 141 CAT assessments had been completed by May 2012. The third behavioural assessment system used by GD is the canine assessment summary (CAS) and this is described and explored further in this chapter and Chapter 9. An advantage of observer rating methods such as CAS compared to applied stimulus tests is, as they are based on observations of the dog in its normal environment, the ratings will reflect its everyday behavior (Meagher, 2009).

As with any characteristic, behavioural traits can only be integrated into a breeding program if they can be accurately measured and if they demonstrate significant genetic variation (Ruefenacht et al, 2002). A large number of studies have suggested that genetic variation exists in many behavioural traits in different breeds of dog, as discussed in Chapter 2. For example Ruefenacht

et al (2002) performed genetic analyses of the results of the field behaviour test of the Swiss German Shepherd Dog breeding club, a standardised test which has been in use since 1949. Specifically, they looked at the test results of 3497 German Shepherd Dogs which had undergone the test between 1978 and 2000. The test in question was an eight-part test with the dog being handled by their owner and assessed by a trained judge who, after observing the dog's behaviour in the eight situations, assigned a score of one (best) to four or five (worst) for eight behavioural traits: self-confidence, nerve stability, reaction to gunfire, "temperament", hardness, sharpness, defence drive and fighting drive. Their heritability estimates ranged from 0.09 for sharpness to 0.24 for reaction to gunfire, with standard errors ranging from 0.04 to 0.06. Positive genetic correlations were found between all eight traits ranging from 0.34 between sharpness and "temperament" to 1.0 between nerve stability and self-confidence (Ruefenacht et al, 2002).

Lindberg et al (2004) performed genetic analyses of the results of a hunting behaviour test carried out by the Swedish Flatcoated Retriever club. They used the results of 800-1150 (depending on trait) dogs aged 12-24 months which had been tested between 1992 and 2000. During the test each dog was exposed to several standardised hunting situations and 10 components were scored by trained assessors. These were: reaction to shot, single marking (locating game which the dog has seen thrown but not where it landed, in open terrain approximately 35 metres from the dog), reaction when game is thrown, interest in search, retrieving, delivery, grip, interest in water retrieving, cooperation and waiting passively in a group. Heritability estimates range from 0.12 for cooperation to 0.74 for waiting passively in a group. Genetic correlations could not be estimated between all traits but those which were estimated ranged from -0.82 between delivery and interest in water retrieving to 0.90 between single marking and cooperation (Lindberg et al, 2004).

Ten behavioural characteristics scored based on the dogs' reactions in seven different test situations had heritability estimates ranging from 0.13±0.05 to

0.37±0.08 in the GSD and 0.15±0.07 to 0.28±0.09 in the Labrador Retriever (Wilsson & Sundgren, 1997b).

The aim of this chapter was to investigate genetic and environmental factors relating to behavioural traits measured by CAS to determine whether there was potential for developing EBVs for any CAS elements.

8.1.1. Canine Assessment Summary (CAS)

CAS is a means of measuring specific behavioural traits, focusing on those areas of behaviour and task performance which indicate potential for success as a guide dog. It was developed as a tool to support GD's objectives of reduced training times and earlier identification of dogs that would be withdrawn from their programme, and came into use in 2002. In total 25 different "dimensions" are scored by trained staff, of which three dimensions are only assessed during puppy walking (PW) and six dimensions are only assessed during early and advanced training. The dimensions and their scoring are shown in more detail in Appendix 9. The dimensions that are assessed across all 3 stages (puppy walking, early training (ET) and advanced training (AT)) are: aggression towards animals, aggression towards people, suspicion, attentiveness, behaviour on transport, behaviour when left, body sensitivity, calmness, confidence, distraction, eagerness, interaction with animals, interaction with people, obedience, toileting routine and stress resilience. These traits are all scored 1-4 with 1 always being the best or most desirable score. The three elements which are only assessed in puppy walking involve task acquisition: handler position in busy areas, handler position in quiet areas and speed control, and these are also scored 1-3 with 1 being the best or most desirable score. Those not assessed in puppy walking are those relating to skills which are only taught in the training phases – these task acquisition traits are kerb work, locating objectives, right shoulder work (the ability to leave enough room on the right hand side for the handler to pass people, obstacles or through doorways without bumping into them), on/off

kerb work, straight line work and traffic (the ability to safely negotiate a path through traffic). These skill acquisition traits are scored 1-7, with 1 being the best or most desirable score. Assessments are carried out by many different GD personnel, all of whom have been trained in using CAS.

CAS assessments were initially completed after each formal visit during puppy walking and then monthly during early and advanced training. However, the work of the Epidemiology of Guide Dog Behaviour team at the University of Nottingham showed that some assessment times were more predictive of later success (qualification) than others (Asher, University of Nottingham, personal communication, 2012). Assessments in puppy walking are now only completed at the first visit and then at 5, 8 and 12 months of age. Monthly assessments in early and advanced training continue. Therefore, based on these findings and to match current practices, it was decided to use for genetic analysis the assessments at the ages and time points which had been shown to be most predictive of success – these were the first assessment in puppy walking and those at 5, 8 and 12 months of age, the first assessment in early training and the first assessment in advanced training.

8.2. Materials and methods

8.2.1. Description of dataset

GD provided a copy of their main database which contained data up to 2nd February 2012. The full CAS dataset (including all CAS assessments between 1st January 2002 and 2nd February 2012) consisted of 141934 records from 11709 dogs of 28 breeds and crosses (see Appendix 8).

8.2.2. Data validation

The data were edited based on two criteria (breed and duplicated records) as explained below. Table 8.1 shows the number of dogs in the data set before and after each round of validation. The final dataset analysed contained 130482 records from 10704 dogs and the breakdown of breeds and crosses in this dataset is shown in Table 8.2.

8.2.2.1. *Breed*

Dogs of any breed except Labrador Retriever, Golden Retriever, GSD and crosses between Labrador Retrievers and Golden Retrievers were excluded from further analyses. This resulted in the removal of 968 dogs.

8.2.2.2. *Duplicated records and date errors*

A number of dogs were found to have negative ages at the time of one of their assessments – these assessments were checked in GDI and the causative date error and age at assessment amended. Any assessments which were undertaken when a dog was greater than 1000 days (2 years and 9 months) were discarded, which resulted in records for 341 dogs being removed (in some cases this resulted in the dog being completely removed). In most cases these assessments had been undertaken after a dog was returned to advanced training after a period working, mostly due to factors relating to the Guide Dog Owner (GDO). Five dogs were ex-breeding stock which had entered training late after a premature end to their breeding career, and some were dogs which had been imported from overseas guide dog schools as adults. Several hundred dogs appeared to have had more than one assessment on the same date – these instances were checked in GDI, where there was a date error this and the age at that assessment were amended. If

it was truly an exact duplicate record, any duplicates were removed such there was only one assessment on a particular date.

Table 8.1 Number of dogs retained and removed at each editing step described in the materials and methods. The percentage expressed is with reference to the number of dogs in the raw data set.

Editing step	Number remaining	Number lost	Percentage remaining
Raw data set	11709	-	-
Breed	10741	968	92%
Duplicate assessments on same date	10704	37	91%

Table 8.2 Number of records available for the breeds and crosses included in subsequent analyses.

Breed	Number of dogs
German Shepherd Dog	626
Golden Retriever	1216
Golden Retriever x (Golden Retriever x Labrador)	36
Golden Retriever x Labrador	4267
Labrador	3522
Labrador x (Labrador x Golden Retriever)	1037
Total	10704

There were insufficient numbers of GSDs for meaningful results to be obtained so analyses were only undertaken in the Labrador Retriever (univariate and bivariate analyses) and Golden Retriever (univariate analyses only).

8.2.3. Extracting CAS assessment scores of interest

MATLAB® programs were used to identify and extract the CAS assessment scores of interest from all the assessments available. In this dataset the median number of CAS assessments per dog was 13 (range 1-27) and there was great variability in the age of dogs at each assessment. This is illustrated in Figure 8.1. To enable some consistency and to be most useful with the current system, assessments which occurred at the approximate age at which they are now performed were extracted. These were the first assessment in puppy walking at between 41 and 71 days old, the first assessment occurring between 139 and 169 days old (approximately 5 months of age), the first assessment falling between 230 and 260 days old (approximately 8 months of age) and the first assessment occurring between 349 and 378 days old (approximately 12 months of age). The first assessments in early and advanced training were also extracted, but these were not linked to age.

At each CAS assessment of interest the following information was available relating to each dog in the dataset: sex, breed, whether or not the dog had been bred by GD, year of birth and assessor identification number. There were 805 assessors, the median number of assessments undertaken by an assessor was 13 (minimum 1, maximum 839). The distribution of number of assessments undertaken by each assessor is shown in Figure 8.2.

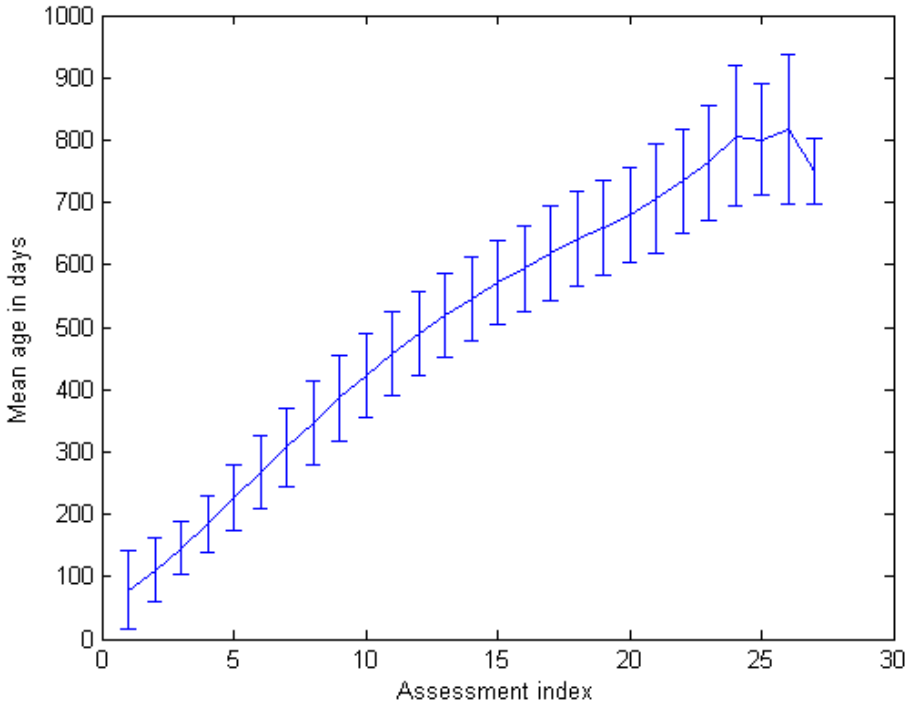

Figure 8-1 Plot of age in days of dogs undergoing CAS assessments against CAS assessment index, showing mean and standard deviation of age at test in days against assessment index (numbered 1 to n for each dog).

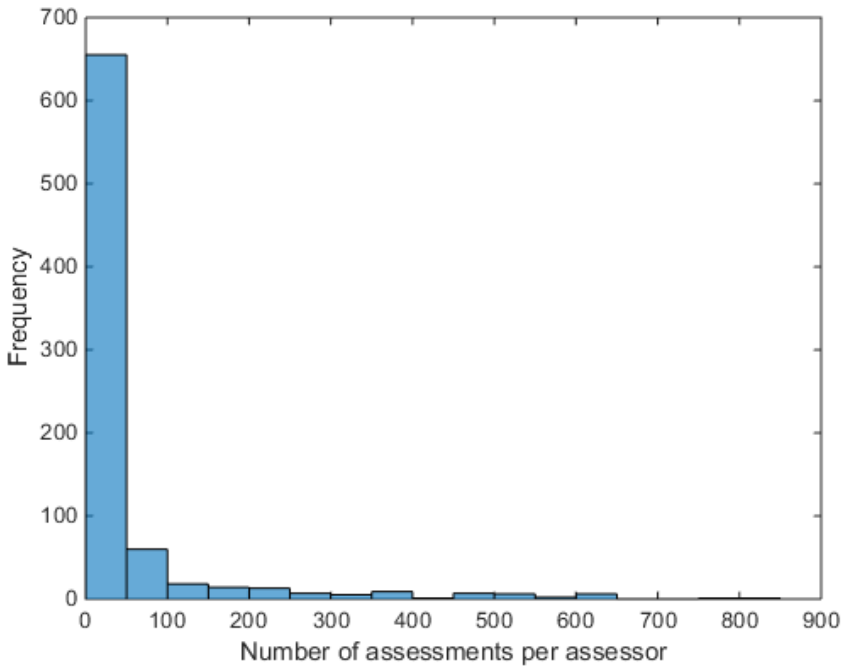

Figure 8-2 Histogram showing the distribution of number of CAS assessments undertaken by the 805 assessors in the dataset.

8.2.4. Statistical analyses

Statistical analysis of the data had the objective of fitting univariate and bivariate mixed linear models using ASReml version 3.0 (Gilmour et al, 2009) to estimate genetic and environmental parameters associated with the traits evaluated in CAS.

The pedigree file used in all analyses was described in Chapter 4.

8.2.4.1. *Univariate linear mixed models*

The general form of the univariate linear mixed model fitted for each CAS trait at each time point was as follows:

$$\mathbf{Y = Xb + Za + Wc + Vd + e}$$

where **Y** is the vector of observations; **X**, **W, V** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \sigma^2_a\mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \sigma^2_c\mathbf{I})$; d is the vector of random assessor effects with the distribution $(0, \sigma^2_d\mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \sigma^2_e\mathbf{I})$; and where **I** denotes an identity matrix of the appropriate size, **A** is the numerator relationship matrix, and σ^2 is a scalar denoting variance. The subscripts *a*, *c, d* and *e* denote additive genetic, litter, assessor and residual variances respectively. The fixed effects included in the model were gender, year of birth, age (in days) at assessment, whether the dog was bred by GD or not, inbreeding coefficient and colour (for Labrador Retrievers only). The random effects fitted were litter, assessor and individual animal effect. Mathematically, the heritability is the ratio of additive genetic variance to phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$.

In order to determine whether the heritability estimates were significantly different from zero Likelihood Ratio Tests (LRTs) were performed between the univariate animal models and null models in which the random effect for the individual had been omitted.

8.2.4.2. *Bivariate linear mixed models*

Bivariate linear models fitted to estimate the genetic correlations between pairs of CAS elements were of the following form:

$$\mathbf{Y = Xb + Za + Wc + Vd + e}$$

where **Y** is the vector of observations; **X**, **W** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \Sigma_a \otimes \mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \Sigma_c \otimes \mathbf{I})$; **d** is the vector of random assessor effects with the distribution $(0, \Sigma_d \otimes \mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \Sigma_e \otimes \mathbf{I})$; **I** is an identity matrix of the appropriate size, **A** is the additive genetic relationship matrix. The subscripts *a*, *c, d* and *e* denote additive genetic, litter and residual (co)variances respectively. The variance terms such as σ^2_a used in the univariate models were replaced by the appropriate bivariate covariance matrices (Σ) for the traits using the Kronecker product e.g. $\Sigma_a \otimes \mathbf{A}$. Thus Σ_a represents the additive genetic covariances of the two CAS elements in the base population. The fixed effects included in the model were the same as for the univariate models, namely: gender, year of birth, age (in days) at assessment, whether the dog was bred by GD or not, inbreeding coefficient and colour (for Labrador Retrievers only). The random effects fitted were litter, assessor and individual animal effect.

In order to determine whether the genetic correlations estimated were significantly different from zero LRTs were performed between the

unconstrained bivariate animal models and null models in which the genetic correlation was constrained to 0.00001.

Due to the smaller size of the datasets for the Golden Retriever, bivariate models were only attempted for the Labrador Retriever.

8.3. Results

8.3.1. Labrador Retriever univariate models

Although there were 3522 Labrador Retrievers in the edited CAS dataset, the number of dogs which had scores at the different time points was considerably lower, ranging from 2331 individuals at the assessment at 5 months of age to 1576 individuals at the first CAS assessment in advanced training. All of the datasets showed a skewed distribution of scores to some extent. The distribution of scores at each of the time points of interest is shown for one trait, calmness, in Appendix 10.

Due to the volume of results, only those in which the heritability estimate was detectably larger than zero are presented. Assessor and litter effects shown in the tables are proportions of total variance.

8.3.1.1. *Aggression towards animals*

The heritability estimates for score for aggression towards animals were detectably larger than zero for the first assessments in early training and advanced training ($p<0.05$), as shown in Table 8.3. Both heritability estimates were low and smaller than the estimate of assessor effect. The assessor effects were larger for the other four time points (for which the heritability estimates were very small and not detectably larger than zero), ranging from 0.31 (s.e. 0.04) for the CAS assessment at 12 months of age to 0.39 (s.e. 0.04) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of the aggression towards animals models.

Table 8.3 Parameter estimates from the two CAS assessments for which the heritability estimates of score for aggression towards animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	Litter effect	Assessor effect
1st in ET	0.05 (0.03)	0.03 (0.02)	0.07 (0.02)
1st in AT	0.06 (0.03)	0 (0)	0.12 (0.02)

8.3.1.2. *Aggression towards people*

The heritability estimates for score for aggression towards people were small but detectably larger than zero for the first assessments in early training and advanced training ($p<0.05$), as shown in Table 8.4. The assessor effects were relatively small for most assessments for this trait ranging from 0.004 (s.e. 0.01) for the CAS assessment at 12 months of age to 0.32 (s.e. 0.04) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of the aggression towards people models.

Table 8.4 Parameter estimates from the two CAS assessments for the which the heritability estimates of score for aggression towards people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	Litter effect	Assessor effect
1st in ET	0.06 (0.03)	0.00 (0.02)	0.01 (0.01)
1st in AT	0.07 (0.03)	0 (0)	0.06 (0.02)

8.3.1.3. *Attentiveness*

Heritability estimates for score for attentiveness were small but detectably larger than zero for the assessment in puppy walking at 8 months of age and for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 8.5. Assessor effects for this trait ranged from 0.10 (s.e. 0.02) for the first CAS assessment in advanced training to 0.59 (s.e. 0.04) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of the attentiveness models.

Table 8.5 Parameter estimates from the three CAS assessments for which the heritability estimates of score for attentiveness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	Litter effect	Assessor effect
8 months of age	0.06 (0.03)	0.01 (0.02)	0.13 (0.02)
1st in ET	0.06 (0.03)	0 (0)	0.16 (0.02)
1st in AT	0.13 (0.05)	0 (0)	0.10 (0.02)

8.3.1.4. *Behaviour on transport*

None of the heritability estimates for score for behaviour on transport were detectably larger than zero. The assessor effect for the trait was generally large, ranging from 0.17 (s.e. 0.03) for the first assessment in advanced training to 0.54 (s.e. 0.04) for the first assessment in puppy walking.

8.3.1.5. *Behaviour when left*

The heritability estimate for behaviour when left was only detectably larger than zero at one CAS assessment – that at 8 months of age, for which it was 0.04 (s.e. 0.02; $p<0.01$), as shown in Table 8.6. As with behaviour on transport, the assessor effect for this trait was generally large ranging from 0.13 (s.e. 0.03) for the first assessment in advanced training to 0.61 (s.e. 0.04) for the first assessment in puppy walking. None of the fixed effects were significant.

Table 8.6 Parameter estimates from the CAS assessment for which the heritability estimate of score for behaviour when left was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS assessment	h^2	Litter effect	Assessor effect
8 months of age	0.04 (0.02)	0 (0)	0.17 (0.03)

8.3.1.6. *Body sensitivity*

The heritability estimates for score for body sensitivity were small but detectably larger than zero for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 8.7. The assessor effects were relatively small for these two CAS assessments but were larger for the other four time points (for which the heritability estimates were very small and not detectably larger than zero), ranging from 0.42 (s.e. 0.04) for the CAS assessment at 8 months of age to 0.68 (s.e. 0.043) for the first CAS assessment in puppy walking. Whether or not a dog was bred by GD was a small but significant fixed effect for the first CAS assessment in advanced training, with an effect of not being GD-bred of -0.16 (s.e. 0.08). None of the other fixed effects were significant in any of the body sensitivity models.

Table 8.7 Parameter estimates from the two CAS assessments for which the heritability estimates of score for body sensitivity were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
1st in ET	0.10 (0.03)	0.01 (0.02)	0.14 (0.02)
1st in AT	0.16 (0.05)	0 (0)	0.09 (0.02)

8.3.1.7. *Calmness*

Heritability estimates for score for attentiveness were small but detectably larger than zero for the assessments in puppy walking at 5, 8 and 12 months of age and for the first assessments in early training and advanced training (all $p<0.01$ except 8 months, $p<0.05$), as shown in Table 8.8. Assessor effects for this trait ranged from 0.09 (s.e. 0.02) for the first CAS assessment in advanced training to 0.41 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect in all six calmness models, with the effect of being male ranging from -0.05 (s.e. 0.02) at 8 months of age to -0.13 (s.e. 0.03) at the first CAS assessment in early training, implying that male dogs tended to receive lower (i.e. better) scores for calmness. None of the other fixed effects were significant.

Table 8.8 Parameter estimates from the five CAS assessments for which the heritability estimates of score for calmness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.08 (0.03)	0.01 (0.01)	0.19 (0.03)
8 months of age	0.10 (0.04)	0.02 (0.02)	0.15 (0.03)
12 months of age	0.03 (0.02)	0 (0)	0.21 (0.03)
1st in ET	0.11 (0.04)	0.00 (0.02)	0.12 (0.02)
1st in AT	0.10 (0.04)	0.05 (0.02)	0.09 (0.02)

8.3.1.8. *Confidence*

The heritability estimate for confidence was only detectably larger than zero at one CAS assessment – the first in early training, for which it was 0.08 (s.e. 0.03; $p<0.01$), as shown in Table 8.9. Assessor effect for this trait ranged from 0.11 (s.e. 0.02) for the first assessment in advanced training to 0.60 (s.e. 0.04) for the first assessment in puppy walking. None of the fixed effects were significant.

Table 8.9 Parameter estimates from the CAS assessment for which the heritability estimate of score for confidence was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training.

CAS assessment	h^2	litter effect	assessor effect
1st in ET	0.08 (0.03)	0 (0)	0.19 (0.02)

8.3.1.9. *Distraction*

The heritability estimates for score for distraction were small but detectably larger than zero for the assessments in puppy walking at 5 ($p<0.05$) and 8 months of age ($p<0.01$) and for the first assessments in early training and advanced training (both $p<0.01$), as shown in Table 8.10. The assessor effects for this trait ranged from 0.09 (s.e. 0.02) for the first CAS assessment in advanced training to 0.65 (s.e. 0.03) for the first CAS assessment in puppy walking. Whether or not a dog was bred by GD was a small but significant fixed effect for distraction at the first CAS assessment in early training, with an effect of not being GD-bred of 0.16 (s.e. 0.05). None of the other fixed effects were significant in any of the distraction models.

Table 8.10 Parameter estimates from the four CAS assessments for which the heritability estimates of score for distraction were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.04 (0.02)	0.02 (0.02)	0.19 (0.03)
8 months of age	0.06 (0.03)	0.00 (0.02)	0.16 (0.03)
1st in ET	0.09 (0.03)	0.01 (0.02)	0.13 (0.02)
1st in AT	0.10 (0.04)	0.03 (0.02)	0.09 (0.02)

8.3.1.10. *Eagerness*

The heritability estimates for score for eagerness were small but detectably larger than zero for the assessments in puppy walking at 8 ($p<0.01$) and 12 months of age ($p<0.01$) and for the first assessments in early training and advanced training (both $p<0.01$), as shown in Table 8.11. The assessor effects for this trait ranged from 0.13 (s.e. 0.03) for the first CAS assessment in advanced training to 0.56 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect for eagerness score at the first CAS assessment in early training with an estimated effect of being male of 0.08 (s.e. 0.03), implying that male dogs tended to receive a higher (i.e. worse) score for eagerness. None of the other fixed effects were significant in any of the distraction models.

Table 8.11 Parameter estimates from the four CAS assessments for which the heritability estimates of score for eagerness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.04 (0.02)	0.02 (0.02)	0.19 (0.03)
12 months of age	0.06 (0.03)	0.00 (0.02)	0.16 (0.03)
1st in ET	0.09 (0.03)	0.01 (0.02)	0.13 (0.02)
1st in AT	0.10 (0.04)	0.03 (0.02)	0.09 (0.02)

8.3.1.11. *Interaction with animals*

Heritability estimates for score for interaction with animals were small but detectably larger than zero for the assessment in puppy walking at 8 months of age and for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 8.12. Assessor effects for this trait ranged from 0.11 (s.e. 0.02) for the first CAS assessment in advanced training to 0.39 (s.e. 0.04) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of these models.

Table 8.12 Parameter estimates from the three CAS assessments for which the heritability estimates of score for interaction with animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.06 (0.03)	0.01 (0.02)	0.21 (0.03)
1st in ET	0.07 (0.03)	0.00 (0.02)	0.13 (0.02)
1st in AT	0.06 (0.05)	0.00 (0.02)	0.11 (0.02)

8.3.1.12. *Interaction with people*

Heritability estimates for score for interaction with people were small but detectably larger than zero for the assessments in puppy walking at 5, 8 and 12 months of age and for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 8.13. Assessor effects for this trait ranged from 0.08 (s.e. 0.02) for the first CAS assessment in advanced training to 0.39 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect in all the interaction with people models except the first assessment in puppy walking, with the effect of being male ranging from -0.08 (s.e. 0.02) at 8 months of age to -0.14 (s.e. 0.03) at the first CAS assessment in early training, implying that male dogs tended to receive lower

(i.e. better) scores for interaction with people. None of the other fixed effects were significant.

Table 8.13 Parameter estimates from the five CAS assessments for which the heritability estimates of score for interaction with people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.08 (0.03)	0.01 (0.01)	0.19 (0.03)
8 months of age	0.10 (0.04)	0.02 (0.02)	0.15 (0.03)
12 months of age	0.03 (0.02)	0 (0)	0.21 (0.03)
1st in ET	0.11 (0.04)	0.00 (0.02)	0.12 (0.02)
1st in AT	0.10 (0.04)	0.05 (0.02)	0.09 (0.02)

8.3.1.13. *Obedience*

Heritability estimates for score for obedience were small but detectably larger than zero for the assessment in puppy walking at 8 months of age and for the first assessments in early training and advanced training ($p<0.05$), as shown in Table 8.14. Assessor effects for this trait ranged from 0.12 (s.e. 0.02) for the first CAS assessment in advanced training to 0.60 (s.e. 0.04) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of the obedience models.

Table 8.14 Parameter estimates from the three CAS assessments for which the heritability estimates of score for obedience were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.02 (0.02)	0.01 (0.02)	0.30 (0.04)
1st in ET	0.03 (0.02)	0.02 (0.01)	0.26 (0.02)
1st in AT	0.06 (0.04)	0.01 (0.02)	0.12 (0.02)

8.3.1.14. *Handler position in busy areas*

Handler position in busy areas is only scored during puppy walking. The heritability estimate for its score was only detectably larger than zero at one CAS assessment – that at 5 months of age, for which it was 0.03 (s.e. 0.02; $p<0.01$), as shown in Table 8.15. The assessor effect for this trait was large ranging from 0.41 (s.e. 0.04) for the assessment at 5 months of age to 0.56 (s.e. 0.04) for the assessment at 12 months of age. Whether or not a dog was bred by GD was a small but significant fixed effect for handler position in busy areas at the CAS assessment at 5 months of age, with an effect of not being GD-bred of 0.09 (s.e. 0.04). None of the other fixed effects were significant.

Table 8.15 Parameter estimates from the CAS assessment for which the heritability estimate of score for handler position in busy areas was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.03 (0.02)	0.02 (0.01)	0.41 (0.04)

8.3.1.15. *Handler position in quiet areas*

Handler position in quiet areas is only scored during puppy walking. The heritability estimate for its score was only detectably larger than zero at one CAS assessment – that at 5 months of age, for which it was 0.03 (s.e. 0.02; $p<0.01$), as shown in Table 8.16. The assessor effect for this trait was large ranging from 0.43 (s.e. 0.04) for the first CAS assessment in puppy walking to 0.58 (s.e. 0.04) for the assessment at 12 months of age. None of the fixed effects were significant.

Table 8.16 Parameter estimates from the CAS assessment for which the heritability estimate of score for handler position in quiet areas was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.03 (0.02)	0.02 (0.01)	0.44 (0.04)

8.3.1.16. *Speed control*

Speed control is only scored during puppy walking. The heritability estimate for its score was only detectably larger than zero at one CAS assessment – that at 5 months of age, for which it was 0.04 (s.e. 0.02; $p<0.01$), as shown in Table 8.17. The assessor effect for this trait was large ranging from 0.41 (s.e. 0.04) for the CAS assessment at 8 months of age to 0.53 (s.e. 0.04) for the assessment at 12 months of age. None of the fixed effects were significant.

Table 8.17 Parameter estimates from the CAS assessment for which the heritability estimate of score for speed control was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.03 (0.02)	0.02 (0.01)	0.44 (0.04)

8.3.1.17. *Stress resilience*

The heritability estimate for score for stress resilience was only detectably larger than zero at one CAS assessment – the first assessment in early training, for which it was 0.04 (s.e. 0.02; $p<0.05$), as shown in Table 8.18. The assessor effect for this trait was generally large ranging from 0.15 (s.e. 0.03) for the first CAS assessment in advanced training to 0.66 (s.e. 0.03) for the first assessment in puppy walking. None of the fixed effects were significant.

Table 8.18 Parameter estimates from the CAS assessment for which the heritability estimate of score for stress resilience was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training.

CAS assessment	h^2	litter effect	assessor effect
1st in ET	0.04 (0.02)	0.00 (0.01)	0.22 (0.02)

8.3.1.18. *Suspicion*

The heritability estimates for score for suspicion were detectably larger than zero for the first assessments in early training and advanced training ($p<0.05$), as shown in Table 8.19. Both heritability estimates were low and smaller than the estimate of assessor effect. The assessor effects generally large for this trait ranging from 0.12 (s.e. 0.03) for the first CAS assessment in advanced training to 0.66 (s.e. 0.03) for the first CAS assessment in puppy walking. None of the fixed effects were significant in any of the suspicion models.

Table 8.19 Parameter estimates from the two CAS assessments for which the heritability estimates of score for suspicion were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.04 (0.02)	0.03 (0.01)	0.38 (0.04)
1st in AT	0.06 (0.03)	0 (0)	0.12 (0.03)

8.3.1.19. *Toileting routine*

The heritability estimate for score for toileting routine was only detectably larger than zero at one CAS assessment – that at 8 months of age, for which it was 0.04 (s.e. 0.02; $p<0.01$), as shown in Table 8.20. The assessor effect for this trait was large and ranged from 0.11 (s.e. 0.03) for the first CAS assessment in advanced training to 0.63 (s.e. 0.04) for the first assessment in puppy walking. None of the fixed effects were significant.

Table 8.20 Parameter estimates from the CAS assessment for which the heritability estimate of score for toileting routine was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.04 (0.02)	0 (0)	0.22 (0.03)

8.3.1.20. *Task acquisition scores*

None of the heritability estimates were detectably larger than zero for the six task acquisition scores (kerb work, locating objectives, right shoulder work, on/off kerb work, straight line work and traffic) at the first assessment in early training and the first assessment in advanced training. The variance component for assessor was low to moderate. It was low for right shoulder work at the first assessment in early training (0.08, s.e. 0.02) and locating objectives at the same assessment (0.07, s.e. 0.02) but for the other scores it ranged from 0.15 for straight line work at the first assessment in early training to 0.39 for on/off kerb work at the first assessment in advanced training.

8.3.2. Golden Retriever univariate models

The CAS datasets were much smaller for the Golden Retriever. Although there were 1216 Golden Retrievers in the edited CAS dataset, the number of individuals which had scores for the traits at each time point ranged from 819 at 5 months of age to 550 at the first assessment in advanced training. Most of the heritability estimates for each trait at the different time points were not detectably larger than zero, probably due to the small size of the datasets, but the 14 significant results are shown in Table 8.21. Estimates of litter effect are not shown as they were all small and not detectably larger than zero.

Table 8.21 Parameter estimates for the 14 traits and time points for which the heritability estimates of score were detectably larger than zero in the Golden Retriever. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

Trait	Time point	h^2	assessor effect
Calmness	12 months of age	0.10 (0.06)†	0.36 (0.05)
Calmness	1st in AT	0.24 (0.10)*	0.10 (0.05)
Confidence	12 months of age	0.15 (0.07)†	0.32 (0.05)
Eagerness	1st in AT	0.25 (0.13)†	0.03 (0.04)
Interaction with people	12 months of age	0.13 (0.07)†	0.28 (0.05)
Interaction with people	1st in ET	0.13 (0.06)*	0.16 (0.04)
Interaction with people	1st in AT	0.24 (0.10)*	0.02 (0.04)
Obedience	1st in ET	0.07 (0.06)†	0.24 (0.04)
Stress resilience	5 months of age	0.13 (0.06)*	0.31 (0.04)
Stress resilience	12 months of age	0.20 (0.09)*	0.26 (0.05)
Suspicion	5 months of age	0.09 (0.06)†	0.30 (0.05)
Suspicion	1st in ET	0.24 (0.10)*	0.22 (0.04)
Suspicion	1st in AT	0.15 (0.09)†	0.09 (0.05)
Toileting routine	1st in ET	0.10 (0.05)*	0.10 (0.03)

* $p<0.01$ †$p<0.05$

The heritability estimates of all but five of these traits and time points were also detectably larger than zero in the Labrador Retriever models. For eight out of those nine traits and time points, the heritability estimates for the Golden Retrievers were approximately twice the size of those for the Labrador Retrievers, the only exception being calmness at 12 months of age for which both heritability estimates were 0.10 (s.e. 0.06 for GR and 0.04 for LR).

8.3.3. Labrador Retriever bivariate models

8.3.3.1. *Within-trait bivariate models*

Bivariate models were run between pairs of time points within each trait dataset for which more than one heritability estimate had been detectably larger than zero. These were aggression towards animals, aggression towards people, attentiveness, body sensitivity, calmness, distraction, eagerness, interaction with animals, interaction with people, obedience and suspicion. Most of the models did not converge, probably because the datasets were insufficiently large, and some others produced genetic correlation estimates which were not detectably larger than zero. The genetic correlation estimate between score for eagerness at the first assessment in early training and the first assessment in advanced training was 0.96 (s.e. 0.15), which was both detectably larger than zero and not detectably smaller than one. The same was true for the genetic correlation estimate between score for interaction with animals at 8 months of age and at the first CAS assessment in early training which was 0.67 (s.e. 0.46), and for the genetic correlation between score for interaction with people at 8 months of age and at the first CAS assessment in advanced training which was 0.75 (s.e. 0.33). The genetic correlation estimates which were detectably larger than zero between scores at different time points for calmness are shown in Table 8.22. These genetic correlation estimates were also not detectably smaller than one.

Table 8.22 Genetic correlation estimates which were detectably larger than zero between scores for calmness at different time points. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

Time point 1	Time point 2	r_G*	p value
5 months of age	8 months of age	0.72 (0.27)	<0.01
5 months of age	1st in ET	0.61 (0.24)	<0.05
5 months of age	1st in AT	0.98 (0.23)	<0.01
8 months of age	1st in ET	0.95 (0.21)	<0.01
1st in ET	1st in AT	0.84 (0.22)	<0.01

* genetic correlation

8.3.3.2. *Across-trait bivariate models*

Across-trait bivariate models were undertaken between any CAS elements which had heritability estimates detectably larger than zero at the same CAS assessment time point. Some of the models did not converge, probably because the datasets were insufficiently large, and some others produced genetic correlation estimates which were not detectably larger than zero. As none of the CAS elements had significant heritability estimates at the first CAS assessment in puppy walking no bivariate models were undertaken for this time point, but the results of the models undertaken for the other time points are presented below.

8.3.3.3. *Bivariate models for the CAS assessment at 5 months of age*

The genetic correlation estimates which were detectably larger than zero between CAS elements at the assessment at 5 months of age are shown in Table 8.23. They were all positive and strikingly large.

Table 8.23 Genetic correlation estimates which were detectably larger than zero between scores for different traits at 5 months of age. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS element 1	CAS element 2	r_G*	p value
Calmness	Distraction	0.72 (0.24)	<0.05
Calmness	Interaction people	0.83 (0.22)	<0.01
Distraction	Interaction people	0.94 (0.21)	<0.01
Distraction	Position busy areas	0.95 (0.27)	<0.01
Distraction	Speed control	0.95 (0.20)	<0.01
Position busy areas	Position quiet areas	0.94 (0.09)	<0.01
Position busy areas	Speed control	0.97 (0.11)	<0.01
Position quiet areas	Speed control	0.99 (0.10)	<0.01

* genetic correlation

8.3.3.4. *Bivariate models for the CAS assessment at 8 months of age*

The genetic correlation estimates which were detectably larger than zero between CAS elements at the assessment at 8 months of age are shown in Table 8.24. All of the estimates were large and positive.

Table 8.24 Genetic correlation estimates which were detectably larger than zero between scores for different traits at 8 months of age. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS element 1	CAS element 2	r_G*	p value
Attentiveness	Calmness	0.97 (0.13)	<0.01
Attentiveness	Interaction animals	0.93 (0.17)	<0.01
Attentiveness	Interaction people	0.86 (0.17)	<0.01
Attentiveness	Obedience	0.98 (0.27)	<0.05
Behaviour when left	Interaction animals	0.60 (0.23)	<0.05
Behaviour when left	Interaction people	0.61 (0.22)	<0.05
Calmness	Distraction	0.93 (0.10)	<0.01
Calmness	Obedience	0.83 (0.31)	<0.01
Distraction	Interaction animals	0.99 (0.12)	<0.01
Distraction	Interaction people	0.76 (0.15)	<0.01
Distraction	Obedience	0.92 (0.23)	<0.01
Interaction animals	Interaction people	0.97 (0.08)	<0.01
Interaction animals	Obedience	0.84 (0.27)	<0.05

* genetic correlation

8.3.3.5. *Bivariate models for the CAS assessment at 12 months of age*

There was only one genetic correlation estimate which was detectably larger than zero between CAS elements at the assessment at 12 months of age, as shown in Table 8.25. The genetic correlation estimate between calmness and interaction with people at this time point was very large and positive.

Table 8.25 The genetic correlation estimate which was detectably larger than zero between scores for different traits at 12 months of age. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS element 1	CAS element 2	r_G*	p value
Calmness	Interaction people	0.99 (0.26)	<0.01

* genetic correlation

8.3.3.6. *Bivariate models for the first CAS assessment in early training*

The genetic correlation estimates which were detectably larger than zero between CAS elements at the first assessment in early training are shown in Table 8.26. All of the estimates were large, and all but two were positive.

Table 8.26 Genetic correlation estimates which were detectably larger than zero between scores for different traits at the first CAS assessment in early training. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS element 1	CAS element 2	r_G*	p value
Aggression animals	Aggression people	0.96 (0.27)	<0.05
Attentiveness	Calmness	0.77 (0.15)	<0.01
Attentiveness	Distraction	0.78 (0.21)	<0.01
Attentiveness	Interaction animals	0.72 (0.19)	<0.01
Attentiveness	Interaction people	0.97 (0.12)	<0.01
Attentiveness	Obedience	0.86 (0.16)	<0.01
Body sensitivity	Confidence	0.43 (0.19)	<0.05
Body sensitivity	Stress resilience	0.76 (0.29)	<0.01
Calmness	Obedience	0.79 (0.17)	<0.01
Confidence	Distraction	-0.45 (0.22)	<0.05
Confidence	Eagerness	0.46 (0.19)	<0.05
Confidence	Stress resilience	0.80 (0.15)	<0.01
Distraction	Interaction animals	0.70 (0.20)	<0.01
Distraction	Interaction people	0.96 (0.14)	<0.01
Distraction	Obedience	0.98 (0.21)	<0.01
Eagerness	Interaction people	-0.46 (0.20)	<0.05
Interaction animals	Interaction people	0.96 (0.12)	<0.01
Interaction animals	Obedience	0.85 (0.23)	<0.05
Interaction people	Obedience	0.99 (0.20)	<0.01

* genetic correlation

8.3.3.7. *Bivariate models for the first CAS assessment in advanced training*

The genetic correlation estimates which were detectably larger than zero between CAS elements at the first assessment in advanced training are shown in Table 8.27. All of the estimates were large and all but one of them was positive.

Table 8.27 Genetic correlation estimates which were detectably larger than zero between scores for different traits at the first CAS assessment in advanced training. Estimates are shown followed by their standard errors in brackets to two decimal places.

CAS element 1	CAS element 2	r_G*	p value
Attentiveness	Distraction	0.69 (0.19)	<0.05
Attentiveness	Obedience	0.77 (0.26)	<0.05
Body sensitivity	Eagerness	0.66 (0.18)	<0.01
Calmness	Eagerness	-0.58 (0.21)	<0.05
Calmness	Interaction animals	0.75 (0.25)	<0.05
Calmness	Interaction people	0.94 (0.21)	<0.01
Calmness	Obedience	0.98 (0.66)	<0.05
Distraction	Interaction people	0.61 (0.26)	<0.05
Interaction animals	Interaction people	0.76 (0.26)	<0.05
Interaction animals	Obedience	0.97 (0.42)	<0.05
Interaction people	Obedience	0.90 (0.47)	<0.05

* genetic correlation

8.4. Discussion

Univariate and bivariate linear mixed models were used to estimate genetic and environmental parameters of, and genetic correlations between, behavioural traits assessed by CAS in GD's Labrador Retrievers and Golden Retrievers. Although the assessor effect was large in many cases, many of the models measured heritability estimates which were detectably larger than zero.

All of the datasets showed a skewed distribution of scores to some extent, with a tail on the less desirable end of the range. The REML method assumes normal distribution of traits, but is quite robust against distribution violations (Liinamo et al, 2007). Several studies have found no difference in heritability estimates for traits which were not normally distributed compared to log-transformed data (e.g. van der Waaij et al, 2008) and therefore untransformed data were used in all analyses.

Although there was a standardised scoring system for each trait, CAS element scores are subjective as they rely on the assessor's perception and judgement and can therefore be influenced by experience and personal views (Meagher, 2009). The number of individuals undertaking CAS assessments was very high and this is problematic. However, it has been suggested that combining ratings from multiple assessors tends to eliminate personal biases or errors in perception not shared by all observers (Meagher, 2009). Previous studies have shown that complex behavioural patterns in dogs can be subjectively evaluated by experienced people (Wilsson & Sundgren, 1997b). Moreover good genetic progress has been made using scores based on the subjective opinions of experienced workers at the Seeing Eye, Inc. (Mackenzie et al, 1985).

Another potential effect of the number of individuals undertaking CAS assessments may be to increase the amount of environmental "noise". Willis (1995) stated that failing to reduce environmental variation or to assess dogs

in a consistent fashion would tend to reduce heritability estimates, thus the estimates measured for the CAS elements at different time points were likely to be underestimates.

Studies have suggested maternal or litter effects on the behaviour of puppies (Scott & Fuller, 1965; Scott & Bielfelt, 1976; Wilsson & Sundgren, 1998) but studies on dogs greater than 1 year old have generally found such effects to be small (Newton et al, 1978; Goddard & Beilharz, 1982; Wilsson & Sundgren, 1997b). None of these studies attempted to separate maternal genetic, maternal permanent environment and litter permanent environmental effects. Strandberg et al (2005) used linear mixed models in ASReml with these 3 elements as random effects in models of 4 canine personality traits (playfulness, chase-proneness, curiosity/fearfulness and aggressiveness) and found little influence of the maternal genetic or maternal permanent environmental effect. They concluded that models including a direct animal effect and a litter effect are acceptable for genetic evaluation of canine personality traits and that omitting the litter effect might results in an upward bias in the additive genetic variance component (Strandberg et al, 2005). Leaving maternal genetic effects out of models for behaviour test component scores was found not to lead to an overestimation of genetic variances in another study (van der Waaij et al, 2008). Litter was included as a random effect in all the CAS models but in all cases the estimates of its effect were very small.

8.4.1. Univariate linear mixed models

Heritability estimates for many CAS elements at different time points were not detectably larger than zero. Those which were detectably larger than zero were all very small to small in Labrador Retrievers, with heritability estimates ranging from 0.02 (s.e. 0.02) for obedience at 8 months of age to 0.16 (s.e. 0.05) for body sensitivity at the first CAS assessment in advanced training. Heritability estimates were more often detectably larger than zero at the first

assessment in early training (12 out of 43) or the first assessment in advanced training (11 out of 43) than at one of the time points in puppy walking. No heritability estimates were detectably larger than zero for CAS elements at the first assessment in puppy walking. For the other puppy walking time points, more heritability estimates were detectably larger than zero for CAS elements at 8 months of age (9 out of 43) than at 5 months of age (7 out of 43) or 12 months of age (4 out of 43).

Far fewer heritability estimates were detectably larger than zero in the Golden Retriever, probably because of the smaller size of the datasets. However those which were detectably larger than zero tended to be larger in the Golden Retriever than in the Labrador Retriever, with heritability estimates ranging from 0.07 (s.e. 0.06) for obedience at the first CAS assessment in early training to 0.25 (0.13) for eagerness at the first assessment in advanced training. The distribution of heritability estimates which were detectably larger than zero between time points was slightly different than that seen in Labrador Retrievers, with 12 months of age, the first CAS assessment in early training and the first assessment in advanced training all having 4 and the assessment at 5 months of age having 2. In this breed no heritability estimates were detectably larger than zero at the first assessment in puppy walking, as was the case in the Labrador Retriever, nor at the assessment at 8 months of age.

The CAS elements relating to skills acquisition in early and advanced training were all found not to be heritable. Those relating to simpler guiding task acquisition in puppy walking, i.e. correct handler position in busy and quiet areas and speed control, all had low heritability estimates of 0.03 (s.e. 0.02) at 5 months of age. Heritability estimates for these three elements at the other 3 time points in puppy walking were not detectably larger than zero.

A study of eight components of behaviour in a colony of working GSDs concluded that there was little additive genetic variation with respect to those components and postulated that this may be due to previous selection for

these behavioural traits (Reuterwall & Ryman, 1973). However, Wilsson & Sundgren (1997b) noted that having many different people assessing the dogs, as in Reuterwall & Ryman's study, inflated the environmental variance component and may lead to lower heritability estimates for the traits in question. It is possible that both factors may be at play in the heritability estimates of scores for CAS elements in the present study. There were many assessors, and GD have been selecting based largely on temperament for decades.

Comparing heritability estimates for CAS element scores with those from other studies of canine behaviour is difficult as the traits measured, sampling procedures, methods of analysis and breeds tested were not the same in every case. Moreover temperamental traits are often poorly defined and two studies using different assessment methods may actually be studying two different sets of traits despite using the same names to describe them (Mackenzie et al, 1985). Goddard and Beilharz (1983) estimated heritabilities of traits scored by guide dog trainers at the Royal Guide Dogs for the Blind Association of Australia on Labrador Retrievers aged 12 to 18 months between 1970 and 1976. Several traits shared names with CAS elements and the definitions were similar. Heritabilities were estimated as 0.10 for suspicion, 0.22 for willingness, 0.08 for distraction and 0.33 for body sensitivity (Goddard & Beilharz, 1983). These estimates were based on the scores of only 249 dogs and were not estimated using an animal model and REML procedures, instead being estimated by least-squares analysis. The estimates presented here for both Labrador Retrievers and Golden Retrievers are based on substantially more data and thus should be more accurate.

Published heritability estimates for traits sharing names with or otherwise resembling CAS elements could not be found for attentiveness, behaviour on transport, behaviour when left, interaction with animals or people, handler position in busy and quiet areas, speed control, stress resilience and toileting routine. Those CAS elements for which comparable heritability estimates

were found in the literature are discussed below, in the order that the traits in question caused withdrawal from GD's programme, as described in Chapter 4.

8.4.1.1. *Distraction*

Distraction accounted for the majority of withdrawals for behavioural reasons of dogs from GD's programme between 1995 and 2012, with 22% (1151 of 5327 dogs) of dogs which were withdrawn for behavioural reasons having been withdrawn due to high levels of distraction. Heritability estimates for this CAS element in Labrador Retrievers ranged from 0.04 (s.e. 0.02) at 5 months of age to 0.10 (s.e. 0.04) at the first CAS assessment in advanced training. EBVs for distraction could therefore possibly be used in GD's Labrador Retrievers, with those for the first CAS assessment in advanced training looking most promising. Goddard and Beilharz (1983) estimated the heritability of distraction, defined as attention and attraction to irrelevant stimuli, as 0.08 in Labrador Retrievers bred by The Royal Guide Dogs for the Blind Association of Australia. Their trait definition was comparable to that used in CAS and the heritability estimate of the trait is similar.

Interestingly those authors estimated a higher heritability of 0.27 for "dog distraction", defined as attention and attraction towards other dogs (Goddard & Beilharz, 1983). The CAS element of distraction is not categorised into different sources of distraction in this way. However, distraction as a withdrawal reason is categorised (Appendix 5). Of the 1151 dogs which were withdrawn from GD's programme between 1995 and 2012, nearly 60% (680 dogs) were withdrawn due to high levels of distraction due to animals or birds. This category would presumably include distraction due to other dogs. GD could consider whether there would be any value in categorising the CAS element of distraction into distraction by animals or birds, or even specifically by other dogs, and distraction by other things. If it was found that dog distraction in GD's dogs is more heritable than distraction due to other things it might be a better selection criterion.

8.4.1.2. *Suspicion*

Suspicion accounted for the second largest number of dogs withdrawn from GD's programme for behavioural reasons, with 16% (832 of 5327 dogs) of dogs which were withdrawn for behavioural reasons having been withdrawn due to high suspicion. The heritability estimates for suspicion were detectably larger than zero at the first CAS assessments in early training and advanced training in Labrador Retrievers, with estimates of 0.04 (s.e. 0.02) and 0.06 (s.e. 0.03) respectively. In Golden Retrievers heritability estimates were detectably larger than zero at these two time points and also at the CAS assessment at 5 months of age, with the largest estimate of 0.24 (s.e. 0.10) at the first CAS assessment in early training. Goddard and Beilharz (1983) estimated the heritability of suspicion, defined as fear mainly shown by approach-withdrawal conflict towards unusual objects, as 0.10 in Labrador Retrievers bred by The Royal Guide Dogs for the Blind Association of Australia. The CAS element of suspicion refers to the degree of anxiety the dog displays towards objects, people, animals, sounds and scents, a much broader trait definition. This broader trait definition may explain the lower heritability estimate in GD's Labrador Retrievers compared to those in Goddard & Beilharz (1983)'s studies although the methods of estimation were also different

8.4.1.3. *Aggression towards animals or people*

Aggression towards people accounted for 11% (562 of 5327 dogs) and aggression towards animals 4% (191 of 5327 dogs) of dogs withdrawn from GD's programme for behavioural reasons between 1995 and 2012. Very low heritability estimates for these two traits were only detectably larger than zero at the first CAS assessment in ET and the first assessment in AT in Labrador Retrievers, ranging from 0.05 (s.e. 0.03) for aggression towards animals at the first CAS assessment in ET to 0.07 (s.e. 0.03) for aggression towards people at the first CAS assessment in AT. These traits had unusually

low assessor effect estimates which ranged from 0.01 (s.e. 0.01) for aggression towards people at the first CAS assessment in ET to 0.12 (s.e. 0.12) for aggression towards animals at the first assessment in AT. None of the heritability estimates for scores for these traits were detectably larger than zero in Golden Retrievers, but the assessor effects were similarly small.

Liinamo et al (2007) reported much higher heritability estimates for owner impressions of human- and dog-directed aggression in the Golden Retriever, with estimates of 0.77 (s.e. 0.09) and 0.81 (s.e. 0.09) respectively, but they warned that these estimates should be approached with caution due to the small size of their dataset. Wilsson and Sundgren (1997a) described a characteristic which they called "sharpness", defined as the tendency to react to any particular test situation with aggression, which was scored in a Swedish dog behaviour test. Heritability estimates for this characteristic, estimated from intraclass correlations between sibs within groups of full and half sibs, were 0.13±0.05 for GSDs and 0.11±0.07 for Labrador Retrievers (Wilsson & Sundgren, 1997b). A later study working with scores from the same Swedish dog behaviour test estimated heritability for "sharpness", using an animal model and REML procedures, as 0.16±0.05 for GSDs and 0.10±0.06 for Labrador Retrievers (van der Waaij et al,2008). These estimates are closer to those estimated in GD's Labrador Retrievers.

8.4.1.4. *Confidence*

Low confidence accounted for 9% (464 of 5327 dogs) of dogs withdrawn from GD's programme due to behavioural reasons between 1995 and 2012. The heritability estimate for confidence in Labrador Retrievers was only detectably larger than zero at the first CAS assessment in early training, at which point it was 0.08 (s.e. 0.03). In Golden Retrievers, the heritability estimate for CAS score for confidence at 12 months of age was 0.15 (s.e. 0.07). C.R. Bartlett reported a heritability estimate of 0.16 for confidence, as judged by the dog's reaction to new people or new environments, in Seeing Eye dogs (reported in

Mackenzie et al, 1986). This definition is relatively similar to that for the trait in CAS, and the heritability estimate is similar to that in GD's Golden Retrievers.

8.4.1.5. *Body sensitivity*

High body sensitivity accounted for 2% (84 of 5327 dogs) of withdrawals from GD's programme for behavioural reasons between 1995 and 2012. The heritability estimates for body sensitivity were low but detectably larger than zero at the first CAS assessment in early training and the first assessment in advanced training, with estimates of 0.10 (s.e. 0.03) and 0.16 (s.e. 0.05) respectively. Goddard and Beilharz (1983) estimated the heritability of body sensitivity as 0.33 in Labrador Retrievers bred by The Royal Guide Dogs for the Blind Association of Australia. Body sensitivity in that study was defined as a strong response to touch and leash corrections and was rated on a 0-5 scale with 0.5-point intervals (Goddard and Beilharz, 1983). C.R. Bartlett reported in a PhD thesis in 1976 a heritability estimate of 0.10 for body sensitivity, as judged by how hard a jerk on a choke-chain the dog could tolerate, in Seeing Eye dogs (reported in Mackenzie et al, 1986). Again, this trait was defined very differently from the CAS definition of body sensitivity. A study of Labrador Retrievers, Golden Retrieves and GSDs bred by Guide Dogs for the Blind (GDB) in California estimated the heritability of body sensitivity in puppies between 8 and 12 weeks of age as 0.16 (Scott & Bielfelt, 1976). In that study the trait described a pup's response to a painful stimulus and scored on a 6-point scale. In those three studies the definition of the trait was quite different from its definition in CAS, in which it refers to the dog's physical acceptance of being in close proximity to or in contact with people or handler, equipment and objects or features within the environment, so the heritabilities are not really comparable.

8.4.1.6. *Obedience*

Poor obedience was not given as a withdrawal reason for any dogs, but may play a part in withdrawals due to unacceptable social behaviour or unacceptable post-qualification habits. Small heritability estimates which were detectably larger than zero were estimated at 8 months of age and at the first CAS assessments in early and advanced training in Labrador Retrievers. They ranged from 0.02 (s.e. 0.02) at 8 months of age to 0.06 (s.e. 0.04) at the first assessment in advanced training. In Golden Retrievers the heritability of obedience at the first CAS assessment in early training was estimated as 0.07 (s.e. 0.06). In a doctoral thesis in 1979, M. Pfleiderer-Högner estimated the heritability of the score for obedience in the Schutzhund test in GSDs as 0.09 (reported in Mackenzie et al, 1986). In the Schutzhund test obedience is scored based on the accuracy and attitude when performing a series of tasks which is very different to the CAS element, defined as the dog's responsiveness to standard commands, including recall.

8.4.1.7. *Calmness*

No dogs were withdrawn due to lack of calmness. Heritability estimates for calmness in Labrador Retrievers ranged from 0.03 (s.e. 0.02) at the CAS assessment at 12 months of age to 0.11 (s.e. 0.04) at the first assessment in early training. In Golden Retrievers heritability estimates were 0.10 (s.e. 0.06) at 12 months of age and 0.24 (s.e. 0.10) at the first assessment in advanced training. The scores for this trait measured a spectrum of responses to stimuli within the environment or situation (excluding people or animals) that range from calmness to excitement. Goddard and Beilharz (1983) estimated the heritability of excitement, defined as high activity, as 0.33 in Labrador Retrievers bred by The Royal Guide Dogs for the Blind Association of Australia, but the trait definition is quite different to that in CAS.

8.4.1.8. *Eagerness*

No dogs were withdrawn due to lack of eagerness. Heritability estimates for eagerness in Labrador Retrievers were small but detectably larger than zero at the CAS assessments at 8 and 12 months of age and at the first assessments in early and advanced training. The largest estimate was 0.10 (s.e. 0.04) at the first CAS assessment in advanced training. In Golden Retrievers the heritability of CAS score for eagerness at the first assessment in advanced training was estimated as 0.25 (s.e. 0.13). This CAS element rates the dog's eagerness to take part in training exercises and activities and willingness to perform the guiding role. Goddard and Beilharz (1983) estimated the heritability of willingness, defined as keenness to work and carry out commands, as 0.22 in Labrador Retrievers bred by The Royal Guide Dogs for the Blind Association of Australia. Their definition of willingness was similar to the CAS definition of eagerness.

8.4.2. Bivariate linear mixed models

8.4.2.1. *Within-trait genetic correlations*

The four traits for which within-trait genetic correlation estimates were detectably larger than zero were calmness, eagerness, interaction with animals and interaction with people. In all four cases the genetic correlations between the scores for these CAS elements at different time points were large and not detectably smaller than one. This implies that these CAS elements, and probably the others, are genetically the same trait at each different time point.

8.4.2.2. *Across-trait genetic correlations*

A high genetic correlation between two or more behavioural traits indicates that there are shared genetics and possibly a common biological mechanism underlying these traits (Saetre et al, 2006). All of the genetic correlation estimates between CAS elements at the different time points of interest which were detectably larger than zero were high and in most cases very high. All but three of the estimates were positive – the three that were negative were that between confidence and distraction at the first CAS assessment in early training with an estimate of -0.45 (s.e. 0.22), between eagerness and interaction with people also at the first CAS assessment in early training with an estimate of -0.46 (s.e. 0.20) and that between calmness and eagerness at the first CAS assessment in advanced training with an estimate of -0.58 (0.21). None of the genetic correlation estimates for these pairs of CAS elements were detectably larger than zero at other time points. These negative correlations suggest that selection for a low (desirable) score for confidence, eagerness and calmness would tend to lead to higher (less desirable) scores for distraction, interaction with people and eagerness respectively. This could be problematic.

The high, positive genetic correlations between many other CAS element scores are interesting and generally make sense that such elements would be related when the CAS element definitions, as shown in Appendix 9, are considered. For example, selecting for low (desirable) scores for attentiveness should also tend to lead to lower (more desirable) scores for calmness, distraction, interaction with animals, interaction with people and obedience.

The genetic correlation between aggression towards animals and aggression towards people at the first CAS assessment in early training was very high at 0.96 (s.e. 0.27), although at the first CAS assessment in advanced training it was smaller (0.69, s.e. 0.36) and not detectably larger than zero. This high genetic correlation suggests that scores for the CAS elements of aggression

towards animals and aggression towards people share the same genetic background. This contrasts with the findings of Liinamo et al (2007) who found a low correlation (0.40) between the EBVs for owner impressions of human- and dog-directed aggression (based on interviews and C-BARQ questionnaires) in Golden Retrievers and suggested that the two traits have a partially different genetic background. Although they did use animal models and REML procedures to estimate the heritabilities of the traits, those authors could not estimate genetic correlations between the traits directly due to the small size of the dataset (325 phenotyped dogs and an additional 865 unphenotyped dogs in the pedigree), instead using Pearson product moment correlation coefficients between EBVs for the two traits. They did comment that the Pearson correlation tends to underestimate the true genetic correlation due to inaccuracies in estimating the breeding values (Liinamo et al, 2007). Thus the high genetic correlation between aggression towards animals and aggression towards people, suggesting that the two traits share the same genetic background, is more likely to be an accurate finding due to the better methodology used for its calculation and the larger sample size. This has implications to the wider dog breeding community, and in fact to the wider community generally, as dogs which are bred to be aggressive towards other dogs (such as those being used in illegal dog-fighting) would thus be likely to be aggressive towards people too with concomitant public health and safety implications.

No genetic correlation estimates were detectably larger than zero between aggression towards animals or people and any other CAS element scores. This mirrors the findings reported by Saetre et al (2006) in GSDs and Rottweilers, based on an applied stimulus test, of genetic correlations between all the behavioural traits measured except those relating to aggression.

8.5. Conclusion

There is certainly genetic variation in scores for the different CAS elements, and so potentially EBVs could be used for some of them. The apparent negative genetic correlations between some of the elements would need to be borne in mind. Heritability estimates were generally higher at the CAS assessments in early and advanced training, although the larger assessor effects at earlier CAS assessments may have played a role in this. Now that all puppies are receiving CAS elements at the time points of interest (beginning of puppy walking, 5 months of age, 8 months of age and 12 months of age) more data at these time points will accrue rapidly and it may be that some of the models which did not work or produced estimates that were not detectably larger than zero would produce better results when the new data is included. Reducing the number of people assigning CAS scores, or working to make the scoring system even more standardised, might also help by reducing the environmental "noise".

Most of the heritability estimates were low, apart from those for calmness, eagerness and interaction with people at the first CAS assessment in advanced training, and that for suspicion at the first CAS assessment in early training all of which were moderate in the Golden Retriever. Therefore selection based on an individual's scores alone would not be very effective. Selection of breeding stock would be much more efficient if based on EBVs for desired CAS element scores. This is particularly true as the scores for most CAS elements seem to be more heritable in early and advanced training and dogs are identified as prospective breeding stock, and begin their breeding careers, without ever reaching these stages.

9. CROSSBREED GENETIC ANALYSIS OF A BEHAVIOURAL SCORING SYSTEM USED BY GUIDE DOGS

9.1. Introduction

The Golden Retriever crossed with the Labrador Retriever has been the most successful of all the breeds and crosses GD have tried, combining the best attributes of both breeds (Freeman, 1991). Derek Freeman first decided to cross the two breeds in the 1960s, in the hope that the offspring would combine the gentleness of the Golden Retriever with the willingness of the Labrador Retriever.

Scott et al (1976) predicted that first-generation (F1) crosses between Labrador Retrievers and Golden Retrievers should achieve higher average success than either parent breed and that there was a good probability that the performance level would be raised even higher with an associated reduction in training time and effort. These predictions appear to have been correct. In a study of GSDs, Labrador Retrievers, Golden Retrievers and Golden Retrievers crossed with Labrador Retrievers born between 1999 and 2004 at The Seeing Eye (TSE), the Golden Retrievers crossed with Labrador Retrievers had the highest probability of qualifying as a guide dog at 59% (Ennik et al, 2006). It was postulated that this could be due both to the benefits of breed differences and heterosis.

Even less has been written about crossbreeding effects on behaviour than on health traits, and most of what has been published relates to behaviour in mice. It was recognised as early as 1964 that it was important to investigate the phenomenon of heterosis in behaviour due to its link with individual adaptation and the adaptation of populations (Winston, 1964). That author found evidence of heterosis for water escape learning in mice, determined by analysing the behaviour of three inbred strains and hybrid crosses between

them, and also that the hybrid crosses were less influenced by infantile trauma than inbred strains. Later, Manosevitz (1972) predicted that heterosis for behavioural traits could be expected when unrelated, inbred lines were crossed and when the trait of interest enhances fitness or adaptation to the environment. He found evidence of heterosis for a complex social behaviour in mice, food competition. Lassalle et al (1979) reprised and refined the studies of Winston (1964) and showed that the heterosis relating to water escape learning in mice reflected behavioural characteristics with potentially adaptive value. The F1's superiority was due to more frequent adoption of efficient behavioural tactics and more rapid learning in this respect than either the inbred parental strains or F2s and not due to physical vigour.

The work of Scott and Fuller investigating the genetics of canine behaviour was discussed in Chapter 2. They crossed Basenjis and American Cocker Spaniels and found that the F1 hybrids outperformed either parent breed in problem-solving situations and recommended that crossbred dogs should be used as working dogs (as they stated that the heterosis lasted only one generation) provided that the purebred lines were well maintained (Scott & Fuller, 1965).

It will have become clear that CAS involves repeated measures on individuals and as such one way of modelling CAS traits would be to use repeatability models. Common environment effects (such as maternal effects among full sibs or maternal half sibs, or litter effects among littermates) may generate similarities of phenotype between relatives that are of equal or even greater magnitude to those due to genetic effects (Kruuk & Hadfield, 2007). As an individual is perfectly related to itself and completely shares its own environment, permanent environment effects can be seen as a very extreme case of the problem of common environment (Wilson et al, 2010). Consequently, not including this source of variance in models when there are repeated models will lead to bias in the estimate of additive variance. Two traits, calmness and eagerness, were selected for use in repeatability models

after considering the results of bivariate models between CAS scores for the two traits at different time points in Chapter 8.

Heterosis, recombination loss and breed effects were explained in Chapter 7, and in that chapter they were quantified for disease conditions in GD's dogs. In this chapter crossbreeding parameters are estimated for CAS elements at the time points of interest. Crossbreeding parameters for behaviour have not been quantified in dogs, although studies have reported evidence of heterosis in the species. In addition the suitability of crossbreed models for producing EBVs for these traits, compared to using single breed models, is evaluated. Univariate linear models are undertaken for CAS elements at the time points of interest and repeatability models are used for two CAS elements, calmness and eagerness.

9.2. Materials and methods

Data acquisition, validation and extraction of CAS assessment scores of interest were as described in Chapter 8.

9.2.1. Estimated crossbreed parameter calculation

The expected heterosis and recombination loss for each individual was calculated from the proportion of Labrador Retriever and Golden Retriever of each animal's sire and dam, after Van der Werf and de Boer (1989). Using this method heterosis was calculated as $h = ½ [(P_S (1-P_D)) + (P_D (1-P_S))]$, and recombination loss as $r = ½ [(P_S (1-P_S)) + (P_D (1-P_D))]$, where P_S and P_D are the proportion of Labrador Retriever in the sire and the dam respectively, and thus the probability of inheriting a Labrador Retriever allele from the sire or dam. The first equation gives the probability that the two alleles inherited from the parents at any one locus originate from different breeds. The second equation gives the probability that any two loci inherited from the same parent originate from different breeds.

The proportion of Labrador Retriever and estimates of heterosis and recombination loss for the different breeds and crosses, together with the number of dogs of each breed or cross, in the dataset are shown in Table 9.1.

Table 9.1 Estimates of heterosis (h) and recombination loss (r loss) for the different breeds and crosses in the dataset and the number (n) and proportion (p) of each.

Breed or cross	Lab*	h	r loss	n	p
Golden Retriever	0	0	0	1216	0.121
Labrador Retriever	1	0	0	3522	0.349
GR x LR	0.5	0.5	0	4267	0.423
GR x (GR x LR)	0.25	0.25	0.125	36	0.004
LR x (GR x LR)	0.75	0.25	0.125	1037	0.103

* Labrador Retriever fraction

9.2.2. Statistical analyses

Statistical analysis of the data had the objective of fitting univariate linear mixed linear models using ASReml version 3.0 (Gilmour et al, 2009) to each CAS component (except the skill and task acquisition elements) at each time point of interest to estimate the heritability and independent regression coefficients of estimated Labrador Retriever fraction, heterosis and recombination loss. Repeatability models were also undertaken for two CAS components, calmness and eagerness, to estimate permanent environmental effects. The significance of estimated effects and regression coefficients from zero was determined using approximate t-tests, with the number of degrees of freedom corresponding to the number of records from which the estimates can be determined.

The pedigree file used in all analyses was described in Chapter 4.

9.2.2.1. *Univariate linear mixed models*

The general form of the univariate linear mixed model fitted for each CAS trait at each time point was as follows:

$$\mathbf{Y = Xb + Za + Wc + Vd + e}$$

where **Y** is the vector of observations; **X**, **W, V** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters (0, $\sigma^2_a\mathbf{A}$); **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters (0, $\sigma^2_c\mathbf{I}$); d is the vector of random assessor effects with the distribution (0, $\sigma^2_d\mathbf{I}$); and **e** is the vector of residuals distributed MVN with parameters (0, $\sigma^2_e\mathbf{I}$); and where **I** denotes an identity matrix of the appropriate size, **A** is the numerator relationship matrix, and σ^2 is a scalar denoting variance. The subscripts *a*, *c, d* and *e* denote additive genetic, litter, assessor and residual variances respectively. The fixed effects included in the model were sex, year of birth, age (in days) at assessment, whether the dog was bred by GD or not and inbreeding coefficient. Labrador Retriever fraction, heterosis and recombination loss were included as covariates. The random effects fitted were litter, assessor and individual animal effect. Mathematically, the heritability is the ratio of additive genetic variance to phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$.

In order to determine whether the heritability estimates were significantly different from zero Likelihood Ratio Tests (LRTs) were performed between the univariate animal models and null models in which the random effect for the individual had been omitted.

9.2.2.2. *Repeatability linear mixed models*

Repeatability models were undertaken for just two traits, calmness and eagerness. These two traits were chosen as estimates of genetic correlations for each of the traits between different time points were very high and not detectably lower than 1 in Labrador Retrievers (see 8.3.3.1 Within-trait bivariate models). Firstly, models were undertaken including CAS score for each trait at each of the six CAS assessments of interest. Secondly reduced models, which only included the CAS scores for each trait at 12 months and at the first CAS assessments in early and advanced training, were undertaken.

The general form of the repeatability linear mixed models was:

$$\mathbf{Y = Xb + Za + Wc + Vd + Tf + e}$$

where **Y** is the vector of observations; **T**, **X**, **W**, **V** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal (MVN), with parameters $(0, \sigma^2_a\mathbf{A})$; **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters $(0, \sigma^2_c\mathbf{I})$; **d** is the vector of random assessor effects with the distribution $(0, \sigma^2_d\mathbf{I})$; **f** is the vector of random permanent non-genetic effects of each individual and distributed MVN with parameters $(0, \sigma^2_f\mathbf{I})$; and **e** is the vector of residuals distributed MVN with parameters $(0, \sigma^2_e\mathbf{I})$; **I** is an identity matrix of the appropriate size, **A** is the additive genetic relationship matrix. The subscripts *a*, *c, d, f* and *e* denote additive genetic, litter, assessor, permanent non-genetic and residual (co)variances respectively. The fixed effects included in the model were the same as for the univariate models, namely: sex, year of birth, age (in days) at assessment, whether the dog was bred by GD or not and inbreeding coefficient. Labrador Retriever fraction, heterosis and recombination loss were included as covariates. The random effects fitted were litter, assessor, individual animal additive genetic effect and individual animal permanent non-genetic effect.

Estimates of variance components contained in the σ^2_a, σ^2_c, σ^2_d, σ^2_f and σ^2_e were used to calculate a number of genetic and phenotypic parameters. Phenotypic variance was calculated by ASReml as $\sigma^2_p = \sigma^2_a + \sigma^2_c + \sigma^2_d + \sigma^2_f + \sigma^2_e$; and for trait *i*, heritability, $(h^2_i) = \sigma^2_a(i,i)/ \sigma^2_p(i,i)$, where (i,i) refers to the i,i element of the matrix; permanent environmental effects $(c^2) = \sigma^2_c(i,i)/ \sigma^2_p(i,i)$; and repeatability = heritability plus permanent environmental effects $(h^2 + c^2)$.

9.3. Results

9.3.1. Univariate models

T the number of dogs which had scores at the different time points was considerably higher in the crossbreed models compared to the single breed models, ranging from 7233 individuals at the first CAS assessment in early training to 4934 individuals at the assessment at 12 months of age. Due to the volume of results, only those in which the heritability estimate was detectably larger than zero are presented. Assessor and litter effects shown in the tables are proportions of total variance.

9.3.1.1. *Aggression towards animals*

The heritability estimates for score for aggression towards animals were detectably larger than zero for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 9.2. Both heritability estimates were low and smaller than the estimate of assessor effect. There was a small but significant sex effect in both models, with estimates of effect of being male of -0.02 (s.e. 0.01) for the first assessment in early training and -0.03 (s.e. 0.01), implying that male dogs tended to receive lower (i.e. better) scores for aggression towards animals at these time points.

Table 9.2 Estimates of heritability, litter effect and assessor effect for the two CAS assessments for which the heritability estimates of score for aggression towards animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	Litter effect	Assessor effect
1st in ET	0.04 (0.01)	0.02 (0.01)	0.05 (0.01)
1st in AT	0.04 (0.01)	0 (0)	0.09 (0.01)

Crossbreeding parameter estimates for aggression towards animals at the first CAS assessment in early and advanced training are shown in Table 9.3. Estimates of heterosis, recombination loss and Labrador fraction were all small and not detectably larger than zero.

Table 9.3 Crossbreeding parameter estimates from the two CAS assessments for which the heritability estimates of score for aggression towards animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
1st in ET	0.00 (0.04)	-0.02 (0.10)	-0.05 (0.04)
1st in AT	-0.08 (0.05)	0.15 (0.14)	0.00 (0.05)

* heterosis † recombination loss § Labrador fraction

9.3.1.2. *Aggression towards people*

The heritability estimates for score for aggression towards people were small but detectably larger than zero for the first assessments in early training and advanced training ($p<0.01$), as shown in Table 9.4. The assessor effects were of the same magnitude as the heritability estimates. Whether or not a dog was bred by GD was a small but significant effect at the first CAS assessment in early training, with an estimate of the effect of being bred by GD of -0.02 (s.e. 0.01). This implies that dogs bred by GD tended to get slightly lower (i.e. better) scores for aggression towards people at this time point, but the

estimate of this effect was not detectably larger than zero at the first CAS assessment in advanced training.

Table 9.4 Estimates of heritability, litter effect and assessor effect for the two CAS assessments for which the heritability estimates of score for aggression towards people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
1st in ET	0.03 (0.01)	0.00 (0.01)	0.03 (0.01)
1st in AT	0.05 (0.02)	0 (0)	0.05 (0.01)

Crossbreeding parameter estimates for aggression towards people at the first CAS assessment in early and advanced training are shown in Table 9.5. Estimates of heterosis, recombination loss and Labrador fraction were all small and not detectably larger than zero.

Table 9.5 Crossbreeding parameter estimates from the two CAS assessments for which the heritability estimates of score for aggression towards people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
1st in ET	-0.01 (0.02)	-0.01 (0.06)	0.01 (0.01)
1st in AT	-0.01 (0.04)	0.11 (0.11)	0.04 (0.04)

* heterosis † recombination loss § Labrador fraction

9.3.1.3. *Attentiveness*

Heritability estimates for score for attentiveness were small but detectably larger than zero ($p<0.01$) at all the time points of interest except the first CAS assessment in puppy walking, as shown in Table 9.6. Assessor effects for this trait ranged from 0.10 (s.e. 0.02) for the first CAS assessment in advanced training to 0.59 (s.e. 0.04) for the first CAS assessment in puppy walking. There was a small but significant negative sex effect in all models except that

at 8 months of age, with estimates of effect of being male ranging from -0.02 (s.e. 0.01) at the CAS assessment at 12 months of age to -0.05 (s.e. 0.01) at the CAS assessment at 5 months of age, implying that male dogs tended to receive lower (i.e. better) scores for aggression towards animals at most time points.

Table 9.6 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for attentiveness were detectably larger than zero.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.02 (0.01)	0.01 (0.01)	0.23 (0.03)
8 months of age	0.04 (0.01)	0.00 (0.01)	0.18 (0.02)
12 months of age	0.03 (0.01)	0.02 (0.01)	0.31 (0.03)
1st in ET	0.08 (0.02)	0.01 (0.01)	0.13 (0.01)
1st in AT	0.10 (0.02)	0 (0)	0.10 (0.01)

Crossbreeding parameter estimates for attentiveness at 5, 8 and 12 months of age and the first CAS assessments in early and advanced training are shown in Table 9.7. Estimates of recombination loss and Labrador fraction were all small and not detectably larger than zero. The estimate of heterotic effect was negative and detectably larger than zero (using an approximate t-test) at the first CAS assessment in early training, suggesting that heterosis tends to produce lower (i.e. better) scores for attentiveness at this time point.

Table 9.7 Crossbreeding parameter estimates from the five CAS assessments for which the heritability estimates of score for attentiveness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.02 (0.05)	0.03 (0.15)	0.05 (0.02)
8 months of age	-0.01 (0.05)	0.01 (0.15)	0.07 (0.06)
12 months of age	-0.08 (0.05)	0.16 (0.14)	0.08 (0.05)
1st in ET	-0.14 (0.06)	-0.07 (0.16)	0.06 (0.07)
1st in AT	-0.02 (0.07)	0.06 (0.20)	0.05 (0.09)

* heterosis † recombination loss § Labrador fraction

9.3.1.4. *Behaviour on transport*

Two heritability estimates for score for behaviour on transport were just detectably larger than zero, with estimates of 0.01 (s.e. 007) at 5 months of age and 0.03 (s.e. 0.13) at the first CAS assessment in advanced training ($p<0.05$), as shown in Table 9.8. These heritability estimates were very small, and very much smaller than the assessor effect estimates at these two time points.

Table 9.8 Estimates of heritability, litter effect and assessor effect for the two CAS assessments for which the heritability estimates of score for behaviour on transport were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.01 (0.01)	0.00 (0.01)	0.31 (0.03)
1st in AT	0.03 (0.01)	0.00 (0.01)	0.15 (0.01)

Crossbreeding parameter estimates for behaviour on transport at 5 months of age and at the first CAS assessment in advanced training are shown in Table 9.9. Estimates of heterosis, recombination loss and Labrador fraction effects were small and not detectably larger than zero except for the estimate of

Labrador fraction effect of -0.15 (s.e. 0.06) at the first CAS assessment in advanced training. This small, negative estimate of Labrador fraction effect implies that increasing Labrador fraction is associated with lower (i.e. better) scores for behaviour on transport at this time point.

Table 9.9 Crossbreeding parameter estimates from the two CAS assessments for which the heritability estimates of score for behaviour on transport were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.01 (0.05)	0.06 (0.14)	-0.01 (0.04)
1st in AT	-0.00 (0.07)	0.39 (0.20)	-0.15 (0.06)

* heterosis † recombination loss § Labrador fraction

8.3.1.5 Behaviour when left

The heritability estimate for behaviour when left was detectably larger than zero at the CAS assessments at 8 months of age and 12 months of age and at the first assessments in early and advanced training, with estimates ranging from 0.02 (s.e. 0.01) at 12 months and at the first assessment in early training to 0.07 (s.e. 0.02) at the first CAS assessment in advanced training, as shown in Table 9.10. As with behaviour on transport, the assessor effect for this trait was generally larger than the heritability estimate, except for the first assessment in advanced training when the two estimates were the same magnitude. There was a small but significant positive effect of being bred by GD at the CAS assessment at 8 months and the first assessment in early training, with estimates of 0.08 (s.e. 0.04) and 0.08 (s.e. 0.03) respectively. These imply that dogs bred by GD tend to get slightly higher (i.e. worse) scores for behaviour when left at these two time points.

Table 9.10 Estimates of heritability, litter effect and assessor effect for the CAS assessments for which the heritability estimate of score for behaviour when left was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.03 (0.01)	0 (0)	0.19 (0.02)
12 months of age	0.02 (0.01)	0 (0)	0.29 (0.03)
1st in ET	0.02 (0.01)	0.02 (0.01)	0.21 (0.02)
1st in AT	0.07 (0.02)	0.00 (0.01)	0.07 (0.01)

Crossbreeding parameter estimates for behaviour when left at 8 and 12 months of age and at the first CAS assessments in early and advanced training are shown in Table 9.11. Estimates of heterosis, recombination loss and Labrador fraction effects were small and not detectably larger than zero except for the estimate of Labrador fraction effect of 0.23 (s.e. 0.08) at 8 months of age and 0.16 (s.e. 0.05) at 12 months of age. These small, positive estimates of Labrador fraction effect implies that increasing Labrador fraction is associated with higher (i.e. worse) scores for behaviour when left at these time points.

Table 9.11 Crossbreeding parameter estimates from the four CAS assessments for which the heritability estimates of score for behaviour when left were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
8 months of age	0.03 (0.08)	-0.43 (0.22)	0.23 (0.08)
12 months of age	-0.11 (0.07)	-0.14 (0.16)	0.16 (0.05)
1st in ET	-0.02 (0.06)	-0.19 (0.17)	0.08 (0.05)
1st in AT	-0.02 (0.08)	0.18 (0.20)	0.10 (0.08)

* heterosis † recombination loss § Labrador fraction

9.3.1.5. *Body sensitivity*

The heritability estimates for score for body sensitivity were small but detectably larger than zero at all the time points of interest except the first CAS assessment in puppy walking, as shown in Table 9.12, although most of the estimates were very low and much smaller than the assessor effects. The highest heritability estimate was 0.13 (s.e. 0.03) at the first CAS assessment in advanced training and at this time point the assessor effect was relatively low at 0.09 (s.e. 0.01). There was a small but significant negative sex effect on the CAS assessments at 8 and 12 months and the first assessments in early and advanced training, with estimates of the effect of being male ranging from -0.03 (s.e. 0.02) at the first assessment in early training to -0.06 (s.e. 0.01) at 8 months of age. This implies that male dogs tend to receive lower (i.e. better) scores for body sensitivity at these 4 time points.

Table 9.12 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for body sensitivity were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.01 (0.00)	0.00 (0.00)	0.47 (0.04)
8 months of age	0.01 (0.01)	0.01 (0.01)	0.41 (0.03)
12 months of age	0.01 (0.01)	0.01 (0.01)	0.46 (0.03)
1st in ET	0.07 (0.02)	0.01 (0.01)	0.15 (0.01)
1st in AT	0.13 (0.03)	0.00 (0.01)	0.09 (0.01)

Crossbreeding parameter estimates for body sensitivity at 5, 8 and 12 months of age and at the first CAS assessments in early and advanced training are shown in Table 9.13. Several estimates were detectably larger than zero. Estimates of heterotic effect were negative and detectably larger than zero (based on approximate t-tests) at 5 and 8 months of age, with estimates of

-0.11 (s.e. 0.05) and -0.22 (s.e. 0.05) respectively, implying that heterosis was associated with lower (i.e. better) scores for body sensitivity at these two time points. The estimate of the effect of recombination loss was just detectably larger than zero at the CAS assessment at 5 months of age, with an estimate of 0.26 (s.e. 0.13) implying that it is associated with higher (i.e. worse) scores for body sensitivity at this time point. Estimates of Labrador fraction effect were detectably larger than zero at 5, 8 and 12 months of age with estimates ranging from -0.12 (s.e. 0.04) to -0.19 (s.e. 0.04) at 8 months of age. These small, negative estimates of Labrador fraction effect implies that increasing Labrador fraction is associated with lower (i.e. better) scores for body sensitivity at these time points.

Table 9.13 Crossbreeding parameter estimates from the five CAS assessments for which the heritability estimates of score for body sensitivity were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	-0.11 (0.05)	0.26 (0.13)	-0.14 (0.03)
8 months of age	-0.22 (0.05)	-0.00 (0.14)	-0.19 (0.04)
12 months of age	-0.06 (0.06)	0.23 (0.15)	-0.12 (0.04)
1st in ET	-0.01 (0.07)	0.07 (0.20)	0.01 (0.09)
1st in AT	0.06 (0.13)	0.20 (0.28)	0.06 (0.13)

* heterosis † recombination loss § Labrador fraction

9.3.1.6. *Calmness*

Heritability estimates for score for calmness were small but detectably larger than zero at all the time points of interest (all $p<0.01$ except the first assessment in puppy walking, $p<0.05$), with estimates ranging from 0.01 (s.e. 0.007) at the first CAS assessment in puppy walking to 0.14 (s.e. 0.03) at the first assessment in advanced training, as shown in Table 9.14. Assessor effects for this trait ranged from 0.11 (s.e. 0.01) for the first CAS assessment in

advanced training to 0.41 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect in all 6 calmness models, with the effect of being male ranging from -0.03 (s.e. 0.02) at the first assessment in early training to -0.08 (s.e. 0.01) at the first CAS assessment in early training, implying that male dogs tended to receive lower (i.e. better) scores for calmness.

Table 9.14 Estimates of heritability, litter effect and assessor effect for score for calmness at each time point of interest. Estimates are shown followed by their standard errors in brackets to two decimal places. PW is puppy walking, ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
1st in PW	0.01 (0.01)	0.00 (0.01)	0.41 (0.04)
5 months of age	0.05 (0.01)	0.01 (0.01)	0.21 (0.03)
8 months of age	0.06 (0.02)	0.02 (0.01)	0.17 (0.02)
12 months of age	0.05 (0.02)	0 (0)	0.20 (0.02)
1st in ET	0.13 (0.02)	0.00 (0.01)	0.12 (0.01)
1st in AT	0.14 (0.03)	0.01 (0.01)	0.11 (0.01)

Crossbreeding parameter estimates for calmness at the CAS assessments of interest are shown in Table 9.15. None of the estimates of heterosis and recombination loss were detectably larger than zero. Estimates of Labrador proportion were detectably larger than zero at all the time points of interest except the CAS assessment at 12 months of age with estimates ranging from 0.17 (s.e. 0.07 and 0.03) at the first assessments in puppy walking and advanced training to 0.20 (s.e. 0.06) at 5 months of age. These small, positive estimates of Labrador fraction effect implies that increasing Labrador fraction is associated with higher (i.e. worse) scores for calmness at these time points.

Table 9.15 Crossbreeding parameter estimates for calmness at the CAS assessments of interest. Estimates are shown followed by their standard errors in brackets to two decimal places. PW is puppy walking, ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
1st in PW	0.13 (0.07)	-0.07 (0.16)	0.17 (0.05)
5 months of age	0.11 (0.06)	-0.13 (0.15)	0.20 (0.06)
8 months of age	0.03 (0.06)	-0.27 (0.17)	0.18 (0.07)
12 months of age	-0.07 (0.06)	0.25 (0.16)	0.13 (0.07)
1st in ET	-0.05 (0.07)	-0.05 (0.18)	0.19 (0.09)
1st in AT	0.08 (0.08)	-0.06 (0.22)	0.17 (0.03)

* heterosis † recombination loss § Labrador fraction

9.3.1.7. *Confidence*

Heritability estimates for confidence were small but detectably larger than zero at all the CAS assessments of interest except the first in puppy walking, with estimates ranging from 0.02 (s.e. 0.01) at 5 and 8 months of age to 0.07 (s.e. 0.02) at the first CAS assessment in early training (all $p<0.01$), as shown in Table 9.16. Assessor effects ranged from 0.10 (s.e. 0.01) for the first assessment in advanced training to 0.35 (s.e. 0.03) at 12 months of age. Sex was a small but significant fixed effect at 5, 8 and 12 months of age, with estimated effects of being male ranging from -0.03 (s.e. 0.01) at 5 months of age to -0.06 (s.e. 0.01) at 8 months of age. These small negative effects imply that being male is associated with a lower (i.e. better) score for confidence at these time points.

Table 9.16 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimate of score for confidence was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.02 (0.01)	0.01 (0.01)	0.34 (0.03)
8 months of age	0.02 (0.01)	0.01 (0.01)	0.29 (0.03)
12 months of age	0.03 (0.01)	0 (0)	0.35 (0.03)
1st in ET	0.07 (0.02)	0.00 (0.01)	0.17 (0.01)
1st in AT	0.05 (0.02)	0.00 (0.01)	0.10 (0.01)

Crossbreeding parameter estimates for confidence at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.17. None of the estimates of heterosis and recombination loss were detectably larger than zero. Estimates of Labrador proportion were detectably larger than zero at the CAS assessments at 5 and 8 months of age, with estimates of -0.17 (s.e. 0.05) and -0.15 (s.e. 0.05) respectively. These small, negative estimates of Labrador fraction effect implies that increasing Labrador fraction is associated with lower (i.e. better) scores for confidence at these time points.

Table 9.17 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for confidence were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	-0.01 (0.05)	0.27 (0.15)	-0.17 (0.05)
8 months of age	-0.10 (0.06)	0.28 (0.15)	-0.15 (0.05)
12 months of age	0.07 (0.06)	0.24 (0.15)	-0.02 (0.06)
1st in ET	0.06 (0.06)	0.06 (0.16)	-0.02 (0.08)
1st in AT	-0.05 (0.07)	-0.07 (0.19)	-0.03 (0.07)

* heterosis † recombination loss § Labrador fraction

9.3.1.8. *Distraction*

The heritability estimates for score for distraction were small but detectably larger than zero at all the CAS assessments of interest except the first in puppy walking, with estimates ranging from 0.02 (s.e. 0.01) at 5 months of age to 0.15 (s.e. 0.03) at the first assessment in advanced training (all $p<0.01$), as shown in Table 9.18. The assessor effects ranged from 0.08 (s.e. 0.01) for the first CAS assessment in advanced training to 0.30 (s.e. 0.03) at 12 months of age. Sex was a small but significant fixed effect at 5 months of age and at the first CAS assessments in early and advanced training, with estimated effects of being male ranging of between -0.05 and -0.06 (s.e. 0.01-0.02). These small, negative estimates imply that being male is associated with lower (i.e. better) scores for distraction.

Table 9.18 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for distraction were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.02 (0.01)	0.00 (0.01)	0.20 (0.02)
8 months of age	0.05 (0.01)	0.01 (0.01)	0.18 (0.02)
12 months of age	0.05 (0.01)	0 (0)	0.30 (0.03)
1st in ET	0.13 (0.02)	0.00 (0.01)	0.12 (0.01)
1st in AT	0.15 (0.03)	0.01 (0.01)	0.08 (0.01)

Crossbreeding parameter estimates for distraction at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.19. Two estimates were detectably larger than zero. The estimate of heterotic effect was negative and detectably larger than zero (based on approximate t-tests) at the first CAS assessment in early training, with an estimate of -0.15 (s.e. 0.07), implying that heterosis was associated with lower (i.e. better) scores for distraction at this time point. The estimate of Labrador

fraction effect was also detectably larger than zero at the first CAS assessment in early training with an estimate of 0.28 (s.e. 0.10). This small, positive estimate of Labrador fraction effect implies that increasing Labrador fraction is associated with higher (i.e. worse) scores for distraction at the first CAS assessment in early training. None of the estimates of recombination loss effect were detectably larger than zero.

Table 9.19 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for distraction were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.02 (0.05)	-0.04 (0.15)	0.10 (0.05)
8 months of age	0.11 (0.06)	-0.24 (0.16)	0.12 (0.06)
12 months of age	-0.01 (0.06)	0.25 (0.16)	0.11 (0.06)
1st in ET	-0.15 (0.07)	-0.32 (0.19)	0.28 (0.10)
1st in AT	-0.07 (0.09)	-0.28 (0.26)	0.12 (0.12)

* heterosis † recombination loss § Labrador fraction

9.3.1.9. *Eagerness*

The heritability estimates for score for eagerness were small but detectably larger than zero all CAS assessments of interest except the first assessment in puppy walking with estimates ranging from 0.01 (s.e. 0.01) at 8 months of age to 0.11 (s.e. 0.02) at the first assessment in advanced training, as shown in Table 9.20. The assessor effects for this trait ranged from 0.08 (s.e. 0.01) for the first CAS assessment in advanced training to 0.60 (s.e. 0.03) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect for eagerness score at the CAS assessment at 5 months of age with an estimated effect of being male of 0.04 (s.e. 0.01), implying that male dogs tended to receive a higher (i.e. worse) score for calmness.

Table 9.20 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for eagerness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.02 (0.01)	0.01 (0.01)	0.32 (0.03)
8 months of age	0.01 (0.01)	0.01 (0.01)	0.30 (0.03)
12 months of age	0.03 (0.01)	0.01 (0.01)	0.36 (0.03)
1st in ET	0.10 (0.02)	0 (0)	0.17 (0.01)
1st in AT	0.11 (0.02)	0.01 (0.01)	0.08 (0.01)

Crossbreeding parameter estimates for eagerness at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.21. Two estimates were detectably larger than zero. The estimate of recombination loss effect was positive and detectably larger than zero (based on an approximate t-test) at the CAS assessment at 5 months of age, with an estimate of 0.31 (s.e. 0.15), implying that recombination loss was associated with higher (i.e. worse) scores for eagerness at this time point. The estimate of the effect of Labrador fraction was just detectably larger than zero at the same CAS assessment, with an estimate of -0.10 (s.e. 0.05) implying that increasing Labrador fraction is associated with lower (i.e. better) scores for eagerness at this time point. None of the estimates of heterotic effect were detectably larger than zero.

Table 9.21 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for eagerness were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	-0.01 (0.05)	0.31 (0.15)	-0.10 (0.05)
8 months of age	-0.09 (0.05)	0.08 (0.14)	-0.02 (0.03)
12 months of age	-0.03 (0.06)	0.04 (0.16)	0.02 (0.06)
1st in ET	-0.03 (0.07)	-0.04 (0.19)	-0.06 (0.09)
1st in AT	0.03 (0.10)	-0.02 (0.27)	-0.18 (0.12)

* heterosis † recombination loss § Labrador fraction

9.3.1.10. *Interaction with animals*

Heritability estimates for score for interaction with animals were small but detectably larger than zero for the assessments in puppy walking at 8 and 12 months of age and for the first assessments in early training and advanced training ($p<0.01$), with estimates ranging from 0.02 (s.e. 0.01) at 12 months of age to 0.06 (s.e. 0.02) at the first assessments in early and advanced training, as shown in Table 9.22. Assessor effects for this trait ranged from 0.13 (s.e. 0.01) for the first CAS assessment in advanced training to 0.41 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect at the first CAS assessments in early and advanced training, with estimated effects of being male at both time points of -0.04 (s.e. 0.01). These small, negative estimates imply that being male is associated with lower (i.e. better) scores for interaction with animals.

Table 9.22 Estimates of heritability, litter effect and assessor effect for the four CAS assessments for which the heritability estimates of score for interaction with animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.04 (0.01)	0.01 (0.01)	0.22 (0.03)
12 months of age	0.02 (0.01)	0 (0)	0.28 (0.03)
1st in ET	0.06 (0.02)	0.01 (0.01)	0.13 (0.01)
1st in AT	0.06 (0.02)	0 (0)	0.14 (0.01)

Crossbreeding parameter estimates for interaction with animals at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.23. None of the estimates of heterotic effect were detectably larger than zero. The estimate of recombination loss effect was positive and detectably larger than zero (based on an approximate t-test) at the first CAS assessment in advanced training, with an estimate of -0.47 (s.e. 0.18), implying that recombination loss was associated with lower (i.e. better) scores for interaction with animals at this time point. The estimates of the effect of Labrador fraction were detectably larger than zero at the CAS assessments at 8 and 12 months of age, with estimates of 0.19 (s.e. 0.06) and 0.12 (s.e. 0.04) respectively, implying that increasing Labrador fraction is associated with higher (i.e. worse) scores for interaction with animals at these time points.

Table 9.23 Crossbreeding parameter estimates for the four CAS assessments for which the heritability estimates of score for interaction with animals were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
8 months of age	0.01 (0.05)	-0.10 (0.14)	0.19 (0.06)
12 months of age	0.00 (0.05)	0.09 (0.13)	0.12 (0.04)
1st in ET	-0.02 (0.06)	-0.07 (0.15)	-0.04 (0.01)
1st in AT	-0.01 (0.07)	-0.47 (0.18)	0.08 (0.07)

* heterosis † recombination loss § Labrador fraction

9.3.1.11. *Interaction with people*

Heritability estimates for score for interaction with people were small but detectably larger than zero at all CAS assessments of interest except the first in puppy walking, with estimates ranging from 0.05 (s.e. 0.01) at 5 months of age to 0.13 (s.e. 0.03) at the first assessment in advanced training ($p<0.01$), as shown in Table 9.24. Assessor effects for this trait ranged from 0.11 (s.e. 0.01) for the first CAS assessment in advanced training to 0.41 (s.e. 0.04) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect in all interaction with people animals, with the effect of being male ranging from -0.06 (s.e. 0.02) at the first CAS assessment in advanced training to -0.11 (s.e. 0.02) at the first assessment in early training, implying that male dogs tended to receive lower (i.e. better) scores for interaction with people.

Table 9.24 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for interaction with people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.05 (0.01)	0.01 (0.01)	0.24 (0.03)
8 months of age	0.07 (0.02)	0.01 (0.01)	0.24 (0.03)
12 months of age	0.07 (0.02)	0 (0)	0.28 (0.03)
1st in ET	0.11 (0.02)	0.01 (0.01)	0.13 (0.01)
1st in AT	0.13 (0.03)	0.01 (0.01)	0.11 (0.01)

Crossbreeding parameter estimates for interaction with people at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.25. None of the estimates of recombination effect were detectably larger than zero. The estimate of heterotic effect was positive and detectably larger than zero (based on an approximate t-test) at the CAS assessment at 12 months of age, with an estimate of -0.17 (s.e. 0.07), implying that heterosis was associated with lower (i.e. better) scores for interaction with people at this time point. The estimate of the effect of Labrador fraction was detectably larger than zero at all of the CAS assessments with heritability estimates which were detectably larger than zero except that at 12 months of age, with estimates ranging from 0.14 (s.e. 0.07) at 5 months of age to 0.30 (s.e. 0.10 and 0.12) at the first assessments in early and advanced training, implying that increasing Labrador fraction is associated with higher (i.e. worse) scores for interaction with people at these time points.

Table 9.25 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for interaction with people were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.03 (0.06)	0.13 (0.17)	0.14 (0.07)
8 months of age	0.03 (0.07)	-0.08 (0.18)	0.23 (0.08)
12 months of age	-0.17 (0.07)	0.28 (0.18)	0.09 (0.08)
1st in ET	-0.05 (0.08)	0.09 (0.21)	0.30 (0.10)
1st in AT	0.12 (0.10)	-0.05 (0.27)	0.30 (0.12)

* heterosis † recombination loss § Labrador fraction

9.3.1.12. *Obedience*

Heritability estimates for score for obedience were small but detectably larger than zero for the assessments in puppy walking at 8 and 12 months of age and for the first assessments in early training and advanced training, with estimates ranging from 0.01 (s.e. 0.007) at the CAS assessment at 8 months of age to 0.06 (s.e. 0.02) at the first assessment in advanced training ($p<0.01$), as shown in Table 9.26. Assessor effects for this trait ranged from 0.15 (s.e. 0.02) for the first CAS assessment in advanced training to 0.62 (s.e. 0.03) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect at the CAS assessment at 8 months of age, with an estimate of effect of being male of -0.03 (s.e. 0.01), suggesting that being male is associated with lower (i.e. better) scores for obedience at this time point.

Table 9.26 Estimates of heritability, litter effect and assessor effect for the four CAS assessments for which the heritability estimates of score for obedience were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
8 months of age	0.01 (0.01)	0.01 (0.01)	0.34 (0.03)
12 months of age	0.02 (0.01)	0.01 (0.01)	0.40 (0.03)
1st in ET	0.04 (0.01)	0.01 (0.01)	0.27 (0.02)
1st in AT	0.06 (0.02)	0.00 (0.01)	0.15 (0.02)

Crossbreeding parameter estimates for obedience at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.27. None of the estimates of the effects of heterosis, recombination loss and Labrador fraction were detectably larger than zero.

Table 9.27 Crossbreeding parameter estimates for the four CAS assessments for which the heritability estimates of score for obedience were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
8 months of age	-0.00 (0.05)	-0.21 (0.14)	0.05 (0.04)
12 months of age	-0.08 (0.05)	-0.01 (0.13)	0.04 (0.04)
1st in ET	-0.07 (0.05)	-0.06 (0.14)	0.08 (0.06)
1st in AT	0.01 (0.07)	-0.20 (0.17)	0.01 (0.07)

* heterosis † recombination loss § Labrador fraction

9.3.1.13. *Stress resilience*

The heritability estimate for score for stress resilience was small but detectably larger than zero at all CAS assessments of interest except the first assessment in puppy walking, with estimates ranging from 0.01 (s.e. 0.006) at 8 months of age to 0.06 (s.e. 0.02) at the first assessment in early training, as shown in Table 9.28. The assessor effect for this trait ranged from 0.12 (s.e.

0.01) for the first CAS assessment in advanced training to 0.66 (s.e. 0.03) for the first assessment in puppy walking. Sex was a small but significant fixed effect at the CAS assessment at 8 months of age, with an estimated effect of being male of -0.07 (s.e. 0.01), implying that being male is associated with lower (i.e. better) scores for stress resilience at this time point.

Table 9.28 Estimates of heritability, litter effect and assessor effect for the CAS assessments for which the heritability estimate of score for stress resilience was detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.03 (0.01)	0.01 (0.01)	0.44 (0.04)
8 months of age	0.01 (0.01)	0.00 (0.01)	0.35 (0.03)
12 months of age	0.03 (0.01)	0.01 (0.01)	0.39 (0.03)
1st in ET	0.06 (0.02)	0.00 (0.01)	0.18 (0.02)
1st in AT	0.04 (0.01)	0.01 (0.01)	0.12 (0.01)

Crossbreeding parameter estimates for stress resilience at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.29. Heterotic effect was only detectably larger than zero at the CAS assessment in puppy walking at 8 months of age, with a small, negative estimate of -0.11 (s.e. 0.05). The estimate of Labrador fraction effect was also only detectably larger than zero at the CAS assessment at 8 months of age, with an estimate of -0.14 (s.e. 0.04). These estimates suggest that heterosis and increasing Labrador fraction are both associated with lower (i.e. better) scores for stress resilience at 8 months of age. The estimate of the effect of recombination loss was only detectably larger than zero at the CAS assessment at 5 months of age, with an estimate of 0.42 (s.e. 0.15), implying that recombination loss is associated with higher (i.e. worse) scores for stress resilience at 5 months of age.

Table 9.29 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for stress resilience were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.00 (0.05)	0.42 (0.15)	-0.09 (0.06)
8 months of age	-0.11 (0.05)	0.27 (0.15)	-0.14 (0.04)
12 months of age	-0.10 (0.06)	0.31 (0.17)	-0.06 (0.06)
1st in ET	-0.02 (0.07)	-0.27 (0.18)	-0.11 (0.08)
1st in AT	-0.04 (0.08)	0.05 (0.21)	-0.02 (0.07)

* heterosis † recombination loss § Labrador fraction

9.3.1.14. *Suspicion*

The heritability estimates for score for suspicion were detectably larger than zero for all of the CAS assessments of interest except the first assessment in puppy walking, with estimates ranging from 0.02 (s.e. 0.01) at 8 months of age to 0.10 (s.e. 0.02) at the first assessment in advanced training ($p<0.01$), as shown in Table 9.30. Assessor effects for this trait ranged from 0.10 (s.e. 0.01) for the first CAS assessment in advanced training to 0.64 (s.e. 0.03) for the first CAS assessment in puppy walking. Sex was a small but significant fixed effect at all CAS assessments of interest, with estimates of effect of being male ranging from -0.05 (s.e. 0.01) at the assessments at 5 and 12 months of age to -0.09 (s.e. 0.01) at the assessment at 8 months of age. This implies that being male is associated with lower (i.e. better) scores for suspicion.

Table 9.30 Estimates of heritability, litter effect and assessor effect for the five CAS assessments for which the heritability estimates of score for suspicion were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.04 (0.01)	0.01 (0.01)	0.36 (0.03)
8 months of age	0.02 (0.01)	0.01 (0.01)	0.35 (0.03)
12 months of age	0.03 (0.01)	0.02 (0.01)	0.38 (0.03)
1st in ET	0.06 (0.02)	0.01 (0.01)	0.17 (0.01)
1st in AT	0.10 (0.02)	0.01 (0.01)	0.10 (0.01)

Crossbreeding parameter estimates for suspicion at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.31. Heterotic effect was detectably larger than zero at the CAS assessments in puppy walking at 8 and 12 months of age, with estimates of -0.15 (s.e. 0.06) and -0.16 (s.e. 0.07) respectively. These estimates suggest that heterosis is associated with lower (i.e. better) scores for suspicion at these time points. Estimates of Labrador fraction effect were detectably larger than zero at all these CAS assessment except the first in advanced training, with estimates ranging from -0.16 (s.e. 0.06) at 5 months of age to -0.23 (s.e. 0.05) at 8 months of age. Thus increasing Labrador fraction appears to be associated with lower (i.e. better) scores for suspicion. None of the estimates of effect of recombination loss were detectably larger than zero for suspicion.

Table 9.31 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for suspicion were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.00 (0.06)	0.28 (0.16)	-0.16 (0.06)
8 months of age	-0.15 (0.06)	0.15 (0.15)	-0.23 (0.05)
12 months of age	-0.16 (0.07)	0.27 (0.17)	-0.18 (0.06)
1st in ET	-0.10 (0.07)	-0.01 (0.19)	-0.21 (0.08)
1st in AT	0.01 (0.09)	0.12 (0.25)	-0.18 (0.11)

* heterosis † recombination loss § Labrador fraction

9.3.1.15. *Toileting routine*

The heritability estimate for score for toileting routine was detectably larger than zero at all CAS assessments of interest except the first in puppy walking, with estimates ranging from 0.02 (s.e. 0.01) at 5 and 12 months of age to 0.12 (s.e. 0.03) at the first assessment in advanced training ($p<0.01$), as shown in Table 9.32. The assessor effect for this trait ranged from 0.11 (s.e. 0.01) for the first CAS assessment in advanced training to 0.61 (s.e. 0.03) for the first assessment in puppy walking.

Table 9.32 Estimates of heritability, litter effect and assessor effect for the CAS assessments for which the heritability estimates of score for toileting routine were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h^2	litter effect	assessor effect
5 months of age	0.02 (0.01)	0 (0)	0.23 (0.03)
8 months of age	0.05 (0.01)	0 (0)	0.18 (0.02)
12 months of age	0.02 (0.01)	0.01 (0.01)	0.30 (0.03)
1st in ET	0.07 (0.02)	0.01 (0.01)	0.15 (0.01)
1st in AT	0.12 (0.03)	0.00 (0.01)	0.11 (0.01)

Crossbreeding parameter estimates for toileting routine at the CAS assessments for which the heritability estimates were detectably larger than zero are shown in Table 9.33. None of the estimates of effect of recombination loss or Labrador fraction were detectably larger than zero. The heterotic effect estimate was detectably larger than zero at the CAS assessment in puppy walking at 5 month of age, with an estimate of 0.18 (s.e. 0.06). This estimate suggests that heterosis is associated with higher (i.e. worse) scores for toileting routine at 5 months of age.

Table 9.33 Crossbreeding parameter estimates for the five CAS assessments for which the heritability estimates of score for toileting routine were detectably larger than zero. Estimates are shown followed by their standard errors in brackets to two decimal places. ET is early training, AT is advanced training.

CAS assessment	h*	r loss†	Lab§
5 months of age	0.18 (0.06)	0.12 (0.17)	0.02 (0.06)
8 months of age	0.06 (0.07)	-0.05 (0.19)	-0.04 (0.08)
12 months of age	0.06 (0.07)	0.19 (0.17)	0.01 (0.06)
1st in ET	0.06 (0.08)	0.04 (0.22)	0.10 (0.10)
1st in AT	0.10 (0.10)	0.04 (0.27)	0.14 (0.12)

* heterosis † recombination loss § Labrador fraction

9.3.2. Repeatability models

Estimates of genetic and environmental parameters for calmness and eagerness using CAS score for each trait at each of the six CAS assessments of interest are shown in Table 9.34. Estimates of genetic and environmental parameters for calmness and eagerness from the reduced models, which only included the CAS scores for each trait at 12 months and at the first CAS assessments in early and advanced training, are shown in Table 9.35.

Table 9.34 Estimates of heritability (h^2), permanent environmental effect (c^2), repeatability (R) and phenotypic variance (σ^2_p) for calmness and eagerness calculated by including CAS scores from all six assessments of interest. Estimates are followed by their standard errors in brackets.

	Calmness	Eagerness
h^2	0.05 (0.01)	0.03 (0.01)
c^2	0.12 (0.01)	0.03 (0.01)
R	0.17 (0.01)	0.07 (0.01)
σ^2_p	0.32 (0.01)	0.48 (0.01)

Table 9.35 Estimates of heritability (h^2), permanent environmental effect (c^2), repeatability (R) and phenotypic variance (σ^2_p) for calmness and eagerness calculated by including just the CAS scores at 12 months and at the first assessments in early and advanced training. Estimates are followed by their standard errors in brackets.

	Calmness	Eagerness
h^2	0.07 (0.02)	0.11 (0.02)
c^2	0.18 (0.02)	0.10 (0.02)
R	0.26 (0.02)	0.22 (0.02)
σ^2_p	0.31 (0.01)	0.44 (0.01)

For both traits, in both full and reduced models, all the estimates were detectably larger than zero (based on approximate t-tests). The permanent environmental effect estimates, also described as the common environmental or non-genetic effect, were larger than the heritability in both the full and reduced model for calmness and were the same as, or close to, the heritability estimates in both models for eagerness. Estimates of phenotypic variance were very close for both calmness and eagerness between the full and reduced models, while estimates of repeatability increased in the reduced models compared to the full models.

9.4. Discussion

Univariate linear mixed models were successfully used to estimate heritabilities and crossbreeding parameters for CAS elements in GD's Labrador Retrievers, Golden Retrievers and crosses between these two breeds. This represents the first attempt to quantify the crossbreeding parameters of heterosis, recombination loss and breed effects for behavioural traits in the dog. In addition repeatability models were used for two CAS elements and produced interesting results.

9.4.1. Univariate models

This type of crossbreed model assumes that the genetic variance for score for each CAS element is broadly the same in Labrador Retrievers and Golden Retrievers. This assumption is not unreasonable given the relatively close relationship between the two breeds. The fairly similar heritability estimates between the two breeds for those CAS elements and time points which were detectably larger than zero also supports this assumption.

Although many heritability estimates for CAS elements at different time points were not detectably larger than zero, this was the case for far fewer models than for the Labrador Retriever and Golden Retriever datasets. This

probably reflects the larger size of the crossbreed datasets. Also, for those heritability estimates which were detectably larger than zero, many more of them were significant at the $p<0.01$ level than was the case for the single breed models. The heritability estimates for the crossbreed dataset were all small, similar to those seen in the Labrador Retriever and smaller than those seen in the Golden Retriever. The lowest heritability estimate which was detectably larger than zero was 0.01 (s.e. 0.00-0.01) for behaviour on transport at 5 months of age, body sensitivity at 5, 8 and 12 months of age, calmness at the first assessment in puppy walking, eagerness at 8 months of age, obedience at 8 months of age and stress resilience at 8 months of age. The largest heritability estimate was 0.15 (s.e. 0.03) for distraction at the first CAS assessment in advanced training. The assessor effects were often large, as seen in the single breed models which may reduce the heritability estimates, as discussed in chapter 8.

Most estimates of heterotic effect were not detectably different from zero. However seven CAS elements had heterosis estimates which were detectably larger than zero at one or more time points of interest. These were -0.14 (s.e. 0.06) for attentiveness at the first CAS assessment in early training; -0.11 (s.e. 0.05) and -0.22 (s.e. 0.05) for body sensitivity at 5 and 8 months of age respectively; -0.15 (s.e. 0.07) for distraction at the first CAS assessment in early training; -0.17 (s.e. 0.07) for interaction with people at 12 months of age; -0.11 (s.e. 0.05) for stress resilience at 8 months of age; -0.15 (s.e. 0.06) and -0.16 (s.e. 0.07) for suspicion at 8 and 12 months of age respectively; and 0.18 (s.e. 0.06) for toileting routine at 5 months of age. All the estimates of heterotic effect which were detectably larger than zero were small to moderate and all but one of them were negative, implying that for alertness, body sensitivity, distraction, interaction with people, stress resilience and suspicion heterosis is associated with lower (i.e. better) CAS scores but for toileting routine heterosis is associated with higher (i.e. worse) CAS scores. These results suggest that the effects of heterosis, when detectable, were largely beneficial in terms of CAS scores and this may relate to the higher

success rate seen in the Golden Retriever cross Labrador Retriever when compared to the two purebred lines.

These effects are smaller than they initially appear as the maximum heterosis probability for any individual is 50% meaning that the values shown should be halved to obtain effect estimates for F1s and quartered to obtain effect estimates for the backcrosses. Heterosis occurs due to dominance, with the amount of heterosis observed following a cross between two breeds depending on the square of the difference of the gene frequency between the two breeds (Falconer & Mackay, 1996). Considering a single locus, there will be no heterosis if the two breeds do not differ in gene frequency and the greatest heterosis will be seen when one allele is fixed in one breed and the other allele is fixed in the other breed. In the situation where multiple loci impact a trait, the absence of detectable heterotic effect is not sufficient evidence for concluding that individual loci show no dominance as if some loci are dominant in one direction and some in the other their effects will tend to cancel each other out.

Even fewer estimates of recombination loss effect were detectably larger than zero than for heterotic effect. The power to detect recombination loss effects is low in this dataset as there are no F2 crosses and only a relatively small number of backcrosses (1073 dogs out of 10078, representing 11% of the dataset). However four recombination loss effect estimates were detectably larger than zero. These were for body sensitivity at the CAS assessment at 5 months of age, with an estimate of 0.26 (s.e. 0.13); eagerness at 5 months of age, with an estimate of 0.31 (s.e. 0.15); interaction with animals at the first assessment in advanced training, with an estimate of -0.47 (s.e. 0.18); and stress resilience at 5 months of age, with an estimate of 0.42 (s.e. 0.15). As with heterotic effects, but to an even larger extent, these estimates are smaller than they initially appear as the maximum recombination loss probability in the CAS dataset was 12.5% (in the backcrosses) and the regression coefficient quoted is scaled to a recombination loss value of one. Recombination loss appears to be associated with a slightly higher (i.e. worse)

CAS score for body sensitivity, eagerness and stress resilience at 5 months of age and a slightly lower (i.e. better) CAS score for interaction with animals at the first assessment in advanced training. The majority of recombination loss estimates which were detectably larger than zero were thus associated with worse scores for CAS elements suggesting that the benefits in behaviour (as measured by CAS scores) seen in the F1 may be at least partially lost in the backcrosses.

The estimates of Labrador fraction effect were detectably larger than zero for more CAS elements and at more time points than the heterosis and recombination loss estimates. This may be because this covariate had representatives at more levels, with individuals having values of 0, 0.25, 0.5, 0.75 and 1. Labrador fraction effect estimates were positive and detectably larger than zero at one or more time points of interest for behaviour when left, calmness, distraction, interaction with animals and interaction with people, with estimates ranging from 0.12 (s.e. 0.04) for interaction with animals at 12 months of age to 0.30 (s.e. 0.10 and 0.12) for interaction with people at the first CAS assessments in early and advanced training. These estimates imply that increasing Labrador fraction is associated with higher (i.e. worse) CAS scores for these elements. Labrador fraction effect estimates were negative and detectably larger than zero at one or more time points of interest for behaviour on transport, body sensitivity, confidence, eagerness, stress resilience and suspicion, with estimates ranging from -0.10 (s.e. 0.05) for eagerness at 5 months of age to -0.23 (s.e. 0.05) for suspicion at the CAS assessment at 8 months of age. This implies that increasing Labrador fraction is associated with lower (i.e. better) CAS scores for these elements. Thus five CAS element scores were detrimentally affected by increasing Labrador fraction and six CAS element scores were beneficially affected by increasing Labrador fraction and overall the benefits and detrimental effects may neutralise each other.

Goddard and Beilharz (1985) recorded 38 measures of fearfulness on offspring of a diallel cross between Labrador Retrievers, GSDs, Boxers and Kelpies and found no evidence of heterosis for fearfulness, based on responses to a battery of tests and to observer ratings over 3 weeks. There is no direct homologue for fearfulness in CAS, but it may be captured at least in part by scores for suspicion and stress resilience; CAS scores for both of which showed evidence of heterosis at one or more time point of interest.

9.4.2. Repeatability models

Repeatability models are used for the analysis of data when multiple measurements of the same trait are recorded on an individual (Mrode, 2014). The models assume for each individual that the genetic correlation between all pairs of records is one, equal variance for all records and equal environmental correlation between all pairs of records. These assumptions were more closely approximated in the reduced models than in the full models for calmness and eagerness. In both full and reduced models for calmness the heritability estimates were lower than the highest estimates seen in the models of different time points, with estimates of 0.05 (s.e. 0.01) in the full model and 0.07 (s.e. 0.02) in the reduced model compared to 0.14 (s.e. 0.03) for the first CAS assessment in advanced training. This may be because, in the models of individual CAS time points, all of the between-individual variance not partitioned due to fixed effects, covariates and other random effects was being portioned as additive variance leading to inflated heritability estimates. In the repeatability models the additive and permanent environment effects were properly separated leading to lower additive variance and hence lower heritability estimates.

Repeatability estimates, which are the proportion of phenotypic variance explained by the dog's genetics and its permanent environment combined, represent the upper limit to the heritability (Mrode, 2014). In the full model for eagerness, the heritability estimate is again lower than the highest

estimate seen in the models at different time points, with an estimate of 0.03 (s.e. 0.01) compared to 0.11 (s.e. 0.02) for the first CAS assessment in advanced training. However in the reduced model for calmness the heritability estimate was the same as that at the first CAS assessment in advanced training i.e. 0.11 (s.e. 0.02). The estimates of permanent environment effects were lower for eagerness than for calmness and this may be part of the reason for this finding. The permanent environmental variance is the variance due to environmental effects which have consistently influenced the dog's behaviour, such as housing, early life influences and maternal effects.

Repeatability estimates were low to moderate, with estimates of 0.17 (s.e. 0.01) in the full model and 0.26 (s.e. 0.02) in the reduced model for calmness and 0.07 (s.e. 0.01) in the full model and 0.22 (s.e. 0.02) in the reduced model for eagerness. A low repeatability may not be particularly surprising for elements that are being scored by different assessors at different time points. The dogs were also undergoing training between CAS assessments which might be expected to change the score received between one assessment and the next, although intuitively calmness and eagerness seem less modifiable than elements such as behaviour on transport or toileting routine. For both calmness and eagerness, permanent environmental effects were larger than the heritability estimates suggesting that permanent environmental effects are more important than genetic influences on these traits as measured by CAS. Thus improvement in CAS scores for calmness and eagerness may be effected more easily by attempting to modify the dogs' permanent environment, for example through attempting to standardise housing and early life experiences, than by selection.

9.5. Conclusion

The results of these analyses suggest that EBVs generated from crossbreed models of some CAS elements and time points could be used for selection in GD's Labrador Retrievers and Golden Retrievers to produce both purebred and crossbred litters. Genetic correlations between different CAS elements in the crossbreed models will need to be estimated (as was undertaken in the Labrador Retriever models) before EBVs for any CAS elements are incorporated into a selection index. CAS scores at the first assessment in advanced training may be the most appropriate to use as they generally have the highest heritability estimates and lowest estimates of assessor effect.

Repeatability models, which were attempted only for calmness and eagerness, found low to moderate estimates of repeatability for both traits. For both traits, permanent environmental effects were larger than the heritability estimates suggesting that permanent environmental effects are more important than genetic influences on these traits. Thus improvement in CAS scores for calmness and eagerness may be achieved more easily by attempting to modify the dogs' permanent environment than by selection.

10. CROSSBREED GENETIC ANALYSIS OF A STANDARDISED BEHAVIOUR TEST FOR POTENTIAL GUIDE DOG PUPPIES.

10.1. Introduction

Guide Dogs (GD) place prospective guide dog puppies with volunteer Puppy Walkers (PWs) at approximately 7 weeks of age and the puppies remain with them until they are around 14 months old, as described in Chapter 3. Throughout this period they undergo regular behavioural assessments, using the Canine Assessment Summary (CAS). Dogs which pass all behavioural and health assessments either enter training or become a brood bitch or stud dog. Dogs entering training, lasting 34 weeks on average, continue to undergo behavioural assessments before commencing work as guide dogs at about 1½ to 2 years old. Selection of individuals for training or breeding could be achieved much earlier, and more accurately, if behavioural test results of young stock were predictive of success as a guide dog, and if such results were shown to be heritable.

Various models of puppy testing have been trialled by working dog organisations internationally in attempts to improve selection for their respective programmes (e.g. Scott & Bielfelt, 1976; Goddard & Beilharz, 1986; Wilsson & Sundgren, 1998; Slabbert & Odendaal, 1999; Russenberger, 2012). It has been suggested that testing puppies at 6 to 8 weeks of age may be advantageous as puppies are motivated to approach unknown people during this period in contrast to usual wariness (Serpell & Jagoe, 1995).

GD has developed a puppy test named the puppy profiling assessment (PPA) to assess the behaviour of puppies prior to placement with puppy walkers, using a series of controlled stimuli. It was developed to be feasible, standardised and its criterion validity has been assessed, under the Taylor and Mills (2006) framework for the development of behavioural tests for dogs.

Asher et al (2013) analysed the results of a pilot study of the PPA involving 587 puppies and showed that five of the PPA stimuli were associated with later success in guide dog training. The PPA was refined based on the findings of Asher et al (2013) and is now used routinely by GD with all puppies at approximately six weeks of age before they are placed with PWs. The aim of this chapter was to investigate genetic and environmental factors, and crossbreeding parameters, relating to the PPA to determine whether there was potential for developing EBVs for any PPA components.

10.1.1. Puppy profiling assessment (PPA)

The PPA stimuli were designed to test either confidence (a positive active response to environmental stimuli) or responsiveness (a positive active response to the human handler). The following situations were scored: 1) following when called; 2) interest in retrieving a toy; 3) response to restraint; 4) response to noise; 5) response to stroking; 6) response to unusual moving object ("squirrel", a piece of soft bedding); 7) response to a tunnel; 8) response to a ramp. One of three assessors, who the puppies had never met before, performed the PPA and undertook the scoring. These individuals were GD staff trained to recognise and assess behaviour in dogs. The puppy's initial reaction to all stimuli was scored on a 7 point scale (with 1 being least confident or responsive and 7 being over confident or responsive), with additional scoring of situations 2, 5 and 6 (also on a 7 point scale, denoted as stimuli 2b, 5b and 6b) for subsequent response to assessor. Thus 2a, 5a and 6a are responses to stimuli and 2b, 5b and 6b are subsequent response to assessor. The test components are summarised in Table 10.1. A score of 4 in each component was considered to be a balanced response likely to indicate puppies most likely to qualify as guide dogs.

Asher et al (2013) found that scores for components 2b (retrieve – response to assessor), 5a (stroking – response to stimulus), 5b (stroking – response to assessor), 6b (squirrel – response to assessor) and 8 (ramp) were associated with later success as guide dogs.

Table 10.1 PPA component descriptions.

PPA component	Description
1	Following
2a	Retrieve – response to stimulus
2b	Retrieve – response to assessor
3	Restraint
4	Noise
5a	Stroking – response to stimulus
5b	Stroking – response to assessor
6a	Squirrel – response to stimulus
6b	Squirrel – response to assessor
7	Tunnel
8	Ramp

10.2. Materials and methods

10.2.1. Description of dataset

GD provided a copy of the PPA test results for all puppies which underwent the test between April 2012 and April 2014. Each puppy was only tested once. The full dataset consisted of PPA test results for 2592 puppies.

10.2.2. Data validation

The data were edited based on two criteria (breed and pedigree availability) as explained below. Table 10.2 shows the number of puppies in the data set before and after each round of validation. The final data set analysed contained 2127 puppies. Coincidentally the two editing steps removed all pups that had not been bred by GD.

10.2.2.1. *Breed*

Only Labrador Retrievers, Golden Retrievers and crosses between these two breeds were included in subsequent analyses, as there were insufficient numbers of individuals of other breeds and crosses. This excluded 337 pups (Table 10.2).

10.2.2.2. *Pedigree availability*

Individuals were excluded from subsequent analyses if their parents were not found in GD's amended pedigree file (as described in Chapter 3). These puppies had all been born since March 2012 thus they were not in the pedigree file which had been used for all previous analyses and therefore had to be linked to it via their parents. This excluded 128 puppies (Table 10.2).

Table 10.2 Number of dogs retained and removed at each editing step described in the materials and methods. The percentage expressed is with reference to the number of dogs in the raw data set.

Editing step	Number remaining	Number lost	Percentage remaining
Raw data set	2592	-	-
Breed	2255	337	87%
Pedigree availability	2127	128	82%

10.2.3. Litter and batch identification number allocation

A MATLAB® program was created to assign litter identification numbers to puppies with dates of birth and parental identification numbers in common. Assessor was poorly recorded and missing for 866 puppies so in order that some of the variability associated with this factor could be captured a variable called “batch” was created based on PPA test date. Most batches included at least 2 litters.

10.2.4. Estimated crossbreed parameter calculation

The expected heterosis and recombination loss for each individual was calculated from the proportion of Labrador Retriever and Golden Retriever of each animal’s sire and dam, after Van der Werf and de Boer (1989). Using this method heterosis was calculated as $h = ½ [(P_S (1-P_D)) + (P_D (1-P_S))]$, and recombination loss as $r = ½ [(P_S (1-P_S)) + (P_D (1-P_D))]$, where P_S and P_D are the proportion of Labrador Retriever in the sire and the dam respectively, and thus the probability of inheriting a Labrador Retriever allele from the sire or dam. The first equation gives the probability that the two alleles inherited from the parents at any one locus originate from different breeds. The second equation gives the probability that any two loci inherited from the same parent originate from different breeds.

The proportion of Labrador Retriever and estimates of heterosis and recombination loss for the different breeds and crosses, together with the number of dogs of each breed or cross, in the dataset are shown in Table 10.3.

Table 10.3 Estimates of heterosis (h) and recombination loss (r loss) for the different breeds and crosses in the dataset and the number (n) and proportion (p) of each.

Breed or cross	Lab*	h	r loss	n	p
Golden Retriever	0	0	0	119	0.06
Labrador Retriever	1	0	0	704	0.33
GR x LR	0.5	0.5	0	950	0.44
GR x (GR x LR)	0.25	0.25	0.125	39	0.02
LR x (GR x LR)	0.75	0.25	0.125	315	0.15

* Labrador Retriever fraction

10.2.5. Statistical analyses

Statistical analysis of the data had the objective of fitting univariate measures mixed linear models using ASReml version 3.0 (Gilmour et al, 2009) to each PPA component at each time point of interest to estimate the heritability and independent regression coefficients of estimated Labrador Retriever fraction, heterosis and recombination loss. The significance of estimated effects and regression coefficients from zero was determined using approximate t-tests, with the number of degrees of freedom corresponding to the number of records from which the estimates can be determined minus one.

The pedigree file used in all analyses was described in Chapter 4.

10.2.5.1. *Univariate linear mixed models*

The general form of the univariate linear mixed model fitted for each PPA component was as follows:

$$\mathbf{Y = Xb + Za + Wc + Vd + e}$$

where **Y** is the vector of observations; **X**, **W, V** and **Z** are known incidence matrices, **b** is the vector of fixed effects, **a** is the vector of random additive genetic effects with the distribution assumed to be multivariate normal

(MVN), with parameters (0, σ^2_a**A**); **c** is the vector of random litter effects with the distribution assumed to be MVN, with parameters (0, σ^2_c**I**); d is the vector of random batch effects with the distribution (0, σ^2_d**I**); and **e** is the vector of residuals distributed MVN with parameters (0, σ^2_e**I**); and where **I** denotes an identity matrix of the appropriate size, **A** is the numerator relationship matrix, and σ^2 is a scalar denoting variance. The subscripts *a*, *c, d* and *e* denote additive genetic, litter, batch and residual variances respectively. The fixed effects included in the model were sex, year of birth and age (in days) at assessment. Labrador Retriever fraction, heterosis and recombination loss were included as covariates. The random effects fitted were litter, batch and individual animal effect. Mathematically, the heritability is the ratio of additive genetic variance to phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$.

In order to determine whether the heritability estimates were significantly different from zero Likelihood Ratio Tests (LRTs) were performed between the univariate animal models and null models in which the random effect for the individual had been omitted.

10.3. Results

The dataset analysed consisted of PPA test results for 2127 puppies, of which 998 (47%) were female and 1129 (53%) were male. Mean age at test was 45.4 days (standard deviation 1.9, minimum 38 days, maximum 51 days). The 2127 puppies came from 298 litters, with a mean of 7.1 pups per litter (standard deviation 2.6, minimum 1, maximum 12). There were 62 unique sires and 229 unique dams.

10.3.1. Univariate linear mixed models

Estimates of heritability, litter effect and batch effects for the PPA elements are shown in Table 10.4. Litter and batch effects are proportions of total variance. Heritability estimates were detectably larger than zero for all PPA

elements except 3 (restraint) and 4 (noise). Four PPA elements (6a, 6b, 7 and 8) had low heritability estimates ranging from 0.09 (s.e. 0.05) for PPA element 6b (squirrel – response to assessor) to 0.16 (s.e. 0.07) for PPA element 7 (tunnel). The remaining 5 PPA elements (1, 2a, 2b, 5a and 5b) had moderate heritability estimates ranging from 0.21 (s.e. 0.07) for PPA elements 2a (retrieve – response to stimulus) and 5b (stroking – response to assessor) to 0.24 (s.e. 0.09) for PPA element 1 (following).

Table 10.4 Estimates of heritability, litter effect and batch effect for the 11 PPA elements. Estimates are shown followed by their standard errors in brackets to two decimal places.

PPA element	h^2	Litter effect	Batch effect
1 - following	0.24 (0.09)*	0.11 (0.04)	0.05 (0.03)
2a - retrieve (stimulus)	0.21 (0.07)*	0.07 (0.03)	0.02 (0.02)
2b - retriever (assessor	0.22 (0.07)*	0.08 (0.03)	0 (0)
3 - restraint	0.11 (0.08)¥	0.15 (0.04)	0.05 (0.03)
4 - noise	0.01 (0.03)¥	0.05 (0.03)	0.11 (0.03)
5a - stroking (stimulus)	0.22 (0.09)*	0.16 (0.05)	0.03 (0.03)
5b - stroking (assessor)	0.21 (0.07)*	0.07 (0.03)	0 (0)
6a - squirrel (stimulus)	0.10 (0.06)†	0.09 (0.03)	0.03 (0.02)
6b - squirrel (assessor)	0.09 (0.05)*	0.08 (0.03)	0.01 (0.02)
7 - tunnel	0.16 (0.07)*	0.13 (0.04)	0.02 (0.02)
8 - ramp	0.12 (0.07)†	0.13 (0.04)	0.01 (0.02)

* $p<0.01$ †$p<0.05$ ¥not significant

Litter effect estimates were all small but detectably larger than zero (based on approximate t-tests) except that for PPA element 4 with estimates ranging from 0.07 (s.e. 0.03) for PPA element 2a (retrieve – response to stimulus) and 5b (stroking – response to assessor) to 0.16 (s.e. 0.05) for element 5a (stroking – response to stimulus). Fixed effect estimates were all not detectably larger than zero in all models except that for PPA element 2a. For PPA element 2a (retrieve – response to stimulus) age at test and sex were both significant, with estimates of 0.04 (s.e. 0.019) for age at test and 0.14 (s.e. 0.05) of being

female. These estimates suggest that greater age at test and being female were both associated with a higher score for PPA element 2a. Batch effect estimates were all small and mostly not detectably larger than zero, except that for PPA element 4 (noise) with an estimate of 0.11 (s.e. 0.03).

Crossbreeding parameter estimates for each of the PPA elements are shown in Table 10.5. The estimate of heterotic effect was just detectably larger than zero for PPA element 6a (squirrel – response to stimulus) with an estimate of -0.35 (s.e. 0.17) implying that heterosis was associated with a lower score for PPA element 6a. Estimates of recombination loss effect were detectably larger than zero for PPA elements 2a (retrieve – response to stimulus) and 7 (tunnel) with estimates of -1.62 (s.e. 0.81) and -1.59 (s.e. 0.73) respectively. These estimates imply that recombination loss is associated with lower scores for PPA elements 2a and 7. However as the maximum recombination loss probability in this dataset was 12.5% (in the backcrosses) the effects are smaller than they initially appear (both at -0.20) because the regression coefficient shown is scaled to a recombination loss value of 1. The estimate of Labrador fraction was only detectably larger than zero for PPA element 3 (restraint), with an estimate of 0.67 (s.e. 0.26), implying that increasing Labrador fraction is associated with a higher score for PPA element 3.

Table 10.5 Crossbreeding parameter estimates for the 11 PPA elements. Estimates are shown followed by their standard errors in brackets to two decimal places.

PPA element	h*	r loss†	Lab§
1 - following	0.03 (0.17)	0.11 (0.79)	-0.26 (0.33)
2a - retrieve (stimulus)	0.09 (0.17)	-1.62 (0.78)	-0.16 (0.34)
2b - retriever (assessor	0.01 (0.18)	-1.27 (0.81)	-0.46 (0.36)
3 - restraint	0.01 (0.17)	-0.27 (0.74)	0.67 (0.26)
4 - noise	-0.06 (0.10)	0.45 (0.43)	0.01 (0.10)
5a - stroking (stimulus)	-0.11 (0.19)	0.00 (0.84)	0.50 (0.35)
5b - stroking (assessor)	0.06 (0.18)	-0.61 (0.84)	-0.59 (0.38)
6a - squirrel (stimulus)	-0.35 (0.17)	-0.61 (0.73)	-0.33 (0.26)
6b - squirrel (assessor)	-0.14 (0.16)	-1.29 (0.69)	-0.39 (0.25)
7 - tunnel	-0.09 (0.17)	-1.59 (0.73)	-0.01 (0.29)
8 - ramp	-0.28 (0.15)	0.33 (0.64)	0.01 (0.24)

* heterosis † recombination loss § Labrador fraction

10.4. Discussion

Univariate linear models were successfully used to estimate heritability and crossbreeding parameters for the eleven components of the PPA. The heritability estimates for the components of the PPA were significantly greater than zero in nine of the 11 scores of behaviour, indicating that performance in these tests has an inherited element. Five of these components (1, 2a, 2b, 5a and 5b) appeared to be moderately heritable (h^2 >0.2). This means that these components should respond to selection to produce puppies which are temperamentally suited to guide blind or partially sighted people. Furthermore these results herald the possibility of calculation of EBVs for these traits, potentially increasing the accuracy of, and so the response to, selection compared to using phenotype.

Most of the crossbreeding parameter estimates were not detectably larger than zero. Exceptions to this were the estimate of heterotic effect for PPA element 6a, with an estimate of -0.35 (s.e. 0.17); estimates of recombination loss effect for element 2a and 7 with estimates of -1.62 (s.e. 0.81) and -1.59 (s.e. 0.73) respectively; and the estimate of Labrador fraction effect for PPA element 3, with an estimate of 0.67 (s.e. 0.26). The PPA dataset was considerably smaller than any of the datasets used for crossbreed evaluations of disease conditions or CAS scores and may have lacked the power to detect crossbreeding effects.

It seems that PPA components were more heritable than CAS components. The highest heritability estimate for a CAS component in the crossbreed models (Chapter 9) was 0.15 (s.e. 0.03) for distraction at the first CAS assessment in advanced training. Six PPA components (1, 2a, 2b, 5a, 5b and 7) had heritability estimates higher than this.

The models of behavioural traits measured by CAS, as described in Chapters 8 and 9, were complicated by the large number of assessors scoring the dogs and the associated large estimates of assessor effect. Only three assessors, working alone or in pairs, perform the PPA so this should reduce this potential source of variance. However, as assessor was poorly recorded at first it was not possible to include assessor as an effect in the models. Instead a proxy, "batch", was used and for all but one (4a, for which the heritability estimate was not detectably larger than zero) PPA component the estimate of batch effect was small and not detectably larger than zero. As more data is accrued it would be interesting to re-run the models, excluding any puppies for which assessor was not recorded, with assessor as a random effect to estimate what proportion of the variance is accounted for by assessor.

Common environment effects (such as maternal effects among full sibs or maternal half sibs, or litter effects among littermates) may generate similarities of phenotype between relatives that are of equal or even greater magnitude to those due to genetic effects (Kruuk & Hadfield, 2007). The

animal model provides an efficient means of modelling such effects although they can become complex, particularly in traits measured in young animals where a characteristic may be considered a trait of either or both the offspring or the dam (for example weaning weight). If traits are governed by direct (individual) genetic and maternal effects, fitting only the direct effects and leaving maternal effects out of the model may lead to an overestimation of heritability (Clément et al, 2001). Strandberg et al (2005) used linear mixed models in ASReml with maternal genetic, maternal permanent environment and litter permanent environmental effects as random effects in models of four canine personality traits (playfulness, chase-proneness, curiosity/fearfulness and aggressiveness) and found little influence of the maternal genetic or maternal permanent environmental effect. They concluded that models including a direct animal effect and a litter effect are acceptable for models for genetic evaluation of canine personality traits and that omitting the litter effect might results in an upward bias in the additive genetic variance component (Strandberg et al, 2005). Leaving maternal genetic effects out of models for behaviour test component scores was found not to lead to an overestimation of genetic variances in another study (van der Waaij et al, 2008). As dams in the PPA dataset had on average 1.3 litters, litter effect was considered a reasonable proxy for maternal effect. Litter was therefore included as a random effect in all the PPA models but in all cases the estimates of its effect were small but detectably larger than zero.

The heritability estimates for the PPA test results compare favourably with the results of other heritability studies of dog behaviour. For example, Wilsson and Sundgren (1998) reported the results of behavioural tests carried out on 554 German Shepherd Dogs at 8 weeks of age. The puppy test focused on characteristics which were considered highly variable among eight-week old puppies including sociability, independence, fearfulness, competitiveness, general activity and exploratory behaviour. The testing yielded 10 scores per puppy and heritability estimates for the different scores were mainly in the range of 0.20-0.27, with 3 higher estimates of 0.42, 0.48 and 0.53 for the

scores described as contact II, tug of war and activity respectively. All the heritability estimates were significantly higher than zero (Wilsson & Sundgren, 1998).

10.5. Conclusion

For the PPA to be useful in identifying and breeding dogs that are temperamentally suitable for guiding work then scores should be both predictive of success in guide dog training and heritable. According to Asher et al (2013) five of the 11 scores of behaviour in the PPA were found to show some association with success in guide dog training (tests 2b, 5a, 5b, 6b and 8). All of these five components had heritability estimates significantly greater than zero. Three of these components (2b, 5a and 5b) had moderate heritability estimates implying these could be good candidate scores to assist with decisions of selection of future breeding stock. This could potentially yield efficiency savings to GD, reducing wastage and allowing more funds from donations to be diverted into providing valuable assistance for blind and partially sighted people in the UK.

11. GENERAL DISCUSSION

The aim of this thesis was to understand which traits were of most importance to Guide Dogs (GD) and which traits measured by GD exhibit substantial genetic variance. It was hoped that there would be substantial overlap between traits of importance and those which exhibit substantial genetic variance such that an improved selective breeding programme, aided by quantitative genetic techniques, could be developed. The population evaluated, the largest population of assistance dogs in the world, consisted of a pedigree of more than 50,000 individuals with data relating to 28 disease conditions and 36 behavioural traits. This work therefore comprises the largest and most comprehensive genetic study performed on the health and temperament of dogs, and the results offer an exciting insight into the prevalence of specific health and temperamental types and their genetic variance. The findings from this study will not only form the basis of selection programmes for GD and potentially other assistance dog organisations, but have significant implications relating to our understanding of prevalence of conditions in pedigree dogs and where resources might therefore be focussed for improving health and behaviour.

11.1. Identification of traits of importance to Guide Dogs

Two approaches were taken in order to clarify which traits were of most importance: reasons for withdrawal of dogs from GD's programme were analysed and a short survey of selection aims was undertaken with key staff in selection of breeding stock. Results of the survey suggested that behavioural and health traits were equally important as selection aims, but analysis of reasons for withdrawal of dogs from GD's programme indicated that behavioural reasons accounted for substantially more withdrawals than health conditions. This discrepancy could potentially indicate that GD have had more success at selecting for healthy dogs than for dogs with the desired

behavioural profile to date, which may reflect the greater difficulty in accurately characterising behavioural traits compared to health traits. In total 68% (5327 of 7892 dogs) of dogs withdrawn from GD's programme between 1995 and 2012 were withdrawn for behavioural reasons, 29% (2257 of 7892 dogs) were withdrawn for health reasons and 3% (308 of 7892 dogs) were withdrawn for other reasons. This was a similar situation to the breakdown of reasons for withdrawal of dogs from their programme reported by other international guide dog organisations (Goddard & Beilharz, 1982; Arata et al, 2010).

Specific behavioural reasons (high distraction, high suspicion, low attentiveness, low stress resilience, aggression towards people, low confidence, unacceptable social behaviour and low willingness) accounted for eight of the 10 most frequent specific reasons for withdrawal, with atopic dermatitis or allergic skin disease and hip dysplasia being the only two individual health conditions in the 10 most frequent specific withdrawal reasons. These two disease conditions appeared of particular importance to GD both in terms of numbers of dogs being withdrawn as a result of the condition being diagnosed and in terms of the results of the survey of selection aims. The three behavioural traits which appeared of equal importance by both measures were aggression towards people, low confidence and low willingness.

One of the key findings of the survey of selection aims was that selection aims were breed-specific, and therefore selection indices will need to be breed-specific. The results of this survey and heritability analyses in subsequent chapters suggest that four different selection indices might be needed: one each for pure Labrador Retrievers, Golden Retrievers and German Shepherd Dogs, to produce both working guide dogs and replacement breeding stock, and one for the production of first-generation (F1) Labrador Retriever cross Golden Retrievers to produce the majority of working guide dogs.

Subsequent to these two pieces of work, GD has instigated a process to attempt to assess the impact of individual health and behavioural traits (data not shown). The intention is that this work will further clarify which traits should become components of the breed-specific selection indices and also to assist with weighting the different traits. Individual attendees of the breed review meeting will be asked to score each trait, identified by the work undertaken in this thesis as both important and heritable, on three measures (each scored 0 to 100): impact on the welfare of an affected dog, impact on a Guide Dog Owner (GDO) if they have an affected dog, cost to GD. A fourth measure will be the percentage of dogs rejected out of those bred each year due to the trait in question. Thus each trait will have a score between 0 and 400 from each respondent. Mean scores will then be calculated and discussed and the traits of interest can then be ranked in order of importance.

One of the challenges facing responsible breeders of pedigree dogs is how to balance and prioritise the different traits impacting their breeding decisions. They struggle to maintain a breed-type whilst also dealing with multiple, complex disorders and the need for dogs with good temperaments. Hedhammar et al (2011) proposed that an overall breeding programme should be developed for each breed, to enable sustainable breeding of healthy dogs, which would consider all traits of importance and take into account population structure and genetic variation. A survey of selection aims among breeders and other stakeholders, such as that undertaken in Chapter 5, could be a useful starting point for each breed. The same process could also be undertaken by breeders of other animals, such as pedigree cats, in which multiple traits without purely economic impacts must be balanced.

11.2. Genetic evaluation of health and behavioural traits

The method used to estimate heritability, genetic correlations and crossbreeding parameters for both health and behavioural traits was restricted maximum likelihood (REML), a powerful method commonly

employed in animal breeding programmes. An alternative approach for heritability and genetic correlation estimation is the Markov-Chain Monte Carlo (MCMC) Bayesian approach using Gibbs sampling. This method has been used in dog genetic studies (e.g. Hamann et al, 2003; Stock et al, 2012) but REML remains popular due to its flexibility and computational ease. Comparative studies of Bayesian and REML approaches to heritability analysis have suggested that both methods are susceptible to bias, especially in cases of high prevalence, high heritability or small sample size (Stock et al, 2007). In univariate models of binary traits, such as those used for disease conditions in Chapters 6 and 7, Gibbs sampling and REML methodology tend to produce similar estimates and the estimated breeding values (EBVs) produced tend to rank animals similarly. In small populations for traits with high heritability and low prevalence, EBVs from Gibbs sampling may be more reliable than those from REML (Stock et al, 2007). For most of the disease conditions investigated in this study, heritability estimates were low to moderate and prevalence was low to moderate thus it can be concluded that REML was a suitable and reliable method of estimation.

The type of crossbreed model used to evaluate crossbreeding parameters for the health and behavioural traits assumes that the genetic variance for each trait is broadly the same in the Labrador Retriever and Golden Retriever. Given the relatively close relationship between the two breeds, with the Labrador Retriever having been used in the early formation of the Golden Retriever breed as discussed in Chapter 2, this assumption does not seem unreasonable.

11.2.1. Health traits

Chapters 6 and 7 dealt with the genetic evaluation of health traits affecting dogs in GD's programme. In Chapter 6, univariate and bivariate linear models were used to estimate heritabilities of individual diseases, and genetic correlations between diseases, in the three breeds used by GD in the largest

numbers, the Labrador Retriever, Golden Retriever and German Shepherd Dog. For many of the health conditions studied this thesis constitutes the first attempt to quantify the genetic contribution to risk in the domestic dog. In this work, heritability estimates have been reported for the first time for panosteitis, sebaceous cysts, entropion, multifocal retinal dysplasia, Horner's syndrome, laryngeal paralysis and diabetes mellitus. These results will be of interest to the wider dog breeding community and herald the potential to select against these conditions. For those conditions previously analysed elsewhere or in other breeds, the results reported here offer validation and interesting comparisons and help shed light on whether the extent of genetic variation in conditions differs in different populations or breeds. Indeed this seems to be the case for some conditions investigated here; for example the heritability estimates for entropion were widely different in the Labrador Retriever and Golden Retriever at 0.11 (s.e. 0.04) and 0.74 (s.e. 0.08) respectively.

11.2.1.1. *Health traits in purebred dogs*

This study has shown that EBVs produced from univariate linear models of some diseases could be used in selection indices for GD's purebred Labrador Retrievers, Golden Retrievers and German Shepherd Dogs. Any disease found to be heritable could potentially be included in a selection index. On this basis atopic dermatitis, cranial cruciate ligament disease, diabetes mellitus, distichiasis, elbow dysplasia, entropion, hip dysplasia, laryngeal paralysis, multifocal retinal dysplasia, panosteitis, patellar luxation and seizures could all be included in a selection index for purebred Labrador Retrievers. However considerations of the relative impact of each condition, apparent prevalence in GD's colony and life stage of onset mean that selection against some of these conditions might be of little value. For example, laryngeal paralysis which had a heritability estimate of 0.11 (s.e. 0.04), making it eminently suitable for inclusion in a selection index on the basis of its heritability, had an apparent prevalence of only 0.03 and tends to occur after dogs have been

retired from guide dog work; thus selection against this condition would not be a priority.

In the Golden Retriever, atopic dermatitis, congenital ichthyosis, entropion, Horner's syndrome and panosteitis could potentially be included in a selection index for purebred dogs. As discussed in Chapters 3 and 6, a DNA test for the mutation which causes congenital ichthyosis has become available since this study began and is now used by GD, thus there would be little value in including this condition in a selection index.

In the German Shepherd Dog, atopic dermatitis, hip dysplasia, panosteitis and sebaceous cysts were disease conditions which might be included in a prospective selection index. However, as for the Labrador Retriever, considerations of impact, prevalence and age at onset may preclude inclusion of some of these conditions in the breed-specific selection indices. Also, as discussed previously in Chapters 6 and 7, GD may be better served by using the EBVs for hip and elbow dysplasia (based on the BVA/KC hip and elbow scheme scores) which are freely available from the Kennel Club (KC) via Mate Select. As they use information from the entire KC-registered population of each breed the EBVs will be much more accurate than those produced from GD's much smaller dataset. These external EBVs could still be combined in a selection index with other traits.

Bivariate models were not attempted for the German Shepherd Dog due to the small size of the available datasets and, although the datasets were slightly larger for the Golden Retriever, none of the bivariate models attempted in that breed produced genetic correlation estimates which were significantly larger than zero. Several bivariate models for the Labrador Retriever, the purebred breed used in the largest numbers by GD with consequently larger datasets, were successfully completed yielding genetic correlation estimates between seven pairs of disease conditions. All of the genetic correlation estimates were positive, which suggests that selecting for

one of the conditions should also assist with selection against the other condition in each pair.

A high, positive genetic correlation was found between hip and elbow dysplasia, which is in contrast to the moderate, positive genetic correlation between hip and elbow score in the BVA/KC Hip and Elbow Schemes reported by Lewis et al (2011b). This suggests that the development of clinical hip and elbow dysplasia is more genetically correlated than the radiographic score of each joint. This may reflect the fact that radiographic scoring of hips and elbows largely measures the development of secondary degenerative changes in the joints rather than the primary pathology. High, positive genetic correlations were also produced between hip and elbow dysplasia and panosteitis. Genetic correlations between these conditions had not been reported before, but as all three are developmental orthopaedic conditions in which rapid growth is thought to play a role and to which the Labrador Retriever is considered predisposed, these results are probably not surprising. One result which was surprising was the high, positive genetic correlation found between elbow dysplasia and seizures which was interesting and unexpected and may warrant further research. However, a simulation study in horses found that genetic correlations between binary traits were generally overestimated by REML (Stock et al, 2007), so some caution is advised with respect to these genetic correlation estimates.

11.2.1.2. *Crossbreed models of health traits*

Crossbreed models were undertaken in Chapter 7 to estimate heritabilities and crossbreeding parameters of diseases in the Labrador Retriever, Golden Retriever and crosses between the two breeds. Crossbreeding parameters for health traits have never been quantified before in the dog and as such will be of interest to the wider dog breeding community. Diseases for which there were more than 100 confirmed cases, and for which a statistically significant heritability estimate had been measured in at least one of the pure breeds,

were investigated further. This yielded heritability and crossbreeding parameter estimates for atopic dermatitis, cranial cruciate ligament disease, elbow dysplasia, entropion, hip dysplasia, Horner's syndrome, panosteitis and seizures. All of these conditions were found to have low to moderate heritability estimates, ranging from 0.06 (s.e. 0.02) for entropion to 0.20 (s.e. 0.04) for hip and elbow dysplasia, and therefore could potentially be included in a selection index for producing crossbred retrievers subject to consideration of the impact, prevalence and age at onset of the individual conditions. EBVs for hip and elbow dysplasia are only produced within each pure breed by the KC, thus EBVs produced by these crossbreed univariate linear models for the clinical diseases as bivariate traits may be a better option for inclusion in the crossbreed selection index.

Most of the crossbreeding parameter estimates for the disease conditions were not detectably larger than zero. It is possible that the effects observed may be larger when the breeds being crossed are less closely related than the Labrador Retriever and Golden Retriever. It would be interesting to attempt crossbreed models between the Golden Retriever and German Shepherd Dog, or the Labrador Retriever and Standard Poodle, if sufficient data accrues.

Heterotic effect estimates were negative and significantly larger than zero for elbow dysplasia, Horner's syndrome and seizures suggesting that heterosis slightly reduced the likelihood of developing any of these three conditions. Estimates of the effect of recombination loss were positive and detectably larger than zero for elbow dyplasia and hip dysplasia suggesting that recombination loss slightly increased the likelihood of developing either of these conditions. Labrador fraction effect estimates were detectably larger than zero and positive for elbow dysplasia, and negative for Horner's syndrome. This suggests that increasing Labrador fraction was associated with a greater probability of developing elbow dysplasia, but a lower probability of developing Horner's syndrome. Overall it seems that there are small health benefits of heterosis for the F1 generation but that these may be lost in the backcrosses (produced by breeding an F1 individual with one of the

pure breeds). Thus it could be advised that GD continue to produce and use large numbers of F1 crosses between the Labrador Retriever and Golden Retriever, but there may be little value (in health terms) of producing backcrosses.

11.2.2. Behavioural traits

Behavioural traits measured by GD in two different behavioural assessments were subjected to genetic evaluation. Firstly, genetic parameters relating to scores in the Canine Assessment Summary (CAS) were estimated in the purebred Labrador Retriever and Golden Retriever in Chapter 8. Subsequently crossbreeding parameters relating to CAS scores were estimated in Chapter 9. Finally heritabilities and crossbreeding parameters relating to components of the Puppy Profiling Assessment (PPA) were estimated in Chapter 10. Key findings of these investigations are discussed below.

11.2.2.1. *CAS models*

Univariate and bivariate linear mixed models were successfully used to estimate genetic and environmental parameters of, and genetic correlations between, behavioural traits assessed by CAS in GD's Labrador Retrievers and Golden Retrievers. Although the assessor effect was large in many cases, likely reflecting both the large number of assessors which undertake CAS assessments and the subjective nature of those assessments, many of the models measured heritability estimates which were detectably larger than zero.

In the Labrador Retriever, heritability estimates which were detectably larger than zero ranged from 0.02 (s.e. 0.02) for obedience at 8 months of age to 0.16 (s.e. 0.05) for body sensitivity at the first CAS assessment in advanced training. Heritability estimates were more often detectably larger than zero

at the first CAS assessment in either early or advanced training than at one of the time points in puppy walking, and no heritability estimates were detectably larger than zero for CAS elements at the first assessment in puppy walking.

Far fewer heritability estimates were detectably larger than zero in the Golden Retriever. Those which were detectably larger than zero tended to be larger in the Golden Retriever than in the Labrador Retriever, with estimates ranging from 0.07 (s.e.0.06) for obedience at the first CAS assessment in early training to 0.25 (s.e. 0.13) for eagerness at the first assessment in advanced training. Heritability estimates were most likely to be detectably larger than zero at the CAS assessment in puppy walking at 12 months of age as well as at the first CAS assessments in early and advanced training.

Comparing heritability estimates for CAS element scores with those from other studies of canine behaviour is difficult as the traits measured, sampling procedures, methods of analysis and breed tested were not the same in every case. There are also often differences in the definition of particular traits even though the same names are used to describe them (Mackenzie et al. 1985). Nevertheless, the heritability estimates in this thesis suggest that there is substantial genetic variation in many of the traits being measured by CAS and as such EBVs for these traits could be included in selection indices. As most of the heritability estimates were low, selection based on a dog's scores alone would not be very effective. Selection of breeding stock would be more efficient if based on EBVs for the desired CAS element scores, especially as the scores for most CAS elements seem to be more heritable during the training stages which dogs identified as prospective breeding stock never reach.

Bivariate models between different CAS elements were only undertaken in the Labrador Retriever as the datasets were largest in this breed. Most of the genetic correlation estimates which were detectably larger than zero were high and positive. However, three quite high negative genetic correlation

estimates were produced: between confidence and distraction at the first CAS assessment in early training, at -0.45 (s.e. 0.22); between eagerness and interaction with people at the first CAS assessment in early training, at -0.46 (s.e. 0.20); and between calmness and eagerness at the first CAS assessment in advanced training with an estimate of -0.58 (0.21). These could be problematic, as they suggest that selection for a low (desirable) score for confidence, eagerness and calmness would tend to lead to higher (less desirable) scores for distraction, interaction with people and eagerness respectively. As no dogs were withdrawn from GD's programme between 1995 and 2012 due to poor interaction with people, low eagerness or low calmness these traits, worse scores for these traits may not be particularly problematic. However, high distraction and low confidence were both given as reasons for withdrawal, accounting for 22% (1151 of 5327 dogs) and 9% (464 of 5327 dogs) respectively of dogs withdrawn from GD's programme for behavioural reasons between 1995 and 2012. Therefore the relatively high genetic correlation estimate of -0.45 (s.e. 0.22) between confidence and distraction at the first CAS assessment in early training would be a particular problem if selection was based on one of these traits. However, selection indices allow optimum selection on multiple traits and can accommodate antagonistic traits such as these so long as the magnitude and direction of the genetic correlation is accounted for correctly (Mrode, 2014).

A particularly interesting finding was the high genetic correlation estimate of 0.96 (s.e. 0.27) between aggression towards animals and aggression towards people at the first CAS assessment in early training. This suggests that scores for the CAS elements of aggression towards animals and aggression towards people share the same genetic background in the Labrador Retriever. If these CAS elements accurately measure these two behavioural traits, and if aggression towards animals and people share the same genetic background in other breeds, this would have implications to the wider community. Dogs which are bred to be aggressive towards other dogs (such as those being used

in illegal dog-fighting) would thus be likely to be aggressive towards people too with concomitant public health and safety implications.

Univariate linear mixed models were also successfully used to estimate heritabilities and crossbreeding parameters for CAS elements in GD's Labrador Retrievers, Golden Retrievers and crosses between these two breeds. This represents the first attempt to quantify the crossbreeding parameters of heterosis, recombination loss and breed effects for behavioural traits in the dog. Many more heritability estimates for CAS elements at different time points were detectably larger than zero, and with a smaller p-value, than for the single breed models, reflecting the larger size of the datasets available for use. All the estimates which were detectably larger than zero were small, ranging from 0.01 (s.e. 0.00-0.01) for many traits at different time points to 0.15 (s.e. 0.03) for distraction at the first CAS assessment in advanced training.

Most of the crossbreeding parameter estimates were not detectably larger than zero. However, seven CAS elements had small to moderate heterosis estimates which were detectably larger than zero at one or more time points of interest, with six of the estimates being negative and one being positive. These results suggest that heterosis is associated with lower (i.e. better) CAS scores for alertness, body sensitivity, distraction, interaction with people, stress resilience and suspicion but with higher (i.e. worse) CAS scores for toileting routine. The largely beneficial heterotic effects may relate to the higher success rate seen in the Labrador Retriever cross Golden Retriever compared to either pure breed. Four estimates of recombination loss effect were detectably larger than zero with all but one of these being positive. Thus it seems that recombination loss is associated with a slightly higher (i.e. worse) CAS score for body sensitivity, eagerness and stress resilience at 5 months of age and a slightly lower (i.e. better) CAS score for interaction with animals at the first assessment in advanced training. This suggests that some of the benefits in behaviour, as measured by CAS scores, seen in the F1 may be at least partially lost in the backcrosses. In terms of the estimates of

Labrador fraction, 11 were detectably larger than zero. Five CAS element scores were detrimentally affected by increasing Labrador fraction and six CAS element scores were beneficially affected by increasing Labrador fraction; overall the benefits and detrimental effects may neutralise each other.

Finally in the analysis of CAS data, repeatability models were used for two CAS elements, calmness and eagerness, to estimate permanent environmental effects. For both traits estimates of permanent environmental effects were larger than heritability estimates suggesting that the permanent environmental effects are more important than genetic influences on these traits. Thus improvement in CAS scores for calmness and eagerness may be more easily effected by attempting to modify the dogs' permanent environment, i.e. environmental effects which consistently influence the dogs' behaviour such as housing, early life influences and maternal effects, than by selection.

11.2.2.2. *PPA models*

Univariate linear models were successfully used to estimate heritability and crossbreeding parameters for the 11 components of the PPA. Nine of the 11 PPA components had heritability estimates which were detectably larger than zero, indicating that performance in these tests had an inherited element. Five components appeared to be moderately heritable with heritability estimates ranging from 0.21 (s.e. 0.07) for PPA components 2a and 5b to 0.24 (s.e. 0.09) for PPA component 1. These results mean that these components should respond to selection and EBVs for these components could potentially be included in the selection index for Labrador Retriever cross Golden Retrievers. Genetic correlations between PPA components remain to be estimated.

Most of the crossbreeding parameter estimates for PPA components were not detectably larger than zero. Exceptions to this were a small, negative estimate of heterotic effect for PPA component 6a, negative estimates of

recombination loss effect for components 2a and 7 and a positive estimate of Labrador fraction effect for PPA component 3. The PPA dataset was considerably smaller than any of the disease condition or CAS datasets used for crossbreed evaluations and may have lacked the power to detect crossbreeding effects.

The PPA looks particularly promising for GD going forward as it has been shown that several components are related to successful qualification as a working guide dog (Asher et al, 2013), and all of those components have now been found within this thesis to be heritable. Thus the inclusion of EBVs for those components in a selection index for Labrador Retriever cross Golden Retrievers should improve selection to produce puppies which are temperamentally suited to guide blind or partially sighted people. This study will also be of interest to the working dog breeding community as, although two other puppy tests have demonstrated associations with training success in working (police) dogs (Slabbert & Odendaal, 1999; Svobodová et al, 2008), the heritability of their test scores were not reported. This is the first study to report that components of a puppy test which have been shown to be related to successful qualification as a working dog are also heritable.

11.3. Limitations of the study

This study was successful in performing preliminary genetic evaluations of traits recorded by GD in their dogs and thus represents the first stage in the implementation of quantitative genetic techniques to improve the accuracy of GD's selection decisions. There are, however, caveats to the analysis and these are discussed below.

11.3.1. Data availability and quality

There were considerable problems with the data for the purposes of genetic evaluations. It is important to remember that the data have not been

collected purposely for use in genetic evaluations. However the huge amount of time which had to be spent validating the data and making them suitable for use in genetic evaluations limited the amount of time which could be spent on the actual genetic evaluations themselves. Thus it was not possible to spend a considerable amount of time comparing different models, for example.

GD's database currently represents the most extensive collection of health and behavioural phenotypic data relating to dogs linked by a pedigree file in the UK if not the world. However when exclusion criteria, necessary to prevent systematic biases, were applied to the health and behavioural datasets their size was quite dramatically reduced. For example, at most 53% (19450 of 36992 dogs) of the dogs in the health record dataset could be included in analyses. Moreover the datasets of phenotyped individuals available for each specific disease or behavioural trait were even smaller. The largest health dataset was 7656 dogs of known phenotype for the crossbreed atopic dermatitis model and the size of the largest behavioural dataset was similar with 7233 dogs having a CAS score in the crossbreed models of the first assessment in early training. These datasets are quite small for the application of quantitative genetic techniques which at least partly explains why some models were unsuccessful.

Guide Dogs Interactive (GDI) has now been superseded by a newer system, known as Guide Dogs Interactive Replacement (GDI-R), which may help reduce the occurrence of some data entry errors. GDI-R is a cloud-based customer relationship management (CRM) software application provided by Salesforce.com. One of its key features which may help to reduce the incidence of some data entry errors is its "autocomplete" feature. As a word is typed into a field, suggestions to complete the word will be offered, thus spelling mistakes and duplicated entries should be less likely to occur.

11.3.1.1. *Pedigree data*

The pedigree data available for the study were limited primarily by the accuracy of recording. Improving the pedigree was a very labour-intensive and time consuming part of the study, taking many months to complete, but was vital as correct pedigree information is paramount in a successful breeding programme. For EBVs produced using the animal model an incorrectly identified parent of one individual will affect the EBV not only of that individual but also all of their relatives (Visscher et al, 2002). Improvements were made to the pedigree with some caution as misidentification of parents in the pedigree have potentially twice the detrimental effect on genetic gain as missing pedigree information (Woolliams, 2006).

GD sometimes has problems obtaining sufficient, accurate pedigree information when dogs are purchased from external breeders (Adams, W., GD, personal communication, 2013). However, five generation pedigrees are available from the KC for anyone who knows the KC-registered name of a dog; it would be advisable that such information should be obtained for every dog for which depth of pedigree supplied is insufficient. It has perhaps not been appreciated before how important it is to have at least this depth of pedigree, which will illuminate cryptic relatedness between dogs, for every dog which GD purchases. This is particularly true as all dogs purchased by GD are considered prospective breeding stock. If such dogs are used for breeding with only one or two generations of pedigree information available GD may inadvertently tend to increase the rate of inbreeding in the colony by mating dogs which are more closely related than they appear.

Pedigree data from purchased dogs has been entered into GD's database by a combination of staff and volunteer workers, and this is the source of many of the errors in the data. Multiple, perhaps poorly trained, individuals entering data has allowed inconsistencies in spelling of names and use of honorifics to

accumulate in the pedigree file. Guidelines must be put in place for how pedigree data are entered in future, such that only the KC-registered name, and none of the honorifics, is entered. More effort must also be taken to ensure that the pedigree file is thoroughly checked to ensure that a dog is not already present in the file before a new entry for it is created. It is hoped that the "autocomplete" feature of GDI-R may particularly help to address this issue.

The amended and improved pedigree file, together with detailed description of all the steps undertaken to produce it, will be provided to GD so that these improvements can be incorporated into their pedigree file. The process may need to be repeated for the two and half years of pedigree information that have accumulated in the meantime.

11.3.1.2. *Health data*

The health data available for use in genetic analyses were limited by two main phenomena: poor accuracy of coding of health records and the large number of dogs which were lost to follow-up when they left GD's programme. The poor accuracy of coding of health records necessitated a huge volume of work to gather as many cases as possible of the health conditions under investigation. This work required careful veterinary interpretation of large amounts of free text which was a slow and laborious process. The accuracy of coding has improved since 2009 (Adams, W., GD, personal communication, 2011) so it is hoped that less time and effort will be necessary to collate cases in this way going forward.

The large number of dogs which were lost to follow-up when they left GD's programme meant that such dogs had to be discounted from any analyses as their disease status for any condition was unknown. This decreased the size of the dataset for the conditions which were investigated thus decreasing their power for quantifying genetic variation. As discussed in Chapter 6, some international guide dog organisations send annual health surveys to all

owners of dogs bred by them which have been removed from their programmes or which have retired. It is recommended that GD consider implementing such a system to enable this valuable health information to be collected.

11.3.1.3. *Behavioural data*

The behavioural datasets did require considerable validation and editing to make them suitable for use in genetic evaluations, but not to the same extent as the pedigree and health data. However, their main limitations were related to the large number of assessors for the CAS assessments and the poor recording of assessors for the PPA. The assessor effect was often larger than the heritability estimate for CAS components, and the heritability estimates may have been reduced by the amount of "noise" in the datasets due to the large number of assessors. Reducing the number of assessors, and attempting to ensure inter-observer reliability, would be of benefit if CAS scores are to be used in genetic evaluations in future.

For the PPA the poor recording of assessor, with assessor identification missing for 41% (866 of 2127 puppies) of puppies which underwent the PPA, meant that this variable could not even be included in the models. Going forward, assessor must be recorded accurately for each puppy that undergoes the PPA. When more data has accrued, puppies with assessor identification missing could be excluded from the dataset and the models re-run to estimate assessor effect. The size of the PPA datasets was limited as it had only recently begun to be applied to all puppies at GD. However, as more than one thousand puppies are undergoing the PPA every year data will accumulate quickly over time.

11.4. Genomic selection

Novel technologies in animal breeding may have potential applications in GD's breeding programme. Genomic selection (GS) is a relatively new method devised by Meuwissen et al (2001) to estimate breeding values using DNA-based information. GS involves genotyping and phenotyping a large "training dataset" sample of the population, so that the effects of each single-nucleotide polymorphism (SNP) in linkage disequilibrium (LD) with a quantitative trait locus (QTL) are estimated. Following this, genomic breeding values (GEBVs) for young animals from the breeding populations can be produced using only genotypic data (Meuwissen et al, 2001), although re-estimation of the SNP effects may be needed every few generations (Sánchez-Molano et al, 2014). Large scale genotyping is required, with associated financial costs, and the size of the reference population needed would require some time to establish. These factors are both inhibitions to the introduction of the technology. Sánchez-Molano et al (2014) estimated that a reference population of 1500-2000 dogs would be necessary to give GEBVs with an accuracy of 0.50 in a breed with an effective population size of approximately 100 such as the Labrador Retriever.

Compared to livestock there are distinctions in the life and breeding programme of guide dogs which mean that genomic technologies could potentially have great impact. The neutering of a large proportion of the population before many selection traits become available, the fact that some of the primary selection traits such as success in guide dog training only become available later in life and the difficulty in measuring traits all limit the genetic progress that can be made. Genomic selection could overcome many of these problems, increasing genetic gain whilst maintaining genetic variation and decreasing the deleterious effects of inbreeding. Another key advantage of GEBVs is that they distinguish between unphenotyped littermates thus allowing selection within families at a very young age (Sánchez-Molano et al, 2014).

Sánchez-Molano et al (2014) compared genomic and phenotypic selection against hip dysplasia as measured by the BVA/KC Hip Scheme in a simulated populated. Genomic selection was show to give greater genetic progress than the phenotypic scheme. However, as in dog populations relatively few progeny are derived from any given parent, compared to the huge numbers sired by dairy bulls for example, it may not be possible to evaluate entirely genomic EBVs for dog with great accuracy (Goddard & Hayes, 2009). Therefore the collection, storage, cleaning and processing of phenotypic and pedigree information will remain necessary for the foreseeable future (Wilson & Wade, 2012).

11.5. Following work

Several key pieces of work remain to be undertaken. Genetic correlations between CAS elements in the crossbreed models must be estimated. Genetic correlations between PPA elements also remain to be estimated. Genetic correlations between the health and behavioural traits identified as potential components of selection indices must also be estimated. All three will be necessary to undertake before selection indices are created to be sure of the impact of selecting on a particular trait on different traits.

Genetic correlations between health and behavioural traits have not been extensively explored in the dog, therefore the results of bivariate models between health and behavioural traits will be of particular interest. Several authors have reported genetic correlations between hip dysplasia and behavioural traits. Bartlett (1976, cited in Mackenzie et al, 1986) estimated genetic correlations between hip dysplasia and several behavioural traits measured in American guide dogs; only that between hip dysplasia and ear sensitivity and was found to be significant, with a positive estimate of 0.52. Subsequently, Mackenzie et al (1985) analysed US Army Biosensor Project data relating to 575 German Shepherd Dogs between 1968 and 1976 using REML methodology and estimated a genetic correlation between hip dysplasia

and temperament scores of -0.33. No other estimates of genetic correlations between health and behavioural traits in dogs were found in the literature.

The final main piece of work to be undertaken is a thorough population structure analysis of GD's colony. Inbreeding coefficients were estimated for all individuals in the pedigree but the lack of dates of birth in the pedigree made it impossible to estimate the rate of inbreeding. Dates of birth are available elsewhere in GDI for dogs bred by GD, but not for most other dogs in the pedigree. It may be possible to augment GD's pedigrees with dates of birth for KC-registered dogs in the pedigree using the KC's own pedigree files; this will be investigated. If this can be achieved then population structure analysis will be undertaken as it is important at least to assess the rate of inbreeding in GD's colony. Because EBVs predicted by best linear unbiased prediction (BLUP) use information from relatives, the correlation among EBVs of relatives tends to be high and thus the probability of co-selecting related animals is increased (Verrier et al, 1993). For example, increased rates of inbreeding have been shown in Danish dairy cattle in which BLUP breeding values are used (Sørensen et al, 2005), although it must be remembered that the number of progeny per dairy sire far exceeds that in dogs. Thus when GD start using BLUP-based selection indices to aid their selection decisions, more active management of genetic diversity may be needed.

11.6. Conclusion

All of the aims of this study were fulfilled. Breeding priorities at GD, the largest breeder of assistance dogs in the world, have been identified although additional work is underway to provide further detail in this area. Heritability estimates have been reported for disease conditions in the three breeds used by GD in the largest numbers, and genetic correlations between disease conditions have been estimated in the Labrador Retriever. Crossbreeding parameters for disease conditions, which have never been investigated in the dog, have also been estimated. Heritability estimates and crossbreeding

parameters have been produced for components of two different behavioural assessments (CAS and PPA) used by GD at different ages, alongside estimates of permanent environmental effects for two traits measured in CAS. This work comprised the largest and most comprehensive genetic study performed to date on the health and temperament of dogs.

This study has met its objectives and provides a platform for the implementation of quantitative genetic techniques to improve the accuracy of GD's selection decisions. Many of the findings of the study will also be of interest to the wider dog breeding community.

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APPENDIX 1: BREEDS IN THE PEDIGREE FILE

Table A1 Number of dogs of each breed and cross in the raw pedigree file.
The breed of sire is listed first.

Breed	Number	Percentage
Akita	1	0.002
Alaskan Malamute	4	0.008
Australian Shepherd	15	0.03
Bernese Mountain Dog	9	0.02
Border Collie (BC)	191	0.39
Border Collie x German Shepherd Dog	2	0.004
Border Collie x Golden Retriever	101	0.21
Border Collie x Labrador	9	0.02
Boxer	24	0.05
Chesapeake Bay Retriever	6	0.01
Crossbreed	34	0.07
Curly Coated Retriever (CCR)	71	0.14
Curly Coated Retriever x Golden Retriever	20	0.04
Curly Coated Retriever x Labrador	187	0.38
(CCR x LR) x Labrador	7	0.40
Dalmatian cross	1	0.002
Dobermann	2	0.004
Flat Coated Retriever	134	0.27
Flat Coated Retriever x Golden Retriever	19	0.04
Flat Coated Retriever x Labrador	8	0.02
German Shepherd Dog (GSD)	2659	5.41
GSD x Bernese Mountain Dog	2	0.004
German Shepherd Dog x Golden Retriever	28	0.06
German Shepherd Dog x Labrador	27	0.05
Golden Retriever (GR)	5131	10.43

Golden Retriever x Border Collie	173	0.35
Golden Retriever x (BC x GR)	1	0.002
Golden Retriever x Flat Coated Retriever	304	0.62
Golden Retriever x German Shepherd Dog	276	0.56
Golden Retriever x (GR x LR)	355	0.72
Golden Retriever x Labrador	8102	16.47
Golden Retriever x (LR x GR)	241	0.49
Hovawart	2	0.004
Irish Water Spaniel	2	0.004
Irish Water Spaniel x Golden Retriever	9	0.02
Irish Water Spaniel x Labrador	27	0.05
Italian Spinone	1	0.002
Italian Spinone x Labrador	15	0.03
Labradoodle	16	0.03
Labradoodle x labradoodle	3	0.006
Labrador (LR)	14775	30.04
Labrador x Border Collie	26	0.05
Labrador x Curly Coated Retriever	133	0.27
Labrador x Golden Retriever	2490	5.06
Labrador x (GR x LR)	1694	3.44
Labrador x (LR x GR)	442	0.90
(LR x GR) x Labrador	66	0.13
(LR x GR) x (LR x GR)	18	0.04
Leonberger	13	0.03
Standard Poodle	25	0.05
Standard Poodle x Golden Retriever	1	0.002
Standard Poodle x Labrador	64	0.13
Tervueren	2	0.004
Weimaraner	2	0.004
Unknown breed – migration purposes only	11219	22.81
Grand Total	49189	100

APPENDIX 2: SURVEY FORM

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We would like to know more about the traits you want your breeding stock to have. We would also like to know how important you consider each of your selected traits to be when selecting new breeding stock. In order to assess this we would like you to allocate 100 points to the traits listed below. You can allocate your 100 points in any way that you like but the total must not exceed 100. The number of points allocated to each trait should reflect how important a selection criterion the trait is to you.

	Labrador Retriever	Golden Retriever	Lab X G. Ret	GSD
Appearance				
Breed conformation and movement	6	6	6	8
Height	4	4	4	4
Weight	4	4	4	4
Coat length	2	5	5	4
Cosmetic appearance	2	2	2	2
Other (please specify)				
Temperament				
Compliance	8	8	8	7
Environmental awareness	8	7	7	7
Willingness	8	7	7	7
Confidence	8	7	7	7
Lack of aggressive behaviour	8	8	8	8
Other (please specify)				
Health				
Acceptable hip score	8	7	7	8
Acceptable score elbow	8	7	7	8
Acceptable shoulder "score"	8	7	7	8
Clear eye examination	8	7	7	8
Clear heart examination	2	7	7	2
Other (please specify) skin disease	8	7	7	8
Other (please specify)				
TOTAL	**100**	**100**	**100**	**100**

If you have any questions, please contact Katy Evans on katy.evans@aht.org.uk .

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Figure A1 Example survey form completed by one of seven respondents for the survey of selection aims.

APPENDIX 3: BREEDS IN THE WITHDRAWAL DATASET

Table A2 Number of dogs of each breed and cross which were withdrawn from GD's programme between 1995 and 2012. The breed of sire is listed first.

Breed	Number	Percentage
Australian Shepherd Dog	6	0.07
Bernese Mountain Dog	2	0.02
Border Collie (BC)	20	0.24
Border Collie x German Shepherd Dog	2	0.02
Border Collie x Golden Retriever	33	0.39
Border Collie x Labrador	4	0.05
Chesapeake Bay Retriever	4	0.05
Crossbreed	8	0.09
Curly Coated Retriever	34	0.40
Curly Coated Retriever x Golden Retriever	7	0.08
Curly Coated Retriever x Labrador	49	0.58
Flat Coated Retriever	34	0.40
Flat Coated Retriever x Golden Retriever	8	0.09
Flat Coated Retriever x Labrador	4	0.05
German Shepherd Dog	627	7.44
German Shepherd Dog x Golden Retriever	5	0.06
German Shepherd Dog x Labrador	11	0.13
Golden Retriever (GR)	1121	13.29
Golden Retriever x Border Collie	27	0.32
Golden Retriever x (BC x GR)	1	0.01
Golden Retriever x Flat Coated Retriever	100	1.19
Golden Retriever x German Shepherd Dog	84	1.00
Golden Retriever x (GR x LR)	24	0.28

Golden Retriever x Labrador	1896	22.49
Golden Retriever x (LR x GR)	5	0.06
Irish Water Spaniel	1	0.01
Irish Water Spaniel x Golden Retriever	4	0.05
Irish Water Spaniel x Labrador	14	0.17
Italian Spinone x Labrador	7	0.08
Labradoodle	2	0.02
Labradoodle x labradoodle	2	0.02
Labrador (LR)	3303	39.17
Labrador x Border Collie	6	0.11
Labrador x Curly Coated Retriever	24	0.28
Labrador x German Shepherd Dog	2	0.02
Labrador x Golden Retriever	514	6.10
Labrador x (GR x LR)	364	4.32
Labrador x (LR x GR)	45	0.53
Leonberger	6	0.07
Standard Poodle	13	0.15
Standard Poodle x Labrador	13	0.15
Tervueren	1	0.01
Weimaraner	2	0.02
Grand Total	8432	100

APPENDIX 4: HEALTH REASONS FOR WITHDRAWAL

Table A3 Reasons for withdrawal for the 2257 dogs withdrawn for health reasons between 1995 and 2012.

Condition	Number	Percentage
Atopic dermatitis or allergic skin disease	407	18.03
Hip dysplasia	353	15.64
Elbow dysplasia	328	14.53
Epilepsy or seizures	96	4.25
Cataract	84	3.72
Cancer	58	2.57
Other forelimb lameness	55	2.44
Death (cause unspecified)	47	2.08
Other disorder of skin	46	2.04
Other musculoskeletal disorder	44	1.95
Trauma or accidental death	43	1.91
Other ophthalmological condition	42	1.86
Arthritis	38	1.68
General health deterioration	33	1.46
Osteochondritis dissecans	32	1.42
Other gastrointestinal disorder	28	1.24
Cruciate ligament disease	27	1.20
Geographic retinal dysplasia	27	1.20
Other disorder of cardiovascular system	26	1.15
Patellar luxation	23	1.02
Urinary incontinence	23	1.02
Physical appearance	21	0.93
Limb deformity	15	0.66
Disorder of spine	14	0.62

Other hindlimb lameness	14	0.62
Respiratory condition	14	0.62
Other neurological condition	13	0.58
Chronic ear disease	12	0.53
Colitis	12	0.53
Discoid lupus erythematosus	12	0.53
Disorder of liver	12	0.53
Multifocal retinal dysplasia	12	0.53
Poor conformation	12	0.53
Renal failure	12	0.53
Ventricular arrhythmia	11	0.49
Aortic or subaortic stenosis	10	0.44
Other	10	0.44
Other autoimmune disease	10	0.44
Congenital disorder of palate	9	0.40
Congenital intrahepatic portosystemic shunt	9	0.40
Deafness	9	0.40
Exocrine pancreatic insufficiency	9	0.40
Infectious disease	9	0.40
Congenital ichthyosis	9	0.40
Travel sickness	8	0.35
Autoimmune haemolytic anaemia	7	0.31
Bacterial overgrowth syndrome	7	0.31
Megaoesophagus	7	0.31
Other congenital abnormalities	7	0.31
Intestinal intussusception	6	0.27
Congenital spinal abnormalities	5	0.22
Panosteitis	5	0.22
Other urological condition	5	0.22
Amputation	4	0.18
Congenital abnormalities of brain	4	0.18

Congenital renal disease	4	0.18
Diabetes insipidus	4	0.18
Exercise-induced collapse	4	0.18
Heart murmur	4	0.18
Hypoadrenocorticism	4	0.18
Hypoplasia of optic nerve	4	0.18
Old age	4	0.18
Retinal degeneration	4	0.18
Diabetes mellitus	3	0.13
Epiphora	3	0.13
Gastric dilatation-volvulus syndrome	3	0.13
Generalised progressive retinal atrophy	3	0.13
Pyrexia of unknown origin	3	0.13
Retinopathy	3	0.13
Short radius syndrome	3	0.13
Other Endocrinological disorder	3	0.13
Grand Total	2257	100

Table A4 Reasons for withdrawal for health by body system affected for the 2257 dogs withdrawn for health reasons between 1995 and 2012 in descending order for the six breeds and crosses under consideration.

Withdrawal reason	GSD	GR	GR x (GR x L)	GR x LR	LR	LR x (LR x GR)	Total
Musculoskeletal	86	99	2	225	529	34	975
Dermatological	46	78	1	164	167	18	474
Ophthalmological	18	39	0	62	52	11	182
Neurological	5	18	1	40	52	4	120
Gastrointestinal	17	11	0	19	32	3	82
Cancer	5	11	1	22	16	2	57
Cardiovascular	5	12	0	18	17	3	55
Death (cause unspecified)	5	8	2	9	22	1	47
Urological	10	7	0	12	15	0	44

Trauma or accidental death	4	8	1	16	10	4	43
Other	1	7	1	10	15	0	34
General health deterioration	2	8	1	5	15	2	33
Hepatic	0	5	0	6	11	1	23
Aural	0	5	0	5	8	3	21
Physical appearance	1	6	0	3	11	0	21
Autoimmune	0	3	1	8	4	1	17
Endocrinological	0	0	0	6	6	3	15
Respiratory	0	0	0	3	11	0	14
Total	205	325	11	633	993	90	2257

APPENDIX 5: BEHAVIOURAL REASONS FOR WITHDRAWAL

Table A5 Reasons for withdrawal for the 5327 dogs withdrawn for behavioural reasons between 1995 and 2012

Withdrawal reason	Number	Percentage
Aggression towards animals	191	3.59
Aggression towards people	562	10.55
Attentiveness – low handler focus	247	4.64
Attentiveness – low task focus	449	8.43
Body sensitivity – high	84	1.58
Confidence – low adaptability	311	5.84
Confidence – low decision-making	153	2.87
Distraction – general	312	5.86
Distraction – people	16	0.30
Distraction – scents or sounds	65	1.22
Distraction – animals or birds	680	12.77
Distraction – food	77	1.45
Social behaviour – coprophagia	82	1.54
Social behaviour – destructive	43	0.81
Social behaviour – noisy when left	8	0.15
Social behaviour – boisterous/hyperactive	99	1.86
Social behaviour – scavenger/scrounger	53	0.99
Social behaviour – not clean in the house	71	1.33
Stress resilience – low	610	11.45
Suspicion – animals	18	0.34
Suspicion – general	348	6.53
Suspicion – objects	99	1.86
Suspicion – people	225	4.22

Suspicion – scents or sounds	142	2.67
Unacceptable post-qualification habits	42	0.79
Willingness – low	340	6.38
Total	5327	100

Table A6 Reasons for withdrawal for the 5327 dogs withdrawn for behavioural reasons between 1995 and 2012 in descending order for the six breeds and crosses under consideration.

Withdrawal reason	GSD	GR	GR x (GR x LR)	GR x LR	LR	LR x (LR x GR)	Total
Distraction – high	113	76	0	329	577	55	1150
Suspicion – high	81	241	3	233	222	52	832
Attentiveness – low	53	70	1	186	356	30	696
Stress resilience - low	21	119	2	206	233	29	610
Aggression towards people	64	85	2	200	178	33	562
Confidence – low	23	62	2	167	184	26	464
Social behaviour - unacceptable	15	19	0	119	176	27	356
Willingness - low	4	64	2	102	150	18	340
Aggression towards animals	31	12	0	68	64	16	191
Body sensitivity - high	0	11	4	31	27	11	84

Unacceptable post-qualification habits	4	2	0	15	18	3	42
Total	409	761	16	1656	2185	300	5327

APPENDIX 6: BREEDS IN THE HEALTH DATASET

Table A5 Number of dogs of each breed and cross in the health dataset. The breed of sire is listed first.

Breed	Number	Percentage
Australian Shepherd Dog	10	0.05
Bernese Mountain Dog	3	0.01
Border Collie (BC)	30	0.15
Border Collie x German Shepherd Dog	1	0.005
Border Collie x Golden Retriever	71	0.34
Border Collie x Labrador	8	0.04
Boxer	18	0.09
Chesapeake Bay Retriever	4	0.02
Crossbreed	8	0.04
Curly Coated Retriever	31	0.15
Curly Coated Retriever x Golden Retriever	20	0.10
Curly Coated Retriever x Labrador	135	0.65
Flat Coated Retriever	74	0.36
Flat Coated Retriever x Golden Retriever	19	0.09
Flat Coated Retriever x Labrador	8	0.04
German Shepherd Dog	1119	5.41
German Shepherd Dog x Golden Retriever	16	0.08
German Shepherd Dog x Labrador	25	0.12
Golden Retriever (GR)	2345	11.35
Golden Retriever x Border Collie	42	0.20
Golden Retriever x (BC x GR)	1	0.005
Golden Retriever x Flat Coated Retriever	293	1.42
Golden Retriever x German Shepherd Dog	252	1.22
Golden Retriever x (GR x LR)	42	0.20
Golden Retriever x Labrador	5801	28.07

Golden Retriever x (LR x GR)	5	0.06
Irish Water Spaniel	2	0.01
Irish Water Spaniel x Golden Retriever	9	0.04
Irish Water Spaniel x Labrador	27	0.13
Italian Spinone	1	0.005
Italian Spinone x Labrador	1	0.005
Labradoodle	12	0.06
Labradoodle x labradoodle	2	0.01
Labrador (LR)	7264	35.15
Labrador x Border Collie	5	0.06
Labrador x Curly Coated Retriever	41	0.20
Labrador x Golden Retriever	1470	7.11
Labrador x (GR x LR)	968	4.68
Labrador x (LR x GR)	114	0.55
Leonberger	11	0.05
Standard Poodle	22	0.11
Standard Poodle x Golden Retriever	1	0.005
Standard Poodle x Labrador	64	0.31
Tervueren	2	0.01
Weimaraner	2	0.01
Unknown breed – migration purposes only	259	1.25
Grand Total	8432	100

APPENDIX 7: YEAR OF BIRTH AND AGE CRITERIA

Table A6 Year of birth and age criteria for cases and non-cases for disease conditions

Condition	
Atopic dermatitis	Records <1 and ≥3, born 1995 to 2009
Chronic degenerative radiculomyelopathy	Records ≤3 and ≥8, born 1995 to 2004
Congenital ichthyosis	Records <1 and ≥4, born 1995 to 2008
Cranial cruciate ligament disease	Records <1 and ≥8, born 1995 to 2004
Diabetes mellitus	Records <1 and ≥8, born 1995 to 2004
Distichiasis	Records <1 and ≥3, born 1995 to 2009
Ectropion	Records <1 and ≥3, born 1995 to 2009
Elbow dysplasia	Records <1 and ≥8, born 1995 to 2004
Entropion	Records <1 and ≥3, born 1995 to 2009
Exocrine pancreatic insufficiency	Records ≤2 and ≥8, born 1995 to 2004
Hip dysplasia	Records <1 and ≥8, born 1995 to 2004
Histiocytoma	Records <1 and ≥8, born 1995 to 2004
Horner's syndrome	Records ≤3 and ≥8, born 1995 to 2004
Hypothyroidism	Records <1 and ≥8, born 1995 to 2004
Juvenile cellulitis	Records <1 and ≥3, born 1995 to 2009
Laryngeal paralysis	Records ≤3 and ≥8, born 1995 to 2004
Mast cell tumour	Records <1 and ≥8, born 1995 to 2004
Multifocal retinal dysplasia	Records <1 and ≥4, born 1995 to 2008
Pancreatitis	Records ≤3 and ≥8, born 1995 to 2004
Panosteitis	Records <1 and ≥3, born 1995 to 2009
Patellar luxation	Records <1 and ≥8, born 1995 to 2004
Posterior polar subcapsular cataract	Records <1 and ≥8, born 1995 to 2004
Renal failure	Records <1 and ≥8, born 1995 to 2004
Sebaceous cyst	Records <1 and ≥8, born 1995 to 2004

Seizures	Records <1 and ≥8, born 1995 to 2004
Spondylosis	Records ≤3 and ≥8, born 1995 to 2004
Umbilical hernia	Records <1 and ≥2, born 1995 to 2010

APPENDIX 8: BREEDS IN THE CAS DATASET

Table A7 Number of dogs of each breed and cross in the CAS dataset of 11709 dogs. The breed of sire is listed first.

Breed	Number	Percentage
Border Collie	9	0.08
Border Collie x Golden Retriever	49	4.18
Border Collie x Labrador	8	0.07
Curly Coated Retriever	24	0.20
Curly Coated Retriever x Golden Retriever	20	0.17
Curly Coated Retriever x Labrador	75	0.64
Flat Coated Retriever	55	0.47
Flat Coated Retriever x Golden Retriever	19	0.16
Flat Coated Retriever x Labrador	8	0.07
German Shepherd Dog	643	5.49
German Shepherd Dog x Golden Retriever	28	0.24
German Shepherd Dog x Labrador	12	0.10
Golden Retriever	1217	10.39
Golden Retriever x Flat Coated Retriever	264	2.25
Golden Retriever x German Shepherd Dog	255	2.18
Golden Retriever x Golden Retriever*	36	0.31
Golden Retriever x Labrador	3405	29.08
Labradoodle	1	0.01
Labradoodle x labradoodle	2	0.02
Labrador	3541	30.24
Labrador x Border Collie	1	0.01
Labrador x Curly Coated Retriever	42	0.36
Labrador x German Shepherd Dog	8	0.07
Labrador x Golden Retriever	862	7.36

Labrador x Golden Retriever*	364	7.96
Labrador x Labrador*	105	0.90
Standard Poodle	15	0.13
Standard Poodle x Labrador	73	0.62
Grand Total	8432	100

APPENDIX 9: CAS DEFINITIONS AND SCORING

The following descriptions are adapted from GD's internal guidelines for completing CAS assessments.

1. Aggression towards animals

1.1 Definition

This CAS element refers to the dog's threatening (growling, posturing, snapping) or hostile (biting) behaviour towards animals.

1.2 Scoring

1. Has not displayed aggression in any form.
2. Has displayed mild threatening behaviour towards animals.
3. Has displayed intense threatening behaviour towards animals.
4. Has displayed frequent, intense threatening behaviour or hostile behaviour towards animals.

2. Aggression towards people

2.1 Definition

This CAS element refers to the dog's threatening (growling, posturing, snapping) or hostile (biting) behaviour towards people.

2.2 Scoring

5. Has not displayed aggression in any form.
6. Has displayed mild threatening behaviour towards animals.
7. Has displayed intense threatening behaviour towards animals.
8. Has displayed frequent, intense threatening behaviour or hostile behaviour towards animals.

3. Attentiveness

3.1 Definition

Attentiveness describes the dog's capacity to focus on the handler when required and to concentrate on the training activity or guiding task.

3.2 Scoring

1. Focuses on the handler when required in any environment.
2. Concentrates on the activity or task in any environment. Usually focuses on the handler when required in most environments. Usually concentrates on the activity or task in most environments.
3. Occasionally focuses on the handler when required but variable in some environments. Occasionally concentrates on the activity or task but variable in some environments.
4. Seldom focuses on the handler. Seldom focuses on the activity or task.

4. Behaviour when left

4.1 Definition

This CAS element refers to the dog's noisiness and destructiveness when left alone in a familiar environment for a period of up to 3 hours.

4.2 Scoring

1. Quiet and non-destructive, no confinement needed when left.
2. Occasionally noisy or destructive but no confinement required.
3. Frequently noisy or destructive or some confinement required.
4. Noisy or destructive at any time and/or confinement required.

5. Behaviour on transport

5.1 Definition

This refers to the dog's confidence when travelling on common forms of transport, i.e. car, bus, train.

5.2. Scoring

1. Confident and relaxed on all forms of transport.
2. Confident and relaxed on some forms of transport.
3. Frequently displays moderate anxiety when travelling on most forms of transport.
4. Consistently displays moderate to high levels of anxiety on all forms of transport.

6. Body sensitivity

6.1 Definition

This CAS element refers to the dog's physical acceptance of being in close proximity to or in contact with people or handler, equipment and objects or features within the environment

6.2 Scoring

1. At a level that does not have an effect on the dog in any of the above aspects.
2. Usually at a level that does not seriously affect the dog in any of the above aspects.
3. Occasionally at a level which adversely affects the dog in some of the above aspects.
4. Consistently at a level which adversely affects the dog in some or all of the above aspects.

7. Calmness

7.1 Definition

This CAS element describes the extent to which the dog physically reacts to stimuli within the environment or social situation (excluding people and animals).

7.2 Scoring

1. Rarely displays excitability. Remains calm and relaxed in all situations.

2. Occasionally displays excitability. Remains calm and relaxed in most situations.
3. Frequently displays excitability. Remains calm and relaxed in some situations.
4. Frequently displays a level of excitability in all situations. Rarely remains calm and relaxed in any situations.

8. Confidence

8.1 Definition

This CAS element refers to the relaxed and positive manner in which the dog acts in a variety of environments, when changing routines and when participating in training activities. In early and advanced training it also refers to the confidence the dog displays when making decisions and performing tasks.

8.2 Scoring

1. Always acts confidently in all of the above aspects.
2. Usually acts confidently in all or most of the above aspects.
3. Occasionally acts confidently in some of the above aspects.
4. Seldom acts confidently in any of the above aspects.

9. Distraction

9.1. Definition

Distraction refers to the degree to which the dog focuses on the stimuli within the environment that interfere with its attention to the handler, training activity or guiding task.

9.2. Scoring

1. Seldom displays any distraction and its attention to the handler, activity or task is easily regained.

2. Occasionally displays a moderate level of distraction but its attention to the handler, activity or task is easily regained.
3. Occasionally displays a moderate to high level of distraction that interferes with its attention to the handler, activity or task.
4. Frequently displays a high level of distraction that significantly interferes with its attention to the handler, activity or task.

10. Eagerness

10.1 Definition

This CAS element rates the dog's eagerness to take part in training exercises and activities and willingness to perform the guiding role.

10.2 Scoring

1. Always willing to take part or perform in any environment. Easily motivated and requires little or no effort to sustain its willingness.
2. Usually eager to take part or perform in any environment. Easily motivated and requires moderate effort to sustain its willingness.
3. Usually eager to take part or perform but only in some environments.
4. Requires effort to motivate and sustain its willingness. Seldom eager to take part or perform in any environment. Difficult to motivate and sustain its willingness.

11. Interaction with animals

11.1 Definition

This CAS element refers to the dog's reaction to and interest in other animals.

11.2 Scoring

1. Displays a relaxed, pleasurable level of interaction.
2. Displays briefly some excitement or tolerant level of interaction.

3. Displays sustained moderate to high excitability or displays mild concern or submission.
4. Displays persistent overreaction and extreme excited interest or intolerance.

12. Interaction with people

12.1 Definition

This CAS element refers to the dog's responsiveness, interest in and cooperation with humans.

12.2 Scoring

1. Displays a relaxed and pleasurable level of interaction.
2. Briefly displays some excitement without jumping, mouthing, vocal behaviour or displays mild disinterest.
3. Displays sustained moderate to high excitability (e.g. jumping, mouthing, vocalisation) or displays moderate disinterest.
4. Displays persistent overreaction and extreme excited interest or displays no interest at all.

13. Obedience

13.1 Definition

Obedience refers to the dog's responsiveness to standard commands, including recall.

13.2 Scoring

1. Responsive to all commands in all situations on and off lead.
2. Occasionally requires reinforcement in some situations in some environments and situations off lead.
3. Inconsistent response, often requires reinforcement in some situation or environments on and off lead.

4. Unacceptable obedience responses in any situation or environment on and off lead.

14. Stress resilience

14.1 Definition

Stress resilience describes the dog's ability to cope with the pressures associated with the training experience or performing the guiding task and with working in all environments.

14.2 Scoring

1. Does not display a level of anxiety that adversely affects its learning, behaviour, performance and adaptability.
2. Occasionally displays a level of anxiety that adversely affects its learning, behaviour, performance and adaptability.
3. Occasionally displays a high level of anxiety that adversely affects its learning, behaviour, performance and adaptability.
4. Frequently displays a high level of anxiety that adversely affects its learning, behaviour, performance and adaptability.

15. Suspicion

15.1 Definition

The CAS element of suspicion refers to the degree of anxiety the dog displays towards objects, people, animals, sounds and scents.

15.2 Scoring

1. Seldom displays anxious reactions to objects etc. Recovers immediately and it does not adversely affect its performance.
2. Occasionally displays anxious reactions to specific objects etc. Recovers immediately and it does not adversely affect its performance.

3. Occasionally displays anxious reactions to specific objects etc. Recovers slowly and it does adversely affect its performance.
4. Consistently displays intense anxious reactions to a range of objects etc. Recovers slowly and it does considerably affect its performance.

16. Toileting routine

16.1 Definition

This CAS element refers to the dog's cleanliness in both social and working situations.

16.2 Scoring

1. Appropriate toileting pattern, including going on command.
2. Toileting pattern not ideal (may be acceptable for the pup's age) but normally indicates appropriately.
3. Toileting pattern is unpredictable with inadequate indication.
4. No established toileting pattern or toileting habits are unacceptable.

17. Puppy walking task acquisition elements

17.1 Definitions

The three tasks which pups are expected to learn during puppy walking are correct handler position in quiet and busy areas and speed control.

17.2 Scoring

1. The pup is consistently responding to the handler's commands and displays the desired behaviour with minimal support.
2. The pup is frequently responding to the handler's commands and displays the desired behaviour with moderate support.
3. The pup is frequently responding to the handler's commands and displays the desired behaviour with moderate to full support.
4. The pup is beginning to respond to the handler's commands and displays the desired behaviour with full support.

18. Skills acquisition for dogs in training

18.1 Definitions

The skills which dogs need to master during early and advanced training are kerb work, locating objectives, on/off kerb work, right shoulder work, straight-line work and traffic.

18.2. Scoring

1. Consistently takes actions/decisions which achieve the desired behaviour across all environments without handler support or with active interference.
2. Consistently takes actions/decisions which achieve the desired behaviour in more demanding environments with minimal handler support or moderate active interference.
3. Consistently takes actions/decisions which achieve the desired behaviour with minimal to moderate handler support or active interference.
4. Consistently takes actions/decisions which achieve the desired behaviour with moderate handler support.
5. Consistently take actions/decisions which approximate to the desired behaviour with moderate to full handler support.
6. Performs the desired behaviour with full support.

APPENDIX 10: DISTRIBUTIONS OF SCORES FOR THE CAS TRAIT CALMNESS

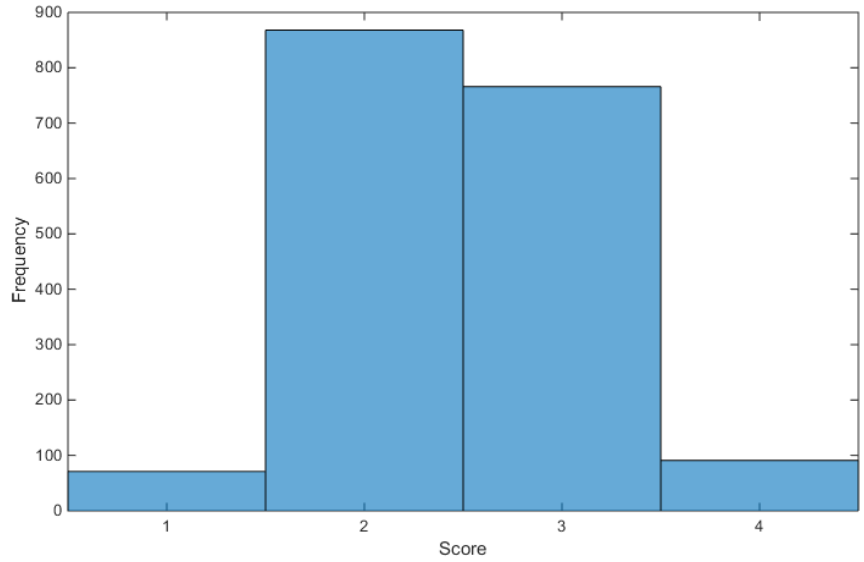

Figure A2 Histogram showing distribution of scores for calmness at the first CAS assessment in puppy walking.

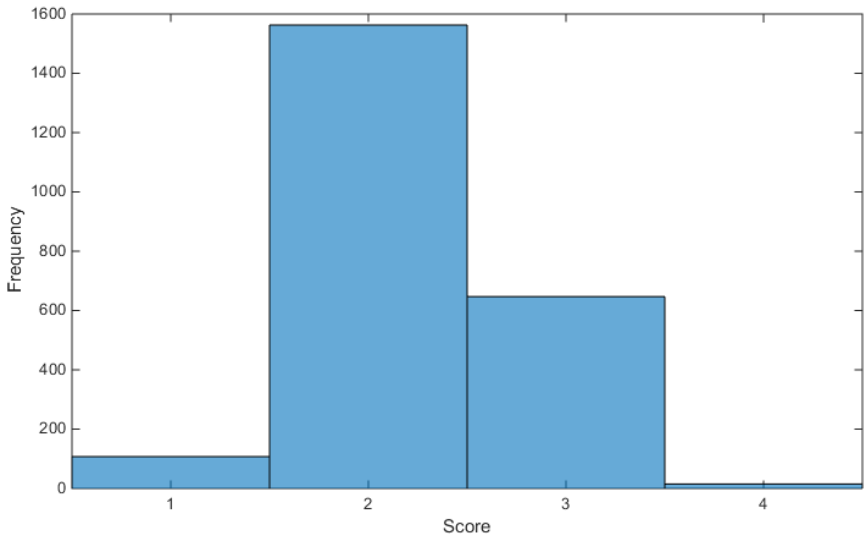

Figure A3 Histogram showing distribution of scores for calmness at the CAS assessment at 5 months of age.

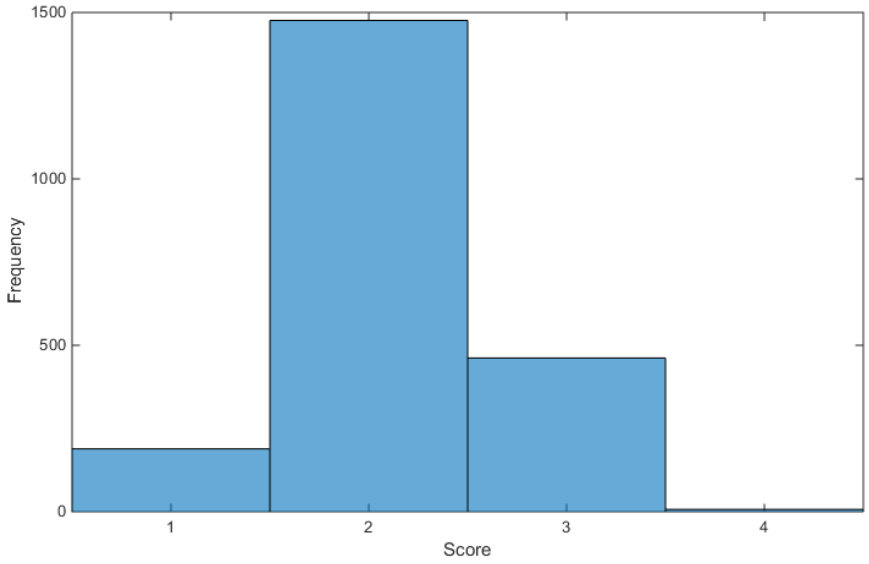

Figure A4 Histogram showing distribution of scores for calmness at the CAS assessment at 8 months of age.

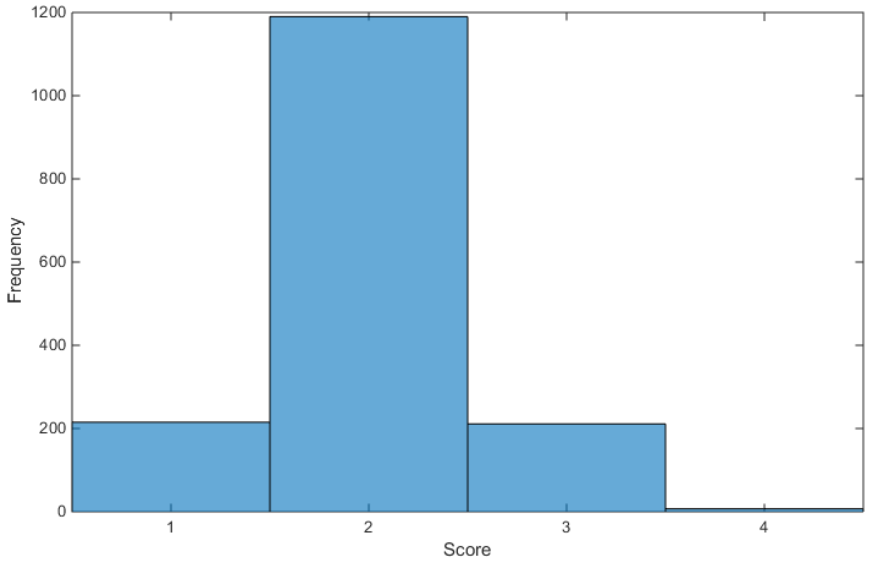

Figure A5 Histogram showing distribution of scores for calmness at the CAS assessment at 12 months of age.

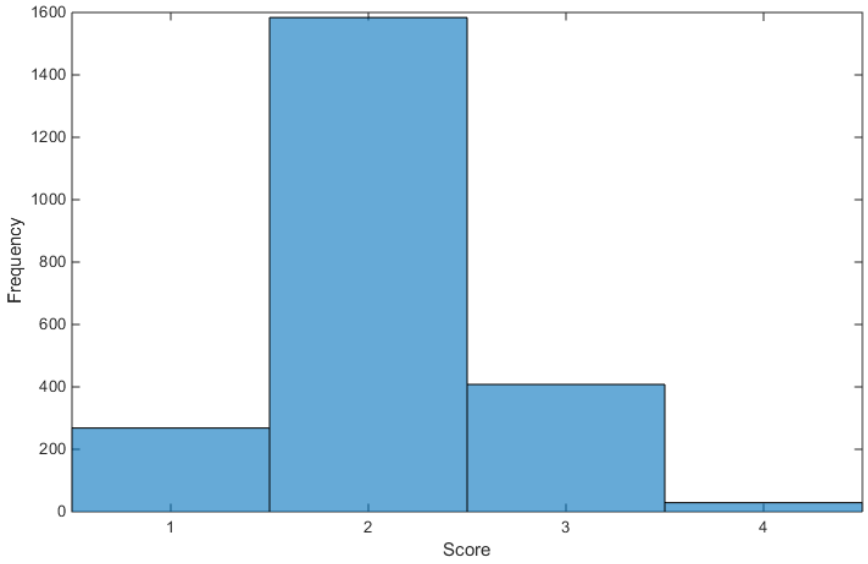

Figure A6 Histogram showing distribution of scores for calmness at the first CAS assessment in early training.

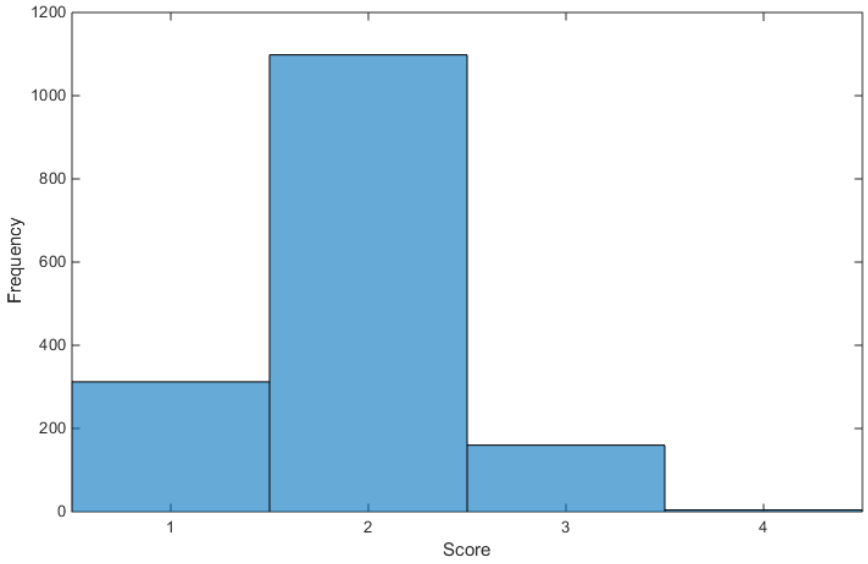

Figure A7 Histogram showing distribution of scores for calmness at the first CAS assessment in advanced training.