DEVELOPMENT OF A MULTIPLEXING STRATEGY FOR WHOLE GENOME SCANS OF THE DOMESTIC DOG AND ANALYSIS OF HEREDITARY DEAFNESS IN THE DALMATIAN

A Dissertation

by

EDWARD JAMES CARGILL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2004

Major Subject: Genetics

DEVELOPMENT OF A MULTIPLEXING STRATEGY FOR WHOLE GENOME SCANS OF THE DOMESTIC DOG AND ANALYSIS OF HEREDITARY DEAFNESS IN THE DALMATIAN

A Dissertation

by

EDWARD JAMES CARGILL

Submitted to Texas A&M University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Approved as to style and content by:

Keith E. Murphy (Chair of Committee)	James N. Derr (Member)
Mark E. Westhusin (Member)	James E. Womack (Member)
Ann B. Kier (Head of Department)	Geoffrey M. Kapler (Chair of Genetics Faculty)

May 2004

Major Subject: Genetics

ABSTRACT

Development of a Multiplexing Strategy for Whole Genome Scans of the Domestic Dog and Analysis of Hereditary Deafness in the Dalmatian. (May 2004)

Edward James Cargill, B.S., University of Nebraska-Lincoln

Chair of Advisory Committee: Dr. Keith E. Murphy

The Dalmatian is affected by deafness more than any other breed of domestic dog, with 30% of the United States population suffering from unilateral or bilateral deafness. The genetic origin of deafness in the Dalmatian is unknown. The objective of this work was to identify, using linkage analysis, any chromosomal region(s) in which the gene(s) responsible for deafness in the Dalmatian may be located. To achieve this objective it was necessary to 1) develop multiplexed microsatellite markers for an efficient whole genome scan, 2) assemble a multigenerational Dalmatian kindred segregating deafness, 3) estimate the heritability of deafness and perform complex segregation analysis, and 4) perform linkage analysis of deafness, and other phenotypic traits, in the Dalmatian kindred.

A set of 172 microsatellite markers, termed Minimal Screening Set 1 (MSS1), was characterized, prior to this work, for whole genome scans of the domestic dog. 155 of the MSS1 markers were multiplexed into 48 multiplex sets. Amplification of the multiplex sets was achieved using a single thermal cycling program. The markers were labeled with fluorescent dyes and optimized for resolution on an ABI 310 Genetic Analyzer or ABI 377 Sequencer.

A kindred of 266 Dalmatians was assembled, of which 199 had been diagnosed using the brainstem auditory evoked response to determine auditory status. Of these, 74.4% (N = 148) had normal hearing, 18.1% (N = 36) were unilaterally deaf, and 7.5% (N = 15) were bilaterally deaf. A heritability of 0.73 was estimated considering deafness a dichotomous trait and 0.75 as a trichotomous trait. Although deafness in the Dalmatian is clearly heritable, the evidence for the presence of a major gene affecting the disorder was not persuasive.

Dalmatians (N = 117) from the assembled kindred were genotyped for the MSS1 markers (149 were polymorphic). Linkage analysis was performed for deafness, eye color, and spot color. The maximum LOD scores for deafness were found with markers Cos15 on CFA17 (LOD = 1.69) and FH2585 on CFA28 (LOD = 1.34). No significant linkage was found with eye color. Significant linkage for spot color was found with marker FH2319 (LOD = 9.7) on CFA11.

For my wife, Holly.

And my son, Ethan.

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Keith Murphy, for his guidance during my graduate training. There are few professors who take care of their students as well as Dr. Murphy. He has provided me with every resource and opportunity to succeed while encouraging me to pursue the challenges expected of graduate level education and research. I will forever be thankful for his support and confidence.

I would like to thank the members of my faculty committee, Dr. James Derr, Dr. Mark Westhusin, and Dr. James Womack, for their support, encouragement, and the challenges they presented to me.

I would like to thank my fellow graduate students and the undergraduate students who were members of the Canine Genetics Laboratory during my tenure from October 1999 to November 2003 for the help they provided in many different ways.

I would like to thank the collaborators for their contributions to this work: Dr. George Strain, of Louisiana State University, for providing the first contact to collect samples for this project and his dedication to the ultimate goal of finding a way to eliminate deafness from all breeds; Dr. Thomas Famula, of the University of California-Davis, for providing insight and technical assistance with statistical analyses in addition to well-timed humor; and Dr. Robert Schnabel, of the University of Missouri, for enormous technical assistance and advice concerning linkage analysis including use of computing resources in the laboratory of his boss, Dr. Jeremy Taylor. I would also like

to thank Dr. David Adelson, of Texas A&M University, for the use of computing resources in his laboratory.

I would like to thank the agencies that provided funding for this work: the American Kennel Club Canine Health Foundation (grants Number 1870 and Number 2264); the National Institutes of Health, National Institute on Deafness and other Communication Disorders (Individual National Research Service Award predoctoral fellowship Number 1 F31 DC05297); and the Dalmatian Club of America Foundation.

I would like to give a special thanks to the Dalmatians, their breeders, owners, and veterinarians who helped contribute the samples for this work by donating their time and effort. Without their trust and cooperation this work would not have been possible.

Finally, I would like to thank my family, my parents, Nan and Bob, and my in-laws, Karen and Nathan.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

> There is no dog more picturesque than this spotted fellow with his slick white coat gaily decorated with clearly defined round spots of jet black, or, in the liver variety, deep brown. He does not look like any other breed, for his markings are peculiarly his own. He is strong-bodied, clean-cut, colorful, and distinctive. His flashy spottings are the culmination of ages of careful breeding. (DCA 2003)

Evolution of dogs and modern breeds

Canis lupus familiaris, the domestic dog, was the first domesticated animal (Clutton-Brock 1995). The dog is descended from the wolf (Wayne 1993) and recent studies point to an East Asian origin of domestication approximately 15,000 years ago (Savolainen et al. 2002). Genetic evidence obtained from fossils show the first dogs in the New World likely traveled across the Bering Strait with humans, rather than being domesticated from wolves indigenous to the American continents, around 12,000 to 14,000 years ago (Leonard et al. 2002). The recognition by humans of certain social-cognitive abilities of wolves was an important factor in the domestication and development of the dog (Hare et al. 2002).

While the first domestic dogs appeared thousands of years ago, the vast majority of their modern day counterparts, which are categorized by morphology and/or specific purpose into breeds, have been developed in the past 250 years (Ostrander and Giniger 1997). The American Kennel Club currently recognizes 150 distinct breeds (AKC 2003), but the World Canine Organization (WCO 2003) recognizes more than 300. The

---

This dissertation follows the style and format of the journal Mammalian Genome.

generation of a specific breed has generally been at the whim of a breeder who wishes to create a dog with specific characteristics (physical or behavioral) not possessed by any existing breed. The unique result has been the rapid creation of breeds suited to a variety of purposes, a feat not observed in any other domesticated species.

Though the diversity of the dog lends itself to multiple functions in human society, ranging from companion to protector to shepherd, the cost of this diversity has been great. That is, selective inbreeding allows the fixation of desirable physical and behavioral traits in each breed, but in conjunction with accompanying population bottlenecks and founder effects, has resulted in the emergence of more than 450 hereditary diseases (Nicholas 2003). The majority of these diseases follow a classic autosomal recessive inheritance pattern, complicating attempts to "breed away from" the deleterious alleles (Ostrander and Kruglyak 2000), because carriers are difficult to identify. In addition, diseases that occur later in life are difficult to prevent because affected individuals most often are bred before their symptoms are evident.

Because of the importance of the dog in society it is only natural that research would be undertaken to treat, if not prevent, many of these diseases. As such, the dog has found a new role in society, which is that of a research model for many human hereditary diseases (Ostrander et al. 2000). Approximately half of the diseases in the dog have clinical presentations similar to human diseases (Nicholas 2003). This fact, coupled with the sheer variety of breeds and well-documented pedigrees, provides a wealth of information for use in genetic studies designed to enhance the quality of life for both dogs and humans.

Evolution of the canine genome map

In order to dissect the genetics underlying hereditary diseases of the dog, a high resolution map of the canine genome needed to be constructed. However this was complicated by the fact that only recently was the karyotype of the dog standardized (Breen et al. 1999a; Breen et al. 1999b). This is because the canine genome is comprised of 38 acrocentric autosomes not easily distinguished by typical cytogenetic methods. Therefore, fluorescence *in situ* hybridization (FISH) paint probes were used to identify individual chromosomes, while whole chromosome-specific FISH paint probes were used to identify regions of conserved synteny in the dog and human (Breen et al. 1999c). The ability to identify each chromosome independently of other chromosomes was a crucial step to begin building linkage and radiation hybrid (RH) maps comprised of gene loci and other genetic markers.

The first meiotic, or linkage, map of the dog actually came before the standardization of the karyotype (Mellersh et al. 1997) and was comprised of 139 microsatellite markers assigned to 30 linkage groups (not specific chromosomes). The construction of canine-rodent hybrid cell lines quickly followed (Langston et al. 1997) to produce the first RH map of the dog (Priat et al. 1998), which was comprised of a total of 400 markers (218 genes and 182 microsatellite markers). The canine linkage and RH maps evolved until the integration of both maps (Breen et al. 2001), which resulted in a map of 1,800 markers. The RH map has continued to be improved and the newest version has 3,270 markers providing 1 Mb resolution (Guyon et al. 2003). While the

linkage and RH maps will continue to be an important resource to the research community, the National Human Genome Research Institute is fully supporting the complete sequencing of the canine genome (NHGRI 2003). Celera recently released a 1.5x sequence of a male Standard Poodle, named Shadow, representing the first canine genome sequencing effort (Kirkness et al. 2003). However, a higher resolution sequence will be needed for utilization in most research. The NHGRI sponsored sequencing project will yield a 6.5x sequence of a female Boxer named Tasha.

Linkage studies in the dog

Due to the existence of more than 450 hereditary diseases, and with many of these having genetic causes and pathologies virtually identical to specific human hereditary diseases (Nicholas 2003), the dog is an ideal model for the study of simple and complex human hereditary diseases. The marked genetic homogeneity and ease with which multigenerational pedigrees can be established are also important advantages of using the dog as a research model. As stated previously, the impediment to the study of canine hereditary diseases and the use of the dog as a model has been the lack of a high-density map of the canine genome. However, the rapid construction of a detailed map allows for tools that are readily available for study of the human (Lapsys et al. 1997, Li et al. 2001, Beekman et al. 2001), murine (Rithidech et al. 1997, Devereux et al. 1998) and bovine genomes (Konfortov et al. 1998, Womack et al. 1997, Grosse et al. 2000) to now be used in examination of the dog.

A set of 172 canine microsatellite markers, termed Minimal Screening Set 1 (MSS1), was characterized by Richman et al. (2001) for use in whole genome scans. The MSS1 is suitable for genome-wide linkage studies because it provides coverage of the canine genome with an estimated average spacing of 10 cM and an average polymorphic information content (PIC) value of 0.74. The MSS1 contains 64 dinucleotide repeats, three trinucleotide repeats, 104 tetranucleotide repeats and one short interspersed nuclear element. While the MSS1 is an extremely useful tool, multiplexing of these markers enhances its utility by allowing for more efficient genotyping. Multiplexing involves combining primer sets for multiple markers in the same reaction to be amplified using the same conditions (e.g., MgCl concentration, etc.), as opposed to amplifying each primer set separately. Multiplexing has already been utilized for linkage studies and verification of lineage in bison (Schnabel et al. 2000), cattle (Schnabel et al. 2000), humans (Beekman et al. 2001) and dogs (Koskinen et al. 1999, Altet et al. 2001).

Hereditary deafness in the Dalmatian

Assessment of auditory capacity in the dog is accomplished using the brainstem auditory evoked response (BAER). BAER allows accurate detection of dogs that are either unilaterally or bilaterally deaf because it elicits an all or none response (Kay et al. 1984; Marshall 1985). A normal functioning ear will produce a specific waveform pattern while a non-functioning ear does not produce a waveform pattern (Strain 2003).

The BAER test has been used to determine the prevalence of deafness in many breeds (Strain 1996, 2004).

Dalmatians are affected by deafness more than any other breed of domestic dog, with 30% of the United States (US) population suffering from unilateral or bilateral deafness (Famula et al. 2001; Holliday et al. 1992; Marshall 1986; Strain 1996). The affected Dalmatians can be subdivided into approximately 20% unilaterally deaf and 10% bilaterally deaf. Previous studies show there is no significant preference for either ear with respect to unilateral deafness (Famula et al. 2001; Greibrokk 1994; Muhle et al. 2002; Strain et al. 1992; Wood and Lakhani 1997). Population studies have shown correlation between deafness and certain phenotypic characteristics in the Dalmatian, and histological studies have shown structural changes supportive of pigmentation associations with deafness, but there are conflicting data from various studies. Therefore, it is necessary to review these data to avoid confusion among them.

Histological studies

Histological studies revealed that inner ear structures develop normally up to and briefly after birth. However, atrophy of the stria vascularis occurs between one and four weeks of age (Anderson et al. 1968; Johnsson et al. 1973). It has been shown that the strial atrophy is not caused by a decreased blood supply to the cochlea (Johnsson et al. 1973). The degeneration of the stria vascularis is followed by collapse of Reissner's membrane, the ductus reuniens, and the wall of the saccule. Subsequently, there is complete degeneration of hair cells involving first the outer hair cells, then inner hair

cells, and finally, degeneration of the eighth cranial nerve afferent neuron (Anderson et al. 1968; Hudson and Ruben 1962; Igarashi et al. 1972; Johnsson et al. 1973; Mair 1976).

Deafness and pigmentation

The distinct coat pattern of a Dalmatian results, in part, from homozygosity of the *extreme piebald* allele of the classical *Piebald* (*S*) gene, which generates a white "mask" over the underlying coat color (Sponenberg and Rothschild 2001). Two additional genes, *ticked* and *nonflecked*, modify *extreme piebald* to create the unique spotting pattern. There are three key findings to suggest that deafness in the Dalmatian is associated with pigmentation. Two studies showed an absence of melanocytes in stria of the affected dogs (Anderson et al. 1968; Johnsson et al. 1973), the first finding to support an association between deafness and pigmentation in the Dalmatian. The function of melanocytes in normal hearing has been investigated (Savin 1965; Steel et al. 1987). More specifically, these cells maintain the ionic composition of the cochlear endolymph, and their absence results in strial atrophy (Steel 1995). If tyrosinase is present, melanocytes produce the pigment melanin (Ito 1998). It is important to note, however, that melanin is not required for normal strial development (Bartels et al. 2001), because in albinism melanocytes do not produce melanin but the condition is not always associated with deafness (Steel 1995). Evidence for association of melanocytic disorders with deafness stems from the fact that melanocytes originate in the neural crest (Weston 1970) and almost all anomalies of pigmentation accompanied by sensorineural

deafness are proposed as neural crest defects (Bartels et al. 2001; Schrott et al. 1990; Steel and Barkway 1989).

A second finding to support an association between pigmentation and deafness is that blue-eyed Dalmatians have a higher prevalence of deafness than brown-eyed Dalmatians (Famula et al. 1996, 2000, 2001; Greibrokk 1994; Muhle et al. 2002; Strain et al. 1992; Wood and Lakhani 1997, Juraschko et al. 2003a). Greibrokk (1994), Wood and Lakhani (1997), Muhle et al. (2002), and Juraschko et al. (2003a) studied Dalmatians in Norway, the United Kingdom (UK), Switzerland, and Germany respectively, and found a lower prevalence of deafness than in the US. This has been attributed to a difference in the official breed standards between the US and certain European countries. That is, the US breed standard allows blue-eyed Dalmatians while Norway, the UK, Switzerland, and Germany do not. Thus, European breeders select against blue eyes and this is a possible explanation for the reduced incidence of deafness.

A third finding to support a deafness-pigmentation association is that Dalmatians with a color patch have a lower prevalence of deafness than Dalmatians without a color patch (Greibrokk 1994; Famula et al. 2000; Muhle et al. 2002; Juraschko et al. 2003a; Strain 2004). Dalmatians are born white and their spots appear during the first few weeks of life. Unlike a spot, a color patch is present at birth and is generally larger than any spot. While a color patch is negatively correlated with deafness, studies indicate that deafness and the color (e.g., black or liver) of a Dalmatian's spots or patch is not associated (Greibrokk 1994; Wood and Lakhani 1998; Famula et al. 2000; Muhle et al. 2002; Strain 2004).

The aforementioned findings support the hypothesis that there is an association between pigmentation and deafness in the Dalmatian. Such an association has also been identified in the human. Waardenburg Syndrome (WS; Waardenburg 1951) is a disorder characterized by sensorineural deafness (unilateral or bilateral), pigmentation anomalies (white forelock, hypopigmentation of the skin), and limb malformations. WS has been proposed as a model for deafness in the Dalmatian (Famula et al. 1996; Hayes et al. 1981; Hudson and Ruben 1962; Mair 1976; Strain 1992). WS is the only known human condition in which unilateral and bilateral sensorineural deafness and pigmentation anomalies are associated. Brenig et al. (2003) excluded a candidate gene implicated in WS, *Pax3*, by comparative sequencing and analysis. Tsai et al. (2004) examined another candidate gene, *c-kit*, which has been implicated in deafness associated with pigmentation in humans (Spritz and Beighton 1998).

In terms of a deafness-pigmentation association in the human and the mouse, there is a correlation that has been established in the human between low amounts of melanin in the inner ear and the presence of blue eyes (Barrenas and Hellstrom 1996, Bonaccorsi 1965). The Dalmatian, unlike the mouse, has ocular structures similar to the human and murine coat color has not been found to be an accurate indicator of strial melanin content (Bartels et al. 2001). Thus, the Dalmatian may serve as a better model for the study of pigmentation-associated sensorineural hearing loss in the human.

In addition to pigmentation, some groups have reported a significant association between deafness and gender (Holliday et al. 1992; Greibrokk 1994; Wood and Lakhani 1998) while others have not found such an association (Marshall 1986; Famula et al.

2001; Muhle et al. 2002; Juraschko et al. 2003a, Strain 2004). Females were found to have a significantly higher prevalence of deafness than males in studies reporting a difference (Holliday et al. 1992; Wood and Lakhani 1998) with the exception of Greibrokk (1994) who reported a higher prevalence of deafness in males. Wood and Lakhani (1998) do state the significantly higher prevalence of deafness they observed in females is a correlation and not indicative of causation.

Heritability and complex segregation analysis

Heritability estimates have been reported in Californian (Famula et al. 2000; Famula et al. 2001), Swiss (Muhle et al. 2002), and German (Juraschko et al. 2003a) Dalmatians. Famula et al. (2000), Muhle et al. (2002), and Juraschko et al. (2003b) also performed complex segregation analysis in order to examine evidence for the presence of a single major locus. Although Famula et al. (2000, 2001) reported heritability estimates and complex segregation analysis in Californian Dalmatians, no study has been performed utilizing a data set of US Dalmatians collected from across the US.

The mode of inheritance for deafness in the Dalmatian has not been determined, but various hypotheses have been proposed as researchers have tried to determine if a single major gene plays a role in the disorder. These hypotheses include transmission by an autosomal recessive, multifactorial gene with incomplete penetrance (Greibrokk 1994), a model of two interacting genes with incomplete penetrance (Strain 1999), a defect in a single major locus with an important role in auditory development but not solely responsible for deafness (Famula et al. 2000), and a recessive allele at a single

biallelic major locus with incomplete penetrance in recessive homozygotes (Muhle et al. 2002). Plausible arguments can be made for each of these hypotheses given certain assumptions, but it is obvious more study is needed to dissect this disorder. Due to the extensive list of potential candidate genes that could be causative for deafness in the Dalmatian (e.g. WS related genes, melanocyte related genes, cochlear specific genes, *etc*.), a whole genome scan approach was undertaken to narrow the possibilities by localizing the specific chromosomes that may harbor such a causative gene(s).

Specific objectives

The genetic origin of deafness in the Dalmatian is unknown. The objective of this work was to identify, using linkage analysis, any region(s) in which the gene(s) responsible for deafness in the Dalmatian may be located. To achieve this objective it was necessary to 1) develop multiplexed microsatellite markers for an efficient whole genome scan, 2) assemble a multigenerational Dalmatian kindred segregating deafness, 3) estimate the heritability of deafness and perform complex segregation analysis, 4) assemble a linkage map based on marker data generated from a whole genome scan of the Dalmatian kindred, and 5) perform linkage analysis of deafness, and other phenotypic traits, in the Dalmatian kindred.

CHAPTER II

MULTIPLEXING OF CANINE MICROSATELLITE MARKERS FOR WHOLE-GENOME SCREENS*

Overview

A set of 172 canine microsatellite markers, termed Minimal Screening Set 1 (MSS1), was recently characterized for use in whole genome screens (Richman et al. 2001). Reported here is the multiplexing of 155 MSS1 markers into 48 multiplex sets. Amplification of the multiplex sets is achieved using a single thermal cycling program. The markers are labeled with fluorescent dyes and optimized for resolution on an ABI 310 Genetic Analyzer or ABI 377 Sequencer. The multiplexing strategy involves amplifying combinations of markers so that no two markers with the same dye and product size overlap. Multiplexing the MSS1 provides an efficient tool for collection of genotypes and streamlines whole genome screens. Screening the canine genome for linkage of markers with various hereditary diseases facilitates identification of affected and carrier individuals, thereby providing researchers and clinicians with an additional diagnostic tool.

Introduction

More than 400 hereditary diseases of the domestic dog have been described. Importantly, more than 200 of these have pathologies resembling specific human hereditary diseases (Ostrander et al. 2000). Furthermore, many canine and human

* Reprinted with permission from Cargill EJ, Clark LA, Steiner JM, and KE Murphy (2002) Multiplexing of Canine Microsatellite Markers for Whole-Genome Screens. Genomics 80(3), 250-253.

hereditary diseases have common genetic etiologies. This fact, combined with the marked genetic homogeneity and the ease with which multigenerational pedigrees can be established, make the dog an ideal model for the study of simple and complex human hereditary diseases. Even so, until very recently, an impediment to the study of canine hereditary diseases and the use of the dog as a model has been the lack of a high-density map of the canine genome. However, advancements towards development of such a resource have come from construction of radiation hybrid (RH) (Priat et al. 1998) and linkage maps (Werner et al. 1999). The subsequent integration of these maps provides coverage of approximately 99% of the canine genome (Mellersh et al. 2000). The most recent advance is an 1800-marker map replete with microsatellite and gene loci (Breen et al. 2001). This rapid development of the canine map allows for tools that are readily available for study of the human (Lapsys et al. 1997, Li et al. 2001, Beekman et al. 2001), murine (Rithidech et al. 1997, Devereux et al. 1998) and bovine genomes (Konfortov et al. 1998, Womack et al. 1997, Grosse et al. 2000) to now be used in examination of the canine.

The MSS1 is suitable for genome-wide linkage studies because it provides coverage of the canine genome with average spacing of 10 cM and an average polymorphic information content (PIC) value of 0.74 (Richman et al. 2001). The MSS1 contains 64 dinucleotide repeats, three trinucleotide repeats, 104 tetranucleotide repeats and one short interspersed nuclear element. While the MSS1 is an extremely useful tool, multiplexing will enhance its utility by allowing for more efficient genotyping. Multiplexing has already been utilized for linkage studies and verification of lineage in

bison (Schnabel et al. 2000), cattle (Schnabel et al. 2000), humans (Beekman et al. 2001) and dogs (Koskinen et al.1999, Altet et al. 2001). To this end, reported here is the multiplexing of 155 MSS1 markers into 48 sets of two to five markers with 151 co-amplified and four co-loaded. The remaining 17 MSS1 markers are amplified and resolved individually.

Materials and methods

DNA from a mixed breed dog was used for optimization of the multiplex sets. Genomic DNA was extracted from whole blood using the PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). DNA is stored at 4^{o}C in 150 µl of rehydration buffer. Concentration of DNA is determined by spectrophotometric analysis and a working solution of 50 ng/µl is maintained.

The microsatellite primers were synthesized using an ABI Expedite Nucleic Acid Synthesis System (PE Biosystems, Foster City, CA, USA). The 5´-end of each forward primer is labeled with one of three fluorescent dyes: 6FAM, HEX, or TET (PE Biosystems). Dyes were selected based on product size ranges (Richman et al. 2001) to evenly distribute dye type and to limit marker overlap.

The multiplex sets and individual markers are amplifiable with stepdown thermal cycling conditions of 2 min 95^{o}C followed by 5 cycles of: 30 s 95^{o}C, 15 s 58^{o}C, 10 s 72^{o}C and an additional 30 cycles of: 20 s 95^{o}C, 15 s 56^{o}C, 10 s 72^{o}C with a final extension of 5 min 72^{o}C. Concentrations for a 10 µl PCR volume are: 0.5 mM each dNTP, 5 ng/µl genomic DNA, 3.0 mM MgCl, 1x *Taq* DNA Polymerase Buffer B (Fisher

Scientific, Pittsburgh, PA, USA), 1x MasterAmp PCR Enhancer (Epicentre Technologies, Madison, WI, USA), and 0.04 units/µl *Taq* DNA Polymerase (Fisher Scientific, Pittsburgh, PA, USA). Primer concentrations vary for each marker. Amplifications are carried out using an Eppendorf Mastercycler (Eppendorf Scientific, Inc., New York, NY, USA). To co-load markers in multiplex sets M47 and M48, individual markers are amplified and equal parts of reaction products are mixed together.

Products of reactions were resolved using an ABI 310 capillary-based Genetic Analyzer or an ABI 377 Sequencer (PE Biosystems) and sized relative to an internal size standard (MAPMARKER HI, Bioventures). The ABI GENESCAN version 3.1 software package (PE Biosystems) was used for analysis of the multiplex sets.

Results

The MSS1 markers have individual amplification conditions and observed product sizes (Richman et al. 2001). A complete list of references for each marker can be found on-line at http://www.cvm.tamu.edu/cgr/multiplex.html. For multiplexing, 53 markers are labeled with 6FAM, 53 markers are labeled with HEX, and 66 markers are labeled with TET. In an effort to further enhance multiplexing, one PCR mix and one thermal cycling program were developed for amplification of the markers. To determine compatibility for multiplexing, markers were amplified in various combinations. Multiplex set optimization was completed by variation of primer concentrations to give approximately equal amplification of each product in a set.

The use of three fluorescent dyes permits overlap of similarly sized markers and 48 multiplex sets of two to five markers were developed. More specifically, 151 markers can be co-amplified and four markers can be co-loaded after individual amplification. This reduces the number of reactions required to generate data for the MSS1 by 60%, from 172 to 69. Table 1 lists each multiplex set as they correspond to each canine linkage group. The multiplex sets, primer concentrations, and fluorescent dyes are listed in Table 2.

A table sorted by canine linkage group listing the MSS1 markers, primer sequences, PIC values, heterozygosity values, marker types, fluorescent dyes, observed allelic sizes, primer concentrations, multiplex sets and marker references is available via the Internet at http://www.cvm.tamu.edu/cgr/multiplex.html. Of the 172 markers, 155 are multiplexed and the remaining 17 are amplified individually because they are not compatible in any multiplex combinations. Eight of these 17 amplify under the same conditions as the multiplex sets (C10.16, FH2200, FH2347, RVCE, FH2165, REN06C11, FH2538, CXX.390.2) while nine do not amplify under the multiplex conditions (ZuBeCa6, FH2149, FH2279, FH2346, N41, FH2457, AHT006, REN51i12, FH2600). The multiplexed MSS1 provides an expedient and cost-effective method for collection of genotype information by reducing the number of reactions, quantities of reagents and time required for whole genome screens of the dog.

Table 1. Multiplex sets as they correspond to each canine linkage group.

Linkage Group	Multiplex Sets	Linkage Group	Multiplex Sets
CFA1	M02 M08 M13 M17	CFY	M32
CFA2	M17 M21 M22 M23 M28 M46	S1/L2	M01 M22 M25 M28 M45
CFA3	M11 M12 M26 M38 M42 M48	S2/L13	M09 M37
CFA5	M14 M25 M27 M43	S3/L14	M09 M15 M44
CFA6	M13 M20 M38 M47	S4/L3	M01 M07 M13 M19 M29 M42 M43
CFA7/L1	M03 M11 M12 M27 M45	S5/L18	M07 M29 M44
CFA8	M03 M08 M21 M27	S6/L12	M24 M32 M39 M44
CFA9	M01 M08 M35	S7/L7	M08 M39 M41
CFA10	M18 M33 M37	S8/L8	M15 M41 M46
CFA12/L4	M32 M38	S9/L5	M08 M28 M40
CFA13,19/L17	M16 M35	S10/L6	M23 M31 M40 M42
CFA15	M14 M20 M21	S11/L9	M14 M29 M34 M36
CFA16	M01 M05 M18	S12/L11	M31 M37 M47
CFA18	M05 M12 M23 M26	S13/L10	M24 M33, M45
CFA20	M01 M40 M41 M43 M48	S14/L15	M30 M32 M35
CFA22	M14 M22 M30 M31 M48	S15/L20	M16 M25 M34 M36
CFA26	M02 M06 M15 M39	S16/L21	M33 M39
CFA29,35/L16	M06	S17/L22	M19 M34
CFA30/L19	M04 M06 M24 M26	L1/L23	M19
CFX	M04 M07	Unlinked	M10 M16 M20 M30 M36 M47

Table 2. Multiplex sets (M01 through M48) with marker primer concentrations[a] and fluorescent dyes[b].

Multiplex	Conc.	Dye	Multiplex	Conc.	Dye	Multiplex	Conc.	Dye	Multiplex	Conc.	Dye
M01						**M25**					
FH2263	0.8	T	**M13**			CPH18	0.8	F	**M37**		
FH2289	0.8	T	C01.673	1.0	H	FH2594	0.8	F	FH2339	1.0	F
CPH16	0.8	F	C06.636	1.0	T	FH2142	0.8	H	FH2312	1.0	H
AHT103	0.8	F	LEI001	0.6	F	CPH2	0.8	T	FH2155	1.0	H
AHT137	0.8	T									
M02			**M14**			**M26**			**M38**		
C01.246	0.8	F	C05.377	1.0	H	FH2531	0.8	F	FH2107	0.8	H
C01.424	0.8	F	CPH5	0.8	F	FH2429	0.8	H	FH2525	0.8	T
FH2016	0.8	T	FH2283	0.8	F	FH2305	0.4	T	FH2223	0.8	F
REN01O23	0.8	H	AHT133	0.6	T						
C26.733	0.8	F							**M39**		
M03			**M15**			**M27**			FH2171	1.0	H
FH2201	1.0	T	FH2130	1.0	H	GLUT4	0.8	T	REN49F22	0.8	H
FH2174	0.8	H	FH2385	1.2	T	C07.1000	0.8	F	CPH10	1.2	T
C08.618	0.8	F	CXX.391	0.6	H	FH2138	0.8	T	FH2566	0.8	F
			M16								
M04			AHT124	0.4	F	**M28**			**M40**		
1F11	0.8	T	AHT127	0.8	T	FH2062	0.8	H	AHTk209	0.8	H
F8C	1.0	T	PEZ2	2.0	H	AHT128	0.8	F	PEZ10	0.8	T
FH2584	1.0	H	AHT106	0.4	T	FH2547	0.6	T	CXX.213	0.8	T
M05			**M17**			**M29**			**M41**		
LEI002	0.8	F	FH2598	1.0	H	CXX.750	0.8	T	PRKCD	1.0	H
FH2356	1.0	T	FH2309	1.0	H	FH2159	0.8	H	REN49C08	1.4	F
FH3010	1.0	T	AHT132	0.8	F	FH2587	0.8	T	CXX.900	1.0	T
M06			**M18**			**M30**			**M42**		
REN48E01	1.0	H	FH2422	0.8	T	C22.745	0.8	T	C03.895	0.6	T
FH2507	0.6	T	FH2293	0.8	T	CXX.176	0.8	T	FH2018	0.4	H
FH2050	0.6	F	PEZ6	0.8	F	CXX.452	0.8	F	AHT140	0.6	F
M07			**M19**			**M31**			**M43**		
FH2548	0.8	H	CXX.873	0.8	T	FH2325	0.8	T	FH2383	1.0	F
FH2985	0.8	H	REN02C20	0.8	F	FH2141	0.8	H	FH2528	0.4	T
FH2096	0.8	F	CXX.672	0.8	F	FH2175	0.8	F	FH2319	0.8	H
FH2079	0.8	T	FH2516	0.8	T						
M08						**M32**			**M44**		
FH2294	0.8	H	**M20**			C12.852	0.8	T	FH2364	1.4	F
C08.410	0.8	F	CPH3	1.2	T	AHT139	0.8	T	FH2261	0.8	F
GALK1	0.8	T	FH2321	0.8	T	FH2585	0.8	F	FH2278	1.0	H
CXX.279	0.8	T	TAT	0.8	F	SRY	0.8	H			
FH2060	0.8	F									
M09			**M21**			**M33**			**M45**		
FH2441	0.8	H	C02.342	0.6	F	FH2537	0.8	H	FH2396	1.0	F
FH2233	1.0	F	FH2144	1.0	H	FH2199	0.8	F	FH2534	0.6	T
REN45F03	0.8	F	Cos15	1.0	T	PEZ8	0.8	H	FH2239	0.6	H
M10			**M22**			**M34**			**M46**		
FH2247	0.8	F	FH2132	1.0	T	CXX.172	0.4	T	C02.864.A	0.8	T
REN01G01	0.8	T	FH2001	0.8	F	REN41D20	0.8	F	FH2394	0.8	T
FH2377	0.8	T	FH2412	0.8	H	FH2244	0.6	T			

Table 2. Continued.

Multiplex	Conc.	Dye	Multiplex	Conc.	Dye	Multiplex	Conc.	Dye	Multiplex	Conc.	Dye
M11			**M23**			**M35**			**M47** [c]		
FH2137	0.8	T	FH2087U	0.8	H	FH2186	1.0	T	FH2119	0.8	H
FH2301	0.8	F	WILMS-TF	0.8	H	FH2206	1.0	T	AHT131	0.8	T
FH2581	0.8	T	FH2526	0.8	T	FH2208	0.8	H	PEZ7 [a]	0.8	F
M12			**M24**			**M36**			**M48** [d]		
FH2302	0.8	T	FH2290	0.8	H	FH2532	0.8	H	FH2316	0.8	H
FH2226	0.8	H	CXX.608	0.8	H	FH2238	0.6	T	REN55P21	0.8	H
AHT130	0.8	F	CXX.642	0.6	T	FH2550	0.8	F	FH2227	0.8	H

[a] Primer concentration in final reaction as μmol/primer, see http://www.cvm.tamu.edu/cgr/multiplex.html.

[b] F = 6FAM, H = HEX, T = TET.

[c] M47 co-amplify FH2119 and AHT131, individually amplify PEZ7, mix products 1:1 for co-loading.

[d] M48 individually amplify FH2316, REN55P21, and FH2227, mix products 1:1 for co-loading.

CHAPTER III

HERITABILITY AND SEGREGATION ANALYSIS OF DEAFNESS IN US DALMATIANS*

Overview

Hereditary loss of hearing affects many breeds of the domestic dog, but the Dalmatian has the highest prevalence. Approximately 30% are affected in the United States (US) population. It is widely accepted that a relationship exists between deafness and pigmentation in the dog and also in other animals. While the Dalmatian exemplifies this relationship, the genetic origin and mode of inheritance of deafness in this breed are unknown. The goals of this study were to: 1) estimate the heritability of deafness in an extended kindred of US Dalmatians, and 2) determine, through complex segregation analysis, whether there is a major segregating locus that has a large effect on the expression of deafness. A kindred of 266 Dalmatians was assembled, of which 199 had been diagnosed using the brainstem auditory evoked response to determine auditory status. Of these, 74.4% (N = 148) had normal hearing, 18.1% (N = 36) were unilaterally deaf, and 7.5% (N = 15) were bilaterally deaf. A heritability of 0.73 was estimated considering deafness a dichotomous trait and 0.75 as a trichotomous trait. Although deafness in the Dalmatian is clearly heritable, the evidence for the presence of a single major gene affecting the disorder is not persuasive.

---

* Reprinted with permission from Cargill EJ, Famula TR, Strain GM, and KE Murphy (2004) Heritability and Segregation Analysis of Deafness in US Dalmatians. Genetics, In Press.

Introduction

The brainstem auditory evoked response (BAER) (Kay et al. 1984; Marshall 1985) allows accurate detection of dogs that are either unilaterally or bilaterally deaf (Strain 2002). The BAER elicits an all or none response; a normal functioning ear will produce a specific waveform pattern while a non-functioning ear produces a flat line (Strain 2002). The prevalence of deafness has been determined in many breeds (Strain 2003). The Dalmatian is most affected with approximately 30% of the United States (US) population exhibiting unilateral or bilateral deafness (Marshall 1986; Holliday et al. 1992; Famula et al. 2001; Strain 2003). Approximately 20% of Dalmatians are unilaterally deaf, with no significant preference for the left or right ear to be affected, and 10% are bilaterally deaf (Greibrokk 1994; Wood and Lakhani 1998; Famula et al. 2001; Muhle et al. 2002; Strain 2003).

Histological studies revealed that inner ear structures develop normally up to and after birth with atrophy of the stria vascularis occurring between one and four weeks of age in affected dogs (Anderson et al. 1968; Johnsson et al. 1973). These studies also showed an absence of melanocytes in stria of the affected dogs (Anderson et al. 1968; Johnsson et al. 1973), the first finding to support an association between deafness and pigmentation in the Dalmatian. The function of melanocytes in normal auditory function has been investigated in the mouse (Savin 1965; Steel et al. 1987). More specifically, these cells maintain the ionic composition of the cochlear endolymph, and their absence results in strial atrophy (Steel 1995).

A second finding supporting an association between deafness and pigmentation is that Dalmatians with at least one blue eye have a higher prevalence of deafness than brown-eyed Dalmatians (Greibrokk 1994; Wood and Lakhani 1998; Famula et al. 2000; Muhle et al. 2002; Juraschko et al. 2003a; Strain 2003). A third finding to support a deafness-pigmentation association is that Dalmatians with a color patch have a lower prevalence of deafness than Dalmatians without a color patch (Greibrokk 1994; Famula et al. 2000; Muhle et al. 2002; Juraschko et al. 2003a; Strain 2003). Dalmatians are born white and their spots appear during the first few weeks of life. Unlike a spot, a color patch is present at birth and is generally larger than any spot. While a color patch is negatively correlated with deafness, studies indicate that deafness and the color (e.g., black or liver) of a Dalmatian's spots or patch is not associated (Greibrokk 1994; Wood and Lakhani 1998; Famula et al. 2000; Muhle et al. 2002; Strain 2003).

Similar associations of deafness with pigmentation have also been identified in the human and one example is that of Waardenburg Syndrome (WS; Waardenburg 1951). WS has been proposed as a model for deafness in the Dalmatian (Hudson and Ruben 1962; Mair 1976; Brenig et al. 2003) and is the only known human condition in which unilateral and bilateral sensorineural deafness and pigmentation are associated.

In addition to pigmentation, some groups have reported a significant association between deafness and gender (Holliday et al. 1992; Greibrokk 1994; Wood and Lakhani 1998) while others have not found such an association (Marshall 1986; Famula et al. 2001; Muhle et al. 2002; Strain 2003). Females were found to have a significantly higher prevalence of deafness than males in studies reporting a difference (Holliday et

al. 1992; Wood and Lakhani 1998) with the exception of Greibrokk (1994) who reported a higher prevalence of deafness in males.

The mode of inheritance for deafness in the Dalmatian has not been determined, but various hypotheses have been proposed as researchers have tried to determine if a single major gene plays a role in the disorder. These hypotheses include transmission by an autosomal recessive, multifactorial gene with incomplete penetrance (Greibrokk 1994), a model of two interacting genes with incomplete penetrance (Strain 1999), a defect in a single major locus with an important role in auditory development but not solely responsible for deafness (Famula et al. 2000), and a recessive allele at a single biallelic major locus with incomplete penetrance in recessive homozygotes (Muhle et al. 2002).

Heritability estimates have been reported in Californian (Famula et al. 2000; Famula et al. 2001), Swiss (Muhle et al. 2002), and German (Juraschko et al. 2003a) Dalmatians. Famula et al. (2000) and Muhle et al. (2002) also performed complex segregation analysis in order to examine evidence for the presence of a single major locus. Although Famula et al. (2000) and Famula et al. (2001) reported heritability estimates and complex segregation analysis in Californian Dalmatians, no study has been performed utilizing a data set of US Dalmatians collected from across the country.

The objectives of the present study were to 1) quantify the inheritance of deafness through the estimation of heritability in a threshold model, and 2) use complex segregation analysis to determine if there is a major segregating locus that has a large

effect on the expression of deafness in a newly assembled kindred representative of the US Dalmatian population.

Materials and methods

Collection of data

BAER results, eye color, spot color, gender, birthdate, number of littermates, and registration pedigree were collected for each dog. Color patch data were not available for a significant portion of kindred members (>50%) and hence were not included. Data from a total of 266 Dalmatians were collected, 199 with auditory status determined by BAER and 67 with unknown auditory status. The phenotypes of the dogs with known auditory status are shown in Table 3.

Dalmatian kindred

A total of 74 matings between parents with known auditory status were present in the kindred; 60 matings occurred between unaffected parents, 13 matings occurred between an unaffected parent and a unilaterally deaf parent, and one mating occurred between two unilaterally deaf parents.

Nine complete litters (litters in which data concerning all offspring from a mating were collected, N = 44) are included in the kindred and contain at least one affected dog in each litter. Both parents and both sets of grandparents are included for each litter (N = 54) and all have known auditory status. Seven litters were the result of matings between two unaffected parents and two litters were the result of matings between two

Table 3. Phenotypes[a] of the 199 Dalmatians with known auditory status.

Phenotype	Male	Female	Total
Hearing/Brown Brown/Black	47	50	97
Hearing/Brown Brown/Liver	19	24	43
Hearing/Brown Blue/Black	3	3	6
Hearing/Brown Blue/Liver	1	1	2
Unilateral/Brown Brown/Black	9	16	25
Unilateral/Brown Brown/Liver	3	6	9
Unilateral/Brown Blue/Black	0	1	1
Unilateral/Blue Blue/Black	0	1	1
Deaf/Brown Brown/Black	1	3	4
Deaf/Brown Brown/Liver	2	3	5
Deaf/Brown Blue/Black	1	4	5
Deaf/Brown Blue/Liver	1	0	1
Total	87	112	199

[a] Auditory status/eye color/spot color.

unaffected sires and unilaterally deaf dams. The remaining dogs (N = 168), including the 67 dogs with unknown auditory status, provided crucial information regarding relationships among the parents and grandparents of the complete litters, as there are multiple common ancestors that create 72 inbreeding loops as identified by LOOPS (1992). There are four halfsib matings, three grandchild by grandparent matings, two niece/nephew by aunt/uncle matings, and one first cousin mating.

One breeder in Louisiana initially provided data for related Dalmatians (N = 16) that did not represent an entire family. Data from additional Dalmatians (N = 31) that are ancestors and offspring of the first dogs provided, were collected from this breeder. Data from the remaining dogs (N = 219) were collected from each dog's respective breeder or owner and represent ancestors and offspring directly and indirectly related to the dogs collected from the breeder in Louisiana. The states where dogs were born are Alabama, California, Florida, Georgia, Kentucky, Louisiana, Massachusetts, Michigan, Mississippi, Missouri, New Hampshire, New Jersey, North Carolina, Tennessee, Texas, and Washington, representing the northern, southern, eastern, and western extents of the US.

The data for Dalmatians collected from the breeder in Louisiana (N = 47) are also included in a data set assembled by Strain (2003). The remaining dogs in the kindred have not been included in any previous studies.

Comparison of kindred to US population

Strain (1999) presented data for 5009 US Dalmatians and has since added an additional 324 Dalmatians (Strain 2003). This is the most comprehensive data set available of US Dalmatians with the limitation that pedigree information was not recorded, precluding complex segregation analysis. However, the data set of Strain (2003) provides a standard for the US Dalmatian population's phenotypic distribution with which to compare the Dalmatians that are part of this study.

The Dalmatians described here did not differ significantly from Strain (2003) when considering deafness as unaffected vs. affected (unilaterally and bilaterally deaf combined) ($P > 0.19$) or unaffected vs. unilaterally deaf vs. bilaterally deaf ($P > 0.40$), nor in terms of eye color ($P > 0.13$). The Dalmatians in this current study did significantly differ from Strain's data set in terms of spot color ($P < 0.0001$). Two factors can explain this result, the first being the smaller sample size of this kindred and the second being a preference by the breeders who contributed samples to this study for liver spotted Dalmatians over black spotted Dalmatians. This simply illustrates the phenotypic composition of the reported Dalmatians in terms of spot color, which has never been shown to correlate with deafness (Greibrokk 1994; Wood and Lakhani 1998; Famula et al. 2000; Muhle et al. 2002; Strain 2003). These results suggest that the kindred of Dalmatians reported here is representative of the US Dalmatian population in terms of deafness and eye color.

Estimation of heritability

The estimation of heritability, as well as subsequent complex segregation analysis, is derived from analysis of a kindred of Dalmatians in which deafness segregates. The BAER is used to determine the auditory function of each ear, providing two possible deafness phenotypes in these dogs. One phenotype would be dichotomous, in which unilaterally deaf and bilaterally deaf dogs would be classified as deaf (i.e., affected vs. unaffected). A second phenotype would be trichotomous, with classes for normal hearing, unilateral deafness and bilateral deafness.

Most data sets utilized in the study of hereditary diseases are constructed around probands, making correction for ascertainment bias necessary; this set of data is no exception. In estimation of heritability, mixed linear models are capable of accommodating non-randomly sampled data (Henderson 1984). Accordingly, the estimation of the heritability of deafness should not be biased by family selection, provided that the animals at the top of the pedigree (those animals with no parents identified) can be considered a random sample of Dalmatians. This is more assumption than assertion because it is not feasible to create or discount a process of selection against deafness or for sampling such animals disproportionately among those animals at the top that have no known auditory status.

Estimation of heritability is conducted through use of threshold models (Falconer and Mackay 1996), an approach typical for study of binary and ordered categorical traits. The observation of deafness is considered as a binary trait y_{ij} (y_{ij} = 0 when unaffected; 1 when affected) for the *j*-th dog (*j*=1,2,…199) of the *i*-th gender (*i* = 1 for males; 2 for

females). In threshold models, this categorical phenotype is assumed to be related to an underlying, unobservable, normally distributed continuous variable, θ, through a set of three fixed thresholds, $[\gamma_0 = -\infty; \gamma_1 = 0; \gamma_2 = \infty]$; γ_1 is set to zero for computational convenience, with no loss in generality or impact on subsequent analysis of data. Specifically, we assume that the combination of continuous genetic and environmental terms thought to control the unobservable θ are translated into a categorical observation through comparison to the fixed thresholds (i.e., observe an unaffected dog when $\gamma_0 \leq \theta < \gamma_1$ or an affected dog when $\gamma_1 \leq \theta < \gamma_2$).

In a later analysis we consider deafness to be a trichotomous trait, in which normal hearing dogs are scored as a zero, unilaterally deaf dogs scored as a one, and bilaterally deaf dogs are scored as a two. Such a characterization of the auditory phenotype requires only minor modification of the threshold model. Specifically we need to add a fourth fixed threshold $[\gamma_0 = -\infty; \gamma_1 = 0; \gamma_2; \gamma_3 = \infty]$, yet in this case γ_2 must be estimated from the available data. Furthermore, normal hearing dogs would be observed when $\gamma_0 \leq \theta < \gamma_1$, unilaterally deaf dogs would be observed when $\gamma_1 \leq \theta < \gamma_2$, and bilaterally deaf dogs would be observed when $\gamma_2 \leq \theta < \gamma_3$.

The model for θ is similar to any that can be used for continuous phenotypes. The algebraic form of the model for this study is:

$$\theta_{ijkl} = \mu + \text{gender}_i + \text{spot}_j + \text{eye}_k + a_l + e_{ijkl} \qquad [1]$$

where θ_{ijkl} is an unobservable continuous variate for the *l*-th (l=1,2,…,199) dog of the *i*-th gender in the *j*-th class of spot color (j = 1 for black; 2 for liver) and the *k*-th eye color

class (k=1 for two pigmented eyes; 2 for one pigmented, one unpigmented eye). The component μ is an unknown constant while $gender_i$ is the contribution of the i-th gender to the expression of deafness. $Spot_j$ and eye_k are similar contributions of these physical characteristics to the liability for deafness; a_l is the additive genetic contribution of the l-th animal and e_{ijkl} is an unknown residual. Both a_l and e_{ijkl} are assumed to be random effects with zero means and variances of σ_a^2 (the additive genetic variance) and σ_e^2 (the residual variance), respectively. The additive genetic effect for each animal accounts for the covariance in phenotypes of relatives and is assumed to be multivariately-normally distributed, with a covariance structure based upon the additive relationships among all 266 animals in the data set. Because the underlying scale is unobservable, the total variance is assumed to be $\sigma_P^2 = \sigma_a^2 + \sigma_e^2$ where $\sigma_e^2 = 1.0$, with no loss of generality (Gianola and Foulley 1983; Harville and Mee 1984; Sorensen et al. 1995). The heritability of deafness, on the unobservable continuous scale, can be estimated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$.

A mixed model Bayesian strategy outlined by Sorensen et al. (1995) was used to arrive at an estimate of σ_a^2. An advantage of Bayesian methods is the ability to arrive at not only a point estimate of the unknown parameters (e.g., heritability), but also a distributional estimate. Though a more complete description of the statistical aspects of this analysis can be found in Sorensen et al. (1995), briefly, the assumed prior densities for the fixed effects (gender, spot, and eye effects) are the uniform density function, what Bayesian modelers refer to as a "flat" prior density. That is, we assume no prior knowledge of the behavior of the fixed effects. For the analysis of deafness as a binary

observation there is no need to estimate the fixed thresholds. However, for the case of the trichotomous deafness, γ_2 must be estimated. The assumed prior distribution for this parameter is the uniform with bounds established by γ_1 and γ_3. As for the random contributions to θ, the additive genetic effects are assumed to be multivariately-normally distributed with a null mean and variance-covariance structure consisting of the numerator relationship matrix times the unknown additive genetic variance, σ_a^2. Similarly the random residuals are assumed to be independently normally distributed with null mean with variance $\sigma_e^2 = 1.0$ (with no loss of generality since θ is an unobservable variate). Finally, given our Bayesian approach to this problem, we also must establish a prior density for the unknown variance σ_a^2. Specifically, we look to the inverted Wishart distribution where the expected prior mean for the additive genetic variance was started at 1.0 and the shape parameter was 20. The shape parameter reflects the degree of certainty we have in the choice of prior mean for the additive genetic variance (the larger the value the more certain). A value of 20, speaking relatively, would be considered large and tend to keep the estimate of the posterior density of the additive genetic variance "close" to the prior density. Analyses were conducted with smaller shape parameters (as well as different starting mean values for the additive genetic variance), but all had the same general behavior of the estimate of the posterior density always returning with a heritability value much higher than the value where we began the search.

Estimation of the distribution of the unknown parameters employs a technique of numerical integration referred to as Gibbs sampling (Geman and Geman 1984). The

algorithm is based on the iterative generation of a sequence of random variables from the known conditional distributions of the parameters, given the likelihood function of the data. Subsequent estimates of the parameters are found in the analysis of this sequence of random numbers, called the Gibbs sample. In this study, a total of 100,000 samples of possible heritability values were generated. The estimate of heritability was taken from the mean of every 25^{th} iterate, after discarding the first 10,000 samples, for a total of 3,600 sample observations (i.e., [100,000-10,000]/25 = 3,600). A more complete description of the Gibbs sampling process and its theoretical justification may be found in Sorensen et al. (1995), and in Van Tassell and Van Vleck (1995), published by the authors of the public domain software, MTGSAM (Van Tassell and Van Vleck 1995), with which this analysis was performed.

Complex segregation analysis

Regressive logistic models developed for complex segregation analysis (Bonney 1986) were used to evaluate the possible segregation of a single major locus with a large effect on deafness in the Dalmatian. A thorough discussion of complex segregation analysis is available (Lynch and Walsh 1998). The technique is intended to integrate Mendelian transmission genetics, allelic frequency, and penetrance with the patterns of covariance expected in polygenic inheritance. Elston et al. (1975) outlined the criteria that must be satisfied before acceptance of the single major locus model. Adherence to these criteria reduces the number of false positives. Evaluation of the models necessary

for complex segregation analysis was conducted with the statistical analysis for genetic epidemiology (S.A.G.E.) software (1997).

S.A.G.E. requires a family structure without "loops" (i.e., a pedigree free of inbreeding). This limitation is not genetic or statistical, but a computational requirement. Currently there is not a software program designed to analyze pedigrees with inbreeding loops to the extent observed in the kindred assembled for this study. Accordingly, the kindred was subdivided into 27 sub-families to remove the loops created by inbreeding. Unfortunately, this may eliminate potentially important genetic information. Creation of the sub-families began with the 199 dogs diagnosed by BAER, and identification of their parents, grandparents and great-grandparents (ignoring ancestors beyond three generations) to build all possible three-generation pedigrees from the kindred. Exclusion of ancestors beyond three-generations for each sub-family represents a compromise between the added genetic information that could be gained by including more than three generations and the increase of inbreeding loops that more generations would introduce. Sub-families still containing inbreeding loops were eliminated as well as families in which the auditory status of all animals was identical (i.e., all normal hearing).

Most dogs were represented in more than one of the 27 families. The duplication was necessary to give the software the impression of two different dogs from what was actually one dog. Though not ideal, this was the only means to evaluate this potentially genetically informative kindred. The impact on the final complex segregation analysis was expected to make the detection of a major locus more difficult because ties that are

known to exist were treated as being unrelated in the analysis. The magnitude of this effect could not be estimated but was assumed to be minor.

Methods to correct for sampling bias begin with an assumption about the sampling process. Employing an inappropriate correction for ascertainment bias can be as damaging to the interpretation of results as ignoring ascertainment bias (Greenberg 1986). For this reason, analyses were done with and without correction for ascertainment bias, with founders as a conditioning subset (Elston and Bonney 1986), an option in the S.A.G.E. software. The results for both analyses were similar so only results from the analysis with correction for ascertainment bias are reported.

For the purpose of estimating heritability, the implication of biased sampling on the evaluation of inheritance must be considered at several levels. The bias should be minimal if the stated assumption of no selection in the animals in this set of data without identified parents is of little effect. Estimation of genetic variances with mixed model methods for data that have been subjected to selection is unbiased when the base population can be considered a random sample (Henderson 1984). The impact of ascertainment bias on complex segregation analysis is less simply evaluated. Because the results are not from a randomly sampled cluster of Dalmatians, but rather a set constructed around several dogs with loss of hearing, this analysis must be corrected for such sampling bias.

Results

Of the 199 dogs (87 males, 112 females) with known auditory phenotypes, 148 dogs (74.4%) had normal hearing, 36 (18.1%) were unilaterally deaf and 15 (7.5%) were bilaterally deaf (Table 3). The relatedness of these dogs complicated generation of a graphical pedigree of all kindred members. A subset of 61 dogs with known auditory status, including six full litters with affected individuals, is shown in Figure 1. As an illustration of the relationships of the dogs, 125 of the total 266 were inbred, with an average inbreeding coefficient of 0.086 as calculated using the program MTGSAM (Van Tassell and Van Vleck 1995).

Table 4 presents a summary of the analysis of the threshold model, including an estimate of the heritability of deafness on the underlying, unobservable scale for the two phenotypic classification schemes (i.e., dichotomous and trichotomous). As shown, the mean heritability of the Gibbs sample is 0.73, with 95% of the values ranging from 0.55 to 0.89 for deafness when measured as a dichotomous trait and 0.75 (with 95% of the values ranging from 0.57 to 0.92) for deafness as a trichotomous trait.

Table 4 also contains evidence for equality in the incidence of deafness across genders. The mean difference in deafness between genders, on the underlying scale, was estimated as -0.49 with an empirical 95% confidence interval from –1.26 to 0.20. An interval that spans zero is evidence that no gender differences exist in the expression of deafness. The only descriptive character with a significant association with loss of hearing was eye color (Table 4), which did not have a confidence interval that spanned zero.

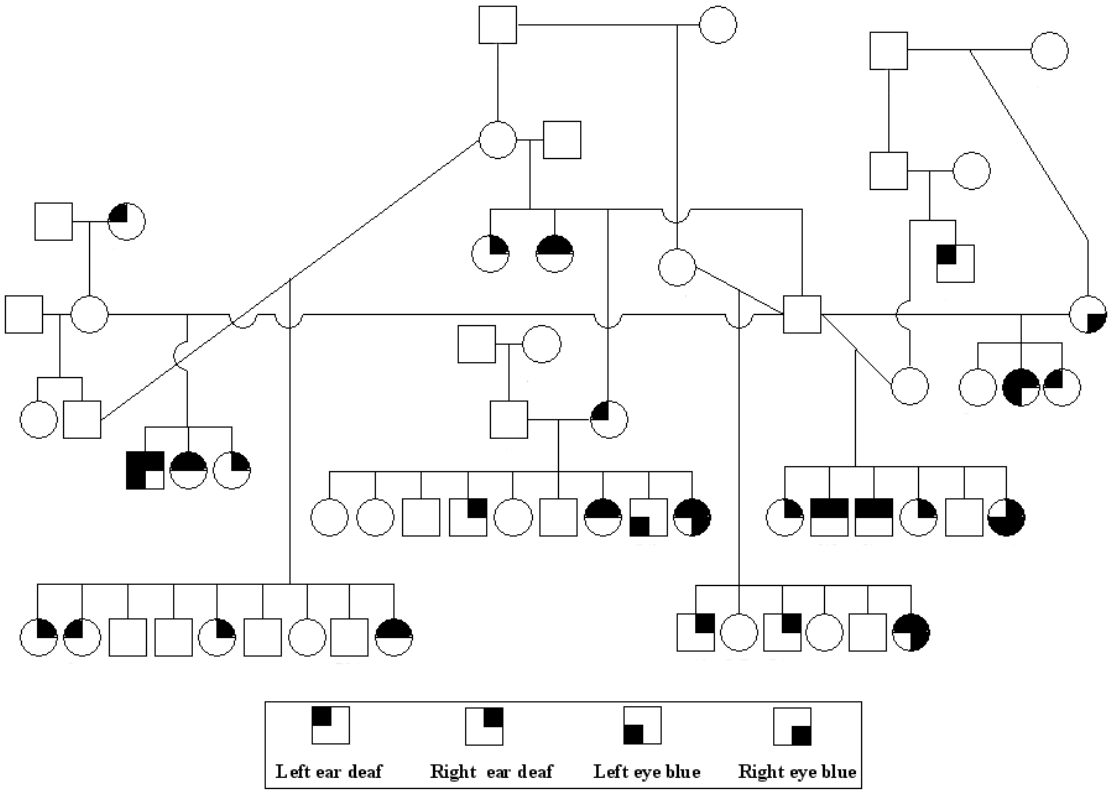

Figure 1. Subset pedigree of 61 Dalmatians with known auditory status, drawn using the software package Progeny (Progeny Software, LLC, South Bend, IN).

Table 4. Estimate of additive genetic variance, heritability, eye color contrast, spot color contrast and gender contrast in a threshold model for deafness measured in two and three categories.[a]

	Mean	Standard Deviation	Empirical 95% Confidence Interval
Dichotomous Trait			
Genetic Variance	3.28	1.99	1.25, 8.49
Heritability	0.73	0.09	0.55, 0.89
Eye Pigmentation[b]	-1.26	0.67	-2.76, -0.08
Black-Liver Spots	0.25	0.40	-0.48, 1.10
Male-Female	-0.49	0.37	-1.26, 0.20
Trichotomous Trait			
Genetic Variance	3.87	3.09	1.30, 11.92
Heritability	0.75	0.09	0.57, 0.92
Eye Pigmentation[b]	-1.97	0.77	-3.81, -0.68
Black-Liver Spots	0.14	0.40	-0.61, 0.97
Male-Female	-0.49	0.38	-1.30, 0.21

[a] Estimates are taken from a Gibbs sample of 3,600 values.
[b] Dogs with two brown eyes contrasted with dogs of one brown eye and one blue eye.

Table 5 presents results of the complex segregation analysis for dichotomous and trichotomous models of deafness with correction for ascertainment bias. The statistical models analyzed were: 1) a no major locus (NML) model, 2) a general major locus model with Mendelian transmission of the putative major allele (major locus Mendelian, MLM), and 3) a general major locus model in which the transmission probabilities are estimated from the pattern of inheritance revealed by the data (major locus arbitrary, MLA).

First, considering deafness as a dichotomous trait the natural log of the likelihood ratio (Table 5) in comparison of the NML and MLM models is calculated as -2(-158.69 - (-148.30)) = 20.78 (3 df, $P < 0.001$). This is a χ^2 statistic with degrees of freedom equal to the difference in number of parameters examined between models (in this case 5 parameters for the NML model and 8 parameters for the MLM model) and the P-values determined by the χ^2 distribution. This result shows that the MLM model provides a significantly better fit to the data than the NML model. However, the natural log of the likelihood ratio in comparison of the MLM and MLA models equals 22.38 (3 df, $P < 0.0001$) showing the MLA model provides a significantly better fit to the data than the MLM model.

Second, considering deafness as a trichotomous trait the natural log of the likelihood ratio in comparison of the NML and MLM models (Table 5) is 7.10 (3 df, $P < 0.07$) showing the NML model does not provide a significantly better fit to the data, at least when using the "standard" Type I error at $P = 0.05$. This result differs from comparison of the same models considering deafness as a dichotomous trait. The natural

Table 5. Parameter (PR) estimates (± standard error, SE) from the logistic regression model in complex segregation analysis of dichotomous and trichotomous deafness in the Dalmatian with correction for ascertainment bias.

	No Major Locus		**Major Locus Mendelian**		**Major Locus Arbitrary**	
	PR	SE	PR	SE	PR	SE
Dichotomous Trait						
P(A)[a]	n/a		0.23	0.09	0.89	0.11
Pooled Base	-1.08	0.47	N/a		n/a	
AA Base	n/a		-3.91	1.83	3.86	1.34
AB Base	n/a		1.18	2.70	-3.34	3.53
BB Base	n/a		-4.78	1.01	-1.61	0.91
τ^{b}_{AA}	n/a		1.00	Fixed	0.48	0.10
τ_{AB}	n/a		0.50	Fixed	0.0	0.0
τ_{BB}	n/a		0.00	Fixed	1.00	0.0
Sex[c]	-1.11	0.55	-1.55	0.44	-4.41	1.19
Eye Color Pattern[d]	-0.42	0.18	-0.07	0.31	-0.84	0.55
Spot Color[e]	-1.03	0.43	-0.31	0.37	-0.62	0.58
Parent[f]	-0.32	0.15	-0.52	0.19	-0.35	0.28
Nat Log of Likelihood	-158.69		-148.30		-137.11	
Trichotomous Trait						
P(A)[a]	n/a		0.29	0.11	0.89	0.11
Pooled Base	-1.71	0.47	n/a		n/a	
AA Base	n/a		-3.84	1.45	3.17	1.32
AB Base	n/a		-4.55	2.99	-4.03	3.42
BB Base	n/a		-0.33	0.78	-2.30	0.90
τ^{b}_{AA}	n/a		1.00	Fixed	0.48	0.10
τ_{AB}	n/a		0.50	Fixed	0.0	0.0
τ_{BB}	n/a		0.00	Fixed	1.0	0.0
Sex[c]	-1.01	0.61	-1.99	0.41	-4.41	1.18
Eye Color Pattern[d]	-0.56	0.21	-0.20	0.26	-0.84	0.55
Spot Color[e]	-0.97	0.40	-0.61	0.33	-0.62	0.57
Parent[f]	-0.38	0.16	-0.48	0.18	-0.35	0.28
Nat Log of Likelihood	-210.68		-207.13		-189.10	

[a] Frequency of the putative major allele A.
[b] Major locus transmission probabilities.
[c] Regression effect for sex (0 for female; 1 for male).
[d] Regression effect for eye color (0 for two brown eyes; 1 for one brown eye, one blue eye).
[e] Regression effect for spot color (0 for black; 1 for liver).
[f] Regression effect for parent's deafness phenotype.

log of the likelihood ratio in comparison of the MLM and MLA models equals 36.06 (3 df, $P < 0.0001$) showing the MLA model provides a significantly better fit to the data, the same result as comparison of the same models considering deafness as a dichotomous trait.

Discussion

Heritability and segregation analysis

It is clear from the results presented in Table 4 that deafness in the Dalmatian is hereditary and is influenced by genetic information passed from parent to offspring. Furthermore, the heritability of deafness is of sufficient magnitude that attempts to select against it are potentially successful. A heritability of this magnitude is suggestive, by itself, of the segregation of a single major locus exerting a large effect. Morton and MacLean (1974) demonstrated that major loci tend to increase the heritability of a trait in a given population and a value greater than 0.70 is comparatively large for many polygenic traits, indicating that deafness in the Dalmatian may not be polygenic. This does not preclude other genes or loci exerting an effect on the major locus.

However, the results of Tables 4 and 5 raise important issues. Firstly, there is the obvious question of which analysis is "correct". The threshold model of heritability in Table 4 and the NML model of Table 5 are conceptually, though not identically, similar. That is both seek to evaluate the inheritance of deafness with explanatory variables of sex, eye color, and spot color. Yet the approach is fundamentally quite different indeed. The threshold model is built around underlying normality in the distributions of

genotypes and environmental contributions (Gianola and Foulley 1983). The complex segregation analysis is derived from logistic regression and the linearity of the log odds of deafness (Bonney 1986).

Conceptually, the threshold model provides a better approach for quantitative genetics analogous to the commonly used mixed models of polygenic continuous phenotypes. Moreover the threshold model permits the inclusion and consideration of all known relationships, including the magnitude of inbreeding present in this kindred. This cannot be said of the logistic regression model for complex segregation analysis. The logistic regression model can only accommodate specific relationships, such as parent-progeny; and inbreeding "loops" cannot be present in families of the data set (S.A.G.E. 1997). Accordingly, owing to limitations of available software (specifically there being no complex segregation analysis packages for dichotomous and trichotomous traits in a threshold model), we have a two-step analysis of the kindred in this data set.

The comparison of the MLM and MLA models in Table 5, considering deafness as either a dichotomous or trichotomous trait, is suggested by Elston et al. (1975) to reduce the probability of falsely declaring the presence of a major locus. Alleles at a genuine major locus should be transmitted from parent to offspring with probabilities that reflect Mendelian transmission. Table 5 demonstrates that a better fit to the data can be provided when the probabilities of transmission are significantly different from those expected under standard Mendelian transmission. Though we can conclude that deafness is highly heritable from the results in Table 4, the exact genetic mechanism that

leads to expression of this disease cannot be stated with certainty based on the results in Table 5. Accordingly, we also conclude that a major locus with an impact on deafness cannot be established with the present data.

Nonetheless, we are encouraged to observe a rough equivalence in the threshold model results of Table 4 with that of the NML models of Table 5. Given the standard errors of Table 5, confidence intervals can simply be constructed (i.e., 95% intervals computed from the parameter estimate ± 1.96 times the standard error) and evaluated for overlap with 0.0. As such, all the logistic regression coefficients are significantly different from zero, with the exception of differences in gender. Note, however, that the parent regression coefficient is negative, implying that normal hearing parents are more likely to have deaf offspring than deaf parents. Figure 1 offers visual support of this result. That is, while only a snapshot of the kindred, there are only three unilaterally deaf dogs that are parents (P04, P14, and R10), all other hearing impaired dogs are without progeny in the figure. As previously stated, there were 74 matings between parents with known auditory status present in the kindred; 60 matings occurred between unaffected parents, 13 matings occurred between an unaffected parent and a unilaterally deaf parent, and one mating occurred between two unilaterally deaf parents. Interestingly, the heritability of hearing loss is still high for dichotomous deafness with a value of 0.73. It is not possible to directly relate the parent regression coefficient of the NML model to the well-recognized parameter of heritability. However, we can see how knowledge of all relationships, made possible in the threshold model, can provide a more thorough evaluation of inheritance than logistic regression.

A manual review of the pattern of inheritance did not support a model of a simple autosomal Mendelian locus. For example, the majority of the affected progeny were the result of matings of two unaffected parents, eliminating models of a single dominant deafness allele. Discarding a model of a single recessive autosomal allele is not possible with the kindred, because there were not any matings of two bilaterally deaf dogs. However, there was a mating of two unilaterally deaf dogs (both deaf in the same ear, with two brown eyes, and with black spots) and the argument can be made that if the auditory phenotype is a dichotomous trait, this mating would support discarding the model of a single recessive autosomal allele because it produced normal hearing offspring. Further support for discarding a single recessive allele is provided by several unrelated matings of bilaterally deaf parents not in this kindred (Strain 1999) that produced normal, unilaterally deaf, and bilaterally deaf offspring.

Famula et al. (2000) reported a heritability estimate of 0.32 in Californian Dalmatians, a value much lower than the estimates presented here. However, Famula et al. (2001) included more records of Californian Dalmatians in a larger data set and reported a heritability estimate of 0.76, a value comparable to the estimates presented here.

Although deafness in the Dalmatian is clearly inherited, the evidence for the presence of a single major gene affecting the disorder is not persuasive with the data from this kindred. Famula et al. (2000) and Muhle et al. (2002) provided evidence of a single major locus of large effect on deafness in Californian and Swiss Dalmatians, respectively. Jarvik (1998), in a review of complex segregation analysis, suggested

prudence in the interpretation of complex segregation analysis until several sets of data had confirmed or rejected the presence of a Mendelian locus.

Future directions

While the absence of a clear mode of inheritance complicates genetic dissection of deafness in the Dalmatian, the assembling of this kindred provides a tool for eventually defining the genetic bases of this disorder. This set of Dalmatians provides a potentially informative group with which to perform a whole-genome scan and the analyses of the kindred described here will assist evaluation of linkage data generated by utilizing a set of multiplexed canine microsatellite markers (Cargill et al. 2002). Due to the uncertainty of the genetic mechanism of deafness, other experimental approaches such as examination of candidate genes may not be as effective as a genome scan. Brenig et al. (2003) reported *PAX3*, a gene implicated in Waardenburg Syndrome, is not responsible for deafness in the Dalmatian. Other candidate genes could be examined, but the possibility exists that the gene responsible for deafness in the Dalmatian has not been characterized in another species. The number of genes associated with deafness in the human and mouse (Steel and Bussoli 1999; Steel and Kros 2001) is quite large. Because of this, the time and cost required to analyze each in the Dalmatian is not justified when tools are available to localize chromosomal regions through a genome scan. Linkage analysis of observed microsatellites in this kindred of Dalmatians will hopefully reveal chromosomal regions harboring the gene(s) causative for deafness in the Dalmatian.

CHAPTER IV

WHOLE GENOME SCAN PART 1:

DNA, MICROSATELLITE MARKERS, & A LINKAGE MAP

Introduction

The first step in performing a whole genome scan in the dog was the characterization of a minimal screening set of microsatellite markers (Richman et al. 2001) that is termed Minimal Screening Set 1 (MSS1). The resolution of this marker set was estimated at 10 cM, but not all of the 172 markers in the set are localized to specific chromosomes by linkage or radiation hybrid (RH) data. Each MSS1 marker's position was reported based on the placement in previous linkage maps (Werner et al. 1999, Mellersh et al. 2000). Only 43.6% (N = 75) of the markers were positioned on a total of 15 chromosomes, including the X chromosome. There were 20 markers positioned on chromosomes (Mellersh et al. 2000). Werner et al. (1999) positioned those same markers in linkage groups. The remaining 77 MSS1 markers were positioned in linkage groups (Werner et al. 1999, Mellersh et al. 2000). Despite the lack of definitive positions for all the markers in the MSS1, statistically 77% of the canine genome was within 10 cM of at least one marker in the MSS1 (Richman et al. 2001). The MSS1 was multiplexed (Cargill et al. 2002) to provide a more efficient method for whole genome scans.

Dukes-McEwan and Jackson (2002) discussed the limitations, not only of the availability of a complete marker screening set, but of the resolution of the genome map itself. Citing the difficulties of conducting linkage in an organism as inbred as the dog, called for a map with a higher marker density because there had not been a major

linkage or RH map update since construction of an integrated linkage/RH map of 1,800 markers (Breen et al. 2001).

An updated RH map comprised of 3,270 markers providing 1 Mb resolution was recently constructed (Guyon et al. 2003). Examination of the locations of the markers present in the MSS1 with respect to this map revealed 68.6% (N = 118) MSS1 markers had been positioned on chromosomes, an increase of 43 markers from Richman et al. (2001). The MSS1 markers are listed in Table 6 according to locations from map of Guyon et al. (2003) and linkage data. Each marker's current location on the RH map is given as well as the marker's previous location as reported (Richman et al. 2001). A reference is also given for each marker's original publication.

In addition to the updated RH map, a second-generation screening set, termed Minimal Screening Set 2 (MSS2), was developed (Guyon et al. 2003). The MSS2 provides 9 Mb resolution of the canine genome achieved with 327 markers, all positioned on the RH map. The benefits of the MSS2 over the MSS1 are obvious (e.g., known marker placement and better coverage of the genome). However, many researchers have invested laboratory finances, time, and effort into making use of the MSS1 and the MSS2 was only recently developed (Guyon et al. 2003). Therefore, mapping of any MSS1 markers not included on the 1 Mb RH map would benefit those using the MSS1.

The multiplexed MSS1 was used in a whole genome scan of a Dalmatian kindred (Cargill et al. 2004). Prior to linkage analysis of traits present in the kindred, the marker data generated by this scan were used to build a linkage map based on the Dalmatian

Table 6. Updated locations of the MSS1 markers based on the latest RH map (Guyon et al. 2003).

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
Markers localized on the RH map Guyon et al. (2003)				
CFA01				
FH2016		CFA01	CFA01	Francisco et al. 1996
C01673		CFA01	CFA01	Ostrander et al. 1995
C01246		CFA01	CFA01	Ostrander et al. 1993
C01424		CFA01	CFA01	Ostrander et al. 1995
FH2309		CFA01	CFA01	Mellersh et al. 1997
FH2598		CFA01	CFA01	Werner et al. 1999
FH2294		CFA01	CFA01	Mellersh et al. 1997
CFA02				
FH2132		CFA02	CFA02	Francisco et al. 1996
FH2062		CFA02	CFA02	Francisco et al. 1996
C02342		CFA02	CFA02	Neff et al. 1999
CFA03				
FH2316		CFA03	CFA03	Mellersh et al. 1997
FH2107		CFA03	CFA03	Francisco et al. 1996
FH2302		CFA03	CFA03	Mellersh et al. 1997
CFA04				
FH2412		CFA04	S1/L2	Werner et al. 1999
FH2142		CFA04	S1/L2	Francisco et al. 1996
AHT103		CFA04	S1/L2	Holmes et al. 1995
FH2457		CFA04	S1/L2	Werner et al. 1999
CFA05				
ZuBeCa6		CFA05	CFA05	Ladon et al. 1998
CPH18		CFA05	CFA05	Fredholm et al. 1995
C05377		CFA05	CFA05	Ostrander et al. 1995
TAT		CFA05	Unlinked	Neff et al. 1999
CFA06				
FH2525		CFA06	CFA06	Werner et al. 1999
FH2119		CFA06	CFA06	Francisco et al. 1996
CFA07				
FH2226		CFA07	CFA07/L1	Mellersh et al. 1997
FH2174		CFA07	CFA07/L1	Francisco et al. 1996
FH2201		CFA07	CFA07/L1	Francisco et al. 1996
FH2581		CFA07	CFA07/L1	Werner et al. 1999
CFA08				
C08410		CFA08	CFA08	Ostrander et al. 1995

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
FH2144		CFA08	CFA08	Francisco et al. 1996
C08618		CFA08	CFA08	Ostrander et al. 1995
CFA09				
GALK1		CFA09	CFA09	Neff et al. 1999
FH2263		CFA09	CFA09	Mellersh et al. 1997
FH2186		CFA09	CFA09	Mellersh et al. 1997
CFA10				
FH2537		CFA10	CFA10	Werner et al. 1999
FH2293		CFA10	CFA10	Mellersh et al. 1997
C1016		CFA10	CFA10	Ostrander et al. 1993
FH2422		CFA10	CFA10	Werner et al. 1999
CFA11				
AHT137		CFA11	S4/L3	Holmes et al. 1995
FH2096		CFA11	S4/L3	Francisco et al. 1996
FH2319		CFA11	S4/L3	Mellersh et al. 1997
C11873	CXX873	CFA11	S4/L3	Neff et al. 1999
LEI001	AHT001	CFA11	S4/L3	Holmes et al. 1993
CFA12				
FH2200		CFA12	CFA12/L4	FHCRC 2003
FH2347		CFA12	CFA12/L4	Mellersh et al. 1997
CFA13				
C13391	CXX391	CFA13	S8/L8	Ostrander et al. 1995
C13900	CXX900	CFA13	S8/L8	Neff et al. 1999
CFA14				
FH2600		CFA14	S16/L21	Werner et al. 1999
FH2060		CFA14	S9/L5	Francisco et al. 1996
PEZ10		CFA14	S9/L5	Neff et al. 1999
CFA15				
REN06C11		CFA15	S6/L12	Jouquand et al. 2000
FH2171		CFA15	S6/L12	Francisco et al. 1996
FH2278		CFA15	S6/L12	Mellersh et al. 1997
AHT139		CFA15	S6/L12	Holmes et al. 1995
CFA16				
FH2155		CFA16	S12/L11	Francisco et al. 1996
FH2175		CFA16	S12/L11	Francisco et al. 1996
CFA17				
Cos15		CFA17	CFA15	Werner et al. 1999
FH2321		CFA17	CFA15	Mellersh et al. 1997
CPH5		CFA17	CFA15	Fredholm et al. 1995

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
PEZ8		CFA17	S16/L21	Neff et al. 1999
CPH10		CFA17	S16/L21	Fredholm et al. 1995
CFA18				
FH3010		CFA18	CFA18	FHCRC 2003
FH2429		CFA18	CFA18	Werner et al. 1999
AHT130		CFA18	CFA18	Holmes et al. 1995
CFA19				
AHT124		CFA19	CFA13,19/L17	Holmes et al. 1994
FH2206		CFA19	CFA13,19/L17	Mellersh et al. 1997
CFA20				
REN55P21		CFA20	CFA20	Jonasdottir et al. 1999
CPH16		CFA20	CFA20	Neff et al. 1999
PRKCD		CFA20	CFA20	Werner et al. 1999
AHTk209		CFA20	CFA20	Thomas et al. 1997
CFA21				
FH2233		CFA21	S2/L13	Mellersh et al. 1997
FH2441		CFA21	S2/L13	Werner et al. 1999
FH2312		CFA21	S2/L13	Mellersh et al. 1997
CFA22				
REN49F22		CFA22	S7/L7	Jouquand et al. 2000
REN49C08		CFA22	S7/L7	FHCRC 2003
C22279	CXX279	CFA22	S7/L7	Ostrander et al. 1993
FH2538		CFA22	S7/L7	Neff et al. 1999
CFA23				
FH2227		CFA23	CFA22	Mellersh et al. 1997
FH2001		CFA23	CFA22	Francisco et al. 1996
CFA24				
FH2159		CFA24	S5/L18	Francisco et al. 1996
FH2261		CFA24	S5/L18	Mellersh et al. 1997
FH2079		CFA24	S5/L18	Francisco et al. 1996
CFA25				
FH2141		CFA25	S10/L6	Francisco et al. 1996
C25213	CXX213	CFA25	S10/L6	Ostrander et al. 1993
CFA26				
REN01O23		CFA26	CFA26	Jouquand et al. 2000
FH2130		CFA26	CFA26	Francisco et al. 1996
REN48E01		CFA26	CFA26	FHCRC 2003
C26733		CFA26	CFA26	Mellersh et al. 1997

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
CFA27				
FH2289		CFA27	CFA16	Mellersh et al. 1997
LEI002	AHT002	CFA27	CFA16	Holmes et al. 1993
FH2346		CFA27	CFA16	Mellersh et al. 1997
PEZ6		CFA27	CFA16	Neff et al. 1999
CFA28				
C28176	CXX176	CFA28	S14/L15	Ostrander et al. 1995
FH2208		CFA28	S14/L15	Mellersh et al. 1997
FH2585		CFA28	S14/L15	Werner et al. 1999
LEI006	AHT006	CFA28	S14/L15	Holmes et al. 1993
REN51i12		CFA28	S14/L15	FHCRC 2003
CFA29				
FH2364		CFA29	S3/L14	Mellersh et al. 1997
REN45F03		CFA29	S3/L14	FHCRC 2003
FH2385		CFA29	S3/L14	Werner et al. 1999
CFA30				
FH2050		CFA30	CFA30/L19	Francisco et al. 1996
FH2290		CFA30	CFA30/L19	Mellersh et al. 1997
LEI-1F11	1F11	CFA30	CFA30/L19	FHCRC 2003
CFA31				
FH2239		CFA31	S13/L10	Mellersh et al. 1997
CFA32				
CPH2		CFA32	S15/L20	Fredholm et al. 1995
REN41D20		CFA32	S15/L20	Jouquand et al. 2000
AHT127		CFA32	S15/L20	Holmes et al. 1995
CFA33				
FH2165		CFA33	CFA29,35/L16	Francisco et al. 1996
CFA34				
FH2377		CFA34	Unlinked	Werner et al. 1999
CFA35				
REN01G01		CFA35	Unlinked	Jouquand et al. 2000
CFA36				
n/a		n/a	n/a	n/a
CFA37				
C37172	CXX172	CFA37	S11/L9	Ostrander et al. 1993
AHT133		CFA37	S11/L9	Holmes et al. 1995
FH2587		CFA37	S11/L9	Werner et al. 1999
FH2532		CFA37	S11/L9	Werner et al. 1999

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
CFA38				
REN02C20		CFA38	S17/L22	Werner et al. 1999
CFAX				
FH2985		CFX	CFX	FHCRC 2003
FH2548		CFX	CFX	Werner et al. 1999
FH2584		CFX	CFX	Werner et al. 1999
F8	F8C	CFX	CFX	FHCRC 2003
CFAY				
SRY		CFY	CFY	Meyers-Wallen et al. 1995
Markers localized by linkage data				
CFA02				
C02864A	CXX864	n/a	CFA02	Neff et al. 1999
AHT132		n/a	CFA02	Neff et al. 1999
FH2087A	FH2087U	n/a	CFA02	Mellersh et al. 1997
CFA03				
C03895		n/a	CFA03	Neff et al. 1999
FH2531		n/a	CFA03	Werner et al. 1999
FH2137		n/a	CFA03	Francisco et al. 1996
CFA05				
FH2594		n/a	CFA05	Werner et al. 1999
GLUT4		n/a	CFA05	Jonsdottir et al. 1999
FH2383		n/a	CFA05	Werner et al. 1999
CFA06				
C06636		n/a	CFA06	Ostrander et al. 1995
CPH3		n/a	CFA06	Fredholm et al. 1995
CFA07				
FH2301		n/a	CFA07/L1	Mellersh et al. 1997
FH2396		n/a	CFA07/L1	Werner et al. 1999
C071000	TETRA	n/a	CFA07/L1	Neff et al. 1999
CFA08				
FH2149		n/a	CFA08	Francisco et al. 1996
CFA10				
FH2339		n/a	CFA10	Mellersh et al. 1997
CFA12				
C12852		n/a	CFA12/L4	Neff et al. 1999
FH2223		n/a	CFA12/L4	Mellersh et al. 1997
CFA16				
RVCE		n/a	CFA16	Molyneux et al. 1994

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
CFA18				
WILMSTF		n/a	CFA18	Neff et al. 1999
FH2356		n/a	CFA18	Mellersh et al. 1997
CFA20				
FH2528		n/a	CFA20	Werner et al. 1999
CFA22				
FH2283		n/a	CFA22	Mellersh et al. 1997
FH2325		n/a	CFA22	Mellersh et al. 1997
C22745		n/a	CFA22	Mellersh et al. 1997
CFA26				
FH2566		n/a	CFA26	Werner et al. 1999
N41		n/a	CFA26	Werner et al. 1999
CFA30				
FH2305		n/a	CFA30/L19	Mellersh et al. 1997
Markers not localized to a chromosome				
FH2138		Unlinked	CFA08	Francisco et al. 1996
FH2279		n/a	CFA13,19/L17	Mellersh et al. 1997
FH2507		n/a	CFA29,35/L16	Werner et al. 1999
CXX672		n/a	L1/L23	Ostrander et al. 1995
FH2516		n/a	L1/L23	Werner et al. 1999
AHT128		n/a	S1/L2	Holmes et al. 1998
FH2534		n/a	S1/L2	Werner et al. 1999
AHT140		n/a	S10/L6	Holmes et al. 1998
FH2526		n/a	S10/L6	Werner et al. 1999
AHT131		n/a	S12/L11	Holmes et al. 1995
FH2199		n/a	S13/L10	Mellersh et al. 1997
CXX642	CXX646	n/a	S13/L10	DogMap 2003
FH2238		n/a	S15/L20	Mellersh et al. 1997
FH2244		n/a	S17/L22	Mellersh et al. 1997
CXX750		n/a	S4/L3	Mellersh et al. 1997
FH2018		n/a	S4/L3	Francisco et al. 1996
C02608	CXX608	n/a	S6/L12	Ostrander et al. 1995
FH2394		n/a	S8/L8	Werner et al. 1999
FH2547		n/a	S9/L5	Werner et al. 1999
CXX3902		n/a	S9/L5	Ostrander et al. 1995
AHT106		n/a	Unlinked	Holmes et al. 1993
CXX452		n/a	Unlinked	Ostrander et al. 1995
FH2247		n/a	Unlinked	Mellersh et al. 1997

Table 6. Continued.

Marker	Alias[a]	Location[b]	Previous Location[c]	Reference[d]
PEZ2		n/a	Unlinked	Neff et al. 1999
PEZ7		n/a	Unlinked	Sutton et al. 1998
FH2550		n/a	Unlinked	Werner et al. 1999

[a] Name of marker as originally published, see marker reference.
[b] Location of marker as reported by Guyon et al. (2003).
[c] Location of marker as reported by Richman et al. (2001).
[d] Earliest available reference to each marker.

kindred in an attempt to place more markers on specific chromosomes.

The specific objectives of this work were to 1) collect genotypes for the MSS1 markers from the Dalmatian kindred and 2) build a genetic linkage map based on the marker data.

Materials and methods

Dalmatian samples

Genomic DNA was isolated from 117 dogs (54 males, 63 females) of the Dalmatian kindred (Cargill et al. 2004) for a whole genome scan. The remaining 149 dogs of the kindred were unavailable for collection of DNA samples. Of the 117 dogs, 77 (65.8%) had normal hearing, 25 (21.4%) were unilaterally deaf, and 15 (12.8%) were bilaterally deaf. Eye color and spot color phenotypes of these dogs are shown in Table 7. DNA was isolated as reported in Chapter II.

Table 7. Phenotypes[a] of the 117 Dalmatians with a DNA sample.

Phenotype	Male	Female	Total
Hearing/Brown Brown/Black	27	25	52
Hearing/Brown Brown/Liver	12	9	21
Hearing/Brown Blue/Black	1	2	3
Hearing/Brown Blue/Liver	1	0	1
Unilateral/Brown Brown/Black	4	12	16
Unilateral/Brown Brown/Liver	2	6	8
Unilateral/Blue Blue/Black	0	1	1
Deaf/Brown Brown/Black	2	2	4
Deaf/Brown Brown/Liver	2	2	4
Deaf/Brown Blue/Black	2	4	6
Deaf/Brown Blue/Liver	1	0	1
Total	54	63	117

[a] Auditory status/eye color/spot color.

Microsatellite markers

Microsatellite markers were amplified and resolved in multiplex sets exactly as described in Chapter II.

Genotype data

The software program Genoprob version 2.0 (Thallman et al. 2001a, 2001b) was used to compute phase and genotype probabilities based on the full pedigree and the linkage map positions. Additionally, Genoprob was used to detect likely genotyping errors. Genotypes with an error probability greater than 0.70 were checked against the original GENESCAN data file and corrections were made when necessary. Genoprob also computes the genotype probability for individuals with an unknown genotype using marker data of related individuals. From the 266 member kindred, 117 dogs (44%) were genotyped. Using a posterior genotype probability of > 0.95, an additional 2% of genotypes were incorporated into the data set.

Marker map

A linkage map for each canine autosome was constructed using CRIMAP v. 2.4 (Green et al. 1990). Initially, each chromosome was built individually using the BUILD option based on markers appearing on the RH map (Guyon et al. 2003). MSS1 markers not appearing on the RH map were localized to chromosomes using the TWOPOINT option and a LOD threshold of 3.0. Unmapped markers showing linkage to a chromosome were incorporated into the map using the BUILD option. The FLIPS

option was used to evaluate local permutations of marker order. Finally, the CHROMPIC option was used to identify spurious double recombinants and to facilitate the correction of genotyping errors.

Results

Of the 173 MSS1 markers, 149 were polymorphic in the Dalmatians, 13 were monomorphic, and 11 could not be amplified despite multiple optimization attempts. Additionally, 119 of the MSS1 markers had previously been placed on the 1 Mb RH map and 109 of these markers were amplified in the Dalmatians. Of the remaining 54 MSS1 markers not placed on the 1 Mb RH map, 28 markers had previously been placed on chromosomes by linkage data (and all 28 were amplified in the Dalmatians). There are 26 markers that had not previously been placed on a chromosome by linkage or RH mapping (25 were amplified in the Dalmatians).

The 13 monomorphic markers were FH2149 (CFA08), FH2339 (CFA10) FH2347 (CFA12), AHT130 (CFA18), FH2528 (CFA20), REN01O23 (CFA26), FH2050 (CFA30), FH2165 (CFA33), C37172 (CFA37), REN02C20 (CFA38), AHT131 (Unlinked), AHT140 (Unlinked), and FH2534 (Unlinked). The 11 markers that could not be amplified were FH2457 (CFA04), ZuBeCa6 (CFA05), FH2226 (CFA07), FH2200 (CFA12), FH2600 (CFA14), FH2206 (CFA19), N41 (CFA26), FH2346 (CFA27), LEI006 (CFA28), REN51i12 (CFA28), and FH2279 (Unlinked).

A linkage map of the polymorphic markers was built with CRIMAP. The map is shown in Table 8. The order of the markers on each chromosome corresponds to the order as they are listed on the 1 Mb RH map (Guyon et al. 2003). In total, 14 MSS1

markers that were not position on the RH map (Guyon et al. 2003) were added to the linkage map.

Seven markers were mapped to the same chromosomes suggested by previous linkage data. Using the Dalmatian data, four markers mapped to different chromosomes than those implicated with previous linkage data. FH2137 (Francisco et al. 1996) and FH2531 (Werner et al. 1999) mapped to CFA03 with C02864A and other markers (FH2316, FH2107, FH2302) already positioned on the RH map. FH2137 and FH2531 had been localized to CFA03 before while C02864A was previously localized to CFA02 (Neff et al. 1999). The markers were placed on CFA03 in the following order: FH2137 – (LOD = 3.93) – FH2531 – (LOD = 4.24) – C02864A – (LOD = 4.96) – FH2316 – (LOD = 5.60) – FH2107 – (LOD = 1.27) – FH2302.

C071000 (Neff et al. 1999), FH2396 (Werner et al. 1999), and FH2301 (Mellersh et al. 1997) mapped to CFA07 with C03895 and other markers (FH2174, FH2201, FH2581) already positioned on the RH map. C071000, FH2396, and FH2301 had been localized to CFA07 before while C03895 was previously localized to CFA03 (Neff et al. 1999). The markers were placed on CFA07 in the following order: C071000 – (LOD = 5.48) – FH2174 – (LOD = 9.91) – FH2201 – (LOD = 3.25) – C03895 – (LOD = 1.82) – FH2396 – (LOD = 4.00) – FH2581 – (LOD = 15.16) – FH2301.

FH2138 (Francisco et al. 1996) mapped to CFA08 (linkage to marker C08618 with a LOD = 9.99) with markers (C08410, FH2144, C08618) positioned on the RH map. WILMSTF (Neff et al. 1999) mapped to CFA18 (linkage to marker FH3010 with a LOD = 6.60) with markers (FH3010, FH2429) positioned on the RH map.

Table 8. Linkage map[a] and radiation hybrid map[b] positions of 113 MSS1 microsatellite markers, which were polymorphic in a kindred of Dalmatians.

CFA	Marker	cM	Mb	CFA	Marker	cM	Mb	CFA	Marker	cM	Mb
1	FH2016	0.0	16.4	10	FH2537	0.0	9.5	22	REN49F22	0.0	2.8
	C01673	6.5	34.3		FH2293	16.5	43.6		REN49C08	15.5	31.6
	C01246	21.5	52.1		C1016	25.5	60.5		C22279	35.7	47.4
	C01424	54.5	68.7		FH2422	44.6	66.4		FH2538	50.4	57.1
	FH2309	78.5	98.7	11	AHT137	0.0	1.4	23	FH2227	0.0	47.9
	FH2598	89.2	127.2		FH2096	0.1	22.2		FH2001	94.4	58.5
	FH2294	105.6	129.4		FH2319	27.9	49.7	24	FH2159	0.0	22.3
2	FH2132	0.0	74.4		CXX750	90.5	n/a		FH2261	17.5	39.8
	FH2062	7.4	81.9		C11873	111.5	80.4		FH2079	39.1	61.4
	C02342	37.3	96.4		LEI001	131.1	83.9	25	FH2141	0.0	38.9
3	FH2137	0.0	n/a	13	C13391	0.0	0.0		C25213	17.9	50.9
	FH2531	28.6	n/a		C13900	53.9	70.5		FH2087A	28.8	n/a
	C02864A	42.4	n/a	14	FH2060	0.0	31.3	26	FH2130	0.0	39.3
	FH2316	63.7	67.7		PEZ10	38.2	71.2		REN48E01	16.8	42.7
	FH2107	76.9	91.0	15	REN06C11	0.0	35.4		C26733	16.8	42.7
	FH2302	104.4	99.5		FH2171	7.0	45.1	27	FH2289	0.0	3.1
4	FH2412	0.0	53.3		FH2278	107.0	71.7		LEI002	47.2	24.1
	FH2142	5.2	61.4		C02608	110.3	n/a		PEZ6	74.5	50
	AHT103	28.2	89.8		AHT139	114.9	73.8	28	C28176	0.0	0.0
5	CPH18	0.0	71.2	16	FH2155	0.0	51.7		FH2208	2.4	24.9
	C05377	19.1	88.9		FH2175	20.5	65.4		FH2585	26.7	36.4
	TAT	19.1	91.3	17	Cos15	0.0	7.1	29	FH2364	0.0	7.5
6	FH2525	0.0	10.8		FH3369	7.4	20.3		REN45F03	37.2	29.0
	FH2119	28.7	66.7		FH2321	9.9	21.5		FH2385	52.1	36.9
7	C071000	0.0	n/a		PEZ2	17.3	n/a	30	FH2290	0.0	30.8
	FH2174	15.4	55.0		CPH5	36.1	36.1		LEI-1F11	20.5	45.1
	FH2201	22.5	70.6		PEZ8	80.1	71.2	31	FH2239	0.0	31.4
	C03895	27.0	n/a		CPH10	87.8	73.8	32	CPH2	0.0	11.7
	FH2396	37.0	n/a	18	WILMSTF	0.0	n/a		REN41D20	3.4	12.8
	FH2581	62.4	76.8		FH3010	10.9	51.6		AHT127	25.0	44.6
	FH2301	74.0	n/a		FH2429	30.5	62.3	34	FH2377	0.0	23.9
8	C08410	0.0	41.9	19	AHT124	0.0	22.3	35	REN01G01	0.0	9.8
	FH2144	2.0	49.8	20	REN55P21	0.0	9.4	37	AHT133	0.0	18.5
	FH2138	49.4	n/a		CPH16	36.1	35.7		FH2587	13.8	30.4
	C08618	55.2	74.4		PRKCD	42.6	43.1		FH2532	32.7	38.8
9	FH2566	0.0	n/a		AHTk209	61.1	64.2				
	GALK1	13.5	1.0	21	FH2233	0.0	8.9				
	FH2263	21.3	9.0		FH2441	28.5	29.7				
	FH2186	50.1	35.9		FH2312	40.7	57.6				

[a] Sex averaged linkage map generated using Dalmatian marker data and CRIMAP; positions in Kosambi centiMorgans (cM).

[b] Radiation hybrid map as reported by Guyon et al (2003); positions in megabases (Mb).

Both FH2138 and WILMSTF had previously been localized to CFA08 and CFA18, respectively.

FH2566 was previously mapped to CFA26 (Werner et al. 1999), but mapped to CFA09 with markers (GALK1, FH2263, FH2186) already positioned on the RH map. Linkage was found with markers GALK1 (LOD = 6.31) and FH2263 (LOD = 8.58). FH2087A was previously mapped to CFA02 (Mellersh et al. 1997), but mapped to CFA25 with markers (FH2141, C25213) previously positioned on the RH map by linkage to marker C25213 (LOD = 4.50).

There were 3 markers that were never mapped to any chromosome by linkage or RH mapping data. CXX750 (Mellersh et al. 1997) had been placed in linkage group S4/L3, but mapped to CFA11 with markers (AHT137, FH2096, FH2319, C11873, LEI001) previously positioned on the RH map by linkage to marker C11873 (LOD = 6.32). CXX608 (Ostrander et al. 1995) had been placed on linkage group S6/L12, but mapped to CFA15 with markers (REN06C11, FH2171, FH2278, AHT139) previously positioned on the RH map by linkage to marker FH2278 (LOD = 14.12). PEZ2 (Neff et al. 1999) had been listed as unlinked, but mapped to CFA17 with markers (Cos15, FH2321, CPH5, PEZ8, CPH10) previously positioned on the RH map by linkage to markers Cos15 (LOD = 3.93), FH2321 (LOD = 15.91), and CPH5 (LOD = 9.33).

Placing these 14 additional markers on the linkage map yields a total of 113 polymorphic markers in this pedigree with known locations. Chromosomes 19, 31, 34, and 35 only have one polymorphic marker while chromosomes 12, 33, 36, and 38 have no polymorphic markers as determined from analysis of this Dalmatian kindred.

Approximately 12 chromosomes (CFA02, CFA04, CFA05, CFA06, CFA07, CFA08, CFA09, CFA13, CFA14, CFA16, CFA19, and CFA23) have significant gaps (> 20 cM) in marker coverage.

Discussion

The placement of 3 MSS1 markers that had never been placed on chromosomes before adds to the utility of the MSS1. Mapping of 7 MSS1 markers confirms previous linkage data. The placement of 4 MSS1 markers on chromosomes different than those determined through previous linkage data, highlights the need to place all of the MSS1 markers on the RH map. Forty MSS1 markers are still not placed on a chromosome by either the 1 Mb RH map (Guyon et al. 2003) or the linkage map based on the marker data from the Dalmatians.

CHAPTER V

WHOLE GENOME SCAN PART 2:

LINKAGE ANALYSIS OF DEAFNESS, EYE COLOR, & SPOT COLOR

Introduction

As previously discussed, the Dalmatian is affected by deafness more than any other breed. Twenty candidate genes for canine congenital sensorineural deafness were recently mapped (Rak et al. 2003). These 20 genes have been found to play a role in auditory function in other species and none, one, or several of these may be involved in deafness in the Dalmatian. The number of candidate genes for deafness prohibits the use of a functional candidate gene approach to identify the causal mutation(s) in the Dalmatian. Therefore, a whole genome scan approach is being employed to identify markers that co-segregate with deafness.

The multiplexed MSS1 is a highly effective tool with which to carry out a genome scan using the assembled kindred of Dalmatians. As previously discussed, there were 149 polymorphic MSS1 markers genotyped. A linkage map was built containing 113 of these markers in order to maximize the informativeness of the MSS1 markers as well as provide a map structure to perform multipoint linkage analysis in addition to twopoint linkage analysis. The remaining 36 polymorphic markers not included in the linkage map would be used for twopoint linkage analysis only.

The specific objective of this work was to identify regions of the genome associated with deafness using twopoint and multipoint linkage analysis with the

phenotypic traits segregating in the Dalmatian kindred (deafness, eye color, and spot color).

Materials and methods

Data source

Materials and methods for the Dalmatian samples, microsatellite markers, genotype data, and the marker map are the same as reported in Chapters II and IV.

Phenotypic traits

All three traits to be examined are qualitative and as such were coded in a binary fashion. For the purposes of traits with three classifications, such as deafness with normal hearing, unilaterally deaf, and bilaterally deaf, there are three possible categories to code the data. The trait can be coded as trichotomous, in which each classification is considered individually. The trait can also be coded as dichotomous type I, in which the two "affected" classifications are combined into one or as dichotomous type II, in which the two "unaffected" classifications are combined into one. Deafness and eye color each had five categories. Deafness consisted of: deafness as trichotomous (normal hearing – 0, unilaterally deaf – 1, bilaterally deaf – 2), deafness as dichotomous type I (normal hearing – 0, unilaterally deaf – 1, bilaterally deaf – 1), deafness as dichotomous type II (normal hearing – 0, unilaterally deaf – 0, bilaterally deaf – 1), the left ear (normal hearing – 0, deaf – 1) and the right ear (normal hearing – 0, deaf –1). Eye color consisted of: eye color as trichotomous (two brown eyes – 0, one brown eye and one

blue eye – 1, two blue eyes – 2), eye color as dichotomous type I (two brown eyes – 0, one brown eye and one blue eye – 1, two blue eyes – 1), eye color as dichotomous type II (two brown eyes – 0, one brown eye and one blue eye – 0, two blue eyes –1), the left eye (brown – 0, blue – 1) and the right eye (brown – 0, blue – 1). Spot color consisted of one category coded as black spots – 0, and liver spots – 1.

Linkage analysis

The program LOKI v2.4.5 (Heath 1997) was used to generate exact estimates of twopoint identity by descent (IBD) and multipoint identity by descent (MIBD) values via Markov Chain Monte Carlo (MCMC) analysis. The sex averaged linkage map generated from CRIMAP was used to estimate MIBD values along each chromosome at 1 cM intervals. A total of 205,000 iterations were run, with the first 5,000 iterations discarded and every 5^{th} iterate collected for a total of 40,000 data points to estimate the IBD and MIBD values.

Variance component interval mapping was performed using the program SOLAR v1.7.4 (Almasy and Blangero 1998) according to the developers' instructions. Twopoint linkage analysis using the IBD estimates obtained from LOKI was conducted using the TWOPOINT command. Multipoint interval analysis using the MIBD estimates obtained from LOKI was conducted at 1 cM intervals using the MULTIPOINT command.

Results

Twopoint linkage analysis

The results of the twopoint linkage analysis using SOLAR are shown in Table 9. The highest LOD scores obtained for each of the five deafness trait categories are listed in Table 9. A marker on CFA17, Cos15, had LOD scores of 1.41, 1.68, 0.86, and 1.01 for deafness as trichotomous, deafness as dichotomous type I, the left ear, and the right ear, respectively. For deafness as trichotomous, the only other LOD score above 1.0 was marker FH2585 on CFA28 with a LOD score of 1.30. For deafness as dichotomous type II, an unlinked marker, FH2238, had a LOD score of 1.32. For the right ear, an unlinked marker, FH2223, had a LOD score of 1.67.

For eye color, there were no LOD scores above 1.0. The highest LOD scores obtained for each of the five eye color trait categories are listed in Table 9. FH2238 had the highest LOD score results of 0.30 and 0.17 for eye color as trichotomous and dichotomous type I, respectively. CPH16 had the highest LOD score results of 0.41 and 0.01 for eye color as dichotomous type II and the right eye, respectively. FH2319 had the highest LOD score of 0.51 for the left eye. For the spot color trait, marker FH2319 on CFA11 had a LOD score of 9.76.

Table 9. Linkage analysis results using SOLAR for twopoint and multipoint analyses of deafness, eye color, and spot color.

	Twopoint			Multipoint		
Trait	**Marker**	**CFA**	**LOD**	**Marker**	**CFA**	**LOD**
Deafness						
Deafness (Trichotomous)	Cos15	17	1.41	Cos15	17	1.43
	FH2585	28	1.30	FH2585	28	1.30
Deafness (Dichotomous I)	Cos15	17	1.68	Cos15	17	1.69
Deafness (Dichotomous II)	FH2238	Unlinked	1.32	Cos15	17	0.48
Left Ear	Cos15	17	0.86	Cos15	17	0.85
Right Ear	FH2223	Unlinked	1.67	Cos15	17	1.02
	Cos15	17	1.01			
Eye Color						
Eye Color (Trichotomous)	FH2238	Unlinked	0.30	n/a	n/a	n/a
Eye Color (Dichotomous I)	FH2238	Unlinked	0.17	n/a	n/a	n/a
Eye Color (Dichotomous II)	CPH16	20	0.41	n/a	n/a	n/a
Left Eye	FH2319	11	0.51	n/a	n/a	n/a
Right Eye	CPH16	20	0.01	n/a	n/a	n/a
Spot Color						
Spot Color	FH2319	11	9.76	FH2319	11	9.70

Multipoint linkage analysis

The results of the multipoint linkage analysis using SOLAR are shown in Table 9. LOKI used the Haldane mapping function for computation of the IBD and MIBD probabilities. Thus, SOLAR results are in Haldane cM. For deafness, the highest LOD scores obtained for each of the five trait categories are listed in Table 9. For deafness as trichotomous, deafness as dichotomous type I, deafness as dichotomous type II, the left ear, and the right ear location 0 cM on CFA17 resulted in the highest LOD score with 1.43, 1.69, 0.48, 0.85, and 1.02 respectively. For deafness as trichotomous, location 32 cM on CFA28 had a LOD score of 1.30. Location 0 cM on CFA17 corresponds to marker Cos15 and location 32 cM on CFA28 corresponds to marker FH2585. The multipoint linkage analysis result for deafness as dichotomous type I on CFA17 is shown in Figure 2.

For eye color, there were no LOD scores above 0.5 from multipoint linkage analysis and thus, are not reported. Spot color yielded a LOD score of 9.70 on CFA11 at position 35 cM that corresponds to marker FH2319. The multipoint linkage analysis result for spot color on CFA11 is shown in Figure 3.

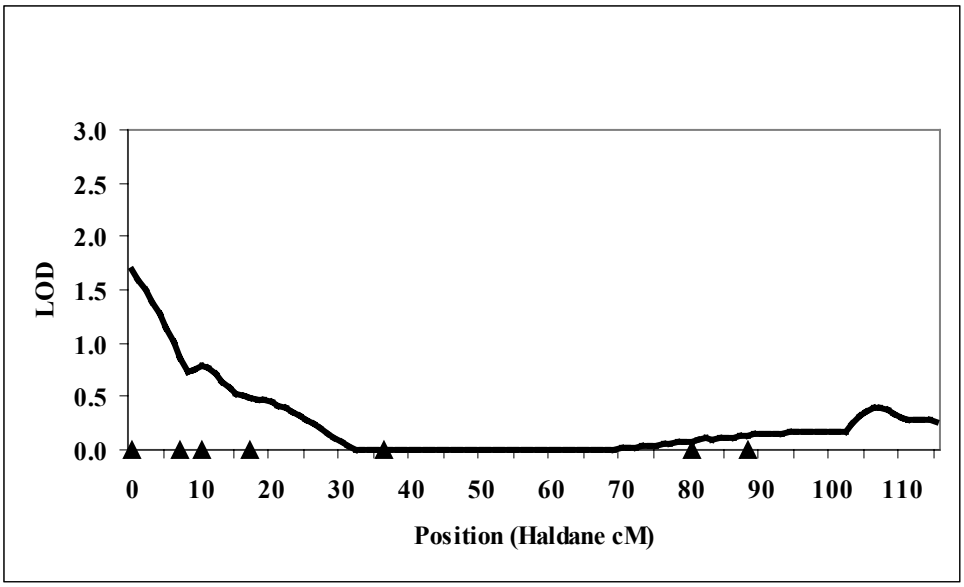

Figure 2. Multipoint linkage analysis for deafness (dichotomous type I) on CFA17 (triangles indicate marker positions).

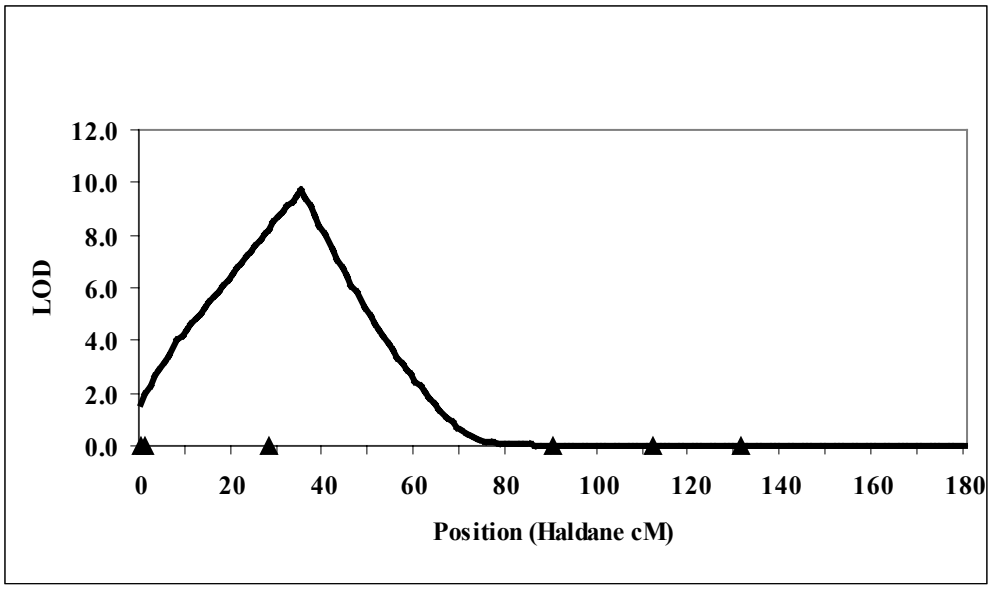

Figure 3. Multipoint linkage analysis for spot color on CFA11 (triangles indicate marker positions).

Discussion

Linkage analysis was performed for the traits deafness, eye color, and spot color using the variance component approach implemented in the SOLAR program. Deafness and eye color were "modeled" in three different categories each as trichotomous, dichotomous type I, and dichotomous type II traits in addition to analyzing each ear and eye individually for the respective trait. Spot color only had one possible category.

Given the linkage analysis results, it is clear that the kindred is sufficient to detect linkage with monogenic traits that follow a Mendelian inheritance pattern. This is evident by the LOD score of 9.76 obtained with marker FH2319 on CFA11 for spot color. FH2319 is 1 Mb from *Tyrosinase related protein 1*, *Tyrp1* (on CFA11), a gene that is the classically named *Brown* (*B*) locus (Schmutz et al. 2002). Given the proximity of marker FH2319 to *Tyrp1*, it is the gene likely responsible for the liver color in the Dalmatian. There was consistency for this finding between the twopoint and multipoint linkage analyses.

Cargill et al. (2004) reported heritability and complex segregation analysis of deafness in the Dalmatian. The complex segregation analysis results did not establish an effect of a major locus causative for deafness. This leaves doubt as to the number of genes and mode of inheritance causing deafness in the Dalmatian. It is likely that deafness is not a monogenic trait, and it is therefore not known if the number of Dalmatians scanned is sufficient to detect linkage at a statistically significant level (Lander and Kruglyak 1995, Gordon et al. 2003) for a polygenic trait. There was consistency between the results obtained for each deafness category and between

twopoint and multipoint linkage analyses. CFA17 exhibits conservation of synteny with HSA02, specifically the p arm from 2p20-p25. There is one human deafness locus in this region, DFNB9 (HHLM 2003). DFNB9 is a deafness locus caused by a recessive mutation in the gene *Otoferlin*, *Otof* (Yasunaga et al. 1999). While DFNB9 appears to be the best functional candidate gene in this region on HSA02, there are 8 to 10 other genes that have known or hypothesized roles in auditory function. *Otof* was a candidate gene mapped in the dog (Rak et al. 2003) with a location of CFA17q13 by FISH, while Cos15 is localized in the 17q11 region (FHCRC 2003).

Despite the attractiveness of *Otof* as a candidate gene, the LOD scores for deafness were not statistically significant compared to the threshold level of 3.0 proposed in human linkage studies (Lander and Kruglyak 1995) and canine linkage studies (Gordon et al. 2003). Therefore another genome scan with more markers, or at least adding markers on CFA17, would be prudent. Since it is not known if the Dalmatians scanned are sufficient to detect linkage with a trait that is not monogenic, adding dogs related to those already in the kindred in addition to more markers is advisable.

CHAPTER VI

CONCLUSIONS

> The dog, as human's favored companion, is unique among animal species in providing new insights into human genetic disease. (Ostrander et al. 2000)

The overall goal of this work and the laboratory in which it was carried out, is to improve the quality of life for the domestic dog and the human. This will be achieved through an increased understanding of canine genetics. The specific aims of this work are to understand the genetic cause of hereditary deafness in the Dalmatian and increase the overall knowledge of normal auditory function.

Deafness affects the Dalmatian more than any other breed, with approximately 30% of the US population suffering from unilateral or bilateral deafness. A unilaterally deaf dog usually learns to compensate for the defect but a bilaterally deaf dog can pose a danger to itself and others. As a result, there is a standing recommendation by the American Kennel Club and Dalmatian Club of America that bilaterally deaf Dalmatians be euthanized. It is obviously distressing to any breeder to have to put down a 6 week old puppy, and this emotional reaction is aggravated by the associated financial loss. There are rescue organizations that work to save bilaterally deaf dogs and place them with dedicated people who are committed to giving the special training required to keep a deaf dog as a pet. While bilaterally deaf dogs that are not euthanized are not used as breed stock, some breeders do use unilaterally deaf dogs for breeding. However, if one wishes to limit or reduce the prevalence of this disease in the breed, using affected dogs for breeding is not recommended because the causative mutation(s) may be passed to the

next generation. Elimination of such dogs is not without consequence. That is, by removing all affected dogs from the breeding pool, especially with such a high percentage of the population affected, desirable alleles may also be removed. Therefore, breeders must weigh the risks of producing affected offspring while maintaining the traits that define the Dalmatian.

Chapter II describes development of a genetic tool suitable for whole genome scans of the dog. A set of multiplexed microsatellite markers was designed (Cargill et al. 2002) for use in canine linkage studies that are increasingly being performed by many laboratories. Richman et al. (2001) characterized a set of markers, termed the MSS1, to provide an estimated 10 cM coverage of the canine genome. In order to carry out a genome scan with the 172 MSS1 markers, 172 PCRs would need to be performed for each sample. By labeling the primers for the markers with fluorescent dyes (6FAM, HEX, and TET), the markers could be co-amplified as long as products in the same size range with the same fluorescent dye do not overlap. The result of multiplexing the markers was a 60% reduction in the number of PCRs required to carry out a genome scan with this marker set.

Chapter III describes the Dalmatian kindred assembled by Cargill et al. (2004) and statistical analyses of the heritability and segregation of deafness in the kindred. Considering deafness as dichotomous or trichotomous, the heritability of deafness in the kindred was 0.73 and 0.75, respectively. A heritability of this magnitude suggests the effect of a major locus on deafness, as major loci tend to increase trait heritability. However, complex segregation analysis of deafness in the kindred did not establish the

effect of a major locus responsible for the inheritance pattern of deafness. Therefore, it is likely that a single major locus is not solely responsible for deafness in the Dalmatian. These results are not entirely unexpected.

DNA samples were collected from 117 Dalmatians of the aforementioned kindred. Chapter IV details the collection of genotypes from these 117 Dalmatians using the multiplexed microsatellites described in Chapter II. In addition to collection of genotypes, a linkage map was built using the program CRIMAP v2.4 (Green et al. 1990). Not all of the MSS1 markers used in the genome scan had been placed on a chromosome. An additional 3 markers which had never been placed on a chromosome by linkage or RH mapping, were placed on chromosomes using the marker data generated by the scan. Another 11 markers that had not previously been mapped on the latest 1 Mb RH map (Guyon et al. 2003) were also placed on chromosomes.

Chapter V describes linkage analysis for deafness, as well as eye color and spot color, which also segregate in the kindred. Twopoint and multipoint linkage analyses were performed using SOLAR v1.7.4 (Almasy and Blangero 1998) and IBDs and MIBDs generated by LOKI v2.4.5 (Heath 1997). Marker Cos15 on CFA17 consistently resulted in the highest LOD score for deafness although no result approached statistical significance (Lander and Kruglyak 1995, Gordon et al. 2003). For this marker, multipoint linkage analysis yielded a LOD of 1.69 for deafness as dichotomous type I. Comparison of the conserved region of CFA17 with HSA02 reveals a human deafness locus in the region (HHLM 2003). This locus, DFNB9, represents the most likely candidate out of roughly a dozen candidate genes on the p arm of HSA02 between 2p20-

p25. In order to confirm the presence of a QTL affecting deafness in this region, additional markers and dogs are necessary for an expanded scan.

A LOD score of 9.70 with marker FH2319 on CFA11 was obtained for spot color in the Dalmatian. This marker is 1 Mb from the gene *Tyrosinase related protein 1*, or the classically named *Brown* locus, on CFA11. Spot color is a monogenic trait and this result provides evidence there is sufficient power with the assembled kindred to detect linkage with simple Mendelian traits. It is not possible to conclude, however, that the assembled kindred is or is not sufficient to detect linkage with a trait as complex as deafness.

The genetic origin of deafness in the Dalmatian remains unknown. The objective of this work was to identify, using linkage analysis, any region(s) in which the gene(s) responsible for deafness in the Dalmatian may be located. To achieve this objective it was necessary to 1) develop multiplexed microsatellite markers for an efficient whole genome scan, 2) assemble a multigenerational Dalmatian kindred segregating deafness, 3) estimate the heritability of deafness and perform complex segregation analysis, 4) assemble a linkage map based on marker data generated from a whole genome scan of the Dalmatian kindred, and 5) perform linkage analysis of deafness, and other phenotypic traits, in the Dalmatian kindred. The results from the completion of these tasks have been reported and discussed.

REFERENCES

1. Almasy L, Blangero J (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62, 1198-1211

2. Altet L, Francino O, Sanchez A (2001) Microsatellite polymorphism in closely related dogs. J Hered 92, 276-279

3. American Kennel Club (AKC) (2003) World Wide Web (http://www.akc.org/), November 2003

4. Anderson H, Henricson B, Lundquist PG, Wedenberg E, Wersall J (1968) Genetic hearing impairment in the Dalmatian dog. An audiometric, genetic and morphologic study in 53 dogs. Acta Otolaryngol Suppl 232:231-234

5. Barrenas ML, Hellstrom PA (1996) The effect of low level acoustic stimulation on susceptibility to noise in blue- and brown-eyed young human subjects. Ear Hear 17, 63-68

6. Bartels S, Ito S, Trune DR, Nuttall AL (2001) Noise-induced hearing loss: The effect of melanin in the stria vascularis. Hear Res 154, 116-123

7. Beekman M, Lakenberg N, Cherny SS, de Knijff P, Kluft CC, et al. (2001) A powerful and rapid approach to human genome scanning using small quantities of genomic DNA. Genet Res 77, 129-134

8. Bonaccorsi P (1965) The color of the iris as a "test" in the quantitative estimation, in man, of the melanin concentration in the stria vascularis. Ann Laringol Otol Rinol Faringol 64, 725-738

9. Bonney GE (1986) Regressive logistic models for familial disease and other binary traits. Biometrics 42, 611-625

10. Breen M, Langford CF, Carter NP, Holmes NG, Dickens HF, et al. (1999a) FISH mapping and identification of canine chromosomes. J Hered 90, 27-30

11. Breen M, Bullerdiek J, Langford CF (1999b) The DAPI banded karyotype of the domestic dog (*Canis familiaris*) generated using chromosome-specific paint probes. Chromosome Res 7, 401-406

12. Breen M, Thomas R, Binns MM, Carter NP, Langford CF (1999c) Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (*Canis familiaris*) and human. Genomics 61, 145-155

13. Breen M, Jouquand S, Renier C, Mellersh CS, Hitte C, et al. (2001) Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. Genome Res 11, 1784-1795

14. Brenig B, Pfeiffer I, Jaggy A, Kathmann I, Balzari M, et al. (2003) Analysis of the 5' region of the canine PAX3 gene and exclusion as a candidate for Dalmatian deafness. Anim Genet 34, 47-50

15. Canine Radiation Hybrid Mapping Project (DogMap) (2003) World Wide Web (http://www-recomgen.univ-rennes1.fr/Dogs/maquette-pnas.html), November 2003

16. Cargill EJ, Clark LA, Steiner JM, Murphy KE (2002) Multiplexing of canine microsatellite markers for whole-genome screens. Genomics 80, 250-253

17. Cargill EJ, Famula TR, Strain GM, Murphy KE (2004) Heritability and segregation analysis of deafness in US Dalmatians. Genetics, In Press

18. Clutton-Brock J (1995) *The domestic dog: Its evolution, behaviour, and interactions with people*. (Cambridge, England: Cambridge University Press)

19. Dalmatian Club of America (DCA) (2003) World Wide Web (http://www.thedca.org), November 2003

20. Devereux TR, Kaplan NL (1998) Use of quantitative trait loci to map murine lung tumor susceptibility genes. Exp Lung Res 24, 407-417

21. Dog Genome Project (FHCRC) (2003) World Wide Web (http://www.fhcrc.org/science/dog_genome/), November 2003

22. Dukes-McEwan J, Jackson IJ (2002) The promises and problems of linkage analysis by using the current canine genome map. Mamm Genome 13, 667-672

23. Elston RC, Namboodiri KK, Glueck CJ, Fallat R, Tsang R, et al. (1975) Study of the genetic transmission of hypercholesterolemia and hypertriglyceridemia in a 195 member kindred. Ann Hum Genet 39, 67-87

24. Elston RC, Bonney GE (1986) Sampling probands in the analysis of family studies. Proceedings of the 13th International Biometrics Conference, Seattle, Washington, 1-14

25. Falconer DS, Mackay TFC (1996) *Introduction to quantitative genetics*. (Essex, England: Longman Group LTD)

26. Famula TR, Oberbauer AM, Sousa CA (1996) A threshold model analysis of deafness in Dalmatians. Mamm Genome 7, 650-653

27. Famula TR, Oberbauer AM, Sousa CA (2000) Complex segregation analysis of deafness in Dalmatians. Am J Vet Res 61, 550-553

28. Famula TR, Oberbauer AM, Williams DC (2001) Gender effects in hearing loss in Dalmatians. Prev Vet Med 48, 15-24

29. Francisco LV, Langston AA, Mellersh CS, Neal CL, Ostrander EA (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. Mamm Genome 7, 359-362

30. Fredholm M, Wintero AK (1995) Variation of short tandem repeats within and between species belonging to the Canidae family. Mamm Genome 6, 11-18

31. Geman S, Geman D (1984) Stochastic relaxation, gibbs distributions, and the bayesian restoration of images. IEEE Trans Pattern Anal 6, 721-741

32. Gianola D, Foulley JL (1983) Sire evaluation for ordered categorical-data with a threshold-model. Genet Sel Evol 15, 201-223

33. Gordon D, Corwin MB, Mellersh CS, Ostrander EA, Ott J (2003) Establishing appropriate genome-wide significance levels for canine linkage analyses. J Hered 94, 1-7

34. Green P, Falls K, Crooks S (1990) Documentation for CRI-MAP, version 2.4. World Wide Web (http://linkage.rockefeller.edu/soft/crimap/)

35. Greenberg DA (1986) The effect of proband designation on segregation analysis. Am J Hum Genet 39, 329-339

36. Greibrokk T (1994) Hereditary deafness in the Dalmatian - Relationship to eye and coat color. J Am Anim Hosp Assoc 30, 170-176

37. Grosse WM, Kappes SM, McGraw RA (2000) Linkage mapping and comparative analysis of bovine expressed sequence tags (ESTs). Anim Genet 31, 171-177

38. Guyon R, Lorentzen TD, Hitte C, Kim L, Cadieu E, et al. (2003) A 1-Mb resolution radiation hybrid map of the canine genome. Proc Natl Acad Sci USA 100, 5296-5301

39. Hare B, Brown M, Williamson C, Tomasello M (2002) The domestication of social cognition in dogs. Science 298, 1634-1636

40. Harville DA, Mee RW (1984) A mixed model procedure for analyzing ordered categorical data. Biometrics 40, 393-408

41. Hayes HM, Wilson GP, Fenner WR, Wyman M (1981) Canine congenital deafness: Epidemiologic study of 272 cases. J Am Anim Hosp Assoc 17, 473-476

42. Heath SC (1997) Markov chain monte carlo segregation and linkage analysis for oligogenic models. Am J Hum Genet 61, 748-760

43. Henderson CR (1984) *Applications of linear models in animal breeding*. (Guelph, Ontario: University of Guelph)

44. Hereditary Hearing Loss Homepage (HHLM) (2003) World Wide Web (http://www.uia.ac.be/dnalab/hhh/), November 2003

45. Holliday TA, Nelson HJ, Williams DC, Willits N (1992) Unilateral and bilateral brainstem auditory-evoked response abnormalities in 900 Dalmatian dogs. J Vet Intern Med 6, 166-174

46. Holmes NG, Humphreys SJ, Binns MM, Curtis R, Holliman A, et al. (1993) Characterization of canine microsatellites. Exs 67, 415-420

47. Holmes NG, Strange NJ, Binns MM, Mellersh CS, Sampson J (1994) Three polymorphic canine microsatellites. Anim Genet 25, 200

48. Holmes NG, Dickens HF, Parker HL, Binns MM, Mellersh CS, et al. (1995) Eighteen canine microsatellites. Anim Genet 26, 132-133

49. Holmes NG, Dickens HF, Neff MW, Mee JM, Sampson I, et al. (1998) Nine canine microsatellites. Anim Genet 29, 477

50. Hudson WR, Ruben RJ (1962) Hereditary deafness in the Dalmatian dog. Arch Otolaryngol 75, 213-219

51. Igarashi M, Alford BR, Cohn AM, Saito R, Watanabe T (1972) Inner ear anomalies in dogs. Ann Otol Rhinol Laryngol 81, 249-255

52. Ito S (1998) *Advances in chemical analysis of melanins*. (New York, NY: Oxford Press)

53. Jarvik GP (1998) Complex segregation analyses: Uses and limitations. Am J Hum Genet 63, 942-946

54. Johnsson LG, Hawkins Jr. JE, Muraski AA, Preston RE (1973) *Vascular anatomy and pathology of the cochlea in Dalmatian dogs*. (Baltimore, MD: University Park Press)

55. Jonasdottir TJ, Dolf G, Sletten M, Aarskaug T, Schelling C, et al. (1999) Five new linkage groups in the canine linkage map. Anim Genet 30, 366-370

56. Jouquand S, Priat C, Hitte C, Lachaume P, Andre C, et al. (2000) Identification and characterization of a set of 100 tri- and dinucleotide microsatellites in the canine genome. Anim Genet 31, 266-272

57. Juraschko K, Meyer-Lindenberg A, Nolte I, Distl O (2003a) Analysis of systematic effects on congenital sensorineural deafness in German Dalmatian dogs. Vet J 166, 164-169

58. Juraschko K, Meyer-Lindenberg A, Nolte I, Distl O (2003b) A regressive model analysis of congenital sensorineural deafness in German Dalmatian dogs. Mamm Genome 14, 547-554

59. Kay R, Palmer AC, Taylor PM (1984) Hearing in the dog as assessed by auditory brainstem evoked potentials. Vet Rec 114, 81-84

60. Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, et al. (2003) The dog genome: Survey sequencing and comparative analysis. Science 301, 1898-1903

61. Konfortov BA, Jorgensen CB, Miller JR, Tucker EM (1998) Characterisation of a bovine/murine hybrid cell panel informative for all bovine autosomes. Anim Genet 29, 302-306

62. Koskinen MT, Bredbacka P (1999) A convenient and efficient microsatellite-based assay for resolving parentages in dogs. Anim Genet 30, 148-149

63. Ladon D, Schelling C, Dolf G, Switonski M, Schlapfer J (1998) The highly polymorphic canine microsatellite ZuBeCa6 is localized on canine chromosome 5q12-q13. Anim Genet 29, 466-467

64. Lander E, Kruglyak L (1995) Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat Genet 11, 241-247

65. Langston AA, Mellersh CS, Neal CL, Ray K, Acland GM, et al. (1997) Construction of a panel of canine-rodent hybrid cell lines for use in partitioning of the canine genome. Genomics 46, 317-325

66. Lapsys NM, Furler SM, Moore KR, Nguyen TV, Herzog H, et al. (1997) Relationship of a novel polymorphic marker near the human obese (OB) gene to fat mass in healthy women. Obes Res 5, 430-433

67. Leonard JA, Wayne RK, Wheeler J, Valadez R, Guillen S, et al. (2002) Ancient DNA evidence for Old World origin of New World dogs. Science 298, 1613-1616

68. Li JL, Deng H, Lai DB, Xu F, Chen J, et al. (2001) Toward high-throughput genotyping: Dynamic and automatic software for manipulating large-scale genotype data using fluorescently labeled dinucleotide markers. Genome Res 11, 1304-1314

69. Lynch M, Walsh B (1998) *Genetics and analysis of quantitative traits*. (Sunderland, MA: Sinauer)

70. Mair IW (1976) Hereditary deafness in the Dalmatian dog. Arch Otorhinolaryngol 212, 1-14

71. Marshall AE (1985) Brain stem auditory-evoked response of the nonanesthetized dog. Am J Vet Res 46, 966-973

72. Marshall AE (1986) Use of brain stem auditory-evoked response to evaluate deafness in a group of Dalmatian dogs. J Am Vet Med Assoc 188, 718-722

73. Mellersh CS, Langston AA, Acland GM, Fleming MA, Ray K, et al. (1997) A linkage map of the canine genome. Genomics 46, 326-336

74. Mellersh CS, Hitte C, Richman M, Vignaux F, Priat C, et al. (2000) An integrated linkage-radiation hybrid map of the canine genome. Mamm Genome 11, 120-130

75. Meyers-Wallen VN, Bowman L, Acland GM, Palmer VL, Schlafer D, et al. (1995) Sry-negative XX sex reversal in the German shorthaired pointer dog. J Hered 86, 369-374

76. Molyneux K, Batt RM (1994) Five polymorphic canine microsatellites. Anim Genet 25, 379

77. Morton NE, MacLean CJ (1974) Analysis of family resemblance. 3. Complex segregation of quantitative traits. Am J Hum Genet 26, 489-503

78. Muhle AC, Jaggy A, Stricker C, Steffen F, Dolf G, et al. (2002) Further contributions to the genetic aspect of congenital sensorineural deafness in Dalmatians. Vet J 163, 311-318

79. National Human Genome Research Institute (NHGRI) (2003) World Wide Web (http://www.genome.gov/11008069), November 2003

80. Neff MW, Broman KW, Mellersh CS, Ray K, Acland GM, et al. (1999) A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. Genetics 151, 803-820

81. Nicholas FW (2003) Online Mendelian Inheritance in Animals. World Wide Web (http://www.angis.org.au/Databases/BIRX/omia/), November 2003

82. Ostrander EA, Sprague GF, Jr., Rine J (1993) Identification and characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. Genomics 16, 207-213

83. Ostrander EA, Mapa FA, Yee M, Rine J (1995) One hundred and one new simple sequence repeat-based markers for the canine genome. Mamm Genome 6, 192-195

84. Ostrander EA, Giniger E (1997) Semper fidelis: What man's best friend can teach us about human biology and disease. Am J Hum Genet 61, 475-480

85. Ostrander EA, Kruglyak L (2000) Unleashing the canine genome. Genome Res 10, 1271-1274

86. Ostrander EA, Galibert F, Patterson DF (2000) Canine genetics comes of age. Trends Genet 16, 117-124

87. Priat C, Hitte C, Vignaux F, Renier C, Jiang Z, et al. (1998) A whole-genome radiation hybrid map of the dog genome. Genomics 54, 361-378

88. Rak SG, Drogemuller C, Leeb T, Quignon P, Andre C, et al. (2003) Chromosomal assignment of 20 candidate genes for canine congenital sensorineural deafness by FISH and RH mapping. Cytogenet Genome Res 101, 130-135

89. Richman M, Mellersh CS, Andre C, Galibert F, Ostrander EA (2001) Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. J Biochem Biophys Methods 47, 137-149

90. Rithidech KN, Dunn JJ, Gordon CR (1997) Combining multiplex and touchdown PCR to screen murine microsatellite polymorphisms. Biotechniques 23, 36-44

91. S.A.G.E. (1997) *Statistical Analysis for Genetic Epidemiology*, Release 3.1, Computer program package available from the Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University, Cleveland

92. Savin C (1965) The blood vessels and pigmentary cells of the inner ear. Ann Otol Rhinol Laryngol 74, 611-622

93. Savolainen P, Zhang YP, Luo J, Lundeberg J, Leitner T (2002) Genetic evidence for an East Asian origin of domestic dogs. Science 298, 1610-1613

94. Schmutz SM, Berryere TG, Goldfinch AD (2002) TYRP1 and MC1R genotypes and their effects on coat color in dogs. Mamm Genome 13, 380-387

95. Schnabel RD, Ward TJ, Derr JN (2000) Validation of 15 microsatellites for parentage testing in North American bison, *Bison bison* and domestic cattle. Anim Genet 31, 360-366

96. Schrott A, Melichar I, Popelar J, Syka J (1990) Deterioration of hearing function in mice with neural crest defect. Hear Res 46, 1-7

97. Sorensen DA, Anderson S, Gianola D, Korsgaard I (1995) Bayesian inference in threshold models using Gibbs sampling. Genet Sel Evol 27, 229-249

98. Sponenberg DP, Rothschild MF (2001) Genetics of coat colour and hair texture. In: *The genetics of the dog*, Eds. A. Ruvinsky and J. Sampson, (New York, NY: CABI Publishing), 61-85

99. Spritz RA, Beighton P (1998) Piebaldism with deafness: Molecular evidence for an expanded syndrome. Am J Med Genet 75, 101-103

100. Steel KP, Barkway C, Bock GR (1987) Strial dysfunction in mice with cochleo-saccular abnormalities. Hear Res 27, 11-26

101. Steel KP, Barkway C (1989) Another role for melanocytes: Their importance for normal stria vascularis development in the mammalian inner ear. Development 107, 453-463

102. Steel KP (1995) Inherited hearing defects in mice. Annu Rev Genet 29, 675-701

103. Steel KP, Bussoli TJ (1999) Deafness genes: Expressions of surprise. Trends Genet 15, 207-211

104. Steel KP, Kros CJ (2001) A genetic approach to understanding auditory function. Nat Genet 27, 143-149

105. Strain GM, Kearney MT, Gignac IJ, Levesque DC, Nelson HJ, et al. (1992) Brainstem auditory-evoked potential assessment of congenital deafness in Dalmatians: Associations with phenotypic markers. J Vet Intern Med 6, 175-182

106. Strain GM (1996) Aetiology, prevalence and diagnosis of deafness in dogs and cats. Br Vet J 152, 17-36

107. Strain GM (1999) Congenital deafness and its recognition. Vet Clin North Am Small Anim Pract 29, 895-907

108. Strain GM (2003) Deafness in dogs and cats. World Wide Web (http://www.lsu.edu/deafness/deaf.htm), November 2003

109. Strain GM (2004) Deafness prevalence and pigmentation and gender associations in dog breeds at risk. Vet J 167, 23-32

110. Sutton MD, Holmes NG, Brennan FB, Binns MM, Kelly EP, et al. (1998) A comparative genetic analysis of the Irish greyhound population using multilocus DNA fingerprinting, canine single locus minisatellites and canine microsatellites. Anim Genet 29, 168-172

111. Thallman RM, Bennett GL, Keele JW, Kappes SM (2001a) Efficient computation of genotype probabilities for loci with many alleles: I. Allelic peeling. J Anim Sci 79, 26-33

112. Thallman RM, Bennett GL, Keele JW, Kappes SM (2001b) Efficient computation of genotype probabilities for loci with many alleles: II. Iterative method for large, complex pedigrees. J Anim Sci 79, 34-44

113. Thomas R, Holmes NG, Fischer PE, Dickens HF, Breen M, et al. (1997) Eight canine microsatellites. Anim Genet 28, 153-154

114. Tsai KL, Guyon R, Murphy KE (2004) Identification of isoforms and RH mapping of canine c-kit. Cytogenet Genome Res, In Press

115. Van Tassell CP, Van Vleck LD (1995) MTGSAM: A set of Fortran programs to apply Gibbs sampling to animal models for variance component estimation. World Wide Web (http://aipl.arsusda.gov/curtvt/mtgsam.html)

116. Waardenburg PJ (1951) A new syndrome combining developmental anomalies of the eyelids, eyebrows, and nose root with pigmentary defects of the iris and head hair and with congenital deafness. Am J Hum Genet 3, 195-253

117. Wayne RK (1993) Molecular evolution of the dog family. Trends Genet 9, 218-224

118. Werner P, Mellersh CS, Raducha MG, DeRose S, Acland GM, et al. (1999) Anchoring of canine linkage groups with chromosome-specific markers. Mamm Genome 10, 814-823

119. Weston JA (1970) The migration and differentiation of neural crest cells. Adv Morphog 8, 41-114

120. Womack JE, Johnson JS, Owens EK, Rexroad CE, 3rd, Schlapfer J, et al. (1997) A whole-genome radiation hybrid panel for bovine gene mapping. Mamm Genome 8, 854-856

121. Wood JL, Lakhani KH (1997) Prevalence and prevention of deafness in the Dalmatian--assessing the effect of parental hearing status and gender using ordinary logistic and generalized random litter effect models. Vet J 154, 121-133

122. Wood JL, Lakhani KH (1998) Deafness in Dalmatians: Does sex matter? Prev Vet Med 36, 39-50

123. World Canine Organization (WCO) (2003) World Wide Web (http://www.fci.be/aboutus.asp?lang=en), November 2003

124. Xie X, Ott J (1992) LOOPS, version 1.30. World Wide Web (ftp://linkage.rockefeller.edu/software/linkage/)

125. Yasunaga S, Grati M, Cohen-Salmon M, El-Amraoui A, Mustapha M, et al. (1999) A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. Nat Genet 21, 363-369

VITA

Name: Edward James Cargill

Permanent Address: 1502 Medina Dr.
College Station, Texas
77840

Education: University of Nebraska-Lincoln
Lincoln, Nebraska
B.S., 1999
Major: Animal Science
Minor: Biological Science

Texas A&M University
College Station, Texas
Ph.D., 2004
Major: Genetics

Selected publications:

Cargill EJ, Baskin LC, Pomp D (1998) Rapid communication: Localization of the porcine carboxypeptidase-E gene by linkage analysis further extends the region of synteny between human chromosome 4 and porcine chromosome 8. J Anim Sci 76, 2211-2212

Cargill EJ, Happold TR, Bertani G, Rocha J, Lou MF, et al. (2001) Localization of a recessive juvenile cataract mutation to proximal chromosome 7 in mice. Hum Hered 52, 77-82

Cargill EJ, Clark LA, Steiner JM, Murphy KE (2002) Multiplexing of canine microsatellite markers for whole-genome screens. Genomics 80, 250-253

Cargill EJ, Famula TR, Strain GM, Murphy KE (2004) Heritability and segregation analysis of deafness in US Dalmatians. Genetics In Press