Genetic characterization of Standard Poodles from the United States and the United Kingdom and how it relates to geography and sebaceous adenitis disease status

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Definitions

Mendelian- The pattern of inheritance of simple genetic traits (traits caused by a mutation in a single gene) is often referred to as Mendelian, following the classic inheritance studies done on the common flowering pea by Gregor Mendel.

Complex genetics – Traits that are caused by the collective effects of numerous genes are referred to as being complex or **polygenic**. The term Mendelian inheritance is not usually applied to complex traits, because Mendel's studies dealt with simple or **monogenic** inheritance.

Heritability- The degree to which a genetic trait is under genetic control. Disorders such as autoimmunity and cancer may only be 30-50% heritable, with epigenetic and environmental triggers playing a role in the remaining disease prevalence.

Epigenetic- Epigenetic changes are alterations in DNA that occur after birth as a result of a variety of extrinsic and intrinsic processes affecting the genetic code. Epigenetic changes, once they occur, are often heritable. Epigenetic changes explain why even identical twins grow more and more dissimilar in appearance, personality, and disease predilection over time.

Locus or loci – A locus is the specific site on a chromosome where a given gene is found.

Single nucleotide polymorphisms (SNPs) - A genetic variation in the sequence of DNA that occurs when a single nucleotide (A, T, C or G nucleotides) is changed is referred to as a SNP (pronounced snip). Mutations in SNPs, such as an A to T or C to G, occur rarely in evolution. A mammalian genome has millions of SNPs, but each SNPs has only two possible alleles.

Short tandem repeat (STR) - A STR is a pattern of two or more nucleotides in the non-coding regions of the genome that are repeated in a sequential manner, e.g., …CGCGCGCGCG… (di STR), …AATAATAATAAT… (tri STR) or …CGGGCGGGCGGGCGG… (tetra STR). Such regions mutate frequently compared to SNPs and are reflected by a change in size (number of repeat elements). STRs are much more polymorphic than SNPs and can have a large number of

alleles. Their polymorphic nature and relatively rapid evolution make them valuable tools to determine genetic changes that have occurred over the last hundred and thousands of years rather than over hundreds of thousands of years.

Mitochondrial DNA (mtDNA) – mtDNA is found in the cytoplasm of cells in structures called mitochondria. mtDNA is passed from cells of the mother to cells of the fetus through the ovum. Sequences from certain regions of mtDNA are used to trace maternal origins.

Y SNPs and Y STRs- The Y chromosome is the most genetically stable of all chromosomes.. Therefore, there are a limited number of SNP and STR differences in coding and noncoding regions that have occurred during the evolution of various male lineages. These STR and SNP differences are powerful tools in tracing more recent as well as ancient paternal lineages.

Genome – The genome contains all of an individual's hereditary information. The dog genome consists of 78 chromosomes; 38 pairs of autosomes and one pair of sex chromosomes (XY or XX).

Genome wide association study (GWAS) - GWAS tests for the presence of genetic variants in one population (case or affected) versus another (control or unaffected). GWAS uses genetic markers (usually SNPs, but sometimes STRs) that are evenly and closely spaced across each chromosome of the genome. If a certain marker is significantly more common in case than control individuals, it strongly suggests that the genetic cause for the trait is linked directly or indirectly to a gene or genes on or near that position of the genome.

Autosomal DNA- An autosome is a chromosome other than the sex chromosomes (X and Y). Autosomes contain the genomic DNA.

Indigenous dogs – Dogs still existing today and loosely attached to villages in under-developed countries throughout the world. Most indigenous dog populations have been randomly breeding for thousands of years and are therefore repositories of the original dog DNA.

Alleles – Each gene is made up of two identical or nearly identical copies (alleles), one inherited from the sire and one from the dam. Alleles often exist in a number of slightly different genetic forms (**polymorphisms**). When the exact same form of a gene is inherited from each parent, the alleles are said to be **homozygous**, and if different, **heterozygous**.

Genotype- Genotype refers to the specific allele makeup of the individual with reference to the specific trait being considered.

Haplotype-A haplotype occurs whenever specific alleles on specific genes are always inherited as a block, i.e., they are linked to each other. Alleles of the three DLA class II genes frequently form three-locus haplotypes. Haplotypes can be involve alleles at a small number of genes or can encompass regions of the genome containing many genes.

Dog leukocyte antigen (DLA) complex- All vertebrate animals possess a large group of genes, usually loosely or tightly linked to each other and on a single chromosome, which code for proteins important in regulating immune responses and disease processes such as autoimmunity. The general term for this region across species is the **major histocompatibility complex** (MHC). The DLA is the name given to the MHC of the dog and it is composed of four major classes of genes, I, II, III, and IV.

DLA class II genes- The DLA class II region on canine chromosome 12 is one part of the larger DLA. The class II region contains a dozen or more genes that are involved with immune recognition. Three genes called ***DRB1, DQA1*** and ***DQB1*** code for proteins that help form cellular receptors important for the recognition of foreign substances by cells of the immune system and the production of antibodies.

Zygosity - Zygosity refers to similarities in alleles at a specific genetic locus or loci (haplotypes). If the two alleles are identical, the alleles are said to be **homozygous**, and if different, **heterozygous**.

Linkage disequilibrium (LD) - LD refers to the randomness of alleles at two or more genetic loci, either within a region of the same chromosome (e.g., the DLA) or on different chromosomes. LD occurs when the genetic type (genotype) at one loci are not inherited independently of each other. The DLA is an example of a region of high LD, because many of the genes and their alleles are inherited dependently (non-randomly) rather than independently (randomly) of each other.

Hardy-Weinberg Equilibrium (HWE) - The HWE principle holds that genetic variation in a population will remain constant from one generation to the next in the absence of factors that disrupt random mate selection. Although an ideal, HWE is seldom achieved because of disruptive pressures (man-made as well as natural) against random mate selection. This is especially true for breed development, regardless of species.

I. Summary

This study has two objectives; 1) to compare genetic diversity within Standard Poodles from the United States (US) and the United Kingdom (UK), and 2) to search for possible genetic associations with sebaceous adenitis in the breed. A total of 233 Standard Poodles (149 from the US, 84 from the UK) were used in the overall study. Pedigrees were analyzed for relatedness and 28% of US dogs and 38% of UK dogs were found to have the same individual or individuals appear more than once within three generations. This was the first indication of ongoing inbreeding. Standard Poodles from the US and UK, regardless of SA status, shared a major matriline (A for US dogs) or matrilines (A and B for UK dogs), and a single patriline (D1D5). Matrilines and patriline were shared with many other modern breeds and with indigenous (village dogs) in SE Asia. Matrilines B and C in US dogs (20% of US population) and F and H in UK dogs (8% of UK population) appeared largely free of SA. UK dogs from matriline B were about one half (13% vs 26%) as likely to be SA affected. Therefore, SA appeared to have entered the breed through matriline A. About one half of the genome (20 chromosomes) was scanned using single tandem repeat (STR) markers, each detecting 3-9 alleles (genetic variants) per locus. Based on comparative allele frequencies at each STR locus, US and UK populations were found to be closely related but genetically distinguishable. Therefore, the two populations share a common gene pool in the relatively recent past and their ancient paternal origin was traced to village dogs in present day Taiwan and the Phillipines. Analysis of the STR markers indicated some degree of either inbreeding or population substructure (i.e., differing bloodlines

based on geography or non-random selection?) within dogs from both the US and UK. Although there were minor genetic differences between US and UK Standard Poodles in general, there were no discernible differences between SA affected and unaffected dogs from the same geographic regions. This observation tends to confirm more detailed analysis of the genomes using 172,000 single nucleotide polymorphism (SNP) markers. These studies also failed to identify genetic differences that would segregate SA affected from healthy dogs. Comparisons were then made in the region on chromosome 12 that contained genes of the major histocompatibility complex (MHC). This region, known as the dog leukocyte antigen (DLA) complex in dogs, contains a large number of genes that are involved with the recognition of foreign substances (antigens), the ability to differentiate self- from non-self-proteins, and genes that regulate the type and intensity of the immune response. A small region of the DLA (dog MHC) contains three genes that regulate the recognition of foreign antigens that evoke an antibody response. These genes are collectively known as the DLA class II genes. Each of the three genes (*DRB1, DQA1* and *DQB1*) contains two possible alleles (genetic variants) – one is inherited from the mother and one from the father. In most purebred dogs, including the Standard Poodle, each of the DLA class II genes are composed of from 4 to 13 different alleles. DRB1 is the most genetically diverse of the class II genes, while DQA1 is the least diverse (i.e., most conserved in evolution). US Standard Poodles were more diverse in the DLA class II genes than UK Poodles. Certain alleles at each of the three DLA class II genes are frequently linked to a specific allele on the other two genes, forming what is known as a DLA class II haplotype. The DLA class II alleles of the Standard Poodles form 14 different haplotypes (i.e., possible combinations of alleles). These haplotypes exist in a heterozygous (the haplotype from one parent is different than the haplotype contributed by the other parent) or homozygous (the haplotype from sire and dam are the same). Ninety four percent of US and 92% of UK Poodles were either heterozygous (~40%) or homozygous (~50%) for a single major DLA class II haplotype (DRB1*01501/DQA1*00601/DQB1*02301), but showed some differentiation in the frequency and geographic distribution of the 13 less common (minor) haplotypes. However, as with the more genome wide association studies, no difference were observed in the distribution of major and minor DLA class II haplotypes between SA affected and unaffected dogs from the same country. This was unexpected, because varying degrees of genetic association is usually found between certain DLA class II haplotypes and various autoimmune disorders in other pure

breeds. Genetic diversity within the DLA region was also tested by a technique called zygosity mapping. Zygosity mapping provides a visual measure of genetic diversity within the DLA region, and in this study, the gold standard for genetic diversity in the DLA was an ancestral outbred population of village dogs from SE Asia. Zygosity maps in the DLA of Standard Poodles show a significant loss of diversity compared to their SE Asian ancestors, with some individual Standard Poodles being virtually identical across the entire region.

Standard Poodles are quite inbred, but no more so than a number of other pure breeds. The degree of inbreeding is made more apparent by studies within the DLA region, and particular in the DLA class II genes. The DLA region, and especially the DLA class II genes, is normally under what is called high linkage disequilibrium (i.e., genes and their alleles tend to be inherited as blocks from each parent rather than as independently segregating entities). Therefore, these regions of the genome are much more susceptible to the effects of inbreeding than other regions of the genome. The high level of homozygosity in the DLA and DLA class II regions of Standard Poodles is a strong indication that similar regions of homozygosity exist in other parts of the Standard Poodle genome. Genes associated with disease traits are frequently found within such regions of homozygosity.

Genetic associations for SA were also not identified in the DLA region as a whole or in the DLA class II region in particular. This was somewhat unexpected, because associations between almost all other autoimmune diseases and the DLA class II region have been previously reported. This can be interpreted in two manners. It is possible that SA is not linked to genes in the DLA or DLA class II regions of the genome, or that an association exists but is present in almost all Standard Poodles (i.e., it is fixed in the breed), making it extremely difficult to detect. This latter possibility was supported by the extremely high prevalence (90%) of a single DLA class II haplotype in both US and UK Poodles.

Although preliminary studies such as this, as well as much denser whole genome scans, have failed to identify a genetic association for SA, circumstantial evidence supports a genetic component to the disease. The heritability of autoimmune disorders in humans, and in several breeds where it has been determined, has ranged from 30-50%. The remaining 50-70% of disease has been associated with epigenetic changes and environmental triggers. Epigenetic changes to DNA occur after birth as a result of aging, radiation, toxic substances, and intrinsic transpositions of genes caused by certain types of inherent processes. Environmental triggers

include things such as infections, traumas, toxic exposures, stresses, etc. To further confound genetic association studies, autoimmune diseases in humans and dogs do not follow a simple Mendelian mode of inheritance, which means that the portion of disease risk attributable to genetic factors is the sum total of risks imposed by a number of genes. Genetic association studies with complex genetic traits require a much greater number of case and control animals, a much larger number of genetic markers, and careful consideration of the confounding effects of population substructure. Unfortunately, the ease with which simple Mendelian traits have been identified in dogs, sometimes with as few as five affected dogs, has led people to believe that identifying genetic associations (and ultimately the development of genetic tests) for complex traits such as autoimmunity and cancer would be equally simple.

Studies not detailed herein demonstrated that Addison's disease and SA are probably not part of the same autoimmune syndrome. SA appears to have entered the breed through dogs from the major maternal haplotype (type A), and is largely free from dogs with minor maternal haplotypes, especially C. However, Addison's disease occurs at similar prevalence in all maternal haplotypes, and selection for C would probably not reduce the Addison's disease prevalence.

Although preliminary studies have not identified a genetic association for either SA or Addison's disease in the Standard Poodle using high density SNP arrays and increased numbers of case and control animals, it does not mean that finding such an association will be impossible. Increasing the numbers of case and controls tested by high density SNP arrays may still yield and association, but the number of case animals may have to be many hundreds and even thousands to demonstrate a significant association. Two alternative approaches may be more viable. The first would be to use a large number of STR markers (>800) across the genome rather than the SNP markers. STR loci are much more polymorphic (variable) and have evolved and changed much more recently than SNP markers. Therefore, they may better reflect genetic mutations and associations that have developed over the last several hundred years. A third possibility would be to use Miniature Poodles for controls, because they are much more likely to be free of the SA trait. If the trait for SA is fixed in Standard Poodles, healthy Miniature Poodles with no history of SA, may be useful controls for identifying the genetic basis of SA in Standard Poodles. However, before doing this, a detailed genetic analysis of Miniature Poodles would have to be done, and only those dogs with close genetic relationships to Standard Poodles should be

included in such a study. Although many people consider Miniature Poodles to be genetically similar to Standard Poodles, differing only in size, evidence from other researchers suggests that they may be more genetically distinct than believed. Regardless of which approach or approaches should be pursued, far more money will be required for research and much better participation will be required from owners of SA affected dogs in submitting DNA.

II. Introduction to SA study in Standard Poodles

The Standard Poodle is known for its temperament, intelligence, and outstanding coat. However, as with most pure breeds, it has its own set of health problems. The Poodle Health Registry database lists over 50 major health disorders of Standard Poodle (http://www.poodlehealthregistry.org), ten of which are of an autoimmune nature. These autoimmune diseases include sebaceous adenitis (SA), Addison's disease (hypoadrenocorticism), immune mediated hemolytic anemia (IMHA), chronic active hepatitis, diabetes mellitus (type I), immune mediated thrombocytopenia (IMTP), masticatory myositis, lupus erythematosus (discoid and systemic), symmetrical lupoid onychodystrophy, and hypothyroidism. The 2010/2011 health survey by the PCA Foundation placed the prevalence of SA in Standard Poodles at 2.7%, Addison's disease 2.5%, hypothyroidism/thyroiditis 1.8%, IMHA 1.0%, chronic active hepatitis 0.7%, and IMTP 0.3%. Assuming that the majority of Standard Poodles suffer from only one autoimmune disease, the overall prevalence of autoimmune disease among US Standard Poodles would be approximately 9%.

Sebaceous adenitis in dogs was first described in detail by Scott (1). The disease has been reportedly recognized in a number of pure breeds of dogs (2), but is most prevalent in Akitas (2, 3), Standard Poodles (3, 4), English Springer Spaniels (4), and Havanese (5). Detailed histopathologic and immunohistopathologic descriptions of lesions of sebaceous adenitis have been reported by Scott (1), Reichler et al (2), Gross and colleagues (6) and Rybnicek et al (7). Lesions often appear as hair loss in the region of the head (face, ears, neck) (Fig. 1). The subsequent disease can evolve slowly or quickly, and be relatively localized or generalized to the body. It can also undergo spontaneous regression at times. A subclinical form also exists, wherein biopsies show characteristic inflammation centered on sebaceous glands but without

outward signs of disease of the coat. Furthermore, there is not always a direct relationship to histologic lesions and outward clinical signs (2).

Dunstan and Hargis (8) were the first to suggest that sebaceous adenitis was a simple Mendelian trait, but no heritability studies were published. However, the patterns of disease occurrence among related individuals and highly variable age at onset (very young to aged dogs) is not entirely compatible with a simple recessive trait. Preliminary GWAS carried out in the UK on 20 SA affected and 28 healthy Standard Poodles using moderately dense SNP arrays (22,362 SNPs) failed to show a simple Mendelian association in any region of the genome with disease (9). A more robust GWAS using SA affected dogs from the US and UK using more than twice the number of dogs and eight times the number of SNPs also failed to find a definitive genetic association with disease (unpublished data, 2011). Although whole genome scanning has so far failed to demonstrate a genetic basis for SA in Standard Poodles, there is little doubt that genetic factors play a role in the disease. Breeders often associate disease risk with certain matings and bloodlines and some blame excessive inbreeding (10) as a factor in the increasing disease prevalence. The most common genetic link with autoimmune disease in both dogs and humans to date has been with genes in the MHC (HLA in humans and DLA in dogs). Autoimmune disorders occur disproportionately in pure breeds and often associate with specific dog leukocyte antigen (DLA) class II haplotypes, especially when they are in the homozygous state (reviewed in 11,12). Human autoimmune disorders are also frequently associated with genes in HLA complex, as well as genes controlling T cell regulation, and genes involved with the production of immunoglobulins (13).

Given the difficulty in identifying a genetic association for SA in Standard Poodles using high density genome wide association studies (GWAS), a decision was made to take a step back and to more thoroughly analyze the basic genetic makeup of Standard Poodle populations in the US and UK. Maternal mtDNA and Y SNP/STR haplotypes for the breed were determined, as well as genetic diversity and population structure based on 24 highly polymorphic STR markers spread across 20 chromosomes. The DLA region, including the DLA class II genes, was also interrogated by high density SNP scan, sequencing of class II alleles, and zygosity mapping. Zygosity maps of the DLA region of SA affected and healthy Standard Poodles were compared to similar maps derived from village dogs of SE Asia, which are living representatives of the

ancestors of modern Standard Poodles. These various genetic parameters were then compared in SA affected and healthy Standard Poodles from both the US and UK.

III. Scientific methods used in study

A. Standard Poodle case and control samples

One hundred forty nine Standard Poodles from the US and 84 dogs from the UK were enrolled in the study. Forty nine dogs from the US and 23 dogs from the UK suffered from SA. DNA containing samples were collected as either 2-5 ml EDTA blood (US dogs) or air dried buccal swabs using cytological brushes (UK dogs).

Pedigrees of all dogs included in the study were screened for relatedness to three generations Pedigrees were either submitted with the sample or downloaded from the American Kennel Club (AKC) and Kennel Club UK websites. After removing dogs related to the level of grandparents, 107/149 dogs remained from the US (36 SA affected and 71 unaffected) and 52/84 from the UK (13 affected and 39 unaffected). Therefore, 28% of dogs from the US and 38% from the UK, regardless of SA status, had the same animal appear at least twice within three breeding generations.

Analyses were performed on randomly related dogs and on the unrelated subset of these dogs (sharing no common relative through the level of grandparents). Analyses were performed on both randomly related dogs and unrelated dogs. However, qualitative results were the same regardless of degree of relatedness; therefore, only analyses based on the full data set of randomly related dogs were presented in most tables and figures.

B. Indigenous Bali street dog samples

DNA was extracted from buccal swabs from 26 randomly selected indigenous dogs from the streets of Bali (14). Bali street dogs are ancient descendants of dogs migrating from SE Asia (14) and maintain the broad genetic diversity of their ancestors (14, 15).

C. DNA extraction

DNA was extracted from whole EDTA blood or cytological brushes using Qiagen Gentra Puregene Blood Kit according to the manufacturer's instructions.

D. Determination of paternal and maternal haplotypes

Y chromosome haplotypes were determined for 17 SA affected and 48 unaffected male Standard Poodles from the US and 12 SA affected and 29 unaffected from the UK using a panel of 11 Y-SNPs (16). The SNPs were assayed using a Sequenom MassARRAY Compact 96 using iPLEX Gold technology. Primer sequences for Y-SNPs were previously reported (16). Ninety one male Standard Poodles from the US, including 30 SA affected and 61 unaffected dogs were also tested with a panel of seven Y-STR markers. Primer sequences and allele sizes for these markers have been previously reported (17).

Mitochondrial DNA (mtDNA) haplotypes were determined for 28 SA affected and 75 unaffected Standard Poodles from the US and 23 SA affected and 58 unaffected dogs from the UK by sequencing 655 bp of the mitochondrial control region between nt 15452 and 16107 as previously described (18). Primer sequences, conditions for PCR, cleaning of PCR products, and sequencing have been previously reported (12).

E. Genetic diversity using STR markers

Twenty four STRs located on 20 different autosomes were used in the study. Repeat motif, chromosome assignment, known allele numbers and allele size range for this set of markers have been previously reported (12).

F. DLA Class II genotyping

Alleles of the DLA class II genes, DRB1, DQA1, and DQB1, were determined for 47 SA affected and 90 unaffected Standard Poodles from the US and 23 affected and 61 unaffected Poodles from the UK by sequence-based typing using published locus-specific intronic primers (20, 21). PCR reactions, purification of PCR products, and sequencing procedures have been previously described (12).

G. DLA wide SNP typing

DNA from 34 SA affected and 24 unaffected dogs from the US, and 23 SA affected and 16 unaffected dogs from the UK were tested on CanineHD Genotyping BeadChips. Data from 150 SNP markers overlapping the DLA region (base 3802975 to 5672682) of CFA12 were extracted from the genome wide association study (GWAS). Thirty five of these SNPs were discarded for being monomorphic, leaving usable data from 115 SNPs across the entire DLA region. Genome and DLA wide SNP associations were determined by PLINK analysis with MAF >0.05, call rate >90%, and 50,000 permutations (21).

H. Data analysis

Haplotype frequencies (mtDNA and DLA) between US and UK populations and between affected and unaffected Standard Poodles were compared using Chi-square tests of independence, with rarer haplotypes pooled to ensure that <20% of expected cell frequencies were <5 cases (22). Calculation of descriptive statistics, expected (H_E) and observed (H_O) heterozygosity, and tests of Hardy Weinberg equilibrium were performed using Arlequin v3.1 (23), as were coefficients of inbreeding (F_{IS}) within populations and fixation indices (F_{ST}) between populations. Tests for gametic ("linkage") disequilibrium were performed using Genepop on the Web (v 4.0.10) (24). Sequential Bonferroni adjustments were applied to P-values to avoid inflation of type I errors due to repeated performance of Hardy-Weinberg and Gametic Equilibrium tests (25). Because numbers of individuals differed between US and UK samples, a rarefaction procedure performed in program HP-rare was used to effectively equalize sample sizes for these estimates based on the lowest numbers of genes sampled from any population and locus (26). Statistical comparison of averages across loci was based on 95% confidence intervals calculated from the Z distribution (21). Principle Coordinate Analysis (PCoA) was performed using GenAlex v6.41 (27).

A Bayesian model-based method that utilizes genotype frequencies, with no prior information on population of origin, was used to assess substructure within the data set (28, 29). The admixture model with correlated allele frequencies was employed.

IV. Results

A. Maternal haplotypes

Seven distinct mtDNA (maternal) haplotypes were identified among 103 randomly related Standard Poodles (28 SA affected, 75 unaffected) from the US and 81 Poodles (23 SA affected and 58 unaffected) from the UK (Table 1). GenBank accession numbers corresponding to the seven mtDNA haplotypes identified in this study are given in Table 1.

Maternal haplotype diversity (1 – sum of squared frequencies) (30) was 0.47 for US dogs and 0.41 for UK dogs; these frequencies were not significantly different between the two countries (F_{ST} = 0.019; Chi square, 2; df = 0.17; P = 0.92). Therefore, dogs from the US and UK countries were then pooled for subsequent comparisons. Haplotype frequencies differed significantly between unaffected and affected dogs (F_{ST} = 0.160; Chi square, 2; df = 6.3; P = 0.04), including a two-fold difference in haplotype diversity between unaffected (0.51) and SA-affected (0.25) dogs. This difference was caused by a greater frequency of the most common haplotype in the SA affected dogs and of minor haplotypes in unaffected dogs (Table 1).

Maternal haplotype frequencies of Standard Poodles used on studies of SA in US dogs were compared to frequencies found for US Standard Poodles with Addison's disease (Table 2). SA appears to have entered the population from dogs with the major A maternal haplotype, while dogs with minor maternal haplotypes B were relatively free of SA and dogs with haplotype C were all healthy. The role of maternal haplotype is not as clear for Addison's disease, i.e., no major or minor haplotype was significantly more or less frequent between affected and healthy dogs.

B. Paternal haplotypes

The 91 SA affected and unaffected dogs from the US and UK shared a single Y SNP haplotype (AGAAGACCTCC), which is found in village dog populations from across SE Asia, a region to which most modern breeds trace their ancestry (15). All of these male dogs also shared an identical Y-STR haplotype. The Y STR markers and their alleles in parentheses were: MS34A (172), MS34B (176), MS41A (206), MS41B (219), 990.35.4 (127), 650.79.2 (120/134), and

650.79.3 (122/124). This haplotype has been designated as D1D5, and is the most common Y-STR haplotype among breed dogs (17).

C. Partial genome scan using 24 STR markers on 20 autosomes

All 24 autosomal STRs were polymorphic in both US and UK Standard Poodles, yielding 172 alleles. The average (across loci) observed heterozygosity (Ho=0.576) was significantly ($P < 0.0001$) lower than average expected heterozygosity (He=0.622), indicating population substructure within the total sample. Allele frequencies differed significantly between US and UK poodles ($F_{ST} = 0.024$; $P < 0.0001$), but not between affected vs. unaffected dogs within either the US ($F_{ST} = 0.001$, $P = 0.19$) or the UK ($F_{ST} = -0.010$, $P = 0.99$).

Six of the 24 STR loci in the US and two loci in the UK were significantly out of Hardy-Weinberg equilibrium, including one locus (INRA21) in both populations (Table 2). After sequential Bonferroni corrections to adjust for differences in sample size, 13 locus pairs in the US and nine different locus pairs in the UK (of 276 pairwise combinations in each population) were out of normal equilibrium. These findings were consistent with inbreeding or population substructure in both US and UK populations. The coefficient of inbreeding was statistically significant in these populations albeit low, with F_{IS} estimated across loci at 0.07 (SE = 0.013) in the US and 0.05 (SE = 0.023) in the UK. To obtain comparisons of allelic richness between populations that were not biased by sample sizes, we rarified estimates to 100 genes (i.e., 50 dogs per population), yielding allelic richness estimates averaged across loci of 5.6 (95% CI: 5.1-6.1) vs. 5.2 (95% CI: 4.7-5.8) alleles per locus in the US and UK, respectively. This difference was not significant across populations. Heterozygosity was also not significantly different between SA affected and healthy Standard Poodles within either the US or UK populations (Table 3).

D. Population structure of US and UK dogs, SA affected and healthy

A principal coordinate analysis (PCoA) plot measuring genetic similarities among randomly related dogs from US and UK populations based on autosomal STRs showed a degree of differentiation by country of origin (Fig. 2a). However, SA affected dogs were indistinguishable

from unaffected dogs from within their own geographic regions (Fig. 2b, 2c). A blind cluster analysis (i.e., the program was not given information on geographical or SA status) was performed in Structure using only unrelated Poodles from the US and UK to investigate patterns of substructure. An analysis using K = 2 was conducted for comparison and the US and UK dogs segregated by geographic origin (Fig. 3). Use of four genetic clusters (K = 4) was indicated as the optimum based on the log probability of the data (Fig. 3), but the analysis at K = 4 failed to differentiate more than the two geographic populations. Therefore, regardless of K, blind analyses provided no evidence that SA affected and unaffected dogs segregated as distinct subpopulations among either US or UK Standard Poodles.

E. Genetic comparisons of the DLA and DLA class II regions of SA affected and healthy dogs from the US and UK

Exon 2 sequencing of DLA-DRB1, -DQA1 and -DQB1 loci was conducted on Standard Poodles from both the US (n=137) and UK (n=84) (Table 3). Twelve DRB1, seven DQA1 and nine DQB1 alleles were identified among US and UK Poodles. One DRB1 allele (tentatively designated drb001v) was unique, but differed from DRB1*00101 by a single nucleotide. This suggested that it occurred as a mutation in the more modern period of breed development.

The known alleles formed fourteen three-locus haplotypes (Table 4). The proportion of heterozygous genotypes (Ho) did not differ significantly between affected and unaffected dogs in the US (Ho = 0.50; Fisher Exact P = 0.16) or UK (Ho = 0.44; Fisher Exact P = 0.25). Nor was there a significant deviation from Hardy-Weinberg equilibrium in the US (He = 0.50; Chi square, 2; df = 0.87; P = 0.65) or the UK (He = 0.47; Chi square, 2; df = 0.54; P = 0.76). There also were no significant differences between haplotype frequencies of SA affected vs. unaffected dogs in the US (Chi-square, 5; df = 1.64; P = 0.90) or in the UK (Chi-square, 5; df = 2.52; P = 0.77). However, haplotype frequencies differed slightly (F_{ST} = 0.016) but significantly between US and UK dogs (Chi-square, 5; df = 24.7; P = 0.0002). This was due mainly to the relative occurrence and frequencies of minor haplotypes (Tables 4, 5).

Although the numbers of individuals with minor haplotypes was too small to render significance, it is noteworthy that several minor haplotypes were found only in unaffected dogs. These haplotypes included 00101/00101/00201 and the novel alternative haplotype

001v/00101/00201 that was created by the mutation of DRB1*00101 to form DRB1*001v (1.67% in US dogs and 1.64% in UK dogs), and 00201/00901/00101, 01101/00201/01302, 010011/00201/01501 (1.68% in US dogs and 0.82% in UK dogs). If dogs possessing these haplotypes were indeed free of SA, hundreds and perhaps thousands more SA affected as well as unaffected dogs would have to be DLA class II haplotyped in order to confirm such an association. Likewise, no significant relationship between minor maternal and DLA class II haplotypes was evident. If such a relationship had existed, it would have lent some credibility to a lack of association with certain minor DLA class II haplotypes and SA.

In order to avoid bias from closely related dogs, DLA class II haplotype zygosity was calculated using only dogs unrelated to three generations. About one-half of all unrelated US and UK Standard Poodles were homozygous for various DLA class II haplotypes (Table 4). These proportions were virtually identical to those of SA affected vs. unaffected dogs from the same countries. Although there were some difference in the types of low frequency haplotypes between US and UK dogs, the 01501*00601*02301 haplotype, either in a homozygous or heterozygous state, was found in about 94% of US and UK dogs (Table 5).

Zygosity mapping was done within a region on CFA12 from base 3802975 to 5672682, which includes the entire DLA, for SA affected and unaffected Standard Poodles from the US (Fig. 4). SA affected and unaffected dogs were each separated into two groups based on zygosity. The first group was largely heterozygous across the DLA. The second group was defined by a large region of homozygosity extending from nucleotide positions 4547874 to 5412195, with relative heterozygosity both upstream and downstream of this region. The DLA-DRB1, -DQA1 and -DQB1 genes are found at approximate positions 5,155,200 to 5,311,100. There was a tendency, although not quite significant, for a greater proportion of SA affected dogs to be in the homozygous group. Fourteen of 24 (58%) unaffected dogs were homozygous for either major or minor alleles across most of the DLA region (Fig. 4), compared to 16/21 (76%) of SA affected dogs. The major 01501*00601*02301/01501*00601*02301 DLA class II haplotype, regardless of SA status, was found almost exclusively among the homozygous dogs (Fig. 4). Similar zygosity mapping was done on affected and unaffected dogs from the UK with virtually identical results to that found for US Standard Poodles (data not shown). Identical zygosity mapping was carried out using the same 115 DLA SNPs on 26 randomly selected indigenous (street) dogs from the Island of Bali, Indonesia (Fig. 4). Bali street dogs showed a

much greater level of heterozygosity across the entire DLA region than SA affected or unaffected Standard Poodles from the US

V. Discussion

A. Breed history and breeding bottlenecks

Difficulties in identifying significant associations by genome wide association studies using modern arrays containing over 172,000 SNPs across the entire genome led us to back-track a step and in order to obtain a better understanding of genetic diversity and population structure between US and UK Standard Poodles, whether healthy or SA affected. Before doing such basic genetic studies, it was important to review the history of the Standard Poodle as a breed to better evaluate our findings. Although Standard Poodles have existed in more or less their present form since the 1600's, the breed has evolved mainly over the last century (31-33). Dogs from the Meadoware, Hill Hurst and Red Brook kennels dominated the breed early in the century, but their contributions were soon supplanted by dogs from other kennels (32). The most noteworthy was the Labory Kennels of Switzerland and a dog named Anderl von Hugelberg. Anderl lived in the 1920's and is perceived as the "Adam" of modern Standard Poodles (32). A further bottleneck occurred with the Wycliffe line, which traces its origins to the late 1950's. This line was created around five Standard Poodles from the then dominant Anderl von Hugelberg line, with only minor contributions from several other lines (33). The Wycliffe line was subsequently enlarged and further refined by extensive inbreeding and became extremely popular around the world. The proportion of Wycliffe ancestry among Standard Poodles in the US, UK and Scandinavia progressively increased to 40-50% by 1980 in dogs and has remained at that level to the present day (33). Two autoimmune disorders, sebaceous adenitis and Addison's disease, parallel the Wycliffe line in time of appearance and increasing popularity (33).

B. Maternal and paternal lineages of modern Standard Poodles (haplotypes)

All Standard Poodles share a single paternal (Y chromosome) haplotype based on a panel of 11 Y SNPs. This particular Y haplotype is rooted deep in village dog populations from across SE

Asia and is common among western dogs regardless of breed (15). The ancient origin of the Y SNP markers limited their usefulness in determining more recent male founders. However, STRs on the Y chromosome have proven to be more useful in resolving modern paternal lineages (15, 17). Sixty seven Y STR haplotypes have been identified among 50 modern breeds of dogs, and D1D5 of Standard Poodles is the most common of these haplotypes (15). D1D5 is also one of the most common haplotypes in the VGL canine forensic database and predominates in breeds that share characteristics with the Standard Poodle, e.g., Airedale Terrier, Maltese Terrier, Bichon Frise, Borzoi, German Short-haired Pointer, Komondor, and Norfolk Terrier. The D1D5 paternal haplotype is also common among village dogs from Taiwan and the Philippines (15).

Sequences within the hypervariable region I of mtDNA have proved useful in determining maternal origins in a number of dog breeds (34). Seven mtDNA haplotypes were identified in this study, US and UK Poodles each possessed five mtDNA haplotypes, three of which were shared and two being unique to each population. Standard Poodles with SA exhibited a higher frequency (88%) of mtDNA haplotype A than did unaffected Poodles (70%), which had a correspondingly higher frequency of rare haplotypes B-H (30%) and, consequently, higher mitochondrial diversity. Although mtDNA polymorphisms have been associated with susceptibility to autoimmune disease in laboratory mice (35), it is more likely that the association is more likely a consequence of autosomal selection.

The predominance of mtDNA haplotype A in US and UK Standard Poodles supports what is known about the recent history of the breed and a bottleneck occurring with the advent of the Wycliffe line in the 1950's. The minor mtDNA haplotypes observed in the present study may be remnants of lines that were more common prior to the 1950's. It is noteworthy that these minor maternal lines remain relatively free of SA, suggesting that SA entered the breed with what has now become the dominant maternal lineage.

C. Genetic diversity and population structure of US and UK Standard Poodles based on autosomal STRs

All approaches of data analysis, *F*-statistics, PCoA, and admixture analysis, suggested that US and UK dogs were closely related but not indistinguishable, as to be expected given their

independently selected breeding over the past 25 to 50 generations. The implication of this degree of population substructure on GWAS using cohorts of Standard Poodles from both the US and UK is unknown. However, indications of population substructure were also seen with the autosomal STR markers. The effect of population substructure could influence the minimum number of case and control dogs from each country required for GWAS, as well as the manner in which data is analyzed.

D. Genetic interrogation of the DLA and DLA class II regions of US and UK Standard Poodles

Studies of the DLA and DLA class II region were conducted with two objectives in mind. The first objective was to identify a DLA class II association with SA, especially because autoimmune diseases in other pure breeds have been almost always associated with specific haplotypes (reviewed in 11, 12). Sequencing of the three DLA class II genes detected 12 DRB1, 7 DQA1 and 9 DQB1 alleles forming 14 three-locus haplotypes. These were among the 245 DRB1, 39 DQA1, and 79 DQB1 alleles and over 200 three-locus haplotypes previously identified from purebred and indigenous dogs around the world (Kennedy LJ, personal communication). The present findings for DLA class II alleles and haplotypes were similar to those reported by Kennedy (36) on 81 Standard Poodles from the US and UK. She reported 9/12 of the same DRB1 alleles, 5 of the 7 DQA1 alleles, and 4/9 of the same DQB1 alleles. The frequency of the various haplotypes was also similar, with 01501/00601/02301 being present in 105/162 (65%) chromosomes in her study. Differences between US and UK dogs, when they occurred, were seen mainly with minor alleles and their relative frequencies. Kennedy reported DLA class II haplotypes and zygosity of Standard Poodles from the US, Canada and the UK (37, 38). Eleven haplotypes were identified among 31 samples from around the world and 10 from among 50 samples submitted by the Animal Health Trust, UK. About one-half of these dogs were homozygous for the DLA class II genes and 90% of these dogs were homozygous for the same major haplotype, DRB1*01501/DQA1*00601/DQB1*02301. Although DLA and DLA class II diversity appeared low in Standard Poodles, it was nonetheless greater than in breeds such as the Italian Greyhound (12) and Pug Dog (11). However, like the Pug Dog, the frequency distributions of alleles and haplotypes were highly skewed because of differences in the number

and frequencies of minor haplotypes. Nonetheless, DLA haplotype diversity in an outbred population of village dogs in Bali, Indonesia (39) displayed heterozygosity in a single locus, DQA1 (Heterozygosity = 0.825) that was 70% higher than observed in this study for the entire tri-locus DLA haplotype of Standard Poodles (0.49) in the present study .

The second objective of studying the DLA was to use a small region of the genome as a window into what may be happening in other regions of the genome. Although the DLA region is normally under high linkage disequilibrium, the degree of homozygosity within both the DLA and the DLA class II regions was much higher than would be expected. This was most noticeable in comparative zygosity mapping between Standard Poodles and their ancestral SE Asian village dog relatives. Zygosity maps of indigenous dogs showed a much greater degree of heterozygosity, much smaller blocks of homozygosity, and a greater use of minor alleles. The loss of genetic diversity in the DLA and DLA class II was mirrored by indications of inbreeding and the occurrence of population substructure between SA affected and healthy dogs, as determined allele frequencies at the 24 autosomal STR loci.

There was no significant association between susceptibility to sebaceous adenitis in Standard Poodles in the present study and any DLA class II haplotype. However, it is possible that a major disease association existed with 01501/00601/02301, in which case it could have gone undetected due to a near fixation of this haplotype within the breed. This would have rendered the numbers of case and control dogs woefully insufficient. Using the SA case and control population from the US in a genetic power calculation (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html), and assuming that the high risk allele (haplotype) frequency was 0.701, the SA prevalence in the breed 2%, the relative risk in the heterozygous state 0.88 and in the homozygous state 1.14, the linkage disequilibrium (D') 0.7, the marker allele frequency 0.701, the number of cases 35, df 1, and the control to case ratio 2.0, 2,629 cases would have been required to yield a probability of 0.05 at 80% power.

E. Complex genetics and SA in Standard Poodles

Finally, the present study did not address complex genetics as it relates to autoimmune diseases such as SA. Autosomal genetic associations in human autoimmune diseases are largely polygenic, a pattern that has also been seen in SLE related disease in Nova Scotia Duck Tolling

Retrievers (41, Addison's disease in Portuguese Water Spaniels (42), and a multiple autoimmune disease syndrome of Italian Greyhounds (12). Therefore, it is not surprising that the genetics of autoimmune diseases in humans has been highly elusive, as elegantly stated by Johannesson and colleagues (43) – "from disease to genes: the monogenic success and the polygenic failure." The discovery of simple Mendelian traits with surprisingly small numbers of case and control dogs has been remarkably easy in dogs, but studies of complex traits such as autoimmunity or epilepsy will likely be as challenging as they have been in humans (12, 41, 42, 44).

F. Where can you find Standard Poodles free of SA?

Trafficking of Standard Poodles between the US, Canada, UK and Scandinavia has obviously been quite extensive throughout the century and therefore dogs in countries where the breed is popular are likely to be quite related. Therefore, in absence of a specific genetic test, it may be important for Standard Poodle breeders to search for remnants of bloodlines, possibly based on minor mtDNA types (or minor DLA class II types if they can be shown to be unaffected), which remain free of autoimmune disorders such as SA and Addison's disease. Such lines may exist in a few older kennels, or more likely, in parts of the world less influenced by the bottlenecks of the 1920's and 1950's. In the absence of definitive genetic markers for SA susceptibility, a recommendation has been made to break this bottleneck by crossing Standard Poodles with Miniature and Toy Poodles (37), which appear to have a much lower prevalence of SA and Addison's disease. However, detailed knowledge of the genetics of Miniature Poodles would be important in the implementation of such a breeding scheme and without tests to identify individuals SA carriers, crossing to Miniature Poodles and then backcrossing to re-establish the desired Standard Poodle phenotype may recreate the original problem. Furthermore, such a breeding scheme, besides requiring careful genetic monitoring, could easily take a decade more and thousands of offspring to prove an effect.

VI. Acknowledgements

Funding for this study was provided by the Poodle Club of America Foundation. We are also grateful for partial matching funds from the Center for Companion Animal Health, UC Davis.

We wish to also thank the staff of the Veterinary Genetics Laboratory (VGL), UC Davis for running STR parentage panels and SRY haplotyping and Angel Del Valle for assisting in mtDNA and DLA class II sequencing. Beth Wictum, head of the Veterinary Forensics Unit of the VGL kindly allowed us access to a large mtDNA sequence database of pure breed dogs. We are also grateful for assistance rendered by the Standard Poodle Club UK.

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Tables

Table 1. The incidence and frequency of maternal or mitochondrial (mtDNA) haplotypes in Standard Poodles

mtDNA Type (GenBank#)	% in VGL forensic data set	US		UK	
		SA (%)	Control (%)	SA (%)	Control (%)
A (AB622536)	0.7	26 (92.9)	56 (77.8)	19 (82.6)	37 (63.8)
B (AB622568)	1.1	0	7 (9.7)	3 (13.0)	15 (25.9)
C (AB622564)	1.6	0	8 (11.1)	0	0
D (AB622557)	1.8	1 (3.6)	1 (1.4)	0	0
F (AF531740)	0	1 (3.6)	0	0	2 (3.5)
G (AY706505)	0	0	0	1 (4.4)	2 (3.5)
H (AB622517)	5.4	0	0	0	2 (3.5)

Table 2. Maternal haplotypes of Standard Poodles from the US used in independent SA and Addison's disease studies. SA appears to have entered the population from dogs with the major A maternal haplotype. Dogs with minor maternal haplotypes B are relatively free of SA, while dogs with haplotype C are all healthy. The role of maternal haplotype is not as clear for Addison's disease; no haplotype is significantly more or less frequent between affected and healthy dogs.

Maternal haplotype (GenBank#)	US Standard Poodles – SA study		US Standard Poodles – Addison's study	
	Sebaceous adenitis (%)	Healthy (%)	Addison's (%)	Healthy (%)
A (AB622536)	41 (91.11)	60 (77.92)	36 (76.6)	76 (78.35)
B (AB622568)	2 (4.44)	8 (10.39)	8 (17.02)	11 (11.34)
C (AB622564)	0	8 (10.39)	2 (4.26)	8 (8.24)
D (AB622557)	1 (2.22)	1 (1.3)	0	2 (2.06)
F (AF531740)	1 (2.22)	0	0	0
G (AY706505)	0	0	0	0
H (AB622517)	0	0	1 (2.13)	0
TOTAL	45	77	47	97

Table 3. Microsatellite locus-specific observed (Ho) and expected (He) heterozygosity, heterozygote deficit (F_{IS}), and rarified (to 100 genes) estimates of Allelic richness (RAR) for Standard Poodles from the US and UK.

	US				UK			
Locus	Ho	He	F_{IS}	RAR	Ho	He	F_{IS}	RAR
AHT121	0.73	0.78	0.06	9.4	0.65	0.76	0.16	9.1
AHT137	0.76	0.78	0.03	6.9	0.65	0.73	0.11	7.0
AHTH130	0.69	0.76	0.09	6.2	0.67	0.81	0.17	6.0
AHTh171-A	0.73	0.71	-0.03	7.9	0.66	0.61	-0.08	5.0
AHTh260	0.46	0.57	0.19*	6.6	0.45	0.52	0.14	6.6
AHTk211	0.39	0.42	0.08	3.7	0.40	0.38	-0.05	3.4
AHTk253	0.70	0.72	0.03	5.0	0.66	0.78	0.15	5.0
C22.279	0.59	0.62	0.04	5.8	0.66	0.68	0.03	5.0
FH2001	0.67	0.72	0.07*	6.3	0.58	0.57	-0.03	4.9
FH2054	0.47	0.56	0.16	6.0	0.52	0.51	-0.03	4.9
FH2328	0.66	0.77	0.15	5.3	0.53	0.79	0.33*	5.7
FH2848	0.19	0.22	0.17*	4.8	0.38	0.40	0.05	6.4
INRA21	0.53	0.62	0.15*	5.8	0.61	0.66	0.07*	4.9
INU005	0.48	0.51	0.05*	3.7	0.53	0.59	0.10	3.8
INU030	0.70	0.69	0.00	5.3	0.64	0.73	0.12	5.0
INU055	0.70	0.69	-0.02	4.8	0.61	0.68	0.10	6.6
LEI004	0.37	0.38	0.03	4.0	0.30	0.32	0.04	4.2
REN105L03	0.49	0.56	0.13	4.6	0.61	0.59	-0.03	4.5
REN162C04	0.47	0.48	0.01	5.8	0.58	0.65	0.11	6.7
REN169D01	0.70	0.72	0.03	6.5	0.61	0.66	0.07	5.9
REN169O18	0.44	0.49	0.10	5.1	0.43	0.42	-0.03	4.5
REN247M23	0.66	0.66	0.01	4.3	0.57	0.53	-0.07	3.6
REN54P11	0.63	0.71	0.12*	4.9	0.79	0.74	-0.07	4.0
REN64E19	0.63	0.65	0.03	5.7	0.80	0.67	-0.19	3.0

*Significant deviation from Hardy-Weinberg equilibrium (HWE) after sequential Bonferroni Correction. Bonerroni correction adjusts for differences in population sizes. HWE is achieved when all individuals in the population are randomly breeding. Significant deviations in HWE at a certain loci may be the result of non-random breeding or population substructure (two or more subpopulations breeding randomly but somewhat independently of the others).

785 Table 4. DLA class II haplotype frequency in all randomly related SA affected and control
786 Standard Poodles. n= the total number of haplotypes with each dog contributing two haplotypes.
787 The percentage of a certain haplotype among individuals in each population is shown in ( ).
788

Haplotype			US		UK	
DRB1	**DQA1**	**DQB1**	**SA** n=94 (%)	**Control** n=180 (%)	**SA** n=46 (%)	**Control** n=122 (%)
01501	**00601**	**02301**	68 (72.34)	124 (68.89)	36 (78.26)	83 (68.03)
01502	**00601**	**02301**	9 (9.57)	17 (9.44)	1 (2.17)	2 (1.64)
01501	**00901**	**00101**	6 (6.38)	12 (6.67)	5 (10.87)	22 (18.03)
02001	**00401**	**01303**	6 (6.38)	12 (6.67)	2 (4.35)	5 (4.1)
01503	**00601**	**02301**	1 (1.06)	5 (2.78)	1 (2.17)	7 (5.34)
00901	**00101**	**008011**	1 (1.06)	3 (1.67)	1 (2.17)	0
01501	**00601**	**04901**	1 (1.06)	1 (0.56)	0	0
02301	**00301**	**00501**	1 (1.06)	0	0	0
00601	**005011**	**00701**	1 (1.06)	0	0	0
001v	**00101**	**00201**	0	2 (1.11)	0	0
00101	**00101**	**00201**	0	1 (0.56)	0	2 (1.64)
00201	**00901**	**00101**	0	1 (0.56)	0	0
01101	**00201**	**01302**	0	1 (0.56)	0	1 (0.82)
010011	**00201**	**01501**	0	1 (0.56)	0	0

789
790

Table 5. Zygosity of DLA class II haplotypes in unrelated Standard Poodles from the US and UK. Homozygous haplotypes are in bold lettering –haplotype from both parent is identical. Hetreozgyous haplotypes are in regular lettering – haplotype from each parent is different.

Haplotype	US		UK	
	SA n=35 (%)	**Control** n=69 (%)	**SA** n=13 (%)	**Control** n=39 (%)
01501*00601*02301/01501*00601*02301	17 (48.57)	29 (42.03)	7 (53.85)	19(48.72)
01501*00601*02301/01502*00601*02301	5 (14.29)	12 (17.39)	1 (7.69)	0
01501*00601*02301/02001*00401*01303	5 (14.29)	8 (11.59)	1 (7.69)	2 (5.13)
01501*00601*02301/01501*00901*00101	4 (11.43)	6 (8.70)	3 (23.08)	11 (28.21)
01501*00601*02301/01503*00601*02301	1 (2.86)	3 (4.35)	0	2 (5.13)
01501*00601*02301/02301*00301*00501	1 (2.86)	0	0	0
01501*00601*02301/010011*00201*01501	0	1 (1.45)	0	0
01501*00601*02301/01501*00601*04901	0	1 (1.45)	0	0
01501*00601*02301/01101*00201*01302	0	1 (1.45)	0	1 (2.56)
01501*00601*02301/00101*00101*00201	0	1 (1.45)	0	1 (2.56)
01501*00601*02301/00201*00901*00101	0	1 (1.45)	0	0
01501*00601*02301/00901*00101*008011	0	2 (2.90)	1 (7.69)	0
001v*00101*00201/001v*00101*00201	0	1 (1.45)	0	0
02001*00401*01303/02001*00401*01303	0	1 (1.45)	0	0
01502*00601*02301/01502*00601*02301	0	1 (1.45)	0	0
01502*00601*02301/00601*005011*00701	1 (2.86)	0	0	0
01502*00601*02301/00901*00101*008011	1 (2.86)	0	0	0
01503*00601*02301/01503*00601*02301	0	1 (1.45)	0	0
01502*00601*02301/01503*00601*02301	0	0	0	1 (2.56)
01501*00901*00101/01501*00901*00101	0	0	0	1 (2.56)
01501*00901*00101/02001*00401*01303	0	0	0	1 (2.56)

Figures

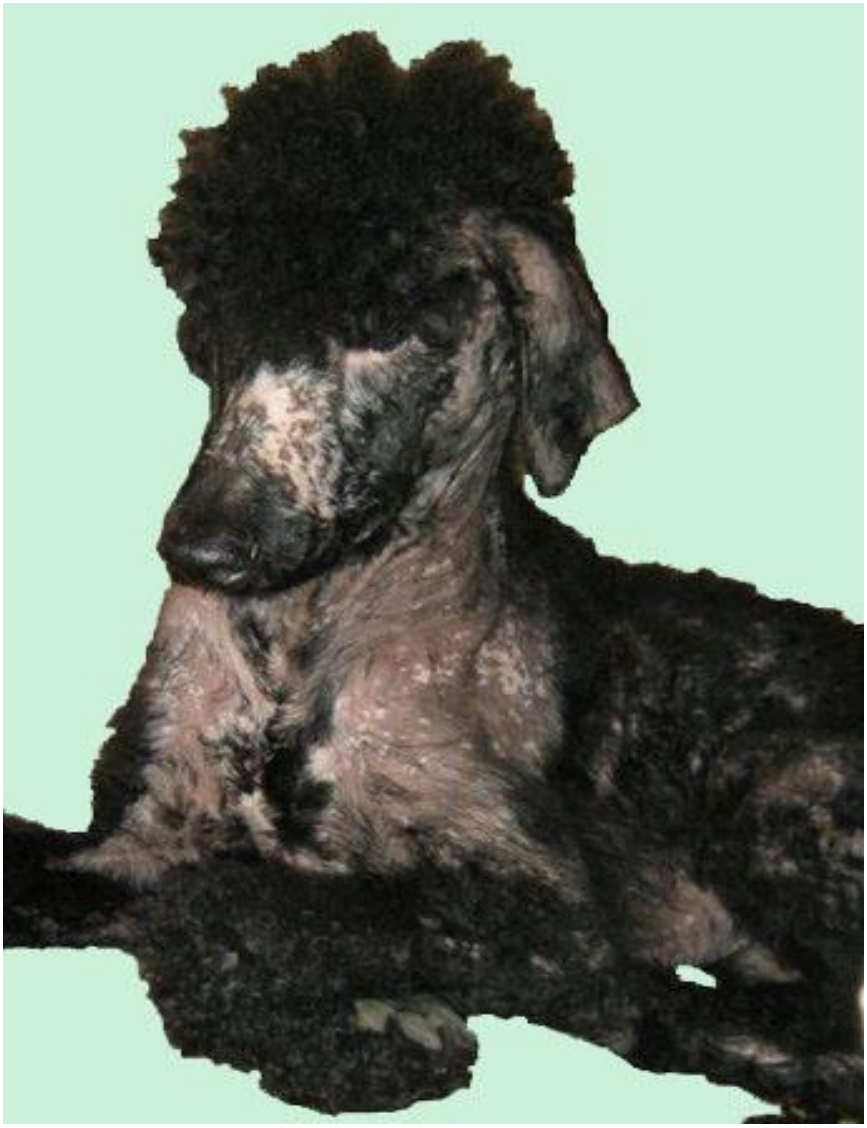
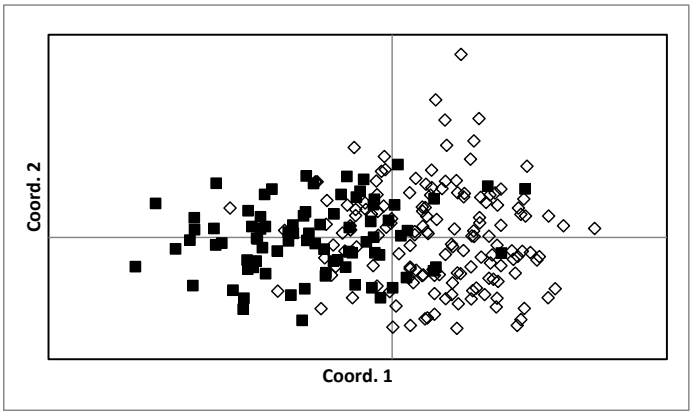
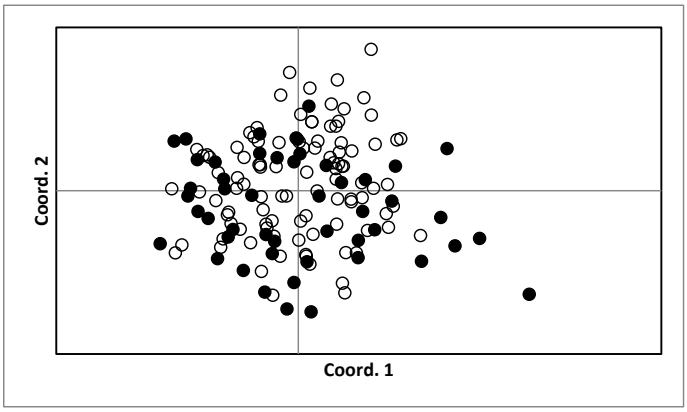


Fig. 1. Standard Poodle suffering from sebaceous adenitis. The disease often starts on the head, neck and ears and can progress to involve all or large parts of the body.

a. All US vs. all UK Standard Poodles



b. SA affected vs. unaffected US Standard Poodles



c. SA affected vs. unaffected UK Standard Poodles

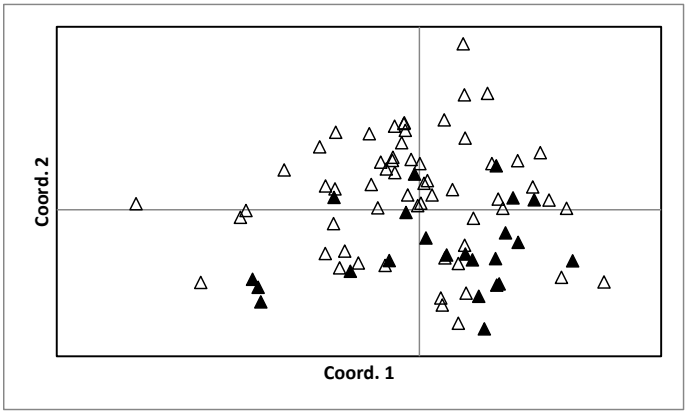


Figure 2. PCoA plot based on STR alleles of randomly related Standard Poodles. a) US (open diamonds) vs. UK (closed squares); b) unaffected (open circles) vs. SA affected dogs (closed circles) from the US; c) unaffected (open triangles) vs. SA affected dogs (closed triangles) from the UK. All of the dogs from the US and UK cluster as two overlapping, yet distinct, populations. SA affected dogs do not segregate from their healthy relatives in either the UK or US.

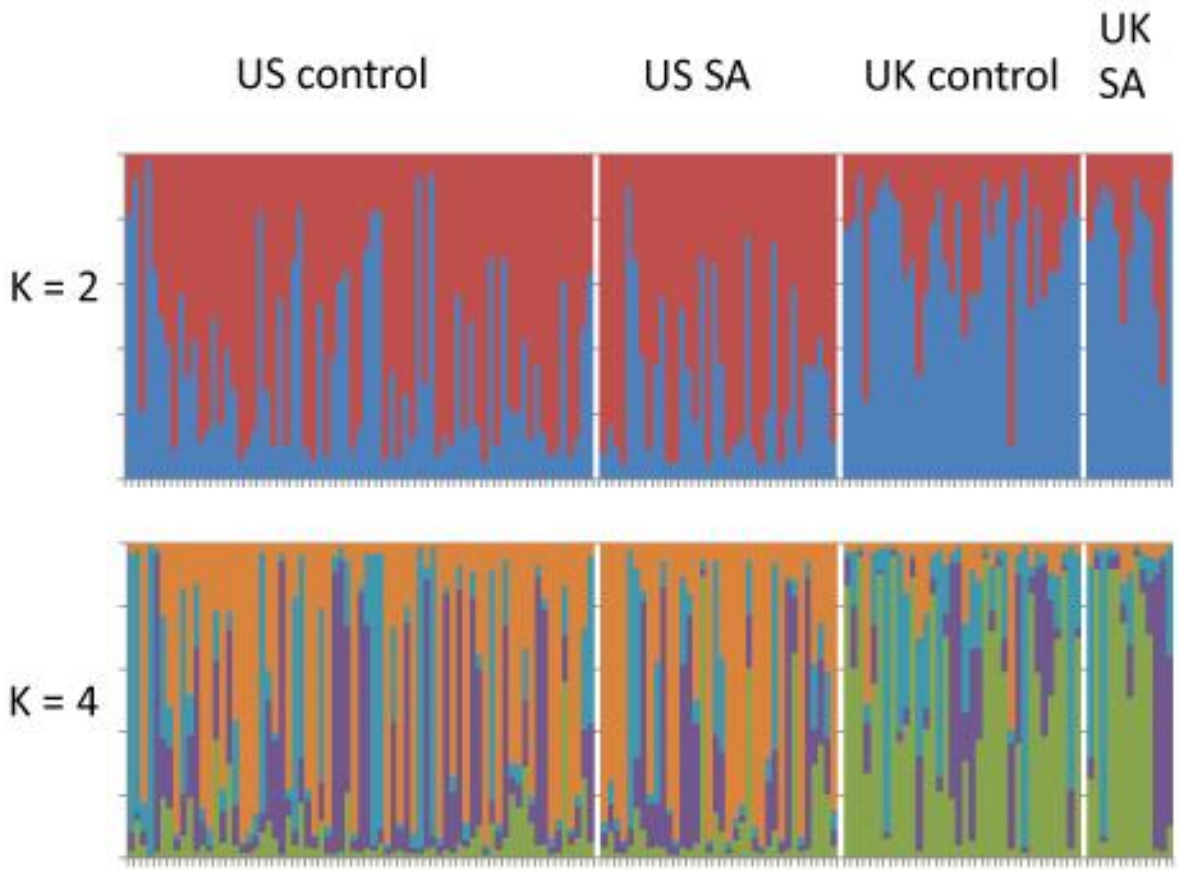


Figure 3. Structure analysis using STRs from unrelated dogs, SA affected and unaffected, from the US and UK. The actual population to which each dog belonged was not listed and the program was "asked" to place each animal into distinct populations based on country of origin and disease status. At K=2, two subpopulations are apparent (red and blue). Blue dominates in the UK dogs while Red dominates in the US population. Attempts to segregate SA affected and healthy dogs from the US and UK (four populations predicted) at K=4 fails to isolate affected from healthy dogs.

Figure 4. Zygosity mapping of across the DLA region of SA affected (left panel) and unaffected (middle panel) unrelated Standard Poodles from the US. The right panel shows the zygosity map for 26 randomly selected indigenous (village) dogs from Bali, Indonesia. Designations of SNPs (far left vertical column) that encompass the DLA class II region are colored grey. The major SNP alleles are colored black, the minor homozygous alleles are colored grey, and all heterozygous alleles are colored white. Individuals possessing the major DLA class II haplotypes 01501*00601*02301/01501*00601*02301 are identified as ++ (second horizontal column at top of figure).