Genetic Diversity Testing for Toy Poodles

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers that will determine genetic diversity within the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to Toy Poodle breeders who wish to determine the amount of genetic diversity that exists in their dogs and to compare diversity with Miniature and Standard Poodles. DLA class I and II haplotypes also provide information on breed founders and relationships within varieties and across breeds.

Breeders and owners have submitted enough DNA samples from 102 Toy Poodles to date. This is sufficient to create an accurate baseline for genetic diversity in the breed. It is possible that additional diversity will be found as more dogs are tested. However, new autosomal STR alleles and DLA class I and II haplotypes will probably be few and their incidence low. The current tables and figures will be updated as more dogs are tested.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across 25 of 39 chromosomes were used to gauge genetic diversity within an individual and across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity.

DLA haplotypes: Seven additional STR loci linked to the DLA class I and II genes were used to identify genetic differences in regions regulating immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with non-genetic factors in the environment, are responsible for autoimmune disease, allergies and reduced resistance to certain infections.

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and cannot be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups. IR values can be adjusted using data from highly random bred village dogs from across the Middle East, SE Asia and Island Pacific nations. Adjusted IR values, known as IRVD, are estimates of the amount of genetic diversity that has been lost over the entire period of breed evolution from when breed ancestors left this region thousands of years ago, over the several hundred years during which the proto-breed (Poodles)evolved.

I. Introduction

A. Breed history

The history of the Toy Poodle is intimately tied up with that of the Standard Poodles. Although it is logical to assume that Standard Poodles came first, miniaturization of dogs has gone on for thousands of years and it is just as likely that various sizes of Poodles evolved in the same time according to different human needs. Standard Poodles would be used as hunting and working dogs, while Miniature and Toy varieties were used primarily for companionship.

The Standard Poodle is thought to have originated as a water fowl retriever in Germany rather than France where it is proclaimed the national dog. The name "Poodle" is the same as the German "pudel", which refers to splashing in water (e.g., water retrieving). Others believe that the Poodle is a descendent of the French Barbet. Regardless of its origin, the Poodle rapidly became one of the most popular dogs in Continental Europe with paintings of dogs identical to Poodles originating in Germany as early as the 15th to 16th century. Small dogs that look like Toy Poodles have been depicted in art going back to the first century.[1] The Standard Poodle was the principal pet dog in Spain in the 18th century and small Poodles were found in many royal households in France during this time.

The Poodle has been bred in several sizes and Standard, Miniature, and Toy Poodles are recognized by the American Kennel Club. The British Kennel Club also recognizes three sizes. The Fédération Cynologique Internationale (FCI) recognizes the three sizes variants but adds in the medium Poodle. The different sizes are not considered breeds, but rather varieties with the miniature and toy being merely smaller versions of the standard. Crosses between various sizes are allowed and offspring registered based on height at the withers. However, the trend is to select parents within the same size range more like breeds.

The Standard Poodle sets the size for the three (or four) different varieties of Poodles. Most kennel clubs state that an adult Standard Poodle must be over 15 inches (38 cm) at the shoulder, while the FCI sets the size for standards at 18-24 inches (45-60 cm). The adult Miniature Poodle must be 11-15 inches (28-38 cm) at the shoulder in registries other than the FCI, where the size range is 11-14 inches (28-35 cm). The slightly smaller size range for Miniature Poodles and higher size range for Standard Poodles in the FCI is to allow for the Medium (Moyen) variety at 14-18 inches (35-45 cm). Toy Poodles are from 7-10 inches (17.8-25.4 cm) in height and 6-9 lbs (3-4 kg) in weight.

There is a theory that Maltese or Havanese may have been crossed to poodles prior to the 1800's to produce Toy Poodles as suggested by the silky coats found in early toys.[1] The present DNA-based study also boosts this theory. The first Toy Poodles depicted in Europe were usually white or white with markings and used to hunt truffles or act as companions. Small white toys from the European continent are thought to have been the foundation for the breed in both the UK and the US. Toy Poodles (which were white) were established as a breed in the United States as far back as 1896 and the first Toy champion was recorded in 1910. Shortly after that time Toy Poodles were accepted as a breed by the AKC, separate from other Poodles. These early Toys were small (3.5 to 5 lbs.) and did not have the same type as the Miniature and Standard Poodles. The breed standard proposed by the International Toy Poodle Club in Philadelphia was published by the AKC in the official book of breed standards in 1929 and had a required weight maximum of 12 pounds. A height maximum of 10 inches was added subsequently to prevent larger dogs from dominating in shows[1].

According to Mackey Irick ("The New Poodle", 1986),[1] many US Toy Poodles can be traced back to CH Happy Chappy, born in 1932 bred by Florence Orsie. Happy Chappy is also the sire of the first colored Toy champion, a silver produced by breeding him to a silver miniature. In 1940, registration papers were revoked for these inter-variety offspring with the argument that these dogs were not true Toy Poodles. Research presented by EE. Ferguson to the AKC resulted in a reversal of this decision, and Toy Poodles were placed within the Poodle breed as a size variety instead of as a separate breed in 1943.

Between the 1940s and the 1980s many Toy to Miniature crosses were done to try to improve the type of the existing Toy Poodles and add new colors. This type of breeding continued until the 1970's or 1980's, after which most breeding was kept mainly within the variety and within colors[1]. Although the result of crossing the original Toys to Miniatures improved head, length of leg, length of body, and coat, it has made it difficult to produce well typed dogs within the desired height of 9.5 to 10 inches at the shoulder. The height issue continues to be one of the biggest challenges for breeders[2]. The Toy Poodle continues to have several different colors that succeed in the show ring and strict within color breeding is no longer the norm.[3] Combinations of red/black, brown/black, black/white, silver/white are more commonly found together in pedigrees than others.

[1]Mackey J. Irick, Jr. "The New Poodle 6th Edition", Chapter Howell Book House, New York, NY 1986

[2]Christi McDonald, "Toy Poodles: 40 years ago and Today", Poodle Variety April-June 2016, p 114

[3]Christi McDonald, "Beyond Black and White", Poodle Variety July-September 2016 p 12.

II. Baseline genetic diversity testing and what it tells us about Toy Poodles

A. Allele frequencies in Toy Poodles at select autosomal STR loci

Autosomal STR loci are highly polymorphic and have great power to determine genetic differences among individuals and between breeds. Table 1 lists the alleles that have been identified in all Toy Poodles tested to date at each of the 33 autosomal STR loci and their incidence in the study population.

Table 1: STR alleles from 33 genomic loci and their frequencies in Toy Poodles

(link to table 1)

B. The use of genomic allele frequencies to determine breed for a breed-wide standard genetic assessment of individual heterozygosity

A standard genetic assessment of heterozygosity proposed by Wright can be determined from alleles and their frequencies for each STR locus in the genome (Table 1) across all 33 loci (Table 2). These measurements include average # alleles/locus (Na), average # effective alleles/locus (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and a coefficient of inbreeding (F) (Table 2). Although a breed may possess a large number of alleles at each locus (Na), a proportion of them may contribute disproportionately to heterozygosity (Ne). A breed with low genetic diversity will often have a low Na and Ne. The observed heterozygosity is the actual diversity that is present in a breed based on the allele frequencies at the 33 STR loci. The expected heterozygosity is the genetic diversity that would exist in a population that is in Hardy-Weinberg equilibrium (i.e., puppies are offspring of the least related individuals in the population being studied). The F value is a coefficient of inbreeding based on He and Ho, i.e. F=1-Ho/He. If Ho and He are equal, F=0 and the population in in HWE. The F value will be positive when there is a deficiency of heterozygotes (i.e., more heterozygotes expected than were observed) and negative where there is an excess of heterozygotes.

The average number of alleles/loci (Na) for the Toy Poodles was 8.42, which is higher than many other pure breeds, but not as high as for the parent breed (Standard Poodle Na=8.91). The average number of alleles/locus (Ne) that contributed most to heterozygosity was 4.09, again higher than many pure breeds that have been studied to this point, and even higher than for Standard Poodle (Ne =3.49). The observed and expected heterozygosity (Ho=0.709 and He=0.729) were also higher than most other breeds. The values for Ho and He yielded a breed-wide coefficient of inbreeding (F) close to zero (0.025). Therefore, standard genetic assessment values indicate that Toy Poodles have a high degree of breed-wide genetic diversity (high Na and Ne) and that breeders are doing a good job of maintaining heterozygosity by choosing (on average) the least possible related parents. However, the standard genetic assessment of Wright provides average values for the population and does not measure the degree of heterozygosity of individual Toy Poodles. This is better determined by measuring the relatedness of a dog's parents based on looking at allele frequencies in a different manner, i.e. internal relatedness (IR).

Table 2. Standard genetic assessment of breed-wide heterozygosity using allele frequencies (Table 1) (Updated October 10, 2019)

	N	Na	Ne	Ho	He	F
Mean	142	8.697	4.179	0.706	0.733	0.037
SE		0.522	0.241	0.016	0.015	0.010

C. Differences in population structure within a breed and between related breeds as determined by principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) compares alleles and their frequency at the 33 STR loci between individual dogs in the population and creates a multi-dimensional graph of their relatedness. Each dog is a point in the graph and the distances between points is a measure of their relatedness to each other. The closer two points are to each other, the more closely they are related. Because it is hard to display results in a multi-dimensional graph, two planes of the graph that most accurately display relationships are chosen to make a two-dimensional graph. This is usually coordinates 1 and 2. Figure 1 shows the relatedness of 102 Toy Poodles. The 102 dogs are obviously of the same breed, because they cluster loosely together. However, the cluster is not tight around the XY axis and there are many outliers. This indicates considerable genetic differences between individuals.

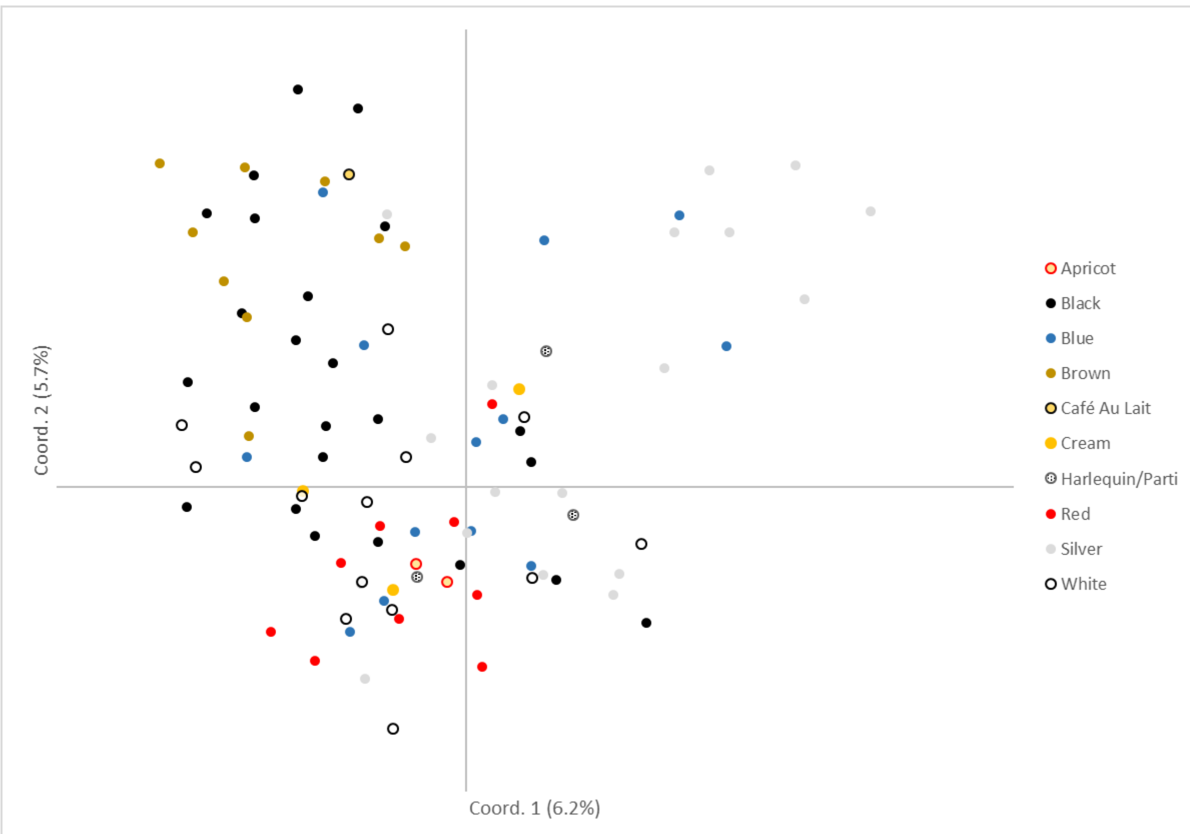


Figure 1. PCoA of Toy Poodles by reported color based on the 33 STRs (click to enlarge)

Principal coordinate analysis can also be used to determine how two populations have genetically differentiated from each other over time. Figure 2 shows a PCoA plot of 102 Toy Poodles and 2003 Standard Poodles using 33 genome-wide STR markers. The two varieties of Poodles are clearly related, given their proximity to each other on the plot, but are genetically distinguishable. However, several Standard Poodles are found within the Toy Poodle population, indicating that they are much more closely related to Standard than Toy Poodles. *(This last statment needs clarification/correction and will be updated.)*

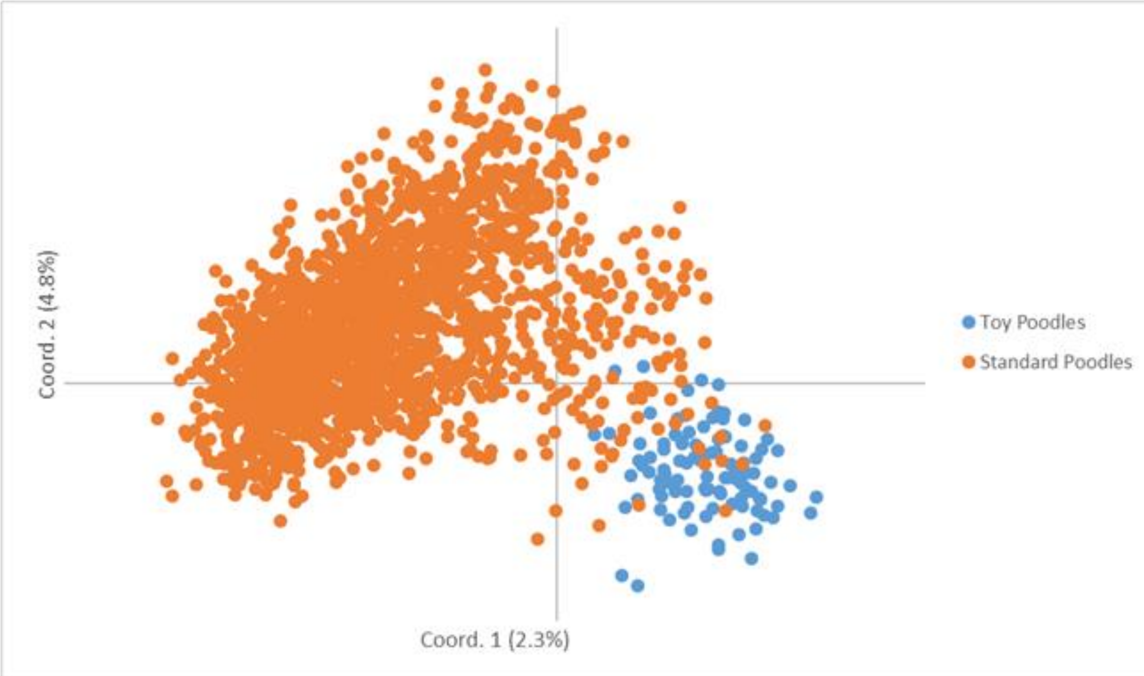


Figure 2. PCoA of Toy Poodles (n=102) combined with Standard Poodles (n=2003) based on the 33 STRs

III. The use of genomic STR loci allele frequencies to determine internal relatedness (IR)

A. Internal relatedness (IR) of individuals and the population as a whole

The genetic assessments given in Tables 1 and 2 refer to the population, and not to individual dogs. Moreover, assessments based on allele frequency alone do not weight the contributions of common vs uncommon alleles to genetic diversity. Internal Relatedness (IR) is a calculation that gives more weight to rare and uncommon alleles and has been often used as a measure of the genetic differences of an individual's parents. IR values are therefore a measure of heterozygosity contributed by each parent. The lower the IR score, the more outbred the individual, and the higher the score, the more inbred. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with a value of -1.0 would come from parents that were totally unrelated at every locus, while dogs with an IR value of +1.0 have parents that were genetically identical at every locus.

The IR curve for Toy Poodle was bi-modal, with a large first peak and second small peak (Fig. 3, red line). The mean of internal relatedness calculated for 102 Toy Poodles from North America and other parts of the worldwas 0.028, with individuals ranging from -.234 to +0.366 (Table 3; Fig. 3). IR values as high as +0.366 were uncommon and most of the breeds had values below +0.20. An IR value of +0.25 would equal offspring of parents that were full siblings, provided that the parents of the full siblings were randomly bred. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred.

Although standard genetic assessment values indicate that Toy Poodles are being randomly bred, the IR values suggest that one-fourth of Toy Poodles have IR values ranging from 0.102 to 0.366. Higher scoring dogs in this population are responsible for the smaller second peak in the IR graph (Fig. 2). The effect of these more inbred dogs is counteracted by an equal population of dogs with IR values below -0.160 to -0.234. This leaves one-half of the population with IR values from -0.060 to +0.102, which represent individuals with reasonably unrelated parents.

Table 3. IR vs IRVD comparison for Toy Poodles (n=102)

	IR	**IRVD**
Min	-0.234	-0.138
1st Qu	-0.060	0.038
Mean	0.028	0.136
Median	0.013	0.132
3rd Qu	0.102	0.204
Max	0.366	0.564

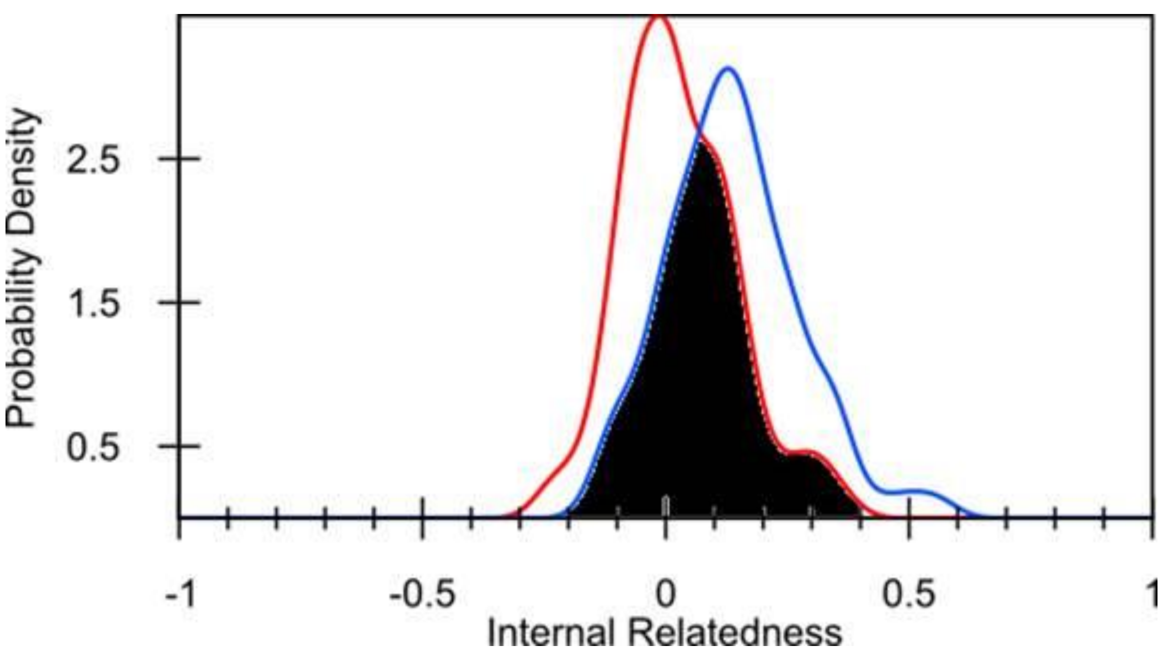


Figure 3. Distribution of IR estimated in Toy Poodles (n=102) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Lost diversity was determined by comparing allele frequencies at the same loci between Toy Poodles and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.

B. IRVD values as a measure of genetic diversity lost during the entire period of breed evolution from earliest ancestors to present

The IR values can be adjusted in such a way as to provide an estimate of total genetic diversity lost from the earliest ancestors of the breed to present time. This is done by using allele frequencies obtained from DNA of present day village dogs from the Middle East, SE Asia and Island Pacific nations. These dogs are the most random bred and genetically diverse population that has been studied to date and is the origin of almost all modern breeds. The adjusted IR value is known as IR-village dogs or IRVD.

The IRVD values for Toy Poodles are shown in Fig. 3 (blue line). The mean IRVD was 0.136 for the population, with individuals ranging from -0.138 to 0.564 (Fig. 3). The shift to the right in IRVD values was not nearly as pronounced as it has been for several other breeds that are participating in genetic diversity testing at the VGL and indicates that Toy Poodles have retained a greater amount of the overall diversity still present in village dogs than other breeds. An estimate of the amount of diversity in dogdom that still exists in Toy Poodles can be made by comparing the blackened area under the two curves with the area of the blue curve. This is around 60%, which is among the highest amount of retained diversity that we have observed and comparable to Miniature Poodles.

III. DLA Class I and II Haplotype frequencies

The DLA consists of four gene rich regions (classes I-IV) making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 4). Groups of genes and their alleles inherited as a block, rather than singly, are called haplotypes. The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and inherited as a single block or haplotype (Table 5). One haplotype comes from each of the parents. Specific class I and II haplotypes are often linked to each other and inherited as a genetic block with limited recombination over time. Therefore, DLA class I and II haplotypes can be viewed as reasonable surrogate markers for breed founders.

Determining DLA class I and II haplotypes is often done by sequencing regions containing the desired alleles. This is a cumbersome and expensive procedure. The use of SNPs for measuring diversity in this region is also extremely difficult, given the high level and complexity of genetic polymorphism between various alleles. Fortunately, STRs are strongly associated with specific genes in the DLA region and can be used instead of SNPs or sequencing to identify and compare various genetic differences (i.e. haplotypes) in these regions. Four STR markers are used to identify DLA class I haplotypes and three STRs for DLA class II haplotypes.

The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes identified in Standard Poodles being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry.

A. DLA class I and II haplotype diversity in Toy Poodles

We have identified 32 distinct DLA Class I and 20 distinct DLA Class II haplotypes in 102 Toy Poodles tested to date (Table 4). These STR-based haplotypes are strongly associated with haplotypes that have been determined by sequencing of DLA-88, DRB1, DQB1, and DQA1 genes. Given the number of dogs tested, it is likely that additional haplotypes will be identified, but few and at low incidence.

Table 4. Toy Poodle DLA class I and class II haplotypes and their frequencies.

DLA Class I Haplotype Frequencies (Updated Oct 10, 2019)		
DLA1 #	**STR types**	**Toy Poodle (n=142)**
1001	380 373 281 182	0.035
1002	380 365 281 181	0.007
1005	389 371 277 181	0.018
1006	387 375 293 180	0.004
1008	386 373 289 182	0.018
1009	382 377 277 184	0.077
1011	376 365 281 180	0.021
1012	388 369 289 188	0.028
1013	392 373 289 186	0.070
1014	375 373 287 178	0.049
1016	382 371 277 178	0.032
1018	375 373 287 186	0.285
1020	388 369 289 184	0.014
1021	380 373 289 186	0.004
1028	376 369 291 186	0.021
1031	382 371 277 186	0.007
1035	386 373 277 184	0.004
1036	389 365 289 180	0.018
1040	380 371 277 186	0.004
1045	376 371 277 186	0.032
1053	382 377 277 186	0.004
1068	380 373 287 181	0.011

1105	382 379 277 178	0.067
1107	376 375 293 183	0.014
1109	381 379 291 186	0.042
1111	387 378 287 182	0.074
1168	382 379 289 186	0.004
1182	382 369 289 176	0.007
1183	382 377 287 182	0.004
1184	386 371 277 178	0.004
1185	388 365 289 188	0.004
1186	389 365 289 186	0.007
1187	389 371 289 176	0.004
1188	395 377 277 184	0.004
1203	378 375 293 180	0.004
1204	378 375 293 181	0.004

DLA Class II Haplotype Frequencies (Updated Oct 10, 2019)

DLA2 #	STR types	Toy Poodle (n=142)
2001	343 324 284	0.007
2003	343 324 282	0.430
2005	339 322 280	0.004
2007	351 327 280	0.004
2008	339 327 276	0.067
2009	351 324 280	0.035
2011	345 322 284	0.021
2012	345 322 280	0.053
2014	339 322 284	0.028
2015	339 327 280	0.035
2016	339 323 284	0.014
2017	343 322 280	0.004
2021	339 324 268	0.067
2022	339 327 282	0.011
2024	343 323 280	0.085
2025	351 321 280	0.018
2027	343 325 284	0.004
2037	341 327 280	0.007
2040	345 327 280	0.028

2050	341 327 284	0.046
2053	343 324 280	0.014
2068	339 327 284	0.014
2094	339 322 276	0.004
2104	341 323 284	0.004

The Toy, Miniature and Standard Poodles have the highest number of DLA class I and II haplotypes of any other pure breeds studied to date. The number of DLA class I and II haplotypes will probably increase a little with the testing of more dogs, but the incidence of any additional haplotypes in the breed is likely to be low. The large number of DLA class I and II haplotypes indicate that many different founders have been involved in the breed's evolution. As expected, the most DLA class I haplotypes are shared with Miniature and Standard Poodles, but the third most class I haplotype sharing is with the Havanese. Class II haplotype sharing is also common with other small breeds such as the Biewer-type terriers, Yorkshire terrier, Bulldog and the larger Flat Coated Retriever (Table 5).

Table 5. Table demonstrating DLA class I and II haplotype sharing between Toy Poodles and several other breeds including the Standard and Miniature Poodles.

DLA Class I Haplotype Frequencies (Updated May 24, 2018)

DLA1 #	STR types	Alaskan Klee Kai (n=459)	Black Russian Terrier (n=109)	Shiloh Shepherd (n=115)	Labrador Retriever (n=125)	Magyar Agar (n=33)	American Akita (n=79)	Japanese Akita (n=308)	Golden Retriever (n=690)	Doberman Pinscher (n=429)	Flat Coated Retriever (n=341)	Havanese (n=374)	Samoyed (n=278)	Miniature Poodle (n=242)	Swedish Vallhund (n=110)	Poodle (n=2083)	Giant Schnauzer (n=158)	Polish Lowland Sheepdog (n=16)	English Bulldog (n=163)	Biewer (n=98)	Biewer Yorshire Terrier (n=50)	Biewer Terrier (n=52)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Italian Greyhound (n=686)	Toy Poodle (n=102)
1001	380 373 281 182	--	--	--	--	--	--	--	--	--	--	--	--	0.014	--	0.266	--	--	--	--	--	--	--	--	--	0.044
1005	389 371 277 181	--	--	--	--	--	--	--	--	--	--	--	--	0.052	--	0.0552	--	--	--	0.031	0.01	0.019	--	--	--	0.02
1008	386 373 289 182	0.06	--	--	0.072	--	--	--	0.0014	--	--	--	--	--	--	0.0194	0.044	--	0.006	0.01	--	--	0.06	--	0.1348	0.015
1009	382 377 277 184	--	--	--	--	--	--	--	--	--	--	--	0.103	0.083	0.05	0.017	--	--	--	--	--	--	--	--	--	0.083
1011	376 365 281 180	0.058	--	--	--	--	--	--	0.0007	--	--	--	0.246	0.004	--	0.0221	0.013	--	--	--	--	--	--	--	--	0.025
1012	388 369 289 188	--	--	--	--	--	--	--	0.0014	--	--	0.017	0.013	0.043	--	0.0106	--	--	0.414	0.184	0.15	0.202	0.28	0.2	0.0087	0.034
1013	392 373 289 186	--	--	--	--	--	--	--	--	--	--	--	--	0.165	--	0.0134	--	--	--	--	--	--	--	--	--	0.059
1014	375 373 287 178	0.365	0.041	--	--	--	--	--	0.0399	--	--	0.033	0.002	0.004	--	0.0074	0.25	--	--	0.02	0.01	0.077	0.09	--	--	0.039
1016	382 371 277 178	--	0.009	--	0.012	--	--	--	0.0014	0.014	--	0.218	--	0.023	--	0.0144	0.038	--	0.095	0.031	0.02	0.01	0.03	--	0.0627	0.029
1018	375 373 287 186	--	--	--	--	--	--	--	--	--	--	0.004	--	0.233	--	0.0084	--	--	--	--	--	--	--	--	--	**0.299**
1020	388 369 289 184	--	--	--	--	--	--	--	--	--	--	--	--	0.002	--	0.005	--	--	--	--	--	--	--	--	--	0.02
1021	380 373 289 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0029	--	--	--	--	--	--	--	--	--	0.005
1028	376 369 291 186	--	--	--	--	--	--	--	--	--	--	--	--	0.05	--	0.0014	--	--	--	--	--	--	--	--	--	0.029
1031	382 371 277 186	--	--	--	--	--	--	--	--	--	--	--	--	0.052	--	0.0012	--	--	--	--	--	--	--	--	--	0.01
1035	386 373 277 184	--	--	0.004	--	--	--	--	--	--	0.003	0.007	--	--	--	0.0002	--	--	--	--	0.01	--	--	--	--	0.005
1036	389 365 289 180	--	--	--	--	--	--	--	--	--	--	--	--	0.045	--	0.0034	--	--	--	--	--	--	--	--	0.0007	0.02
1040	380 371 277 186	0.221	--	--	--	--	0.013	--	0.0007	0.013	--	0.023	--	0.006	--	0.0005	--	0.59	0.04	0.107	0.22	0.038	--	--	0.1006	0.005
1045	376 371 277 186	--	--	0.004	0.012	--	0.006	--	--	--	--	--	--	0.004	--	0.0014	--	--	--	--	--	--	--	--	--	0.044
1053	382 377 277 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0005	--	--	--	--	--	--	--	--	0.0955	0.005
1068	380 373 287 181	--	--	0.248	0.044	--	--	--	0.05	--	0.265	0.016	0.029	0.014	0.359	--	0.035	--	--	--	0.01	--	--	--	--	0.015
1105	382 379 277 178	--	--	--	0.068	--	--	--	--	0.001	--	--	--	0.064	--	0.0017	--	--	--	--	--	--	--	0.3	--	0.029
1107	376 375 293 183	--	--	--	--	--	--	--	--	--	--	--	--	0.029	--	--	--	--	--	--	--	--	--	--	--	0.015
1109	381 379 291 186	--	--	--	--	--	--	--	--	--	--	--	--	0.014	--	--	--	--	--	--	--	--	--	--	--	0.044
1111	387 378 287 182	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	--	--	--	--	--	--	--	--	--	0.059
1168	382 379 289 186	--	--	--	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	0.005
1182	382 369 289 176	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01
1183	382 377 287 182	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005
1184	386 371 277 178	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005
1185	388 365 289 188	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005
1186	389 365 289 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01
1187	389 371 289 176	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005
1188	395 377 277 184	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005

DLA Class II Haplotype Frequencies (Updated May 24, 2018)

DLA2 #	STR types	Alaskan Klee Kai (n=459)	Black Russian Terrier (n=109)	Shiloh Shepherd (n=115)	Labrador Retriever (n=125)	Magyar Agar (n=33)	American Akita (n=79)	Japanese Akita (n=308)	Golden Retriever (n=690)	Doberman Pinscher (n=429)	Flat Coated Retriever (n=341)	Havanese (n=374)	Samoyed (n=278)	Miniature Poodle (n=242)	Swedish Vallhund (n=110)	Poodle (n=2083)	Giant Schnauzer (n=158)	Polish Lowland Sheepdog (n=16)	English Bulldog (n=163)	Biewer (n=98)	Biewer Yorshire Terrier (n=50)	Biewer Terrier (n=52)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Italian Greyhound (n=686)	Toy Poodle (n=102)
2003	343 324 282	--	--	--	0.02	--	--	--	0.0225	--	0.128	0.223	0.013	0.504	--	0.0903	0.038	--	0.598	0.23	0.2	0.231	0.38	0.2	0.0066	**0.441**
2005	339 322 280	--	0.018	--	0.06	--	--	--	0.0159	--	0.422	0.003	--	--	--	0.0228	0.006	--	0.015	0.026	0.01	0.096	--	--	--	0.005
2008	339 327 276	--	--	--	--	--	--	--	--	--	--	--	--	0.083	--	0.017	--	--	--	--	--	--	--	--	--	0.074
2009	351 324 280	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	0.0113	--	--	--	--	--	--	--	--	--	0.044
2011	345 322 284	--	--	--	--	--	--	--	--	0.001	--	--	--	0.004	--	0.0223	--	--	--	--	--	--	--	--	--	0.025
2012	345 322 280	0.062	--	--	--	0.12	--	0.002	0.0007	--	--	0.005	--	0.066	--	0.0048	0.013	--	--	--	--	--	--	--	--	0.059
2014	339 322 284	0.073	--	--	--	--	--	--	--	--	0.032	0.009	--	0.023	--	0.0132	0.003	0.59	0.092	--	--	--	--	--	--	0.029
2015	339 327 280	--	--	--	--	--	--	--	--	--	--	--	0.011	0.052	--	0.0065	--	--	--	--	--	--	--	--	0.0087	0.049
2016	339 323 284	--	--	--	--	0.06	--	--	--	--	--	0.013	--	0.029	--	0.0024	--	--	--	0.082	0.09	0.067	0.09	--	--	0.015
2017	343 322 280	--	--	0.383	--	0.35	0.019	0.005	0.042	--	0.001	0.009	--	--	--	0.0029	--	0.38	0.215	--	--	--	--	--	0.2157	0.005
2021	339 324 268	0.214	--	--	0.02	--	--	--	0.0942	--	--	0.001	--	0.064	--	0.0019	--	--	--	--	--	--	--	--	--	0.029
2022	339 327 282	--	--	0.057	0.088	--	--	--	0.0007	0.002	0.125	0.115	0.092	0.002	0.05	0.0002	0.006	--	0.015	--	--	--	--	--	--	0.015
2024	343 323 280	--	--	--	0.064	--	--	--	--	0.001	--	0.004	0.014	0.01	--	0.0002	--	--	0.006	--	--	--	--	--	--	0.074
2025	351 321 280	--	--	--	--	--	--	--	--	--	--	--	--	0.045	--	0.0034	--	--	--	--	--	--	--	--	--	0.02
2037	341 327 280	0.365	0.289	--	--	--	0.165	0.258	--	--	--	0.033	--	0.008	--	--	0.32	--	--	0.02	0.01	0.077	0.09	--	0.0109	0.005
2040	345 327 280	--	--	--	--	--	--	--	--	--	--	0.004	--	0.004	--	--	--	--	--	0.082	0.09	0.019	0.03	--	0.0022	0.039
2050	341 327 284	--	--	--	--	--	--	--	0.0399	--	--	--	0.002	--	--	--	0.161	--	--	--	--	--	--	--	--	0.039
2053	343 324 280	--	--	0.317	0.04	--	--	--	0.029	--	0.139	0.039	0.613	0.014	0.5	--	0.038	--	--	--	0.01	--	--	--	--	0.015
2068	339 327 284	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.015
2104	341 323 284	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005

The 1018 DLA class I and 2003 class II haplotypes are higher than expected by chance in Toy Poodles (also in Miniature Poodles), and are in linkage disequilibrium (i.e., inherited together). However, the numerous remaining haplotypes are much more random in their incidence. A founder or founder line with the 1018/1023 haplotypes has obviously played an important role in maintaining predominant phenotypes of the Miniature and Toy Poodle breeds and has been highly conserved. The question is whether dogs with these haplotypes have equilibrated within the breed or still enjoy a selection advantage. This can be answered by doing a standard genetic assessment on the frequency of alleles at the seven STR loci that determine the DLA class I and II haplotypes.

B. A standard genetic assessment of breed heterozygosity using STRs that define the DLA Class I and II regions

Another way to assess genetic diversity in the DLA class I and II regions is to apply the same types of statistics used to assess diversity across the genome as reported in Table 2 but compare allele frequencies at the seven STR loci that define the DLA Class I and II regions. Toy Poodles possess somewhat fewer average alleles per locus than Standard Poodles (7.00 vs. 7.86), but the average number of effective alleles per loci is higher (3.53 vs. 2.76). Therefore, a greater proportion of alleles are contributing to genetic diversity in the DLA in Toy Poodles than in Standard Poodles. The observed and expected heterozygosity (Ho and He) are higher in Toy Poodles than in Standard Poodles and are virtually identical in value, giving an inbreeding coefficient (F) in these DLA regions that is nearly zero (0.021). Therefore, it can be concluded that the common DLA class I and II haplotypes are not under positive selection at this time, but rather have become equilibrated in the population over time.

Table 6. Assessment of genetic diversity within DLA region using the frequencies of alleles for each of the 4 STR loci associated with DLA class I and the 3 STR loci associated with class II. (Updated October 10, 2019)

	N	Na	Ne	Ho	He	F
Mean	142	7.286	3.569	0.685	0.705	0.027
SE		0.897	0.334	0.019	0.023	0.009

V. Heritable diseases problems in the Toy Poodle

Toy Poodles, like many small dogs, are long-lived (avg. 15 years). The breed suffers from several complex genetic traits that are common among miniature breeds such as patellar luxation, aseptic necrosis of the femoral head, collapsing trachea, and periodontal disease. Hip dysplasia occurs but is uncommon. Cushing’s syndrome and heart valve degeneration increase in frequency with age. Obesity is a problem in the breed and increases with age. Hypoglycemia can be a problem in the smaller members of the breed.

Autoimmune disorders, such as sebaceous adenitis and Addison's disease are common in breeds such as the Standard Poodle, but much less common in Miniature and Toy Poodles. However, Toy Poodles can suffer from type 1 diabetes, immune mediated thrombocytopenia or hemolytic anemia, and granulomatous meningoencephalitis. Epilepsy is increasing incidence in many breeds in parallel with increasing incidence of autoimmune disorders. Otitis externa is a problem as it is in any dogs with this type of coat, drooping ears, hair growth down into the ear canal and skin allergies. Skin tumors, usually benign, are also a problem in the breed.

Progressive retinal atrophy and von Willebrand's disease type 1 are genetic disorders in the breed. Cataracts often occur within the first three years of life, also suggesting a heritable origin.

One of the emerging problems in Toy and Miniature Poodles is intravertebral disc disease (IVDD), something that is common in shorter and longer backed breeds such as the Dachshund and Corgi. The Toy and Miniature Poodle had two different body types: 1) "Correct or square", with legs long enough for their body height equal their length, and 2) short legs and a long back. The latter body type is much more susceptible to IVDD. Shortening of the legs was first thought to involve a single mutation in what is known as the FGF4 gene. The common mutation leads to what is called chondrodysplasia, a condition that leads to a moderate shortening of the legs and not associated with IVDD. This mutation has been introduced into many breeds of dogs to decrease the length of long bones and torso and cause some bowing of the front legs. Many Miniature Poodles possess this mutation and its associated phenotype. A second mutation in FGF4 has been recently described (see reference below) that causes a further shortening of the legs and lengthening of the back, but also causes weakness of the cartilage of the spinal discs (Brown et al., 2017). Breeds such as the Dachshund and Corgi have both mutations, explaining the extraordinary shortening of the legs and elongation of the back. The FGF4 mutation has been identified in Miniature Poodles that have suffered IVDD (Brown et al., 2017).

Brown EA, Dickinson PJ, Mansour T, Sturges BK, Aguilar M, Young AE, Korff C, Lind J, Ettinger CL, Varon S, Pollard R, Brown CT, Raudsepp T, Bannasch DL., 2017. FGF4 retrogene on CFA12 is responsible for chondrodystrophy and intervertebral disc disease in dogs. Proc Natl Acad Sci U S A. 114(43):11476-11481.

VI. Interpretation of DNA-based genetic testing

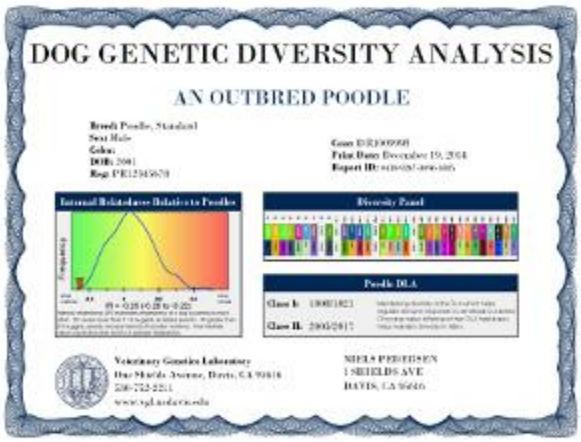
Toy Poodles have among the greatest amount of genetic diversity of any breed tested to date. This diversity is evident in the genomic autosomes as well as in important regions such as the DLA. This genetic (genotypic) diversity can be attributed to the tremendous phenotypic diversity found between individuals of the "variety." This genetic diversity is a result of a large founder population and introgressions from several breeds other than Standard or Miniature Poodle varieties; breed popularity and large population size favoring random mate selection; a relatively loose standard including different coats, coat colors; and a range of sizes and body types. Breeders have been also diligent in selecting the least related parents available to them, as indicated by the small amount of allele and haplotype sharing.

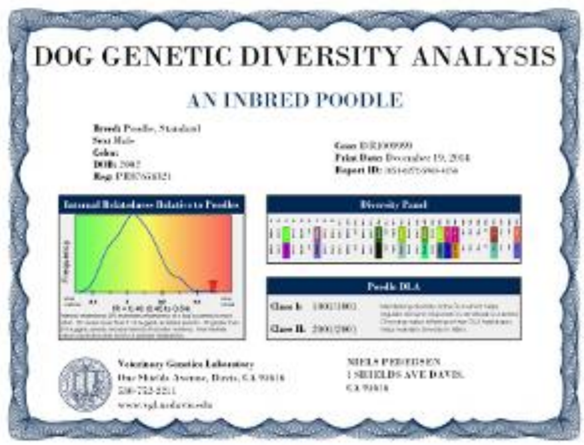
Although breed-wide standard genetic assessments indicate that the breed is very heterogeneous, internal relatedness (IR) scores indicate that there is still a proportion of individual Toy Poodles that are offspring of parents that are quite related, sometimes to the equivalent level of full siblings. It is important to identify such individuals or bloodlines prior to breeding to find mates that will produce puppies that have much lower IR scores. This should not be a great problem as the breed has great genetic diversity from which to select the best mates.

VII. How to use apply this information

A. Certificates for sharing of genetic information

After a sample is submitted for genetic testing, the identity of the dog and owner will be replaced by a laboratory barcode identifier. This identifier will be used for all subsequent activities. After testing, each owner will be provided with a certificate that reports the internal relatedness, genomic STR genotypes and DLA class I and II haplotypes for the dog(s) tested. The diversity of alleles at each of the genomic STR loci and their frequency in the population were used to determine the genetic relatedness and diversity. The internal relatedness value for the dog being tested is related to the population as a whole.





B. How are DNA based genetic markers best used by breeders?

The goal for Toy Poodle breeders should be to maintain the large amount of genetic diversity that exists in the breed and to continue to randomly select mating pairs to avoid the low proportion of highly inbred individuals. IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. A breeding pair with identical IR values can have genetically distinct parents and produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter is much different in genomic allele and allele frequencies and DLA haplotypes, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents because of genetic recombination. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

<u>In brief, potential sires and dams should be first screened for genetic differences in the genome and in the DLA regions by first comparing allele differences at each STR locus, and then at the DLA class I and II haplotypes. Some thought should be given to rare vs common alleles. This information is included on all certificates and on the website. This preliminary comparison will identify promising pairings and if desired, genetic information on the potential sires and dams can then be used to calculate actual IR expectations for their puppies. Puppies, once born, should be tested for their individual IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals but improving genetic diversity in puppies should be paramount.</u>

Genetic information on Miniature Poodles and Standard Poodles will be extremely useful for those breeders interested in outcrossing between the two varieties of Poodle. Outcrossing would most benefit Standard Poodles and may be useful in creating desired medium sized dogs. Outcross pairs should be chosen to provide maximum genetic differences in puppies over parents (i.e. low IR scores) and genetic diversity testing used to identify which puppies would be most valuable for further outcrossing or backcrossing. The goal of outcrossing and backcrossing should be to regain the desired phenotype, while maintaining the greatest amount of new diversity.