Genetic Diversity Testing for Italian Greyhounds

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 across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to Italian Greyhound (IG) breeders who wish to track and increase genetic diversity of the Italian Greyhound breed as a long term goal.

For **other breeds**, please see Enrolling a Breed

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across the genome 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, and breed-wide allele frequency is provided.

DLA haplotypes: 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.

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.

I. Introduction

A. Breed history

The Italian Greyhound is the smallest of the sighthound breeds, typically weighing from 8 to 15 lb (3.6 to 6.8 kg) and 13 to 15 inches (33 to 38 cm) tall at the shoulder. They have been placed in the toy group in the UK and US, but in the sighthound group in the Fédération Cynologique Internationale (FCI). The breed has an ancient history and mummified similar appearing dogs have been found in Egypt and pictures of small Greyhounds have been found in the ruins of Pompeii. Dogs like Italian Greyhounds were reportedly pets in the early Roman imperial courts

[1]. Although the breed has been named after its well-known connections to Italy, it is believed to have originated in Greece and Turkey, where small greyhound-like dogs were pictured in early pottery and wall art from these countries. Italian greyhound was widespread in Southern Europe and were highly favored by Italians of the sixteenth century. The breed was well established in England and other European countries in the seventeenth century.

Italian Greyhounds (IG) in the USA began with a small number of founders imported from Europe, with limited introduction of new dogs in the subsequent decades resulting in narrow breeding choices. Geographic separation and limited founder numbers, coupled with differences in phenotypic preferences, has led to a distinct genetic separation to the level of varieties between North American and European Italian Greyhounds [2]. These genetic bottlenecks were enhanced by a series of popular sires and their progeny. Each period of inbreeding was followed by an extended period of close linebreeding. The most famous and important genetic influence on the breed in the USA and Canada was a champion stud named Dasa King of the Mountain. His occurrence in pedigrees rapidly rose from 0 to 99.7% from 1970 to 2010 [3]. The resultant inbreeding along Dasa lines has reduced genetic diversity and increased homozygosity in the most popular contemporary show lines in North America. This has resulted in a greater susceptibility to a wide range of autoimmune diseases [3]. and inadvertent positive selection of other complex and simple deleterious traits [4]. Much less is known about the situation in Europe, but it is certain that Dasa lines have not had near the influence as in North America. However, Italian Greyhounds from Continental Europe appear to suffer from many of the same common heritable health problems as IG from the USA but at an unreported incidence.

II. Baseline genetic diversity

A. Population genetics based on existing alleles and their frequency at 33 STR loci on 25 chromosomes

1. Identification of alleles and their frequency at each of the 33 genomic STR loci

As of Oct 1, 2018, 739 Italian greyhound from around the world have been tested (Table 1). The loci contained from 3 to 15 alleles. One to three alleles occurred at frequencies of 20% or higher at each locus, and 11 loci had a single allele that occurred at a frequency of 50% or greater. One locus (C22.279) had a single allele that was present in 93.1% of dogs tested and a second locus (INU030) had an allele found in 83.2% of dogs. These single high frequency alleles were obviously highly conserved in the breed and were most likely associated with phenotypic traits that were both highly favored and strongly conserved.

Table 1. Allele frequencies for 33 STR markers in Italian greyhound Table 1 Link

2. Standard genetic assessment of heterozygosity based on allele frequencies

Allele and allele frequencies were used to determine basic genetic parameters related to heterozygosity, such as the number of alleles found at each STR locus (Na), the number of alleles that contribute most to heterozygosity (Ne), the average observed or actual heterozygosity (Ho) that was found in the population, and the heterozygosity that would be expected (He) if the

existing population was in Hardy Weinberg equilibrium (HWE). HWE is achieved when the selection of mates is entirely random and subject to no positive or negative human selection pressure. The value F is a coefficient of inbreeding derived from the Ho and He values. A value of +1.0 would occur only if every individual were genetically indistinguishable at each of the 33 STR loci, while a value of -1.0 would be seen when all the dogs were completely different at each of the 33 loci

The genetic information used to formulate the enclosed tables and graphs came from DNA samples of 329 dogs from North America, the UK, and Continental Europe including outlying countries such as Romania, Russia, and the Ukraine. We believe that the dogs tested represent almost all the genetic diversity that still exists in the breed. This data will be updated as more dogs are tested, so allele and DLA haplotype frequencies may change to a limited extent over time.

a. A comparison of heterozygosity values between Italian greyhound from USA and Europe

Average heterozygosity values were determined for 213 Italian greyhound known to have come from the USA and 174 from Europe (Table 2). The average number of alleles per locus was similar in both varieties and was typical for many pure breeds of dogs. Heterozygosity was similar in the two populations, although Ho was somewhat higher in European (Ho=0.624) than USA dogs (Ho=0.607). The expected heterozygosity was virtually identical in both populations, but the resultant inbreeding coefficient (Fis or F) was higher in USA dogs (F=0.053) than for European dogs (F=0.020). Therefore, USA dogs were somewhat more inbred, on average, than European dogs.

Table 2. Genetic assessment of Italian Greyhound confirmed to be from the USA (n=213) or Continental Europe (n=174) using 33 genomic STR markers. Mean value plus or minus one standard error (SE).

	N		Na	Ne	Ho	He	FIS
USA	213	**Mean**	6.452	3.368	0.607	0.641	0.053
		SE	0.431	0.226	0.026	0.026	0.008
EU	174	**Mean**	6.262	3.089	0.624	0.638	0.020
		SE	0.386	0.150	0.021	0.021	0.010

b. Heterozygosity values for all Italian greyhounds (n=735) tested to date

Italian greyhound breeders from many different countries have participated in DNA based genetic diversity testing over the last 5 years and there has been increased interest in increasing breed-wide genetic diversity by crossing between varieties and decreasing inbreeding within certain bloodlines by selecting sires and dams for significant differences at both genome-wide and DLA-associated loci. Table 3 lists the current results from 735 dogs tested as of Oct 1, 2018.

Data from this larger and more diverse population of dogs is not significantly different from either the USA or European populations shown in Table 2. The exception is an increase in the average number of alleles found at each locus from 6.45/6.26 to 7.17. This is a 13% increase in genetic diversity than in the earlier and smaller populations of Italian greyhound. It is unlikely that any additional genetic diversity will be found over time, and if it is, it will be for alleles occurring at very low incidence in the population.

Table 3. Summary of Standard Genetic Assessment for Italian Greyhound using 33 STR loci

	N	Na	Ne	Ho	He	F
Mean	735	7.12	3.37	0.61	0.65	0.06
SE		0.45	0.23	0.03	0.03	0.01

Analyses reported in Tables 2 and 3 indicate that Italian greyhound are randomly breeding, as F values are low. However, these figures are misleading because they are only averages for the population and do not look at inbreeding in individual dogs. Average values can result when the contribution of highly inbred dogs in the population are canceled out by a similar group of highly outbred dogs. The level of inbreeding in individual dogs is better measured by internal relatedness (IR) calculations (see below).

B. Differences in population structure as determined by principal coordinate analysis (PCoA)

We tested Italian greyhound from the USA. PCoA is a graphic portrayal of how closely individual dogs in a defined population are related to each other. The actual presentation is a sphere, which is difficult to portray. Therefore, the relationships are usually graphed in the two planes or coordinates, usually 1 and 2, that most accurately depicts relationships between individuals. The Italian greyhound tested clearly belong to a single breed, but with clear genetic differentiation by region of origin (USA vs. Europe). Some minor genetic differentiation is also seen within the European population, with dogs from the UK, Belgium and Germany segregating more closely with the North American dogs. Dogs from Poland, Ukraine and Finland are somewhat more distant, while dogs from France are central to the European population.

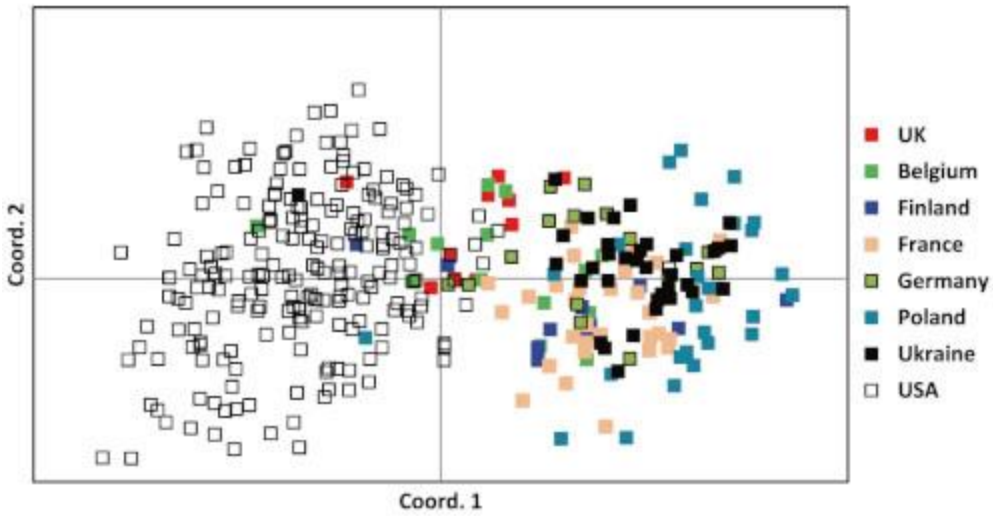

Fig. 1. PCoA plot showing population structure of IG from the USA and from several countries in Continental Europe and the UK. Found in: https://cgejournal.biomedcentral.com/articles/10.1186/s40575-015-0030-9.

Many more Italian greyhound have been tested since 2015 [2] when the dogs shown in Fig. 1 were sampled. The PCoA derived from this larger collection of 735 dogs tends to form a single and genetically diffuse population (Fig. 2). However, the tendency for the population to segregate into two groups to the right and left of the midline is still evident, but not as distinct as in Fig. 1. The tendency for all the dogs to cluster as a single breed and not two geographic varieties is due to the inclusion of more outcrosses between USA and European varieties. It is hoped that outcrossing between the two geographic disparate populations will continue to increase. The increased genetic diversity that is occurring because merging USA and European dogs is apparent.

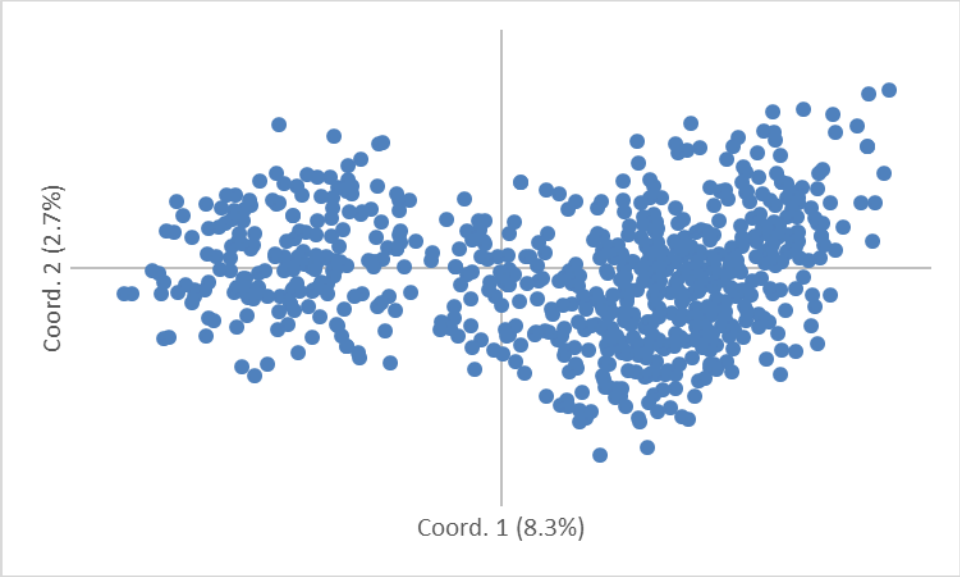

Fig. 2. PCoA plot of all (n=735) Italian greyhound from around the world tested as of October 1, 2018.

III. The use of genomic allele frequencies to determine internal relatedness

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

1. IR values

Genetic assessments such as those presented in Tables 2-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity of individuals within the population. The genetic diversity of an individual dog is largely determined by the diversity inherited from each of its parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which an individual dog's parents were related (Table 4). The IR calculation takes into consideration homozygosity at each locus and gives more importance to rare and uncommon alleles. Rare and uncommon alleles would presumably be present in less related individuals. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0 (red lines). A dog with a value of -1.0 would have parents that were totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical at all loci. An IR value of +0.25 is expected for offspring of full sibling parents from a random breeding population. IR values >0.25 are expected when the parents were themselves highly inbred. IR scores for individual dogs can be graphed (Figs. 3, 4).

The IR scores for Italian greyhound from the US ranged from a low of -0.25 (most outbred or most unrelated parents) to 0.50 (most inbred or most related parents) (Fig. 3). A small proportion of the population had IR scores of 0.25 to 0.50, which would be comparable to village dog puppies born to parents that were either siblings from random breeding parents or offspring of even closer related parents. The IR curve for the European Italian greyhound was more shifted to the left with no dogs having IR scores ranging from -0.30 to >0.38 (Fig. 4). Therefore, IR values more accurately portrayed the level of inbreeding (or outbreeding) among individual dogs in the breed and confirmed that Italian greyhounds from the USA were more likely to be highly inbred or outbred than dogs from Europe.

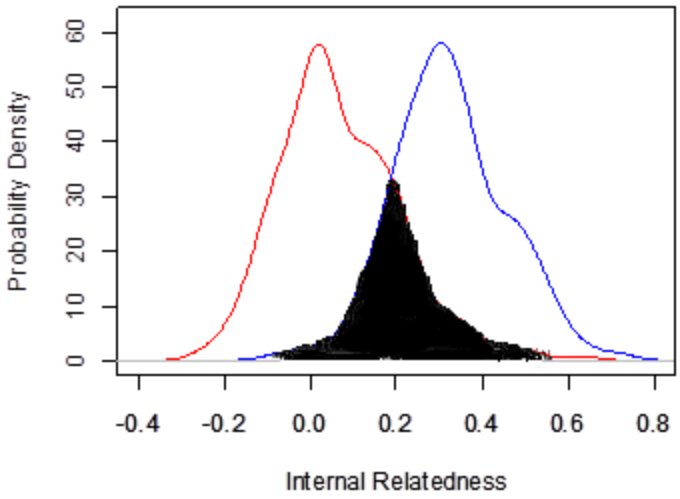

Figure 3. Distribution of IR estimates in 212 Italian Greyhound from USA based on intra-breed diversity (solid line or Red line), compared with IR adjusted for diversity lost during breed development (dashed line or blue line). Diversity lost because of breed development was determined by comparing allele frequencies at the same loci between Italian Greyhound and randomly breeding village dogs from the Middle East, SE Asia, and the Pacific Islands. These village dogs are the most genetically diverse population that has been studied and are the ancestors of Italian Greyhound. The area of overlap (black=27%) between the IR and IRVD curves represents the amount of available canine genetic diversity that has been retained in the breed up to the present day.

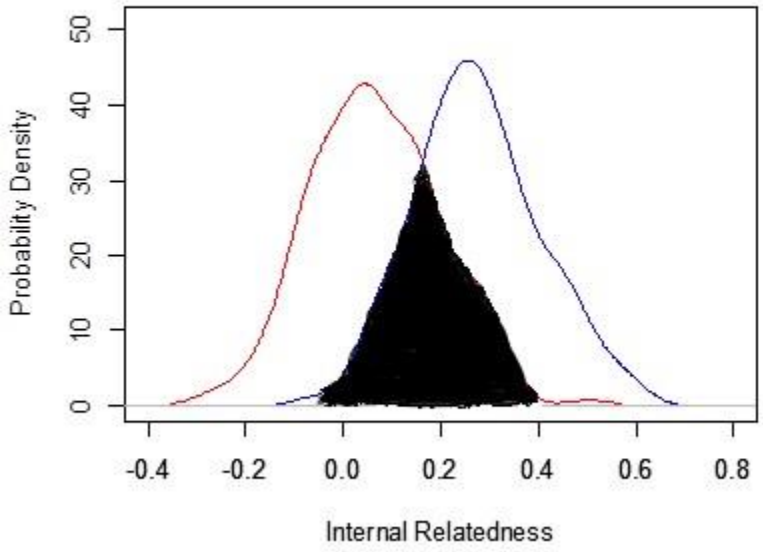

Figure 4. Distribution of IR estimates in 174 Italian Greyhound from Europe based on intra-breed diversity (solid line or Red line), compared with IR adjusted for diversity lost during breed development (dashed line or blue line). Diversity lost because of breed development was determined by comparing allele frequencies at the same loci between Italian Greyhound and randomly breeding village dogs from the Middle East, SE Asia, and the Pacific Islands. These village dogs are the most genetically diverse population that has been studied and are the ancestors of Italian Greyhound. The area of overlap (black=54%) between the IR and IRVD curves represents the amount of available canine genetic diversity that has been retained in this population up to the present day.

IR values were also calculated for the 738 Italian greyhound that were tested as of Oct 1, 2018 Table 7). Individual IR values for the 738 dogs are graphed in Fig. 5. DNA samples came from many parts of the world for testing and the IR values calculated for the entire population without regards to origin (Table 4). IR values for this population ranged from -0.325 to 0.708. This was an extreme degree of internal relatedness and the rare and most inbred dogs (0.708) were even more closely related than offspring of sibling parents from a random breeding population. The parents of such dogs would have come from the same highly inbred lines. On the opposite extreme, were dogs with scores of -0.313 that were obviously offspring of very unrelated parents.

Table 4. IR vs IRVD comparison for Italian Greyhound (n=738)

	IR	IRVD
Min	-0.313	-0.109
1st Qu	-0.034	0.199
Mean	0.060	0.293
Median	0.046	0.296
3rd Qu	0.149	0.372
Max	0.708	0.786

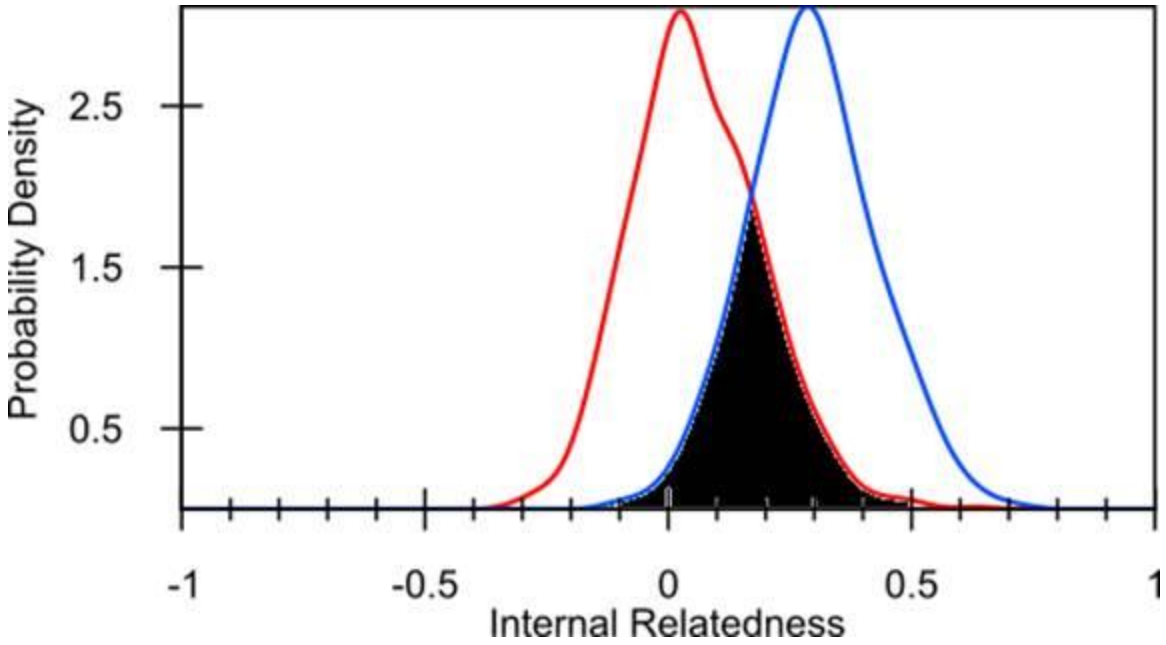

Fig. 5. Distribution of IR estimated in Italian Greyhound (n=735) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Shared genetic diversity (37%) was determined by comparing allele frequencies at the same loci with village dogs from the Middle East, SE Asia, and the Islands Pacific.

2. IRVD values

The IR values can also be used to give an approximation of how much of the genetic diversity that exists in all dogs has been lost during early (proto-breed) breed evolution and subsequent events that occurred after the breed was registered and closed to further introgressions. This is done by comparing the frequency of a given allele in Italian greyhound with the frequency of the same alleles in a population of village dogs from the Middle East, SE Asia, Taiwan and other Pacific island nations such as Brunei and the Philippines. Contemporary village dogs are largely unchanged from the ancestors of almost all modern dog breeds. The resultant frequencies are then used to calculate the IRVD curves (Figs. 2-4). An estimate of retained (shared) genetic diversity can be obtained by determining the percentage of the IR curve that is overlaid by the IRVD curve. This percentage is 27% for Italian greyhound from the USA (Fig. 3) and 56% for European Italian greyhounds (Fig. 4). The larger worldwide population shared 37% of their genetic diversity with village dogs, which was intermediate as would be expected from a mixture of USA and European dogs (Fig. 5). The additional loss of genetic diversity by Italian greyhound from the USA reflects the limited number European founders used to establish the breed in the USA. This is a well-known artificial genetic bottleneck for many breeds that have been re-established in the USA. These results parallel those obtained from the PCoA graphs, i.e. the overall population is much more genetically diverse than any of its parts. Overall, contemporary European Italian greyhound about one-half of the genetic diversity found in modern village dogs, which is higher proportion of available genetic diversity than in many other breeds. However, USA dogs have retained even less diversity. At the lowest extreme, the Swedish Vallhund has retained only 7% of canine genetic diversity, the Doberman Pinscher 15% and the Shiloh

Shepherd 27%, while at the highest level the Miniature Poodle has retained 51%, the Golden Retriever 50.4% in Labrador retriever 54%, and Toy Poodle 60%.

IV. DLA Class I and II Haplotype frequencies and genetic diversity

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.

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.

1. DLA class I and II haplotypes existing in Italian greyhound

Dog DLA and STR haplotype diversity.

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and II haplotypes, forming unique "extended DLA class I-II haplotypes. Extended class I-II haplotypes are inherited as a single block of genes.

a. DLA haplotypes in USA vs. European Italian greyhound

We have identified 19 different STR-associated DLA Class I and 18 DLA Class II haplotypes in Italian Greyhounds (Tables 3-5). Three DLA class I (1040, 1044, 1052) are found in 76% of all European Italian Greyhound, while three haplotypes (1008, 1044, 1052) are found in 61% of USA dogs. Similarly, three DLA class II (2017, 2034, 2035) are found in 56% of USA Italian greyhounds and two (2017, 2034, 2039) in 79% of European dogs. The sharing of the dominant 1044, 1052, 2017 and 2034 haplotypes reflects the importance of founders or founder possessing these haplotypes in the origin of the breed in ancient time. The haplotypes that were unique to either European or North American Italian greyhounds were either gained or lost in more recent times (Tables 3, 4). The numbers (18/19) of DLA class I and II haplotypes found across North American and European dogs was much higher than Swedish Vallund (6,4) and Shiloh shepherd (7, 6); somewhat higher than Giant Schnauzer (14, 15), Samoyed (13,12) and Shiba Inu (16/15); and lower than Golden Retriever (26,23) and Miniature Poodle (33, 23).

Table 3. STR-associated DLA class I haplotype frequencies among Italian Greyhound from Continental Europe (*n*=174 dogs and 348 haplotypes) and the USA (*n*=213 dogs and 426 haplotypes)

VGL #	Class I Haplotype	EU	US
1008	386/373/289/182	0.03	0.21
1012	388/369/289/188	0.01	0.01
1016	382/371/277/178	0.10	0.06
1030	380/373/293/178	0.01	0.03
1036b	389/365/289/180	0.003	0.00
1040	380/371/277/186	0.24	0.04
1044	375/373/291/178	0.32	0.21
1048a	380/370/289/184	0.00	0.02
1049a	380/370/289/186	0.00	0.002
1050a	380/371/289/182	0.00	0.002
1051b	380/371/289/184	0.01	0.00
1052	380/372/289/184	0.20	0.19
1053	382/377/277/186	0.03	0.11
1054a	382/379/277/184	0.00	0.01
1055a	386/373/289/180	0.00	0.002
1056	386/373/289/190	0.01	0.01
1058b	387/378/287/186	0.01	0.00

1059	390/371/291/182	0.03	0.09
1065b	380/371/277/181	0.003	0.00

a=present only in IG from USA
b=present only in IG from Continental Europe

Table 4. STR-associated DLA class II haplotype frequencies among Italian Greyhound from Continental Europe (*n*=174 dogs and 348 haplotypes) and the USA (*n*=213 dogs and 426 haplotypes). Recognized DRB1/DQA1/DQB1 haplotypes based on exon 2 sequencing that correspond with STR-associated haplotypes are listed when known

VGL #	Haplotype	EU	US
2003	343/324/282	0.01	0.01
2015b	339/327/280	0.00	0.002
2017	343/322/280	0.23	0.22
2023	341/323/282	0.01	0.03
2029	337/324/268	0.03	0.09
2030b	339/322/268	0.00	0.002
2031	339/322/282	0.10	0.06
2032b	339/323/280	0.00	0.09
2033b	339/323/282	0.00	0.01
2034	341/322/280	0.32	0.21
2035	341/323/280	0.03	0.13
2036b	341/327/276	0.00	0.09
2037b	341/327/280	0.00	0.01
2038a	345/324/280	0.03	0.00
2039	345/327/276	0.24	0.05
2040a	345/327/280	0.06	0.00
2041a	349/321/280	0.003	0.00
2044a	343/324/268	0.003	0.00

a=present only in IG from Continental Europe
b=present only in IG from USA

Table 5 lists the DLA class I and II haplotypes that were found after the addition of 336 additional Italian greyhound without indication of origin. The number of DLA class I haplotypes has increased from 19 to 21, and the number of DLA class II haplotypes from 18 to 19. These increases were due to the recognition of haplotypes that had a low incidence in the population. It

is likely that the known number of DLA class I and II haplotypes for the breed have been identified among these 723 dogs. The frequency of each haplotype in the population has not significantly changed.

Table 5. DLA class I and II haplotypes and their frequency for 723 Italian greyhound from around the world.

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)		
DLA1 #	**STR types**	**Italian Greyhound (n=882)**
1008	386 373 289 182	0.1304
1012	388 369 289 188	0.0079
1016	382 371 277 178	0.0595
1030	380 373 293 178	0.0249
1036	389 365 289 180	0.0006
1040	380 371 277 186	0.0901
1044	375 373 291 178	0.2494
1048	380 370 289 184	0.0119
1049	380 370 289 186	0.0006
1050	380 371 289 182	0.0011
1051	380 371 289 184	0.0023
1052	380 372 289 184	0.1899
1053	382 377 277 186	0.0941
1054	382 379 277 184	0.0147
1055	386 373 289 180	0.0006
1056	386 373 289 190	0.0062
1058	387 378 287 186	0.0079
1059	390 371 291 182	0.1026
1065	380 371 277 181	0.0006
1104	386 373 289 186	0.0023
1169	375 373 277 186	0.0006
1228	390 373 289 176	0.0017
DLA Class II Haplotype Frequencies (Updated Oct 9, 2019)		
DLA2 #	**STR types**	**Italian Greyhound (n=882)**
2003	343 324 282	0.0062
2014	339 322 284	0.0017
2015	339 327 280	0.0091
2017	343 322 280	0.2211

2023	341 323 282	0.0249
2029	337 324 268	0.1026
2030	339 322 268	0.0006
2031	339 322 282	0.0590
2032	339 323 280	0.0431
2033	339 323 282	0.0051
2034	341 322 280	0.2494
2035	341 323 280	0.0896
2036	341 327 276	0.0714
2037	341 327 280	0.0085
2038	345 324 280	0.0096
2039	345 327 276	0.0941
2040	345 327 280	0.0017
2041	349 321 280	0.0006
2044	343 324 268	0.0006
2102	341 327 268	0.0011

Genetic polymorphisms in DLA class I and II genes have been linked to an increased risk of autoimmune disorders in several dog breeds [3]. The first study of a multiple autoimmune disease syndrome in Italian greyhound from North America looked for DLA associations with specific polymorphisms in the DLA class II genes, DRB1, DQA1 and DQB1 [3]. This study identified two alleles of the DRB1 gene, *00203 and *02901, as significant risk factors for autoimmune disease. These alleles are present within the DLA class II 2029, 2036, and 2037 haplotype regions defined by STRs [2]. **However, these and other class I and II haplotypes were not found to confer significantly greater risk for autoimmune disease in a subsequent study** [2]. Therefore, more studies are required to confirm whether genetic polymorphisms with the DLA region are associated with an increased risk of autoimmune disease in Italian greyhound as in other dog breeds and humans [3]. Although autoimmunity in North American Italian greyhound could not be strongly associated with specific gene polymorphisms in the DLA region, both studies confirmed the significant contribution of inbreeding to disease risk [2,3]. The highest incidence of autoimmune disease occurred in the one-third of the North American Italian greyhound population that was most inbred as determined by a relative lack of genetic diversity and high degree of allele and haplotype sharing (homozygosity). **Therefore, the best way to reduce the incidence of autoimmune disease is to rebalance both genomic and DLA diversity by outcrossing the dominant and less diverse bloodlines with less common and more genetically diverse lines and not to concentrate on specific genomic STR alleles or DLA class I and II haplotypes.**

IV. What does this assessment of genetic diversity and heterozygosity tell us about contemporary Italian Greyhounds

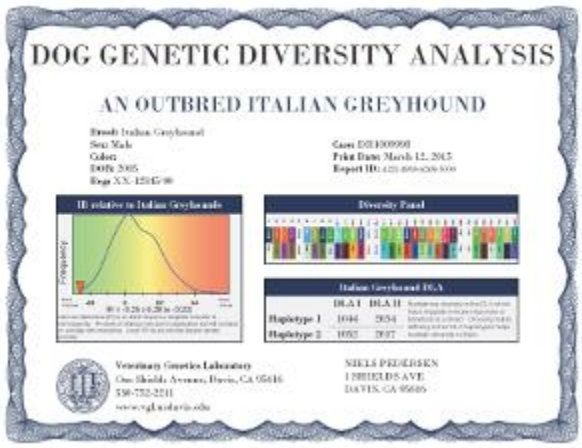
The breed as it exists world-wide appears to have reasonable breed-wide diversity, but this diversity exists across two genetically distinct varieties in North America and Europe as a recent study has demonstrated [2]. The European population appears to be more heterozygous (less inbred) than dogs from North America and has retained a greater proportion of the genetic diversity known to exist in modern village dogs (54% vs 27%). The secondary loss of genetic diversity in Italian greyhound from the USA compared to Europe was most likely to have been associated with a limited importation of founders. However, it is possible that some of this loss may be due to other genetic bottlenecks such as the Dasa popular sire effect [2].

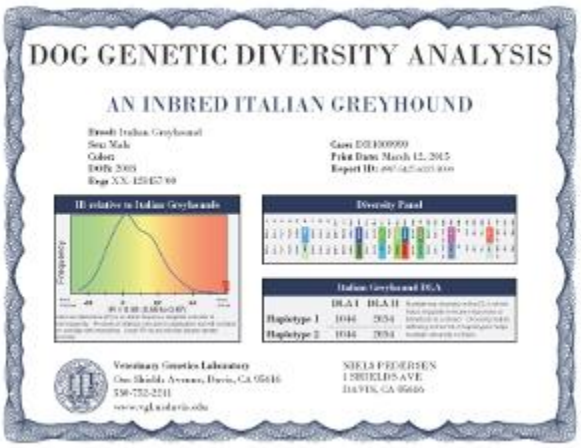
Decreased heterozygosity in North American Italian greyhound appears to be due in large part to the over-representation of more highly inbred lines within the North American population because of Dasa influence [2]. Therefore, a majority of North American Italian greyhound possess a minority of the existing genetic diversity, while a minority of dogs possess a majority. This has resulted in an increased incidence of known simple recessive disease traits (e.g., PRA, glaucoma, enamel hypoplasia) and a wide variety of autoimmune diseases [3,4]. Additional disorders of a heritable nature exist in the breed, but the exact form of inheritance and causative mutations are yet to be determined. These disorders include spontaneous limb bone fractures in young dogs, Legg-Perthe's disease (aseptic necrosis of the femoral head), patellar luxation, hip dysplasia, congenital megesophagus, progressive periodontal disease, hepatoportal shunts, masticatory myopathy, vitreous degeneration, cataracts, lens luxation, color dilution alopecia, epilepsy, and allergies. These various disorders appear to have resulted from both ancient and relatively new mutations that have been concentrated in certain lines because of inbreeding. The hope is that breeders will use genetic diversity testing, along with pedigrees, to re-establish genetic diversity across the breed by careful mate selection, while continuing to investigate diseases that appear to have a genetic basis.

V. Interpretation of genetic diversity test data for Italian Greyhound

A. How will you be given the results of DNA-based genetic diversity testing on your dog?

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 and 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 internal relatedness value for the dog being tested is related to the population.





B. How to apply results of DNA testing

The goal for IG breeders should be to produce a greater and greater proportion of puppies with IR scores less than 0, and with time even lower scores. There appears to be ample genetic diversity in the breed to achieve this goal over several generations. This will require using different combinations of breeding stock, including even those from inbred lines with high IR values. IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may 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 has few alleles and DLA haplotypes in common, 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. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

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. It is important to rebalance diversity for both the genomic and DLA types. 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 actual 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.

Out-crossing between North American and European Italian greyhound is apparently becoming more acceptable and the impact of such crossing is apparent (Fig. 1, 2). Increasing genetic diversity by outcrossing, coupled with eliminating highly inbred lines by careful line breeding, should significantly reduce the incidence of heritable disorders. Further efforts should be made, however, to identify the genetic basis for serious heritable diseases such as autoimmune disorders, congenital megesophagus and spontaneous long bone fractures in young dogs. The

genetic causes of three simple autosomal recessive disorders (PRA, glaucoma, enamel hypoplasia) have been discovered, and the role of inbreeding and DLA types in autoimmune disease better understood, by collaborative research between breeders and academia [2-7].

VI. Health problems of a heritable nature

The Italian Greyhound has a median lifespan of 13.5 in a 2004 UK Kennel Club survey. UK dogs are primarily of the European type. However, a 1993 US breed club survey, presumably involving USA dogs, gives an average lifespan of 9 years, but more than a quarter of the dogs had "accidents" recorded as cause of death. This suggests that Italian greyhound from the USA are less healthy. These figures may be biased, as health problems of Italian greyhound from the USA have been more extensively reported than for European dogs. Nonetheless, there is a feeling that both populations suffer from many of the same disorders, although not as frequently in the European population [2,3]. The breed suffers from more disorders of suspect or proven heritability than most other breeds.

Disorders of unknown heritability are presumed to be of a complex genetic nature and associated risk factors (genetic polymorphisms?) most likely entered the breed by descent from founders or through subsequent outcrosses. Complex heritable disorders such as Legg-Perthe's disease, patellar luxation, and liver shunts are strongly associated with small breeds. Epilepsy and autoimmune disorders increase in frequency in all breeds as they become more inbred. Risk factors for these types of complex genetic disorders have probably accumulated over the much longer period from when dogs first associated with people and increasingly subjected to human-directed selection. Heritable disorders such as congenital megesophagus and spontaneous long-bone fractures are serious problems in the breed and there is evidence that congenital megesophagus entered the breed through English terriers. Color dilution alopecia appeared with the introduction of the dilute and black coat genes, most likely from surreptitious outcrossing rather than spontaneous mutations. There are at least four disorders that have a simple autosomal recessive mode of inheritance that appear to be breed specific. The mutations causing vonWillebrand's disease are numerous and have occurred spontaneously in many dog breeds. Mutations for PRA, closed angle glaucoma and enamel hypoplasia have apparently occurred within the breed.

A. Health problems that have been reported in the breed

Disorders of unknown and apparently complex heritability that may have been inherited by descent from founders:

1. Epilepsy
2. Legg-Perthes disease (aseptic necrosis femoral head)
3. Patellar Luxation
4. Cataracts
5. Vitreous degeneration
6. Liver shunts
7. Multiple autoimmune diseases [2,3]; thyroiditis, Addison's disease, autoimmune hemolytic anemia, pure red cell aplasia, autoimmune thrombocytopenia, SLE and SLE-like syndromes, sterile (idiopathic) meningitis, masticatory myositis, myasthenia gravis, orchitis (sterility), cerebellitis, pemphigus, rheumatoid arthritis, pansteatitis
8. Periodontal disease (gingivitis/ periodontitis with gum recession and early tooth loss)

Disorders of unknown and apparently simple or complex heritability that may have entered the breed through more recent outcrosses:

1. Congenital megesophagus
2. Spontaneous or minimal stress fractures of long bones in young dogs?
3. Color dilution alopecia (disorder of blues, blue fawns) [4].

Disorders associated with autosomal recessive mutations from within the breed:

1. von Willebrand's disease (mild or inapparent bleeding disorder)?
2. Progressive retinal atrophy IGa, IGb, IGc (autosomal recessive with late onset) [5]
3. Closed angle glaucoma [6]
4. Ameleogenesis imperfecta (simple autosomal recessive) [7]

VII. References

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