Genetic Diversity Testing for Great Dane

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 to determine genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel is useful to dog breeders who wish to use DNA-based testing to track and distribute genetic diversity as a supplement to in-depth pedigrees. Information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions in order to improve the overall genetic health of a breed.

Genetic diversity testing of Great Dane is now in the preliminary results phase. During this phase, we will continue to test more registered dogs to build the genetic database necessary to provide an accurate assessment of genetic diversity within the breed. This report is based on 43 registered Great Danes from Australia (n=3), Canada (n=4), and USA (n=36). Although results reported herein are preliminary, this cohort of individuals should provide a reasonable picture of genetic diversity in the breed. Allele and DLA haplotype frequencies will be updated as more dogs are tested. It is anticipated that new alleles at the 33 STR loci and additional DLA class I and II haplotypes will be identified in the future for the Great Dane, but these will likely be of lower frequency than those detected in this initial population.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from carefully selected regions of the genome were used to assess genetic heterogeneity and existing genetic diversity within an individual as well as across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity and genetic diversity in individuals as well as breed-wide.

DLA haplotypes: Seven STR loci linked to the DLA class I and II genes were used to identify genetic differences in a region that regulates 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 susceptibility to infectious agents.

Internal Relatedness: The IR value is a measure of the genetic relatedness of an individual's parents. The value 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; two individuals from different sources may have identical IR values, but a quite different genetic makeup.

I. Introduction to the Great Dane

A. Breed History [1-3]

The Great Dane can be traced back to mid-16th century crosses between English Mastiffs and Irish Wolfhounds, exported all over Europe from England. These dogs were used primarily to hunt large game such as bear and boar by European nobility. In the 17th century, German nobility started breeding these dogs in order to improve their hunting ability, thus calling them "*Englische Dogge*" (or English Dog). With the increased use of guns for hunting, the Englische Dogge was no longer needed in order to chase and pin down bears and boar; subsequently, they started being bred for companionship. Therefore, during the 1800's German breeders sought friendly, calm and good-natured dogs to serve as "Kammerhunde", or chamber dogs.

In 1878, a committee of German dog breeders and show judges changed the name of the breed to "Deutsche Dogge", later known as German Mastiff. Despite no contributions from Denmark to the development of the breed, it later became known as Great Dane due to tensions between the Germans and the other countries during the war. The English market rejected the name German Mastiff, and the dog became known as the "Grand Danois" (Great Dane), a name given by French naturalist Compte de Buffon. The first Chancellor of Germany, Otto von Bismarck, was an enthusiast of the breed, having owned several Great Danes throughout his life (**Figure 1**). His dogs were nicknamed "Reichshunde", or "dogs of the Empire".

Figure 1. Otto von Bismarck with Tyras II and Rebecca in 1891.

The Great Dane was recognized as a breed by the AKC in 1887, and a breed standard was set the following year (1888) by the German Deutsche Doggen-Club. The Great Dane Club of America was formed in 1899. It is currently a relatively popular breed in the USA, ranked 16 of 204 in popularity among the AKC registries.

B. Appearance [1-3]

According to the AKC's breed standard, male Great Danes should not be shorter than 30 inches at the shoulders, but preferred height is 32 inches or more. Females should not be shorter than 28 inches at the shoulders, but height for bitches is preferred to be 30 inches or more. Individuals under these minimum height requirements are disqualified. Males should appear more massive than females, with larger frame and heavier bone structure; weight usually ranges between 140 and 175 pounds for males, and between 110 and 140 pounds for bitches. In the ratio between length and height, the Great Dane should be square. One of the most discernible features of Great Danes, aside from their stature, is their long and rectangular head. The coat is short, thick and glossy and can present many colors, markings, and patterns. The AKC's breed standards list them as follows:

Brindle
Color: Yellow gold, brindled with black cross stripes.
Patterns/Markings: Black chevron pattern with a black mask. Black should appear on the eye rims and eyebrows and may appear on the ears and tail tip. Preference is given to intense base color with distinct and even brindling. Too much or too little brindling, white markings on the chest or toes, and dirty colored brindles are undesirable.

Fawn
Color: Yellow gold.
Patterns/Markings: Black should appear on the eye rims and eyebrows with a black mask and may appear on the ears and tail tip. Deep yellow gold is preferred. White markings on the chest or toes and dirty colored fawns are undesirable.

Blue
Color: Pure steel blue.
Patterns/Markings: White markings on the chest or toes are not desirable.

Black
Color: Glossy black.
Patterns/Markings: White markings on the chest or toes are not desirable.

Harlequin
Color: White with black torn patches. Merle patches are normal.
Patterns/Markings: Black torn patches are well distributed over the body, usually with whole or partial white neck. Black pigment may be seen on the skin in white areas. Large patches are undesirable. Eligible but less desirable, are black hairs showing through the white base coat ("salt and pepper").

Mantle
Color: Black and white with a black blanket extending over the body.
Patterns/Markings: Black skull with white muzzle (white blaze is optional), usually with whole or partial white neck. White is seen on the chest, whole or part of the forelegs and hind legs, and on the tip of the tail.

Merle
Color: A pale gray to dark gray merle base color with black torn patches within.
Patterns/Markings: Solid Merle (white on chest and toes is permissible) or Merle with a Mantle Pattern.

Color disqualifications: Merlequin (white dog with only patches of merle) and any color other than the seven described above.

The VGL currently offers a genetic test for the Harlequin Pattern in Great Danes: https://vgl.ucdavis.edu/test/harlequin-pattern-great-danes. Harlequin results from the complex interaction of the Merle (*PMEL17*) and Harlequin (*PSMB7*) genes on black pigment. The dominant Merle gene by itself produces dark spots on a dilute background on black dogs. If a merle dog also inherits one copy of the Harlequin gene, the dark spots increase in size and the background pigment is removed altogether. Dogs that are not merle, or only have red pigment, cannot express the Harlequin gene. Two copies of Harlequin have not been observed and is presumed to be embryonic lethal, thus all Harlequin patterned dogs have only one copy of the mutation.

C. Temperament [2,3]

The Great Dane must be spirited, courageous, always friendly, dependable and never timid or aggressive. Despite their gentle nature, Great Danes are alert home guardians. The breed is known for their patience and friendly demeanor towards children.

D. Health of the Great Dane [4-6]

1. Lifespan

The breed's average lifespan is 7 to 10 years of age, which is typical for large dog breeds.

2. Diseases

2.1. Gastric Dilatation-Volvulus (GDV)

Also called "bloat", GDV is an extremely painful and life-threatening condition that occurs in higher frequency in large, deep-chested dog breeds. The Great Dane has the highest lifetime risk of GDV among dog breeds, estimated at 36.7%. This disorder is characterized by the accumulation of gas in the stomach, which leads to its dilatation and oftentimes rotation on its axis (volvulus). This rapid expansion of the stomach may cause compression of other organs (such as the heart) and blood vessels; if not treated immediately, GDV can lead to death due to cardiogenic shock. The causes of GDV are not fully understood, but several factors have been implicated in this disorder such as breed, age, diet, exercise patterns, stress, and temperament. Previous research identified three risk alleles for GDV in the immune system genes *DLA88*, *DRB1*, and *TLR5* in Great Danes. Prophylactic gastropexy, or preventative tack, is a surgical procedure that can help reduce the mortality from GDV in Great Danes by 29.6-fold. Other preventative measures include offering multiple small meals per day and not exercising the dog immediately after a meal.

2.2. Dilated Cardiomyopathy (DCM)

Dilated cardiomyopathy is an inherited, potentially fatal heart disorder. In affected dogs, the left ventricle is often dilated, resulting in a progressive thinning of the wall and irregular heartbeat, thus decreasing overall cardiac function and output. This lack of adequate circulation can lead to fluid accumulation in the lungs as well as other parts of the body. Great Danes often also have atrial fibrillation. Affected dogs can show progressive deterioration leading to death or can be relatively asymptomatic and then die suddenly, usually of congestive heart failure. Great Danes are known to be one of the highest predisposed breeds to DCM, together with Doberman Pinschers. Research is ongoing to characterize the genetic mechanisms of DCM in Great Danes. The OFA recommends a cardiac evaluation by board certified cardiologist, including an echocardiogram, for all breeding stock.

2.3.Inherited Myopathy of Great Danes (IMGD)

Inherited myopathy of Great Danes (IMGD) is a rapidly progressive muscle myopathy with an age of onset around six months; both sexes are affected. Affected dogs exhibit exercise intolerance and progressive muscle atrophy. Research data suggest that only 20% of affected dogs survive to adulthood with acceptable quality of life, and all recorded cases were either fawn or brindle. This hereditary disease has an autosomal recessive mode of inheritance: dogs with one normal and one mutated *BIN1* gene (carriers) are unaffected, but breeding two carriers together would be predicted to produce 25% affected offspring and 50% carriers. Testing for IMGD assists owners and breeders in identifying affected and carrier dogs. Breeders can use results from the test as a tool for selection of mating pairs to avoid producing affected dogs. The VGL offers a genetic test for IMGD: https://vgl.ucdavis.edu/test/imgd.

2.4. Leukoencephalomyelopathy (LEMP)

Leukoencephalomyelopathy (LEMP) is a neurodegenerative disorder of the central nervous system characterized by a generalized, progressive loss of balance with increasing immobility. Signs of LEMP often appear prior to 1 year of age, typically presenting as gait abnormalities including dragging of paws and knuckling. The disease is progressive, moving from front to back limbs but is not associated with pain. This disorder, initially discovered in Rottweilers, is inherited in an autosomal recessive fashion, which means that males and females are equally affected and that two copies of the defective gene are needed to cause LEMP. Dogs with one normal and one affected gene (carriers) are normal and show no signs of the disease. This variant is also present in Great Danes, therefore testing for this breed is advisable since matings between carriers are expected to produce 25% of affected puppies. The VGL offers a genetic test for LEMP: https://vgl.ucdavis.edu/test/lemp.

2.5.Hip Dysplasia (HD)

As is the case of most large and giant dog breeds, Great Danes can also suffer from hip dysplasia. HD results from an unstable hip socket and subsequent degenerative arthritic changes that result from this instability. HD can affect young puppies, but more frequently leads to a degenerative, sometimes crippling, arthritis in mature dogs. Clinical manifestations vary greatly; some dogs do

not show clear symptoms, whereas for others the disease is completely debilitating. Hip dysplasia examinations are recommended for breeding dogs (OFA, PennHIP).

2.6. Wobblers Syndrome

Also called "wobbler disease", this condition originates from malformation and/or instability of the neck vertebrae, which in turn causes compression of the spinal cord. The disease is more prevalent in large dog breeds such as Doberman Pinschers and Great Danes, and is thought to have a genetic component. Additionally, external factors such as a high-protein diet or neck trauma might also result in this condition. Symptoms tend to appear progressively and include weakness, ataxia, dragging of the toes, and a "drunken" gait. Treatment is usually done with corticosteroids and movement restriction, or through surgery to correct the spinal cord compression.

2.7. OFA-CHIC Health Testing Requirements for the Great Dane

Hip dysplasia, eye examination, autoimmune thyroiditis, and cardiac evaluation. More information on https://www.ofa.org/recommended-tests?breed=GD.

II. Preliminary Results on Genetic Diversity of 41 Great Danes

A. Population genetics based on 33 STR loci on 25 canine chromosomes

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes [7,8]. Each STR locus contains 7 to 29 different alleles (average of 15.4 alleles/locus) in the breeds tested at the VGL so far. Each breed, having evolved from a small number of founders and having been exposed to artificial genetic bottlenecks, will end up with only a portion of the total available diversity. Artificial genetic bottlenecks can include phenomena such as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity which can lead to increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 43 Great Dane individuals in this study are listed in **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Great Dane (n=43). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
80 (0.02)	129 (0.01)	117 (0.02)	**219 (0.29)**	238 (0.02)	87 (0.04)
94 (0.10)	131 (0.12)	119 (0.17)	221 (0.02)	242 (0.01)	89 (0.32)
98 (0.29)	133 (0.05)	121 (0.28)	225 (0.05)	244 (0.35)	**91 (0.43)**
100 (0.01)	137 (0.11)	123 (0.04)	227 (0.21)	**246 (0.43)**	95 (0.22)
102 (0.05)	141 (0.18)	125 (0.01)	229 (0.17)	248 (0.12)	
104 (0.01)	**147 (0.51)**	**127 (0.32)**	233 (0.18)	250 (0.05)	
106 (0.13)	151 (0.01)	131 (0.07)	235 (0.07)	254 (0.01)	
110 (0.07)		133 (0.09)			

112 (0.04)
114 (0.22)
118 (0.05)

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
286 (0.24)	116 (0.06)	128 (0.01)	148 (0.04)	232 (0.10)	91 (0.05)
288 (0.52)	**118 (0.56)**	132 (0.10)	**156 (0.40)**	**234 (0.59)**	**95 (0.44)**
290 (0.21)	120 (0.11)	136 (0.04)	160 (0.17)	238 (0.22)	99 (0.33)
292 (0.02)	122 (0.05)	**144 (0.61)**	164 (0.18)	240 (0.09)	101 (0.16)
	124 (0.22)	148 (0.18)	168 (0.11)	242 (0.01)	105 (0.02)
		152 (0.04)	172 (0.04)		
		158 (0.02)	176 (0.06)		

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.02)	144 (0.05)	204 (0.05)	85 (0.18)	227 (0.05)	192 (0.09)
122 (0.09)	146 (0.01)	208 (0.07)	**95 (0.73)**	231 (0.24)	194 (0.01)
124 (0.32)	**150 (0.74)**	210 (0.23)	97 (0.01)	233 (0.12)	202 (0.27)
126 (0.56)	152 (0.20)	212 (0.21)	107 (0.01)	**235 (0.37)**	204 (0.12)
132 (0.01)		**216 (0.37)**	111 (0.06)	241 (0.07)	206 (0.15)
		218 (0.07)		245 (0.15)	**208 (0.37)**

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.05)	164 (0.05)	268 (0.26)	222 (0.01)	139 (0.06)	12 (0.15)
212 (0.16)	166 (0.15)	**272 (0.74)**	226 (0.17)	143 (0.12)	13 (0.04)
216 (0.46)	**168 (0.41)**		228 (0.16)	145 (0.17)	19.2 (0.10)
220 (0.33)	170 (0.38)		232 (0.05)	147 (0.15)	20.2 (0.01)
	172 (0.01)		234 (0.24)	**149 (0.34)**	21.2 (0.02)
			238 (0.37)	153 (0.16)	22.2 (0.12)
					23.2 (0.29)
					24.2 (0.26)
					25.2 (0.01)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
12 (0.04)	12 (0.01)	15 (0.01)	14 (0.01)	9 (0.28)	13 (0.22)
14 (0.05)	13 (0.07)	18 (0.06)	15 (0.09)	13 (0.02)	14 (0.01)
17.1 (0.02)	**14 (0.44)**	19 (0.06)	**16 (0.26)**	14 (0.02)	15 (0.07)
18.1 (0.02)	15 (0.28)	21 (0.04)	17 (0.07)	**15 (0.61)**	16 (0.04)
19.1 (0.10)	16 (0.07)	22 (0.01)	19 (0.20)	16 (0.06)	**17 (0.45)**
20.1 (0.27)	17 (0.07)	25 (0.01)	20 (0.17)		18 (0.18)
21.1 (0.27)	18 (0.02)	26 (0.15)	21 (0.05)		19 (0.02)
22.1 (0.15)	19 (0.02)	**27 (0.46)**	22 (0.16)		
23.1 (0.06)		28 (0.04)			
24.1 (0.02)		29 (0.15)			
		31 (0.01)			

VGL2918	VGL3008	VGL3235
11 (0.01)	13 (0.04)	12 (0.26)

13 (0.55)	14 (0.04)	13 (0.02)
14 (0.18)	15 (0.04)	**14 (0.60)**
15 (0.04)	16 (0.16)	16 (0.01)
16 (0.05)	**17 (0.28)**	17 (0.04)
18.3 (0.09)	18 (0.26)	18 (0.07)
19.3 (0.04)	19 (0.07)	
21.3 (0.05)	20 (0.05)	
	21 (0.07)	

The number of alleles found for each STR locus in the Great Dane ranged from two (REN247M23) to 11 (AHT121, VGL1165). Allele distribution within each of the 33 autosomal STR loci is typical of most pure breeds of dogs, in which one allele is observed at higher frequency than others (bold on Table 1). However, unlike other breeds where one allele predominates (i.e., occurs at a disproportionately higher frequency), Great Dane allele frequencies are more evenly distributed in each locus. This indicates that sire and dam selection has tended to use the complete range of available genetic diversity at the time of breed development. Additional alleles for these 33 STR markers may be identified as more individuals are tested, but likely at low number and frequency.

B. Assessment of population diversity using standard genetic parameters

Alleles for each of the 33 STR loci listed in Table 1 and their respective frequencies are used to determine basic genetic parameters for the population (**Table 2**). These parameters include the number of alleles found at each locus (**Na**); the number of effective alleles (**Ne**) per locus (i.e., the number of alleles that contribute most to genetic differences/heterozygosity); observed heterozygosity (**Ho**); expected heterozygosity (**He**) if the existing population was in Hardy-Weinberg equilibrium (i.e., random breeding); and the coefficient of inbreeding (**F**) derived from Ho and He values.

Table 2. Genetic Assessment of 43 Great Danes based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	**Ne**	**Ho**	**He**	**F**
Mean	6.5	3.49	0.63	0.68	0.07
SE	0.4	0.21	0.03	0.02	0.02

The average number of STR alleles identified in this cohort of 43 Great Danes (Na = 6.5) corresponds to approximately 42% of the average number of alleles known to exist at each of these loci across breeds (6.5 out of 15.4 – see section IIA). This means that around 58% of the known canid genetic diversity at these 33 STR loci has been lost up to this point in time in Great Danes. However, the average number of effective alleles (Ne) constitutes a more important metric for diversity, since these alleles have the greatest genetic influence on heterozygosity. The average number of effective alleles per locus in this cohort was 3.49, indicating that most of the heterozygosity was determined by approximately one-half of the alleles segregating in the breed.

The mean observed heterozygosity (Ho) for this cohort was estimated at 0.63 while the expected heterozygosity (He) was slightly higher at 0.68, yielding a coefficient of inbreeding (F) of 0.07.

This means that this group of Great Danes was randomly breeding (Hardy-Weinberg equilibrium), (*i.e.*, sires and dams were as unrelated as possible), and only 7% of the study cohort were more inbred than that. Although the standard genetic assessment values indicate that Great Danes constitute a random mating population, this conclusion is based on the cohort as a whole and not on individual dogs making up the population. Internal Relatedness (IR) scores provide a better picture of heterozygosity for each dog and should be used by breeders to select the most unrelated mates possible (see section E below).

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each of the 33 autosomal STR loci used in this study (**Table 3**). This provides an estimate of genetic similarities in the specific regions of the genome that are associated with each STR marker. The average number of effective alleles (Ne) per locus ranged from 1.59 (REN247M23) to 5.78 alleles (VGL1828). Average observed heterozygosity (Ho) for an individual STR locus ranged from 0.256 (REN247M23) to 0.878 (VGL1828), while average expected heterozygosity (He) ranged from 0.369 (REN247M23) to 0.827 (VGL1828) (**Table 3**).

Loci with the lowest Ho values contributed the least to heterozygosity levels across the breed; they are most likely associated with inherited traits that are important for the breed's phenotypic standard. Conversely, high Ho values for a particular locus means that it shows greater genetic diversity across the breed, and that these loci can be associated with phenotypic variation among individuals. Additionally, high inbreeding coefficients ($F > 0.1$) were estimated for 13 of the 33 STR loci (40%, shaded in gray on **Table 3**), which suggests that these loci have been under strong positive selection since breed development. However, these are balanced by STR loci with F values around or below zero, which results in the F value estimated for the cohort as a whole (**Table 2**). Taken together, these results suggest that this cohort was as unrelated as possible and that Great Dane breeders have kept a good balance in inbreeding levels across these genomic regions through careful sire and dam selection.

Table 3. Standard Genetic Assessment of individual STR loci for 43 Great Danes. Individual STR loci with high inbreeding coefficients (F > 0.1) are shaded in gray.

Locus	Na	Ne	Ho	He	F
AHT121	11	5.76	0.81	0.83	0.026
AHT137	7	3.05	0.65	0.67	0.031
AHTH130	8	4.39	0.62	0.77	0.198
AHTh171-A	7	5.01	0.66	0.8	0.177
AHTh260	7	3.04	0.69	0.67	-0.028
AHTk211	4	3.02	0.65	0.67	0.026
AHTk253	4	2.65	0.54	0.62	0.137
C22.279	5	2.63	0.56	0.62	0.094
FH2001	7	2.39	0.63	0.58	-0.090
FH2054	7	4.12	0.71	0.76	0.066
FH2848	5	2.48	0.43	0.6	0.282
INRA21	5	3.04	0.66	0.67	0.018
INU005	5	2.36	0.42	0.58	0.281
INU030	4	1.68	0.37	0.41	0.099
INU055	6	4.11	0.83	0.76	-0.096
LEI004	5	1.75	0.34	0.43	0.201
REN105L03	6	4.14	0.74	0.76	0.019
REN162C04	6	4.01	0.78	0.75	-0.040
REN169D01	4	2.79	0.49	0.64	0.239
REN169O18	5	3	0.74	0.67	-0.107
REN247M23	2	1.59	0.26	0.37	0.307
REN54P11	6	4	0.85	0.75	-0.138
REN64E19	6	4.74	0.68	0.79	0.135
VGL0760	9	5.02	0.73	0.8	0.086
VGL0910	10	5.43	0.73	0.82	0.103
VGL1063	8	3.46	0.59	0.71	0.177
VGL1165	11	3.73	0.73	0.73	0.000
VGL1828	8	5.78	0.88	0.83	-0.062
VGL2009	5	2.2	0.54	0.55	0.015
VGL2409	7	3.42	0.63	0.71	0.103
VGL2918	8	2.86	0.59	0.65	0.100
VGL3008	9	5.36	0.81	0.81	0.011
VGL3235	6	2.33	0.61	0.57	-0.070

D. Differences in population structure as determined by Principal Coordinate Analysis (PCoA)

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (usually coordinates 1 and 2). The closer individuals cluster together around the XY axis, the more closely related they are to each other. The 43 Great Danes in this study clustered

as expected for a population composed of genetically similar individuals (i.e., a breed) in the PCoA. Individual dogs were reasonably dispersed across all four quadrants of the graph with several dogs appearing as outliers (i.e., more genetically diverse), seen on the periphery of the plot (**Figure 3**). A few individuals towards the center of the plot (circled in red) were more closely related to each other than the population-at-large, as suggested by their tight clustering pattern.

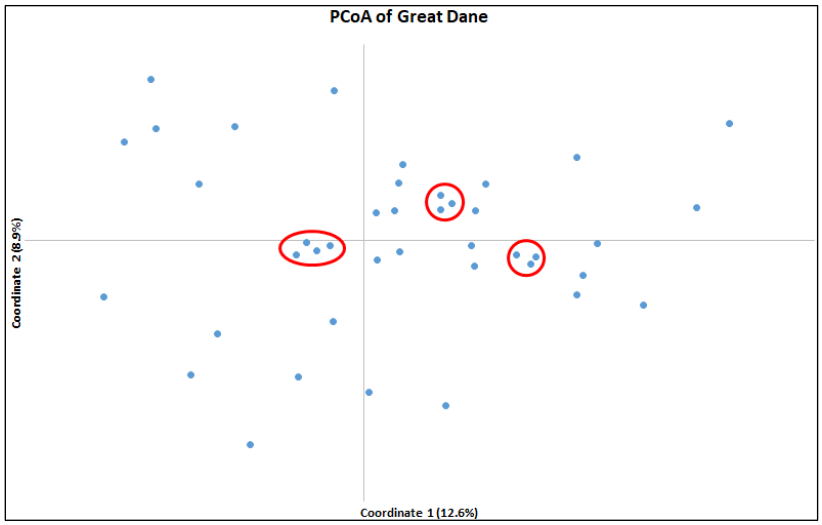


Figure 3. PCoA of Great Dane (n=43) based on alleles and allele frequencies at 33 autosomal STR loci. Three groups of closely related dogs are circled in red.

The degree of intra- and inter-breed relatedness can be further assessed by comparing the 43 Great Danes in this study with a closely related breed, the English Mastiff [9] (**Figure 4**).

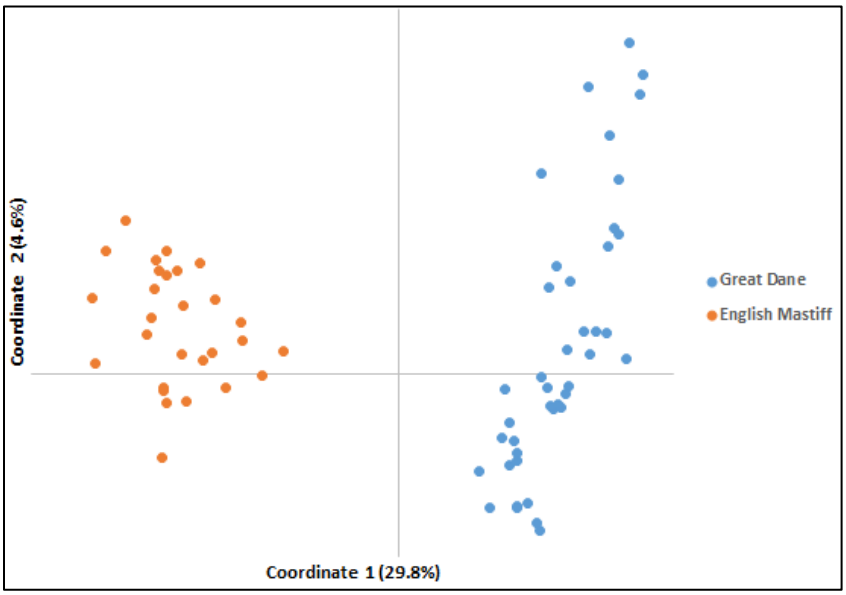


Figure 4. PCoA plot comparing intra- and inter-breed relatedness of Great Dane (blue dots; n=43) with English Mastiff (orange dots; n=50).

The relatively high level of genetic diversity in Great Danes becomes apparent when compared to English Mastiff. The 43 Great Danes analyzed in this study are more dispersed on the PCoA (especially along the vertical or Y axis) than English Mastiffs, which form a much tighter cluster of individuals. The more dispersed along the PCoA plot individuals from a breed are, the more genetically diverse the breed is.

E. Internal relatedness (IR) scores for Great Dane

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree of relatedness of parents of an individual dog. The IR calculation takes into consideration homozygosity at each of the 33 STR loci in this study and gives more weight to rare and uncommon alleles, which would presumably be identified 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. A dog with an IR value of -1.0 would have parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that are genetically identical at all loci. An IR value of +0.25 would be found among offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 43 Great Danes analyzed herein.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies for 33 STR loci in 43 Great Danes.

	IR	IRVD
Minimum	-0.2518	-0.1500
1st Quartile	-0.0107	0.1513
Mean	0.0651	0.2375
Median	0.0682	0.2281
3rd Quartile	0.1111	0.3170
Maximum	0.4039	0.6184

The most outbred Great Dane had an IR score of -0.25, while the most inbred dog had an IR score of 0.4, with a mean IR of 0.06. **Table 4** shows that 25% of the Great Dane cohort had IR values between 0.1 and 0.4, suggesting that they are products of closely related parents. Therefore, although standard genetic metrics (**Tables 2 and 3**) indicate that this group of Great Danes was the product of random mating, IR values for individual dogs suggest that this population is actually composed of inbred and outbred dogs. This wide range of IR values shows that the degree of parental relatedness varies greatly in the study cohort, a typical finding for almost all pure breeds of dogs.

This wide range of IR values can also be represented graphically (**Figure 5**). Note that the IR curve for Great Danes (red line) contains a main peak (left) representing the ~75% of the cohort

composed of outbred dogs, and two smaller peaks (right) which contain the most inbred individuals in the population (~25%).

2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed development

The IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to the present day. Village dogs that exist throughout the SE Asia, the Middle East and the Island Pacific region are randomly breeding descendants of dogs from which most modern breeds evolved. The known STR alleles and their frequencies of a given breed can be compared with the same alleles and their frequency in modern village dogs to yield an adjusted IR score (IR-village dog or IRVD) (**Table 4** and **Figure 5**, blue line). IRVD scores approximate how the IR score for a Great Dane would compare to other village dogs if its parents were also village dogs.

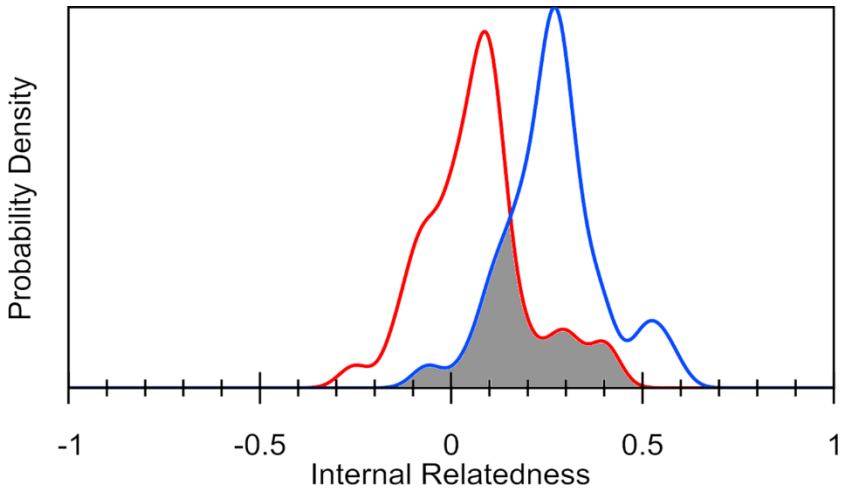


Figure 5. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Great Dane (n=43). The overlap between the curves (gray area) shows that Great Danes retain 37.1% of the genetic diversity existing in randomly breeding village dogs.

The Great Dane IRVD curve (blue line) is similarly shaped but shifted to the right of their actual IR scores (red line), with an area of overlap of 37.1% (gray area). This overlap indicates that Great Danes retain 37.1% of the genetic diversity found in contemporary village dogs from the region considered the ancestral home of most modern breeds. This is slightly higher than the approximately 30% retained genetic diversity calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL (**section IIB**). Moreover, roughly half of this cohort has IRVD scores of 0.25 or greater (**Table 4**, **Figure 5**), which means that if they were village dogs, they would be considered inbred to the level of at least offspring of full sibling parents. This not uncommon as pure breeds of dogs are developed from relatively small founder lines and therefore have limited genetic diversity from the time registries were created and closed.

F. DLA class I and II haplotype frequencies and genetic diversity

The DLA consists of four gene-rich regions that make up a small portion of 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, which can cause autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases. Breeds that lack genetic diversity in the DLA region are often more prone to autoimmune disorders.

The Class I region contains several genes, but only one, *DLA88*, is highly polymorphic (i.e., contains many alleles) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with *DLA88* are linked in various combinations, forming specific haplotypes (**Table 5**). Groups of genes (and consequently their alleles) inherited as a block 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 these three loci associated with the three class II genes are strongly linked, and often inherited as a single haplotype (**Table 6**). An individual inherits one haplotype from each of the parents. It is common for different dog breeds to share common and even rare haplotypes for these loci, depending on common ancestry.

1. DLA class I and II haplotypes existing in the Great Dane

Fifteen DLA class I and eleven DLA class II haplotypes were identified in this cohort (**Table 5**). This number is higher than the one found in the closely related English Mastiff (6 DLA I and 6 DLA II haplotypes) and in Doberman Pinschers, for example (12 and 11, respectively). It is lower however than that of more popular (and diverse) breeds such as Golden Retrievers (24 and 19, respectively).

DLA-I haplotypes 1030, 1066, and 1016 were the most predominant in Great Danes, being identified in 22%, 18%, and 17% of the dogs tested, respectively. Similarly, DLA-II haplotypes 2046, 2023, and 2033 were also identified in more than half of the study cohort, with frequencies of 29%, 22%, and 21% respectively (**Table 5**). Since the aforementioned DLA-I/DLA-II haplotypes were identified in similar frequencies in Great Danes, it can be inferred that they are in linkage disequilibrium (i.e., inherited together). Therefore, a founder (or founder line) with these combinations of DLA-I/DLA-II haplotypes (such as 1066/2046) has played an important role in establishing important phenotypic trait(s) in the Great Dane, and thus they have been retained in the breed.

Table 5. DLA class I and II haplotypes identified in Great Danes (n=43) and their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 haplotype	STR types	Frequency
1006	387 375 293 180	0.02
1009	382 377 277 184	0.07
1016	**382 371 277 178**	**0.17**
1030	**380 373 293 178**	**0.22**
1066	**376 375 277 178**	**0.18**
1068	380 373 287 181	0.01
1091	381 371 277 181	0.09
1094	395 375 277 176	0.04
1129	382 371 277 181	0.07
1273	376 375 277 176	0.01
1274	385 371 277 181	0.01
1275	387 371 277 181	0.04
1276	392 373 281 182	0.02
1277	394 369 277 183	0.01
1278	399 373 277 181	0.02
DLA2 haplotype	**STR types**	**Frequency**
2003	343 324 282	0.01
2005	339 322 280	0.07
2007	351 327 280	0.02
2014	339 322 284	0.05
2022	339 327 282	0.07
2023	**341 323 282**	**0.22**
2033	**339 323 282**	**0.21**
2046	**339 329 280**	**0.29**
2053	343 324 280	0.01
2086	339 329 284	0.01
2136	353 324 280	0.02

The DLA-I/DLA-II haplotypes identified in Great Danes are shared with 43 different dog breeds/varieties (**Table 6**). DLA haplotypes tend remain mostly unchanged over the generations, and can be used to assess the shared influence of founder lines in different breeds. As expected, the most predominant Great Dane DLA haplotypes are also found in higher frequency in the English Mastiff, such as the 1066/2046 DLA haplotype (53% and 52%, respectively). Most of the DLA-I and DLA-II haplotypes found in Great Danes were also identified in a number of other breeds. However, almost half of the DLA-I haplotypes identified in Great Dane (6 out of 15) are unique to the breed so far; these are 1273, 1274, 1275, 1276, 1277, and 1278. Collectively, they occur in 11% of the study cohort. Similarly, DLA-II haplotype 2136, identified in 2% of the individuals in this study, is so far unique to Great Danes (**Table 6**).

Table 6. Sharing of DLA class I and II haplotypes between Great Danes (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=43).

DLA1 #	STR types	Great Dane (n=41)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Akita (n=158)	Alaskan Klee Kai (n=615)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=129)	Black Russian Terrier (n=148)	Biewer (n=123)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=200)	Yorkshire Terrier (n=16)	Borzoi (n=144)	Collie (n=49)	Doberman Pinscher (n=1031)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=19)	Flat Coated Retriever (n=750)	Golden Retriever (n=837)	Giant Schnauzer (n=305)
1006	387 375 293 180	0.02	--	--	0.082	--	--	--	0.07	0.047	--	--	--	--	0.146	--	--	0.003	--	0.03	--	0.0149	0.051
1009	382 377 277 184	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1016	382 371 277 178	0.17	0.008	0.04	--	--	--	--	0.047	0.01	0.024	0.019	0.013	0.03	--	0.04	0.0199	0.095	0.15	0.16	--	0.0048	0.046
1030	380 373 293 178	0.22	0.008	0.03	--	--	0.029	0.008	0.012	--	0.455	0.349	0.495	0.31	--	--	0.096	--	0.08	--	--	0.0012	--
1066	376 375 277 178	0.18	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.53	0.45	0.0007	0.2826	--
1068	380 373 287 181	0.01	--	--	--	--	--	0.221	0.062	--	--	0.009	--	--	--	--	0.0005	--	--	--	0.282	0.0484	0.056
1091	381 371 277 181	0.09	--	--	--	--	--	--	--	0.507	--	--	--	--	--	--	0.0019	--	--	--	--	--	0.034
1094	395 375 277 176	0.04	--	--	--	--	--	--	0.143	0.014	--	--	--	--	0.01	--	0.7459	--	--	--	--	--	--
1129	382 371 277 181	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	0.002
1273	376 375 277 176	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1274	385 371 277 181	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1275	387 371 277 181	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1276	392 373 281 182	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1277	394 369 277 183	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1278	399 373 277 181	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 (cont'd)	STR types	Great Dane (n=41)	Havanese (n=770)	Italian Greyhound (n=1183)	Irish Setter (n=59)	Irish Red and White Setter (n=71)	Lakeland Terrier (n=126)	Labrador Retriever (n=270)	Llewellin Setter (n=107)	Magyar Agar (n=77)	Polish Lowland Sheepdog (n=56)	Toy Poodle (n=200)	Miniature Poodle (n=382)	Poodle (n=4442)	Rat Terrier (n=40)	Samoyed (n=191)	Saint Bernard (n=83)	Scottish Collie (n=112)	Shiba Inu (n=134)	Greater Swiss Mountain Dog (n=40)	Shiloh Shepherd, ISSA (n=240)	Swedish Vallhund (n=387)	Cardigan Welsh Corgi (n=43)	Whippet (n=95)
1006	387 375 293 180	0.02	0.0461	--	--	0.07	--	0.028	--	--	--	0.002	0.003	0.0467	--	0.005	--	0.036	--	--	--	0.255	--	--
1009	382 377 277 184	0.07	0.0006	--	--	--	--	--	--	--	--	0.075	0.088	0.0156	0.05	0.123	--	--	--	--	--	0.044	--	--
1016	382 371 277 178	0.17	0.1968	0.0596	--	--	0.008	0.017	0.023	--	0.036	0.025	0.025	0.0206	0.05	--	--	0.098	--	0.09	--	--	--	0.121
1030	380 373 293 178	0.22	0.0032	0.0287	--	--	--	0.039	0.028	--	--	--	--	0.0024	0.03	--	--	--	--	--	--	--	--	--
1066	376 375 277 178	0.18	--	--	--	--	--	0.004	--	0.084	--	--	--	--	--	--	--	--	--	--	--	--	0.12	0.2
1068	380 373 287 181	0.01	0.0136	--	0.008	--	--	0.031	--	--	--	0.01	0.013	--	--	0.042	0.169	--	--	--	0.267	0.376	--	--
1091	381 371 277 181	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.235	--	--	--	--	--
1094	395 375 277 176	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--
1129	382 371 277 181	0.07	0.0013	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1273	376 375 277 176	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1274	385 371 277 181	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1275	387 371 277 181	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1276	392 373 281 182	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1277	394 369 277 183	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1278	399 373 277 181	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Great Dane (n=41)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Akita (n=158)	Alaskan Klee Kai (n=615)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=129)	Black Russian Terrier (n=148)	Biewer (n=123)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=200)	Yorkshire Terrier (n=16)	Borzoi (n=144)	Collie (n=49)	Doberman Pinscher (n=1031)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=19)	Flat Coated Retriever (n=750)	Golden Retriever (n=837)	Giant Schnauzer (n=305)
2003	343 324 282	0.01	0.016	0.04	--	--	0.132	0.057	--	--	0.24	0.208	0.248	0.38	--	--	0.0005	0.598	--	--	0.14	0.0203	0.031
2005	339 322 280	0.07	--	--	--	--	--	0.008	0.147	0.014	0.045	0.009	0.025	--	--	--	--	0.015	--	--	0.4187	0.0143	0.008
2007	351 327 280	0.02	--	--	0.082	0.0122	--	--	0.078	0.047	--	--	--	--	0.149	--	--	--	--	--	--	0.0149	0.051
2014	339 322 284	0.05	0.008	0.03	--	0.0691	--	--	--	--	--	--	--	--	--	--	--	0.092	0.15	0.16	0.0287	--	0.005
2022	339 327 282	0.07	0.024	0.21	--	--	--	0.008	--	--	--	--	--	--	0.594	--	0.001	0.015	--	--	0.1173	0.0006	0.003
2023	341 323 282	0.22	0.008	0.03	--	--	0.029	0.008	--	0.003	0.455	0.349	0.498	0.31	--	--	0.096	--	0.08	--	--	0.0012	--
2033	339 323 282	0.21	0.008	--	0.013	--	--	0.008	--	0.51	--	--	--	--	--	--	0.0015	--	--	--	--	--	0.036
2046	339 329 280	0.29	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.52	0.45	0.0007	0.2581	--
2053	343 324 280	0.01	0.226	0.12	--	--	--	0.074	0.062	--	--	0.009	--	--	--	--	0.0015	--	--	--	0.1487	0.0293	0.066
2086	339 329 284	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.02	--	--	0.0155	--
2136	353 324 280	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 (cont'd)	STR types	Great Dane (n=41)	Havanese (n=770)	Italian Greyhound (n=1183)	Irish Setter (n=59)	Irish Red and White Setter (n=71)	Lakeland Terrier (n=126)	Labrador Retriever (n=270)	Llewellin Setter (n=107)	Magyar Agar (n=77)	Polish Lowland Sheepdog (n=56)	Toy Poodle (n=200)	Miniature Poodle (n=382)	Poodle (n=4442)	Rat Terrier (n=40)	Samoyed (n=191)	Saint Bernard (n=83)	Scottish Collie (n=112)	Shiba Inu (n=134)	Greater Swiss Mountain Dog (n=40)	Shiloh Shepherd, ISSA (n=240)	Swedish Vallhund (n=387)	Cardigan Welsh Corgi (n=43)	Whippet (n=95)
2003	343 324 282	0.01	0.211	0.0076	--	--	0.766	0.022	--	--	--	0.412	0.514	0.104	0.11	0.013	--	0.018	--	0.91	0.006	--	0.23	0.095
2005	339 322 280	0.07	0.0026	--	0.085	0.099	0.004	0.056	0.785	--	--	0.002	--	0.0178	0.08	--	0.066	--	--	--	--	--	--	--
2007	351 327 280	0.02	0.0481	--	--	0.155	--	0.031	--	--	--	0.002	0.001	0.0158	--	0.005	0.006	0.004	--	--	--	0.258	0.12	--
2014	339 322 284	0.05	0.0052	0.0017	--	--	0.008	0.002	--	--	0.732	0.023	0.024	0.019	0.03	--	0.108	--	--	--	--	--	--	--
2022	339 327 282	0.07	0.1247	--	0.153	--	0.171	0.056	0.093	--	--	0.007	0.003	0.0002	0.15	0.107	--	--	--	--	0.048	0.044	--	--
2023	341 323 282	0.22	0.0032	0.0287	--	--	--	0.039	0.019	--	--	--	--	0.0024	0.03	--	0.38	--	--	--	--	--	--	--
2033	339 323 282	0.21	0.0013	0.0059	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	0.116
2046	339 329 280	0.29	--	--	--	--	--	0.004	--	0.019	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2053	343 324 280	0.01	0.0312	--	0.008	--	--	0.03	--	--	--	0.013	0.013	--	--	0.56	0.151	--	--	--	0.335	0.523	--	--
2086	339 329 284	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2136	353 324 280	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

2. Heterozygosity in the DLA region

Due to their physical proximity in canine chromosome 12, the seven loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to other parts of the genome. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will be inherited randomly as well. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and averaged across all loci (**Table 8**).

The highest number of alleles (Na) identified at each DLA locus for Great Danes was 10 (DLA I-3CCA) and the lowest was 3 (5BCA). However, as observed in the 33 STR markers across the genome, the number of effective alleles (Ne) per locus was lower, ranging from 1.73 (locus DLA I-4BCT) to 4.85 (DLA I-3CCA). Also according to the expectation that this region would be in equilibrium with other loci in the genome, we can observe that coefficients of inbreeding (F) for individual DLA loci range from high (F=0.1 in the case of DLA I-3CCA) to low (F=-0.03 for 5ACA) (**Table 7**). Therefore, when averaged across DLA loci, the inbreeding coefficient estimated for this region (F=0.03, **Table 8**) is similar to that estimated for Great Danes using 33 STR loci across the genome (F=0.07, **Table 2**). This suggests that only a small subpopulation (around 3%) of Great Danes are more inbred than the population as a whole based on DLA haplotypes. Therefore, the over-representation of three linked DLA-I/DLA-II haplotypes (Table 5) probably occurred during the earliest origins of the breed, followed by a longer period of somewhat random breeding. Alternatively, given the amount of genetic diversity found in Great Danes, this can also mean that the original population was more genetically diverse than it currently is, and that artificial bottlenecks during the evolution of the breed have led to such over-representation of DLA haplotypes.

Table 7. Standard genetic assessment for Great Dane using each of the 7 STRs in the DLA class I and II regions (n=43).

DLA Locus	Na	Ne	Ho	He	F
DLA I-3CCA	10	4.85	0.71	0.79	0.109
DLA I-4ACA	5	3.42	0.68	0.71	0.034
DLA I-4BCT	4	1.73	0.42	0.42	0.018
DLA1131	7	2.52	0.56	0.6	0.07
5ACA	5	1.82	0.46	0.45	-0.03
5ACT	5	3.31	0.66	0.7	0.057
5BCA	3	2.23	0.56	0.55	-0.02

Table 8. Summary of standard genetic assessment for Great Danes using 7 STRs in the DLA class I and II regions (n=43). SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	5.57	2.84	0.58	0.6	0.034
SE	0.81	0.39	0.04	0.05	0.017

Following the assumption that the DLA region would behave in a similar manner as the genome-at-large as a proxy for genetic diversity in the breed, the number of alleles (Na) and number of effective alleles (Ne) was similar between the 33 STR loci (Table 2) and the DLA loci (Table 8). Overall, the observed heterozygosity (Ho) for this region was just slightly lower than the expected heterozygosity (He), suggesting that according to diversity metrics calculated for the DLA region, this cohort of Great Danes is randomly breeding (F=0.03). The number of alleles identified in this region can increase (albeit at lower frequencies) as more individuals are tested.

III. What does this assessment of genetic diversity tell us about the Great Dane

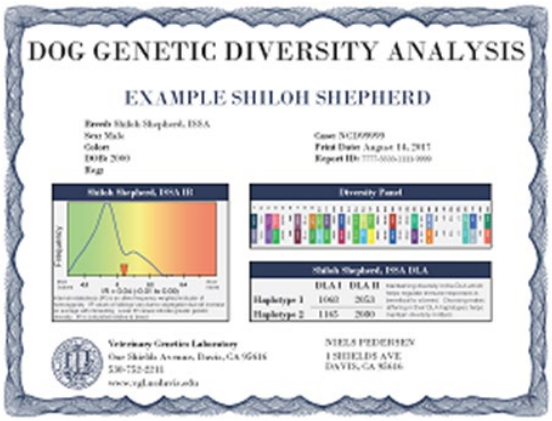
Great Danes have managed to retain a significant amount of genetic diversity compared to some other breeds. However, there is evidence that certain founders or founder lines have had a disproportionately high genetic influence on the breed, and that the genetic imbalance originating from that is being maintained to some degree by artificial selection (breeding practices). The positive news is that Great Dane breeders have a good amount of genetic diversity to work with through mate selection, making it possible to redistribute diversity in such a way as to maintain and improve the genetic health of the breed. This preliminary report shows that, indeed, Great Dane breeders have done a good job so far in choosing sires and dams that are as unrelated as possible. It is expected that more diversity in the form of novel alleles will be identified in the breed as more individuals are tested over time, although at lower frequencies.

The goal for breeders is to maintain existing genetic diversity by breeding the least related parents possible. Breeders should be aware of this when selecting mates for their breeding programs, in order to redistribute the diversity that currently exists in the breed. The goal is to produce dogs with IR scores lower than zero.

IV. Results of VGL Canine Diversity Testing

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 reported in relation to others in the population. The alleles at each of the 33 STR loci are presented as numbers that correspond to those found in Table 1. Each locus will have two alleles, which can be different (heterozygous) or the same (homozygous). Each allele is inherited from one of the parents. Dogs from closely related parents will be homozygous for more alleles at each locus, or in regions of the genome that are under strong positive selection for phenotypic trait or traits mostly favored in the breed. Dogs with a predominance of rare (i.e., low frequency) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high frequency) alleles. A sample genetic diversity report is shown below.



B. What should you do with this information?

The goal for breeders should be to continue to produce puppies with IR scores close to zero, and as informed breeding decisions are made, even lower scores. Mates should be preferably selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype; moreover, mating of dogs with less frequent genomic alleles or DLA haplotypes is encouraged. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity. However, because IR values reflect the unique genetics of individuals, they cannot be used as the primary criterion for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, breeding dogs with high IR values (providing they are genetically different) may produce puppies with much lower IR scores than either parent. A mating between a dog with a high IR value and one with 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 could 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.

The next step is to compare the DLA class I and II haplotypes of the mates. You want to avoid breeding dogs that will produce puppies homozygous for the same haplotypes; once again, less common haplotypes may increase breed diversity in relation to common ones.

Breeders who would like to predict the genetic outcome of puppies of certain sires and dams should screen them for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Rare alleles should be favored over common ones. This information is included on all certificates and on the breed-wide data found on the VGL website.

V. References

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