Genetic Diversity Testing for German Pinscher

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 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 will be useful to dog breeders who wish to use DNA-based testing to track and increase genetic diversity as a supplement to in-depth pedigrees. DNA based information on genetic heterogeneity and diversity, along with genetic testing results for desired phenotypes and health traits, can aid in informing breeding decisions.

Genetic diversity testing in the German Pinscher has been established, and almost all existing alleles at the 33 STR loci and 7 DLA class I and II regions have potentially been identified. As of November of 2023, 97 German Pinschers from the USA (n=96) and Canada (n=1) were tested to assess genetic diversity in the breed. We will continue to add new alleles and haplotypes if they are found in the breed, and their respective frequencies will be updated if necessary.

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 and 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 German Pinscher
A. Breed History [1-4]

The German Pinscher, or '*Deutscher Pinscher*', is a dog breed developed in the mid- to late-19th century in Germany to act as a ratter in carriages and/or barns. The name 'Pinscher' derives either from the English 'pinch' or the French 'pincer', both of which describe what these dogs do to rodents. The breed was first recorded in the *Deutschen Hundestammbuch* in the 1880's as a smooth-coated Pinscher, as served as a foundation breed for subsequent development of the Miniature Schnauzer, Standard Schnauzer, Doberman Pinscher, and the Miniature Pinscher. Initially, the breed was composed of two different 'varieties': the wire-haired Pinscher and the smooth-haired Pinscher. A breed society called the *Pinscher-Schnauzer-Klub* was established in 1895, which included both varieties of German Pinschers. In the early 1900's these varieties were officially divided into different breeds, with the wire-haired Pinscher being renamed as Schnauzer, whereas the smooth-haired variety kept the original breed name.

After World War II, the German Pinscher almost disappeared; there were no litters registered in West Germany from 1949 to 1958. The breed was saved from extinction by Werner Jung, who acted as head breed-warden for the *Pinscher-Schnauzer Klub* in the 1950's. In an attempt to reestablish the breed, Jung used a black and red female smuggled from East Germany (Kitti) and four oversized Miniature Pinschers: Jutta, a black female; Illo, a black dog; Fürst, a red dog; and Onzo, a chocolate dog (**Figure 1**). Most, if not all, modern German Pinschers descend from these five animals.
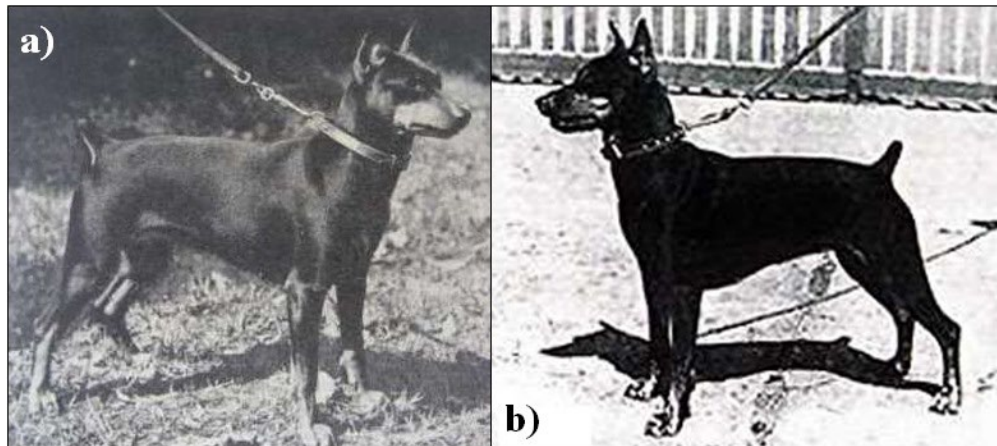

Figure 1. Kitti **(a)** and Jutta **(b)**, the two females that Werner Jung used to reestablish the German Pinscher in the late 1950's after its near extinction following World War II.

In the late 1970's and early 1980's, German Pinschers began to be imported into the United States and bred in small numbers. The population has been growing slowly but steadily since the breed was accepted into the American Kennel Club in 2003. However, the German Pinscher is considered an endangered breed due to its small population worldwide. In 2016 and 2017, there were just over 50 litters registered per year. The German Pinscher currently ranks 145 of 200 in the AKC Breed Popularity ranking.

B. Appearance [1-3]

The German Pinscher is a medium-sized breed. The ideal height at the withers for both males and females is 17 to 20 inches. Ideally, the height at the highest point of the withers should equal the

length of the body from the sternum to the rump. According to the AKC breed standard, size should be penalized in accordance with the degree it deviates from the ideal range. Their weight can range from 25 to 45 pounds. The coat is short, dense, smooth, and shiny. Accepted colors are fawn, red, stag red (red with intermingling of black hairs), black, and blue with red/tan markings. In red dogs, a rich vibrant medium to dark shade is preferred. In bi-colored dogs, sharply demarcated markings (dark red or tan) are desirable. Pencil marks on the toes are acceptable; however, any white markings are undesirable (a few white hairs do not constitute a marking).

Dogs are muscular with a powerful and elongated skull, muscular and flat cheeks, and medium-sized dark eyes. The total length of the head from the tip of the nose to the occiput is ideally one-half the length from the withers to the base of the tail, resulting in a ratio of approximately 1:2. The ears are set high and symmetrical. If cropped, they must be carried erect; if uncropped, they are V-shaped with a folding pleat. The nose and lips are black. Overshot or undershot bites and the absence of primary molars constitute faults.

Their bodies are compact and strong, with a short and level back. The length of their legs is ideally equal to the depth of their body. The chest is moderately wide and oval from the front, and their belly is moderately drawn up. An excessive tuck up constitutes a fault. Their tail is moderately set and carried above the horizontal; it is usually docked between the second and third joints.

C. Temperament [1-3]
The German Pinscher is very intelligent and eager to please, and therefore easy to train. They are fearless and have very high energy levels. German Pinschers are alert, vigilant, and watchful of strangers. They are not excessive barkers, but if threatened they show courage and tenacity. According to the AKC breed standard, aggressive behavior towards another dog is not deemed viciousness, and German Pinschers should not be shy, as this constitutes a fault.

II. Genetic Diversity of German Pinscher
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 [5, 6]. Each STR locus is known to contain 7 to 29 different alleles (average of 15.4 alleles/locus) in the canine 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 97 German Pinschers are listed in **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in German Pinscher (n=97). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
92 (0.206)	**131 (0.670)**	**121 (0.479)**	219 (0.284)	238 (0.227)	87 (0.119)
94 (0.021)	137 (0.103)	127 (0.103)	225 (0.036)	246 (0.031)	89 (0.144)
96 (0.144)	147 (0.216)	129 (0.418)	**227 (0.521)**	**250 (0.716)**	**91 (0.732)**
98 (0.015)	149 (0.005)		233 (0.119)	252 (0.026)	95 (0.005)
102 (0.026)	151 (0.005)		237 (0.041)		
104 (0.165)					
106 (0.418)					
110 (0.005)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
286 (0.015)	116 (0.258)	132 (0.314)	148 (0.082)	232 (0.222)	**99 (0.500)**
288 (0.216)	**118 (0.381)**	**144 (0.371)**	**152 (0.428)**	236 (0.052)	101 (0.500)
290 (0.418)	120 (0.021)	148 (0.077)	156 (0.351)	238 (0.062)	
292 (0.351)	124 (0.340)	152 (0.237)	160 (0.026)	**240 (0.649)**	
			168 (0.113)	244 (0.015)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.052)	144 (0.170)	210 (0.335)	85 (0.237)	227 (0.005)	202 (0.088)
122 (0.005)	148 (0.170)	212 (0.268)	95 (0.098)	231 (0.165)	204 (0.289)
124 (0.804)	150 (0.206)	214 (0.015)	**97 (0.665)**	233 (0.134)	**206 (0.588)**
132 (0.139)	**152 (0.454)**	**218 (0.381)**		**235 (0.531)**	208 (0.005)
				239 (0.119)	210 (0.031)
				241 (0.046)	

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
212 (0.041)	162 (0.036)	**268 (0.686)**	**222 (0.376)**	139 (0.263)	12 (0.077)
216 (0.175)	**164 (0.443)**	270 (0.015)	226 (0.258)	145 (0.237)	21.2 (0.082)
220 (0.784)	168 (0.077)	272 (0.299)	232 (0.278)	**147 (0.495)**	**22.2 (0.454)**
	170 (0.443)		234 (0.088)	153 (0.005)	23.2 (0.381)
					24.2 (0.005)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
17.1 (0.093)	8 (0.320)	15 (0.046)	15 (0.284)	11 (0.026)	14 (0.067)
18.1 (0.216)	13 (0.021)	20 (0.036)	16 (0.031)	13 (0.108)	**15 (0.500)**
19.1 (0.134)	**14 (0.428)**	23 (0.036)	17 (0.093)	14 (0.015)	16 (0.371)
20.1 (0.165)	15 (0.021)	24 (0.273)	18 (0.005)	**15 (0.448)**	18 (0.062)
21.1 (0.088)	17 (0.211)	26 (0.036)	19 (0.098)	16 (0.387)	
22.1 (0.021)		**27 (0.433)**	20 (0.021)	17 (0.015)	
23.1 (0.010)		28 (0.052)	21 (0.067)		
24.1 (0.170)		30 (0.088)	**22 (0.289)**		
25.1 (0.103)			23 (0.113)		

VGL2918	VGL3008	VGL3235
12 (0.010)	**13 (0.593)**	13 (0.170)
13 (0.737)	14 (0.010)	14 (0.005)
14 (0.139)	15 (0.144)	15 (0.144)
15 (0.108)	17 (0.021)	**16 (0.655)**
16 (0.005)	18 (0.144)	18 (0.026)
	19 (0.036)	
	20 (0.036)	
	21 (0.010)	
	22 (0.005)	

The number of alleles identified in the 33 STR loci included in this study was low in German Pinschers, ranging from nine (VGL0910, VGL1828, and VGL3008) to two (INRA21). These numbers are amongst the lowest found in any breed analyzed at the VGL to date. This finding is not surprising, given the history of the breed and the extreme genetic bottlenecks it went through during its development. Similar to other pure dog breeds, a single allele predominated at most loci (bolded in **Table 1**), while the other alleles were identified at lower frequencies. However, one allele predominated at an especially high frequency (greater than 70%) in five STR loci (AHTh260, AHTk211, INU005, REN169D01, and VGL2918). This might indicate that the genomic regions harboring these STR loci have been under strong positive selection since the formation of the breed and can be associated with highly valued traits. It is possible that additional alleles will be identified as more dogs are tested by the VGL, albeit at a low 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); the observed or actual heterozygosity (**Ho**) that was found; the heterozygosity that would be expected (**He**) if the existing population was in Hardy-Weinberg equilibrium (i.e., random breeding); and the coefficient of inbreeding (**F**) derived from the Ho and He values.

Table 2. Standard Genetic Assessment of 97 German Pinschers based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	4.91	2.74	0.597	0.595	0
SE	0.31	0.177	0.02	0.02	0.01

The mean number of alleles (Na) identified in this cohort was low (Na=4.91). This number corresponds to only 31.8% of alleles known to exist on average at each of these loci in all canids tested at the VGL (Na=15.4 alleles/locus). This number is similar to that of breeds with low genetic diversity such as the Lakeland Terrier (30%) and the Flat-Coated Retriever (35%). The average number of effective alleles (Ne) constitutes a more important metric for diversity, since it corresponds to the number of alleles contributing to heterozygosity. This number was estimated to be 2.74 in German Pinschers, or approximately 56% of the total number of alleles segregating in

the breed. This phenomenon is typical of most pure dog breeds. The observed (actual) heterozygosity was estimated at 0.597, which was similar to the expected heterozygosity calculated for a population in Hardy-Weinberg equilibrium (i.e., state of total random breeding; He=0.595). This yielded a coefficient of inbreeding (F) of zero, thus indicating that the mean distribution of alleles was a result of near-random selection. Therefore, it appears that despite the lack of standing genetic diversity in the breed, the average German Pinscher is a product of random selection.

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 specific regions of the genome that are associated with each STR marker. Loci with low Ho and He values contribute the least to heterozygosity among individuals, and are most likely associated with traits that define the breed's phenotypic standards. Conversely, loci with high Ho and He values are more genetically variable and can be associated with phenotypic variation among individuals within the breed. Moreover, the F value is a coefficient of inbreeding based on Ho and He; if these two values are equal, F=0 which means that the population is in HWE. The F value will be positive when there is a deficiency of heterozygotes (i.e., fewer heterozygotes than expected), whereas negative F values correspond to an excess of heterozygotes within the population.

As described in **Table 1**, the number of alleles (Na) identified in individual STR loci for this cohort ranged from 2 to 9 alleles per locus. The number of effective alleles (Ne), on the other hand, ranged from 1.47 (INU005) to 6.74 (VGL0910) alleles per locus. Observed heterozygosity (Ho) values displayed a wide range across individual STR loci, being estimated between 0.34 (REN169D01) and 0.87 (VGL0910). Expected heterozygosity (He) values followed the same distribution, ranging from 0.33 (INU005) to 0.85 (VGL0910) (**Table 3**). As for inbreeding coefficient (F) values, the number of loci with positive F-values (n=16) is similar to those with negative F-values (n=17), which results in the F value of zero estimated for the cohort as a whole (**Table 2**). Additionally, high inbreeding coefficients (F > 0.1) were estimated for four of the 33 STR loci (bolded on **Table 3**), which suggests that these loci have been under strong positive selection since breed development.

Table 3. Standard Genetic Assessment of individual STR loci (n=33) for 97 German Pinschers.

Locus	Na	Ne	Ho	He	F
AHT121	8	3.756	0.67	0.734	0.087
AHT137	5	1.974	0.536	0.493	-0.09
AHTH130	3	2.411	0.567	0.585	0.031
AHTh171-A	**5**	**2.71**	**0.57**	**0.63**	**0.1**
AHTh260	**4**	**1.77**	**0.38**	**0.43**	**0.12**
AHTk211	4	1.752	0.433	0.429	-0.01
AHTk253	**4**	**2.9**	**0.58**	**0.66**	**0.12**
C22.279	4	3.048	0.66	0.672	0.018
FH2001	4	3.347	0.691	0.701	0.015
FH2054	5	3.065	0.649	0.674	0.036
FH2848	5	2.093	0.495	0.522	0.053

INRA21	2	2	0.485	0.5	0.031
INU005	4	1.496	0.351	0.331	-0.06
INU030	4	3.266	0.742	0.694	-0.07
INU055	4	3.032	0.722	0.67	-0.08
LEI004	3	1.969	0.495	0.492	-0.01
REN105L03	6	2.913	0.67	0.657	-0.02
REN162C04	5	2.287	0.557	0.563	0.011
REN169D01	3	1.547	0.34	0.354	0.038
REN169O18	4	2.498	0.619	0.6	-0.03
REN247M23	3	1.787	0.412	0.44	0.064
REN54P11	4	3.411	0.742	0.707	-0.05
REN64E19	4	2.701	0.66	0.63	-0.05
VGL0760	5	2.747	0.722	0.636	-0.14
VGL0910	9	6.738	0.866	0.852	-0.02
VGL1063	5	3.024	0.742	0.669	-0.11
VGL1165	8	3.591	0.711	0.721	0.014
VGL1828	9	4.984	0.845	0.799	-0.06
VGL2009	6	2.752	0.639	0.637	-0
VGL2409	4	2.525	0.649	0.604	-0.08
VGL2918	5	1.74	0.402	0.425	0.055
VGL3008	9	2.523	0.66	0.604	-0.09
VGL3235	**5**	**2.09**	**0.44**	**0.52**	**0.15**

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

PCoA measures the genetic relatedness of individuals in 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 97 German Pinschers clustered as expected for a closed breed in the PCoA, with individual dogs (represented by blue dots) reasonably dispersed across all four quadrants (**Figure 2**). Some of the individuals clustered together (red circles), which suggests that they are genetically more closely related to each other than to the cohort at large.
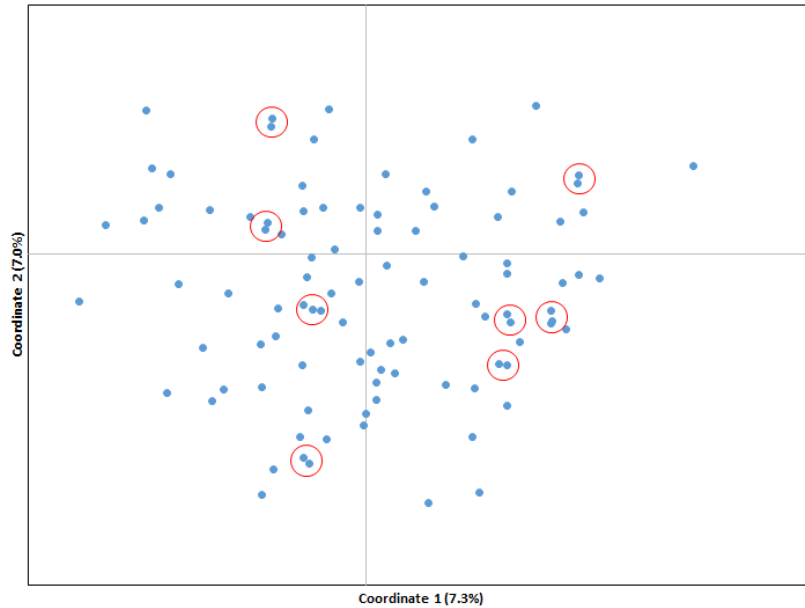
Figure 2. PCoA of German Pinscher (n=97) based on alleles and allele frequencies at 33 autosomal STR loci. The closely related individuals are circled in red.

The degree of relatedness of individuals within the breed can be further emphasized by comparing the 97 German Pinschers with a genetically distinct but somewhat closely related breed (the Standard Schnauzer), and a more distantly related breed (Italian Greyhound) (**Figure 3**).

Inter-breed clustering shows separate and well-defined populations, thus indicating that the breeds are genetically distinct as expected. However, this type of comparison accentuates the relatedness of individuals within a breed. The German Pinschers (blue dots) included in this study form a tighter cluster when compared to individuals from the other two breeds, thus corroborating the relative lack of genetic diversity of the German Pinscher (**Figure 3**).
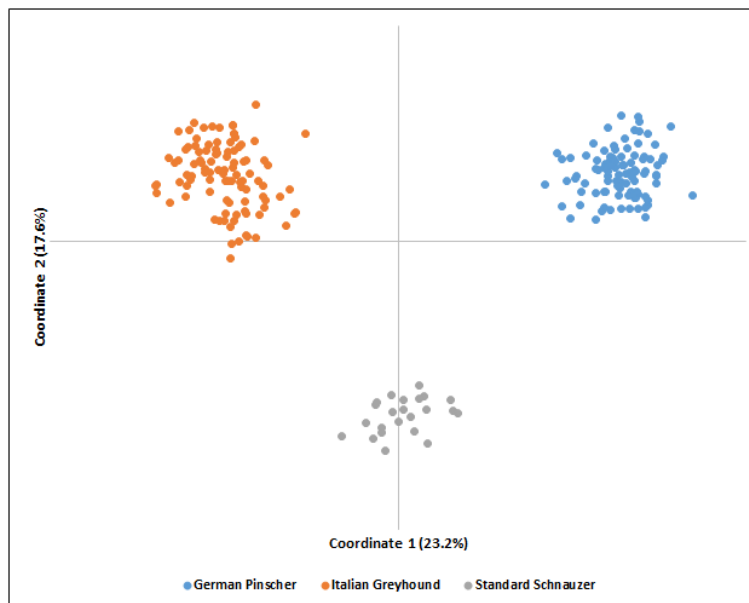

Figure 3. PCoA graph comparing intra- and inter-breed relatedness of German Pinscher (n=97, blue dots), Standard Schnauzer (n=23, grey dots) and Italian Greyhound (n=100, orange dots).

Another useful parameter to consider when evaluating within-breed relatedness based on PCoA is parental origin of the North American dogs included in this study. According to the database, the sires and dams of individuals in the study cohort originated from 14 different countries: Australia, Austria, Belarus, Canada, Finland, France, Germany, Italy, Netherlands, Norway, Poland, Russia, Sweden, and UK. No clear genetic differentiation can be observed within this cohort of German Pinschers based on parental country of origin when all countries are included (**Figure 4**). In this study, "mixed" dogs (i.e., parents born in different countries) were not included in the PCoA analysis; however, this group will be included in subsequent analyses as more individuals are tested.
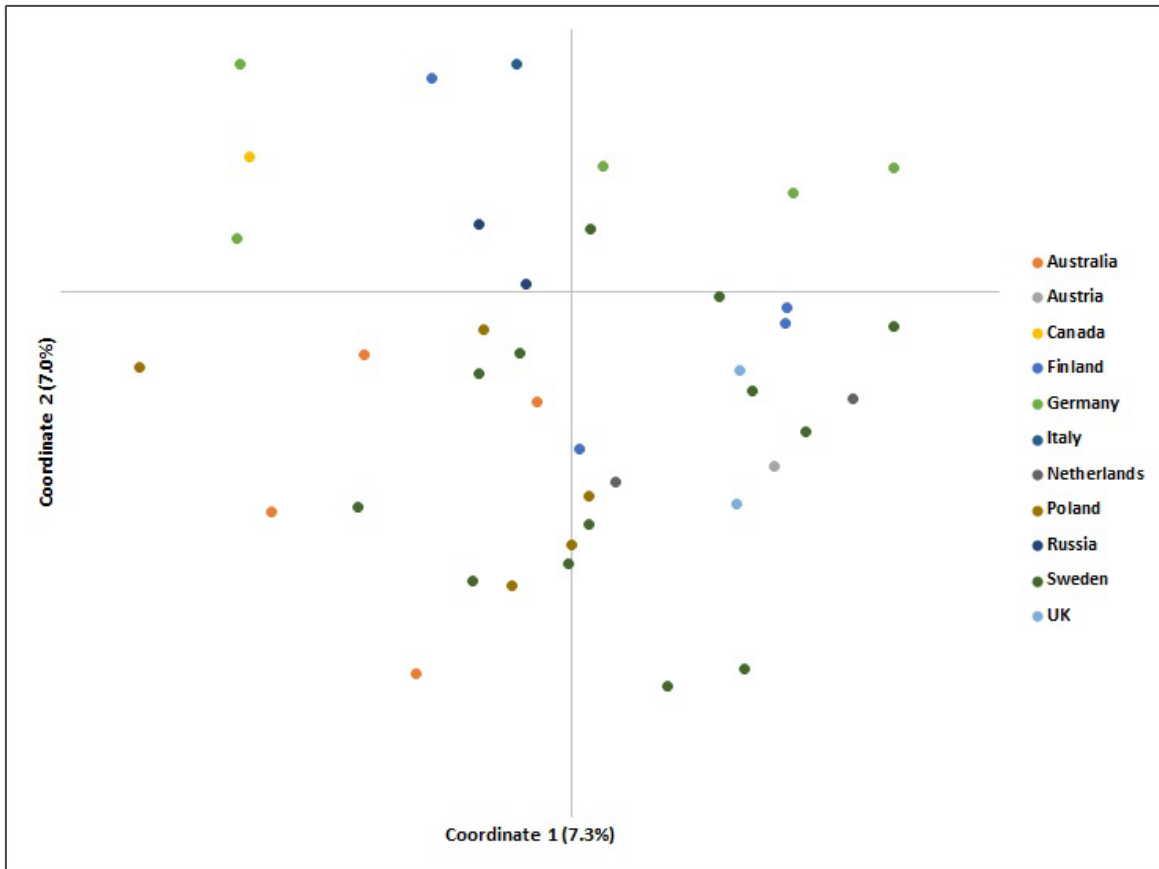

Figure 4. PCoA graph showing within-breed genetic relatedness of North American German Pinschers based on parental country of origin. "Mixed" dogs (i.e., parents born in different countries) were not included in this analysis.

When considering German Pinschers born in North America from parents originating from Germany and Sweden compared to other countries, we observe an overall tendency of the different groups of dogs to cluster as genetically distinct subpopulations (circled in red on **Figure 5**). However, a few dogs cluster away from their main group and blend into the opposite subpopulation, thus indicating a certain degree of interrelatedness. However, this genetic structure is not yet to the level of varieties, such as seen with American and Japanese Akita, for example. Since Germany and Sweden were the countries with the most number of parents represented in this cohort (43% of the total), it is possible that this level of genetic substructure will be identified for other countries of origin as more individuals are tested.
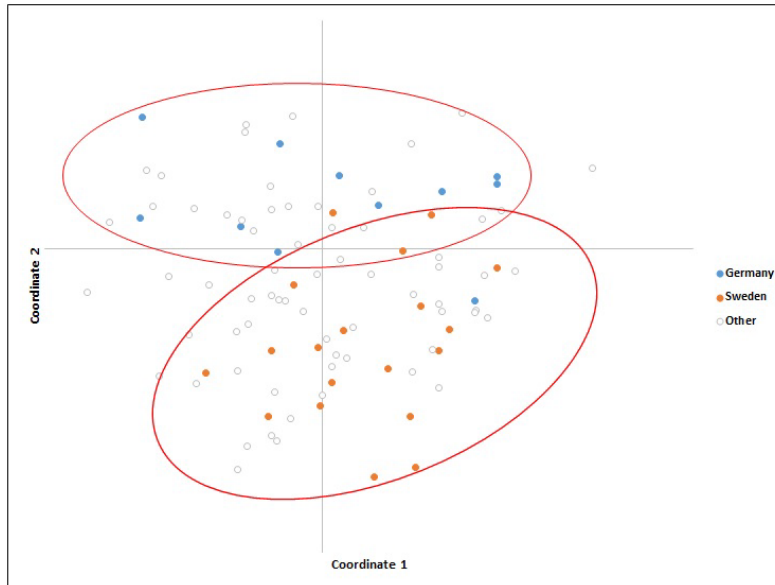
Figure 5. PCoA of North American German Pinschers born from dogs originating from Germany (blue dots), Sweden (orange dots), and other countries (white dots) based on alleles and allele frequencies at 33 autosomal STR loci. Red circles represent the two genetically distinct subpopulations based on parental country of origin (Germany and Sweden).

Finally, a PCoA analysis can be also be done considering the coefficient of inbreeding (COI) of dogs included in this study, compared to their STR alleles and allele frequencies. For that, individual 8-generation COI were estimated between 1.15% (most outcrossed dog) to 21.82% (most inbred dog), with a mean COI of 6.60% and a median COI of 4.95% for the cohort (data not shown). For the PCoA, dogs were grouped in three categories based on individual COI: 1-5%, 5.01-10%, and above 10.01%. No genetic differentiation was observed within this cohort of German Pinschers based on COI, with dogs from each COI range reasonably dispersed across all quadrants of the graph (**Figure 6**).
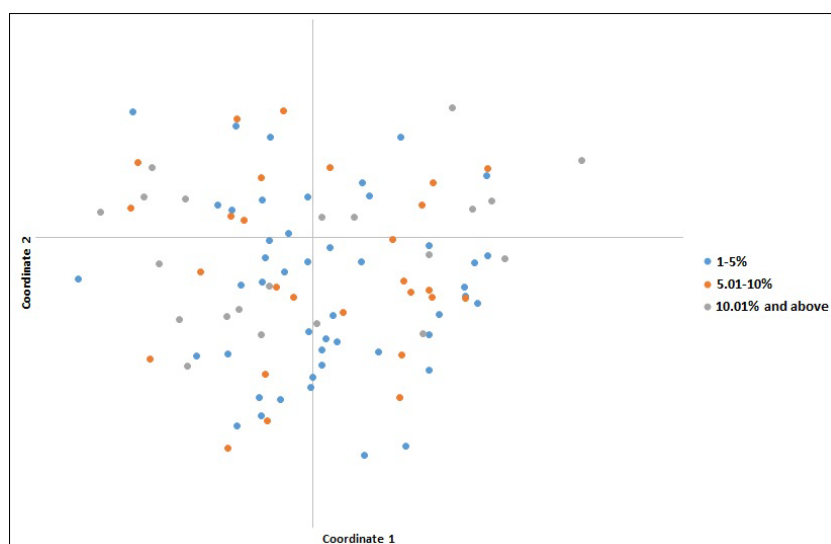

Figure 6. PCoA of German Pinschers based on coefficient of inbreeding (COI) compared to alleles and allele frequencies at 33 autosomal STR loci. Blue dots: COI between 1 and 5%; orange dots:

COI between 5.01% and 10%; grey dots: COI ranging between 10.01% and 21.82%. No genetic differentiation was observed within this cohort of German Pinschers based on COI.

E. Internal relatedness (IR) of individuals and the population as a whole
        1. IR testing
Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide (average 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 to which the parents of an individual dog are related. The IR calculation takes into consideration homozygosity at each locus and gives more weight to rare and uncommon alleles. Rare and uncommon alleles would presumably be present in more genetically diverse 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 a 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 above 0.25, the more closely related are the parents and grandparents of the sibling parents. **Table 4** summarizes the IR values for the 97 German Pinschers tested.

Table 4. Internal relatedness (IR) values calculated using allele numbers and frequencies in 97 German Pinschers. The IR values can be adjusted to reflect how these same dogs would score if they were to exist in a large population of village dogs (IRVD).

	IR	IRVD
Minimum	-0.2859	-0.0234
1st Quartile	-0.0807	0.2265
Mean	-0.0026	0.3042
Median	-0.0125	0.3041
3rd Quartile	0.0833	0.3789
Maximum	0.3444	0.5736

According to the table, the most outbred dog in this cohort had an IR score of -0.28, while the most inbred dog had an IR score of +0.34. This wide range of IR values shows that the degree of parental relatedness varies greatly in this cohort, a typical finding for almost all pure breeds of dogs. Additionally, 25% of the dogs had IR scores of 0.083 or greater; this means that this cohort is comprised of inbred dogs (IR>0.25) which is balanced by an equally sized group of strongly outbred dogs (IR scores ranging from -0.28 to -0.08). Therefore, IR scores provide a more accurate representation of heterozygosity in individual dogs than the breed-wide averages obtained from the standard genetic assessment.

        2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed evolution.
The IR values obtained from known 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 Pacific Islands are randomly breeding descendants of dogs from which most modern breeds evolved. The

known 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).

Figure 7 shows that the curve representing IRVD scores for the 97 German Pinscher (blue line) is shifted to the right of their actual IR scores (red line), which is typical of most pure dog breeds. Roughly 75% of this cohort have IRVD values of 0.22 or greater (**Table 4**, **Figure 7**), which means that if they were found among village dogs, they would all be considered offspring of at least full sibling parents. This is a result of the low amount of available canid genetic diversity retained in German Pinschers.

In addition, the gray area in **Figure 7** represents the overlap between IR and IRVD curves, which is an estimate of the amount of genetic diversity in present-day randomly breeding village dogs that still exists in contemporary German Pinschers. This amount of genetic diversity (22%, **Figure 7**) is very similar to the retained genetic diversity (25%) found in all canids tested at the VGL to date (section IIB).
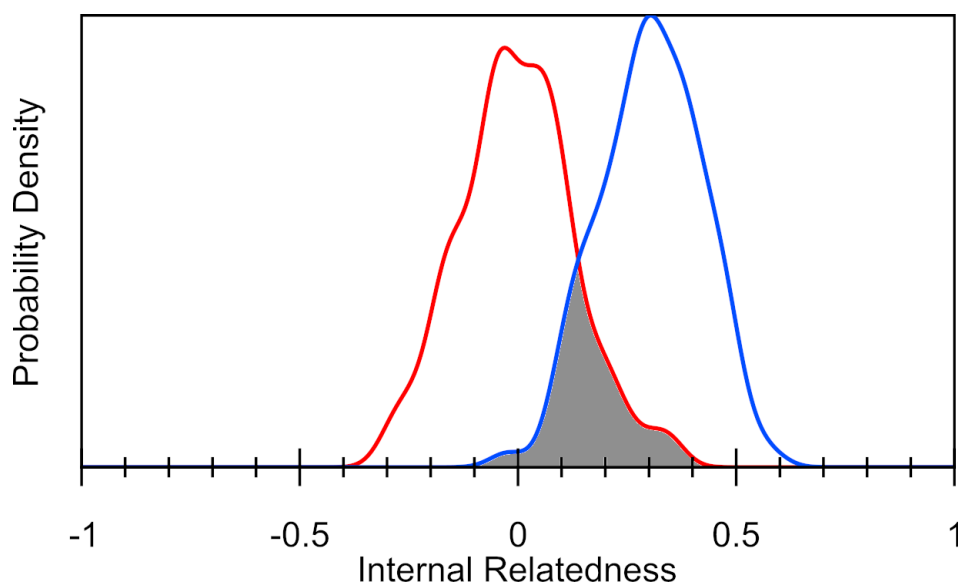

Figure 7. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for German Pinschers (n=97). The overlap between the curves (gray) represents the degree of allele sharing (22%) between this breed and village dogs.

The low proportion of available canid genetic diversity retained in German Pinschers can be explained by the dramatic genetic bottleneck suffered by the breed in the 1950's, when only five dogs were used to establish the entire population existing today. Moreover, since only dogs from North America were used in this study, an additional contributor to this phenomenon might have been the subsequent genetic bottleneck that occurred during the development of the North American subpopulation of German Pinschers (see section IA).

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 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, which can cause autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases.

The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (i.e., 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 5**). Haplotypes are groups of genes (and consequently their alleles) inherited as a block, rather than individually.

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 STR 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. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking: class I haplotypes (originally identified in Standard Poodles) are named 1001, 1002, and so on; class II haplotypes are named 2001, 2002, etc. 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 German Pinscher

Seven DLA class I and seven DLA class II haplotypes were identified in this study cohort (**Table 5**). This is a relatively low number of DLA haplotypes when compared to other breeds tested at the VGL. DLA I haplotype 1036 was the most frequent, being identified in 53% of the dogs tested; the most frequent DLA II haplotype was 2041, also identified at a frequency of 53%. The remaining DLA I and DLA II haplotypes were identified at lower frequencies in the population. DLA haplotypes 1036/2041, 1062/2021, and 1052/2100 are in linkage, and being transmitted as larger extended haplotypes in the population. The predominance of the 1036/2041 extended haplotype (occurring in more than one-half of the cohort) is typical of pure dog breeds, and once again highlights the profound influence of a founder (or a founder line) in the development of the breed.

Table 5. DLA class I and II haplotypes identified in German Pinschers (n=97) with their respective frequencies. The haplotype with the highest frequency for each class is bolded.

DLA I haplotype	STR types	Frequency (%)
1030	380 373 293 178	8.2
1036	**389 365 289 180**	**53.1**
1052	380 372 289 184	5.7
1062	382 371 277 183	12.9
1073	388 369 289 186	0.5
1094	395 375 277 176	1
1293	386 373 291 182	18.6

DLA II haplotype	STR types	Frequency (%)
2021	339 324 268	12.9
2023	341 323 282	2.6
2041	**349 321 280**	**53.1**
2054	343 322 268	0.5
2067	343 322 284	24.2
2089	343 331 276	1
2100	341 324 282	5.7

Moreover, DLA haplotype analysis showed DLA I and DLA II haplotype sharing with 36 other dog breeds tested at the VGL (**Table 6**). DLA I haplotype 1293, found in almost 19% of German Pinschers, is unique to the breed; similarly, DLA II haplotype 2100 (5.7% frequency) is only shared with Miniature Poodle, albeit at an extremely low frequency (0.1%). Interestingly, the only closely related breed with which the German Pinscher shares DLA haplotypes is the Doberman Pinscher. The extended 1094/2089 DLA haplotype found in 1% of German Pinschers is highly prevalent in Doberman Pinschers, being found in approximately 75% of the population (**Table 6**). No DLA haplotype sharing was found between German Pinschers and Schnauzers, but this might change as more dogs are tested and additional low frequency DLA haplotypes are identified in the breed.

Table 6. Sharing of DLA class I and II haplotypes between German Pinschers (highlighted in blue) and other dog breeds tested at the VGL (n=36).

DLA1 #

DLA1 #	STR types	German Pinscher (n=97)	American Eskimo, Standard (n=65)	American Eskimo, Miniature (n=38)	American Hairless Terrier (n=186)	Alaskan Klee Kai (n=657)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=148)	Bernese Mountain Dog (n=151)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorkshire Terrier (n=54)	Biewer Terrier (n=219)	Yorkshire Terrier (n=16)	Doberman Pinscher (n=1242)	English Bulldog (n=163)	Great Dane (n=74)	Golden Retriever (n=880)	German Shepherd (n=38)
1030	380 373 293 178	0.082	0.008	0.03	--	--	0.029	0.008	--	0.013	--	0.449	0.352	0.493	0.31	0.0982	--	0.264	0.0011	--
1036	389 365 289 180	0.531	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.47
1052	380 372 289 184	0.057	--	--	0.022	0.2291	--	0.015	0.105	--	--	--	--	--	--	0.0008	0.187	--	0.0858	--
1062	382 371 277 183	0.129	--	--	0.169	--	--	--	--	--	--	--	--	--	--	--	0.172	--	--	--
1073	388 369 289 186	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1094	395 375 277 176	0.01	--	--	--	--	--	--	--	0.142	0.013	--	--	--	--	0.7379	--	0.034	--	--
1293	386 373 291 182	0.186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 # (cont'd)

DLA1 # (cont'd)	STR types	German Pinscher (n=97)	Havana Silk (n=46)	Havanese (n=998)	Italian Greyhound (n=1406)	Irish Wolfhound (n=78)	Lakeland Terrier (n=137)	Labrador Retriever (n=295)	Llewellin Setter (n=152)	Newfoundland (n=139)	Toy Poodle (n=234)	Miniature Poodle (n=419)	Poodle (n=5193)	Rat Terrier (n=44)	Saint Bernard (n=90)	Scottish Collie (n=120)	Shiba Inu (n=162)	Shikoku (n=85)	Shiloh Shepherd (n=69)	Shiloh Shepherd, ISSA (n=273)
1030	380 373 293 178	0.082	0.04	0.003	0.0302	--	--	0.044	0.02	--	--	--	0.00212	0.02	--	--	--	--	--	--
1036	389 365 289 180	0.531	--	--	0.0007	--	--	--	--	--	0.011	0.043	0.00164	--	--	--	--	--	--	--
1052	380 372 289 184	0.057	--	0.0105	0.1863	0.333	0.011	0.012	--	--	--	--	0.00029	0.07	0.061	--	--	--	0.304	0.383
1062	382 371 277 183	0.129	--	0.0005	--	--	--	0.012	--	0.007	--	--	--	0.07	--	--	--	--	--	--
1073	388 369 289 186	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1094	395 375 277 176	0.01	--	--	--	0.192	--	--	--	--	--	--	--	--	--	0.004	--	--	--	--
1293	386 373 291 182	0.186	--	--	--	--	--	--	--	0.018	--	--	--	--	--	--	--	--	--	--

DLA2 #

DLA2 #	STR types	German Pinscher (n=97)	American Eskimo, Standard (n=65)	American Eskimo, Miniature (n=38)	American Hairless Terrier (n=186)	Alaskan Klee Kai (n=657)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=148)	Bernese Mountain Dog (n=151)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorkshire Terrier (n=54)	Biewer Terrier (n=219)	Yorkshire Terrier (n=16)	Doberman Pinscher (n=1242)	English Bulldog (n=163)	Great Dane (n=74)	Golden Retriever (n=880)	German Shepherd (n=38)
2021	339 324 268	0.129	--	--	0.151	0.226	--	0.008	--	0.043	--	0.004	0.352	0.004	--	--	--	--	0.0858	--
2023	341 323 282	0.026	0.008	0.03	--	--	0.029	--	0.013	--	0.003	0.449	--	0.495	0.31	0.0982	--	0.264	0.0011	--
2041	349 321 280	0.531	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2054	343 322 268	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2067	343 322 284	0.242	--	--	0.022	--	--	0.069	0.895	--	--	--	--	--	--	0.7717	--	--	--	0.04
2089	343 331 276	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2100	341 324 282	0.057	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 # (cont'd)

DLA2 # (cont'd)	STR types	German Pinscher (n=97)	Havana Silk (n=46)	Havanese (n=998)	Italian Greyhound (n=1406)	Irish Wolfhound (n=78)	Lakeland Terrier (n=137)	Labrador Retriever (n=295)	Llewellin Setter (n=152)	Newfoundland (n=139)	Toy Poodle (n=234)	Miniature Poodle (n=419)	Poodle (n=5193)	Rat Terrier (n=44)	Saint Bernard (n=90)	Scottish Collie (n=120)	Shiba Inu (n=162)	Shikoku (n=85)	Shiloh Shepherd (n=69)	Shiloh Shepherd, ISSA (n=273)
2021	339 324 268	0.129	--	0.0045	--	0.295	--	0.012	--	0.007	0.077	0.05	0.00366	0.02	0.061	--	--	--	--	--
2023	341 323 282	0.026	0.04	0.003	0.0302	--	--	0.044	0.013	--	--	--	0.00212	0.02	0.394	--	--	--	--	--
2041	349 321 280	0.531	--	--	0.0007	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2054	343 322 268	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2067	343 322 284	0.242	--	--	0.0004	--	0.011	--	--	--	--	0.006	--	--	--	--	0.216	0.012	--	--
2089	343 331 276	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	0.004	--	--	--	--
2100	341 324 282	0.057	--	--	--	--	--	--	--	--	--	0.001	--	--	--	--	--	--	--	--

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 measured by the 33 autosomal STR markers. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will also be inherited randomly. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and averaged across all loci (**Table 8**).

Analysis of individual DLA STR loci shows that observed heterozygosity (Ho) values were higher than expected heterozygosity (He) values estimated for each locus, indicating a higher than expected degree of heterozygosity in the DLA region compared to other parts of the genome. This is also corroborated by the negative F values found for all DLA loci (**Table 7**). A similar conclusion can be reached when considering mean values for observed *vs.* expected heterozygosity and inbreeding coefficient (F) across the region (**Table 8**). These findings suggest that, on average, this cohort of German Pinschers was estimated be 4% more outbred than a random mating population (F=-0.04) based on DLA loci, and that the DLA region is apparently reaching equilibrium with other loci in the genome as values from **Table 8** are close to those reported on **Table 2.**

Table 7. Standard genetic assessment for German Pinschers (n=97) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	6	2.837	0.67	0.648	-0.04
DLA I-4ACA	6	2.676	0.639	0.626	-0.02
DLA I-4BCT	4	2.427	0.629	0.588	-0.07
DLA1131	7	2.915	0.68	0.657	-0.04
5ACA	4	2.69	0.639	0.628	-0.02
5ACT	5	2.643	0.649	0.622	-0.05
5BCA	5	2.736	0.649	0.635	-0.02

Table 8. Summary of standard genetic assessment for German Pinschers (n=97) using 7 STRs in the DLA class I and II regions.

	Na	Ne	Ho	He	F
Mean	5.29	2.704	0.651	0.629	-0.04
SE	0.39	0.054	0.006	0.008	0.006

III. What does this assessment of genetic diversity tell us about contemporary German Pinschers

This study confirmed that this cohort constituted a single breed based on alleles and their frequencies for the 33 autosomal STR loci, as well as intra- and inter-breed genetic clustering based on PCoA. However, it also showed that the German Pinscher is lacking in genetic diversity, most likely due to the genetic bottleneck(s) the breed underwent in the last half century. Although it is possible that additional diversity will be discovered as more dogs from diverse regions are tested, any additional autosomal STR alleles or DLA class I and II haplotypes will be at a

comparatively low incidence. In addition, this group of 97 dogs is entirely from North America and may not reflect the genetic diversity existing in dogs from other parts of the world.

It is important to note that limited genetic diversity is not in itself a bad thing. If the founding population was genetically healthy, the original breed standard strictly adhered to, a reasonable population size sustained, and HWE maintained as is the case here, the breed should survive in good health. Breeds with low genetic diversity are more prone to genetic disorders as well as autosomal recessive mutations, especially when subjected to periods of strong artificial selection for desired conformation traits. Many of these types of disorders involve popular sire effects. A low level of genetic diversity also makes it more difficult, and even impossible, to eliminate genetic mutations from a breed.

Therefore, the goal for German Pinscher breeders should be to find mates as unrelated as possible, and avoid popular sire lines. This will help maintain and re-distribute the existing genetic diversity in the breed (see section IV-B below).

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.
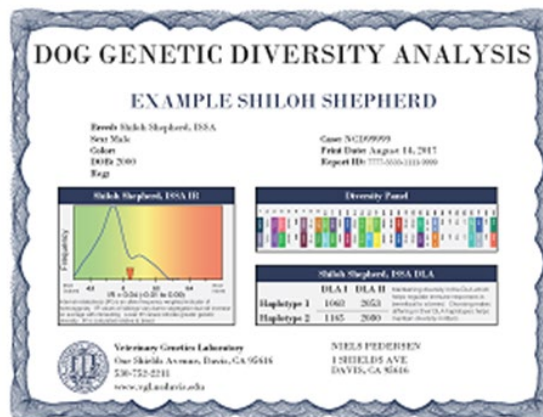
B. What should you do with this information?

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

1. Wikipedia. German Pinscher. https://en.wikipedia.org/wiki/German_Pinscher.
2. American Kennel Club. German Pinscher. https://www.akc.org/dog-breeds/german-pinscher/.
3. The German Pinscher Club of America. https://germanpinscher.org/.
4. German Pinscher History: A Breed Saved From Extinction. https://www.akc.org/expert-advice/dog-breeds/german-pinscher-history/
5. Pedersen NC, Pooch AS, Liu H. A genetic assessment of the English bulldog. *Canine Genet Epidemiol.* 2016; 3:6. doi: 10.1186/s40575-016-0036-y.
6. Pedersen NC, Shope B, Liu H. An autosomal recessive mutation in *SCL24A4* causing enamel hypoplasia in Samoyed and its relationship to breed-wide genetic diversity. *Canine Genet Epidemiol.* 2017; 4:11. doi: 10.1186/s40575-017-0049-1.

This report was generated by Felipe Avila and Shayne Hughes on 12/12/2023.