Genetic Diversity Testing for Polish Lowland Sheepdog

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 Polish Lowland Sheepdog 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 in this study. This report is based on data from 103 registered Polish Lowland Sheepdogs from five countries: United States (n=83), Sweden (n=14), Finland (n=4), Poland (n=1), and Canada (n=1).

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 Polish Lowland Sheepdog

A. Breed History [1-4]

The Polish Lowland Sheepdog has been known in its present form in Poland as far back as the thirteenth century. The breed is known as *Polski Owczarek Nizinny* in its native country, thus the widely used nickname "PON". The breed is thought to descend from ancient breeds from Eastern Europe and Asia such as the Puli, Tibetan Terrier, and Huns herding dog. In Poland, the breed was almost driven to extinction during World War II. However, thanks to the effort of Dr. Danuta Hryniewicz (**Figure 1**), the breed was rescued. Her dog, *Smok* ("Dragon"), sired the first ten litters in the 1950's. Therefore, all modern Polish Lowland Sheepdogs in the world can be traced back to *Smok*, who Dr. Hryniewicz considered the epitome of the restored breed based on his appearance and temperament.

Figure 1. Dr. Danuta Hryniewicz with her Polish Lowland Sheepdogs.

The Polish Lowland Sheepdog may be ancestral to several breeds in Scotland and England. As the story goes, Kazimierz Grabski, a Polish merchant, traded a shipment of grain for sheep in Scotland in 1515 and brought six Polish Lowland Sheepdogs with him to help move the sheep. A Scottish shepherd was so impressed with the herding ability of these dogs that he traded a ram and two ewes for one male and two females. These dogs were purportedly bred with indigenous dogs to produce the Scottish herding dog (Bearded Collie). The Bearded Collie was, in turn, an important ancestor to the Old English Sheepdog that appeared in the 18th and 19th centuries. However, the Old English Sheepdog more closely resembles the Polish Lowland Sheepdog than the Bearded Collie.

The first official standard for the Polish Lowland Sheepdog was accepted by the *Fédération Cynologique Internationale* (FIC) in 1959. The American Kennel Club recognized the Polish Lowland Sheepdog as a breed in 2001, including it in the Herding group. Currently, the breed ranks 181 of 201 in the AKS Breed Popularity Ranking.

B. Appearance [1-4]

The Polish Lowland Sheepdog has a similar appearance and muscular frame, but is only about half the size of the Old English Sheepdog. It is a medium-sized dog; males are 45–50cm (18-20 inches) in height at the withers, while females are 42–47cm (17–19 inches). Males typically weigh between 18 and 23kg (40–50lb), and females 14-18kg (30–40lb). It is not desirable to diminish the size below the breed standard. Their topcoat is rough and either straight or wavy, but not curly, while the undercoat is soft and dense. Facial hair typically covers the eyes. The coat can be of any color or pattern; white, gray, and brown are most common, with black, gray, or brown markings. Faults include a curly, short, or silky coat; a lack of undercoat; a thin, wispy coat; and any coat that appears to be sculpted. The Polish Lowland Sheepdog must be shown naturally, with an "unkempt" but clean appearance - any trimming of the coat is severely penalized. The tail is either naturally short or docked. European countries have banned docking for the most part, and many Polish Lowland Sheepdogs now have tails of varying lengths. Additional disqualifications include blue or yellow eyes, and an overshot or undershot bite.

C. Temperament [1-3]

Polish Lowland Sheepdogs are described as stable, confident, intelligent, affectionate, playful, and good with children. However, they can be headstrong, thus requiring a dominant master and consistent training. They adapt well to various conditions and are popular companion dogs for apartment dwellers in their native Poland. Polish Lowland Sheepdogs need to be kept busy, as bored dogs may engage in excess barking and destructive or self-destructive behaviors. They also require a moderate amount of time for daily exercise and grooming. Their territorial behavior and barking tendencies make them good watchdogs.

Although no longer used to herd sheep, they can compete in dog agility trials, obedience, rally, showmanship, flyball, tracking, and herding events. Herding instincts and trainability can be measured at noncompetitive herding tests and individuals with basic herding instincts can be trained to compete in herding trials.

D. Health of the Polish Lowland Sheepdog [5-12]

1. Lifespan

The breed’s average lifespan is 12 to 14 years of age.

2. Disorders

Hip dysplasia is a recognized problem in the Polish Lowland Sheepdog, and often leads to debilitating secondary degenerative joint disease in older dogs. This disorder is common in

medium and large sized pure breeds, and has a complex heritability that is also influenced by environmental factors. Dogs should have their hips evaluated radiographically by experts starting when over one year of age and the severity scored by recognized methods. According to the Orthopedic Foundation for Animals (OFA) Health Survey for the breed, 16% of dogs evaluated (n=114) had dysplastic hips.

Progressive retinal atrophy (PRA) is a recognized cause of blindness in Polish Lowland Sheepdog, as it is in many other breeds. It is a late-onset degenerative disease with slow progression. There is early loss of rod function by 4.5 years of age, while cone deterioration occurs later. The rcd4 mutation in the *C2ORF71* gene has been identified in at least 90% of affected dogs from Sweden and USA. The OFA Health Survey reports that 16.7% of all dogs tested (n=63) had this condition, and 51% were carriers for the rcd4 mutation. The VGL offers tests for PRA (https://vgl.ucdavis.edu/test/progressive-retinal-atrophy-rcd4-pra). Besides PRA, 6.7% of dogs tested by the OFA had other ocular disorders.

Other health problems reported in Polish Lowland Sheepdog include hypothyroidism (a common autoimmune disorder occurring in many pure breeds), cataracts, neuronal ceroid lipofuscinosis, and patent ductus arteriosus (PDA).

II. Results on Genetic Diversity of Polish Lowland Sheepdog

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 [6,7]. For each STR locus included in this study, an average of 17 different alleles have been identified across all 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 (i.e., the total number of alleles). 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 Polish Lowland Sheepdogs included in this study are listed in **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Polish Lowland Sheepdogs (n=103). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
100 (0.432)	131 (0.320)	121 (0.044)	219 (0.117)	240 (0.146)	87 (0.243)
102 (0.466)	135 (0.005)	123 (0.034)	225 (0.010)	244 (0.024)	**91 (0.709)**
104 (0.019)	**145 (0.670)**	127 (0.427)	233 (0.053)	**246 (0.680)**	95 (0.049)
106 (0.083)	149 (0.005)	**131 (0.481)**	**237 (0.796)**	250 (0.150)	
		133 (0.015)	239 (0.010)		
			243 (0.015)		
AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**

284 (0.097)	116 (0.248)	**132 (0.966)**	152 (0.024)	232 (0.306)	95 (0.087)
288 (0.005)	118 (0.248)	144 (0.034)	156 (0.214)	234 (0.005)	**101 (0.913)**
290 (0.553)	**124 (0.505)**		160 (0.024)	**236 (0.466)**	
292 (0.345)			164 (0.019)	240 (0.039)	
			168 (0.670)	242 (0.184)	
			172 (0.039)		
			176 (0.010)		

INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
124 (0.704)	**144 (0.553)**	204 (0.252)	**95 (0.854)**	227 (0.039)	204 (0.194)
126 (0.010)	150 (0.296)	**210 (0.466)**	97 (0.146)	**231 (0.549)**	**206 (0.694)**
128 (0.262)	152 (0.150)	218 (0.282)		233 (0.408)	210 (0.112)
132 (0.024)				241 (0.005)	

REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
210 (0.053)	164 (0.432)	264 (0.005)	**228 (0.335)**	147 (0.194)	14 (0.039)
212 (0.214)	166 (0.010)	266 (0.063)	232 (0.257)	149 (0.117)	**15 (0.631)**
216 (0.650)	168 (0.005)	**268 (0.830)**	236 (0.170)	**153 (0.689)**	16 (0.010)
220 (0.083)	**170 (0.553)**	272 (0.102)	238 (0.228)		20.2 (0.199)
			240 (0.010)		21.2 (0.121)

VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
13 (0.044)	14 (0.073)	17 (0.262)	19 (0.243)	10 (0.184)	15 (0.053)
14 (0.306)	15 (0.218)	18 (0.005)	**20 (0.306)**	11 (0.165)	16 (0.010)
15 (0.097)	**16 (0.335)**	25 (0.005)	21 (0.189)	13 (0.010)	**17 (0.641)**
16 (0.155)	17 (0.131)	**26 (0.427)**	22 (0.044)	**14 (0.519)**	18 (0.121)
19.1 (0.073)	18 (0.214)	27 (0.039)	23 (0.214)	15 (0.107)	19 (0.175)
20.1 (0.141)	19 (0.029)	28 (0.053)	24 (0.005)	16 (0.015)	
21.1 (0.175)		29 (0.005)			
22.1 (0.010)		30 (0.184)			
		31 (0.019)			

VGL2918	**VGL3008**	**VGL3235**
12 (0.777)	**15 (0.350)**	13 (0.097)
13 (0.010)	16 (0.068)	**14 (0.621)**
15 (0.019)	17 (0.243)	16 (0.282)
17.3 (0.194)	18 (0.150)	
	19 (0.189)	

The number of alleles found for each STR locus in this cohort of Polish Lowland Sheepdogs was low, ranging from 2 (FH2001, INRA21, LEI004) to 9 (VGL1165). Moreover, a single allele was predominant (found in 50% or more of the cohort) at 23 out of the 33 STR loci (bolded in **Table 1**), which indicates that these alleles were present in the foundation stock and were retained in high frequency in the population due to being linked to breed-defining phenotypic traits. For some STR markers such as FH2001 and INRA21, a single allele was identified in over 90% of the dogs tested. This finding indicates a lack of genetic diversity in the breed, so the goal for breeders should be to

re-distribute allele frequencies for the 33 STR markers by conserving and breeding rare lines/families.

The number of alleles and their respective frequencies at each locus also indicate that a great deal of inbreeding has gone into the evolution of this breed. As stated above, the average number of known alleles for these 33 autosomal STR loci is 17, while the average number of alleles per locus (Na) for Polish Lowland Sheepdogs was 4.4 (**Table 2**). Therefore, Polish Lowland Sheepdogs have only retained 25.9% of the known canid diversity for these markers. This amount of retained genetic diversity is lower than almost all other breeds tested at the VGL so far, being similar to that of the Irish Wolfhound (24.8%) and Shiloh Shepherd (26%).

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 103 Polish Lowland Sheepdogs based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	4.39	2.429	0.533	0.526	-0.01
SE	0.28	0.172	0.032	0.03	0.013

The average number of STR alleles identified in this study cohort (Na = 4.4) corresponds to approximately 26% of the average number of alleles identified by the VGL across breeds (see **section IIa**). This means that 74% of the genetic diversity known to exist at these 33 STR loci has been lost in the modern Polish Lowland Sheepdog. However, the number of effective alleles (Ne) constitutes a more relevant metric for genetic diversity, since these alleles have the greatest genetic influence on heterozygosity levels. The average number of effective alleles per locus (Ne) was estimated at 2.4 for this cohort; this indicates that most of the heterozygosity was determined by one-half of the alleles segregating in the breed (**Table 2**).

The observed (actual) heterozygosity in this group of dogs was 0.53, while the expected heterozygosity (He) for a population in Hardy-Weinberg equilibrium (HWE) was 0.52, yielding a coefficient of inbreeding (F) of -0.01 (i.e., 1% more outbred than predicted for HWE). These standard genetic assessment values indicate that the parents of these 103 Polish Lowland Sheepdogs were more heterozygous, on average, given the existing population size and genetic diversity.

Although these findings provide an estimate of genetic diversity at the breed level, a better picture of heterozygosity at an individual level can be obtained by looking at Internal Relatedness (IR) scores. This diversity metric should be used by breeders to select the most unrelated mates possible

in order to continue redistributing the genetic diversity currently existing in the breed (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 assessment provides an estimate of genetic diversity in the specific regions of the genome that harbor each STR marker. Again, it is important to assess the number of effective (Ne) alleles, as these have greater impact on heterozygosity. **Table 3** shows that the number of alleles (Na) ranged from a low of 2 to a high of 9, whereas the number of effective alleles (Ne) ranged from 1.07 (FH2001) to 5.4 (VGL0910). Based on allele frequencies per locus, observed heterozygosity (Ho) values ranged from 0.068 (FH2001) to 0.854 (VGL0910); locus-specific expected heterozygosity (He) values were in general similar to their respective Ho values, ranging from 0.066 (FH2001) to 0.815 (VGL0910).

The loci with low Ho values were under a greater degree of positive selection than those with high values, and were in areas of the genome that that were associated with strong breed specific phenotypic traits. Loci with high Ho values were more likely to be associated with phenotypic variation within the breed.

Based on Ho and He values estimated for each STR locus, inbreeding coefficients (F) for Polish Lowland Sheepdogs ranged from -0.138 (INU055) to 0.199 (AHT137) (**Table 3**). Twenty-one loci had negative F values and 12 were positive. The higher number of loci with negative F values suggests that, despite the overall lack of genetic diversity estimated for the breed, this cohort of 103 dogs was carefully selected to emphasize unrelatedness over relatedness.

Table 3. Standard Genetic Assessment of individual STR loci for 103 Polish Lowland Sheepdogs. Individual STR loci with high inbreeding coefficients (F>0.1) are bolded.

Locus	Na	Ne	Ho	He	F
AHT121	4	2.433	0.631	0.589	-0.071
AHT137	4	1.813	0.359	0.449	**0.199**
AHTH130	5	2.4	0.621	0.583	-0.065
AHTh171-A	6	1.537	0.282	0.349	**0.194**
AHTh260	4	1.975	0.476	0.494	0.036
AHTk211	3	1.774	0.447	0.436	-0.023
AHTk253	4	2.302	0.592	0.566	-0.047
C22.279	3	2.649	0.641	0.623	-0.029
FH2001	2	1.07	0.068	0.066	-0.035
FH2054	7	2.01	0.495	0.502	0.015
FH2848	5	2.888	0.67	0.654	-0.025
INRA21	2	1.19	0.175	0.159	-0.096
INU005	4	1.77	0.398	0.435	0.085
INU030	3	2.4	0.583	0.583	0.002
INU055	3	2.776	0.728	0.64	-0.138

LEI004	2	1.331	0.252	0.249	-0.014
REN105L03	4	2.134	0.515	0.531	0.031
REN162C04	3	1.88	0.524	0.468	-0.12
REN169D01	4	2.09	0.544	0.522	-0.042
REN169O18	4	2.028	0.476	0.507	0.062
REN247M23	4	1.422	0.291	0.297	0.018
REN54P11	5	3.855	0.767	0.741	-0.036
REN64E19	3	1.9	0.505	0.474	-0.066
VGL0760	5	2.202	0.563	0.546	-0.032
VGL0910	8	5.413	0.854	0.815	-0.048
VGL1063	6	4.369	0.767	0.771	0.005
VGL1165	9	3.448	0.757	0.71	-0.067
VGL1828	6	4.24	0.767	0.764	-0.004
VGL2009	6	2.917	0.641	0.657	0.025
VGL2409	5	2.18	0.573	0.541	-0.058
VGL2918	4	1.559	0.369	0.359	-0.029
VGL3008	5	4.095	0.709	0.756	0.062
VGL3235	3	2.106	0.563	0.525	-0.072

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 103 Polish Lowland Sheepdogs used in this study clustered as expected for a pure breed in the PCoA, with dogs reasonably dispersed across all four quadrants and no evidence of central clustering (**Figure 2**).

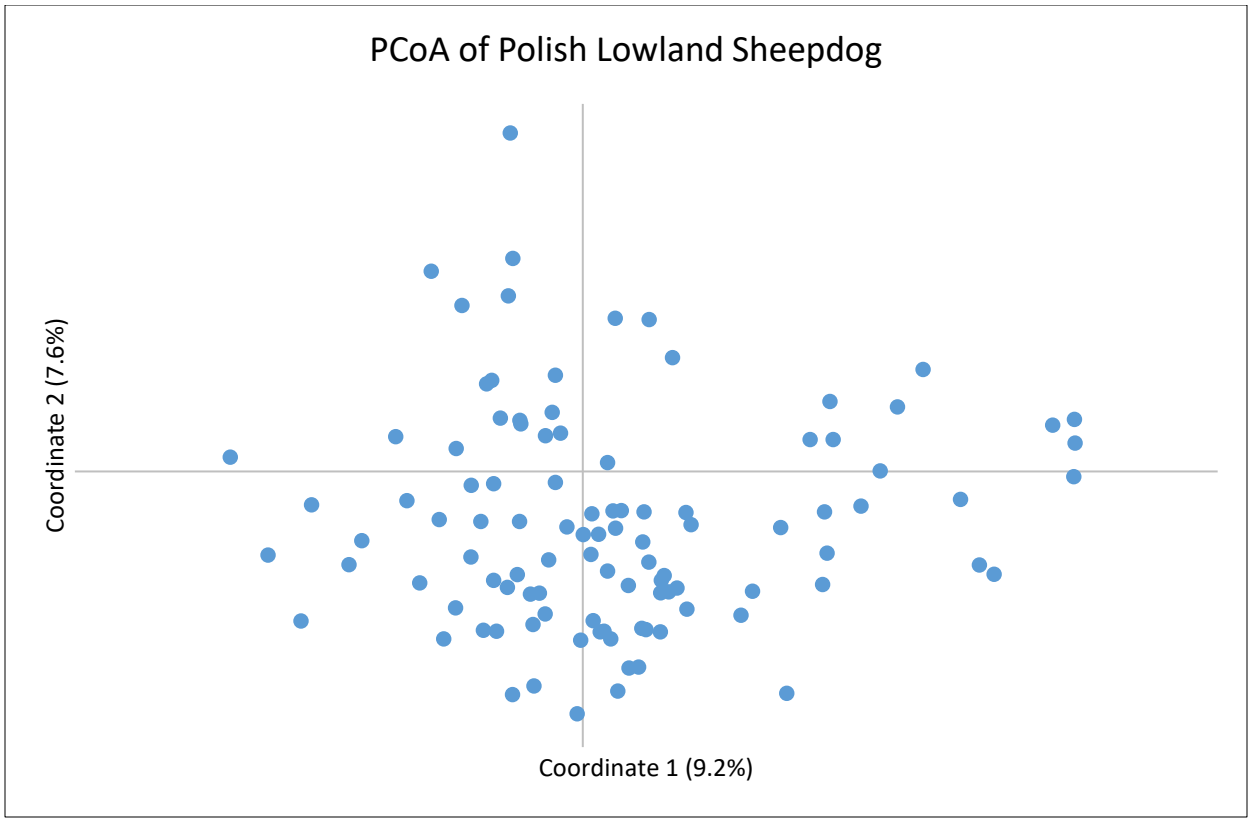


Figure 2. PCoA of Polish Lowland Sheepdog (n=103) based on STR alleles and their frequencies at 33 autosomal loci.

The degree of intra- and inter-breed relatedness can be further assessed by comparing this cohort of Polish Lowland Sheepdogs with other breeds, such as Border Collie and Shiloh Shepherd, ISSA (**Figure 3**). Inter-breed clustering shows separate and well-defined populations, thus indicating that the breeds are genetically distinct as expected. Clustering of each breed in the plot highlights that Border Collie is more genetically diverse than Shiloh Shepherd and Polish Lowland Sheepdog, due to a relatively more "diffuse" scattering of individuals on the plot.

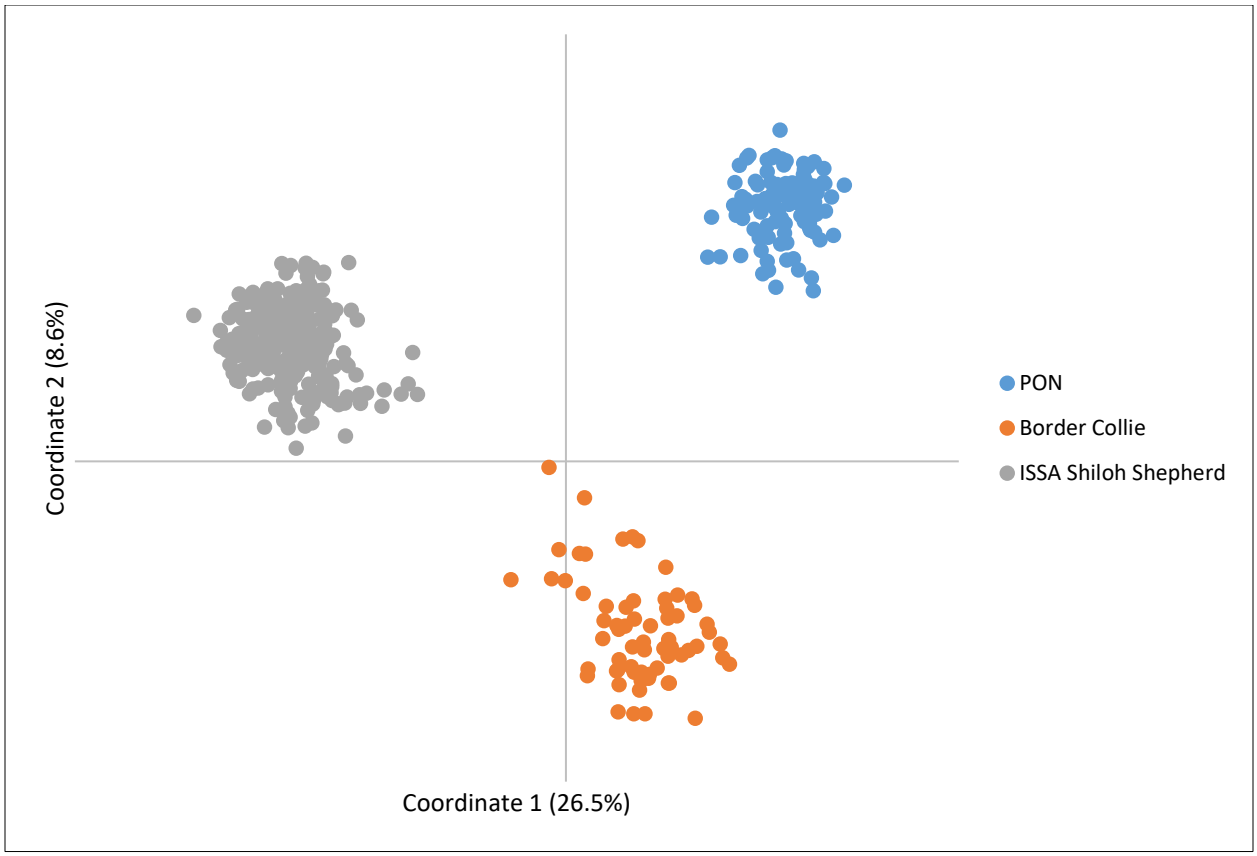


Figure 3. PCoA plot comparing intra- and inter-breed relatedness of Polish Lowland Sheepdog (blue dots; n=103) with Border Collie (orange dots; n=66) and Shiloh Shepherd, ISSA (grey dots; n=277).

III. Internal relatedness (IR) scores for Polish Lowland Sheepdog

a. 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 and adjusted village-dog IR (IRVD – see **section e-2**) values for the cohort analyzed in this study.

Table 4. Internal relatedness (IR) and adjusted village-dog IR (IRVD) values calculated using the number of alleles and their respective frequencies for 33 STR loci in 103 Polish Lowland Sheepdogs.

	IR	IRVD
Minimum	-0.3012	0.0888
1st Quartile	-0.1255	0.3520
Mean	-0.0016	0.4213
Median	-0.0033	0.4114
3rd Quartile	0.0933	0.5046
Maximum	0.4058	0.6860

The most outbred dog in the population had an IR score of -0.30, while the most inbred dog in the group had an IR score of 0.40. The mean (average) IR score for the group was approximately zero (**Table 4**). Around 25% of animals tested were moderately to highly inbred, i.e., they were as inbred as offspring of full sibling parents (IR > 0.25). Therefore, IR values give a different picture than seen with the average scores determined by the standard genetic assessment (**Table 2**). While the standard genetic assessments indicated a population in HWE (in other words, random breeding), the IR scores showed a population of individuals that ranged from very outbred to highly inbred. The most inbred dogs are balanced by the most outbred dogs, making it appear that the overall population were products of least related parents. This is a common feature of all dog breeds.

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

Internal relatedness values can also be represented graphically (**Figure 4**). The IR curve (red line) for the breed peaks around zero, with approximately half of the population on either side. Furthermore, the IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that was lost when a breed was developed from its oldest common ancestors. 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 4**, blue line). The mean IRVD value for this cohort was 0.42, with three-quarters of the dogs scoring 0.35 or higher (**Table 4**). Therefore, if this group of dogs were village dogs, more than three-fourths of them would be considered more inbred than even the offspring of full sibling parents. The IRVD curve for the Polish Lowland Sheepdogs tested was shifted to the right of the IR curve and the area of overlap (shaded in grey) was 11.4%, which is amongst the lowest ever estimated for any dog breed tested at the VGL. The average retained genetic diversity calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL is 30% (**section IIb**).

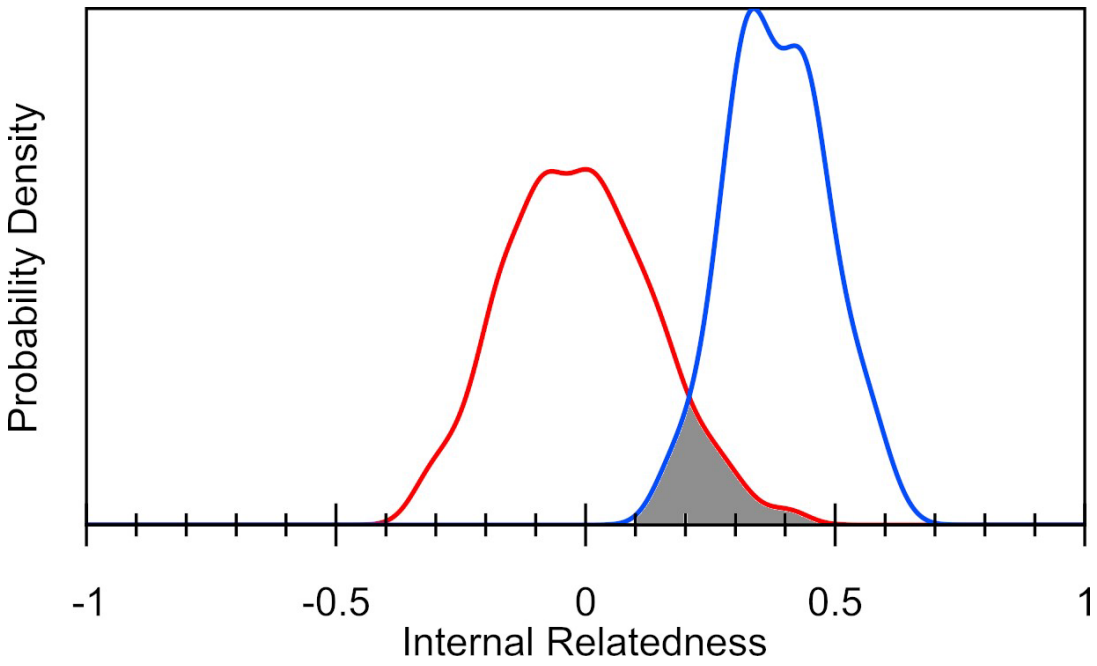


Figure 4. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Polish Lowland Sheepdogs (n=103). The overlap between the curves (grey area) shows that the breed retains 11.4% of the total genetic diversity existing in randomly breeding village dogs.

c. 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. Breeds that lack genetic diversity in the DLA region are often more prone to autoimmune disorders.

The Class I region contains several genes, but 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 (see **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 breed history and common ancestry.

d. DLA class I and II haplotypes in Polish Lowland Sheepdogs

Only 5 DLA class I and 5 DLA class II haplotypes were identified in this group of Polish Lowland Sheepdogs, which is a low number when compared to other breeds tested at the VGL so far (**Table 5**). These numbers are lower than those found in the English Mastiff (6 DLA I and 6 DLA II haplotypes) and Shiloh Shepherd (7 DLA I and 6 DLA II), for example. They are much lower than those of more popular and diverse breeds such as Golden Retrievers (24 DLA I and 19 DLA II) haplotypes) and Miniature Poodle (33 and 23, respectively).

Table 5. DLA I and DLA II haplotypes identified in the 103 Polish Lowland Sheepdogs, as well as their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 haplotype	STR types	Frequency (%)
1016	382 371 277 178	2.5
1040	**380 371 277 186**	**66.8**
1067	376 373 277 178	29.8
1068	380 373 287 181	0.5
1156	380 373 291 178	0.5
DLA2 haplotype	**STR types**	**Frequency (%)**
2014	**339 322 284**	**68.9**
2017	343 322 280	29.6
2053	343 324 280	0.5
2067	343 322 284	0.5
2098	343 323 282	0.5

One class I (1040) and one class II (2014) haplotype occurred in almost 69% of the dogs tested, while another class I (1067) and class II (2017) haplotype were observed in about 30% dogs (**Table 5**). These high incidence haplotypes were in strong linkage disequilibrium, forming larger 1040/2014 and 1067/2017 haplotypes that were found collectively in over 95% of the dogs tested. Therefore, these DLA-I/DLA-II haplotypes were present in founding stock and have been retained in the breed since its development.

The DLA-I/DLA-II haplotypes identified in Polish Lowland Sheepdog are shared with 55 different dog breeds/varieties tested at the VGL (**Table 6**). DLA haplotypes found in a breed can be used to investigate the founder lines and different breeds that were used to create and refine a breed. DLA haplotype sharing is a reflection common distant ancestry. DLA I haplotype 1156, found in 0.5% of Polish Lowland Sheepdogs tested, is unique to this breed. Interestingly, the linked 1067/2017 extended haplotype identified in 30% of Polish Lowland Sheepdogs is also present in Akita, Flat Coated Retriever, and Giant Schnauzer (albeit at extremely low frequencies). The highly prevalent 1040/2014 linked haplotype (identified in almost 70% of Polish Lowland Sheepdogs) was not seen in any other breed (**Table 6**).

Table 6. Sharing of DLA class I and II haplotypes between Polish Lowland Sheepdogs (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=55).

DLA1 #	STR types	Polish Lowland Sheepdog (n=103)	American Eskimo, Standard (n=66)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=186)	American Akita (n=160)	Japanese Akita (n=584)	Alaskan Klee Kai (n=658)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=152)	Bernese Mountain Dog (n=153)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorshire Terrier (n=54)	Biewer Terrier (n=219)	Yorkshire Terrier (n=16)	Borzoi (n=154)	Chinook (n=36)	Collie (n=49)	Doberman Pinscher (n=1282)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=878)	Great Dane (n=74)	German Pinscher (n=97)	Golden Retriever (n=888)	Whippet (n=110)
1016	382 371 277 178	0.025	0.008	0.04	0.11	0.005	--	--	--	--	--	--	0.042	0.01	0.022	0.019	0.011	0.03	--	--	0.04	0.0218	0.095	0.15	0.14	--	0.209	--	0.0045	0.105
1040	380 371 277 186	0.668	--	--	--	0.043	0.006	--	0.2128	--	--	--	--	--	0.095	0.204	0.078	--	--	--	--	0.0094	0.04	--	--	--	--	--	0.0006	0.086
1067	376 373 277 178	0.298	--	--	--	--	0.009	0.0034	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	--	--	0.045	--
1068	380 373 287 181	0.005	--	--	--	0.11	--	--	--	--	0.208	--	0.062	--	--	0.009	--	--	--	0.06	--	0.0004	--	--	--	0.2716	0.007	--	0.0479	--
1156	380 373 291 178	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 #	STR types	Polish Lowland Sheepdog (n=103)	German Shepherd (n=41)	Giant Schnauzer (n=332)	Havana Silk (n=53)	Havanese (n=1024)	Italian Greyhound (n=1444)	Irish Setter (n=60)	Irish Red and White Setter (n=109)	Irish Wolfhound (n=78)	Lakeland Terrier (n=141)	Labrador Retriever (n=300)	Llewellin Setter (n=152)	Magyar Agar (n=78)	Newfoundland (n=139)	Toy Poodle (n=234)	Miniature Poodle (n=424)	Poodle (n=5291)	Rat Terrier (n=44)	Samoyed (n=193)	Saint Bernard (n=90)	Scottish Collie (n=125)	Shiba Inu (n=164)	Shikoku (n=115)	Greater Swiss Mountain Dog (n=59)	Shiloh Shepherd (n=76)	Shiloh Shepherd, ISSA (n=277)	Swedish Vallhund (n=539)	Cardigan Welsh Corgi (n=43)
1016	382 371 277 178	0.025	--	0.045	0.094	0.1938	0.0637	--	--	--	0.007	0.015	0.03	--	0.327	0.024	0.028	0.02145	0.05	--	--	0.088	--	--	0.076	--	--	--	--	--
1040	380 371 277 186	0.668	--	--	0.019	0.0156	0.0783	--	--	--	0.177	--	--	--	0.277	0.002	0.005	0.00057	0.17	--	0.011	--	--	--	--	--	--	--	--	--
1067	376 373 277 178	0.298	--	0.002	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1068	380 373 287 181	0.005	0.29	0.054	0.236	0.0122	--	0.008	--	0.128	--	0.032	--	--	0.104	0.009	0.012	--	--	0.041	0.167	--	--	--	--	0.474	0.26	0.3618	--
1156	380 373 291 178	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Polish Lowland Sheepdog (n=102)	American Eskimo, Standard (n=66)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=186)	American Akita (n=160)	Japanese Akita (n=584)	Alaskan Klee Kai (n=658)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=152)	Bernese Mountain Dog (n=153)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorshire Terrier (n=54)	Biewer Terrier (n=219)	Yorkshire Terrier (n=16)	Borzoi (n=154)	Chinook (n=36)	Collie (n=49)	Doberman Pinscher (n=1282)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=878)	Great Dane (n=74)	German Pinscher (n=97)	Golden Retriever (n=888)	Whippet (n=110)
2014	339 322 284	0.689	0.008	0.03	0.07	0.005	--	--	0.0669	--	--	--	--	--	--	--	--	--	--	--	--	--	0.092	0.15	0.14	0.0279	0.034	--	--	--
2017	343 322 280	0.296	--	--	--	0.005	0.009	0.0034	--	0.029	0.169	0.102	--	--	--	--	--	--	0.006	0.01	--	--	0.215	0.21	0.29	0.0006	--	--	0.0389	0.241
2053	343 324 280	0.005	0.22	0.12	0.04	0.005	--	--	--	--	0.069	--	0.062	--	--	0.009	--	--	--	0.06	--	0.0012	--	--	--	0.1469	0.007	--	0.0287	--
2067	343 322 284	0.005	--	--	--	0.022	--	--	--	--	0.069	0.898	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.242	--	--
2098	343 323 282	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.013	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Polish Lowland Sheepdog (n=102)	German Shepherd (n=41)	Giant Schnauzer (n=332)	Havana Silk (n=53)	Havanese (n=1024)	Italian Greyhound (n=1444)	Irish Setter (n=60)	Irish Red and White Setter (n=109)	Irish Wolfhound (n=78)	Lakeland Terrier (n=141)	Labrador Retriever (n=300)	Llewellin Setter (n=152)	Magyar Agar (n=78)	Newfoundland (n=139)	Toy Poodle (n=234)	Miniature Poodle (n=424)	Poodle (n=5291)	Rat Terrier (n=44)	Samoyed (n=193)	Saint Bernard (n=90)	Scottish Collie (n=125)	Shiba Inu (n=164)	Shikoku (n=115)	Greater Swiss Mountain Dog (n=59)	Shiloh Shepherd (n=76)	Shiloh Shepherd, ISSA (n=277)	Swedish Vallhund (n=539)	Cardigan Welsh Corgi (n=43)
2014	339 322 284	0.689	--	0.005	--	0.0039	0.0014	--	--	--	0.007	0.002	--	--	0.349	0.021	0.027	0.01966	0.02	--	0.117	--	--	--	--	--	--	--	--	--
2017	343 322 280	0.296	0.43	0.002	--	0.0107	0.2109	--	--	--	0.028	--	--	0.449	--	0.002	0.001	0.00208	0.02	--	--	--	--	--	--	0.349	0.381	--	0.19	
2053	343 324 280	0.005	0.28	0.063	0.255	0.0273	--	0.008	--	0.128	--	0.03	--	--	0.104	0.011	0.012	--	--	0.56	0.144	--	--	--	--	0.493	0.336	0.5297	--	
2067	343 322 284	0.005	0.05	--	--	--	0.0003	--	--	--	0.018	--	--	--	--	--	0.006	--	--	--	--	--	0.213	0.009	--	--	--	--	--	--
2098	343 323 282	0.005	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	0.094	--	0.012	--	--	--	--	--	--	--

e. 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, in modern dog breeds, these loci have achieved an equilibrium with other loci across the genome over time due to theoretical random breeding. This assumption can be tested through a standard genetic assessment of each DLA locus (**Table 7**), as well as through an estimate averaged across all loci (**Table 8**).

This study indicates that the STR loci within the DLA region are less heterogeneous than the genome-at-large in Polish Lowland Sheepdogs, as evidenced by comparing observed heterozygosity values (Ho=0.29 for DLA and Ho=0.53 for the genome, **Tables 2 and 8**). A standard genetic assessment of allele frequencies in the 7 STR loci in the DLA region also indicates that more genetic diversity has been lost in the DLA region than in other parts of the genome (**Tables 3 and 7**). The F value for the DLA region was estimated at 0.07 (**Table 8**), and -0.01 for other regions of the genome (**Table 2**). Although this suggests that there is less randomness to the selection of the dogs included in this study in regard to their DLA types than for other measures of relatedness (e.g. COI), the values are still close to zero. Therefore, even though founders of the breed were inadvertently selected for a small number of DLA types, the DLA region is now in genetic equilibrium with other parts of the genome.

Table 7. Standard genetic assessment for Polish Lowland Sheepdogs (n=103) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	3	1.83	0.41	0.45	0.101
DLA I-4ACA	2	1.75	0.39	0.43	0.093
DLA I-4BCT	3	1.02	0.02	0.02	-0.01
DLA1131	3	1.81	0.42	0.45	0.07
5ACA	2	1.75	0.39	0.43	0.093
5ACT	3	1.02	0.02	0.02	-0.01
5BCA	3	1.75	0.38	0.43	0.114

Table 8. Summary of standard genetic assessment for Polish Lowland Sheepdogs (n=103) using 7 STRs in the DLA class I and II regions. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	2.71	1.56	0.29	0.32	0.07
SE	0.17	0.13	0.06	0.07	0.02

f. What does this assessment of genetic diversity tell us about Polish Lowland Sheepdog

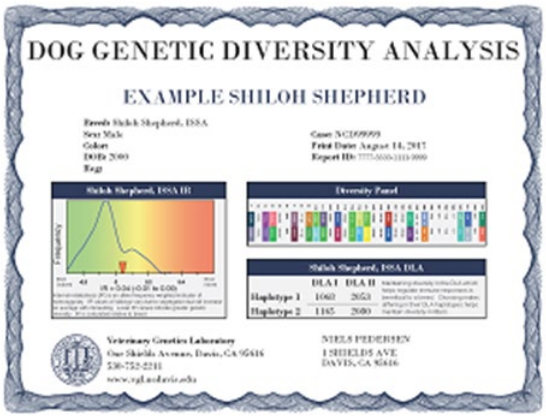
Genetic diversity in Polish Lowland Sheepdogs is among the lowest of all breeds tested to date. Based on allele frequencies, Polish Lowland Sheepdogs have retained only 26% of genetic diversity known to exist in all canids tested at the VGL and 11% of village dogs. Breeds that lack genetic diversity must be managed much more diligently to avoid further loss of genetic diversity. In this case, breeders have less leeway in dealing with simple recessive or complex polygenic disorders found in the breed, due to the low number of alleles segregating in the population.

Despite low genetic diversity, the Polish Lowland Sheepdog appears to be reasonably long-lived and of average health compared to other pure breeds. The question then becomes, is it essential to increase genetic diversity in the breed? The genetic diversity of a breed cannot be increased from within, but only by introgressions from outside the breed. As outcrossing is universally resisted, the only things that can be done is make sure that no additional diversity is lost and to rebalance/redistribute the diversity that is left. 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. DNA testing would allow accurate selection of mates that would have the greatest impact on their puppies, both in the genome-at-large and in the DLA region.

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.

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