

Genetic Diversity Testing for Berger Picard 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 DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions.

Genetic diversity in Berger Picard has been established, and we feel that almost all existing alleles at the 33 STR loci and 7 DLA class I and II regions have been identified. We will continue to add new alleles and haplotypes if they are found, and allele frequencies will be adjusted if necessary. As of September of 2020, 101 Berger Picard from six different countries were used to assess genetic diversity in the breed: USA (88 dogs), Great Britain (6 dogs), The Netherlands (2 dogs), Canada (2 dogs), Denmark (2 dogs), and France (1 dog). Of those, 65 were involved in showing and 18 also participated in performance activities.

Results are described below.

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 Berger Picard

A. Breed history

1. Origin: The origin of the Berger Picard beyond the Picardy region of France is unknown. One possibility is that direct ancestors of the breed were brought to northern France during the second Celtic invasion of Gaul around 400 BC. Sheepdogs resembling Berger Picards have been depicted for centuries in tapestries, engravings and woodcuts. The most accurate portrayal of dogs like the

Berger Picard is from *Livre de chasse* (Book of the Hunt) by Gaston Phoebus, in 1387. The picture, entitled *Chien de Ferme* (farm dogs), portrays dogs closely resembling Picard with large upright ears and J-tails. Another theory is that the Picardy Shepherd did not evolve strictly in the Picardy region of France. Harsh-coated sheep and cattle dogs were typical throughout north Western Europe and some experts believe that the Berger Picard is related to the well-known Briard and Beauceron, which may be related in turn to Dutch and Belgian Shepherds [1-3]. A genetic study reported in 2018 suggested that a common herding dog existed across Europe prior to 1859 and gave rise to the French Berger Picard, 5 Italian herding breeds and the German Shepherd [4]. What is certain is that dogs of similar type were well known in France by the middle of the 19th century. These dogs were of several types, long hair (Berger de Brie or Briard) and short hair (Berger de Beauce or Beaceron). Dogs with a mid-length coat were ignored for some time, but finally became recognized as the Berger de Picardie (or Picard). The Berger Picard made its first appearance as a breed in a French dog show in 1863, and was judged in the same class as Beaucerons and Briards.

2. Recent history [1-3] In spite of some success in dog shows, the breed did not enjoy great popularity outside of farms and was further decimated by the ravages of World War I. Dogs were concentrated on the farms of north-eastern France and the trench warfare in the Somme reduced the original stock to near extinction. However, the breed managed a small resurgence on farms, dog shows and herding trials, being officially recognized by the French Shepherd Club in 1925. World War II brought food rations and made it difficult to feed large size dogs outside of farms and dogs maintained by peasants were not usually registered. After WWII, breeders of the Bouvier des Flanders sought to rebuild their breed by searching Picardy for typical subjects for breeding. Registration records showed that Radjah de la Bohème was bred to Wax de la Bohème in early 1950 to produce a fawn male (Yucca des Hauts-Chesnaux) and a brindle female (Yasmina des Hauts-Chesnaux). These two dogs became important founders of the restored Berger Picard breed. The Club Les Amis du Berger Picard was officially recognized in 1959 and a new breed standard for the Berger Picard was approved by the Société Centrale Canine in 1964. The Les Amis du Berger Picard now has over 250 members with breed clubs in Switzerland, Belgium, the Netherlands, and Germany. Picard breeders can also be found in Austria, Denmark, Sweden, Norway, Italy, Great Britain, the Czech Republic, Canada and the United States. The breed was accepted by the Fédération Cynologique Internationale (FCI) in 1995, and the present FCI standard was drawn up in 2008. The Berger Picard Club of America was formed in 2006 to help promote and protect this breed [2], and it was fully recognized in the herding group by the American Kennel Club in 2015 [3]. It is currently ranked 144 of 196 breeds in popularity among the AKC registries [3]. The Berger Picard is also recognized by the Canadian Kennel Club in the Herding Group. The interim breed standard for the Picardy Sheepdog was approved, and the breed was accepted to the import register of the United Kennel Club in 2014. There are currently around 3500 Berger Picard in France, 500 in Germany, and 400 in the United States and Canada.

The Berger Picard, like breeds such as the Collie, German Shepherd and Dalmatian, has gained recent fame and an associated boost in popularity through modern movies. The expressive look of the Berger Picard has led them to starring roles in three major movies, *Daniel and the Superdogs* (2004), *Because of Winn-Dixie* (2005), and *Are We Done Yet?* (2007) [1].

B. Appearance [1-3]

The Berger Picard is a medium-sized, well-muscled dog, with large erect ears, tousled weather resistant mid-length coat, long tail and thick eyebrows, and an expressive face. Males are 23-½ to 25-½ inches (60-65 cm) at the withers, females 21-½ to 23-½ inches (55-60 cm), and weight 50-

70 lb. (23-32 kg) [2]. Five key points of the breed include: 1). athletically built dog of rustic appearance; 2). efficient gait with effortless, fluid movement; 3) head with ears held naturally erect, possessing an intelligent and alert expression; 4). crisp, harsh outer coat; 5) long tail, reaching the hock, ending with a J shaped hook [1, 2].

The coat of the Berger Picard goes through at least 4 stages from puppyhood to adulthood [2]. The puppy coat is fine and soft with dark markings on head and neck. It does not mat and requires little brushing. The juvenile coat is thick and luscious, the dark head and neck markings remain, and long unruly hair (griffonage) on face is starting to grow. The adolescent coat around 12 months to 2 years has a wiry texture. There may be dark undertone appearing in the fawns. Eyebrows and beard fully grown. The adult coat is thick with a crunchy texture, while the undercoat is fine and dense. The topcoat is somewhat stiff and harsh with slight clumping when not brushed out. The correct length of coat is around 3 inches. If not brushed regularly, the coat can become too long and unkempt. Aged Berger Picard often develop graying of the muzzle and eyebrows.

The Picard coat comes in two basic colors, fawn and gray (a darker mixture of browns, grays, silver and black) [2]. The dark multicolored gray is referred to as brindle. A dark fawn (fawn with black or gray points on topcoat and grayish undercoat) is the most popular color, while the paler or true fawn has lost popularity.

C. Temperament [1-3]

Berger Picard tend to be laid back and mellow but can be reserved towards strangers. They are energetic and intelligent with a sensitive and assertive disposition that responds quickly to obedience training. Although energetic, they are not excessive barkers. Berger Picard are playful and mischievous, making them endearing companions. However, like many herding breeds, they require considerable human companionship. Their enthusiasm towards other people and animals can require obedience training and plenty of positive socialization during the first two years of life.

II. Genetic diversity studies of contemporary Berger Picard

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

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 [10, 11]. Each STR locus is made up of 7 to 27 different alleles (avg. 15.4 alleles/locus) when tested across many breeds of dogs. 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 include such things 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 101 Berger Picard individuals are listed in Table 1.

Table 1. Alleles and their frequencies for 33 STR markers in Berger Picard (n=101). Alleles found in $\geq 90\%$ of dogs are bolded.

AHT121	AHT137	AHTH130	AHTH171-A	AHTH260	AHTk211
96 (0.064)	131 (0.030)	121 (0.163)	219 (1.000)	242 (0.579)	91 (0.307)
98 (0.426)	137 (0.084)	123 (0.173)		244 (0.233)	95 (0.139)
100 (0.401)	147 (0.703)	127 (0.005)		248 (0.188)	97 (0.554)
102 (0.109)	149 (0.183)	131 (0.441)			

137 (0.218)

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
284 (0.762)	116 (0.252)	132 (0.074)	156 (0.035)	236 (0.475)	95 (0.297)
286 (0.020)	118 (0.723)	144 (0.079)	164 (0.084)	238 (0.421)	97 (0.262)
288 (0.064)	124 (0.025)	148 (0.079)	168 (0.010)	240 (0.104)	101 (0.441)
292 (0.153)		152 (0.767)	172 (0.376)		
			176 (0.490)		
			180 (0.005)		

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.292)	144 (0.020)	210 (0.960)	85 (0.540)	233 (0.842)	200 (0.030)
122 (0.495)	146 (0.109)	214 (0.010)	95 (0.460)	241 (0.158)	206 (0.752)
126 (0.213)	150 (0.871)	220 (0.030)			208 (0.218)

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
212 (0.025)	164 (0.158)	268 (0.851)	222 (0.005)	147 (0.069)	13 (0.015)
214 (0.144)	168 (0.366)	270 (0.025)	226 (0.020)	151 (0.005)	14 (0.005)
218 (0.005)	170 (0.475)	272 (0.005)	232 (0.678)	153 (0.030)	15 (0.089)
220 (0.827)		274 (0.119)	234 (0.297)	155 (0.896)	16 (0.005)
					20.2 (0.005)
					21.2 (0.668)
					22.2 (0.198)
					23.2 (0.015)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
16.1 (0.005)	8 (0.485)	16 (0.020)	18 (0.050)	9 (0.292)	15 (0.015)
17.1 (0.490)	10 (0.054)	17 (0.366)	19 (0.950)	11 (0.540)	17 (0.178)
18.1 (0.054)	14 (0.020)	18 (0.005)		13 (0.168)	18 (0.678)
19.1 (0.030)	18 (0.030)	20 (0.470)			18.1 (0.010)
20.1 (0.010)	19 (0.317)	26 (0.020)			19 (0.119)
21.1 (0.411)	20 (0.094)	27 (0.035)			
		28 (0.084)			

VGL2918	VGL3008	VGL3235
13 (0.158)	14 (0.074)	14 (0.955)
14 (0.139)	15 (0.594)	16 (0.045)
15 (0.005)	18 (0.218)	
17.3 (0.693)	19 (0.109)	
18.3 (0.005)	20 (0.005)	

The most striking finding was the low number of alleles (1-6) found at most loci (except for VGL0760 and VGL1165). Of particular interest is AHTh171-A, where all 101 dogs tested shared

the same allele (**Table 1**). For three other loci (INU055, VGL1828, and VGL3235), a single allele also occurred in >90% of dogs tested (**Table 1**). These four alleles are most likely associated with regions of the genome involving traits critical for maintaining the breed standard. Additional alleles may be discovered within the breed 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); 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. F values close to 0 indicate that all dogs tested have completely different alleles at each of the 33 loci, whereas F values close to 1 indicate that every individual would be genetically indistinguishable at each of the 33 STR loci.

Table 2. Standard Genetic Assessment of a population consisting of 101 Berger Picard based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	3.85	2	0.44	0.44	0.005
SE	0.27	0.11	0.04	0.03	0.014

The average number of alleles (Na) identified in this group of 101 Berger Picard represented 25% of alleles known to exist at each of these loci in all canids tested at the VGL (3.85 out of 15.4). This is the lowest amount of retained canid genetic diversity observed in any breed to date. It is even lower than the retained canid-wide genetic diversity of the Swedish Vallhund (31.9%), Irish Red and White Setter (34.8%) and Flat Coated Retriever (38.6%). It is also less than half of the retained canid genetic diversity of the most genetically diverse breeds such as the Golden Retriever (54.5%), Toy Poodle (55.6%) and Standard Poodle (58%).

The 101 Berger Picard had an average of 3.85 alleles per locus (Na), while the Ne in this group of dogs averaged 2 alleles per locus. This means that 52% of the alleles (2 out of 3.85) found in this cohort accounted for most of the existing heterogeneity (heterogeneity = genotypic variation = phenotypic variation). This is typical for most pure breeds of dogs.

The observed (actual) heterozygosity of this group of 101 dogs was 0.44, which was the same value as the expected heterozygosity (He) calculated for a population in Hardy-Weinberg equilibrium (HWE). This yielded a coefficient of inbreeding (F) of 0.005, thus indicating a 0.5% excess in population-wide homozygosity over the expected for a random breeding population. Therefore, it appears that breeders have done a good job in maintaining HWE by selecting lesser related parents from the existing population.

C. Standard genetic assessment values for individual STR loci

The allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each of the 33 autosomal STR loci (**Table 3**). This provides an estimate of genetic similarities in the specific regions of the genome that are associated with each STR marker. Phenotypic differences equate to genotypic differences. Therefore, alleles that are widely shared across the population are indicators that positive selection is occurring for certain desired traits. The number of alleles (Na) found in individual STR loci for this cohort ranged from 1 to 8 alleles per locus, while the Ne ranged from 1.0 to 3.352 alleles per locus. The observed heterozygosity (Ho) for an

individual STR locus ranged from 0 to 0.792, while He ranged from 0 to 0.702 (**Table 3**). Loci with the lowest Ho and He values contributed the least to heterozygosity and are possibly involved with traits that are most important in maintaining standard breed characteristics. Conversely, loci with high Ho and He values are more genetically variable and thus associated with phenotypic variation within the breed.

Of the 33 loci, 17 had values of $F \geq 0.00$, whereas 16 had negative F values. The loci with positive F values were under greater positive selection and therefore within regions of the genome that tend to be associated with desired breed-specific traits. However, the influences of these various inbred, neutral and outbred regions of the genome defined by these 33 STR loci have been kept in good balance by Berger Picard breeders as evidenced by the nearly zero average F value for the population as a whole (**Table 2**).

Table 3. Standard Genetic Assessment of individual STR loci for 101 Berger Picard

Locus	N	Na	Ne	Ho	He	F
AHT121	101	4	2.79	0.66	0.64	-0.03
AHT137	101	4	1.87	0.46	0.46	0.019
AHTH130	101	5	3.35	0.79	0.7	-0.13
AHTh171-A	101	1	1	0	0	0
AHTh260	101	3	2.35	0.49	0.58	0.156
AHTk211	101	3	2.38	0.6	0.58	-0.04
AHTk253	101	4	1.64	0.33	0.39	0.164
C22.279	101	3	1.7	0.42	0.41	-0.01
FH2001	101	4	1.65	0.4	0.39	-0.01
FH2054	101	6	2.56	0.6	0.61	0.01
FH2848	101	3	2.42	0.64	0.59	-0.1
INRA21	101	3	2.85	0.61	0.65	0.054
INU005	101	3	2.66	0.6	0.62	0.033
INU030	101	3	1.3	0.26	0.23	-0.13
INU055	101	3	1.08	0.08	0.08	-0.03
LEI004	101	2	1.99	0.41	0.5	0.183
REN105L03	101	2	1.36	0.26	0.27	0.035
REN162C04	101	3	1.63	0.38	0.39	0.024
REN169D01	101	4	1.42	0.29	0.3	0.028
REN169O18	101	3	2.6	0.61	0.62	0.002
REN247M23	101	4	1.35	0.24	0.26	0.087
REN54P11	101	4	1.82	0.47	0.45	-0.03
REN64E19	101	4	1.24	0.21	0.19	-0.09
VGL0760	101	8	2.02	0.47	0.51	0.08
VGL0910	101	6	2.42	0.61	0.59	-0.05
VGL1063	101	6	2.87	0.72	0.65	-0.11
VGL1165	101	7	2.74	0.62	0.64	0.019
VGL1828	101	2	1.1	0.08	0.09	0.158
VGL2009	101	3	2.47	0.6	0.6	-0.02
VGL2409	101	5	1.98	0.47	0.49	0.058

VGL2918	101	5	1.91	0.52	0.48	-0.08
VGL3008	101	5	2.39	0.61	0.58	-0.05
VGL3235	101	2	1.09	0.09	0.09	-0.05

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 more closely individuals cluster together around the XY axis, the more related they are to each other.

The 101 Berger Picard formed a single population (i.e., a breed) in the PCoA (**Figure 1**). Individual dogs in the group were reasonably dispersed across all four quadrants of the graph, with the exception of three groups that graphed together and are therefore more closely related than the others (red circles). Several other individuals appeared as outliers from the main population, especially on the right side of the PCoA. Therefore, it can be assumed that this group of 101 dogs (except for these three groups) were as unrelated as possible given the low amount of existing genetic diversity in the cohort.

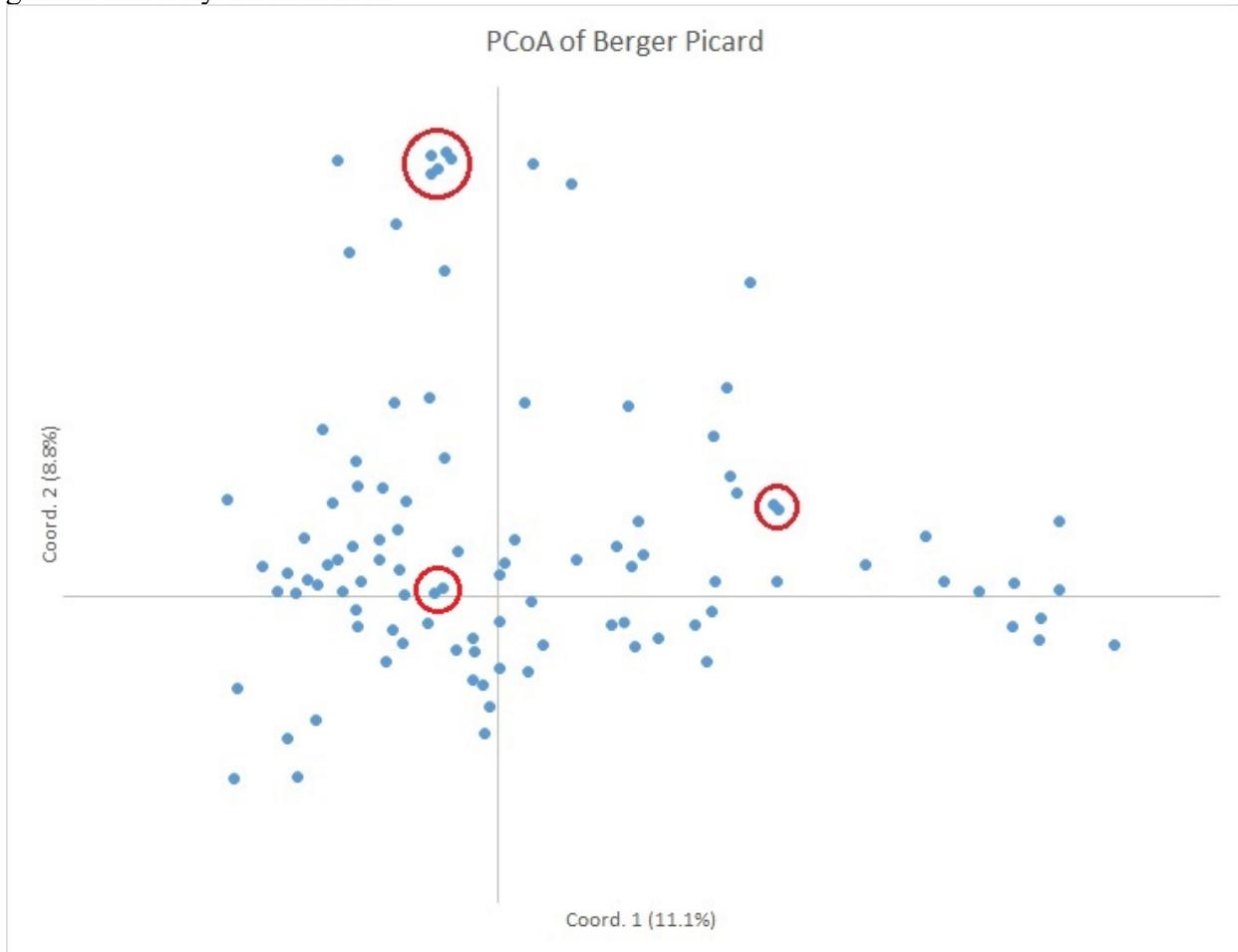


Figure 1. PCoA of Berger Picard (n=101) based on alleles and allele frequencies at 33 autosomal STR loci. Three closely related groups of dogs are circled.

The degree of relatedness of individuals within a breed can be further emphasized by comparing the 101 Berger Picard with genetically distinct breeds, such as the Italian Greyhound (**Figure 2**). This comparison shows the two breeds to be genetically distinct, as would be expected. However, this type of comparison also accentuates the degree of relatedness of individuals within a breed by grouping them more closely around the XY axis. The greater genetic diversity of the Italian Greyhounds is also apparent, based on the wider dispersal of individual Italian Greyhounds across the graph.

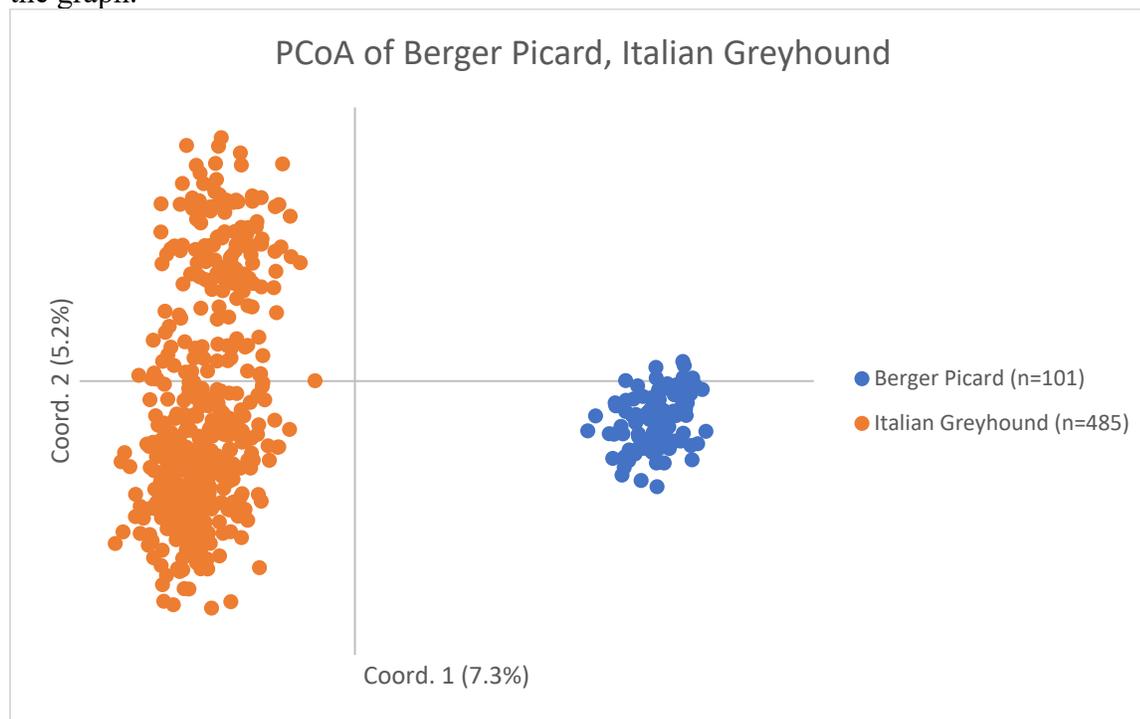


Figure 2. PCoA of Berger Picard (n=101) and Italian Greyhound (n=485, selected at random) based on 33 autosomal STR loci.

D. 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 (mean/average) heterozygosity, and do not reflect the genetic diversity given to individuals by their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which the two parents of an individual dog are related. The IR calculation takes into consideration homozygosity at each locus and gives more importance to rare and uncommon alleles. Rare and uncommon alleles would presumably be present in less related individuals. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. 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 101 Berger Picard tested. The most outbred dog in the population had an IR score of -0.52, while the most inbred dog in the group had an IR score of

0.39. The mean IR score for the cohort was 0.0053. Therefore, this group appeared to contain small and equal proportions of dogs with parents that were as unrelated (most outbred) or related as possible (most inbred) given the genetic makeup of the population. The existence of both highly inbred and outbred individuals is a typical finding for almost all pure breeds of dogs.

Table 4. Internal relatedness (IR) values calculated using allele numbers and frequencies in 101 Berger Picard. 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
Min	-0.5200	0.1586
1st Qu	-0.1071	0.3678
Mean	0.0053	0.4813
Median	0.0124	0.4742
3rd Qu	0.1280	0.5763
Max	0.3937	0.7917

2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed evolution from time of origin to the present time.

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 Island Pacific region 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). Therefore, the IRVD score approximates how a Berger Picard's IR score would compare to other village dogs if its parents were also village dogs.

The IR values listed in Table 4 are most easily studied in a graph form (**Figure 3**). The IRVD scores for the 101 Berger Picard (blue line) is shifted to the right of their IR scores. Almost all 101 Berger Picard from this cohort have IRVD values of 0.25 or greater (**Table 4, Figure 3**), which means that if they were found among village dogs, they would all be judged offspring of at least full sibling parents. The darkened area in **Figure 3** represents the overlap between IR and IRVD curves (11.7%) and is an estimate of the amount of genetic diversity in present-day randomly breeding village dogs that still exists in contemporary Berger Picard. This amount of genetic diversity is less than one half of the retained genetic diversity (25%) found in the comparison with all canids tested at the VGL to date (section IIB).

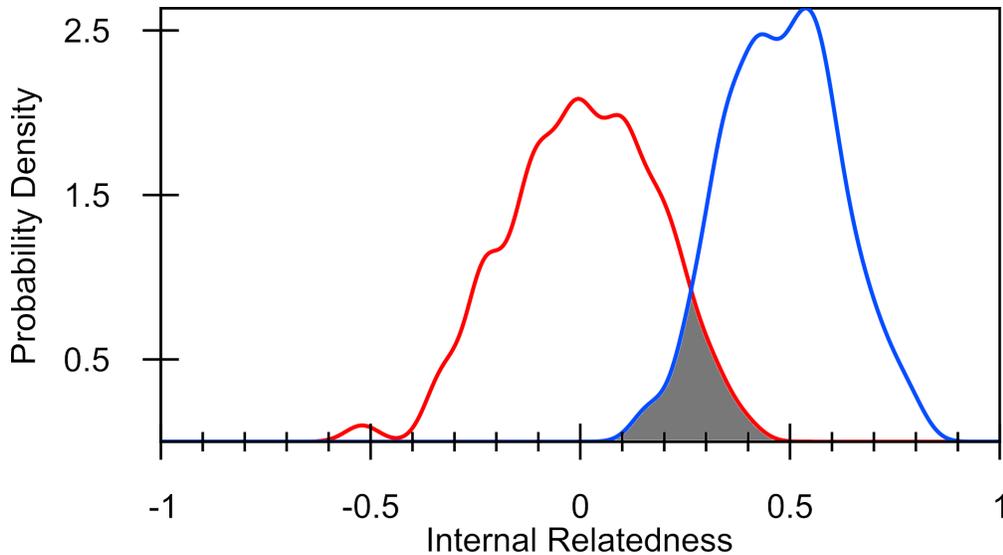


Fig. 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Berger Picard ($n=101$). The overlap between the curves (gray) represents the degree of allele sharing (11.7%) between Berger Picard and village dogs.

Regardless of reference populations, it is apparent that Berger Picard have retained only a small amount of available canid genetic diversity. The first and greatest loss of diversity probably occurred when founders were selected and after the registry was closed, either in 1863 or from breed restoration after WWII. Further loss of diversity may have occurred from a range of artificial genetic bottlenecks such as geographic isolation, natural and man-made catastrophes, breed refinement, popular sire and dam effects, change in interpretation of breed standard, etc. All these types of genetic bottlenecks have been documented at one time or another in the history of the Berger Picard.

E. 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**). Groups of genes (and consequently their alleles) inherited as a block (rather than singly) are called haplotypes.

The class II region also contains several genes, three of which are highly polymorphic: DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at 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 Berger Picard

Only two DLA class I and two DLA class II haplotypes were identified in the 101 Berger Picard (Table 5). DLAI haplotype 1227 and DLAII haplotype 2067 were both identified in 87% of the dogs tested, and the other two haplotypes occurred at a frequency of 13%. One of the class I haplotypes (1227) was unique to the breed, whereas the three remaining DLA haplotypes were shared with several other breeds (Table 6).

The number of DLA class I and II haplotypes (2 and 2) found in these 101 Berger Picard was the lowest found in a dog breed studied to date. It was even lower than breeds with limited genetic diversity such as the Swedish Vallhund (6 and 4, respectively), Shiloh shepherd (7 and 6), Giant Schnauzer (14 and 15), Samoyed (13 and 12) and Shiba Inu (16 and 15). This discrepancy becomes more evident when the number of DLA haplotypes found in Berger Picard is compared to more genetically diverse breeds such as the Golden Retriever (26 and 23) and Miniature Poodle (33 and 23). If these 101 dogs are representative of the breed, it would suggest that a single founder or individuals belonging to a closely related bloodline played a dominant role in breed creation.

Table 5. DLA class I and Class II haplotypes identified in Berger Picard and their respective frequencies (n=101).

DLA1 haplotype	STR alleles	Berger Picard
1052	380 372 289 184	0.129
1227	380 372 289 186	0.871

DLA2 haplotype	STR alleles	Berger Picard
2017	343 322 280	0.129
2067	343 322 284	0.871

2. DLA haplotype sharing with other dog breeds

DLA haplotypes are more conserved than other regions of the genome, and are inherited as blocks of linked genes, one from each parent. Recombination within and between these blocks of genes tends to be low, allowing them to remain mostly unchanged over the generations. Therefore, the DLA haplotypes found in a breed can be used to investigate the founder/founder lines that were used to create a breed, as well as to assess the impact of various founders in subsequent breed evolution.

DLAI haplotype number 1227 is unique to Berger Picard among all breeds tested by the VGL, thus reflecting a unique founder line (Table 6). In comparison, the 1052 DLAI haplotype is found in several different breeds tested by the VGL, most commonly in the Shiloh Shepherd and Irish Wolfhound. The 2017 and 2067 DLAII haplotypes are found in several breeds. The 2017 haplotype is particularly common in the Polish Lowland Sheepdog, Shiloh Shepherd, and Magyar Agar, whereas the 2067 haplotype is common in the Shiba Inu.

Given that DLA haplotypes 1227 and 2067 are found at the same frequency (87%) in the Berger Picard cohort, it appears that they are in strong linkage disequilibrium (LD), thus forming a unique (and possibly breed-specific) extended haplotype. Similarly, since haplotypes 1052 and 2017 are found at the same frequency (13%), they are likewise in LD in Berger Picard. DLA haplotype linkage is also found in the Lakeland Terrier, Shiloh Shepherd, and Border Collie (Table 6).

Table 6. Sharing of specific DLA class I and II haplotypes between Berger Picard (highlighted in blue) and various breeds.

DLA Class I Haplotype Frequencies																										
DLA1 #	STR types	Rat Terrier (n=37)	Lakeland Terrier (n=101)	Doberman Pinscher (n=768)	Flat Coated Retriever (n=599)	Havanese (n=546)	Shiba Inu (n=118)	Polish Lowland Sheepdog (n=26)	Borzoi (n=130)	English Bulldog (n=163)	Whippet (n=66)	Italian Greyhound (n=1001)	Shiloh Shepherd, ISSA (n=218)	Magyar Agar (n=77)	English Mastiff (n=22)	American Akita (n=142)	Japanese Akita (n=432)	Blend Akita (n=59)	Berger Picard (n=101)	Golden Retriever (n=737)	Irish Wolfhound (n=55)	Border Collie (n=54)	Miniature Poodle (n=307)	Barbet (n=68)	Poodle (n=3310)	Toy Poodle (n=167)
1052	380 372 289 184	--	0.015	0.0013	--	0.0055	--	--	--	--	--	0.1883	0.362	0.052	--	--	--	--	0.129	--	0.355	0.009	--	--	--	--
1227	380 372 289 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.871	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies																										
DLA2 #	STR types	Rat Terrier (n=37)	Lakeland Terrier (n=101)	Doberman Pinscher (n=768)	Flat Coated Retriever (n=599)	Havanese (n=546)	Shiba Inu (n=118)	Polish Lowland Sheepdog (n=26)	Borzoi (n=130)	English Bulldog (n=163)	Whippet (n=66)	Italian Greyhound (n=1001)	Shiloh Shepherd, ISSA (n=218)	Magyar Agar (n=77)	English Mastiff (n=22)	American Akita (n=142)	Japanese Akita (n=432)	Blend Akita (n=59)	Berger Picard (n=101)	Golden Retriever (n=737)	Irish Wolfhound (n=55)	Border Collie (n=54)	Miniature Poodle (n=307)	Barbet (n=68)	Poodle (n=3310)	Toy Poodle (n=167)
2017	343 322 280	0.03	0.035	--	0.0008	0.0064	--	0.33	0.008	0.215	0.25	0.2178	0.362	0.455	0.18	0.011	0.005	0.017	0.129	0.0407	--	0.204	--	0.029	0.0024	0.003
2067	343 322 284	--	0.015	--	--	--	0.216	--	--	--	--	0.0005	--	--	--	--	--	0.017	0.871	--	--	0.009	0.008	--	--	--

3. 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 LD 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 through random mating and/or over time. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and across all loci (**Table 8**). The distribution of alleles at the seven loci in the DLA class I and II regions in Berger Picard is unique for any breed tested at the VGL to date. Three of four class I and two of three class II loci were homozygous for a single allele, while only two alleles were identified for the remaining two loci (**Table 7**). Therefore, heterozygosity (H_o and H_e) and inbreeding coefficients (F) could not be accurately determined for these loci. Although in theory it is possible that the 101 individuals tested herein did not constitute a random representation of the breed, this is highly unlikely given the pattern of diversity demonstrated by standard genetic assessment and PCoA results for the 33 autosomal STRs. The more likely explanation is that the breed was restored from a founder line containing these alleles which existed prior to modern restoration.

Table 7. Standard genetic assessment for Berger Picard using each of the 7 STRs in the DLA region.

Locus	N	Na	Ne	Ho	He	F
DLA I-3CCA	101	1	1	0	0	0
DLA I-4ACA	101	1	1	0	0	0
DLA I-4BCT	101	1	1	0	0	0
DLA1131	101	2	1.29	0.16	0.22	0.29
5ACA	101	1	1	0	0	0
5ACT	101	1	1	0	0	0
5BCA	101	2	1.29	0.16	0.22	0.29

Table 8. Summary of standard genetic assessment for Berger Picard using 7 STRs in the DLA region.

	Na	Ne	Ho	He	F
Mean	1.29	1.083	0.045	0.064	0.084
SE	0.17	0.049	0.027	0.038	0.05

III. What does this assessment of genetic diversity tell us about contemporary Berger Picard

This study confirmed that the cohort tested constituted a single breed based on alleles and allele frequencies for the 33 autosomal STR loci. However, the estimated amount of retained genetic

diversity for Berger Picard (11.7%) corresponded to approximately half of that of other breeds tested by the VGL (25%). Although this was the lowest diversity of any breed studied to date, breeders have managed the remaining diversity in the best possible manner: standard genetic assessment for heterozygosity and PCoA graphing indicate that the average dog in the population is a product of parents that are as unrelated as possible. However, internal relatedness (IR) scores also show that average values can be misleading, as some individuals were much more inbred or outbred than the breed average.

The most striking finding was the almost total loss of genetic diversity in the DLA regions of the genome. This region is in relatively high linkage, and the two extended haplotypes tend to be inherited by descent, with limited recombination, from each parent. The most likely cause of this pattern of lost DLA diversity is found in the breed history. Registration records showed that Radjah de la Bohème was bred to Wax de la Bohème in early 1950 to produce a fawn male (Yucca des Hauts-Chesnaux) and a brindle female (Yasmina des Hauts-Chesnaux). These two dogs reportedly became important founders of the restored Berger Picard breed [1]. A recent study indicated that the Berger Picard is also closely related to several older Italian herding breeds [6]. Therefore, it can be hypothesized that modern Berger Picard either 1) represent a remnant of a line of dogs that existed prior to the restoration and that shared a unique DLA type resulting from earlier genetic bottlenecks, or 2) result from a very small number of founders that were extensively backcrossed to a specific subpopulation of these founders.

A lack of genetic diversity is not inherently bad, providing the founder population was relatively free of deleterious genetic traits and breeders have been judicious in avoiding a loss or imbalance in the original diversity. This is corroborated by the fact that the breed is surprisingly clear of simple breed-specific heritable disease traits, and enjoys a reasonable lifespan compared to other large dog breeds. The health problems that exist are of a complex genetic basis and are common to many dog breeds and even mongrels [7]. These traits were most likely inherited from generation to generation as dogs underwent intense human-directed artificial selection.

The greatest problems with low genetic diversity often arise when a breed becomes popular as pets, for shows, or both [9-11]. Popularity creates a rapid demand for more dogs, which in turn leads to less attention to proper breeding management practices, and in particular overuse of certain sires. Likewise, showing can often lead to changes in interpretation of breed standards and bouts of inbreeding to modify the desired trait [9]. Such changes often involve popular sires, but also popular dams and related bloodlines. If the gene pool (i.e., number of individuals used for breeding) is small, spontaneous mutations that occur in areas of high selection pressures in the genome can be inadvertently swept up and propagated throughout the breed [9-11].

The extreme lack of genetic diversity in the DLA class I and II regions identified in the Berger Picard cohort in this study is concerning, but its biological meaning for the genetic health of the breed is uncertain. Certain DLA class I and II haplotypes have been associated with specific autoimmune diseases in certain breeds [8], but autoimmune disorders are not seen as a problem in Berger Picard. Therefore, the strongly shared DLA region of Berger Picard does not appear to have a strong negative effect on self/non-self-recognition. Nevertheless, it is important that breeders maintain as much diversity and heterozygosity in the DLA region as possible.

Breeds that lack genetic diversity must be managed much more closely to avoid further loss of diversity, which in turn leads to less leeway to deal with simple recessive or complex polygenic disorders that might arise [10, 11]. Disease mutations are usually autosomal recessive in nature, and in some cases occur in linkage with a region of the genome under strong positive selection for a phenotype that is deemed desirable in the show ring [9-11]. Elimination of such deleterious

mutations through selective breeding may result in loss of genetic diversity, especially when diversity is already limited when compared to other breeds as is the case of Berger Picard [9-11].

IV. Health of the Berger Picard

A. Lifespan

The breed is reasonably long-lived, and mortality from 6 months to five years of age tends to be low [5]. Mortality rises at 6 years of age and peaks around 13 years, with some dogs living 15+ years [5]. The AKC lists the average life expectancy as 12-13 years [2]. Berger Picards are relatively healthy and over 75% of owners reported no significant health problems [5].

B. Disease problems of heritable nature

1. Orthopedic problems. Only 9% of owners reported orthopedic problems to the OFA [5]. Eighty percent of Berger Picard registered by the OFA were reported to have normal hips on testing, and 90% had normal elbows [3]. However, clinical signs of hip and elbow dysplasia were reported in only 3.1% and 0.3% of dogs, respectively. Anterior cruciate ligament ruptures were reported in 3.4% of dogs.

2. Eye disorders. Eight percent of Berger Picard reported to the OFA had an eye disorder of some type and only 2% manifested retinal dysplasia or retinal atrophy [5]. Progressive retinal atrophy (PRA) is present in the breed and may occur in at least 2 different forms [12]: 1) early onset, with clinical signs beginning to appear at about 3-4 years of age, and 2) late onset form with clinical signs appearing at 8-9 years of age [2]. Berger Picard do not have any of the common mutations for PRA that occur in other breeds. Canine multifocal retinopathy (CMR) has been recognized in the breed [13], but it does not progress to complete blindness as it does in breeds such as Great Pyrenees or Coton de Tulear. A causative mutation for CMR has not been identified in the Berger Picard as in other breeds, but a search is underway [14]. Other eye disorders seen in Berger Picard include punctate and suture line cataracts or opacities; distichiasis, persistent pupillary membranes; ectropion, retinal dysplasia and eye infections (conjunctivitis) [12, 13].

3. Cancer. Cancer occurred in 9.3% of Berger Picard reported to the OFA [5]. This appears lower than the 27% average for all pure breeds of dogs and more in the range of small breeds such as the Shetland Sheepdog and Yorkshire and Jack Russell Terriers [6]. The most common forms were hemangiosarcoma, squamous cell carcinoma, lymphoma, gastric and pancreatic carcinoma, and testicular cancer [5].

4. Allergies and autoimmune diseases. Allergies (intestinal, skin) and autoimmune disorders occur in Berger Picard at relatively low frequency (7% and 1.4%, respectively) [5]. Symmetrical lupoid onychodystrophy. (SLO) of the nails is a specific form of autoimmune disease seen in Berger Picard and several unrelated breeds and associated with specific DLA class II (DRB1, DQA1, DQB1) haplotypes [15].

5. Gastrointestinal disorders. Vague gastrointestinal issues occur with greater than expected frequency and manifested by inappetence, difficulty in maintaining weight and soft stools.

6. Reproductive issues [2] - The Berger Picard breed is known to be difficult to breed, which may be one of the reasons that it continues to be a rare breed even in its country of origin. Breeders through the years and around the world have reported that some Berger Picard simply refuse to breed. Most of the attention has been focused on the females, who may have either chemical or mechanical issues. Mechanical issues associated with the female Picard is what the French call “Vulve Barree” which translates into a locked vulva or recessed vulva. In the more severe cases, the fur at the tip of the vulva looks as if it was coming right out of the lower part of the belly and looks like a very juvenile vulva. In the case of a completely locked vulva, surgical correction is

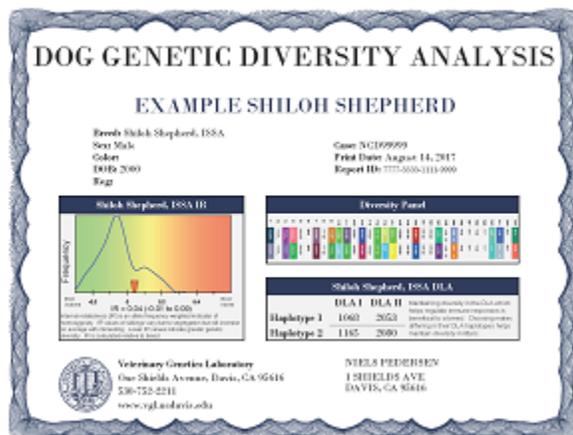
required. A second mechanical problem is with vaginal malformations or altered anatomical structures such as imperforate hymen (where the hymen is solid) or dorsoventral septum (where the vagina has a vertical dividing membrane or wall). This congenital issue has been noted by the French club since the 1990's. Vaginal strictures are not visible, which is a good reason for a pre-breeding exam. The bitch can have one or many strictures in the form of fibrous bands at the vulva-vestibule junction or in the vagina.

Uterine inertia is another reproductive problem of the breed and is either primary or secondary. In primary inertia the cervix may dilate but the uterus never contracts, and the puppies eventually die in utero. If the inertia is not recognized and treated, sepsis can set in and can compromise the ability to breed and, in a worst-case scenario, the health of the mother. Secondary uterine inertia describes when the bitch has gone into labor, delivers one or more pups, and then stops contracting.

V. Results of 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 use of DNA for testing genetic diversity in the Berger Picard has confirmed that the breed lacks genome-wide genetic diversity as well as in the DLA region, most likely resulting from a small number of related founders or founder lines used to create the breed. This circumstance is surprisingly like that of the Cheetah, a species made up of individuals that share 95% of their genomes and almost all the MHC class II genes [16]. This sharing was a result of two distinct artificial bottlenecks that occurred in ancestors of the modern Cheetah >100,000 and 11,084-

12,589 years ago (as opposed to tens or hundreds of years ago as in the Berger Picard). Nonetheless, this bottleneck has not doomed either the Cheetah or the Berger Picard. However, the low amount of genetic diversity means that both the Cheetah [13] and the Berger Picard will have to be much more carefully managed than species or breeds with large amounts of genetic diversity (such as the Golden Retriever or the Standard Poodle, for example). Therefore, it is important to closely monitor existing diversity into the future. In the meantime, searches should continue for dogs with the desired breed phenotype but that have different genotypes; this way, the existing gene pool can be increased. We believe that this can be most accurately done with DNA testing as a supplement to in-depth pedigrees. If the breed were to consider increasing genetic diversity by further genetic introgressions, DNA testing of dogs intended for such introgressions would also be essential to preclude deleterious mutations and to assure that the added DNA is properly incorporated into the existing population.

The goal for breeders should be to continue to produce puppies with IR scores lower than zero, and with time, even lower scores. Although most of the individuals tested in this study were randomly bred, small subpopulations of dogs were found to be more inbred or outbred than the cohort at large. This finding can provide breeders with tools to better balance genetic diversity in Berger Picard. 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 common 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, like what is being done by many Standard Poodle breeders. However, because IR values reflect the unique genetics of each individual, 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. However, the extreme lack of genetic diversity in the DLA region of Berger Picard limits the extent of success of this approach, as well as its benefit to the breed.

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.

Puppies should be tested for their IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals but maintaining and/or improving genetic diversity in puppies should be paramount.

Results from this study can also contribute the genetic information from Berger Picard to a web repository. This information could be incorporated into a mate selection online service that will

allow a breeder to identify, among all the dogs tested, potential mates that would be most suitable to increase genetic diversity in their litters.

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