Genetic Diversity Testing for Borzoi

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

Genetic diversity testing of Borzoi is now in the data collection phase. During this phase, we will continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. This report is based on testing of 117 Borzoi. This number of dogs is probably sufficient to do a near-final genetic assessment of the breed. Allele and DLA haplotype frequencies will be updated as more Borzoi are tested. It is anticipated that new alleles at the 33 STR loci and additional DLA class I and II haplotypes will be identified in the future, but these will tend to be of much lower incidence than those detected in the present population.

Results reported as:
Short tandem repeat (STR) loci: A total of 33 STR loci from across the genome were used to gauge genetic diversity within an individual and across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity, and breed-wide allele frequency is provided.

DLA haplotypes: STR loci linked to the DLA class I and II genes were used to identify genetic differences in regions regulating immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with non-genetic factors in the environment, are responsible for autoimmune disease.

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and cannot be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

I. Introduction

A. History

Borzoi are descendants of dogs that originated in central Asian countries and eventually made their way to Russia [1]. Borzoi in Russia are known as Russkaya psavaya borzaya and one of 10 popular breeds of Russian origin [2]. Similar Russian breeds to the Borzoi originated in the steppe (Stepnaya borzaya or Stepnoi) and Crimea (Krimskaya borzaya or Krimskoi). The breed resembles the greyhound and central Asian breeds such as the Afghan hound and Saluki. Borzoi are placed in the hound group by the AKC [3] and the sighthound and Pariah group by the UKC [4, 5].

The history of the Borzoi parallels that of many hunting breeds that were maintained almost exclusively by European aristocracy from the 13th through 19th centuries [3,4]. Hunting with sighthounds was a passion of the Russian nobility during the centuries of Romanov rule in Russia, and ritualized hunts and festivals were frequently held on their vast estates and a special treat for their guests. The Russian revolution led to the downfall of both the aristocracy and their aristocratic hounds and set back Russian breeding of Borzoi for much of the 20th century. Fortunately, dog fanciers in Europe, England and America had begun the importation of Borzois to their shores prior to the revolution, thus preventing the breed's extinction. In most of the English-speaking world, the breed name was originally Russian Wolfhound. This changed starting in America in 1936 when the breed name became Borzoi (Russian borzyi or swift).

Borzoi ranks 103/193 in popularity in the AKC registries [3]. Although slightly losing popularity in the US [4], registration numbers in the UK have fallen steadily from 300 per year to less than 100 in the period between 1980 and 2015 [6].

B. Appearance

Borzoi are often referred to as long-haired greyhounds and come in a wide range of colors such as black, brindle, cream, red, sable and white [3, 4]. The Borzoi coat is silky, and can be straight, wavy or curly. The long topcoat has varying degrees of waviness or curliness. The soft undercoat thickens during winter or in cold climates but is shed in hot weather to prevent overheating. In its texture and distribution over the body, the Borzoi coat is unique with a frill or mane on the neck and feathering on its front legs, hindquarters and tail.

Borzoi males frequently weigh more than 75-100 lbs. (34-45kg) and stand at least 28 inches (71 cm) at the shoulder, while females are 60-85 lbs. (27-39 kg) and around 26 inches (66 cm) in height [3]. Their body structure is described as streamlined and graceful with a curvy shapeliness but with an appearance of compact strength.

C. Temperament

The Borzoi is viewed by many as one of the most beautiful of all dogs and is cherished for their calm (gentle, quiet), agreeable (respectful, intelligent, quiet) temperament [3-5]. Their behavior is referred to as cat-like, and like the cat, they can be stubborn (independent), and training is best accomplished with patience, consistency and good humor [3]. They are affectionate family dogs but can be reserved with strangers. They do enjoy outdoor runs and energetic play with other sighthounds. The breed still retains some of their ancestral hunting tendency, and they are known to pursue small animals on the run such as cats or squirrels.

II. Genetic diversity studies of contemporary Borzoi

A. Population genetics based on 33 STR loci on 25 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. Each of these STR loci is known to contain from 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 and resulting increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies were determined for 117 Borzois and are listed in Table 1.

Table 1. Allele frequencies for 33 STR markers in Borzoi(n=117)

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
94 (0.145)	131 (0.688)	119 (0.009)	217 (0.543)	242 (0.209)	87 (0.038)
98 (0.098)	135 (0.038)	121 (0.081)	219 (0.286)	244 (0.077)	89 (0.744)
100 (0.226)	143 (0.077)	125 (0.244)	221 (0.034)	246 (0.013)	91 (0.175)
104 (0.068)	147 (0.004)	127 (0.372)	223 (0.026)	248 (0.598)	95 (0.043)
106 (0.380)	149 (0.192)	129 (0.115)	229 (0.038)	252 (0.103)	
108 (0.073)		131 (0.004)	231 (0.017)		
110 (0.004)		133 (0.175)	233 (0.056)		
112 (0.004)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
282 (0.038)	114 (0.064)	132 (0.090)	148 (0.098)	234 (0.034)	95 (0.479)
286 (0.269)	116 (0.389)	136 (0.192)	152 (0.068)	236 (0.483)	97 (0.244)
288 (0.132)	118 (0.158)	140 (0.120)	156 (0.346)	238 (0.402)	99 (0.047)

290 (0.128)	120 (0.030)	144 (0.051)	160 (0.013)	244 (0.081)	101 (0.197)
292 (0.081)	124 (0.009)	148 (0.380)	164 (0.132)		103 (0.034)
294 (0.338)	126 (0.350)	152 (0.145)	168 (0.231)		
296 (0.013)		156 (0.021)	172 (0.064)		
			176 (0.047)		

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.043)	144 (0.179)	210 (0.868)	85 (0.252)	229 (0.274)	192 (0.107)
124 (0.547)	148 (0.017)	214 (0.115)	95 (0.218)	231 (0.077)	194 (0.004)
126 (0.389)	150 (0.688)	220 (0.017)	97 (0.056)	233 (0.209)	202 (0.564)
130 (0.021)	154 (0.115)		107 (0.278)	235 (0.440)	204 (0.274)
			111 (0.167)		206 (0.004)
			113 (0.017)		208 (0.047)
			117 (0.013)		

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.150)	162 (0.376)	266 (0.363)	226 (0.009)	139 (0.265)	12 (0.017)
208 (0.017)	164 (0.316)	268 (0.368)	230 (0.184)	141 (0.145)	14 (0.132)
210 (0.026)	166 (0.038)	270 (0.073)	232 (0.085)	145 (0.415)	15 (0.004)
212 (0.098)	168 (0.038)	272 (0.030)	234 (0.261)	147 (0.175)	16 (0.060)
216 (0.141)	170 (0.231)	274 (0.038)	236 (0.278)		18.2 (0.004)
218 (0.060)		278 (0.128)	238 (0.184)		19 (0.004)
220 (0.376)					19.2 (0.201)
222 (0.132)					20.2 (0.205)
					21.2 (0.205)
					22.2 (0.124)
					23.2 (0.021)
					24.2 (0.021)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
13 (0.038)	8 (0.184)	14 (0.081)	15 (0.038)	10 (0.004)	13 (0.034)
16.1 (0.021)	10 (0.132)	20 (0.013)	16 (0.346)	11 (0.026)	15 (0.128)
17.1 (0.132)	12 (0.051)	21 (0.004)	17 (0.026)	13 (0.551)	16 (0.197)
18.1 (0.017)	14 (0.308)	23 (0.017)	18 (0.009)	14 (0.393)	17 (0.299)
19.1 (0.120)	15 (0.009)	24 (0.026)	19 (0.004)	15 (0.026)	18 (0.291)
20.1 (0.231)	17 (0.013)	25 (0.073)	20 (0.564)		19 (0.009)
21.1 (0.120)	18 (0.278)	26 (0.047)	22 (0.013)		20 (0.038)
22.1 (0.303)	19 (0.017)	27 (0.453)			21 (0.004)
23.1 (0.017)	20 (0.009)	28 (0.274)			
		29 (0.013)			

VGL2918	VGL3008	VGL3235

12 (0.090)	15 (0.085)	13 (0.603)
13 (0.517)	16 (0.167)	14 (0.009)
14 (0.107)	17 (0.124)	15 (0.218)
15 (0.013)	18 (0.274)	16 (0.047)
17.3 (0.004)	19 (0.179)	17 (0.030)
18.3 (0.252)	20 (0.137)	18 (0.021)
19.3 (0.017)	21 (0.034)	19 (0.009)
		20 (0.064)

B. Assessment of population diversity using standard genetic parameters

Allele and allele frequencies at each of the 33 STR loci are listed in Table 1 and used to determine basic genetic parameters (Table 2) such as the number of alleles found at each STR locus (Na); the number of effective alleles (Ne) per locus (i.e., the number of alleles that contribute most to genetic differences); the observed or actual heterozygosity (Ho) that was found; and the heterozygosity that would be expected (He) if the existing population is being randomly bred. The value F is a coefficient of inbreeding derived from the Ho and He values. A value of +1.0 would occur only if every individual were genetically indistinguishable at each of the 33 STR loci, while a value of -1.0 would be seen when all the dogs were completely different at each of the 33 loci. A value of 0.00 would be seen if the selection of sires and dams was entirely random in reference to the existing gene pool.

The allele frequency data obtained from the 33 STR panels can be used to assess heterozygosity within a population (Table 2). Using the 33-marker panel, the 117 Borzois had an average of 6.42 alleles/loci (Na). This is higher than for the Shiloh Shepherd (Na=4.0), Lakeland Terrier (Na=4.24), Swedish Vallhund (Na=4.91) and Irish Red and White Setter (Na=5.09); similar to the Llewellin Setter (Na=5.94) and Flat-coated Retriever (Na=5.94); but lower than large and genetically diverse breeds such as the Labrador Retriever (Na=7.33), Golden Retriever (Na=8.39) and Miniature Poodle (Na=8.91). However, the average number of alleles is less important than the number of alleles that have the greatest genetic influence on heterozygosity, a figure known as average effective alleles/loci or Ne. The Ne in this group of dogs averaged 3.42 effective alleles per locus. The observed (actual) heterozygosity of this group of 117 dogs was 0.62, while the expected heterozygosity (He) for a population in Hardy-Weinberg equilibrium (HWE) was 0.67, yielding a coefficient of inbreeding (F) of 0.064 (i.e., 6.4% more inbred than predicted for HWE). These standard genetic assessment values indicate that the breed has good genetic diversity, which has been sustained in a relatively random manner across the breed's history.

Table 2: Standard genetic assessment of 117 Borzoi based on allele frequencies at 33 genomic STR loci on 25 chromosomes. Values are expressed as means (averages) with one standard error (SE).

	N	Na	Ne	Ho	He	F
Mean	117	6.42	3.42	0.62	0.67	0.064
SE		0.35	0.21	0.02	0.02	0.009

B. Standard genetic assessment values for individual STR loci

The allele frequencies can be also used to do a standard genetic assessment of heterozygosity at each STR locus (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 Na values for an individual STR locus for this population of 117 Borzois ranged from a low of 3 to a high of 10 alleles per locus, while the Ne ranged from 1.31 to 6.16 alleles per locus. It is important to remember that each STR locus can have from 7-27 different alleles (avg. 15.4 alleles/locus) when testing across all dogs. The observed heterozygosity (Ho) for an individual STR locus ranged from 0.23 to 0.87, while He ranged from 0.21 to 0.80 (Table 3). Twenty-five loci had positive F values and 6 were negative. The loci with positive F values were under a greater degree of positive selection than those with negative F values and were presumably areas of the genome more strongly 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 breeders as evidenced by only a slightly positive F value (Table 2).

On average, the alleles identified in this group of 117 dogs represented 6.42/15.4=40.9% of alleles known to exist in all canids tested at the VGL. This is higher than the Swedish Vallhund (31.9%), Flat-coated Retriever (38.6%), Irish Red and White Setter (34.8%); identical to the Magyar Agar (40.4%); but lower than breeds such as the Golden Retriever (54.5%), Toy Poodle (55.6%) and Standard Poodle (58%).

Table 3: Standard genetic assessments of individual STR loci of 117 Borzoi.

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	117	8	4.22	0.78	0.76	-0.019
2	**AHT137**	117	5	1.93	0.43	0.48	0.114
3	**AHTH130**	117	7	4.03	0.69	0.75	0.079
4	**AHTh171-A**	117	7	2.61	0.54	0.62	0.127
5	**AHTh260**	117	5	2.39	0.54	0.58	0.074
6	**AHTk211**	117	4	1.70	0.42	0.41	-0.014
7	**AHTk253**	117	7	4.37	0.71	0.77	0.08

8	**C22.279**	117	6	3.29	0.61	0.70	0.128
9	**FH2001**	117	7	4.38	0.74	0.77	0.037
10	**FH2054**	117	8	4.73	0.80	0.79	-0.008
11	**FH2848**	117	4	2.49	0.56	0.60	0.07
12	**INRA21**	117	5	3.03	0.64	0.67	0.043
13	**INU005**	117	4	2.21	0.47	0.55	0.141
14	**INU030**	117	4	1.93	0.48	0.48	0.004
15	**INU055**	117	3	1.31	0.21	0.23	0.123
16	**LEI004**	117	7	4.56	0.69	0.78	0.113
17	**REN105L03**	117	4	3.14	0.61	0.68	0.11
18	**REN162C04**	117	6	2.46	0.50	0.59	0.15
19	**REN169D01**	117	8	4.64	0.72	0.79	0.085
20	**REN169O18**	117	5	3.36	0.74	0.70	-0.047
21	**REN247M23**	117	6	3.44	0.65	0.71	0.084
22	**REN54P11**	117	6	4.55	0.77	0.78	0.014
23	**REN64E19**	117	4	3.40	0.71	0.71	-0.005
24	**VGL0760**	117	12	6.16	0.75	0.84	0.102
25	**VGL0910**	117	9	5.15	0.73	0.81	0.099
26	**VGL1063**	117	9	4.42	0.72	0.77	0.072
27	**VGL1165**	117	10	3.39	0.64	0.71	0.090
28	**VGL1828**	117	7	2.27	0.52	0.56	0.068
29	**VGL2009**	117	5	2.18	0.55	0.54	-0.013
30	**VGL2409**	117	8	4.32	0.67	0.77	0.132
31	**VGL2918**	117	7	2.85	0.62	0.65	0.039
32	**VGL3008**	117	7	5.64	0.79	0.82	0.044
33	**VGL3235**	117	8	2.39	0.59	0.58	-0.014

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 it is often presented in the two dimensions that most closely represent its three-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 117 Borzoi formed a single population (i.e., breed) divided into two genetically distinguishable groups by PCoA (Fig. 1). As expected, the main population clustered around of the XY axis, while a smaller but genetically diffuse population of 19 dogs clustered to the right of the XY axis. This smaller group contained dogs from a single popular kennel and a bloodline based on an outstanding foundation bitch. Her desired traits have been maintained by strict line breeding. It is uncommon for bloodlines to differentiate to the degree of varieties within a breed. Examples of varieties would be Japanese and American Akita, or black vs. pepper-and-salt Giant Schnauzers.

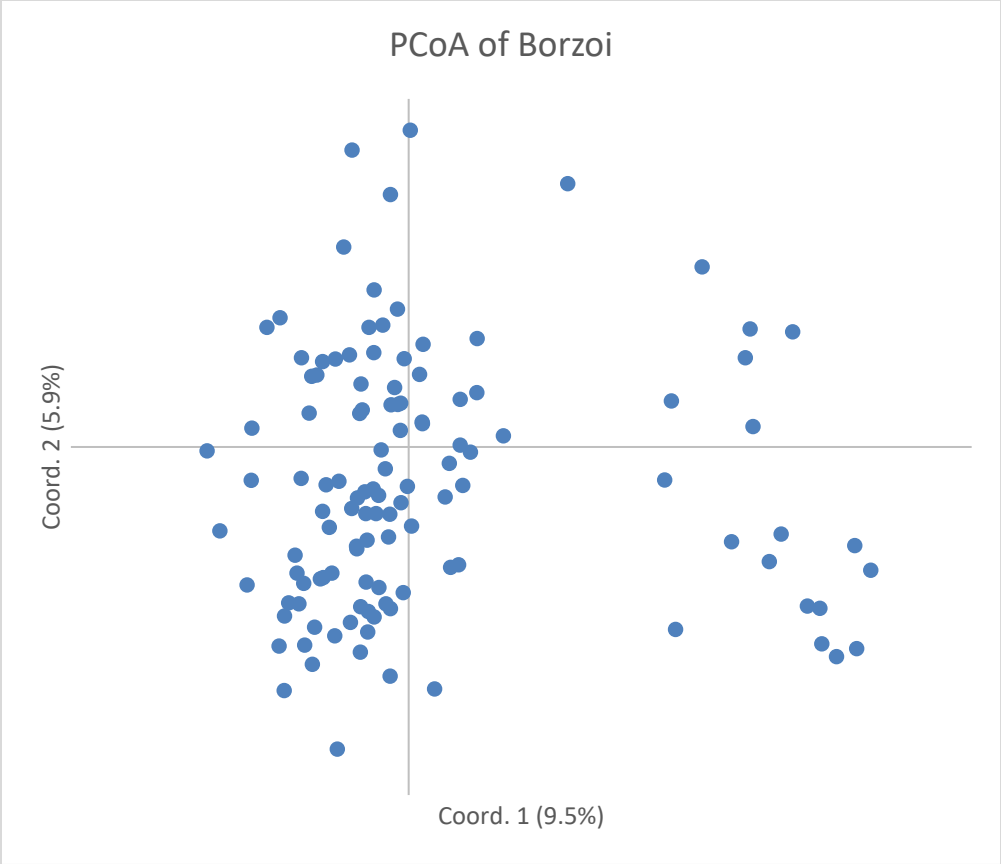


Figure 1. PCoA graph portraying the genetic relatedness of 117 Borzois

The degree of relatedness of individuals within a breed can be further emphasized by comparing the 117 Borzoi with genetically distinct breeds, such as the Irish Setter and Irish Red and White Setter (Fig. 2). These breeds have parallel histories and are from the same region. This comparison shows the three breeds to be genetically distinct, but with tightening in the relationship of individuals within each breed. The two varieties seen in Figure 1 have now been merged into a single cluster or breed.

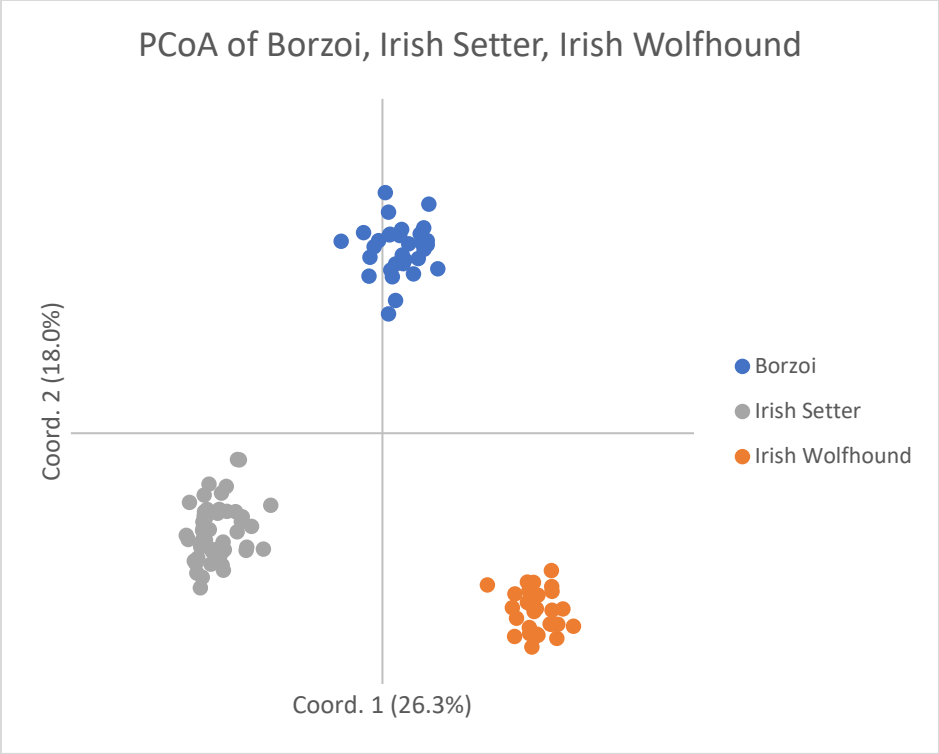


Figure 2. PCoA comparing Borzoi (n=117), Irish Setter (n=50), and Irish Wolfhound (n=55) based on the 33 STRs

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 heterozygosity and do not reflect the genetic diversity being provided 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 were 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 were totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical at all loci. An IR value of +0.25 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 were themselves highly inbred. The higher the IR value above 0.25 the more closely related were the parents and grandparents of the siblings.

Table 4 lists the IR values for the 117 Borzoi that were initially tested. The 25% of most outbred dog in the population had an IR scores of -0.271 to -0.302, while the 25% of most inbred dog in the group had an IR score of 0.154 to 0.351, while the mean (average)

IR score for the group was 0.034. Therefore, IR values give a different picture than seen with the population average scores from the standard genetic assessment (Table 2). While the standard genetic assessments indicated a population in HWE, the IR scores showed a population of individuals that ranged from reasonably outbred to highly inbred. The more inbred dogs are balanced by outbred dogs, making it appear that the overall population was in HWE. This is a common feature of all pure breed dogs.

Table 4. Internal relatedness (IR) values calculated using allele numbers and frequencies for 117 Borzoi (redline). 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-blue line).

	IR	IRVD
Min	-0.302	-0.092
1st Qu	-0.027	0.159
Mean	0.065	0.257
Median	0.062	0.242
3rd Qu	0.154	0.351
Max	0.428	0.635

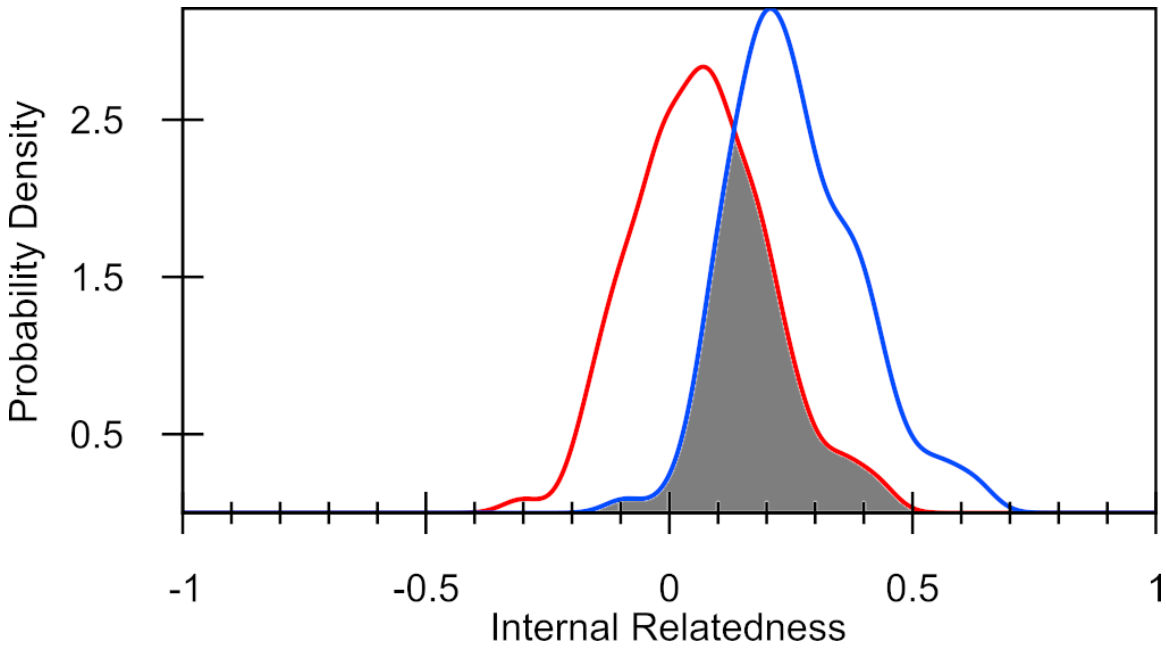


Fig. 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Borzoi (n=117). The area under the curve (black) represents the degree of allele sharing (48.4%) between Borzoi and village dogs.

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

It is possible to determine the amount of canid genetic diversity a breed has retained as it evolved to present day. This is done by assuming that individual Borzoi were members of the current village dog population found in the Middle East, SE Asia and the Island Pacific nations. The IR values and IR values adjusted to village dogs (IRVD) (Table 4) can then be graphed and the graphs overlaid (Fig. 3). One half of the dogs have IRVD scores greater than 0.257 and one half with IRVD scores less than 0.257. Therefore, if this group of dogs were found among modern village dogs, one-half of them would be considered equally or more inbred than offspring of full sibling village dog parents.

The IRVD curve for the Borzois tested was shifted to the right of the IR curve, and the area of overlap was 48.4% (Fig. 3). This figure is close to the 40.9% of retained genetic diversity calculated from a somewhat different population, i.e., all canids ever tested at the VGL (Tables 1, 2). This level of retained village dog genetic diversity is lower than the 60% or so retained diversity observed in the Miniature/toy Poodle or 54% in Labrador Retriever, and much higher than the 23% for Irish Wolfhound, 15% in Doberman Pinchers and 7% in Swedish Vallhund. All pure breeds of dogs have come from relatively small founder populations, which has limited genetic diversity from the time registries were created and closed. Greatly varying amounts of genetic diversity may have been lost subsequently through artificial genetic bottlenecks such as cataclysmic events (e.g., world wars) or inbreeding for a specific show conformation (e.g., popular sire effects).

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

The DLA consists of four gene rich regions making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases.

The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 4). Groups of genes and their alleles inherited as a block, rather than singly, are called haplotypes.

The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at the three STR loci associated with the three Class II genes are strongly linked and also inherited as a single block or haplotype (Table 6). One haplotype comes from each of the parents.

Specific class I and II haplotypes are often linked to each other and inherited as a genetic block. However, there is enough distance between these two regions to allow for a degree

of recombination resulting in unusual class I/II combinations. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes identified in Standard Poodles being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry.

1. DLA class I and II haplotypes existing in Borzoi

The 117 Borzoi in this study possessed 15 DLA class I and 11 DLA class II haplotypes (Table 5). Six class I (1215-1217, 1219, 1223, 1241) and one class II (2116) were unique to the breed and the rest shared with several other breeds (Table 6). One class I (1160) and one class II (2022) haplotypes occurred in 59% of the dogs tested, while another class I (1006) and class II (2007) haplotype were observed in 15% of dogs. These high incidence haplotypes found in 73% of the dogs were in strong linkage disequilibrium (LD), forming larger 1160/2022 and 1006/2007 haplotypes. All the remaining haplotypes occurred at incidences ranging from 1-9%.

The number of DLA class I and II haplotypes found in these 117 Borzoi was low compared to many other breeds studied to date. The numbers of DLA class I (n=10) and II (n=9) haplotypes found in Borzois were higher than the Swedish Vallhund (6/4) and Shiloh Shepherd (7/6); somewhat lower than Giant Schnauzer (14/15), Samoyed (13/12) and Shiba Inu (16/15); and much lower than Golden Retriever (26/23) and Miniature Poodle (33/23). If these 117 dogs represent the ancestry of the breed, two founder dogs or closely related bloodlines have played dominant roles in breed creation and/or evolution. However, the fact that several low incidence haplotypes appear to be unique to the breed, indicates that additional dogs were also important to the breed's standard and their genes closely preserved.

Table 6. DLA class I and Class II haplotypes and their frequencies (n=117). Updated April 8, 2020

DLA1 #	STR types	Borzoi (n=117)
1006	387 375 293 180	0.145
1033	382 379 277 181	0.038
1058	387 378 287 186	0.009
1059	390 371 291 182	0.017
1094	395 375 277 176	0.013
1111	387 378 287 182	0.013
1159	395 379 277 181	0.043
1160	386 369 289 176	0.590
1206	381 379 277 180	0.013
1215	376 365 281 182	0.047
1216	376 379 277 182	0.038
1217	386 369 277 184	0.009

1219	390 371 291 178	0.013
1233	382 379 277 180	0.004
1241	380 379 277 184	0.009

DLA2 #	STR types	Borzoi (n=117)
2006	339 325 280	0.085
2007	351 327 280	0.150
2017	343 322 280	0.009
2022	339 327 282	0.598
2024	343 323 280	0.013
2029	337 324 268	0.060
2047	339 331 280	0.013
2066	339 324 280	0.009
2072	339 325 282	0.013
2098	343 323 282	0.013
2116	337 324 282	0.038

2. DLA haplotype sharing with other dog breeds

DLA haplotypes are much more conserved than most other regions of the genome and each DLA region inherited as a block of linked genes from each parent and passed on by descent. Therefore, the number and incidence of DLA haplotypes found in a breed can be used to estimate the founder/founder lines that were used to create a breed and the importance of the various lines in subsequent breed evolution. The DLA class I and II regions are frequently shared between breeds, reflecting common distant ancestry and inheritance by descent (Table 6). One class I (1160) and one class II (2022) haplotype occurred in 59% of the Borzoi tested, while class I (1006) and class II (2007) haplotypes were observed in 15% of dogs. These haplotypes, which were found collectively in 73% of the Borzoi tested, were in linkage disequilibrium (LD), forming extended 1160/2022 and 1006/2007 haplotypes. These same haplotypes were found in several breeds, but were most prevalent in Black Russian Terriers, Havanese, Irish Red and White Setter, Labrador Retriever, Doberman Pinschers, Samoyed, Giant Schnauzer, Shiba Inu, English Bulldog and American Akita.

It is also noteworthy that DLA haplotype sharing was much broader with class I haplotypes than with class II haplotypes. This pattern has not been seen to date in other breeds. The reason for this is the presence of many unique class I haplotypes. These unique class I haplotypes originated from founders that were not commonly found in the indigenous (Russian?) dogs from which Borzoi evolved.

Table 6. Sharing of specific DLA class I and II haplotypes between Borzoi and various breeds.

DLA1 #	STR types	Black Russian Terrier (n=138)	Lakeland Terrier (n=82)	Labrador Retriever (n=185)	Irish Red and White Setter (n=61)	Doberman Pinscher (n=658)	Flat Coated Retriever (n=562)	Havanese (n=481)	Samoyed (n=189)	Saint Bernard (n=61)	Shiba Inu (n=108)	Giant Schnauzer (n=231)	Polish Lowland Sheepdog (n=19)	Borzoi (n=117)	English Bulldog (n=163)	Italian Greyhound (n=959)	Alaskan Klee Kai (n=563)	Shiloh Shepherd (n=5)	Shiloh Shepherd, ISSA (n=196)	Magyar Agar (n=77)	English Mastiff (n=20)	Mastiff (n=5)
1006	387 375 293 180	0.043	--	0.038	0.057	--	--	0.046	0.005	--	--	0.05	--	0.145	0.003	--	--	--	--	--	--	0.1
1033	382 379 277 181	--	--	0.003	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	0.097	--	--
1058	387 378 287 186	--	--	--	--	--	--	--	--	--	--	--	--	0.009	--	0.0073	--	--	--	0.104	--	--
1059	390 371 291 182	--	--	--	--	--	--	--	--	--	--	--	--	0.017	--	0.1022	--	--	--	--	--	--
1094	395 375 277 176	0.014	--	--	--	0.7637	--	--	--	--	--	--	--	0.013	--	--	--	--	--	--	--	--
1111	387 378 287 182	--	--	--	--	--	--	--	--	--	--	--	--	0.013	--	--	--	--	--	--	--	--
1159	395 379 277 181	--	0.006	--	--	0.0008	--	--	--	--	--	0.175	--	0.043	--	--	--	--	--	--	--	--
1160	386 369 289 176	0.033	--	--	--	--	--	--	0.016	0.098	0.014	--	--	0.59	--	--	--	--	--	--	--	--
1206	381 379 277 180	--	--	--	--	--	--	--	--	--	--	--	--	0.013	--	--	--	--	--	0.013	--	--
1215	376 365 281 182	--	--	--	--	--	--	--	--	--	--	--	--	0.047	--	--	--	--	--	0.006	--	--
1216	376 379 277 182	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--
1217	386 369 277 184	--	--	--	--	--	--	--	--	--	--	--	--	0.009	--	--	--	--	--	--	--	--
1219	390 371 291 178	--	--	--	--	--	--	--	--	--	--	--	--	0.013	--	--	--	--	--	--	--	--
1233	382 379 277 180	--	--	--	--	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--
1241	380 379 277 184	--	--	--	--	--	--	--	--	--	--	--	--	0.009	--	--	--	--	--	--	--	--
2006	339 325 280	--	--	--	--	--	--	0.003	--	--	0.005	0.158	--	0.085	--	--	--	--	--	0.195	--	--
2007	351 327 280	0.043	--	0.043	0.156	--	--	0.049	0.005	0.008	--	0.05	--	0.15	--	--	0.0133	--	--	--	--	--
2017	343 322 280	--	0.03	--	--	--	0.0009	0.007	--	--	--	--	0.32	0.009	0.215	0.2185	--	0.3	0.36	0.455	0.13	0.2
2022	339 327 282	--	0.177	0.073	--	0.0015	0.1219	0.119	0.108	--	--	0.004	--	0.598	0.015	--	--	--	0.059	--	--	--
2024	343 323 280	--	--	0.051	--	0.0008	--	0.003	0.016	--	--	--	--	0.013	0.006	--	--	--	--	--	--	--
2029	337 324 268	--	--	--	--	--	--	--	--	--	--	--	--	0.06	--	0.1022	--	--	--	0.006	--	--
2047	339 331 280	--	--	--	--	--	--	--	--	--	--	--	--	0.013	--	--	--	--	--	0.091	--	--
2066	339 324 280	--	--	--	--	--	0.0009	0.186	--	--	--	--	--	0.009	--	--	--	--	--	--	--	--
2072	339 325 282	--	--	--	--	0.0015	--	0.014	--	--	--	--	--	0.013	--	--	--	--	--	--	--	--
2098	343 323 282	--	--	0.003	--	--	--	--	--	0.107	0.014	--	0.03	0.013	--	--	--	--	--	--	--	--
2116	337 324 282	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--

3. Heterozygosity in the DLA region

The 7 loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium that other parts of the genome that are measured by the 33 autosomal STR markers. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome with random mating and over enough time. This can be tested by doing a standard genetic assessment of each of locus (Table 7), as well as all of the loci taken together (Table 8). Standard genetic assessment of each of the 7 loci demonstrates values for F (inbreeding coefficient) that range from slightly negative to slightly positive (Table 7). The F values tend to balance out in the overall standard genetic assessment of all 7 loci, where F is 0.00 (Table 8). These values indicate that the DLA region is in equilibrium with the rest of the genome and that the proportionately high incidence of certain haplotypes occurred at the foundation of the breed and has come into equilibrium over the breed's existence. In other words, the imbalance in DLA haplotypes has become a fixed feature of the breed. Such DLA imbalances are commonly found in many pure breeds of dogs.

Table 7. Standard Genetic Assessment for Borzoi using 7 STRs in the DLA region

#	Locus	N	Na	Ne	Ho	He	F
1	**DLA I-3CCA**	117	8	2.51	0.58	0.60	0.03
2	**DLA I-4ACA**	117	6	2.45	0.58	0.59	0.02
3	**DLA I-4BCT**	117	6	2.50	0.60	0.60	0.00
4	**DLA1131**	117	7	2.44	0.61	0.59	-0.03
5	**5ACA**	117	4	1.82	0.46	0.45	-0.02
6	**5ACT**	117	6	1.72	0.40	0.42	0.04
7	**5BCA**	117	3	1.93	0.50	0.48	-0.03

Table 8. Summary of Standard Genetic Assessment for Borzoi using 7 STRs in the DLA region

	N	Na	Ne	Ho	He	F
Mean	117	5.71	2.195	0.532	0.533	0.002
SE		0.6	0.124	0.028	0.028	0.01

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

The Borzoi tested constituted a single breed, albeit with limited genetic diversity and some intra-breed variation to the level of varieties. Some of this variation could be explained by relative isolation, e.g., the popularity of certain bloodline(s). The breed originated from dogs that shared many of their genomic alleles with village dogs currently found in the Middle East, SE Asia and Island Pacific nations. Their DLA haplotypes are also shared with several other breeds that evolved in both Europe and Asia. However, a portion of the Borzoi genome also came from founders that are not represented in any other breed tested to date.

The Borzoi lack diversity in the DLA regions. Seventy three percent of the individuals tested possessed one or two copies of the 1006/2007 or 1160/2022 class I/II linked haplotypes. This is evidence that two founders, or closely related founder lines, have contributed disproportionately to the breed's evolution. However, the fact that these haplotypes are in equilibrium with the rest of the genome indicates that this imbalance occurred at the time of the founding and has become fixed/equilibrated over the time, and not from some more recent genetic bottleneck.

Although it appears that contemporary Borzoi have a relatively narrow genetic base, a lack of genetic diversity is not in itself bad, providing the founder population was relatively free of deleterious genetic traits and breeders have been judicious in avoiding any artificial genetic bottlenecks that may cause either a loss or imbalance of original diversity. The breed is surprisingly clear of breed-specific heritable disease traits and enjoys a good 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 [11]. These traits common to modern dogs have most likely been inherited from

generation to generation as dogs underwent progressively more intense human-directed artificial selection.

The lack of genetic diversity in the DLA class I and II region of these 117 Borzoi is noteworthy, but it is uncertain what it means. Certain DLA class I and II haplotypes have been associated with specific autoimmune diseases in certain breeds [13], but autoimmune disorders other than hypothyroidism have not been documented as serious problems in Borzoi. 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 genetic diversity and have less leeway in dealing with simple recessive or complex polygenic disorders that might arise. Elimination of deleterious traits may result in loss of genetic diversity, especially when diversity is limited.

IV. Heritable disorders of Borzoi

A. Lifespan

The lifespan of Borzois has been reported to be 9-14 years [3] or 10-12 years [4]. A study from The Kennel Club reported a wide range of ages at death, usually from 1-13 years of age, with a median at 6 years and a maximum of 21 years [7].

B. Major disease problems

Borzoi are judged as a healthy breed by many owners, but a few diseases of a heritable nature have been described [7-9]. Panosteitis and hypertrophic osteodystrophy occur in adolescent Borzoi, as in several other larger breeds. Older dogs may suffer from the effects of osteochondritis dissecans, hip dysplasia, and elbow dysplasia that appear at a younger age. Immunologic disorders, including autoimmune thyroiditis and allergies, occur in the breed but appear to be reasonably managed. Cancer of various types occur in older Borzoi, as it does in most breeds. Cardiomyopathy and fatal arrhythmias of unknown genetic bases occur in the breed. Borzoi also may suffer from acute bloat, like other large and deep-chested breeds.

The most comprehensive study of disease incidence in Borzoi comes from a survey conducted by The Kennel Club [7]. Bloat was the single most reported disease condition in 6.2% of Borzoi, while bone cancer was the most common reported cause of death. Cancers of various types were reported in 25.6% of individuals collectively, which is in the range of most other breeds. Cancer types were unspecified (4.6%), or involved bone (3.1%), brain (1.5%), heart (1.5%), liver (3.0%), and mammary gland (1.5%) [7]. Renal failure occurred in 3.1% of the breed, cervical spondylomyelopathy (1,5%), heart disease (1.5%), and intervertebral disc disease in 1.5%.

C. Testing for heritable conditions

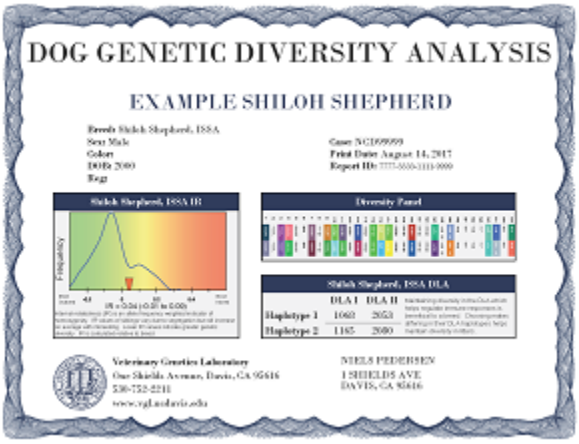
The Orthopedic Foundation of America recommends testing for the following heritable disorders: 1) eye examination by a boarded veterinary ophthalmologist, 2) elbow dysplasia, 3) hip dysplasia, 4) degenerative myelopathy, 5) autoimmune thyroiditis [8].

The only disorder that currently requires DNA testing is degenerative myelopathy (DM). A test is available from several veterinary laboratories for the SOD-1 gene mutation that has been associated with the condition when in a homozygous state [12]. A study of 787 Borzoi found 0.17% to be homozygous and at high risk for DM and 26.6% to be heterozygous carriers [12]. This mutation occurs at a similar frequency in many breeds of dogs [12] and is presumably of ancient origin and inherited by descent as affected breeds were created.

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 related to the entire 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 are either different (heterozygous) or the same (homozygous). Each allele is inherited from each of the parents. More of the alleles at each locus will be homozygous in dogs from closely related parents or that in regions of the genome that are under strong positive selection for some favored phenotypic trait or traits. Dogs with a predominance of rarer (i.e., low incidence) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high incidence) alleles.


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE SHILOH SHEPHERD

Breed: Shiloh Shepherd, ISSA
Sex: Male
Color:
DOB: 2010
Reg:

Case: NG109999
Print Date: August 14, 2017
Report ID: 7777-XXXX-1111-9999

Shiloh Shepherd, ISSA III

Diversity Panel

Shiloh Shepherd, ISSA DLA

	DLA I	DLA II
Haplotype 1	1060	2053
Haplotype 2	1165	2080

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B. What should you do with this information?

The use of DNA for testing genetic diversity in the Borzoi has confirmed that the breed lacks genetic diversity genome-wide and in the DLA region, most likely from a small number of founder individuals/lines. It is more important, therefore, to closely monitor existing diversity into the future. 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.

The goal for breeders should be to continue to produce puppies with IR scores less than 0, and with time, even lower scores. Although most of the individuals tested were randomly bred, there were small subpopulations of dogs that were much more inbred or outbred than the rest of the population. Therefore, there is a possibility to better balance genetic diversity in the breed. Mates should be selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype and encourage the use of dogs with less common genomic alleles or DLA haplotypes. 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, IR values, because they reflect the unique genetics of individuals, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

The next step is to compare the DLA class I and II haplotypes. You want to avoid breeding pairs that will produce puppies that will be homozygous for the same haplotypes, and once again, less common haplotypes may offer more diversity than common ones.

Breeders who do not have access to computer programs to predict the outcome of pairings based on IR values of sire and dam can also compare values by manual screening. Potential sires and dams should be first screened for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Some extra weight should be given to rare v*s* common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.

Puppies, once born, should be tested for their actual IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices

for genetic diversity should be balanced with other breeding goals but maintaining and/or improving genetic diversity in puppies should be paramount.

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