Genetic Diversity Testing for American Eskimo Dog

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers to determine genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel is useful to dog breeders who wish to use DNA-based testing to track and 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 testing of the American Eskimo Dog is now in the preliminary results phase. During this phase, we will continue to test more registered dogs to build the genetic database necessary to provide an accurate assessment of genetic diversity within the breed. This report is based on genetic testing of **60 standard**, **38 miniature**, and **14 toy** American Eskimo Dogs from the USA and Canada (total = 112 individuals). Although results reported herein are preliminary, this selection of individuals should provide a reasonable picture of genetic diversity in the standard American Eskimo Dog. Allele and DLA haplotype frequencies will be updated as more dogs from each of the size divisions are tested. It is anticipated that new alleles at the 33 STR loci and additional DLA class I and II haplotypes will be identified in the future for the American Eskimo Dog, but these will likely to be of much lower frequency than those detected in this initial population.

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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 American Eskimo Dog

A. Breed History [1-4]

The American Eskimo Dog was originally bred in Germany as a multipurpose farm dog. It belongs to the Spitz ("*sharp point*" in German) family of Nordic breeds, being closely related to the German, Japanese and Italian Spitz, and the Pomeranian (among others). European immigrants brought the first German Spitz individuals to the United States in the early 1900's, where the white variety became hugely popular even though Spitz are also found in black, chocolate, and red. Sometime after World War I, the breed name was changed to American Eskimo Dog when Mr. and Mrs. F.M. Hall first registered the breed with the United Kennel Club (UKC) using their kennel name, "American Eskimo". In the 1930's, American Eskimo Dogs became popular attractions in circuses across the US, including the famous Barnum and Bailey Circus. Due to their popularity with the public, many circuses started selling American Eskimo Dog puppies. Because of that, many of today's "Eskies" (as the breed has been nicknamed) can trace their lineage back to the most popular circus dogs from that time.

In 1969, the studbook was closed and the National American Eskimo Dog Association was founded. Subsequently, in 1985 the club name was changed to American Eskimo Dog Club of America (AEDCA) to conform to AKC guidelines. The breed was recognized by the AKC in 1995 and by the Canadian Kennel Club in 2006; it is not recognized anywhere else in the world.

Currently, the breed is ranked 122 of 196 in popularity among the AKC registries.

B. Appearance [1-4]

The American Eskimo Dog shares features with Japanese Spitz, Danish Spitz, Volpino Italiano, German Spitz and Samoyed. It is a small to medium-size Nordic type dog that comes in three sizes: Toy, Miniature, and Standard. According to the breed standard, height at the withers for the three size divisions are: Toy – 9 inches to and including 12 inches (22.9-30.5 cm); Miniature – over 12 inches to and including 15 inches (30.5-38.1 cm); and Standard – over 15 inches to and including 19 inches (38.1-48.3 cm). Although weight is not specified by the breed standard, toy dogs can range from 5 to 10 lbs. (2.3-4.5 kg), miniature dogs from 10 to 20 lbs. (4.5-9 kg), and standard-sized dogs from 15 to 40 lbs. (6.8-18.1 kg). The Toy, Standard, and Miniature American Eskimo Dogs are considered identical except for their height, similar to the varieties of Poodles. Therefore, larger than accepted miniatures are registered as standards, shorter standards as miniatures, etc. American Eskimo Dogs are always white, or white with biscuit cream markings. The double coat consists of a short and dense undercoat, with longer guard hair forming the straight outer coat. The coat is thicker and longer around the neck and chest forming a ruff or mane, especially in males. The rump and hind legs down to the hocks are also covered with thicker, longer hair. Their tail is covered profusely with long hair. The face is Nordic type with erect triangular ears, and distinctive black points (lips, nose, and eye rims). Dark to medium brown is the preferred eye color. The breed standard lists three disqualifications for the American Eskimo Dog: any color besides white or biscuit cream, blue eyes, and height under 9 inches or over 19 inches.

Temperament [2]

The American Eskimo Dog is intelligent, alert, friendly, and eager to please. It is never overly shy nor aggressive – these traits are penalized in the show ring. It is kid-friendly and an excellent watchdog, barking at strangers to protect its home and family.

II. Preliminary Results on Genetic Diversity of American Eskimo Dogs

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

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 autosomal STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes [5, 6]. Each STR locus contains 7 to 29 different alleles (average of 15.4 alleles/locus) in the breeds tested at the VGL so far. Each breed, having evolved from a small number of founders and having been exposed to artificial genetic bottlenecks, will end up with only a portion of the total available diversity. Artificial genetic bottlenecks can include phenomena such as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity which can lead to increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 60 **standard,** 38 **miniature**, and 14 **toy** American Eskimo Dogs are listed in **Table 1, Table 2**, and **Table 3** respectively.

Assessment of allelic diversity in the standard American Eskimo Dog shows a relatively low number of alleles per locus when compared to more popular dog breeds (**Table 1**). This number ranged from 3 alleles (LEI004) to 11 alleles (VGL1165) per locus in the 60 individuals tested. As expected for a purebred breed, one or two alleles predominated (i.e., were found at a higher frequency) at the 33 STR loci, with the remaining alleles reasonably dispersed at lower frequencies. However, in the case of three markers (AHTk211, VGL1828, and AHTH130), a single allele occurred at a high frequency (95.8%, 87.5%, and 84.2%, respectively). These findings suggest that the genomic regions harboring these three loci have been under strong positive selection since breed formation and are most likely associated with prominent phenotypic traits.

Table 1. Alleles and their frequencies for 33 STR markers in standard American Eskimo Dogs (n=60). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
94 (0.017)	**131 (0.325)**	119 (0.008)	**219 (0.500)**	238 (0.125)	87 (0.017)
96 (0.017)	141 (0.117)	**121 (0.842)**	221 (0.220)	242 (0.008)	89 (0.017)
98 (0.254)	145 (0.117)	127 (0.092)	227 (0.195)	244 (0.308)	**91 (0.958)**
102 (0.458)	147 (0.283)	129 (0.058)	229 (0.085)	246 (0.217)	97 (0.008)
104 (0.042)	151 (0.158)			248 (0.008)	
106 (0.017)				**250 (0.333)**	
108 (0.178)					
112 (0.017)					
AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**
284 (0.008)	**116 (0.644)**	132 (0.051)	**152 (0.508)**	232 (0.183)	**95 (0.758)**
286 (0.008)	118 (0.297)	144 (0.424)	156 (0.008)	238 (0.067)	97 (0.108)
288 (0.305)	124 (0.051)	**148 (0.432)**	168 (0.034)	**240 (0.617)**	99 (0.033)
290 (0.085)	126 (0.008)	152 (0.093)	172 (0.068)	242 (0.058)	101 (0.100)
292 (0.593)			176 (0.381)	244 (0.075)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.233)	144 (0.208)	208 (0.008)	**85 (0.483)**	227 (0.217)	**200 (0.458)**
122 (0.242)	**150 (0.458)**	210 (0.083)	95 (0.258)	231 (0.225)	202 (0.217)
124 (0.500)	152 (0.017)	214 (0.225)	107 (0.258)	233 (0.092)	204 (0.183)
126 (0.008)	156 (0.317)	**218 (0.683)**		235 (0.100)	206 (0.075)
132 (0.017)				239 (0.083)	210 (0.067)
				241 (0.283)	
REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.567)	162 (0.325)	**268 (0.700)**	226 (0.034)	139 (0.067)	**12 (0.408)**
210 (0.025)	164 (0.125)	270 (0.008)	**232 (0.703)**	**145 (0.808)**	13 (0.333)
216 (0.033)	166 (0.158)	272 (0.275)	234 (0.068)	147 (0.067)	15 (0.008)
218 (0.275)	**168 (0.383)**	274 (0.017)	236 (0.017)	149 (0.042)	20.2 (0.042)
220 (0.008)	170 (0.008)		238 (0.119)	153 (0.017)	21.2 (0.042)
222 (0.008)			240 (0.059)		22.2 (0.033)
224 (0.083)					23.2 (0.108)
					24.2 (0.025)
VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
14 (0.008)	8 (0.017)	14 (0.050)	16 (0.042)	9 (0.008)	13 (0.367)
16.1 (0.017)	**13 (0.517)**	16 (0.008)	17 (0.008)	10 (0.008)	**14 (0.383)**
17.1 (0.067)	14 (0.117)	17 (0.008)	18 (0.058)	11 (0.008)	15 (0.008)
18.1 (0.275)	15 (0.017)	19 (0.008)	**19 (0.875)**	13 (0.283)	16 (0.208)
19.1 (0.042)	18 (0.075)	20 (0.075)	20 (0.017)	**14 (0.500)**	17 (0.033)
20.1 (0.025)	19 (0.075)	**21 (0.692)**		15 (0.192)	
21.1 (0.525)	20 (0.183)	22 (0.033)			
22.1 (0.033)		26 (0.008)			
23.1 (0.008)		27 (0.017)			
		28 (0.050)			
		29 (0.050)			
VGL2918	**VGL3008**	**VGL3235**			
12 (0.017)	10 (0.017)	13 (0.300)			
13 (0.750)	15 (0.017)	**14 (0.558)**			
14 (0.208)	16 (0.008)	15 (0.017)			
15 (0.008)	17 (0.267)	16 (0.025)			
19.3 (0.017)	**18 (0.508)**	18 (0.083)			
	19 (0.183)	19 (0.017)			

A low number of alleles was also identified in miniature American Eskimo Dogs (**Table 2**), ranging from 3 (AHTk211, VGL1828) to 10 alleles (AHT121, VGL0760, and VGL0910). However, contrary to the standard size group, miniature American Eskimo Dogs showed more evenly distributed allelic frequencies for the 33 autosomal STR loci. Predominance of a single allele was identified in only one marker (VGL1828), with the major allele occurring at a frequency of 96%. These findings corroborate the trend observed in other dog breeds comprised of different

size varieties (such as the Poodle), and can be explained by the outcrossing needed to attain the desired heights for the miniature and toy varieties.

Table 2. Alleles and their frequencies for 33 STR markers in miniature American Eskimo Dogs (n=38). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
92 (0.03)	131 (0.20)	113 (0.17)	219 (0.17)	238 (0.07)	87 (0.26)
94 (0.16)	137 (0.12)	121 (0.24)	221 (0.21)	242 (0.04)	89 (0.09)
96 (0.17)	141 (0.08)	127 (0.24)	225 (0.01)	244 (0.18)	**91 (0.64)**
98 (0.07)	145 (0.07)	**129 (0.34)**	227 (0.03)	246 (0.21)	
100 (0.01)	**147 (0.53)**	133 (0.01)	**229 (0.25)**	248 (0.05)	
102 (0.22)	151 (0.01)		231 (0.11)	**250 (0.45)**	
104 (0.17)			233 (0.14)		
106 (0.05)			235 (0.08)		
108 (0.05)					
110 (0.07)					
AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**
284 (0.01)	**116 (0.50)**	132 (0.25)	**152 (0.61)**	232 (0.09)	**95 (0.43)**
286 (0.01)	118 (0.39)	136 (0.08)	156 (0.17)	**234 (0.37)**	97 (0.12)
288 (0.21)	120 (0.03)	**144 (0.36)**	160 (0.05)	236 (0.05)	99 (0.01)
290 (0.26)	124 (0.08)	148 (0.24)	168 (0.16)	238 (0.26)	101 (0.41)
292 (0.50)		152 (0.08)	172 (0.01)	240 (0.13)	103 (0.03)
				242 (0.01)	
				244 (0.08)	
INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
110 (0.04)	**144 (0.53)**	208 (0.09)	85 (0.22)	227 (0.20)	**200 (0.53)**
122 (0.32)	146 (0.01)	**210 (0.54)**	**95 (0.37)**	229 (0.01)	202 (0.16)
124 (0.55)	150 (0.33)	214 (0.05)	97 (0.03)	231 (0.13)	204 (0.29)
126 (0.04)	152 (0.01)	218 (0.32)	105 (0.14)	**233 (0.49)**	206 (0.03)
132 (0.05)	156 (0.12)		107 (0.24)	235 (0.03)	
				241 (0.14)	
REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.22)	**162 (0.51)**	**268 (0.54)**	222 (0.14)	139 (0.30)	12 (0.09)
210 (0.03)	164 (0.17)	270 (0.03)	**232 (0.64)**	145 (0.22)	13 (0.05)
212 (0.13)	166 (0.11)	272 (0.25)	234 (0.03)	**147 (0.39)**	15 (0.04)
216 (0.30)	168 (0.13)	274 (0.01)	236 (0.03)	149 (0.01)	18.2 (0.11)
218 (0.20)	170 (0.08)	276 (0.17)	240 (0.16)	153 (0.07)	19.2 (0.01)
222 (0.01)					20.2 (0.01)
224 (0.11)					21.2 (0.05)
					22.2 (0.16)
					23.2 (0.34)
					24.2 (0.13)
VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**

12 (0.01)	8 (0.01)	17 (0.01)	16 (0.01)	11 (0.04)	13 (0.26)
15.1 (0.01)	**13 (0.36)**	19 (0.07)	18 (0.03)	**13 (0.45)**	**14 (0.36)**
16.1 (0.04)	14 (0.30)	**21 (0.72)**	**19 (0.96)**	14 (0.43)	15 (0.09)
17.1 (0.05)	15 (0.12)	27 (0.08)		15 (0.08)	16 (0.04)
18.1 (0.09)	16 (0.01)	28 (0.09)			17 (0.13)
19.1 (0.45)	18 (0.08)	29 (0.03)			18 (0.11)
20.1 (0.05)	19 (0.08)				19 (0.01)
21.1 (0.20)	20 (0.04)				
22.1 (0.07)					
23.1 (0.03)					

VGL2918	**VGL3008**	**VGL3235**
12 (0.03)	**15 (0.30)**	13 (0.04)
13 (0.29)	16 (0.04)	14 (0.24)
14 (0.42)	17 (0.09)	16 (0.14)
15 (0.01)	18 (0.18)	17 (0.12)
17.3 (0.16)	19 (0.11)	**18 (0.46)**
19.3 (0.01)	21 (0.26)	
20.3 (0.07)	22 (0.01)	
21.3 (0.01)		

Initial assessment of allelic diversity for toy American Eskimo Dogs also identified a low number of alleles for the 33 STR markers (**Table 3**). This number ranged from 3 to 7 alleles, and comparably to the miniature size division, allele frequencies were more evenly distributed across all markers when compared to standard American Eskimo Dogs. Since this assessment was made using only 14 individuals, it is expected that additional alleles will be identified for each STR marker in toy American Eskimo Dogs as more individuals are tested.

Table 3. Alleles and their frequencies for 33 STR markers in toy American Eskimo Dogs (n=14). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
92 (0.04)	131 (0.18)	113 (0.25)	219 (0.14)	244 (0.18)	87 (0.21)
94 (0.29)	137 (0.14)	121 (0.18)	**221 (0.21)**	246 (0.29)	89 (0.21)
96 (0.18)	141 (0.21)	127 (0.11)	225 (0.11)	248 (0.04)	**91 (0.57)**
98 (0.04)	145 (0.11)	**129 (0.46)**	229 (0.18)	**250 (0.50)**	
102 (0.32)	**147 (0.36)**		231 (0.11)		
104 (0.04)			233 (0.07)		
110 (0.11)			235 (0.18)		

AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**

286 (0.07)	**116 (0.43)**	132 (0.21)	**152 (0.64)**	232 (0.18)	**95 (0.57)**
288 (0.25)	118 (0.39)	136 (0.11)	156 (0.14)	**234 (0.39)**	97 (0.11)
290 (0.25)	120 (0.04)	**144 (0.43)**	160 (0.04)	236 (0.04)	101 (0.32)
292 (0.43)	124 (0.07)	148 (0.25)	164 (0.04)	238 (0.18)	
	126 (0.07)		168 (0.11)	240 (0.07)	
			172 (0.04)	242 (0.07)	
				244 (0.07)	

INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
122 (0.18)	**144 (0.61)**	208 (0.21)	85 (0.21)	227 (0.14)	**200 (0.54)**
124 (0.75)	150 (0.32)	**210 (0.43)**	**95 (0.50)**	231 (0.21)	202 (0.14)
126 (0.07)	156 (0.07)	218 (0.36)	105 (0.14)	**233 (0.64)**	204 (0.25)
			107 (0.14)		206 (0.04)
					210 (0.04)

REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.32)	**162 (0.68)**	**268 (0.68)**	222 (0.07)	139 (0.14)	12 (0.07)
210 (0.07)	164 (0.11)	270 (0.07)	**232 (0.75)**	**145 (0.43)**	13 (0.14)
212 (0.21)	166 (0.18)	272 (0.21)	236 (0.07)	147 (0.36)	18.2 (0.11)
216 (0.21)	168 (0.04)	276 (0.04)	240 (0.11)	153 (0.07)	21.2 (0.11)
218 (0.07)					22.2 (0.07)
224 (0.11)					**23.2 (0.50)**

VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
16.1 (0.07)	12 (0.04)	19 (0.07)	16 (0.04)	11 (0.04)	13 (0.21)
17.1 (0.04)	**13 (0.36)**	**21 (0.61)**	17 (0.04)	13 (0.25)	**14 (0.43)**
18.1 (0.21)	14 (0.25)	26 (0.04)	**19 (0.93)**	**14 (0.57)**	15 (0.21)
19.1 (0.43)	15 (0.18)	27 (0.18)		15 (0.14)	17 (0.07)
20.1 (0.04)	18 (0.11)	28 (0.11)			18 (0.04)
21.1 (0.07)	19 (0.04)				20 (0.04)
22.1 (0.14)	20 (0.04)				

VGL2918	**VGL3008**	**VGL3235**
12 (0.04)	15 (0.21)	13 (0.04)
13 (0.21)	16 (0.04)	14 (0.25)
14 (0.57)	17 (0.04)	16 (0.04)
15 (0.04)	18 (0.07)	17 (0.11)
17.3 (0.04)	19 (0.07)	**18 (0.57)**
19.3 (0.04)	20 (0.04)	
20.3 (0.07)	**21 (0.54)**	

B. Assessment of population diversity using standard genetic parameters

Alleles for each of the 33 STR loci listed in Tables 1 and 2, as well as their respective frequencies, are used to determine basic genetic parameters for the population. These parameters include the average number of alleles found at each locus (**Na**); the average number of effective alleles (**Ne**) per locus (i.e., the number of alleles that contribute most to genetic differences/heterozygosity); observed heterozygosity (**Ho**); expected heterozygosity (**He**) if the existing population was in

Hardy-Weinberg equilibrium (HWE, or random breeding); and the coefficient of inbreeding (**F**) derived from Ho and He values. **Tables 4**, **5**, and **6** break down the genetic parameters for the standard, miniature, and toy American Eskimo Dogs tested in this study, respectively.

Table 4. Genetic Assessment of standard American Eskimo Dogs (n = 60) based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	5.61	2.57	0.536	0.564	0.068
SE	0.29	0.146	0.031	0.028	0.019

Table 5. Genetic Assessment of miniature American Eskimo Dogs (n = 38) based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	5.848	3.341	0.661	0.66	-0.01
SE	0.318	0.204	0.025	0.024	0.015

Table 6. Genetic Assessment of toy American Eskimo Dogs (n = 14) based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	4.818	2.955	0.645	0.621	-0.03
SE	0.252	0.178	0.033	0.023	0.031

The number of effective alleles (Ne) is greater for both miniature (3.34) and toy (2.95) American Eskimo Dogs when compared to the standard sized cohort (2.57). The miniature and toy groups also show higher observed (Ho) and expected (He) heterozygosity, which yield negative inbreeding coefficients (-0.01 and -0.03, respectively) compared to a value of 0.068 for the standard cohort. Therefore, standard genetic assessment values indicate that miniature and toy American Eskimo Dogs have higher genetic diversity than the standard size variety for the 33 autosomal STR loci (which is expected due to outcrossing). Overall, genetic assessment values calculated for American Eskimo Dogs are within the range of most pure breeds that have been tested at VGL to date. They also show that breeders are maintaining diversity in the breed by choosing (on average) the least possible related parents within each cohort. However, this is an average of the entire population and does not measure the degree to which an individual dog is inbred (see IR values below). It is worth noting that the average and effective number of alleles for the toy division are expected to increase as more individuals are tested, especially those occurring at lower frequency.

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each of the 33 autosomal STR loci used in this study. This provides an estimate of genetic similarities in the specific regions of the genome that are associated with each STR marker.

Loci with low Ho and He values in each population contribute the least to heterozygosity among individuals, and are most likely associated with traits that define the population's phenotypic standards. Conversely, loci with high Ho and He values are more genetically variable and can be associated with phenotypic variation among individuals within the breed. Moreover, the F value

is a coefficient of inbreeding based on Ho and He; if these two values are equal, F=0 which means that the population is in HWE. The F value will be positive when there is a deficiency of heterozygotes (i.e., fewer heterozygotes than expected), whereas negative F values correspond to an excess of heterozygotes within the population.

In standard American Eskimo Dogs, the observed heterozygosity (Ho) for individual STR loci ranged from 0.05 to 0.83, while the expected heterozygosity (He) ranged from 0.17 to 0.81 (**Table 7**). High inbreeding coefficients (F>0.1) were calculated for 13 of the 33 STR markers analyzed (40%), which suggests that alleles at these loci are responsible for a substantial proportion of the genetic diversity (bolded on **Table 7**) and have been under strong positive selection since breed development. Additionally, strong positive F values for these loci suggest that these dogs were selected from a more inbred subpopulation and may be more conforming to breed-defining phenotypic standards.

Table 7. Standard Genetic Assessment of individual STR loci for 60 standard American Eskimo Dogs. Bolded loci correspond to those with high inbreeding coefficients (F>0.1).

#	Locus	Na	Ne	Ho	He	F
1	**AHT121**	9	3.32	0.75	0.7	-0.07
2	**AHT137**	5	4.2	0.73	0.76	0.037
3	**AHTH130**	4	1.39	0.22	0.28	**0.225**
4	**AHTh171-A**	5	2.94	0.53	0.66	**0.191**
5	**AHTh260**	6	3.72	0.8	0.73	-0.09
6	**AHTk211**	4	1.09	0.05	0.08	**0.383**
7	**AHTk253**	5	2.21	0.57	0.55	-0.03
8	**C22.279**	5	2.03	0.45	0.51	**0.111**
9	**FH2001**	4	2.64	0.52	0.62	**0.168**
10	**FH2054**	6	2.48	0.55	0.6	0.079
11	**FH2848**	5	2.34	0.52	0.57	0.098
12	**INRA21**	4	1.67	0.4	0.4	0.005
13	**INU005**	5	2.75	0.68	0.64	-0.07
14	**INU030**	4	2.83	0.67	0.65	-0.03
15	**INU055**	4	1.91	0.45	0.48	0.053
16	**LEI004**	3	2.72	0.57	0.63	**0.105**
17	**REN105L03**	6	4.92	0.83	0.8	-0.05
18	**REN162C04**	5	3.33	0.68	0.7	0.023
19	**REN169D01**	7	2.47	0.53	0.59	**0.103**
20	**REN169O18**	5	3.41	0.62	0.71	**0.127**
21	**REN247M23**	4	1.77	0.37	0.43	**0.155**
22	**REN54P11**	7	1.99	0.42	0.5	**0.163**
23	**REN64E19**	5	1.51	0.32	0.34	0.057
24	**VGL0760**	8	3.39	0.7	0.71	0.007
25	**VGL0910**	9	2.78	0.65	0.64	-0.02
26	**VGL1063**	7	3.07	0.73	0.67	-0.09
27	**VGL1165**	11	2.03	0.48	0.51	0.046
28	**VGL1828**	5	1.3	0.17	0.23	**0.272**

29	**VGL2009**	6	2.72	0.58	0.63	0.078
30	**VGL2409**	5	3.07	0.6	0.67	**0.11**
31	**VGL2918**	5	1.65	0.38	0.39	0.026
32	**VGL3008**	6	2.75	0.7	0.64	-0.1
33	**VGL3235**	6	2.44	0.48	0.59	**0.181**

In miniature American Eskimo Dogs, the observed heterozygosity (Ho) for individual STR loci ranged from 0.08 to 0.89, while the expected heterozygosity (He) ranged from 0.07 to 0.85 (**Table 8**). High inbreeding coefficients (F>0.1) were calculated for 4 of the 33 STR markers (12%), which suggests that alleles at these loci are mostly responsible for the genetic diversity found in this cohort and have been under strong positive selection due to their putative association with desired phenotypic traits (bolded on **Table 8**).

Table 8. Standard Genetic Assessment of individual STR loci for 38 miniature American Eskimo Dogs. Bolded loci correspond to those with high inbreeding coefficients (F>0.1).

#	Locus	Na	Ne	Ho	He	F
1	**AHT121**	10	6.73	0.9	0.85	-0.05
2	**AHT137**	6	2.94	0.61	0.66	0.082
3	**AHTH130**	5	3.87	0.66	0.74	**0.113**
4	**AHTh171-A**	8	5.71	0.82	0.83	0.011
5	**AHTh260**	6	3.48	0.66	0.71	0.077
6	**AHTk211**	3	2.03	0.58	0.51	-0.14
7	**AHTk253**	5	2.75	0.68	0.64	-0.08
8	**C22.279**	4	2.42	0.55	0.59	0.059
9	**FH2001**	5	3.89	0.74	0.74	0.008
10	**FH2054**	5	2.36	0.58	0.58	0
11	**FH2848**	7	4.17	0.76	0.76	0
12	**INRA21**	5	2.7	0.66	0.63	-0.04
13	**INU005**	5	2.43	0.58	0.59	0.017
14	**INU030**	5	2.5	0.66	0.6	-0.1
15	**INU055**	4	2.49	0.74	0.6	-0.23
16	**LEI004**	5	3.8	0.76	0.74	-0.04
17	**REN105L03**	6	3.17	0.74	0.69	-0.08
18	**REN162C04**	4	2.59	0.71	0.61	-0.16
19	**REN169D01**	7	4.77	0.71	0.79	**0.101**
20	**REN169O18**	5	3.06	0.66	0.67	0.022
21	**REN247M23**	5	2.61	0.58	0.62	0.061
22	**REN54P11**	5	2.16	0.55	0.54	-0.03
23	**REN64E19**	5	3.31	0.66	0.7	0.058
24	**VGL0760**	10	5.37	0.74	0.81	0.094
25	**VGL0910**	10	3.85	0.74	0.74	0.004
26	**VGL1063**	8	4.06	0.82	0.75	-0.08
27	**VGL1165**	6	1.84	0.4	0.46	**0.135**

28	**VGL1828**	3	1.08	0.08	0.08	-0.03
29	**VGL2009**	4	2.52	0.61	0.6	0
30	**VGL2409**	7	4.27	0.74	0.77	0.038
31	**VGL2918**	8	3.43	0.61	0.71	**0.146**
32	**VGL3008**	7	4.63	0.87	0.78	-0.11
33	**VGL3235**	5	3.28	0.71	0.7	-0.02

In toy American Eskimo Dogs, the observed heterozygosity (Ho) for individual STR loci ranged from 0.14 to 1, while the expected heterozygosity (He) ranged from 0.13 to 0.84 (**Table 9**). High inbreeding coefficients (F>0.1) were calculated for 5 of the 33 STR loci (bolded on **Table 9**).

Table 9. Standard Genetic Assessment of individual STR loci for 14 toy American Eskimo Dogs. Bolded loci correspond to those with high inbreeding coefficients (F>0.1).

#	Locus	Na	Ne	Ho	He	F
1	**AHT121**	7	4.31	0.71	0.77	0.07
2	**AHT137**	5	4.22	0.86	0.76	-0.12
3	**AHTH130**	4	3.11	0.57	0.68	**0.158**
4	**AHTh171-A**	7	6.32	1	0.84	-0.19
5	**AHTh260**	4	2.74	0.64	0.64	-0.01
6	**AHTk211**	3	2.39	0.64	0.58	-0.11
7	**AHTk253**	4	3.19	0.86	0.69	-0.25
8	**C22.279**	5	2.86	0.79	0.65	-0.21
9	**FH2001**	4	3.29	0.64	0.7	0.077
10	**FH2054**	6	2.23	0.57	0.55	-0.04
11	**FH2848**	7	4.26	0.93	0.77	-0.21
12	**INRA21**	3	2.27	0.57	0.56	-0.02
13	**INU005**	3	1.67	0.21	0.4	**0.465**
14	**INU030**	3	2.1	0.57	0.52	-0.09
15	**INU055**	3	2.8	0.64	0.64	0
16	**LEI004**	4	2.97	0.64	0.66	0.031
17	**REN105L03**	3	2.09	0.64	0.52	-0.24
18	**REN162C04**	5	2.69	0.71	0.63	-0.14
19	**REN169D01**	6	4.61	0.71	0.78	0.088
20	**REN169O18**	4	1.98	0.5	0.5	-0.01
21	**REN247M23**	4	1.95	0.5	0.49	-0.03
22	**REN54P11**	4	1.71	0.21	0.42	**0.485**
23	**REN64E19**	4	2.97	0.86	0.66	-0.29
24	**VGL0760**	6	3.29	0.71	0.7	-0.03
25	**VGL0910**	7	3.81	0.79	0.74	-0.07
26	**VGL1063**	7	4.22	0.79	0.76	-0.03
27	**VGL1165**	5	2.39	0.64	0.58	-0.11
28	**VGL1828**	3	1.16	0.14	0.14	-0.06
29	**VGL2009**	4	2.44	0.71	0.59	-0.21

30	**VGL2409**	6	3.53	0.79	0.72	-0.1
31	**VGL2918**	7	2.61	0.43	0.62	**0.306**
32	**VGL3008**	7	2.88	0.57	0.65	**0.125**
33	**VGL3235**	5	2.48	0.71	0.6	-0.2

Taken together, these findings corroborate that miniature and toy American Eskimo Dogs have higher genetic diversity than the standard size variety for the 33 autosomal STR loci. This is expected due to the outcrossing needed to obtain these smaller size varieties, and similar to the trend observed in other dog breeds with different size divisions such as the Poodle.

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

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (usually principal coordinates 1 and 2). The closer individuals cluster together around the XY axis, the more closely related they are to each other. Additionally, this approach can be used to determine how different subpopulations have genetically differentiated from each other over time. The three varieties of American Eskimo Dogs are clearly related given their proximity to each other on the plot, with individual dogs reasonably dispersed across all four quadrants of the graph (as expected for a pure breed). Expectedly, the miniature and toy divisions cluster together and are genetically distinguishable from the standard cohort (**Figure 1**). Interestingly, two individuals registered as standard American Eskimo Dogs (red circles) were found among the miniature/toy subpopulation, indicating that they are more closely related to the smaller sized cohort. These might represent individuals who have miniature/toy ancestors at some point in their pedigrees.

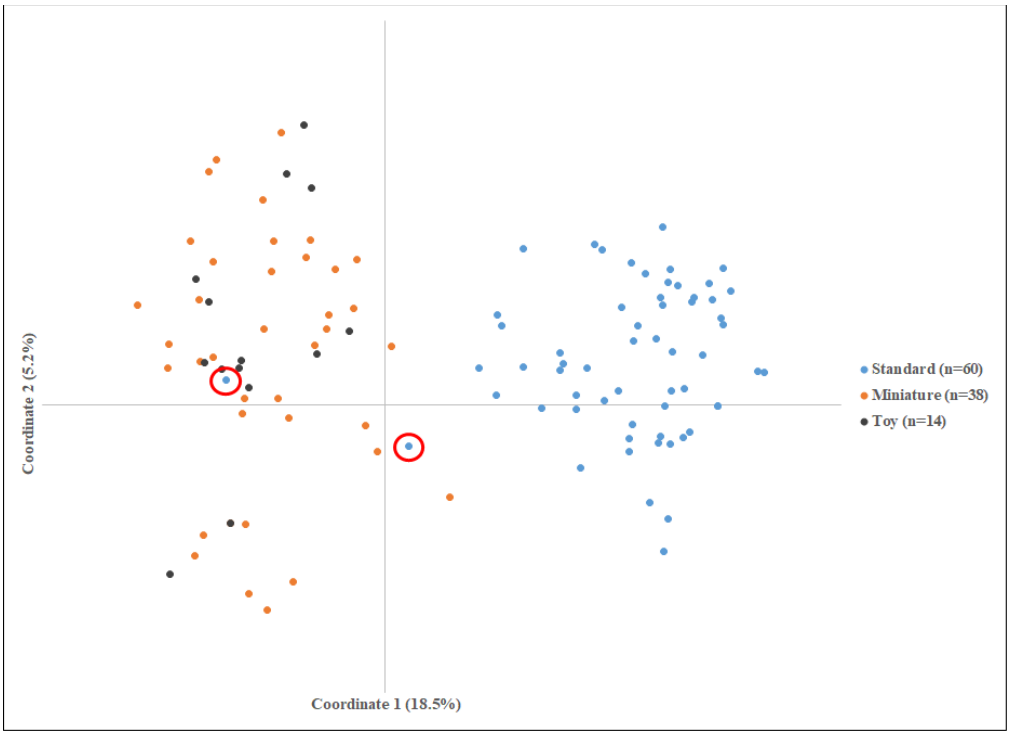


Figure 1. PCoA of American Eskimo Dogs (n= 112 total) based on alleles and allele frequencies at 33 autosomal STR loci. The two standard individuals clustering among the miniature/toy subpopulation are circled in red.

The degree of relatedness among individuals within a breed, and between closely related breeds, can be further enhanced by comparing them to breeds more distantly related. Therefore, 112 standard, miniature and toy American Eskimo Dogs were compared to related (Samoyed and Giant Schnauzer) and unrelated (Italian Greyhound) breeds [7] (**Figure 2**).

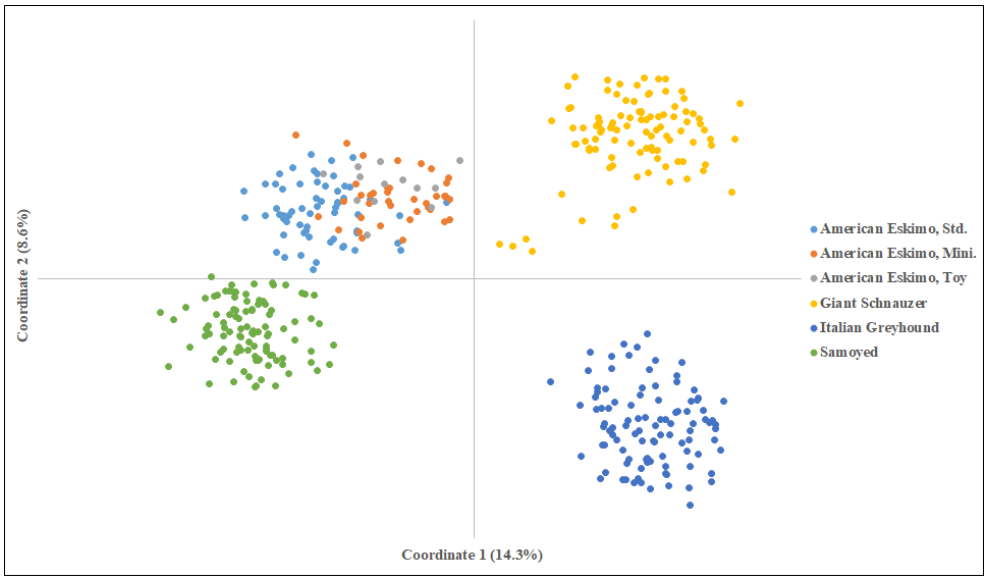


Figure 2. PCoA plot comparing intra- and inter-breed relatedness of American Eskimo Dog (n=112) with Samoyed (n=100), Giant Schnauzer (n=100) and Italian Greyhound (n=100).

As expected, PCoA shows that the three sizes of American Eskimo Dogs are more closely related to the Samoyed and Giant Schnauzer than to the Italian Greyhound. This comparison also confirmed the origins of the miniature and toy from their standard sized ancestors. As expected, standard-sized American Eskimo Dogs group closer to the Samoyed than their smaller-sized counterparts. Interestingly, miniature and toy American Eskimo dogs shared more ancestry with Giant Schnauzers than did their standard sized ancestors.

E. Internal relatedness (IR) of individuals and the population as a whole

1. IR testing

Genetic assessments such as those presented in Tables 4-6 are indicators of population-wide heterozygosity and do not reflect the genetic diversity given to individuals by their parents. Internal Relatedness (IR) is a calculation that determines the degree to which parents of an individual dog are related. This calculation takes into consideration homozygosity at each locus and gives more importance to rare and uncommon alleles, which would presumably be present in less related individuals within a breed. 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 completely unrelated at all 33 autosomal STR loci, whereas a dog with an IR value of +1.0 has parents that are genetically identical at all loci. An IR value of +0.25 would be found among offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents are themselves highly inbred. The higher the IR value is above 0.25, the more closely related are the parents and grandparents of the sibling parents. **Tables 10**, **11**, and **12** summarize the IR values for the 60 standard, 38 miniature, and 14 toy American Eskimo Dogs tested, respectively.

Table 10. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies in 60 standard American Eskimo Dogs.

	IR	IRVD
Min	-0.1983	0.0175
1st Qu	0.0555	0.2514
Mean	0.1456	0.3405
Median	0.1455	0.3539
3rd Qu	0.2503	0.4459
Max	0.4643	0.6263

The most outbred dog in the standard subpopulation had an IR score of -0.198, while the most inbred dog in the group had an IR score of 0.464. The mean IR score for the cohort was 0.145 (**Table 10**). This wide range of IR values shows that the degree of parental relatedness varies greatly in this cohort, a typical finding for almost all pure breeds of dogs. It is important to note that 25% of the standard American Eskimo Dogs tested have IR values between 0.25 and 0.464 (3rd quartile), which indicates that, in a random breeding population, they would be offspring of full sibling parents with highly inbred parents themselves. This is another reflection of relatively low genetic diversity in the cohort.

Table 11. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies in 38 miniature American Eskimo Dogs.

	IR	IRVD
Min	-0.1476	-0.0688
1st Qu	-0.0446	0.0980
Mean	0.0276	0.1689
Median	0.0061	0.1813
3rd Qu	0.0980	0.2450
Max	0.2777	0.3568

The mean IR score for the miniature American Eskimo Dog cohort was 0.027, and the most inbred dog had an IR of 0.277 (**Table 11**). These values reflect the higher genetic diversity of this group in relation to the standard size division, since a negligible portion of the miniature subpopulation has IR values that suggest offspring of full sibling parents with highly inbred parents. A similar trend of higher genetic diversity can be observed from IR values for the 14 toy American Eskimo Dogs used in this study (**Table 12**).

Table 12. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies in 14 toy American Eskimo Dogs.

	IR	IRVD
Min	-0.0757	0.0261
1st Qu	-0.0199	0.1034
Mean	0.0414	0.1896
Median	0.0568	0.2123
3rd Qu	0.0877	0.2568
Max	0.1996	0.3568

In the toy cohort, the range of IR values was narrower with an IR score of -0.075 calculated for the most outbred dog, and 0.199 for the most inbred dog (mean IR = 0.041). These values indicate that this subpopulation is comprised of individuals with reasonably unrelated parents. Collectively, these results indicate that miniature and toy American Eskimo Dogs are being randomly bred.

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

The IR values obtained from known alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to the present day. Village dogs that exist throughout the SE Asia, the Middle East and the Pacific Islands are randomly breeding descendants of dogs from which most modern breeds evolved. The alleles and respective frequencies of a given breed (or subpopulation within a 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 the IR score for a standard American Eskimo Dog would compare to other village dogs if its parents were also village dogs. **Figure 3** shows the curve corresponding to IRVD scores for the 60 standard American Eskimo Dogs (blue line) in relation to their actual IR scores (red line), which represents loss of genetic diversity during breed development.

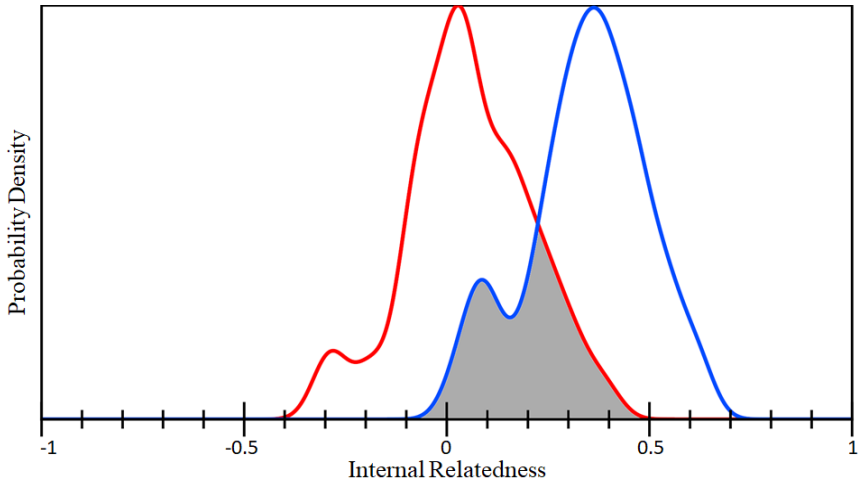


Figure 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for standard American Eskimo Dogs (n=60). The overlap between the curves (shaded in gray) represents the degree of allele sharing (33%) between this cohort and village dogs.

The bimodal distribution of the IRVD values depict the different degrees of parental relatedness listed on **Table 10**. The first peak represents approximately 25% of the standard-sized population with IRVD values below +0.25. Roughly 75% of this cohort (second peak) have IRVD values of +0.25 or greater, which means that if they were village dogs, they would all be considered offspring of at least full sibling parents. Moreover, the gray area in **Figure 3** indicates that standard American Eskimo Dogs retain 33% of the genetic diversity existing in present-day randomly breeding village dogs. This is typical of purebred dog breeds, and similar to the approximately 30% retained genetic diversity calculated for all canids tested at VGL to date (**section IIB**).
In contrast, IRVD values of +0.25 or greater were obtained for approximately 25% of the miniature American Eskimo Dogs tested (**Table 11**, **Figure 4**). The shift to the right in IRVD values (blue line) was not nearly as pronounced as in the standard group, indicating that the miniature size division has retained a greater amount of the genetic diversity present in village dogs (40.4%).

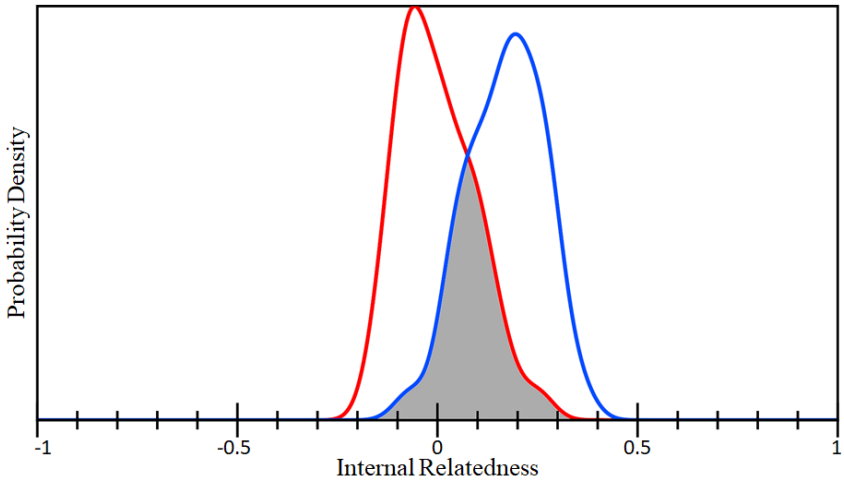


Figure 4. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for miniature American Eskimo Dogs (n=38). The overlap between the curves (shaded in gray) represents the degree of allele sharing (40.4%) between this cohort and village dogs.

Similar findings were obtained for toy American Eskimo Dogs: roughly 25% of the cohort tested had IRVD values of +0.25 or greater, which corresponds to the portion of the population that would be considered offspring of at least full sibling parents if they were village dogs (**Table 12**, **Figure 5**). Approximately 25% of the genetic diversity presently existing in village dogs (represented by the gray are in **Figure 5**) has been retained in the toy cohort, which is less than the standard size division as well as the majority of canids tested at the VGL. However, this value is expected to increase as additional toy American Eskimo Dogs are tested. As in the standard variety, the IRVD curve for this group was also bimodal, with roughly half of the population represented by each peak (IRVD values lower and greater than +0.19).

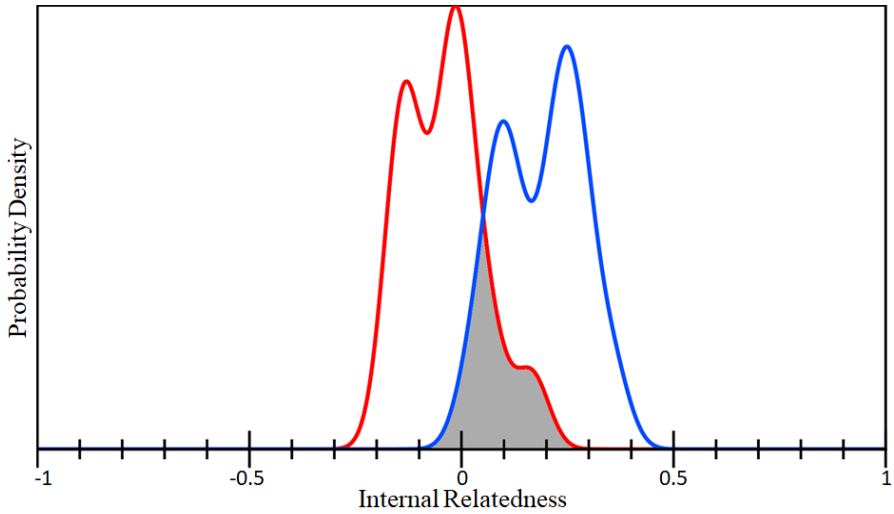


Figure 5. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for toy American Eskimo Dogs (n=14). The overlap between the curves (shaded in gray) represents the degree of allele sharing (25.3%) between this cohort and village dogs.

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

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

The DLA 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 DLA88 are linked (or inherited together) in various combinations, forming specific haplotypes.

The DLA class II region also contains several genes, three of which are highly polymorphic: DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at three STR loci associated with these three class II genes are strongly linked, and often inherited as a single haplotype. An individual inherits one haplotype for both class I and class II genes from each of the parents. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking: class I haplotypes 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 American Eskimo Dog

In total, 11 DLA1 and 10 DLA2 haplotypes were identified in the standard cohort; 11 DLA1 and 12 DLA2 were identified in the miniature cohort; and 7 DLA1 and 9 DLA2 were found in the toy size division (**Table 13**). Expectedly, many of these were shared among the different size varieties. These numbers are similar to those identified in somewhat closely related breeds, such as Giant Schnauzer (14 DLA1 and 15 DLA2 haplotypes) and Samoyed (13 and 12).

In standard American Eskimo Dogs, one DLA1 haplotype (1002) and one DLA2 haplotype (2001) were found at a disproportionally high frequency (both at 65.8%). This suggests that these haplotypes are linked, and that founders possessing these haplotypes have played an important role in maintaining predominant or desirable phenotypes of the breed; therefore, these haplotypes have been highly conserved by descent over time, a typical finding for many pure dog breeds. The 1002/2001 linkage was also identified in miniature and toy American Eskimo Dogs, as well as 1153/2053, which indicates that these haplotypes are being inherited together as a larger extended block (**Table 13**). Contrary to the standard size group, none of the class I or class II haplotype frequencies appear to dominate others in the miniature and toy cohorts. However, those found in higher frequencies (bolded on **Table 13**) were also likely present in the founder population and are being inherited by descent due to their putative association with desirable traits in these subpopulations. Given the number of dogs tested, it is likely that additional haplotypes will be identified (especially in the toy variety), albeit at low frequency.

Table 13. DLA class I and II haplotypes identified in each American Eskimo Dog size division with their respective frequencies. The haplotype with the highest frequency in each cohort is bolded.

DLA1 Haplotype	STR Types	Standard (n=60)	Miniature (n=38)	Toy (n=14)
1002	380 365 281 181	**0.658**	0.14	0.11
1011	376 365 281 180	--	0.01	--
1012	388 369 289 188	0.008	0.04	--
1014	375 373 287 178	0.033	**0.28**	**0.32**
1016	382 371 277 178	0.008	0.04	0.11
1030	380 373 293 178	0.008	0.03	--
1054	382 379 277 184	0.008	0.21	**0.32**
1087	380 371 277 178	0.008	--	--
1093	386 379 277 180	0.008	0.05	0.07
1153	389 373 287 183	0.233	0.11	0.04
1215	376 365 281 182	0.008	0.08	0.04
1261	376 379 277 186	0.017	--	--
1268	380 371 277 184	--	0.01	--

DLA2 Haplotype	STR Types	Standard (n=60)	Miniature (n=38)	Toy (n=14)
2001	343 324 284	**0.658**	0.14	0.11
2003	343 324 282	0.008	0.04	--
2014	339 322 284	0.008	0.03	0.07
2022	339 327 282	0.025	0.21	**0.32**
2023	341 323 282	0.008	0.03	--

2032	339 323 280	--	0.05	0.07
2033	339 323 282	0.008	--	--
2037	341 327 280	0.033	**0.26**	0.25
2050	341 327 284	--	0.01	0.07
2053	343 324 280	0.233	0.12	0.04
2066	339 324 280	0.008	--	--
2080	339 325 276	--	0.08	0.04
2084	339 323 268	--	0.01	0.04
2096	351 322 280	0.008	0.01	--

Further analysis showed that American Eskimo Dogs share DLA-I and DLA-II haplotypes with 38 other dog breeds (**Table 14**). DLA-I haplotype 1261 (which occurs in 1.7% of the study cohort) is unique to standard American Eskimo Dogs, whereas DLA-I haplotype 1268 was found exclusively in 1% of miniature American Eskimo Dogs. No unique DLA-II haplotypes were found in American Eskimo Dogs of any size variety. Interestingly, DLA-I haplotype 1153 (occurring in 23% of standard, 11% of miniature, and 4% of toy American Eskimo Dogs) was found to be shared exclusively with Samoyed (1.8%) – a breed known to share common ancestry with Spitz breeds [7]. Similarly, DLA-II haplotype 2096 (identified in 0.8% of standard and 1% of miniature American Eskimo Dogs) is also shared uniquely with Samoyed (11.3%) and Border Collie (3.3%) (**Table 14**). Class I and II haplotype sharing was also common with other small breeds, such as Havanese, Rat Terrier, and Miniature and Toy Poodle; this might suggest that miniaturization in these breeds might have been achieved through the use of closely related founders.

Finally, DLA haplotype sharing corroborates the PCoA clustering of American Eskimo Dog and Samoyed (**Figure 2**). Although it is not possible to exclude more recent introgressions between the two breeds, this analysis indicates that that American Eskimo Dog and Samoyed share several common ancestors and that this relationship goes back quite a way in their breed's evolution. This is because these breeds only share two unique DLA haplotypes, found at relatively low frequency in each population.

Table 14. Sharing of specific DLA class I and II haplotypes between three size varieties of American Eskimo Dogs (highlighted in different shades of blue) and other dog breeds tested at the VGL (n=43).

DLA1 #	STR types	American Eskimo, Standard (n=60)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Eskimo, (n=155)	Irish Setter (n=49)	Japanese Akita (n=505)	Irish Red and White Setter (n=71)	Alaskan Klee Kai (n=587)	Barbet (n=68)	Irish Wolfhound (n=56)	Lakeland Terrier (n=114)	Border Collie (n=60)	Labrador Retriever (n=207)	Bernese Mountain Dog (n=100)	Ulewellin Setter (n=98)	Black Russian Terrier (n=145)	Magyar Agar (n=77)	Biewer (n=121)	Polish Lowland Sheepdog (n=30)	Biewer Yorshire Terier (n=53)	Toy Poodle (n=178)	Miniature Poodle (n=330)	Biewer Terrier (n=185)	Miniature Poodle (n=330)	Yorkshire Terrier (n=16)	Poodle (n=3798)	Biro Biewer (n=3)	Rat Terrier (n=39)	Borzoi (n=135)	Samoyed (n=191)	Collie (n=49)	Doberman Pinscher (n=854)	Saint Bernard (n=71)	Scottish Collie (n=102)	English Bulldog (n=163)	Shiba Inu (n=125)	English Mastiff (n=26)	Flat Coated Retriever (n=680)	Shikoku (n=63)	Golden Retriever (n=767)	Giant Schnauzer (n=284)	Havanese (n=678)	Italian Greyhound (n=1122)
1002	380 365 281 181	0.658	0.14	0.11	--	--	--	--	--	0.066	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1011	376 365 281 180	--	0.01	--	--	--	--	--	0.0579	--	--	0.075	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0007	--	--	--	--	--
1012	388 369 289 188	0.008	0.04	--	--	--	--	--	--	--	--	0.058	--	--	--	--	0.19	--	0.151	0.227	0.28	0.2	--	--	0.0006	0.414	--	--	--	--	0.0013	--	0.014	0.0085	--	--	--	--	--	--	--	--	--	--	--
1014	375 373 287 178	0.033	0.28	0.32	--	--	--	--	0.3739	--	--	0.075	--	--	--	0.034	0.029	0.029	0.009	0.059	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0404	0.241	0.0258	--	--	--
1016	382 371 277 178	0.008	0.04	0.11	--	--	--	--	--	--	--	--	0.025	0.01	0.025	0.019	0.014	0.03	--	--	0.04	0.03	--	--	0.0181	0.095	0.15	--	0.002	0.044	0.2028	0.0615	--	--	--	--	--	--	--	--	--	--	--	--	--
1030	380 373 293 178	0.008	0.03	--	--	--	--	--	--	0.029	0.008	0.015	--	--	--	0.499	0.349	0.495	0.31	0.5	--	--	--	--	0.0989	--	0.1	--	0.0007	--	0.0022	0.0299	--	--	--	--	--	--	--	--	--	--	--	--	--
1054	382 379 277 184	0.008	0.21	0.32	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1087	380 371 277 178	0.008	--	--	0.013	--	--	--	--	--	0.162	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	0.1118	--	0.004	0.0029	--	--	--	--	--	--	--	--	--	--	--	--
1093	386 379 277 180	0.008	0.05	0.07	--	--	--	--	--	--	--	--	--	--	--	--	0.083	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0192	--	--	--	--	--	--	--	--	--	--	--	--
1153	389 373 287 183	0.233	0.11	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1215	376 365 281 182	0.008	0.08	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1261	376 379 277 186	0.017	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1268	380 371 277 184	--	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 #	STR types	American Eskimo, Standard (n=60)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Eskimo, (n=155)	Irish Setter (n=49)	Japanese Akita (n=505)	Irish Red and White Setter (n=71)	Alaskan Klee Kai (n=587)	Barbet (n=68)	Irish Wolfhound (n=56)	Lakeland Terrier (n=114)	Border Collie (n=60)	Labrador Retriever (n=207)	Bernese Mountain Dog (n=100)	Ulewellin Setter (n=98)	Black Russian Terrier (n=145)	Magyar Agar (n=77)	Biewer (n=121)	Polish Lowland Sheepdog (n=30)	Biewer Yorshire Terier (n=53)	Toy Poodle (n=178)	Miniature Poodle (n=330)	Biewer Terrier (n=185)	Miniature Poodle (n=330)	Yorkshire Terrier (n=16)	Poodle (n=3798)	Biro Biewer (n=3)	Rat Terrier (n=39)	Borzoi (n=135)	Samoyed (n=191)	Collie (n=49)	Doberman Pinscher (n=854)	Saint Bernard (n=71)	Scottish Collie (n=102)	English Bulldog (n=163)	Shiba Inu (n=125)	English Mastiff (n=26)	Flat Coated Retriever (n=680)	Shikoku (n=63)	Golden Retriever (n=767)	Giant Schnauzer (n=284)	Havanese (n=678)	Italian Greyhound (n=1122)	Swedish Vallhund (n=328)	Whippet (n=77)
1002	380 365 281 181	0.658	0.14	0.11	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	0.002	0.166	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.045
1011	376 365 281 180	--	0.01	--	0.1	0.028	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.039	0.003	0.0188	0.05	0.272	0.07	--	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1012	388 369 289 188	0.008	0.04	--	--	--	--	--	--	0.768	--	--	--	--	--	--	--	--	--	--	--	0.022	0.005	0.0133	0.05	0.013	--	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.052
1014	375 373 287 178	0.033	0.28	0.32	0.37	0.035	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.07	0.003	0.0093	0.01	0.003	--	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1016	382 371 277 178	0.008	0.04	0.11	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.03	0.028	0.027	0.0213	0.05	--	--	0.088	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.104
1030	380 373 293 178	0.008	0.03	--	--	--	--	--	--	--	--	0.051	0.026	--	--	--	--	--	--	--	--	--	--	0.0022	0.03	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1054	382 379 277 184	0.008	0.21	0.32	0.18	--	--	--	--	--	--	0.072	0.087	0.143	--	--	--	--	--	--	--	--	0.002	--	0.01	--	--	--	--	0.348	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006
1087	380 371 277 178	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1	--	--	0.015	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1093	386 379 277 180	0.008	0.05	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.593	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1153	389 373 287 183	0.233	0.11	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.018	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1215	376 365 281 182	0.008	0.08	0.04	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1261	376 379 277 186	0.017	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1268	380 371 277 184	--	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	American Eskimo, Standard (n=60)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	America n Akita (n=155)	Japanese Akita (n=505)	Alaskan Klee Kai (n=587)	Barbet (n=68)	Border Collie (n=60)	Bernese Mountain Dog (n=100)	Black Russian Terrier (n=145)	Biewer (n=121)	Biewer Yorshire Terrier (n=53)	Polish Lowland Sheepdog (n=30)	Toy Poodle (n=178)	Miniature Poodle (n=330)	Biewer Terrier (n=185)	Yorkshir e Terrier (n=16)	Poodle (n=3798)	Bro Biewer (n=3)	Rat Terrier (n=39)	Borzoi (n=135)	Samoyed (n=191)	Collie (n=49)	Doberman Pinscher (n=854)	Saint Bernard (n=71)	Scottish Collie (n=102)	English Bulldog (n=163)	Shiba Inu (n=125)	English Mastiff (n=26)	Flat Coated Retriever (n=680)	Shikoku (n=63)	Golden Retriever (n=767)	Giant Schnauzer (n=284)	Havanese (n=678)	Italian Greyhound (n=1122)
2001	343 324 284	0.658	0.14	0.11	--	--	--	0.199	0.142	0.058	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2003	343 324 282	0.008	0.04	--	--	--	--	0.132	0.058	--	--	--	--	--	--	--	0.231	0.208	0.246	0.38	0.2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2014	339 322 284	0.008	0.03	0.07	--	--	0.0698	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.092	0.15	0.0294	--	0.005	0.0059	0.0018	--	--	--	--	--	--	--	--	--
2022	339 327 282	0.025	0.21	0.32	--	--	--	--	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.593	--	0.0012	0.015	--	0.1176	0.0007	0.004	0.118	--	--	--	--	--	--	--	--	--	--	--
2023	341 323 282	0.008	0.03	--	--	--	--	0.029	0.008	--	--	0.003	0.459	0.349	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0988	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2032	339 323 280	--	0.05	0.07	--	--	--	0.162	--	--	--	0.083	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2033	339 323 282	0.008	--	0.013	--	--	--	--	0.008	--	--	0.51	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2037	341 327 280	0.033	0.26	0.25	0.181	0.2525	0.3739	--	0.075	--	--	0.283	0.029	0.009	0.009	0.059	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2050	341 327 284	--	0.01	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2053	343 324 280	0.233	0.12	0.04	--	--	--	--	0.075	0.065	--	--	--	0.009	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	--	--	0.1404	0.0306	0.058	--	--	--	--	--	--	--	--	--	--	--
2066	339 324 280	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.007	--	--	--	--	--	--	0.0007	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2080	339 325 276	--	0.08	0.04	--	--	--	--	--	--	--	--	0.004	0.009	--	--	--	0.03	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0332	0.1785	--	--	--	--	--	--	--	--	--	--
2084	339 323 268	--	0.01	0.04	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2096	351 322 280	0.008	0.01	--	--	--	--	--	0.033	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	American Eskimo, Standard (n=60)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	Irish Setter (n=49)	Irish Red and White Setter (n=71)	Irish Wolfhound (n=56)	Lakeland Terrier (n=114)	Labrador Retriever (n=207)	Ulewellin Setter (n=98)	Magyar Agar (n=77)	Polish Lowland Sheepdog (n=30)	Toy Poodle (n=178)	Miniature Poodle (n=330)	Biewer Terrier (n=185)	Miniature Poodle (n=330)	Yorkshire Terrier (n=16)	Poodle (n=3798)	Rat Terrier (n=39)	Borzoi (n=135)	Samoyed (n=191)	Scottish Collie (n=102)	Shiba Inu (n=125)	Shikoku (n=63)	Shiloh Shepherd (n=11)	Shiloh Shepherd, ISSA (n=231)	Swedish Vallhund (n=328)	Whippet (n=77)
2001	343 324 284	0.658	0.14	0.11	--	--	--	--	0.002	--	0.071	--	0.006	0.012	--	0.6048	0.01	--	--	--	--	0.014	--	0.012	--	--	--	0.004	--	0.065
2003	343 324 282	0.008	0.04	--	--	--	--	0.768	0.014	--	--	--	0.424	0.521	0.0981	0.1	0.013	--	0.02	--	--	--	--	--	--	--	--	0.006	--	0.117
2014	339 322 284	0.008	0.03	0.07	--	--	--	0.009	0.002	--	--	0.68	0.025	0.026	0.0195	0.03	--	0.085	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2022	339 327 282	0.025	0.21	0.32	0.18	--	0.384	0.162	0.072	0.087	--	--	0.008	0.002	0.0001	0.15	0.107	--	0.162	--	--	--	--	--	0.05	0.05	0.047	--	--
2023	341 323 282	0.008	0.03	--	--	--	--	--	0.051	0.015	--	--	--	--	0.0022	0.03	--	0.401	--	--	--	--	--	--	--	--	--	--	--	--
2032	339 323 280	--	0.05	0.07	--	--	--	--	--	--	--	--	--	0.011	--	--	--	--	--	--	--	--	0.008	--	--	--	--	--	--	--
2033	339 323 282	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.143
2037	341 327 280	0.033	0.26	0.25	0.37	--	--	--	--	--	0.013	--	0.008	0.006	0.0001	0.06	--	--	--	0.005	--	--	--	--	--	--	--	--	--	--
2050	341 327 284	--	0.01	0.07	--	--	--	--	--	--	--	--	0.065	--	--	0.01	0.003	--	--	--	--	--	--	--	--	--	--	--	--	--
2053	343 324 280	0.233	0.12	0.04	0.01	--	0.08	--	0.039	--	--	--	0.011	0.015	--	--	0.56	0.162	--	--	--	--	--	--	0.5	0.329	0.506	--	--
2066	339 324 280	0.008	--	--	--	--	--	--	--	--	--	--	--	0.029	0.0001	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2080	339 325 276	--	0.08	0.04	--	--	--	--	0.196	--	--	--	--	--	--	--	--	0.07	0.02	--	--	--	--	--	0.23	0.223	--	--	--
2084	339 323 268	--	0.01	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.175	--	--
2096	351 322 280	0.008	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	0.113	--	--	--	--	--	--	--	--	--	--	--	--

2. Heterozygosity in the DLA region

Due to their physical proximity in canine chromosome 12, the seven loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to other parts of the genome as measured by the 33 autosomal STR markers. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will be inherited randomly as well. This assumption can be tested through a standard genetic assessment of each locus (**Tables 15-17**) and average values across all loci (**Tables 18-20**).

In standard American Eskimo Dogs, the number of alleles (Na) at each locus ranged from 3 to 8 (mean=5.14), but like the 33 STR markers across the genome, the number of effective alleles (Ne) per locus within this region was low (mean=1.74) (**Tables 15** and **18**). The lower Na and Ne values for alleles in the DLA class I and II regions in this cohort may be the result of an over-representation of founders or founder lines with the 1002/2001 and 1153/2053 extended haplotypes. The average observed (Ho) and expected (He) heterozygosity values for the DLA region were similar (0.39 and 0.4, respectively), yielding an inbreeding coefficient (F) of 0.05 (**Table 18**). The resulting F value indicates a 5% excess in inbreeding calculated from DLA alleles, slightly lower than that obtained from the 33 autosomal STR markers, calculated at 6.8% (**Table 4**). Therefore, it can be concluded that: 1) the DLA region has less genetic diversity than the other regions of the genome defined by the 33 STR loci; 2) it has nonetheless reached an equilibrium with other parts of the genome over the period of breed development; 3) the population of 60 standard dogs tested is somewhat more inbred than the population from which it was selected; and 4) this excess of inbred dogs may have been selected from among those possessing the dominant DLA class I/II haplotype(s).

Similar correlations between genetic assessments utilizing the 33 autosomal STR loci and the 7 DLA loci were found in the miniature **(Tables 16** and **19**) and toy (**Tables 17** and **20**) cohorts. Genetic diversity in the DLA region was higher in these subpopulations than that calculated for the standard variety, with values mirroring those obtained for the 33 autosomal markers (**Tables 5** and **6**). However, in miniature American Eskimo Dogs the average inbreeding coefficient calculated for the 7 DLA STR loci (5.8%) was significantly higher than the F value obtained using autosomal STR markers (close to zero). This implies that this small subpopulation is more inbred in the DLA region than in other parts of the genome covered by the 33 STR loci. This degree of positive artificial selection is most likely inadvertent, caused either by the relatively small number of dogs tested herein or by the presence of a highly desired trait(s) in dogs possessing certain haplotypes. Finally, testing of more individuals of the toy variety is warranted in order to obtain a more comprehensive and accurate picture of genetic diversity within the DLA class I and II regions in this subpopulation of American Eskimo Dogs.

Table 15. Standard genetic assessment for standard American Eskimo Dogs using each of the 7 STRs in the DLA class I and II regions (n=60).

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	7	1.952	0.483	0.488	0.009
DLA I-4ACA	5	1.917	0.483	0.478	-0.01
DLA I-4BCT	5	1.93	0.5	0.482	-0.04
DLA1131	8	2.033	0.483	0.508	0.049
5ACA	4	1.228	0.15	0.186	0.192
5ACT	4	1.206	0.15	0.171	0.123
5BCA	3	1.897	0.467	0.473	0.013

Table 16. Standard genetic assessment for miniature American Eskimo Dogs using each of the 7 STRs in the DLA class I and II regions (n=38).

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	7	5.085	0.816	0.803	-0.016
DLA I-4ACA	5	3.378	0.579	0.704	0.178
DLA I-4BCT	5	3.293	0.632	0.696	0.093
DLA1131	7	4.734	0.711	0.789	0.099
5ACA	4	3.04	0.632	0.671	0.059
5ACT	5	2.9	0.658	0.655	-0.004
5BCA	5	3.156	0.684	0.683	-0.002

Table 17. Standard genetic assessment for toy American Eskimo Dogs using each of the 7 STRs in the DLA class I and II regions (n=14).

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	6	3.267	0.857	0.694	-0.235
DLA I-4ACA	4	3.187	0.786	0.686	-0.145
DLA I-4BCT	3	2.513	0.786	0.602	-0.305
DLA1131	6	3.267	0.786	0.694	-0.132
5ACA	3	2.435	0.714	0.589	-0.212
5ACT	5	2.215	0.643	0.548	-0.172
5BCA	5	3.379	0.857	0.704	-0.217

Table 18. Summary of standard genetic assessment for standard American Eskimo Dogs using 7 STRs in the DLA class I and II regions (n=60).

	Na	Ne	Ho	He	F
Mean	5.14	1.738	0.388	0.398	0.048
SE	0.62	0.125	0.057	0.053	0.029

Table 19. Summary of standard genetic assessment for miniature American Eskimo Dogs using 7 STRs in the DLA class I and II regions (n=38).

	Na	Ne	Ho	He	F
Mean	5.43	3.655	0.673	0.715	0.058
SE	0.4	0.307	0.027	0.02	0.025

Table 20. Summary of standard genetic assessment for toy American Eskimo Dogs using 7 STRs in the DLA class I and II regions (n=14).

	Na	Ne	Ho	He	F
Mean	4.57	2.895	0.776	0.645	-0.203
SE	0.45	0.17	0.027	0.022	0.021

III. What does this assessment of genetic diversity tell us about the American Eskimo Dog.

American Eskimo Dogs, like many other less populous breeds, have a relatively **low** level of genetic diversity, with miniature and toy size divisions being relatively more genetically diverse than the standard-sized variety. This lack of genetic diversity is even greater in the important DLA region than in other parts of the genome. These findings indicate that the breed was developed from a relatively small number of founders, and that closely related founder lines have contributed significantly in relation to others over time. Nonetheless, similar to other dog breeds comprised of smaller size varieties such as the Poodle, the use of outcrossing to achieve miniaturization might have contributed to the greater genetic diversity found for the 33 autosomal STR loci in miniature and toy American Eskimo Dogs. Overall, the dogs tested herein represent a good cross-section of the breed, with some tendency to include more inbred dogs that possess the dominant DLA class I/II haplotype. However, more individuals of the toy division need to be tested in order to obtain a more comprehensive and reliable picture of genetic diversity within this group. The relative lack of genetic diversity in American Eskimo Dogs probably exists since the earliest time of breed development, including periods when prototypes of the breed became popular as circus dogs in the 1930's and 1940's. The closing of the studbook in 1969 terminated further phenotypic and genotypic changes. There has also been limited opportunity over the last 50 years, given the small number of individuals and relative obscurity of the breed, for the creation of further genetic bottlenecks. The breed has entered a new era of popularity that often fosters genetic bottlenecks. However, a lack of genetic diversity is not inherently bad if the original founding stock lacked heritable diseases and if a further loss or imbalance in the existing diversity has been avoided. So far, no breed-specific inherited diseases have been reported in the American Eskimo Dog and most individuals of the breed have a long-life expectancy (even though some canine diseases are known to occur in the breed – see next section). Nonetheless, it is more important for less populous breeds, even if relatively healthy, to maintain existing genetic diversity by breeding the least related parents possible.

As observed in other pure dog breeds, the standard subpopulation consisted of many dogs that were much more inbred than others based on IR values: approximately 25% of the dogs tested were as inbred as offspring equivalent to full sibling parents from within the breed. Therefore, breeders of standard American Eskimo Dogs should be aware of this when selecting mates for their breeding programs, in order to redistribute the diversity that currently exists in this group – the goal is to produce dogs with IR scores lower than zero. As for the miniature and toy groups, distribution of IR values across the individuals tested herein show that a negligible fraction of these cohorts is highly inbred (IR values greater than +0.25). This indicates that breeders are doing a

good job of maintaining diversity in these varieties by choosing the least possible related parents for their mating programs.

It will be very important for breeders to not only test more dogs of the standard variety, but also individuals of the miniature and toy types. Genetic diversity can be increased in the breed as a whole by finding outliers that significantly differ genetically from the major population, or alternatively by outcrossing to breeds with similar phenotypes. Based on this study, more closely related Samoyeds could serve as a potential source of genetic diversity if this was ever a goal of the American Eskimo Dog breeders. However, outcrossing must be well planned to avoid introduction of additional genetic disorders or risking dilution or loss of existing diversity. The use of modern DNA technology makes it much easier to identify new diversity and as well as to safely introduce it into a breed.

Health of the American Eskimo Dog

A. Lifespan

The breed's average lifespan is 13 to 15 years [2].

B. Diseases

1. Progressive Retinal Atrophy (PRA)

Progressive retinal atrophy (PRA) is a medical classification that represents several inherited forms of retinal degeneration that are caused by mutations in different genes. One of these disease forms that affects American Eskimo Dogs is called Progressive rod-cone degeneration (PRCD). The age of onset and rate of progression vary among breeds, but retinal changes can be identified by screening performed by a veterinary ophthalmologist from adolescence to early adulthood. Most PRCD-affected dogs have noticeable visual impairment by 4 years of age, typically progressing to complete blindness. A genetic test for PRCD-PRA at any age, as well as an OFA or CERF eye exam after the age of 24 months, are listed as part of the AEDCA's health clearances and requirements [8]. The VGL offers genetic testing for the mutation that causes PRCD in several dog breeds (https://vgl.ucdavis.edu/test/pra-prcd).

2. Hip Dysplasia (HD)

As is the case of most medium and large sized dog breeds, American Eskimo Dogs can also suffer from hip dysplasia. HD results from an unstable hip socket and leads to a degenerative, sometimes crippling, arthritis in mature dogs. An OFA hip exam after the age of 24 months is listed on the AEDCA's health clearances and requirements [8].

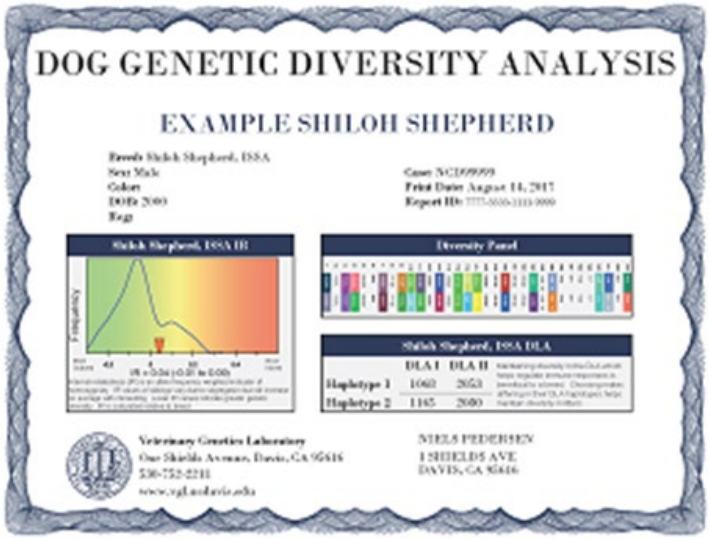
3. Diabetes mellitus (DM)

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia (high levels of blood sugar), glycosuria (presence of glucose in urine) and weight loss. These symptoms are the result of an absolute or relative deficiency in the hormone insulin. Genetic factors play an important role in breed susceptibility to this disorder; associations between DM and mutations in different genes have been found in various breeds of dogs [9], but not yet in the American Eskimo Dog. A recent study reported an estimated heritability of 62% for DM using a group of 156 American Eskimo Dogs, all of which belonged to an extended inter-breeding family. The authors hypothesized that DM transmission follows a polygenic inheritance pattern (i.e., mutations in several genes contribute to the disease) in American Eskimo Dogs, although the genes have yet to be identified [10].

V. Results of VGL Canine Diversity Testing

A. How will you be given the results of DNA-based genetic diversity testing on your dog?

After a sample is submitted for genetic testing, the identity of the dog and owner will be replaced by a laboratory barcode identifier. This identifier will be used for all subsequent activities and each owner will be provided with a certificate that reports the internal relatedness, genomic STR genotypes and DLA class I and II haplotypes for the dog(s) tested. The internal relatedness value for the dog being tested is reported in relation to others in the population. The alleles at each of the 33 STR loci are presented as numbers that correspond to those found in Table 1. Each locus will have two alleles, which can be different (heterozygous) or the same (homozygous). Each allele is inherited from one of the parents. Dogs from closely related parents will be homozygous for more alleles at each locus, or in regions of the genome that are under strong positive selection for phenotypic trait or traits mostly favored in the breed. Dogs with a predominance of rare (i.e., low frequency) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high frequency) alleles. A sample genetic diversity report is shown below.


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE SHILOH SHEPHERD

Shiloh Shepherd, ISSA

Sex: Male

Color:

DOB: 2/03

Reg:

Case: SGD09999

Print Date: August 14, 2017

Report ID: ????-????-????-????

Shiloh Shepherd, ISSA III

Diversity Panel

Shiloh Shepherd, ISSA DLA

	DLA I	DLA II
Haplotype 1	1060	2053
Haplotype 2	1165	2000

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B. What should you do with this information?

DNA testing for genetic diversity in the American Eskimo Dog shows a low level of genome-wide genetic diversity, and inbreeding in a fraction of the study cohort (especially in the standard variety). The number of DLA1 and DLA2 haplotypes identified in this cohort was relatively low when compared to other breeds. This is an indication that a single founder or closely related founder lineage has had a strong influence on the contemporary American Eskimo Dog. Nonetheless, as expected, diversity was greater in this region in smaller sized varieties. Our results indicate that breeding of American Eskimo Dogs needs to be carefully managed with the goal of maintaining and/or redistributing the existing diversity more evenly across the population. It is important to monitor existing diversity into the future both across the genome and in the DLA region. We believe that this can be most accurately done with DNA testing and the use of interrelatedness scores and DLA-I/II haplotypes to better balance and maintain genetic diversity and as a supplement to in-depth pedigrees.

If the breed were to consider increasing genetic diversity by further genetic introgressions or outcrossing, DNA testing of dogs intended for such practices would also be essential in order to

avoid deleterious mutations and to ensure 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 close to zero, and as informed breeding decisions are made, even lower scores. Mates should be preferably selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype; moreover, mating of dogs with less frequent genomic alleles or DLA haplotypes is encouraged. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity. However, because IR values reflect the unique genetics of individuals, they cannot be used as the primary criterion for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, breeding dogs with high IR values (providing they are genetically different) may produce puppies with much lower IR scores than either parent. A mating between a dog with a high IR value and one with low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies could have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

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

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

VI. References

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