Genetic Diversity Testing for Havana Silk 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 distribute genetic diversity as a supplement to in-depth pedigrees. Information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions in order to improve the overall genetic health of a breed.

Genetic diversity testing of the Havana Silk Dog ("Havana Silk") 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 29 individuals registered with the Havana Silk Dog Association of America (HSDAA). Although results reported herein are preliminary, this cohort of individuals should provide a reasonable picture of genetic diversity in the breed. Allele and DLA haplotype frequencies will be updated as more dogs are tested.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from different regions of the genome were used to assess genetic heterogeneity and existing genetic diversity within an individual as well as across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity and genetic diversity in individuals as well as breed-wide.

DLA haplotypes: Seven STR loci linked to DLA class I and II genes were used to assess genetic diversity within a region that regulates immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with environmental factors, are responsible for autoimmune disease, allergies, and susceptibility to infectious agents.

Internal Relatedness (IR): 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 Havana Silk

A. Breed History [1-3]

As their name implies, the Havana Silk Dog is derived from the Havanese breed of bichon dogs, originated in Cuba in the 1700's. Efforts to develop the Havana Silk began in 2000, when a group of Havanese breeders from the United States and Canada decided to start only breeding dogs with long, straight forelegs. In order to accomplish this, stock from Russia and post-revolutionary Cuba were also used. This decision was made due to two reasons: first, in order to re-create the original look of the Havanese breed based on older paintings, sculptures, and manuscripts. Second, and most importantly, these breeders aimed to breed away from osteochondrodysplasia (a form of dwarfism that is found in modern Havanese from the United States and Europe) and its many associated health issues. In addition to straight legs and sound health, in just a few generations these dogs displayed leaner bone structure, flatter and silkier coat, smaller ears, a longer muzzle, and a more 'elegant' body.

In 2007, breeders involved in the development of the Havana Silk Dog decided to split from Havanese breeders and created the Havana Silk Dog Association of America (HSDAA) with the purpose of preventing further interbreeding between the two breeds. In order to maintain the strict health and conformation guidelines established for the breed, the Havana Silk Dog Association of America requires an OFA/CHIC number, a DNA profile, and a physical evaluation with the goal to exclude dogs displaying short or bowed forelegs and other deviations from the breed's standard from the breeding stock. The Havana Silk Dog is not recognized as a breed by the American Kennel Club (AKC).

It is important to note that development of the Havana Silk Dog involves two major genetic bottlenecks, or founder effects. A genetic bottleneck can be described as a sudden and pronounced decline in the number of reproductively active individuals from a population. The first genetic bottleneck experienced by this breed occurred around 40 years before its inception: modern Havanese, which originated the Havana Silk, stem from only 11 individuals that were brought to the United States by upper class Cubans fleeing the Cuban Revolution. Subsequently, a second genetic bottleneck was introduced in 2007 when a small subpopulation of individuals with the desired phenotypic traits was selected to develop the Havana Silk.

B. Appearance [2-3]

According to the HSDAA breed standard, dogs are small but sturdy, elegant but athletic, and have long, flat, silky hair of different colors. Their height varies between 9 and 11 inches. The body is a little longer than the height, with the tail carried high and arched over the back. The coat is dense with no undercoat present, and any color or combination of colors is accepted as long as the nose is black (or dark brown in the case of chocolate dogs). The HSDAA lists the following disqualifications: dilute pigment on the body and/or nose, merle pattern, blue eyes, and bowing of the forelegs or valgus deviation of the carpus.

C. Temperament [2,3]

The Havana Silk Dog is a typical companion breed, and as such, its temperament is outgoing and friendly. They are intelligent, gentle, patient, and good with children. Their reservation towards people and other dogs should resolve upon introduction.

D. Health of the Havana Silk Dog [2-3]

1. Lifespan

The Havana Silk is a very healthy breed, with a lifespan of 10 to 15 years.

2. Diseases

The Havana Silk Dog Association of America (HSDAA) is one of the few dog registries in the world that requires all breeding dogs to pass designated health clearances in order to register a litter. Results of these required exams should be submitted to OFA. These include screening for congenital patella luxation and congenital heart defects at 8 weeks old (dogs must be re-screened at 12 months of age). In addition, after 12 months of age, breeding dogs should be screened for hip dysplasia, eye disease, hearing status, and submit soaped photos for the evaluation of straight legs, proper proportions, shoulders, structure and angulation of the dog as well as a physical conformation evaluation. They also need have a DNA profile recorded for research purposes as well as bank their DNA with OFA. They must submit a CBC and Blood Chemistry Panel as well as paired Bile Acids showing normal liver function.

In December of 2021, the HSDAA reported the following disease frequencies in the Havana Silk based on OFA statistics for the breed:

1) Congenital patella luxation: 98.3% normal screenings.
2) Congenital heart defects: 100% normal screenings.
3) Hearing – BAER exam: 99.1% normal screenings.
4) Vision – CERF exam: 99.6% normal screenings.
5) Hip dysplasia – 82.4% normal screenings.
6) Serum Bile Acid: 100% normal screenings.

II. Preliminary Results on Genetic Diversity of 29 Havana Silk Dogs

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

STR markers are multiallelic, 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 [4,5]. Each STR locus contains 7 to 29 different alleles (average of 15.4 alleles/locus) in the breeds tested at the VGL so far. Dog breeds, having evolved from a small number of founders and having been exposed to artificial population bottlenecks, will end up with

only a portion of the total available genetic diversity found in canids. Artificial genetic bottlenecks can include phenomena such as sire effects, geographic isolation, catastrophes, outbreaks of disease, and variation in popularity, which can lead to a decrease in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 29 Havana Silk individuals in this study are listed in **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Havana Silk Dogs (n=29). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
92 (0.10)	131 (0.19)	119 (0.24)	219 (0.19)	244 (0.07)	**87 (0.38)**
96 (0.03)	137 (0.14)	121 (0.14)	225 (0.05)	246 (0.29)	89 (0.28)
100 (0.22)	**141 (0.40)**	125 (0.24)	227 (0.09)	**248 (0.40)**	95 (0.34)
102 (0.07)	143 (0.02)	**127 (0.28)**	**229 (0.40)**	250 (0.10)	
104 (0.22)	147 (0.24)	129 (0.07)	231 (0.26)	254 (0.14)	
106 (0.07)	149 (0.02)	131 (0.03)	233 (0.02)		
108 (0.28)					
AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**
286 (0.09)	116 (0.10)	**132 (0.74)**	152 (0.03)	232 (0.17)	91 (0.03)
288 (0.55)	**118 (0.33)**	144 (0.21)	**156 (0.36)**	**236 (0.40)**	95 (0.24)
290 (0.09)	120 (0.02)	148 (0.02)	160 (0.02)	238 (0.09)	**97 (0.48)**
292 (0.28)	124 (0.33)	152 (0.03)	164 (0.17)	240 (0.10)	99 (0.03)
	126 (0.09)		168 (0.26)	242 (0.24)	101 (0.19)
	130 (0.14)		172 (0.03)		103 (0.02)
			176 (0.12)		
INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
124 (0.74)	**144 (0.55)**	**210 (0.36)**	**85 (0.93)**	227 (0.02)	202 (0.05)
126 (0.10)	148 (0.02)	212 (0.29)	95 (0.03)	229 (0.03)	204 (0.12)
128 (0.02)	150 (0.09)	214 (0.22)	107 (0.03)	231 (0.31)	206 (0.38)
132 (0.03)	152 (0.34)	218 (0.12)		233 (0.16)	**208 (0.40)**
138 (0.10)				237 (0.14)	210 (0.05)
				241 (0.34)	
REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.12)	160 (0.07)	268 (0.22)	222 (0.16)	139 (0.05)	12 (0.02)
210 (0.33)	**162 (0.62)**	**270 (0.31)**	**226 (0.22)**	143 (0.02)	13 (0.09)
212 (0.03)	164 (0.19)	272 (0.29)	228 (0.07)	145 (0.05)	14 (0.03)
214 (0.12)	166 (0.05)	276 (0.12)	232 (0.22)	147 (0.24)	18.2 (0.14)
216 (0.21)	168 (0.03)	278 (0.05)	236 (0.22)	149 (0.22)	20.2 (0.03)
218 (0.05)	170 (0.03)		238 (0.10)	**153 (0.36)**	21.2 (0.07)
220 (0.14)				155 (0.05)	**22.2 (0.34)**
					23.2 (0.21)
					24.2 (0.07)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
16.1 (0.10)	8 (0.16)	18 (0.14)	15 (0.09)	11 (0.26)	14 (0.02)
17.1 (0.34)	12 (0.05)	19 (0.17)	**16 (0.45)**	12 (0.03)	**16 (0.34)**
18.1 (0.05)	13 (0.05)	21 (0.12)	19 (0.07)	13 (0.19)	17 (0.29)
19.1 (0.21)	14 (0.10)	**26 (0.34)**	20 (0.10)	**14 (0.34)**	18 (0.29)
21.1 (0.19)	15 (0.05)	27 (0.09)	21 (0.29)	15 (0.17)	20 (0.05)
22 (0.03)	17 (0.12)	28 (0.09)			
22.1 (0.05)	18 (0.05)	29 (0.05)			
23.1 (0.02)	19 (0.03)				
	20 (0.21)				
	21 (0.07)				
	22 (0.10)				

VGL2918	VGL3008	VGL3235
12 (0.05)	13 (0.03)	**13 (0.60)**
13 (0.64)	15 (0.02)	14 (0.12)
14 (0.12)	17 (0.03)	15 (0.10)
16 (0.07)	18 (0.26)	18 (0.09)
18.3 (0.05)	19 (0.22)	19 (0.09)
22.3 (0.07)	20 (0.02)	
	21 (0.38)	
	22 (0.03)	

Allele distribution within the 33 autosomal STR loci is typical of most dog breeds, in which one or two alleles are observed at higher frequency than others (bold on **Table 1**). The number of alleles found for each STR locus in the Havana Silk Dog was quite polymorphic, ranging from three (AHTk211 and LEI004) to 11 (VGL1063) with an average of 5.8 alleles per locus (**Table 2**). However, unlike other breeds in which one allele occurs at a disproportionately higher frequency due to a strong founder effect, STR allele frequencies per locus are more evenly distributed in Havana Silk Dogs. This indicates that mate selection has been aimed at distributing the genetic diversity available at the time of breed development. Predominance of a single allele was identified in only one marker (LEI004), with a frequency of 93% (**Table 1**). This near fixation of a single allele indicates that this locus is associated with a breed defining trait that has been strongly conserved throughout breed development. Since only 29 individuals were genotyped as part of this study, we expect additional alleles to be identified but likely at low number and frequency as more individuals are tested.

B. Assessment of population diversity using standard genetic parameters

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

Weinberg equilibrium (i.e., randomly breeding); and the coefficient of inbreeding (**F**) derived from Ho and He values.

Table 2. Genetic Assessment of 29 Havana Silk Dogs based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	5.82	3.67	0.73	0.69	-0.06
SE	0.29	0.23	0.03	0.03	0.018

The average number alleles (Na) known to exist at the 33 STR loci across breeds, based on all dog breeds tested at the VGL so far, is 15.4 (see section IIA). In the case of the Havana Silk Dog, this number was estimated at 5.82 (**Table 2**). This means that approximately 37.8% of the available allelic diversity has been retained in Havana Silk Dogs. This proportion of retained canid-wide genetic diversity is comparable to that of Irish Red and White Setter (34.8%) and Flat Coated Retriever (38.6%), for example. However, the average number of effective alleles (Ne) constitutes a more important metric for diversity, since these alleles have the greatest genetic influence on heterozygosity. This number was estimated at 3.67 for this cohort, indicating that the bulk of genetic diversity for the dogs tested was determined by approximately 50% of the alleles segregating in the breed. This is typical for most pure breeds of dogs.

The mean observed heterozygosity estimated for this group of dogs (Ho = 0.73) was higher than the estimated expected heterozygosity (He = 0.69). This yielded a negative coefficient of inbreeding (F = -0.06), an outcrossed group of dogs. Therefore, this cohort was carefully selected for maximal unrelatedness, and it appears that Havana Silk Dog breeders are efficiently distributing the available genetic diversity through mate selection.

However, the aforementioned values were estimated for the entire cohort and not for individual dogs making up the population. Internal Relatedness (IR) scores provide a better picture of heterozygosity for each dog and should be used by breeders to select the most unrelated mates possible (see section E below).

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each STR locus (**Table 3**). This provides an estimate of genetic similarities in the genomic regions associated with each STR marker. The average number of effective alleles (Ne) per locus across individuals ranged from 1.15 (LEI004) to 8.37 alleles (VGL1063). The lowest average observed heterozygosity (Ho) for an individual STR locus was 0.14 (LEI004), whereas the highest was estimated at 0.97 (VGL1063 and VGL1165). Average expected heterozygosity (He) values ranged from 0.13 (LEI004) to 0.88 (VGL1063) (**Table 3**).

Loci with the lowest Ho values contribute the least to heterozygosity levels across the breed; they are most likely associated with inherited traits that are important for the breed's phenotypic standard. Conversely, high Ho values for a particular locus means that it shows greater genetic diversity across the breed, and that these loci can be associated with phenotypic variation among individuals. The values for Ho and He are used to calculate what is known as inbreeding coefficient

(or F), which is a measure of how near that locus is to Hardy-Weinberg equilibrium (HWE). HWE is zero when a population is randomly breeding, (no artificial selection). Positive values of F indicate non-random selection (inbreeding), while negative values indicate outbreeding.

Table 3. Standard Genetic Assessment of individual STR loci for 29 Havana Silk Dogs. Individual STR loci with high inbreeding coefficients (F > 0.1) are shaded in gray.

Locus	Na	Ne	Ho	He	F
AHT121	7	5.05	0.72	0.8	0.097
AHT137	6	3.69	0.9	0.73	-0.23
AHTH130	6	4.6	0.79	0.78	-0.01
AHTh171-A	6	3.7	0.62	0.73	0.149
AHTh260	5	3.6	0.9	0.72	-0.24
AHTk211	3	2.95	0.83	0.66	-0.25
AHTk253	4	2.53	0.62	0.61	-0.03
C22.279	6	3.97	0.76	0.75	-0.01
FH2001	4	1.68	0.41	0.41	-0.02
FH2054	7	4.08	0.86	0.76	-0.14
FH2848	5	3.8	0.72	0.74	0.017
INRA21	6	3.03	0.72	0.67	-0.08
INU005	5	1.75	0.41	0.43	0.032
INU030	4	2.32	0.66	0.57	-0.15
INU055	4	3.55	0.83	0.72	-0.15
LEI004	3	1.15	0.14	0.13	-0.06
REN105L03	6	3.85	0.86	0.74	-0.17
REN162C04	5	3.12	0.72	0.68	-0.07
REN169D01	7	4.95	0.9	0.8	-0.12
REN169O18	6	2.32	0.59	0.57	-0.03
REN247M23	5	4.01	0.76	0.75	-0.01
REN54P11	6	5.26	0.79	0.81	0.021
REN64E19	7	4.03	0.86	0.75	-0.15
VGL0760	9	4.99	0.86	0.8	-0.08
VGL0910	8	4.65	0.72	0.79	0.077
VGL1063	11	8.37	0.97	0.88	-0.1
VGL1165	7	5.01	0.97	0.8	-0.21
VGL1828	5	3.23	0.72	0.69	-0.05
VGL2009	5	3.96	0.79	0.75	-0.06
VGL2409	5	3.41	0.79	0.71	-0.12
VGL2918	6	2.29	0.62	0.56	-0.1
VGL3008	8	3.77	0.59	0.74	0.202
VGL3235	5	2.47	0.59	0.6	0.016

According to **Table 3**, high inbreeding coefficients (F > 0.1) were only estimated for two of the 33 STR loci (VGL3008 and AHTh171-A, shaded in gray), which suggests that these loci have been under strong positive selection since breed development and might be associated with breed-

defining phenotypic traits. Conversely, F values around or below zero were estimated for the remaining 31 loci, which again suggests that this cohort corresponds to an outcrossed population.

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

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (usually coordinates 1 and 2). The closer individuals cluster together on the plot, the more closely related they are to each other. This group of 29 Havana Silk Dogs clustered as expected for a breed, with individual dogs reasonably dispersed across all four quadrants of the graph. Some individuals, seen on the periphery of the plot, are more genetically diverse when compared to the cohort-at-large. Conversely, two pairs of individuals (red circles) were closely related to each other, as suggested by their tight clustering pattern (**Figure 3**).

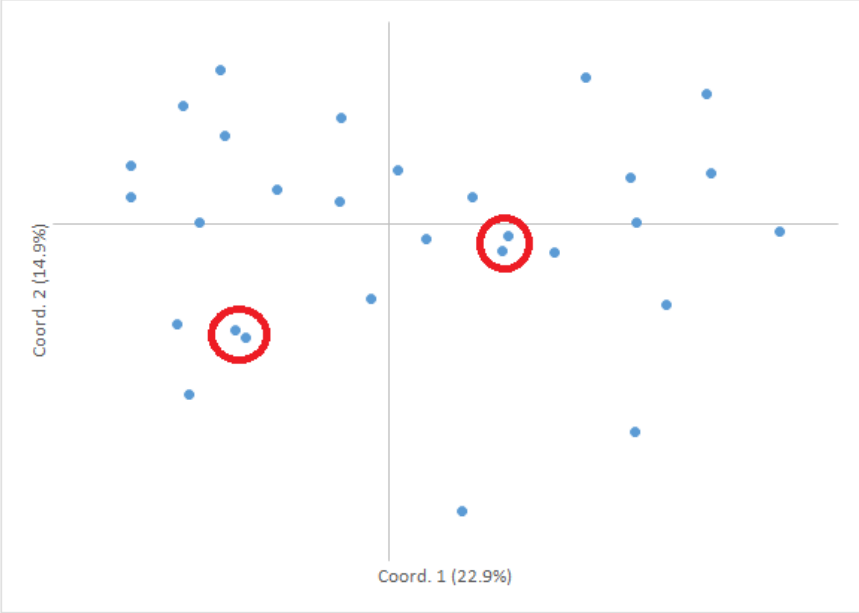


Figure 3. PCoA of Havana Silk Dog (n = 29) based on alleles and allele frequencies at 33 autosomal STR loci. Two pairs of closely related dogs are circled in red.

The degree of intra- and inter-breed relatedness can be further assessed by generating a PCoA of the 29 Havana Silk Dogs with a closely related breed (the Havanese) and a somewhat unrelated breed with relatively similar levels of genetic diversity (the Flat-Coated Retriever) [6,7] (**Figure 4**).

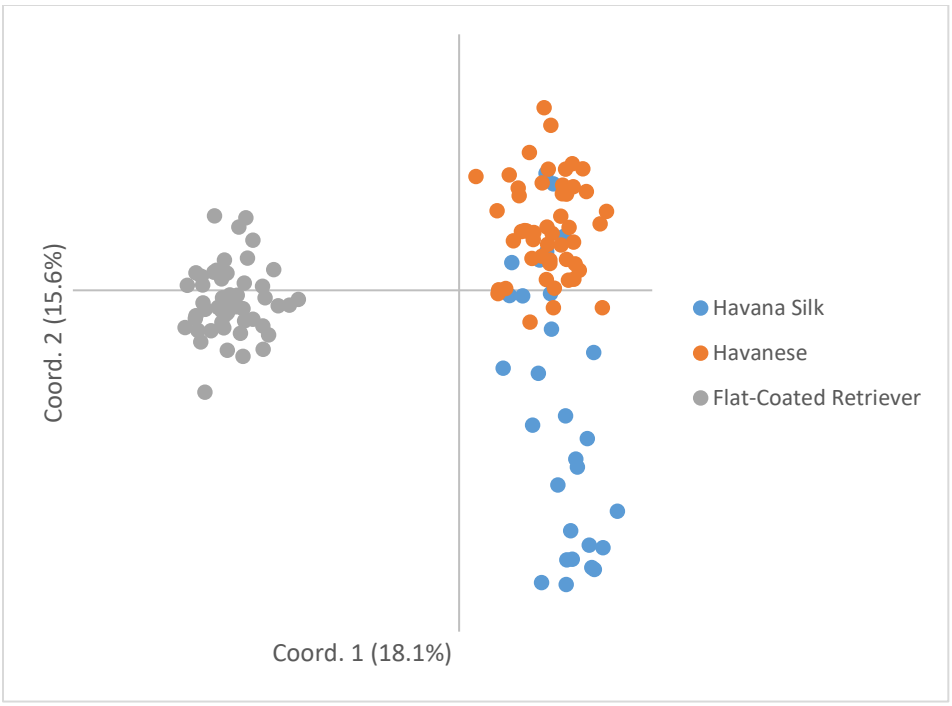


Figure 4. PCoA plot of Havana Silk Dog (blue dots; n=29), Havanese (orange dots; n=50), and Flat-Coated Retriever (gray dots, n=50).

Two observations can be made based on **Figure 4**. First, Havana Silk Dog and Havanese are clearly closely related given their proximity on the plot, but they are differentiating into genetically distinct populations. Clustering of Havana Silk Dog and Havanese on the PCoA was similar to that of subpopulations of the same breed (such as Scottish Collie and Collie; or North American and European Italian Greyhounds), but they are not yet as genetically distinct as varieties within breeds, such as American and Japanese Akita, or black and salt and pepper Giant Schnauzers.

The overlap of some Havana Silk individuals with the Havanese cohort (**Figure 4**; top right quadrant) suggests that these dogs are more closely related to the Havanese than to individuals from their own breed based on data from the 33 STR loci. This observation is expected given that the Havana Silk Dog was developed from the Havanese less than two decades ago.

The second observation is that the distribution of Havana Silk Dogs on the PCoA suggests a higher level of genetic differentiation within the cohort when compared to its founding breed (the Havanese, orange dots) and to Flat-Coated Retrievers (gray dots). The 29 Havana Silk individuals are more dispersed on the PCoA (especially along the vertical or Y axis) than the other breeds, which form much tighter clusters. The more dispersed along the PCoA plot individuals from a breed are, the more genetically diverse the breed is.

E. Internal relatedness (IR) scores for Havana Silk Dogs

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree of relatedness of parents of an individual dog. The IR calculation takes into consideration homozygosity at each of the 33 STR loci in this study and gives more weight to rare and uncommon alleles, which would presumably be identified in less related individuals. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with an IR value of -1.0 would have parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that are genetically identical at all loci. IR values above +0.25 occur when the parents of the full sibling parents are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 29 Havana Silk Dogs.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies for 33 STR loci in 29 Havana Silk Dogs.

	IR	IRVD
Minimum	-0.2637	-0.0973
1st Quartile	-0.1427	-0.0065
Mean	-0.0600	0.1253
Median	-0.0911	0.1472
3rd Quartile	0.0214	0.2394
Maximum	0.2090	0.4262

The most outbred dog had an estimated IR score of -0.2637, while the most inbred dog had an IR score of +0.2, with a mean IR of -0.06 for the cohort. **Table 4** shows that none of the Havana Silk Dogs sampled in this study had IR values above +0.25, thus indicating that this group was the product of random mating and mostly composed of outbred dogs. Finally, the wide range of IR values indicate genetic heterogeneity in the cohort, a typical finding for dog breeds. Given that this is a preliminary assessment of IR values across 29 Havana Silk Dogs, testing of more individuals will provide a more comprehensive picture of the distribution of IR values across the breed.

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

The IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to the present day. Village dogs that exist throughout the SE Asia, the Middle East and the Island Pacific region are randomly breeding descendants of dogs from which most modern breeds evolved. The known STR alleles and their frequencies of a given breed can be compared with the same alleles and their frequency in modern village dogs to yield an adjusted IR score (IR-village dog or IRVD) (**Table 4** and **Figure 5**, blue line). IRVD scores approximate how the IR

score for a Havana Silk Dog would compare to other village dogs if its parents were also village dogs.

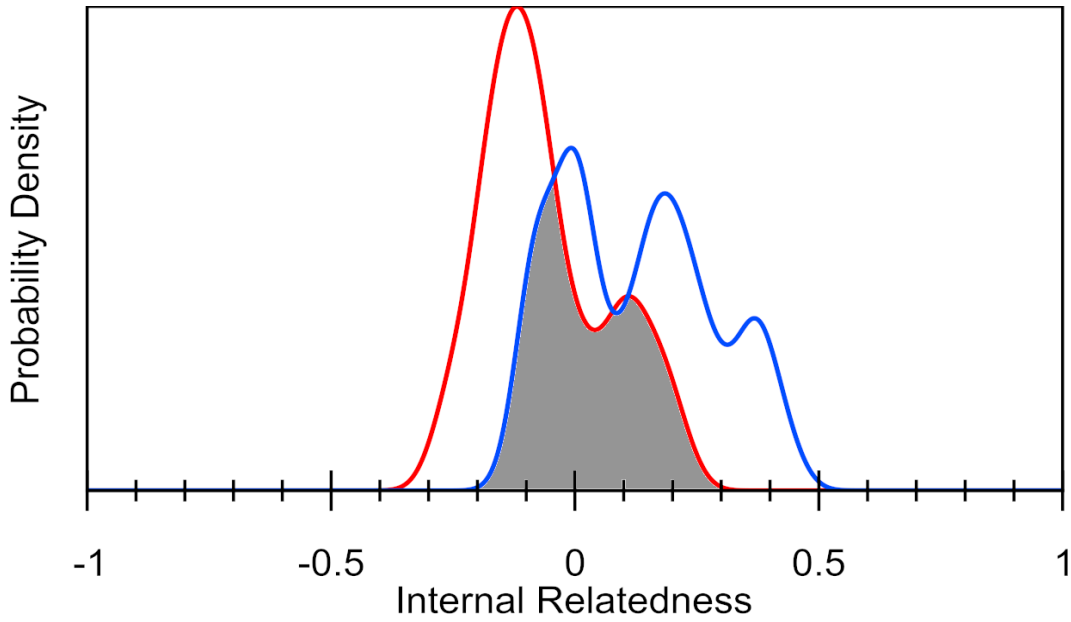


Figure 5. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Havan Silk Dog (n=29). The overlap between the curves (gray area) shows that the breed retains 55.8% of the genetic diversity existing in randomly breeding village dogs.

The IR curve (red line) is bimodal (two peaks) and supports standard genetic assessment values for Havana Silk Dogs, showing that roughly 75% of the cohort is composed of relatively outbred dogs (left peak), whereas the other 25% of dogs (right peak) have slightly higher IR values and thus are mildly inbred. The IRVD curve (blue line) is shifted to right of the IR curve indicating that some genetic diversity has been lost since breed development, which is expected. The curve is trimodal (three peaks), and each peak represents IRVD values for roughly one-third of the cohort: from outbred to moderately inbred, to highly inbred. This means that, if they were village dogs, 25% of the Havana Silk Dogs tested herein would be considered inbred to the level of at least offspring of full sibling parents. This is a typical finding for breeds developed from a relatively small number of individuals or founder lines, as was the case of the Havana Silk Dog.

The gray area corresponding to the overlap between the IR and IRVD curves shows that Havana Silk Dogs retain 55.8% of the existing genetic diversity of randomly breeding village dogs, one of the highest values ever found for a pure breed analyzed at the VGL. It is slightly higher than the 54% retained diversity estimated for the Labrador Retriever, which is considered a genetically diverse breed. Additionally, this figure is almost two times higher than the 30% retained genetic diversity calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL (**section IIB**).

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 chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses, which can cause autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases. Breeds that lack genetic diversity in the DLA region are often more prone to autoimmune disorders.

The Class I region contains several genes, but only one, *DLA88*, is highly polymorphic (i.e., contains many alleles) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with *DLA88* are linked in various combinations, forming specific haplotypes (**Table 5**).

The class II region also contains several genes, three of which are highly polymorphic: *DLA-DRB1*, *DLA-DQB1* and *DLA-DQA1*. Specific alleles at these three loci associated with the three class II genes are strongly linked, and often inherited as a single haplotype (**Table 6**). An individual inherits one haplotype from each of the parents. It is common for different dog breeds to share common and even rare haplotypes for these loci, depending on common ancestry.

1. DLA class I and II haplotypes existing in the Havana Silk Dog

Twelve DLA class I and eleven DLA class II haplotypes were identified in this cohort (**Table 5**). This number is considerably lower than that found in the Havanese, with 35 DLA class I and 27 DLA class II haplotypes. This loss of genetic diversity in both DLA regions when compared to the Havanese is expected, given the history of the Havana Silk Dog breed.

DLA-I haplotypes 1068 (24%) and 1092 (21%) were the most predominant in Havana Silk; similarly, DLA-II haplotypes 2003 (26%) and 2053 (24%) occurred in higher frequency than other haplotypes (bolded on **Table 5**). The higher frequency of these DLA class I and II haplotypes suggests that they have been retained from founder lines and/or may have remained in the population after artificial genetic bottleneck(s). Again, either hypothesis is plausible given the history of the Havana Silk Dog. As more dogs are tested, we expect to find additional DLA class I and class II haplotypes, albeit at low frequency.

Table 5. DLA class I and II haplotypes identified in Havana Silk Dog (n = 29) and their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 Haplotype	STR types	Frequency
1003	387 375 277 186	0.07
1006	387 375 293 180	0.02
1016	382 371 277 178	0.1
1030	380 373 293 178	0.05
1054	382 379 277 184	0.07
1068	**380 373 287 181**	**0.24**
1092	**376 379 277 181**	**0.21**

1093	386 379 277 180	0.02
1115	386 371 277 182	0.05
1116	380 365 289 186	0.05
1117	376 373 277 180	0.09
1262	382 377 289 180	0.03
DLA2 Haplotype	**STR types**	**Frequency**
2001	343 324 284	0.07
2003	**343 324 282**	**0.26**
2005	339 322 280	0.03
2007	351 327 280	0.02
2022	339 327 282	0.07
2023	341 323 282	0.05
2032	339 323 280	0.02
2053	**343 324 280**	**0.24**
2066	339 324 280	0.1
2070	347 324 282	0.05
2074	341 324 284	0.09

The Havana Silk Dog shares DLA class I and class II haplotypes with 48 dog breeds/varieties (**Table 6**). Expectedly, all DLA class I and II haplotypes found in Havana Silk Dogs were also identified in the Havanese; among those, DLA class I haplotype 1262 (3%) and DLA class II 2070 (5%) were shared exclusively with Havanese. Interestingly, the linked 1117/2074 haplotype (9%) was shared exclusively with Havanese (5%) and Biewer Terrier (0.2%), suggesting common ancestry among these three breeds.

Table 6. Sharing of DLA class I and II haplotypes between Havana Silk Dog (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=48).

DLA1 #	STR types	Havana Silk (n=29)	Havanese (n=781)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Akita (n=158)	Japanese Akita (n=552)	Alaskan Klee Kai (n=616)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=130)	Black Russian Terrier (n=149)	Biewer (n=128)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=200)	Yorkshire Terrier (n=16)	Borzoi (n=145)	Collie (n=50)	Doberman Pinscher (n=1051)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=19)	Flat Coated Retriever	Great Dane (n=43)	Golden Retriever (n=842)
1003	387 375 277 186	0.07	0.0474	--	--	--	--	--	--	0.125	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1413
1006	387 375 293 180	0.02	0.0455	--	--	--	0.082	--	--	--	--	0.069	0.047	--	--	--	--	0.145	--	--	0.003	--	0.03	--	0.02	0.0148
1016	382 371 277 178	0.1	0.1972	0.008	0.04	0.11	--	--	--	--	--	0.046	0.01	0.023	0.019	0.013	0.03	--	0.04	0.0205	0.095	0.15	0.16	--	0.16	0.0048
1030	380 373 293 178	0.05	0.0032	0.008	0.03	--	--	--	--	0.029	0.008	0.012	--	0.457	0.349	0.495	0.31	--	--	0.0966	--	0.08	--	--	0.22	0.0012
1054	382 379 277 184	0.07	0.1312	0.008	0.21	0.32	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1118	--	--
1068	380 373 287 181	0.24	0.0134	--	--	--	--	--	--	--	0.221	0.065	--	--	0.009	--	--	--	--	0.0005	--	--	--	0.2829	0.01	0.0481
1092	376 379 277 181	0.21	0.0941	--	--	--	--	0.308	--	0.037	0.008	--	0.252	--	0.038	--	0.06	--	--	--	0.006	--	--	0.0007	--	--
1093	386 379 277 180	0.02	0.0179	0.008	0.05	0.07	--	--	--	0.162	--	--	0.081	--	--	--	--	--	--	--	--	--	--	--	--	--
1115	386 371 277 182	0.05	0.0935	--	--	--	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--
1116	380 365 289 186	0.05	0.098	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.001	--	--	--	--	--	--
1117	376 373 277 180	0.09	0.0499	--	--	--	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--
1262	382 377 289 180	0.03	0.0013	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 # (cont'd)	STR types	Havana Silk (n=29)	Giant Schnauzer (n=306)	Italian Greyhound (n=1189)	Irish Setter (n=59)	Irish Red and White Setter (n=73)	Irish Wolfhound (n=75)	Lakeland Terrier (n=126)	Labrador Retriever (n=272)	Llewellin Setter (n=115)	Magyar Agar (n=77)	Polish Lowland Sheepdog (n=58)	Toy Poodle (n=204)	Miniature Poodle (n=382)	Poodle (n=4536)	Rat Terrier (n=40)	Samoyed (n=191)	Saint Bernard (n=86)	Scottish Collie (n=113)	Shiba Inu (n=134)	Shikoku (n=71)	Greater Swiss Mountain Dog (n=47)	Shiloh Shepherd, ISSA (n=242)	Swedish Vallhund (n=390)	Cardigan Welsh Corgi (n=43)	Whippet (n=96)
1003	387 375 277 186	0.07	--	--	--	--	--	--	0.002	--	0.058	--	--	0.009	0.1695	--	--	--	--	--	--	--	--	--	--	--
1006	387 375 293 180	0.02	0.051	--	--	0.068	--	--	0.028	--	--	--	0.002	0.003	0.0466	--	0.005	--	0.035	--	--	--	--	0.254	--	--
1016	382 371 277 178	0.1	0.046	0.0597	--	--	--	0.008	0.017	0.022	--	0.034	0.025	0.025	0.022	0.05	--	--	0.097	--	--	0.07	--	--	--	0.12
1030	380 373 293 178	0.05	--	0.029	--	--	--	--	0.039	0.026	--	--	--	--	0.0023	0.03	--	--	--	--	--	--	--	--	--	--
1054	382 379 277 184	0.07	0.003	0.0147	0.153	--	--	--	0.055	0.087	0.143	--	0.002	0.001	0.0001	0.01	--	--	--	0.354	0.007	--	--	--	--	0.005
1068	380 373 287 181	0.24	0.056	--	0.008	--	0.12	--	0.031	--	--	0.009	0.01	0.013	--	--	0.042	0.163	--	--	--	--	0.267	0.378	--	--
1092	376 379 277 181	0.21	0.265	--	--	--	--	--	--	--	--	--	--	--	0.0002	0.06	--	--	--	--	--	--	--	--	--	--
1093	386 379 277 180	0.02	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1115	386 371 277 182	0.05	--	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--
1116	380 365 289 186	0.05	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1117	376 373 277 180	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1262	382 377 289 180	0.03	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Havana Silk (n=29)	Havanese (n=781)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Akita (n=158)	Japanese Akita (n=552)	Alaskan Klee Kai (n=616)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=130)	Black Russian Terrier (n=149)	Biewer (n=128)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=200)	Yorkshire Terrier (n=16)	Borzoi (n=145)	Collie (n=50)	Doberman Pinscher (n=1051)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=19)	Flat Coated Retriever	Great Dane (n=43)	Golden Retriever (n=842)
2001	343 324 284	0.07	0.0512	0.661	0.14	0.11	--	--	--	0.199	0.148	0.012	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1419
2003	343 324 282	0.26	0.2119	0.016	0.04	--	--	--	--	0.132	0.057	--	--	0.246	0.208	0.248	0.38	--	--	0.0005	0.598	--	--	0.1414	0.01	0.0202
2005	339 322 280	0.03	0.0026	--	--	--	--	--	--	--	0.008	0.146	0.013	0.043	0.009	0.025	--	--	--	--	0.015	--	--	0.4164	0.07	0.0143
2007	351 327 280	0.02	0.0474	--	--	--	0.082	--	0.0122	--	--	0.077	0.047	--	--	--	--	0.148	--	--	--	--	--	--	0.02	0.0148
2022	339 327 282	0.07	0.1255	0.024	0.21	0.32	--	--	--	--	0.008	--	--	--	--	--	--	0.597	--	0.001	0.015	--	--	0.1171	0.08	0.0006
2023	341 323 282	0.05	0.0032	0.008	0.03	--	--	--	--	0.029	0.008	--	0.003	0.457	0.349	0.498	0.31	--	--	0.0966	--	0.08	--	--	0.22	0.0012
2032	339 323 280	0.02	0.0224	--	0.05	0.07	0.003	--	--	0.162	--	--	0.081	--	--	--	--	--	--	--	--	--	--	--	--	--
2053	343 324 280	0.24	0.0314	0.226	0.12	0.04	--	--	--	--	0.074	0.065	--	--	0.009	--	--	--	--	0.0014	--	--	--	0.148	0.01	0.0291
2066	339 324 280	0.1	0.1735	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	0.007	--	--	--	--	--	0.0007	--	--
2070	347 324 282	0.05	0.0973	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2074	341 324 284	0.09	0.0499	--	--	--	--	--	--	--	--	--	--	0.004	0.019	0.002	--	--	--	--	--	--	--	--	--	--

DLA2 # (cont'd)	STR types	Havana Silk (n=29)	Giant Schnauzer (n=306)	Italian Greyhound (n=1189)	Irish Setter (n=59)	Irish Red and White Setter (n=73)	Irish Wolfhound (n=75)	Lakeland Terrier (n=126)	Labrador Retriever (n=272)	Llewellin Setter (n=115)	Magyar Agar (n=77)	Polish Lowland Sheepdog (n=58)	Toy Poodle (n=204)	Miniature Poodle (n=382)	Poodle (n=4536)	Rat Terrier (n=40)	Samoyed (n=191)	Saint Bernard (n=86)	Scottish Collie (n=113)	Shiba Inu (n=134)	Shikoku (n=71)	Greater Swiss Mountain Dog (n=47)	Shiloh Shepherd, ISSA (n=242)	Swedish Vallhund (n=390)	Cardigan Welsh Corgi (n=43)	Whippet (n=96)
2001	343 324 284	0.07	0.007	--	--	--	--	--	0.002	--	0.071	--	0.005	0.013	0.599	0.01	--	0.017	--	0.015	--	--	0.004	--	--	0.083
2003	343 324 282	0.26	0.031	0.0076	--	--	--	0.766	0.022	--	--	--	0.422	0.514	0.1033	0.11	0.013	--	0.018	--	--	0.93	0.006	--	0.23	0.099
2005	339 322 280	0.03	0.008	--	0.085	0.103	0.193	0.004	0.055	0.791	--	--	0.002	--	0.0174	0.08	--	0.064	--	--	--	--	--	--	--	--
2007	351 327 280	0.02	0.051	--	--	0.151	--	--	0.031	--	--	--	0.002	0.001	0.0163	--	0.005	0.006	0.004	--	--	--	--	0.258	0.12	--
2022	339 327 282	0.07	0.003	--	0.153	--	0.36	0.171	0.055	0.087	--	--	0.007	0.003	0.0006	0.15	0.107	--	--	--	--	--	0.048	0.044	--	--
2023	341 323 282	0.05	--	0.029	--	--	--	--	0.039	0.017	--	--	--	--	0.0023	0.03	--	0.401	--	--	--	--	--	--	--	--
2032	339 323 280	0.02	--	0.0387	--	--	--	--	--	--	--	--	--	0.009	--	--	--	--	--	0.007	--	--	--	--	--	--
2053	343 324 280	0.24	0.065	--	0.008	--	0.12	--	0.029	--	--	--	0.012	0.013	--	--	0.56	0.145	--	--	--	--	0.335	0.524	--	--
2066	339 324 280	0.1	--	--	--	--	--	--	--	--	--	--	--	0.026	0.0001	--	--	--	--	--	--	0.07	--	--	--	--
2070	347 324 282	0.05	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2074	341 324 284	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

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. 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 (**Table 7**) and averaged across all loci (**Table 8**).

The number of alleles (Na) identified at each DLA locus ranged from 3 (5BCA) to 6 (DLA I-4ACA and DLA1131). As observed in the 33 STR loci, the number of effective alleles (Ne) per locus was lower, ranging from 1.49 (5ACT) to 3.85 (DLA I-3CCA) (**Table 7**). Similarly to the standard genetic assessment using STR loci, inbreeding coefficients (F) for 6 out of 7 loci were estimated to be around or lower than zero. Therefore, when averaged across DLA loci, the inbreeding coefficient for this region (F = -0.04, **Table 8**) is similar to that estimated genome-wide (F = -0.06, **Table 2**). This suggests that indeed these DLA loci have achieved an equilibrium with other loci in the genome over time in Havana Silk Dogs, also indicating that this constitutes an outbred group of dogs. Low heterozygosity (high inbreeding) was only observed for DLA locus 5ACT (F = 0.268).

Table 7. Standard genetic assessment for Havana Silk Dog (n=29) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	5	3.85	0.9	0.74	-0.21
DLA I-4ACA	6	3.77	0.76	0.74	-0.03
DLA I-4BCT	4	2.3	0.55	0.57	0.024
DLA1131	6	3.69	0.79	0.73	-0.09
5ACA	5	2.53	0.59	0.6	0.03
5ACT	4	1.49	0.24	0.33	0.268
5BCA	3	2.62	0.79	0.62	-0.28

Table 8. Summary of standard genetic assessment for Havana Silk Dogs (n=29) using 7 STRs in the DLA class I and II regions. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	4.71	2.893	0.66	0.617	-0.04
SE	0.39	0.314	0.077	0.051	0.063

Table 8 shows that, as stated above, both DLA and genomic markers are under random selection in this cohort of dogs. The number of alleles identified in this region can increase (albeit at lower frequencies) as more individuals are tested.

III. What does this assessment of genetic diversity tell us about the Havana Silk Dog

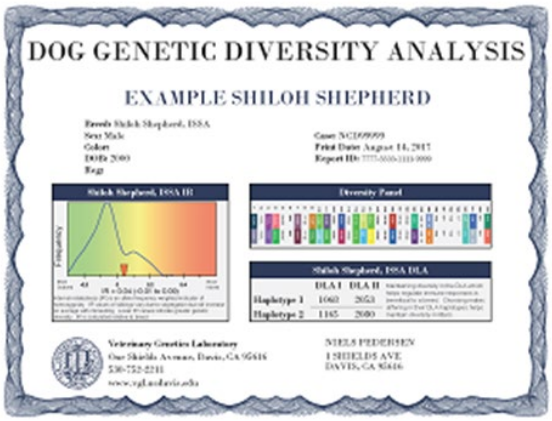
Given that the Havana Silk Dog was derived from the Havanese only around 20 years ago, it is not surprising that a great deal of genetic similarity was found between these breeds. Also expectedly, the Havana Silk has great genetic diversity as does the Havanese, despite the numerous and somewhat recent genetic bottlenecks the breed has experienced and the strict breeding rules established by the HSDAA. This might be due to the many founders from different breeds used to establish the Havanese, as well as the wide range of phenotypic diversity that has been allowed in that breed. The genetic similarity between the Havanese and Havana Silk Dog can be observed on the PCoA plot, which indicates that the latter can still be considered a genetic subpopulation of the former. However, the overall tendency is for genetic divergence to slowly increase over time between the two breeds.

A low level of inbreeding is occurring in the cohort of 29 Havana Silk Dogs used in this study, but this finding is not concerning and needs to be further evaluated by testing additional dogs. Havana Silk Dog breeders have been doing an excellent job in maintaining and distributing the genetic diversity existing in the breed through mate selection. The goal for breeders is to maintain existing genetic diversity by breeding the least related parents possible by using IR scores to select their breeding stock. The goal is to produce dogs with IR scores lower than zero.

IV. Results of VGL Canine Diversity Testing

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

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



B. What should you do with this information?

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

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

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

V. References

1. Wikipedia. Havanese dog. https://en.wikipedia.org/wiki/Havanese_dog.

2. The Havana Silk Dog Association of America (HSDAA). https://havanasilkdog.org/.

3. Wag! Havana Silk. https://wagwalking.com/breed/havana-silk.

4. Pedersen NC, Pooch AS, Liu H. A genetic assessment of the English bulldog. *Canine Genet Epidemiol.* 2016; 3:6. doi: 10.1186/s40575-016-0036-y.

5. Pedersen NC, Shope B, Liu H. An autosomal recessive mutation in *SCL24A4* causing enamel hypoplasia in Samoyed and its relationship to breed-wide genetic diversity. *Canine Genet Epidemiol.* 2017; 4:11. doi: 10.1186/s40575-017-0049-1.

6. Parker HG, Dreger DL, Rimbault M, Davis BW, Mullen AB, Carpintero-Ramirez G, Ostrander EA. Genomic Analyses Reveal the Influence of Geographic Origin, Migration, and Hybridization on Modern Dog Breed Development. *Cell Rep.* 2017; 19(4):697-708. doi: 10.1016/j.celrep.2017.03.079.

7. Veterinary Genetics Laboratory. Genetic Diversity Testing for Havanese. https://vgl.ucdavis.edu/canine-genetic-diversity/havanese.

This report was generated by Felipe Avila and Shayne Hughes on 05/12/2022 and revised on 06/06/2022.