Genetic Diversity Testing for Chinook

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 Chinook 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 33 registered Chinook from the United States (n=31) and Switzerland (n=2). 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. It is anticipated that new alleles at the 33 STR loci and additional DLA class I and II haplotypes will be identified in the future, but these will likely be of lower frequency than those detected in this initial population.

Results reported as:

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

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

Internal Relatedness: The IR value is a measure of the genetic relatedness of an individual's parents. The value takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog; two individuals from different sources may have identical IR values, but a quite different genetic makeup.

I. Introduction to the Chinook

A. Breed History [1-6]

The Chinook breed originated in New Hampshire in the early 1900's. At a time when quality sled dogs were not yet common in New England, sled dog driver, author, dog breeder and innkeeper Arthur Treadwell Walden developed a distinctly tawny colored, American sled dog that had power, endurance, speed, and trainability, along with a friendly gentle nature. The rich history behind the Chinook breed has earned it the distinction of being named the State Dog of New Hampshire.

Figure 1. Kim and Ningo, Chinook's Sire and Dam.

All Chinooks trace their ancestry back to one dog named Chinook who was born on Walden's farm in Wonalancet, New Hampshire in 1917. Chinook's sire was a large, tawny, thick coated, farm dog, likely from Canada, and his dam was a black and white Husky bitch - the granddaughter of Polaris, Admiral Robert Peary's lead dog from his 1908-09 Arctic polar expedition. With Chinook as his foundation sire, Walden developed an American-bred, all- purpose sled dog whose function was both drafting and sled dog racing. Pedigree documents show that Walden bred Chinook to Murra, a Belgian Shepherd of "work class type, father brought back from the war", and to Erika, an AKC registered German Shepherd, also "work class type". His goal was to produce faster offspring for racing, while continuing to breed for intelligence, freighting, and trainability. It is noteworthy to mention that the Belgian Shepherd breed has not been identified in any Chinook genetic testing. A German Shepherd historian postulated that perhaps Murra was labeled Belgian Shepherd due to the strong anti-German sentiment surrounding her time around World War One, substituting "German" for "Belgian" Shepherd.

Figure 2. Arthur Treadwell Walden and Chinook in 1922.

In 1925, Arthur Walden's Chinook sled dog team was the first dog team to summit Mt. Washington, the highest peak in the northeast United States. Moreover, Walden and his dog team (with Chinook in lead) are credited with bringing the sport of sled dog racing to New England. In 1927, Walden was appointed to lead the Dog Department for Admiral Richard Byrd's first Antarctic Expedition (BAE 1). Walden, with Chinook and 15 of Chinook's sons, joined Admiral Byrd's expedition, and the Chinook dogs hauled the expedition freighting sleds.

It was in Antarctica that Chinook, nearing his twelfth birthday, wandered away from camp and was never found.

When Walden came back from Antarctica, he transferred full interest in his Chinook Kennels to Milton and Eva "Short" Seeley who took Walden's Chinook stock and kennel name, and moved the Chinook Kennels a short way down the road from Walden's farm. The Seeley's bred Malamutes and Siberians, and Mrs. Seeley was an AKC judge. The complete list of dogs inventoried at the Seeley's Chinook Kennels on August 30, 1933, listed an assortment of breeds (with an emphasis on Labrador Huskies), but just one Chinook was listed, a Chinook X Labrador Husky cross named Smokey.

The continuation of the breed rested with Julia Lombard, of Wonalancet-Hubbard Kennel (related to Old Mother Hubbard foods), and later Perry Greene, who purchased the remaining Chinooks from Lombard and moved them to his Perry Greene Kennel in Maine. In 1965, the Guinness Book of World Records recorded the Chinook for the first of three times as the rarest dog, with just 125 Chinook dogs alive. In 1981, only 11 Chinooks, useful for breeding, were housed at Maine's Sukee Kennel; subsequently, these Chinooks were dispersed to breeding homes in California, Ohio and Maine.

The United Kennel Club (UKC) recognized the Chinook in 1991, in their Northern Group. The American Kennel Club (AKC) accepted the Chinook in their Foundation Stock Service in 2003, and fully recognized the breed in AKC's Working Group in 2013. The Chinook is one of AKC's rarer breeds, ranking 180 out of 200 in the AKC's 2023 Breed Popularity Ranking.

In the 1980's and 1990's, some Chinook breeders had concerns over a genetic bottleneck, with some outcrossing Chinooks with other breeds or mixed breeds. The United Kennel Club approved a crossbreeding program with the Chinook's UKC parent club, and Chinooks from some of these matings are now registered purebreds with the AKC and UKC and are found in many of the breed's pedigrees. In 2008, a study on genetic diversity of the Chinook breed conducted by Mars Veterinary in conjunction with the CCA/COA reported that Chinook mixes are genetically similar to the Chinook pures.

In 2017, the UKC parent club, the Chinook Owners' Association, instituted the Chinook Breed Conservation Program, which promotes a second Chinook crossbreeding program. As of August of 2023, purebred Chinooks have been bred to a Tamaskan Dog, a Bernese Mountain Dog, a Labrador Retriever, and a Seppala Siberian dog. These new lines, crossing Chinooks with other breeds and back to purebreds, are currently being bred.

B. Appearance [1-4]

Features that set the Chinook apart from the ancient Spitz/sledding breeds are a more modern pedigree dating from the early 1900's, a tawny, close fitting coat, a variety of ears including down, up, helicoptered, or mismatched ears, a saber tail that does not touch the back, and a dependent temperament. "Helicopter" is a historic breed term used to describe ears that are similar to flying or propeller ears, with the fold being maintained when at attention. Perry Greene Kennel sent a

Chinook named Charger to An Khe, South Vietnam in 1966 to serve as the mascot for the first operational Chinook Helicopter unit in Vietnam. Charger fittingly had "helicopters". "Tawny" is a breed term, which is called sable in other breeds. A tawny Chinook coat has a golden color of varying intensity of shades, with single darker tawny hairs, and usually including single black hairs, interspersed in the coat. A Chinook's coat will never be monotone as there will always be distinct shadings of color, whether tawny or dilute tawny. In addition to tawny and dilute tawny, Chinooks also may show white to buff, black, and black and tan coats. The AKC and the UKC breed registries have their own Chinook standards describing coat color descriptions. The Chinook head is distinctive: dark markings around the eye that accentuate the eye and give character are desirable while extended black pigment in an apostrophe shape at the inner corner of each eye is preferred. Dark brown eyes are preferred. It is also desirable for the ears and muzzle to have darker coloring than the body. The Chinook's head is impressive and in balance with the size of the dog. Ears may be mismatched, but matching, down ears are preferred. The Chinook is not a giant breed but must be large enough in size and muscle to have the strength and stamina to do the job it was bred to do. It may take a few years for a Chinook to reach maturity in body and in mind. Young Chinooks may appear gangly and may not show the muscle, chest, and weight that they will have when mature. There is a natural range in size in the breed, with males appearing unquestionably masculine and females having a distinct feminine look.

C. Temperament [1-4]

Chinooks are affectionate, playful and easy to train. Unlike some other sledding breeds, Chinooks tend to be reliable off-lead. Chinooks are friendly and confident toward strangers, though some may be shy. Chinooks are not good watchdogs and are poor protection dogs. Although not big barkers, Chinooks may be vocal, often talking, howling, or whining when excited. Chinooks are inside dogs who crave the company of their family. If left alone, some Chinooks develop separation anxiety. Chinooks have energy to burn and thrive with exercise, training and play. Some may be highly food motivating making training easy.

D. Health of the Chinook [1-4]

1. Lifespan

The breed's average lifespan is 12 to 15 years of age. Some Chinooks have lived over 17 years.

2. Disorders

Disorders found in the Chinook breed include hip dysplasia, cryptorchidism, epilepsy/seizures, fetal edema, cataracts, dwarfism, MDR1, vaccine reactions, gastrointestinal disorders, atopy, and allergies. There are currently no cancer trends identified in the Chinook, though hemangiosarcoma has been reported. Some Chinooks have an unusual form of seizure disorder called "Chinook seizures", described as a movement disorder rather than a true seizure.

Recommended health tests found on the OFA CHIC site, as of August 1, 2023:

→ Hip dysplasia – one of the following tests: OFA Evaluation or PennHIP Evaluation;

→ Eye Examination – performed by a Board Certified ACVO Veterinary Ophthalmologist;
→ Multi-Drug Resistance 1 (MDR1) – DNA test for the MDR1 mutation performed by an approved laboratory;
→ Chondrodysplasia – DNA test performed by an approved laboratory.

Both normal and abnormal results for these tests should be recorded with the Orthopedic Foundation for Animals (OFA). Additional testing and participation are suggested for the following:

- OFA Elbow Dysplasia Evaluation
- OFA Patellar Luxation Evaluation
- AKC DNA Profile
- Cardiac Evaluation: basic and/or advanced exam by a veterinary cardiologist
- CHIC DNA Repository (by blood sample or cheek swab)
- Degenerative Myelopathy (DM): DNA based test by an approved laboratory
- CDDY/IVDD1 (Intervertebral Disc Disease): DNA based test by an approved laboratory
- Autoimmune Thyroiditis: testing by an approved laboratory.

E. Preliminary Results on Genetic Diversity of 33 Chinooks

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

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes [7,8]. Each STR locus contains, on average, 15.4 alleles 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 33 Chinook individuals included in this study are listed in **Table 1**.

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

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
100 (0.05)	**131 (0.42)**	115 (0.02)	219 (0.18)	238 (0.09)	87 (0.21)
102 (0.27)	137 (0.35)	**121 (0.70)**	**221 (0.65)**	**244 (0.83)**	89 (0.35)
104 (0.09)	141 (0.11)	123 (0.08)	223 (0.03)	246 (0.08)	**91 (0.39)**
108 (0.59)	145 (0.12)	127 (0.11)	227 (0.14)		97 (0.05)
		129 (0.11)			
AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**
288 (0.39)	116 (0.17)	136 (0.02)	152 (0.20)	238 (0.29)	95 (0.30)
290 (0.05)	118 (0.15)	**148 (0.68)**	156 (0.15)	240 (0.21)	**97 (0.44)**
292 (0.56)	**124 (0.68)**	156 (0.30)	**168 (0.65)**	**242 (0.32)**	99 (0.06)

				244 (0.18)	101 (0.20)
INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
124 (0.41)	144 (0.06)	**210 (0.79)**	85 (0.45)	231 (0.02)	**200 (0.79)**
126 (0.59)	**150 (0.94)**	216 (0.06)	**95 (0.50)**	233 (0.02)	202 (0.02)
		220 (0.15)	97 (0.02)	**235 (0.80)**	208 (0.20)
			107 (0.03)	241 (0.17)	
REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
212 (0.15)	162 (0.08)	**268 (0.89)**	222 (0.03)	**145 (0.74)**	**12 (0.53)**
220 (0.85)	**168 (0.83)**	270 (0.02)	234 (0.06)	147 (0.06)	13 (0.11)
	170 (0.09)	272 (0.08)	**236 (0.91)**	149 (0.20)	20.2 (0.02)
		278 (0.02)			21.2 (0.33)
					22.2 (0.02)
VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
13 (0.80)	11 (0.18)	16 (0.18)	16 (0.12)	9 (0.21)	15 (0.09)
18.1 (0.06)	12 (0.05)	19 (0.11)	**19 (0.50)**	11 (0.12)	16 (0.11)
20.1 (0.14)	**13 (0.39)**	20 (0.02)	20 (0.36)	14 (0.03)	**17 (0.80)**
	14 (0.14)	**22 (0.44)**	21 (0.02)	**15 (0.64)**	
	18 (0.23)	25 (0.02)			
	19 (0.02)	28 (0.23)			
		29 (0.02)			
VGL2918	**VGL3008**	**VGL3235**			
13 (0.08)	10 (0.15)	**14 (0.61)**			
14 (0.24)	13 (0.12)	15 (0.14)			
17.3 (0.08)	15 (0.06)	16 (0.17)			
19.3 (0.55)	16 (0.20)	17 (0.09)			
21.3 (0.06)	**17 (0.30)**				
	18 (0.17)				

The number of alleles found for each STR locus in Chinook was extremely low, ranging from two (INU005, INU030, and REN169D01) to seven (VGL1165). Allele distribution within each of the 33 autosomal STR loci is typical of most pure breeds of dogs, in which one allele is observed at higher frequency than others (bold on **Table 1**). One of the consequences of bottleneck effects that happen upon development and/or along the history of a breed is a disproportionately high frequency estimated for a single allele at most STR loci, as seen in Chinook (**Table 1**). These alleles have been inherited from one or few founding dog(s) whose phenotypes (and consequently genotypes) were highly valued, and therefore have been positively selected and maintained at high frequency over time. In Chinook, a single allele was identified in 50% or more of the cohort at 24 out of the 33 loci (bold on **Table 1**), which indicates that these alleles were present in the foundation stock and are linked to breed-defining phenotypic traits. Additionally, the low number of alleles found across STR loci in the study population reflects a lack of genetic diversity in the breed, which can be explained by the low number of individuals associated with the population bottlenecks that happened over the course of the breed's 100-year history. Additional alleles for the 33 STR markers will be identified as more individuals are tested, but likely at low number and frequency.

B. Assessment of population diversity using standard genetic parameters

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

Table 2. Genetic Assessment of 33 Chinooks based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	3.76	2.21	0.48	0.48	0
SE	0.2	0.15	0.03	0.03	0.02

The mean number of STR alleles identified in this Chinook cohort (Na = 3.76) corresponds to only approximately 25% of the average number of alleles identified by the VGL at each of these loci across breeds (3.76 out of 15.4 – see **section IIA**). This means that 75% of the genetic diversity known to exist at these 33 STR loci has been lost in the Chinook. 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. The average number of effective alleles per locus (Ne) was estimated at 2.21, indicating that most of the heterozygosity was determined by a little over one-half of the alleles segregating in the breed (**Table 2**).

Both the mean observed (Ho) and expected (He) heterozygosity values were estimated at 0.48 for this cohort, thus yielding an inbreeding coefficient (F) of zero (**Table 2**). This means that this group of 33 Chinooks was a product of random breeding (also called Hardy-Weinberg equilibrium, where sires and dams of the study population were as unrelated as possible). Therefore, even though genetic diversity is low in this breed, these results indicate that Chinook breeders appear to be doing a good job of evenly distributing the existing diversity by mating individuals as unrelated as possible.

The conclusion above is based on the study cohort as a whole (n=33), and not on 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 in order to continue redistributing the genetic diversity currently existing in Chinook (see **section E** below).

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each of the 33 autosomal STR loci used in this study (**Table 3**). This provides an estimate of genetic similarities in the specific regions of the genome that harbor each STR marker. The average number of effective alleles (Ne) per locus ranged from 1.14 (INU030) to 5.01 alleles (VGL3008).

Additionally, mean observed heterozygosity (Ho) for an STR locus ranged from 0.12 (INU030) to 0.82 (VGL1063 and VGL3008), while average expected heterozygosity (He) ranged from 0.11 (INU030) to 0.8 (VGL3008) (**Table 3**).

Loci with the lowest Ho values contribute the least to heterozygosity levels across the breed, because they are most likely associated with genetic traits that are important for the breed's phenotypic standard. Conversely, a high Ho value means that a locus has greater genetic diversity (variability) across the breed. These loci can be associated with phenotypic variation found among individuals.

Based on Ho and He values estimated for each STR locus, inbreeding coefficients (F) ranged from -0.18 (REN169D01) to 0.27 (REN169O18) (**Table 3**). High F values (F > 0.1) were estimated for nine of the 33 STR loci (or 27%, bolded on **Table 3**). These high levels of inbreeding suggest that these loci have been under strong positive selection since breed development, and thus lack diversity. However, these are balanced by the STR loci with F values around or below zero (thus indicating outbreeding), leading to the mean F value of zero estimated for the cohort (**Table 2**).

Table 3. Standard Genetic Assessment of individual STR loci for 33 Chinooks. Individual STR loci with high inbreeding coefficients (F > 0.1) are bolded.

Locus	Na	Ne	Ho	He	F
AHT121	4	2.31	0.64	0.57	-0.12
AHT137	4	3.06	0.64	0.67	0.054
AHTH130	5	1.95	0.52	0.49	-0.06
AHTh171-A	4	2.1	0.49	0.52	0.073
AHTh260	3	1.41	0.33	0.29	-0.14
AHTk211	**4**	**3.09**	**0.58**	**0.68**	**0.149**
AHTk253	3	2.12	0.58	0.53	-0.09
C22.279	3	1.94	0.55	0.48	-0.13
FH2001	**3**	**1.8**	**0.39**	**0.44**	**0.111**
FH2054	3	2.06	0.58	0.51	-0.12
FH2848	4	3.81	0.7	0.74	0.055
INRA21	4	3.06	0.7	0.67	-0.04
INU005	**2**	**1.94**	**0.39**	**0.48**	**0.185**
INU030	2	1.13	0.12	0.11	-0.07
INU055	3	1.55	0.33	0.35	0.055
LEI004	4	2.19	0.55	0.54	-0.01
REN105L03	4	1.49	0.33	0.33	-0.02
REN162C04	**3**	**1.52**	**0.3**	**0.34**	**0.109**
REN169D01	2	1.35	0.3	0.26	-0.18
REN169O18	**3**	**1.41**	**0.21**	**0.29**	**0.272**
REN247M23	4	1.24	0.18	0.2	0.066
REN54P11	**3**	**1.2**	**0.15**	**0.17**	**0.103**
REN64E19	3	1.68	0.46	0.41	-0.12

VGL0760	**5**	**2.48**	**0.49**	**0.6**	**0.186**
VGL0910	**3**	**1.5**	**0.27**	**0.33**	**0.181**
VGL1063	6	3.84	0.82	0.74	-0.11
VGL1165	7	3.45	0.73	0.71	-0.02
VGL1828	4	2.52	0.64	0.6	-0.06
VGL2009	**4**	**2.15**	**0.46**	**0.53**	**0.149**
VGL2409	3	1.51	0.36	0.34	-0.08
VGL2918	5	2.69	0.7	0.63	-0.11
VGL3008	6	5.01	0.82	0.8	-0.02
VGL3235	4	2.37	0.67	0.58	-0.15

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

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (usually coordinates 1 and 2). The closer individuals cluster together around the XY axis, the more closely related they are to each other. The 33 Chinooks used in this study clustered as expected for a pure breed in the PCoA, with dogs reasonably dispersed across all four quadrants (**Figure 2**). Two pairs of closely related individuals (red circles) can be observed in the cohort.

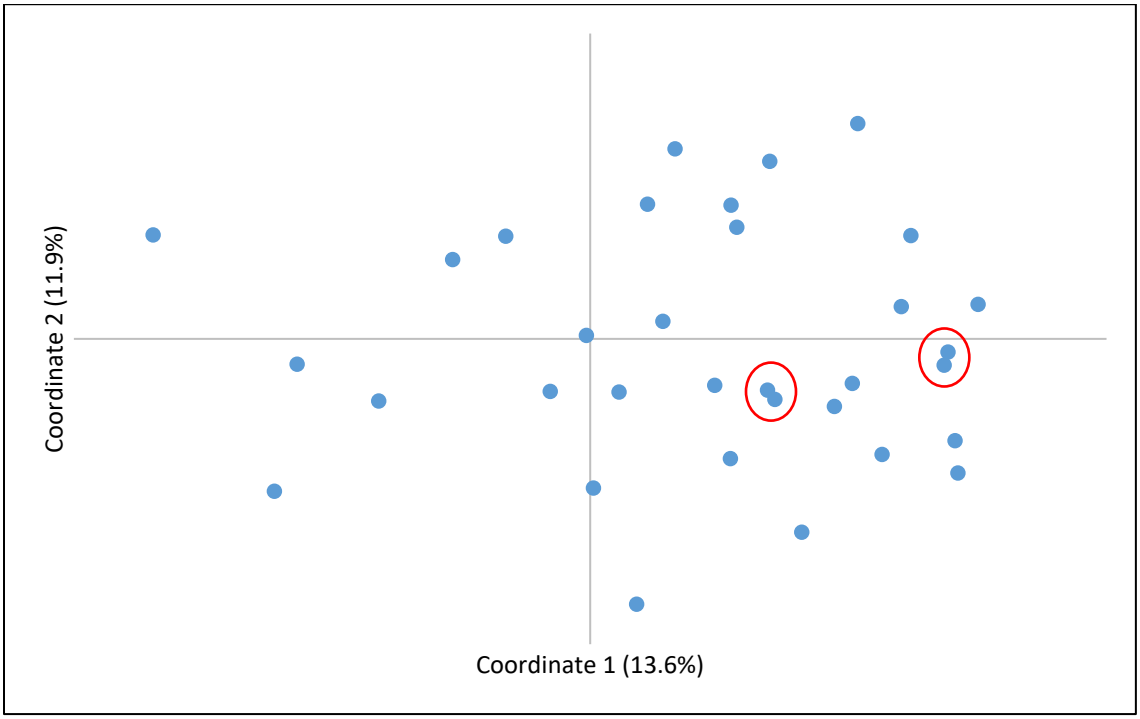


Figure 2. PCoA of Chinook (n=33) based on alleles and their frequencies at 33 autosomal STR loci. Red circles indicate closely related individuals.

The degree of intra- and inter-breed relatedness can be further assessed by comparing the 33 Chinooks in this study with other breeds. Based on the breeds being utilized in the Chinook Breed Conservation Program (see Section I-A), the Chinook was compared to Bernese Mountain Dog, Labrador Retriever, and German Shepherd (**Figure 3**).

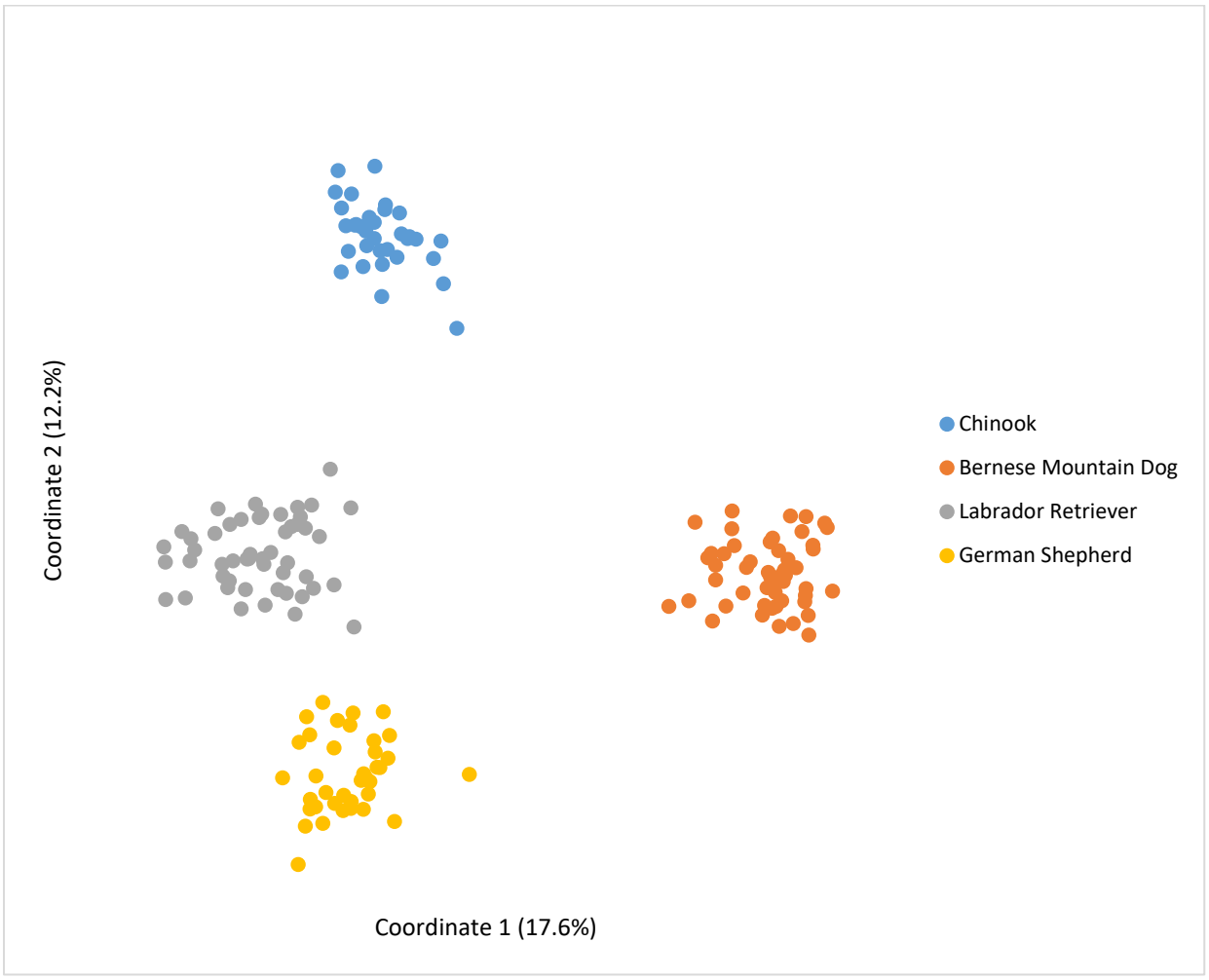


Figure 3. PCoA plot comparing intra- and inter-breed relatedness of Chinook (blue dots; n=33) with Bernese Mountain Dog (orange dots; n=50), German Shepherd (yellow dots; n=50), and Labrador Retriever (grey dots; n=50).

Inter-breed clustering shows separate and well-defined populations, thus indicating that the breeds are genetically distinct as expected. This comparison of four breeds caused more closely related breeds and individuals to cluster closer to each other. Although individuals in each breed did form tighter groups, the breed relationships persisted. This plot also highlights that some Chinooks are more distinct genetically from the cohort at large (blue dots, bottom of the breed cluster).

E. Internal relatedness (IR) scores for Chinooks

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. An IR value of +0.25 would be found among offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 33 Chinooks analyzed in this study.

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

	IR	IRVD
Minimum	-0.2898	0.2438
1st Quartile	-0.0511	0.4009
Mean	0.0024	0.4580
Median	0.0248	0.4505
3rd Quartile	0.0863	0.5186
Maximum	0.2203	0.6707

The most outbred Chinook of the study cohort had an IR score of -0.29, while the most inbred dog had an IR score of 0.22, with a mean IR of 0.002. This wide range of IR values shows that the degree of parental relatedness varies greatly in the study cohort, a typical finding for almost all pure breeds of dogs. Additionally, these numbers show that one fourth of the population had IR scores from -0.05 to -0.29, and one fourth from 0.08 to 0.22. Although the standard genetic assessments made from allele frequencies indicated that the population was randomly breeding, IR values suggest that this assumption is misleading because more outbred dogs are cancelling out more inbred dogs. In truth, close to one-fourth of Chinooks tested were products of closely related parents.

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

The wide range of IR values estimated for this cohort can also be represented graphically (**Figure 4**). The IR curve for Chinooks (red line) is bimodal (two peaks), with a small peak on the left

representing the ~25% most outbred dogs and a large peak (right) which contain the remaining 75% of the cohort.

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 4**, blue line). IRVD scores approximate how the IR score for a Chinook would compare to other village dogs if its parents were also village dogs.

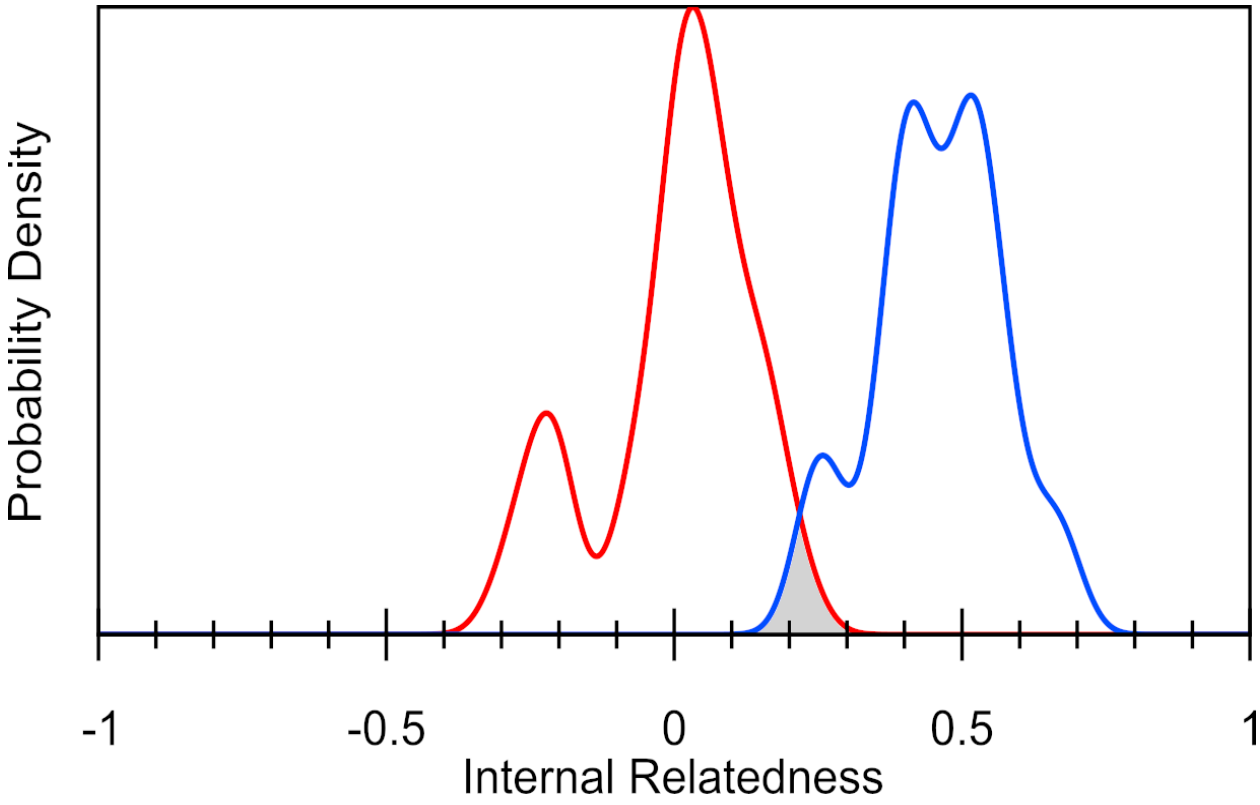


Figure 4. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Chinooks (n=33). The overlap between the curves (gray area) shows that the Chinook retains 3.9% of the genetic diversity existing in randomly breeding village dogs.

The Chinook IRVD curve (blue line) is similarly shaped but shifted to the right of their actual IR scores (red line), with values ranging from 0.24 to 0.68 and an area of overlap of 3.9% (gray area). This indicates that Chinooks have only maintained 3.9% of the total genetic diversity thought to still exist among village dogs from the region considered the ancestral home of most modern breeds. This is the lowest amount of available genetic diversity found in any of the breeds tested by the VGL to date. In the breed with the second lowest amount of retained diversity, the Swedish Vallhund, this figure is estimated at 7%; moreover, the average retained genetic diversity

calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL is 30% (**section IIB**).

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 one (*DLA88*) is highly polymorphic (i.e., contains many alleles) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with *DLA88* are linked in various combinations, forming specific haplotypes (**Table 5**). Groups of genes (and consequently their alleles) inherited as a block are called haplotypes.

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

1. DLA class I and II haplotypes in Chinooks

Only 6 DLA class I and 7 DLA class II haplotypes were identified in this group of Chinooks, which is amongst the lowest number ever reported by the VGL and in line with the low amount of genetic diversity estimated across the genome (**Table 5**). These number are equal to those found in the English Mastiff (6 DLA I and 6 DLA II haplotypes), and much lower than those of more popular (and diverse) breeds such as Golden Retrievers (24 and 19, respectively).

Table 5. DLA class I and II haplotypes identified in Chinooks (n=33) and their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 haplotype	STR types	Frequency (%)
1012	388 369 289 188	14
1045	**376 371 277 186**	**36**
1052	380 372 289 184	2
1068	380 373 287 181	6
1160	**386 369 289 176**	**30**
1291	385 371 291 180	12
DLA2 haplotype	**STR types**	**Frequency (%)**
2003	343 324 282	14
2012	**345 322 280**	**30**

2017	343 322 280	2
2024	**343 323 280**	**30**
2028	345 327 288	6
2053	343 324 280	6
2072	339 325 282	12

DLA-I haplotypes 1045 and 1160 were the most predominant in Chinooks, being identified in 66% of the dogs when combined. Similarly, DLA-II haplotypes 2012 and 2024 were identified 60% of the cohort (**Table 5**). Since most DLA-I haplotypes were identified in identical frequencies as DLA-II haplotypes, it can be inferred that they are in linkage disequilibrium (i.e., inherited together). Therefore, a founder (or founder line) with these combinations of DLA-I/DLA-II haplotypes (such as 1012/2003 and 1291/2072) has played an important role in establishing the breed (as corroborated by the breed's history), and those haplotypes have remained intact over time. We anticipate that additional DLA-I and DLA-II haplotypes will be identified as more Chinooks are tested for genetic diversity at the VGL.

The DLA-I/DLA-II haplotypes identified in Chinooks are shared with 54 different dog breeds/varieties (**Table 6**). As mentioned above, DLA haplotypes tend to remain unchanged across generations, and thus can be used to assess the shared influence of founder lines in a breed.

DLA-I haplotype 1291 (12% frequency) was found exclusively in Chinooks, whereas no DLA-II haplotypes were unique to the breed. Interestingly, the most predominant DLA-I haplotypes in Chinooks (1045 and 1160) were also found in relatively high frequency in Collie breeds such as Collie, Border Collie, and Scottish Collie. Most of the DLA-II haplotypes found in Chinooks were also identified in a number of other breeds (**Table 6**).

Table 6. Sharing of DLA class I and II haplotypes between Chinooks (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=54).

DLA1 haplotype	STR types	Chinook (n=33)	American Eskimo, Standard (n=65)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=186)	America n Akita (n=159)	Japanese Akita (n=579)	Blend Akita (n=66)	Alaskan Klee Kai (n=651)	Barbet (n=68)	Border Collie (n=64)	Berger Picard (n=146)	Bernese Mountai n Dog (n=148)	Black Russian Terrier (n=150)	Biewer (n=136)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=215)	Yorkshire Terrier (n=16)	Borzoi (n=149)	Collie (n=49)	Doberman Pinscher (n=1218)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=856)	Great Dane (n=70)	Golden Retriever (n=874)	Giant Schnauzer (n=329)
1012	388 369 289 188	0.14	0.023	0.04	--	0.005	--	--	--	--	--	0.055	--	--	--	0.228	0.151	0.221	0.28	--	--	0.0008	0.414	--	--	--	--	0.0011	--
1045	376 371 277 186	0.36	--	--	--	--	0.003	--	--	--	--	0.156	--	--	--	--	--	--	--	--	0.96	0.0004	--	--	--	--	--	--	--	--
1052	380 372 289 184	0.02	--	--	--	0.022	--	--	--	--	--	0.016	0.103	--	--	--	--	--	--	--	--	0.0008	--	0.02	--	--	--	--	--	--
1068	380 373 287 181	0.06	--	--	--	0.11	--	--	--	--	--	0.211	--	0.064	--	--	0.009	--	--	--	--	0.0004	--	--	--	0.2728	0.007	0.0463	0.055
1160	386 369 289 176	0.3	--	--	--	--	0.003	--	--	--	--	--	--	0.534	0.03	--	--	--	--	0.581	--	0.0012	--	--	--	--	--	--	--	--
1291	385 371 291 180	0.12	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 haplotype	STR types	Chinook (n=33)	Havana Silk (n=43)	Havanese (n=969)	Italian Greyhoun d (n=1358)	Irish Setter (n=59)	Irish Red and White Setter (n=92)	Irish Wolfhoun d (n=78)	Lakeland Terrier (n=135)	Labrador Retrieve r (n=292)	Llewelli n Setter (n=152)	Magyar Agar (n=78)	Newfoundlan d (n=137)	Polish Lowland Sheepdo g (n=81)	Toy Poodle (n=232)	Miniatur e Poodle (n=413)	Poodle (n=5117)	Rat Terrier (n=44)	Samoyed (n=192)	Saint Bernard (n=89)	Scottish Collie (n=119)	Shiba Inu (n=154)	Shikoku (n=84)	Greater Swiss Mountai n Dog (n=59)	Shiloh Shepher d (n=57)	Shiloh Shepher d, ISSA (n=268)	Swedish Vallhun d (n=495)	Cardigan Welsh Corgi (n=43)	Whippet (n=110)
1012	388 369 289 188	0.14	--	0.0124	0.0077	--	--	--	0.763	--	--	--	--	--	0.022	0.074	0.01456	0.06	0.013	--	0.008	--	--	0.924	--	--	--	0.23	0.036
1045	376 371 277 186	0.36	--	--	--	--	--	--	--	0.005	0.003	--	--	--	0.019	0.005	0.00068	--	--	--	0.723	--	--	--	--	0.002	--	0.33	0.005
1052	380 372 289 184	0.02	--	0.0108	0.1859	--	--	0.333	0.011	--	--	0.051	--	--	--	--	0.00029	--	--	--	--	--	--	--	0.316	0.382	--	--	--
1068	380 373 287 181	0.06	0.24	0.0129	--	0.008	--	0.128	--	0.033	--	--	0.106	0.006	0.009	0.012	--	--	0.042	0.163	--	--	--	--	0.518	0.265	0.368	--	--
1160	386 369 289 176	0.3	--	--	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	0.016	0.09	0.008	0.013	--	--	--	--	--	--	--	--
1291	385 371 291 180	0.12	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 haplotype	STR types	Chinook (n=33)	American Eskimo, Standard (n=65)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=186)	America n Akita (n=159)	Japanese Akita (n=579)	Blend Akita (n=66)	Alaskan Klee Kai (n=651)	Barbet (n=68)	Border Collie (n=64)	Berger Picard (n=146)	Bernese Mountai n Dog (n=148)	Black Russian Terrier (n=150)	Biewer (n=136)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=215)	Yorkshire Terrier (n=16)	Borzoi (n=149)	Collie (n=49)	Doberman Pinscher (n=1218)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=856)	Great Dane (n=70)	Golden Retriever (n=874)	Giant Schnauzer (n=329)
2003	343 324 282	0.14	0.023	0.04	--	0.263	--	--	--	--	0.132	0.055	--	--	--	0.265	0.208	0.242	0.38	--	--	0.0004	0.598	--	--	0.146	0.007	0.0195	0.03
2012	345 322 280	0.3	--	--	--	0.016	--	0.0009	--	0.0576	--	--	--	0.003	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	0.009
2017	343 322 280	0.02	--	--	--	0.005	0.009	0.0035	0.015	--	0.029	0.172	0.103	--	--	--	--	--	--	0.007	--	--	0.215	0.21	0.29	0.0006	--	0.0389	0.002
2024	343 323 280	0.3	--	--	--	0.008	--	--	--	--	--	--	--	0.527	--	--	--	--	--	0.01	--	0.0004	0.006	--	--	--	--	--	--	--
2028	345 327 288	0.06	--	--	--	0.011	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.037	--	--	--	--	--	--	--
2053	343 324 280	0.06	0.223	0.12	0.04	0.005	--	--	--	--	--	0.07	--	0.064	--	--	0.009	--	--	--	--	0.0012	--	--	--	0.1472	0.007	0.028	0.064
2072	339 325 282	0.12	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.02	--	0.0008	--	--	--	--	--	--	--	--

DLA2 haplotype	STR types	Chinook (n=33)	Havana Silk (n=43)	Havanese (n=969)	Italian Greyhoun d (n=1358)	Irish Setter (n=59)	Irish Red and White Setter (n=92)	Irish Wolfhoun d (n=78)	Lakeland Terrier (n=135)	Labrador Retrieve r (n=292)	Llewelli n Setter (n=152)	Magyar Agar (n=78)	Newfoundlan d (n=137)	Polish Lowland Sheepdo g (n=81)	Toy Poodle (n=232)	Miniatur e Poodle (n=413)	Poodle (n=5117)	Rat Terrier (n=44)	Samoyed (n=192)	Saint Bernard (n=89)	Scottish Collie (n=119)	Shiba Inu (n=154)	Shikoku (n=84)	Greater Swiss Mountai n Dog (n=59)	Shiloh Shepher d (n=57)	Shiloh Shepher d, ISSA (n=268)	Swedish Vallhun d (n=495)	Cardigan Welsh Corgi (n=43)	Whippet (n=110)
2003	343 324 282	0.14	0.24	0.2147	0.007	--	--	--	0.763	0.022	--	--	0.004	--	0.422	0.511	0.10289	0.13	0.013	--	0.017	--	--	0.924	--	0.009	--	0.23	0.086
2012	345 322 280	0.3	--	0.0031	--	0.051	0.19	--	--	--	0.056	0.083	--	--	0.052	0.085	0.00704	0.01	--	--	0.008	--	0.018	--	--	--	--	--	--	--
2017	343 322 280	0.02	--	0.0114	0.2117	--	--	--	0.03	--	--	0.449	--	0.278	0.002	0.001	0.00205	0.02	--	--	--	--	--	--	0.316	0.382	--	0.19	0.241
2024	343 323 280	0.3	--	0.0046	--	--	--	--	--	0.087	--	--	--	--	0.078	0.01	0.00059	--	0.016	--	--	--	--	--	--	--	--	--	--	--
2028	345 327 288	0.06	--	0.001	--	--	--	--	--	--	--	--	--	--	--	0.005	0.00059	0.1	--	0.011	--	--	--	--	--	--	--	--	--	0.086
2053	343 324 280	0.06	0.26	0.0284	--	0.008	--	0.128	--	0.031	--	--	0.106	0.006	0.011	0.012	--	--	0.56	0.14	--	--	--	--	0.535	0.338	0.534	--	--	--
2072	339 325 282	0.12	--	0.017	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

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 due to theoretical randomized breeding. This assumption can be tested through a standard genetic assessment of each DLA locus (**Table 7**) and averaged across all loci (**Table 8**).

Table 7 shows that the highest number of alleles (Na) identified for a DLA locus was 6 (DLA1131) and the lowest was 3 (5ACA and 5BCA). However, in line with the genome-at-large, the number of effective DLA alleles (Ne) per locus was lower, ranging from 1.81 (5BCA) to 3.83 (DLA1131). Therefore, the DLA region appears to be in equilibrium with other loci in the genome in Chinooks. Further evidence can be gathered by estimated coefficients of inbreeding (F) for individual DLA loci, which range from high (F=0.12 for 5ACA) to relatively low (F=0.01 for DLA I-3CCA) (**Table 7**). The number of alleles identified in this region can increase (albeit at lower frequencies) as more individuals are tested.

Table 7. Standard genetic assessment for Chinooks (n=33) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	5	3.8	0.73	0.74	0.01
DLA I-4ACA	4	2.32	0.55	0.57	0.04
DLA I-4BCT	4	2.8	0.58	0.64	0.1
DLA1131	6	3.83	0.73	0.74	0.02
5ACA	3	2.43	0.52	0.59	0.12
5ACT	5	4	0.73	0.75	0.03
5BCA	3	1.87	0.46	0.47	0.02

When averaged across all DLA loci, the inbreeding coefficient estimated for Chinooks (F=0.05, **Table 8**) is 5% greater than that estimated across the genome (F=0, **Table 2**). Based on that, it can be suggested that 5% of tested Chinooks are more inbred in the DLA region than the population as a whole. Taken together with the low number of DLA-I/DLA-II haplotypes identified in the breed and their relative frequencies, it can be hypothesized that the over-representation of six linked DLA-I/DLA-II haplotypes (**Table 5**) occurred at the time of breed development or upon one of the various population bottlenecks, followed by a longer period of random breeding.

Table 8. Summary of standard genetic assessment for Chinooks (n=33) using 7 STRs in the DLA class I and II regions. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	4.29	3.01	0.61	0.64	0.05
SE	0.39	0.3	0.04	0.04	0.02

F. What does this assessment of genetic diversity tell us about the Chinook

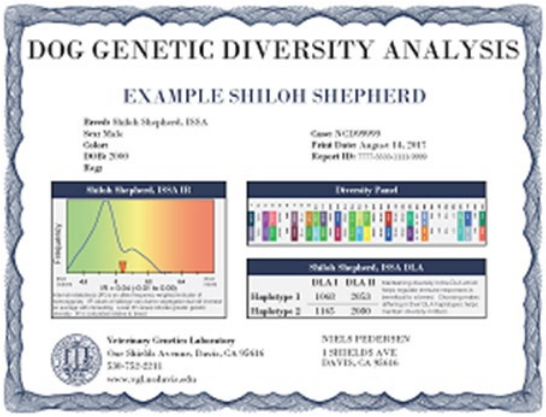
Based on the results of this study, it is evident that a founder or founder lines have had a disproportionately high genetic influence on the modern Chinook, and that the genetic imbalance originating from that phenomenon is being maintained to some degree by breeding practices. However, despite the low amount of genetic diversity found in the study cohort, our results also indicate that Chinook breeders have done a good job in choosing sires and dams that are as unrelated as possible and thus redistributing the available diversity by maintaining overall inbreeding levels close to zero. Since this study is preliminary and based on genotyping data from only 33 individuals, we anticipate that more diversity in the form of novel STR and DLA alleles will be identified in the breed as more individuals are tested over time.

The goal for breeders is to maintain existing genetic diversity by breeding the least related parents possible. Breeders should be aware of this when selecting mates for their breeding programs, in order to redistribute the diversity that currently exists in the breed. The goal is to produce dogs with IR scores lower than zero.

IV. Results of VGL Canine Diversity Testing

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

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



B. What should you do with this information?

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

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

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

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