

## Genetic Diversity Testing for Scottish Collie (SCPS registered)

### Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers that will assess genetic diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to breeders who wish to track and increase genetic diversity of their breed as a long-term goal.

Genetic diversity testing of the Scottish Collie is now in the preliminary results phase. During this phase, we continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity. This report is based on testing of 43 dogs from the Scottish Collie Preservation Society registry. Many of these dogs are also registered with the earlier Old-time Scotch Collie (OTSC) Association or as Collie with the American Kennel Club. Although this number of dogs is probably not sufficient in number or geographic location to do a final assessment of the breed, this selection of individuals should provide a reasonable picture of putative 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 tend to be of much lower incidence than those detected in the present population.

**Price: \$50.**

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#### **Results reported as:**

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

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

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

## I. Introduction

### A. History of Collies

The Scottish Collie, also known as Scotch Collie, Highland Collie, Old-time Scotch Collie, Farm Collie, Old Farm Collie and Old Scotch Farm Collie [1-4, 13, 21], should not be confused with primarily conformation breeds such as the (Rough) Collie, a popular breed recognized by the AKC [5], the Collie (Smooth) a breed recognized by The Kennel Club [6], or the Smooth and Rough Collies recognized by the FIC [7, 8].

Collie-type dogs in Scotland were used for centuries to aid in herding, protecting and driving flocks of sheep., Ancestors of these dogs included native Celtic dogs, Roman cattle dogs and Viking herding Spitz [1, 26]. Dogs resembling modern Collie-type breeds were frequently described in 17th century literature in Scotland [3], and in spite of their landrace origins, they ultimately developed a breed-like uniformity. The origin of the breed name Collie is obscure and may have been in either Scotland or England. "Coll" is the Anglo-Saxon name for coal (black) [31] and "Collie" is Gaelic for "little dog, one who follows another constantly, a loungeur, or one who hunts for a dinner" [2]. However, neither the name nor the breed was widely known or used outside of Scotland before the early 1800s [2]. Their fame spread in the Victorian era (1837-1901), possibly bolstered by the queen herself [31], and they became highly sought after outside of their native land, initially in England for herding and driving stock as well as for dog shows, and later throughout the world [3]. Early aficionados of these dogs, most of whom were English, referred to them as Scotch Shepherd, Scotch Sheep Dog or Highland Sheep Dog to differentiate them from the common English sheep dogs of the period. By the end of the nineteenth the name "Collie" had become commonplace [2].

### B. Conformation (show) vs. Performance (field)

The Scottish Collie was a rage on both sides of the Atlantic by the turn of the twentieth century. People in cities wanted them because they were fashionable, royalty owned them, dog showmen loved to campaign them, farmers used them for various tasks around the farm and more were being imported from Britain all the time. At that time all Collies were of one breed, the show people, the city pet owners and the farmers and ranchers all had Scottish Collies, and, for the most part they all had some degree of working ability. As time went on, through the artificial constraints of the show ring, the fancy dogs began to lose a lot of what had made them great in the first place. As the 20th century approached people also began to notice changes in their physical appearance, the most notable being an increase in length and narrowing of the head, decreased size of the eyes, change in the coat and a loss of intelligence and working ability were also observed [3]. An article from 1892 described this best [3] - *"That they are beautiful dogs in their way nobody can deny, but to speak of them as lovely collies is another matter, for the majority of them are no more fitted to perform the work of the old Scotch collie than is the Laird, the lion of some London drawing room, to pursue the avocation of his Highland shepherd. There is no reason why beauty should not go hand in hand with utility, but at present unfortunately such is not the case"* (The Fanciers' Journal, January 1892). However, the herding ability was not

lost forever, but retained in the form of another Collie breed - the Border Collie [3] or in breeds such as the English Shepherd, Australian Cattle Dog, Australian Koolie, and McNab dog [26, 8].

The 1920's have been seen as the start of a golden age for the Collie breed in shows and as pets. The height of this golden age was brought about with the movie *Lassie Come Home* in 1943. Lassie was a Rough Collie named Pal, the first of several generations of Lassies, that would be known for their beautiful sable and white coat and "superdog" attributes [9]. Sales of Collies rose by 40% after the release of this film, an effect that purportedly lasted for a decade [10]. An increase in heritable disorders has been seen as one effect of this increased popularity [10].

### **C. The revival of the Old-Time Scottish Collie**

As pedigreed Collies became more popular, their relatives on farms were increasingly replaced by conformation type Collies or Border Collie and Australian or English Shepherds [1-3]. By the 1980s, decades of neglect had decimated the old-time Scottish Collie population on both sides of the Atlantic, and only a few remote pockets of these dogs existed [26]. Their landrace origins and phenotypic variability were not acceptable for show Collies and survivors were either not registered and allowed to die out or registered as Australian and English Shepherds.

At least three groups are working towards a similar goal, the restoration of the original farm Collie of Scotland as a breed. These organizations included: 1) Scottish Collie Preservation Society (SCPS) [4], 2) Old-Time Scotch Collie (OTSC) association [1, 2, 26], and 3) the Old Time Farm Shepherd (OTFS) organization [2]. The latter two groups have tended to merge over time and now share a common registry managed by the OTSC association [21, 23]. The common goal is to restore the breed to its original form, health, and function (e.g. shepherding, hunting) by means of careful breeding of genetically sound dogs representing the earliest definitions and descriptions of the original Scottish farm shepherds. The challenge is to define a landrace type of dog that was never a registered breed, but which possessed a breed like consistency of form and function. The official breed standard for the SCPS was drawn directly from the three Collie Standards of 1885, 1898, and 1910 [4, 23] and they rely on these early blueprints largely in their original form to guide selection for the historical form and function of the breed. The SCPS (and original OTFS association) is more focused on gathering bonified remnants of these dogs from around the world, in order to include a maximum amount of genetic diversity in the restored breed. In contrast, the OTSC Association asserts that Scottish farm collies were always a somewhat genetically and phenotypically diverse breed. Therefore, their "*standard*" *does not seek to change that but rather to define the breed and distinguish it from other breeds that have descended from the Scotch Collie. Towards maintaining the Old-Time Scotch Collie as a landrace breed this standard is intended as a broad set of descriptions with plenty of room for individual breeders' preferences....* [23].

The history of the "contemporary" Scottish-type farm shepherd is somewhat complicated by the efforts of an American dog breeder, J. Richard McDuffie (1923-2006) [20]. McDuffie and followers began a serious attempt to revive the original Scottish Collie in the 1980s and 1990s by searching out small number of dogs scattered across Appalachia based on their perception of the original farm and hunting type shepherds of Scotland. McDuffie, and later Chandler Strunk, and their Old Time Farm Shepherds (OTFS) struggled for over a decade to gain acceptance of their

"Old-Time Scotch Shepherds", but this ultimately failed, and their cause was incorporated into the OTSC association [25] and an actual registry (database) for this type of dog created [21, 23]. The OTSC database is open to any farm Collie type dog regardless of prior registration and currently includes 1515 total dogs, 238 registered OTSC dogs, and is accessed by 643 dog owners and 439 users [23]. The SCPS association database currently consists of 3568 dogs, some of which are found in other registries. Interestingly, many dogs that meet the common standards have been accepted by the SCPS and OTSC Associations, although each organization tends to remain circumspect about some of the other's dogs.

#### **D. Appearance**

The original Scottish Collie was a rugged working dog, able to cope with various situations and environments easily, sound in body and mind (Fig. 1) [1]. It is reasonable to expect that the Scottish Collie should show this same heartiness and versatility whether they are used as hard-working farm dogs or as faithful companions.



**Figure 1a.** Section of a painting entitled "A highland Shepherd" by Charles Jones (1836-1892).



**Figure 1b.** Picture of highland dogs herding sheep (Richard Ansdell 1815-1885) [3]. The dogs in these pictures are typical of the multi-use working dogs of the period.

Original farm shepherds of Scotland were of moderate length, neither too long like modern Rough Collies, nor too short like some lines of English Shepherd, with a moderate and well-defined stop (Fig. 1a and 1b). The overall emphasis of the restored breed is on moderation with no part exaggerated or out of proportion like the long nose or superabundant coat of the modern Rough Collie. The SCPS breed standard preserves the language of the early breed standards “in order to restore the most authentic Scottish Collie possible. It requires bitches to measure 20 to 22 inches and weigh between 40 and 55 pounds [24] while the OTSC breed standard allows for greater range, 17 to 23 inches and weight of 32 to 70 pounds. [1]. Likewise, the SCPS breed standard calls for dogs to measure 22 to 24 inches and weigh between 45 and 65 pounds, while the OTSC standard allows 19 to 25 inches in height, and 35 to 85 pounds in weight. Both breed standards call for a dog that is long bodied, athletic, muscular, intelligent and biddable. They both require a moderate head without cheekiness, with fair sized eyes, good expression, a slight stop and a scissors bite. [1, 24]. Ears differ in the breed standards, where the SCPS calls for semi-erect ears when alert and the OTSC standards allows erect or partially erect. Tails, too, are different, where the OTSC allows for gay tails and partial tails, whereas the SCPS standard does not.

Both standards describe the Scottish Collie coat as double, with an outer coat straight and harsh to the touch and a soft and furry undercoat. The OTSC standard emphasizes moderation in the coat, neither too thin nor overabundant [1]. The SCPS standard allows for all colors, though discourages all white or all red and prefers a showily marked dog [24]. The OTSC accepts all colors and markings traditionally found on Collies, with the most common color being sable, often with white markings (Fig. 1a). Black with white and/or tan markings (Fig. 1b) are also common, as is blue merle.

The Scottish gait should be single track and effortless [24]. The stride as viewed from the side should be reasonably long, smooth and even, with the backline firm and level... The drive from hindlegs should be more powerful [1].

## E. Temperament

The original Scottish farm Collie has been best described as “steady, bold, sensible, quick to learn and responsive - content to lie about when not needed but ready to spring into action at a minute's notice, reserved with strangers, yet never snappy, vicious or shy. Trainability, intelligence and reciprocity are hallmarks of the breed, while hyper-activity, shyness, cowardice, inane stock chasing, excessive barking, lack of watchfulness or otherwise low intelligence are serious faults [4].”

## II. Preliminary genetic diversity studies of 43 dogs from the Scottish Collie Preservation Society registry

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

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes. Each of these STR loci is known to contain from 7 to 27 different alleles (avg. 15.4 alleles/locus) when tested across many breeds of dogs. Each breed, having evolved from a small number of founders and having been exposed to artificial genetic bottlenecks will end up with only a portion of the total available diversity. Artificial genetic bottlenecks include such things as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity and resulting increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies as determined for 43 Scottish Collie registered in the SCPS (some also in the OTSC Association registry) are listed in Table 1.

**Table 1.** Allele frequencies for 33 autosomal STR markers in Scottish Collie (n=43). One to three alleles (highlighted) at each locus contribute 70-98% of the genetic diversity.

AHT121	AHT137	AHTH130	AHTH171-A	AHTH260	AHTk211
94 (0.17)	131 (0.03)	119 (0.01)	219 (0.07)	238 (0.06)	89 (0.74)
98 (0.02)	133 (0.01)	121 (0.02)	221 (0.35)	246 (0.64)	91 (0.01)
100 (0.36)	137 (0.63)	123 (0.60)	223 (0.23)	248 (0.03)	95 (0.17)
102 (0.07)	139 (0.02)	125 (0.01)	225 (0.02)	250 (0.15)	97 (0.08)
104 (0.31)	147 (0.11)	127 (0.31)	233 (0.30)	252 (0.13)	
106 (0.02)	151 (0.19)	129 (0.02)	235 (0.02)		
108 (0.02)		131 (0.02)	237 (0.01)		
110 (0.02)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
284 (0.02)	116 (0.22)	128 (0.01)	148 (0.03)	232 (0.10)	91 (0.07)
286 (0.01)	118 (0.10)	132 (0.22)	152 (0.01)	234 (0.28)	95 (0.13)
288 (0.67)	124 (0.50)	136 (0.24)	156 (0.08)	236 (0.10)	97 (0.47)
292 (0.30)	126 (0.17)	140 (0.02)	160 (0.01)	238 (0.42)	99 (0.02)
	128 (0.01)	144 (0.42)	168 (0.09)	240 (0.02)	101 (0.32)
		148 (0.08)	172 (0.06)	242 (0.05)	
		152 (0.01)	176 (0.67)	244 (0.01)	
			180 (0.05)	246 (0.01)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.01)	144 (0.05)	210 (0.74)	85 (0.01)	227 (0.28)	200 (0.05)
122 (0.01)	146 (0.32)	212 (0.06)	95 (0.94)	233 (0.27)	202 (0.13)
124 (0.48)	150 (0.02)	214 (0.02)	101 (0.01)	235 (0.24)	204 (0.33)
126 (0.50)	152 (0.61)	218 (0.18)	107 (0.03)	237 (0.02)	206 (0.36)
				241 (0.18)	208 (0.14)

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.02)	162 (0.74)	266 (0.43)	222 (0.52)	143 (0.02)	12 (0.01)
212 (0.82)	164 (0.01)	268 (0.51)	226 (0.44)	145 (0.33)	13 (0.15)
216 (0.11)	166 (0.01)	270 (0.01)	232 (0.03)	147 (0.57)	14 (0.39)
218 (0.01)	168 (0.23)	272 (0.01)		151 (0.02)	18.2 (0.01)
220 (0.03)	170 (0.01)	278 (0.03)		153 (0.06)	19.2 (0.11)
					20.2 (0.22)
					21.2 (0.03)
					22.2 (0.06)
					23.2 (0.02)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
16.1 (0.06)	8 (0.08)	19 (0.01)	15 (0.01)	9 (0.17)	15 (0.07)
17.1 (0.23)	9 (0.01)	20 (0.07)	16 (0.69)	10 (0.30)	16 (0.02)
18.1 (0.09)	12 (0.16)	21 (0.27)	17 (0.15)	11 (0.03)	17 (0.02)
19.1 (0.17)	13 (0.06)	23 (0.02)	19 (0.01)	13 (0.25)	18 (0.84)
20.1 (0.07)	14 (0.11)	24 (0.03)	20 (0.08)	14 (0.05)	19 (0.05)
21.1 (0.33)	15 (0.02)	25 (0.01)	21 (0.06)	15 (0.07)	
22.1 (0.03)	16 (0.02)	26 (0.52)		16 (0.13)	
23.1 (0.02)	17 (0.06)	27 (0.05)		17 (0.01)	
	18 (0.01)	28 (0.01)			
	19 (0.08)				
	20 (0.32)				
	21 (0.05)				
	22 (0.02)				



VGL2918	VGL3008	VGL3235
11 (0.01)	10 (0.02)	12 (0.01)
12 (0.23)	13 (0.03)	14 (0.25)
13 (0.59)	15 (0.51)	15 (0.03)
14 (0.11)	16 (0.08)	16 (0.68)
15 (0.02)	17 (0.17)	19 (0.02)
18.3 (0.03)	18 (0.05)	
	19 (0.05)	
	21 (0.03)	
	22 (0.05)	
	23 (0.01)	

Allele and allele frequencies at each locus indicates that a great deal of inbreeding has gone into the evolution of this group of 43 dogs. One to three alleles at each locus are found in 70-98% of individuals and 94% of the dogs shared the same allele (95) at the LE1004 locus. The average number of known alleles for these 33 autosomal STR loci is 15.4, while the average number of alleles per locus ( $N_a$ ) for Scottish Collie was 6.12 (Table 2). Therefore, the 43 Scottish Collie possessed only  $6.12/15.4 = 39.7\%$  of known canid diversity for these markers. In other words, over 60% of known canid genetic diversity at these 33 loci has been lost up to this point in time. The amount of retained genetic diversity is higher than the Swedish Vallhund (31.9%); similar to the Flat Coated Retriever (38.6%), Irish Red and White Setter (34.8%) and Magyar Agar (40.4%); but lower than popular breeds such as the Golden Retriever (54.5%), toy Poodle (55.6%) and Standard Poodle (58%).

## B. Assessment of population diversity using standard genetic parameters

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

The observed (actual) heterozygosity in this group of 43 dogs was 0.59, while the expected heterozygosity ( $H_e$ ) for a population in Hardy-Weinberg equilibrium (HWE) was 0.58, yielding a coefficient of inbreeding ( $F$ ) of -0.02 (i.e., 2% more outbred or heterozygous than predicted for HWE). The standard genetic assessment values indicate that the parents of these 43 Scottish Collie were as heterozygous as possible given the existing population size and genetic diversity.



However, this is the conclusion based on the 43 dogs taken as a group. Internal relatedness (IR) scores will provide a better picture of heterozygosity on an individual basis.

**Table 2.** Standard Genetic Assessment values for 43 Scottish Collies based on 33 autosomal STR loci

	<b>N</b>	<b>Na</b>	<b>Ne</b>	<b>Ho</b>	<b>He</b>	<b>F</b>
<b>Mean</b>	43	6.12	2.76	0.59	0.58	-0.022
<b>SE</b>		0.37	0.2	0.03	0.03	0.016

A standard genetic assessment was made on 55 conformation Collies for the purpose of breed comparison (Table 3). The Scottish Collie were more genetically diverse than Collie ( $N_a = 6.12$  vs  $5.33$ ) and had higher observed heterozygosity ( $H_o = 0.59$  vs.  $0.44$ ). The average effective alleles per locus was higher ( $N_e = 2.76$ ) in Scottish Collie than Collie ( $N_e = 2.12$ ). The Scottish Collie were 2.2% more heterozygous (more outbred) than expected for a random breeding population ( $F = -0.022$ ), while the Collie were 9% less heterozygous (more inbred) than expected ( $F = 0.09$ ). The F values for the two groups of Scottish Collie and Collie were close to zero (i.e. HWE) and likely reflect the heterozygosity of both breeds as a whole and not a small amount of selection bias.

**Table 3.** Standard Genetic Assessment values for 55 conformation Collies based on 33 autosomal STR loci.

	<b>N</b>	<b>Na</b>	<b>Ne</b>	<b>Ho</b>	<b>He</b>	<b>F</b>
<b>Mean</b>	55	5.33	2.12	0.45	0.49	0.09
<b>SE</b>		0.36	0.13	0.03	0.03	0.02

## **B. Standard genetic assessment values for individual STR loci**

The allele frequencies can be also used to do a standard genetic assessment of heterozygosity at each STR locus (Table 4). This provides an estimate of genetic similarities in specific regions of the genome associated with each STR marker. Phenotypic differences equate to genotypic differences - therefore, loci that have alleles shared by a large proportion of individuals, are under strong positive selection and are most likely regions of the genome that are associated with breed-specific phenotypes (traits). The  $N_a$  values for an individual STR locus for this population of 43 Scottish Collies ranged from a low of 3 to a high of 13 alleles per locus, while the  $N_e$  ranged from 1.37 to 6.13 alleles per locus. It is important to remember that each STR locus can have from 7-27 different alleles (avg. 15.4 alleles/locus) when tested across all dog breeds. The observed heterozygosity ( $H_o$ ) for an individual STR locus ranged from 0.12 to 0.86, while  $H_e$  ranged from 0.11 to 0.84 (Table 3). Fourteen loci had positive F values and 19 were negative. The loci with positive F values were under a greater degree of positive selection than those with negative F values and therefore areas of the genome that are more strongly associated with desired breed-specific traits. However, the influences of these various inbred, neutral and outbred regions of the genome defined by these 33 STR loci have been kept in good balance by breeder as evidenced by only a slightly negative F value ( $-0.22$  or 2.2% excess heterozygosity) across all loci (Table 2).

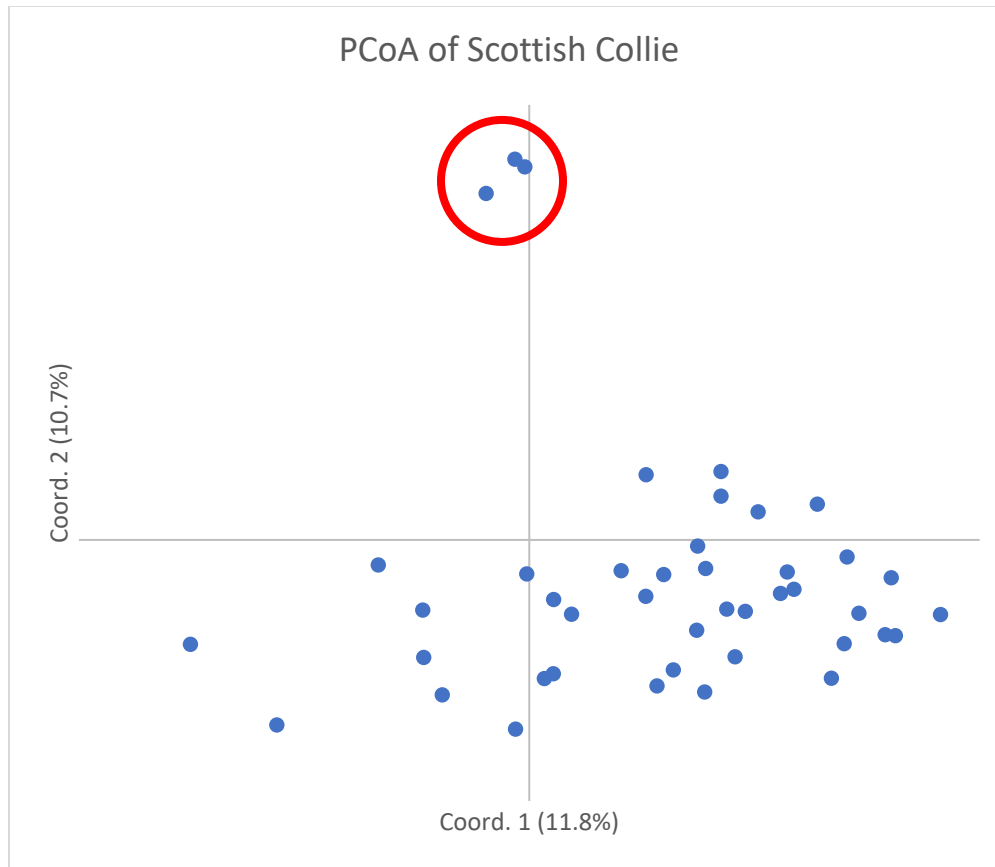
**Table 4.** Standard Genetic Assessment of individual autosomal STR loci for 43 Scottish Collies

#	Locus	N	Na	Ne	Ho	He	F
1	AHT121	43	8	3.86	0.77	0.74	-0.035
2	AHT137	43	6	2.18	0.56	0.54	-0.03
3	AHTH130	43	7	2.15	0.58	0.53	-0.088
4	AHTh171-A	43	7	3.73	0.7	0.73	0.047
5	AHTh260	43	5	2.21	0.54	0.55	0.025
6	AHTk211	43	4	1.74	0.4	0.43	0.072
7	AHTk253	43	4	1.85	0.54	0.46	-0.163
8	C22.279	43	5	2.92	0.72	0.66	-0.096
9	FH2001	43	7	3.55	0.74	0.72	-0.036
10	FH2054	43	8	2.17	0.54	0.54	0.007
11	FH2848	43	8	3.54	0.79	0.72	-0.102
12	INRA21	43	5	2.90	0.58	0.66	0.113
13	INU005	43	4	2.09	0.61	0.52	-0.157
14	INU030	43	4	2.08	0.47	0.52	0.104
15	INU055	43	4	1.74	0.37	0.43	0.124
16	LEI004	43	4	1.13	0.12	0.11	-0.044
17	REN105L03	43	5	4.06	0.86	0.75	-0.142
18	REN162C04	43	5	3.66	0.65	0.73	0.104
19	REN169D01	43	5	1.48	0.37	0.32	-0.155
20	REN169O18	43	5	1.69	0.44	0.41	-0.081
21	REN247M23	43	5	2.23	0.54	0.55	0.03
22	REN54P11	43	3	2.12	0.63	0.53	-0.19
23	REN64E19	43	5	2.30	0.54	0.57	0.053
24	VGL0760	43	9	4.25	0.79	0.77	-0.034
25	VGL0910	43	8	4.81	0.72	0.79	0.09
26	VGL1063	43	13	6.13	0.81	0.84	0.027
27	VGL1165	43	9	2.87	0.70	0.65	-0.071
28	VGL1828	43	6	1.94	0.51	0.48	-0.058
29	VGL2009	43	8	4.83	0.79	0.79	0.003
30	VGL2409	43	5	1.37	0.28	0.27	-0.025
31	VGL2918	43	6	2.40	0.65	0.58	-0.115
32	VGL3008	43	10	3.17	0.61	0.68	0.116
33	VGL3235	43	5	1.92	0.49	0.48	-0.022

#### **D. Differences in population structure as determined by principal coordinate analysis (PCoA)**

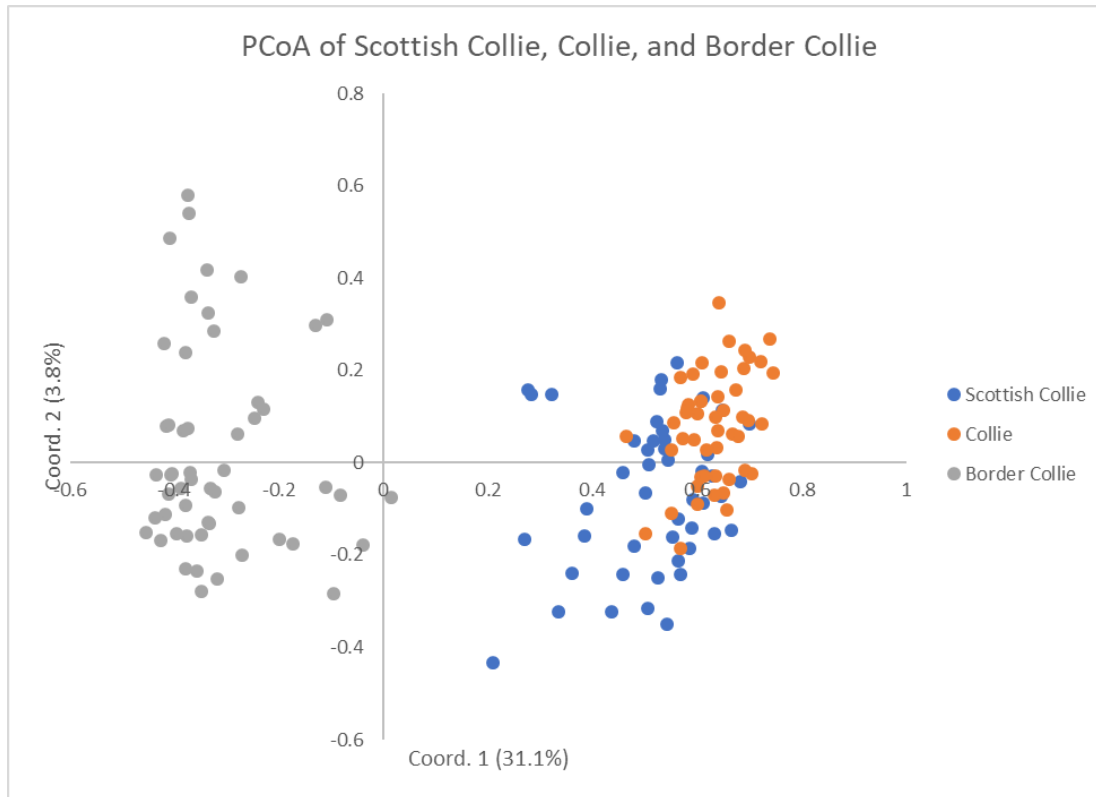
PCoA measures the genetic relatedness of individuals in a population. The data is computed in a spherical form, but it is often presented in the two dimensions that most closely represent its three-dimensional form (usually coordinates 1 and 2). The more closely individuals cluster together around the XY axis, the more related they are to each other. Forty of the 43 Scottish Collies formed a single population (i.e., breed) in PCoA (Fig. 2). However, three individuals

were outliers from the main population, of sufficient distance to constitute a distinct "variety" of the breed or outcrosses with more distantly related dogs.



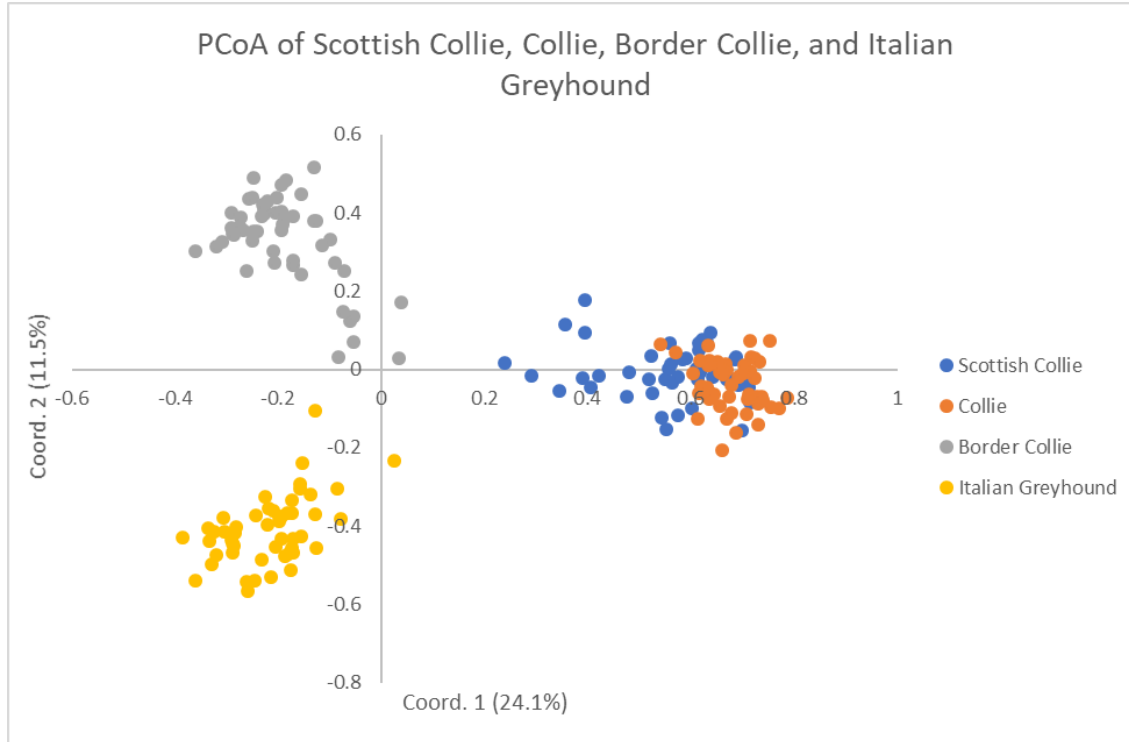
**Fig. 2.** PCoA graph of Scottish Collie (n=43) based on the 33 STRs. Three dogs appear to be closely related to each other but less to the other dogs (circled) and form what are known as genetic outliers.

The relatedness of Scottish Collie to Collie and Border Collie was compared on a separate PCoA graph (Fig. 3). The Scottish Collie and Collie were starting to differentiate into genetically distinct populations (i.e., subpopulations) - more like North American and European Italian Greyhounds or performance and conformation types of Golden Retrievers, but not yet to the level of varieties, such as seen with American and Japanese Akita, or Black and Salt and Pepper Giant Schnauzers. The Border Collies were genetically distinct from the Scottish Collie and Collie and manifested a surprising degree of genetic diversity between individuals, similar to Labrador and Golden Retrievers.



**Fig. 3.** PCoA graph comparing relatedness of Scottish Collie (n=43), Collie (n=55), and Border Collie (n=57).

A third PCoA comparison was made by adding in a fourth breed, the Italian Greyhound (Fig. 4). The Italian Greyhound clusters with the Collie in the genetically related herding-sighthound group. This comparison of four breeds caused related breeds and individuals to cluster closer to each other. Although individuals in each breed did form tighter groups, the breed relationships persisted. Scottish Collie and Collie clustered together with the latter forming a distinct subpopulation within the Scottish Collie. The Border Collie, Italian Greyhound and Scottish Collie/Collie remained genetically distinct breeds, although the high level of genetic diversity in Border Collie and Scottish Collie was still apparent.



**Fig. 4.** PCoA comparison of the Italian Greyhound, Scottish Collie, Collie and Border Collie.

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

### 1. IR testing

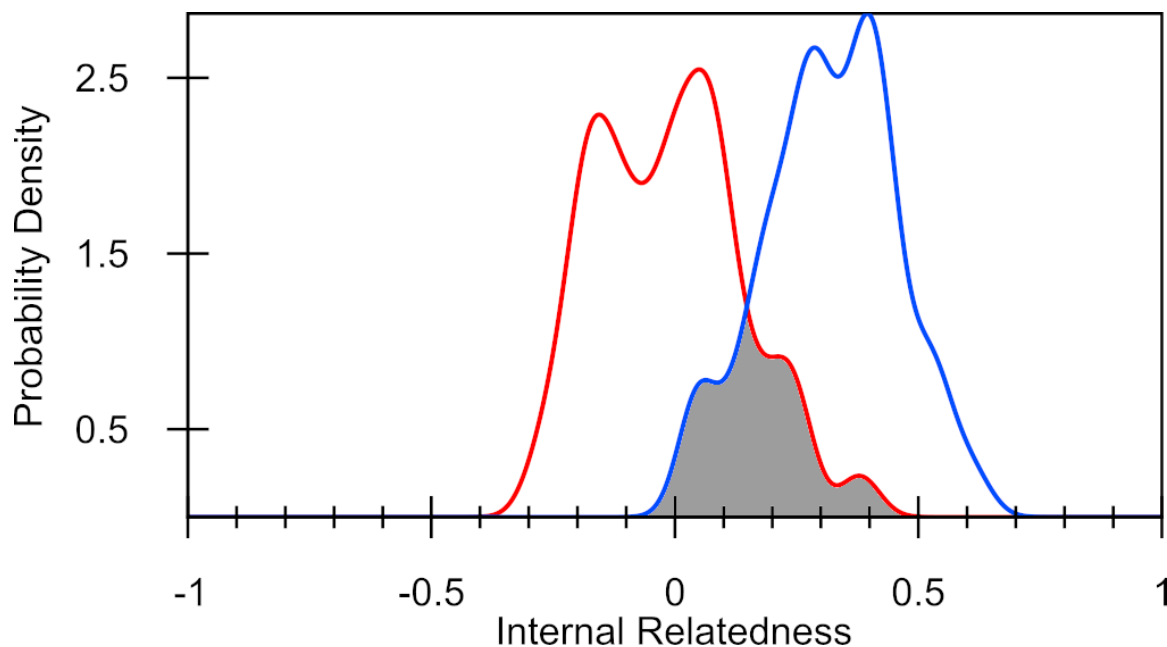
Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity being provided to individuals by their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which the two parents of an individual dog were related. The IR calculation takes into consideration homozygosity at each locus and gives more importance to rare and uncommon alleles. Rare and uncommon alleles would presumably be present in less related individuals. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with a value of -1.0 would have parents that were totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical at all loci. An IR value of +0.25 would be found among offspring of full sibling parents from a random breeding population. IR values  $>0.25$  occur when the parents of the full sibling parents were themselves highly inbred. The higher the IR value above 0.25 the more closely related were the parents and grandparents of the siblings.

Table 5 lists the IR values for the 43 Scottish Collies that were initially tested. The most outbred dog in the population had an IR score of -0.274, while the most inbred dog in the group had an IR score of 0.380, while the mean (average) IR score for the group was -0.011. The IR curve created from this data was also somewhat biphasic, with one group having IR scores  $< 0.082$  and a second group with scores  $< -0.136$  (Fig. 5). This latter group contained a small proportion of dogs that was more inbred ( $IR > 0.25$ ) than offspring of full sibling parents. Therefore, IR values

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

**Table 5.** Internal relatedness (IR) values calculated using allele numbers and frequencies for 43 Scottish Collie (redline). The IR values can be adjusted to reflect how these same dogs would score if they were to exist in a large population of village dogs (IR village dog or IRVD)

	IR	IRVD
<b>Min</b>	-0.274	-0.027
<b>1st Qu</b>	-0.136	0.175
<b>Mean</b>	-0.011	0.294
<b>Median</b>	-0.007	0.336
<b>3rd Qu</b>	0.082	0.411
<b>Max</b>	0.380	0.569



**Fig. 5.** Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Scottish Collie (n=43). The area under the curve (black) represents the degree of allele sharing (26.8%) between Scottish Collie and village dogs.

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

It is possible to determine the amount of canid genetic diversity a breed has retained as it evolved to present day. This is done by assuming that individual Scottish Collies are actual members of the current village dog population found in the Middle East, SE Asia and the Island Pacific nations. The IR values and IR values adjusted to village dogs (IRVD) (Table 5) can then be graphed and the graphs overlaid (Fig. 5). The IRVD graph, similar to the IR graph, tended to be bimodal with one quarter of the dogs having IRVD scores  $>0.411$  and one half with scores  $>0.366$  (Table 5). Therefore, if this group of dogs were village dogs, more than one-half of them would be considered more inbred than offspring of full sibling parents.

The IRVD curve for the Scottish Collies tested was shifted to the right of the IR curve, and the area of overlap was 26.8% (Fig. 5). This figure is comparable to the 39.7% of retained genetic diversity calculated using a somewhat different population, i.e., all canids ever tested at the VGL (Tables 1, 2). This level of retained village dog genetic diversity is lower than the 60% or so retained diversity observed in the Miniature/Toy Poodle or 54% in Labrador Retriever, but much higher than the 23% for Irish Wolfhound, 15% in Doberman Pinschers and 7% in Swedish Vallhund. All pure breeds of dogs have come from relatively small founder populations and have therefore had limited genetic diversity from the time registries were created and closed. Greatly varying amounts of genetic diversity may have been lost subsequently through artificial genetic bottlenecks such as cataclysmic events (e.g., world wars) or inbreeding for a specific show conformation (e.g., popular sire effects).

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

The DLA consists of four gene rich regions making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. The region of DLA class I that contains the DLA-88 gene is associated with four STR loci (Table 6). The DLA II region, which contains three polymorphic genes (DQA1, DRB1, and DRB2) is defined by three STR loci (Table 6).



**Table 6.** Specific STR loci that are associated with the DLA class I and II regions, including alleles identified at each locus and their frequencies. Allele numbers and frequencies are used to assess heterozygosity within the DLA region.

#	STR loci	N	Na	Ne	Ho	He	F
<b><u>DLA Class I STRs</u></b>							
1	<b>DLA I-3CCA</b>	43	6	1.60	0.42	0.37	-0.12
2	<b>DLA I-4ACA</b>	43	4	1.10	0.09	0.10	-0.03
3	<b>DLA I-4BCT</b>	43	3	1.10	0.09	0.09	-0.04
4	<b>DLA1131</b>	43	5	1.58	0.42	0.37	-0.14
<b><u>DLA Class II STRs</u></b>							
5	<b>5ACA</b>	43	4	1.61	0.42	0.38	-0.11
6	<b>5ACT</b>	43	4	1.15	0.14	0.13	-0.05
7	<b>5BCA</b>	43	5	1.65	0.44	0.39	-0.12
	<b>Avg.</b>		<b>4.43</b>	<b>1.40</b>	<b>0.29</b>	<b>0.26</b>	<b>-0.09</b>

### ***1. Heterozygosity in the DLA region***

The expectation is that allele and allele frequencies for STR loci in the DLA region are in equilibrium with other STR loci due to random mating over a long period of time. This can be tested by doing a standard genetic assessment of STR loci in other parts of the genome (Table 4 as well as in the DLA region (Table 6). This comparison indicates that the DLA region is significantly less heterogeneous than the rest of the genome (0.29 vs. 0.45), as would be expected given the high proportion of two specific extended haplotypes. The proportion of alleles ( $N_e$ ) that define heterozygosity was also lower than observed for the 33 autosomal STR loci (1.40 vs. 2.76) and the observed heterozygosity at each locus is much lower (0.29 vs. 0.45). However, a standard genetic assessment of the population of 43 dogs taken as a whole showed similar F values for autosomal STRs of -0.02 and DLA STRs -0.09. These values indicate that the DLA region, although appearing to be greatly unbalanced based on haplotype frequencies and heterogeneity, is actually in equilibrium with the rest of the genome. Therefore, the proportionately high incidence of certain haplotypes in this group of 43 Scottish Collies did not result from non-random selection in recent times, but rather at least 40/43 dogs tested are remnants of a "single founder or closely related founder population" that lived in the distant past and still exists through its genes to this day.

### ***2. DLA class I and II haplotypes in Scottish Collie***

Specific alleles at the three STR loci associated with the DLA class I region and the four STR loci that define the DLA class II region are in strong linkage disequilibrium (LD) forming specific haplotypes (Table 7). A haplotype is a group of genes within an organism that is inherited together from a single parent. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes identified in Standard Poodles being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class

II haplotypes. It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry.

**Table 7.** DLA class I and Class II haplotypes and their frequencies in 43 Scottish Collies

<b>DLA1 #</b>	<b>STR types</b>	<b>Scottish Collie (n=43)</b>
1006	387 375 293 180	0.02
1008	386 373 289 182	0.01
1016	382 371 277 178	0.13
1045	376 371 277 186	0.78
1087	380 371 277 178	0.02
1222	386 379 277 181	0.01
1223	380 371 289 181	0.01
1224	385 371 277 182	0.01
<b>DLA2 #</b>		
2003	343 324 282	0.01
2007	351 327 280	0.01
2031	339 322 282	0.02
2039	345 327 276	0.77
2065	339 325 286	0.01
2081	343 322 282	0.01
2091	343 327 288	0.10
2119	343 324 286	0.01
2120	343 327 286	0.03
2121	351 327 282	0.01

The 43 Scottish Collies in this study possessed 8 DLA class I and 10 DLA class II haplotypes (Table 7). Three minor class I (1222-1224) and three minor class II (2119-2121) are unique to the breed, to date, and the rest shared with a number of other breeds (Table 9). One class I (1045) and one class II (2039) haplotypes occurred in 77-78% of the dogs tested, while another class I (1016) and class II (2091) haplotype were observed in 10-13% of dogs. Collectively, these high incidence haplotypes were in strong linkage disequilibrium, forming larger 1045/2039 and 1016/2091 haplotypes and were found collectively in almost 90% of the 43 Scottish Collies tested. All of the remaining haplotypes occurred at incidences ranging from 1-3%.

Genetic diversity within the DLA region can also be compared between populations and breeds. The existing DLA class I and II haplotypes in Scottish Collie make up 8/205=3.9% and 10/112=8.9%, respectively, of DLA haplotypes currently identified by the VGL. The numbers of DLA class I (n=8) and II (n=10) haplotypes found in Scottish Collie were higher than the Swedish Vallhund (6/4) and Shiloh shepherd (7/6); somewhat lower than Giant Schnauzer (14/15), Samoyed (13/12) and Shiba Inu (16/15); and much lower than Golden Retriever (26/23) and Miniature Poodle (33/ 23).

### 3. DLA class I and II haplotype sharing between breeds

DLA haplotypes are much more conserved than most other regions of the genome and each DLA region inherited as a block of linked genes from each parent and passed on by descent over many generations. Therefore, DLA haplotypes can be used to estimate the founder/founder lines that were used to create a breed and the importance of the various lines in subsequent breed evolution. DLA haplotypes can also be used to determine possible common ancestry between breeds.

#### a. DLA haplotype sharing between Scottish Collie and Collie

DLA class I and II haplotypes found in Scottish Collie (Table 7) were compared with the haplotypes found in 49 conformation class Collies (Table 8). Only two DLA class I and three class II haplotypes were identified in these 49 Collies (Table 8). DLA class I 1045 and DLA class II 2097 haplotypes were in linkage disequilibrium forming an extended 1045/2039 haplotype that was found in 95% of the Collies tested. This same DLA class I/II haplotype was found in only 14.7% of Scottish Collie (Table 7).

**Table 8.** DLA class I and II haplotypes found in conformation Collies (n=49)

DLA1 #	STR types	Collie (n=49)
1016	382 371 277 178	0.04
1045	376 371 277 186	0.96
DLA2 #		
2039	345 327 276	0.95
2091	343 327 288	0.04
2097	343 327 276	0.01

#### b. DLA class I and II haplotype sharing with other breeds

The Scottish Collie shares DLA class I and II haplotypes with over 30 different breeds, all originating in the United Kingdom (Table 9). The greatest number of shared haplotypes was with the Irish Red and White Setter; Toy, Miniature and Standard Poodles; and the Swedish Vallhund. The highest incidence sharing of a single haplotype was with the Borzoi, Labrador Retriever, Giant Schnauzer, Havanese, and the Biewer, Yorkshire and Lakeland Terriers.

**Table 9.** DLA class I and II haplotype sharing between Scottish Collie and other breeds that have been tested by the VGL

		DLA Class I Haplotype Frequencies (Updated Dec 6, 2019)																																	
DLA I #	STR Types	Black Russian Terrier (n=144)	Labrador Retriever (n=75)	Irish Red and White Setter (n=69)	Doberman Pinscher (n=618)	Flat Coated Retriever (n=47)	Havanese (n=48)	Samoyed (n=189)	Saint Bernard (n=12)	Clint Schaefer (n=220)	Polish Lowland Sheepdog (n=19)	Borzoi (n=8)	English Bulldog (n=182)	Bleu de France (n=121)	Bleu de Picardie (n=107)	Yorkshire Terrier (n=16)	Italian Greyhound (n=943)	Alaskan Klee Kai (n=548)	Shiloh Shepherd (n=183)	English Mastiff (n=20)	Irish Setter (n=49)	Lowell Setter (n=94)	Akita (n=101)	Japanese Akita (n=366)	Golden Retriever (n=718)	Collie (n=49)	Irish Wolfhound (n=55)	Border Collie (n=51)	Miniature Poodle (n=288)	Scottish Collie (n=47)	Barbet (n=4)	Swedish Vallhund (n=225)	Poodle (n=284)	Toy Poodle (n=142)	
1006 387 375 293 180	...	0.045	...	0.075	0.081	...	0.047	0.005	...	0.05	...	0.14	0.003	...	...	...	...	...	...	...	0.11	0.069	...	0.059	0.0139	...	...	0.03	0.02	...	0.264	0.9468	0.004		
1008 386 373 289 182	...	...	0.004	0.083	...	...	...	...	...	0.045	...	...	0.006	0.008	...	...	0.06	0.1267	0.0011	...	0.021	...	...	0.0014	...	...	0.01	...	0.018	0.018	0.018	0.018	0.018	0.018	
1016 382 371 273 178	...	0.011	0.013	0.021	0.0139	...	0.212	...	...	0.036	0.03	...	0.095	0.025	0.019	0.03	0.0569	...	...	0.003	0.13	...	...	0.0021	0.08	...	0.024	0.12	...	0.0164	0.022	0.022	0.022	0.022	
1045 376 371 277 178	...	...	0.008	...	...	...	...	...	...	...	...	...	...	0.004	...	...	...	...	...	...	...	0.005	0.005	...	0.96	...	0.157	0.003	0.79	...	0.001	0.012	0.012	0.012	
1087 380 371 277 178	...	...	...	...	...	...	0.001	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.015	...	...	...	...	0.01	...	0.02	...	0.02	...	0.02	0.02	
1222 386 379 273 185	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	...	0.01	...	0.01	0.01	
1223 380 371 289 181	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	...	0.01	...	0.01	0.01	
1224 385 371 277 182	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.01	...	0.01	...	0.01	0.01	
		DLA Class II Haplotype Frequencies (Updated Dec 6, 2019)																																	
DLA II #	STR Types	Black Russian Terrier (n=144)	Labrador Retriever (n=75)	Irish Red and White Setter (n=69)	Doberman Pinscher (n=618)	Flat Coated Retriever (n=47)	Havanese (n=48)	Samoyed (n=189)	Saint Bernard (n=12)	Clint Schaefer (n=220)	Polish Lowland Sheepdog (n=19)	Borzoi (n=8)	English Bulldog (n=182)	Bleu de France (n=121)	Bleu de Picardie (n=107)	Yorkshire Terrier (n=16)	Italian Greyhound (n=943)	Alaskan Klee Kai (n=548)	Shiloh Shepherd (n=183)	English Mastiff (n=20)	Irish Setter (n=49)	Lowell Setter (n=94)	Akita (n=101)	Japanese Akita (n=366)	Golden Retriever (n=718)	Collie (n=49)	Irish Wolfhound (n=55)	Border Collie (n=51)	Miniature Poodle (n=288)	Scottish Collie (n=47)	Barbet (n=4)	Swedish Vallhund (n=225)	Poodle (n=284)	Toy Poodle (n=142)	
2003 343 324 282	...	0.74	0.017	...	0.1444	0.217	0.013	...	0.036	...	0.147	...	0.598	0.231	0.208	0.252	0.38	0.0069	...	0.008	...	...	0.023	...	0.019	...	0.029	0.516	0.01	0.129	...	0.091	0.43	0.43	
2007 351 327 280	...	0.045	...	0.041	0.158	...	0.05	0.005	0.01	0.05	...	0.147	...	...	...	...	...	...	...	0.0137	...	...	...	0.059	...	0.0139	...	0.02	0.02	0.02	0.02	0.02	0.02	0.02	
2011 339 322 282	...	0.034	...	0.008	...	0.008	...	...	...	0.036	...	...	0.0583	...	...	...	0.0961	...	...	0.003	...	...	...	0.099	0.094	...	0.95	0.147	...	0.78	...	0.001	0.001		
2039 345 327 276	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2065 339 325 286	...	...	...	...	...	...	...	...	...	...	...	...	0.006	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2086 339 325 276	...	...	0.207	...	...	...	...	...	0.087	...	...	...	...	0.004	0.009	...	0.03	...	...	0.243	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
2081 343 322 282	...	...	...	...	...	...	...	...	...	...	...	...	...	0.052	0.019	0.005	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2091 343 327 288	...	...	...	0.0138	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.04	...	...	...	...	...	...	...	...	...	
2119 343 324 286	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2120 341 327 286	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2121 351 327 282	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

### III. What does this assessment of genetic diversity tell us about the contemporary Scottish Collie

#### A. What is meant by "breed restoration."

Restoration of the older, working-type, regional breed is becoming increasingly more common. However, such modern restorations are not new and have occurred in a number of older breeds, but at a time when DNA testing was not available. The objective of breed restoration is to discover as many remaining representatives of the breed as possible. If they cannot be found, or not found in sufficient numbers, phenotypically similar dogs with known historic (pedigree) or confirmed (DNA) relationships can be included in the founder population to assure as much genetic diversity as possible. Once a number of genetically and/or phenotypically desired dogs are found, the registry will presumably be closed. DNA will provide a much better measure of the genetic diversity that is present in the founder population than pedigrees, especially when other registered breeds are included in the restoration. If collected and stored properly, DNA can maintain intact for hundreds or even thousands of years and provide a highly accurate database for all subsequent genetic testing, parent selection, maintenance and balancing of genetic diversity, monitoring further genetic introgressions or for tracing and eliminating any heritable disorders that may arise.

#### B. What is a Scottish Collie?

A simple and plausible explanation for the origin of the working dogs of Scotland has been published - *"The Collie originated as a shepherd's dog in the Highlands of northern Scotland where it functioned as an all-purpose farm dog. ... Scotland was subject to conquest, invasion and immigration from various peoples in the centuries before the Collie appears in written history. Notable among these people who came to live in Scotland, no doubt bringing their dogs with them, were Celts, Romans, Norse, Irish and English. Dogs from all these peoples no doubt contributed genetic material to the dogs of Great Britain as a whole. ... By the 1700s all this canine genetic material had settled into pockets where it was most useful for different jobs. The Scottish Deerhound for example was used for hunting deer and running down stags, the Scottish Terrier was used to hunt smaller animals like badgers and foxes, the Gordon Setter was used for hunting birds and the Collie was used for herding sheep."* [3]

The 43 Scottish Collies initially tested possessed genetic markers in the DLA class I and II regions that were found in 30 other breeds, almost all originating in Victorian England. This confirms the belief that Scottish farm-type shepherds are a mixture of many types of dogs of the region. It also makes sense that a large number of DLA haplotypes were also shared between Scottish Collie and the Irish Red and White Setter and Poodle, breeds of similar stature with shared histories of farm work and hunting. The highest incidence sharing of a single haplotype was with the Borzoi, Labrador Retriever, Giant Schnauzer, Havanese, and the Biewer, Yorkshire and Lakeland terriers. These genetic relationships, best defined by shared regions within the DLA, reflect the common ancestry of dog breeds in general, but also the propensity of British dog breeders to create and refine new breeds over the last 200 years. One interesting breed appeared in the DLA ancestry of the Scottish Collie, the Borzoi. The evolution of Collies from performance to conformation type occurred during the late 1800s and early 1900s, and it is noteworthy that some breeders of the time were accused of introducing Borzoi into the breed [3]. The sharing of a single extended DLA class I/II haplotype, 1006/2009, and the similar head shape support this accusation. The close relationship of the Collie and Borzoi has been also noted in other studies, where both are found in the herding-sighthound breeds [27].

The closest DLA class I/II haplotype sharing was between Scottish Collie and Collie, as might be expected. However, the pattern of sharing was unexpected. Only two DLA class I and three class II haplotypes were identified among all show-type Collie tested, DLA class I/II 1045/2039 and 1016/2091. These same two haplotypes were found collectively in 91% of Scottish Collie. This strongly suggests that dogs defined as Collies may have evolved primarily from two founder lines, one possessing the extended DLA class I/II 1045/2039 haplotype and the other the 1006/2009 haplotype. The extreme loss of genetic diversity in one or more genes of the DLA class II region has been previously reported for the rough Collie [28] and Bearded Collie [29].

## **B. Restoration of farm type Collies of Scotland**

The 43 dogs that were studied herein are being evaluated as potential foundation stock for the modern restoration of the original farm-type Collie of Scotland. Forty of these 43 dogs were closely related based on the 33 autosomal STR markers and 7 DLA class I and II associated STRs. This particular group of dogs was also closely related to the conformation-type Collie based on strong sharing of two major DLA class I /II haplotypes. This relationship occurred at the variety rather than breed level. However, this relationship was opposite of what was expected. The 43 Scottish Collie were more genetically diverse than the Collies and possessed a number of minor DLA haplotypes not found in the Collie. There are two differing explanations for this occurrence: 1) The 43 Scottish Collies were indeed representatives of a somewhat genetically diverse population of original farm-type Collie that still exists, and that contemporary Collie have evolved by more severe inbreeding into a small subpopulation of this population; or 2) that the 43 Scottish Collies contained a number of outcrossed and outcross/backcrossed dogs that were brought into the breed over the last decades as a surreptitious attempt to counter the severe inbreeding perceived by some breeders. Evidence for the latter scenario came from three of the 43 dogs that were genetically outliers and presumably close outcrosses. Regardless, the 43 dogs in this study met the requirements of the new standard. However, closing the new registry with this type of dog would limit genetic diversity in the breed from the onset, and it might be

wise to consider including a degree of outcrossing with phenotypically similar, but genotypically diverse and distinct breeds. The Border Collie meets those requirements as shown by the PCoA comparisons in Figure 2. Indeed, there are some individual Border Collies and Scottish Collies that appear to be more closely related to each other in the four-breed comparison in Figure 3.

The dominant objective of contemporary Scottish Collie breeders is to stay as close to pure Collies as possible, while preserving the working instinct, size and conformation of the classic farm Collie. “[*The SCPS*] intends to return the Scottish Collie to its original form, health, and vigor, with form fitted to its function of sheepherding by means of careful breeding, which provides for genetic diversity and selection of genetically-tested dogs and bitches resembling the dogs described in the earliest Standards of the breed [4].” This is a viable approach if the desired phenotypic traits still exist in an overt or hidden form among a sub-population of modern registered Collie or can be obtained through outcrossing. This goal is in line with statements from some prominent breeders, “*We are trying to restore the old fashioned Scotch Collies, it is a desperate situation but I believe this battle is not lost, we can take back genetic material from various sources where it has gone in past decades* [18].” Both the SCPS and the OTSC have organizational mechanisms for outcrossing should it be necessary or desirable. If there is evidence of too little genetic variability in the final selection of founders, some outcrossing may be considered in the future [18].

Outcrossing, if contemplated as the best way to increase genetic diversity, is not as simple as it might seem but fortunately facilitated by modern DNA testing. The first, and most important thing, is that the added diversity must not mimic existing diversity, nor can new genotypes replace existing genotypes. However, the linkage of genotypic and phenotypic traits may make this more difficult. The same phenotypes in other breeds may or may not add as much to genotypic diversity as desired. Secondly, it is important that heritable disorders not be introduced into the breed through the outcrosses. This will also require extensive DNA screening, although not all heritable disorders have identifiable genotypes. Genetic diversity brought in by outcrossing has to be rapidly and widely distributed among existing dogs.

## **IV. Heritable disorders of the Scottish Collie**

### **A. General health and lifespan**

The Scottish Collie is purportedly of good health [13, 14, 30]. The average lifespan of the Scottish Collie was given as 12-16 years in one report [13] and 10-14 yrs. (with some 15-16 yrs.) for the Smooth and Rough Collie [30]. Common disorders of dogs such as hip dysplasia are reportedly uncommon among Scottish Collies [14].

### **B. Heritable disease problems**

#### ***1. Heritable disorders of primary concern [30]***

***a. Collie eye*** - Collie eye anomaly (CEA) is a congenital, inherited, eye disease of dogs affecting the retina, choroid, and sclera [15]. CEA is inherited as an autosomal recessive mutation in the NHEJ1 gene with complete penetrance and localized to canine chromosome 37 [16]. Therefore, it

can only affect a dog's vision if both parents are carriers of the gene. The disorder can be mild or cause blindness, depending on the individual. It is known to occur in Scottish Collies (smooth and rough Collies), Shetland Sheepdogs, Australian Shepherds, Border Collies, Lancashire Heelers, and Nova Scotia Duck Tolling Retrievers [16]. The incidence of CEA tends to be higher in conformation type Collies than in related breeds of performance type (e.g., Border Collie, Nova Scotia Duck Tolling Retriever, farm type Scottish Collie). CEA is reportedly rare in the Old-Time Scotch Collie breed [14], whereas the incidence in the Collie breed in the US has been estimated to be as high as incidence of 80 to 85 percent [15]. The CEA phenotype can be identified with ophthalmoscopic examination in puppies by 7 weeks of age and a DNA based test is available for detection of carriers.

**b. Multiple drug resistance** - Multiple drug resistance type 1 (MDR1) is an autosomal recessive condition that is common in dogs of the Collie lineage [17]. This malady renders the dog more sensitive to the negative effects of certain medications including ivermectin, a popular deworming treatment. Vets have alternative medications they can use for MDR1 effected dogs. A DNA test can check for this issue.

## **2. Heritable disorders of secondary concern [30]**

This is a group of autosomal recessive diseases that occur at low incidence in the breed and are often handled by detecting carriers and avoiding breeding of heterozygotes. This is often done in preference to elimination of the mutations from the breed, which can lead to a significant loss of genetic diversity in breeds that already lack diversity.

**a. Cyclic neutropenia** - Affected dogs have a gray coat color, hence the name gray Collie syndrome. Dogs homozygous for this mutation are vulnerable to infections during periods of low neutrophil counts. Clinical signs include fever, diarrhea, inflamed lymph nodes, gingivitis, lameness and mild bleeding episodes. Affected dogs usually die before 2 years of age. A DNA test is available for detecting mutation in the ELANE gene.

**b. Degenerative myelopathy**- Degenerative Myelopathy (DM) is caused by an autosomal recessive mutation in SOD1 that is found in many breeds of dog, including the Scottish Collie. The average age of onset of degenerative myelopathy is approximately nine years of age. A DNA test is available

**c. progressive rod-cone disease (PRCD)**- A progressive rod-cone dysplasia caused by an autosomal recessive mutation in the RD3 gene occurs at low incidence in the breed. Reliable genetic testing is important for either eliminating this mutation from breeding lines or in producing affected dogs by breeding of carrier parents. One or more types of progressive retinal atrophy (PRA), yet to be defined, may also occur in the breed. Therefore, selection against PRCD may not exclude PRA in the pedigree.

**e. Hyperuricosuria**- Dogs with mutations in both copies of the SLC2A9 gene are predisposed to have elevated levels of uric acid in the urine. Uric acid can form crystals and/or stones (uroliths) in the urinary tract. Urinary stones in the bladder can cause urinary tract infections or more seriously, urethral blockage. Urethral blockage is more common in males due to differences in



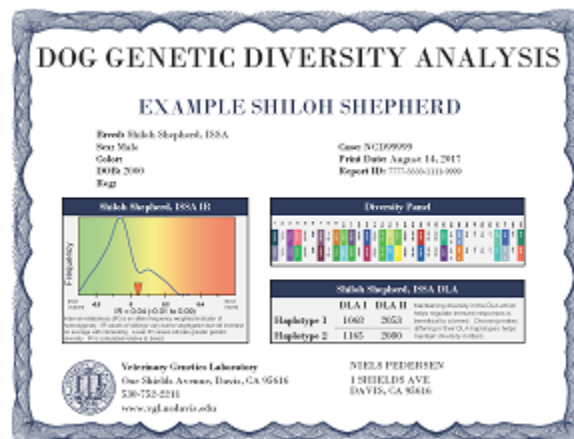
anatomy. Not all dogs homozygous for the SLC2A9 gene mutation will have clinical signs of disease, though they will have increased uric acid excretion in the urine.

**d. Von Willebrand's disease Type II-** VWDII is an inherited blood-clotting disease. Clinical signs include easy bruising, frequent nosebleeds, lots of bleeding from teething, and excessive bleeding from surgery, trauma (accidental, nail clipping). VWD involving identical or different mutations in the Von Willebrand factor gene occur in many breeds of dogs and are usually tolerated and seldom a cause of life-threatening hemorrhage.

## V. Results of Diversity testing

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

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



### B. What should you do with this information?

Breeders of contemporary Scottish Collie are at a crossroad and must decide whether to select foundation stock from within the existing Collie breed or to further increase genetic diversity by allowing outcrosses with other breeds. This is an important decision, because once the founders are identified and the registry closed, no more genetic diversity can be added.

If the selected path is to stick with founders from within the Collie breed, the goal for breeders should be to rebalance the existing genetic diversity. This can be done by utilizing DNA to select parents that will produce puppies with IR scores less than 0, and with time, even lower scores. Mates should be selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype and encourage the use of dogs with less common genomic alleles or DLA haplotypes. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity, similar to what is being done by many Standard Poodle breeders. However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

The next step is to compare the DLA class I and II haplotypes. You want to avoid breeding pairs that will produce puppies that will be homozygous for the same haplotypes, and once again, less common haplotypes may offer more diversity than common ones. Re-establishing a balance in of existing frequencies of existing DLA haplotypes will be more difficult because of the preponderance of two extended DLA class I/II haplotypes. However, it is fortunate that many Scottish Collies in the 43 dogs tested possess unrelated, and sometimes unique, DLA haplotypes at low frequency. Selecting parents that will lower the frequency of the two major haplotypes and encourage heterozygosity in these regions of the DLA can rebalance existing DLA diversity over relatively few generations. What effect this will have on the phenotypes of the breed (performance, health or appearance) is difficult to predict, but hopefully it will be positive.

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

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

A more effective use of this study is to contribute the genetic information to a web repository. The best format for such a repository and testing has been provided by Standard Poodle breeders. This information could be incorporated into a mate selection service that will allow a breeder to identify, among all the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters-<https://www.betterbred.com/>.

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