Genetic Diversity Testing for Border Collie

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 and heterozygosity 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 Border 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 57 registered Border Collie from the USA. 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 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.

ORDER TEST KITS

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. Breed history

The Border collie is one of the breeds that has evolved from the landrace (indigenous) Scottish farm shepherds of the 18th century that became to be known as Coll, Colley, Colly, Coaley, Coaly or Collie in the 19th century [1-4]. The exact origin of the name Collie is unknown, but its roots are presumed to be in Old Scottish or Gaelic/Celtic languages. Collie, or similar words, can mean coal or black, little dog, one who follows another constantly, a lounger, one who hunts for a dinner; or useful [1-4]. The name Border Collie refers to a type of dog that was strongly associated with the sheep herding in the border regions of Northumbria and the Scottish Lowlands [34, 35].

Ancestors of the farm dogs of the North of England and Scotland included native Celtic dogs, Roman cattle dogs and Viking herding Spitz [2-4]. Dogs resembling modern Collie-type breeds were frequently described in 17th century literature in Scotland and in spite of their landrace origins, they ultimately developed a breed-like uniformity (Fig. 1). However, the breed was not widely known outside of Scotland before the early 1800s [2]. Their fame actually spread in the Victorian era (1837-1901), possibly bolstered by the queen herself [5]. Collie-type dogs were actually popularized in the England and was rapidly exported to its colonies and to North America [3, 6]. Early English breeders initially 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] and breeds such as the Bearded Collie and Border Collie came into existence.

Figure 1. 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.

The rapid increase in popularity of Collie-type dogs in the late 19th and early 20th centuries involved showing, pet ownership, and farm work. However, the evolution of Border Collie was even more closely linked to sheep herding trials or "trialling" [35]. There was a particular need for sheep herding dogs of the Collie type not only in the sheep raising regions of UK but in places like Australia and New Zealand. "Collies" were listed as imports to New Zealand as early as 1858, but the type was not specified [5]. In 1915, James Reid, Secretary of the International Sheep Dog Society (ISDS) in the United Kingdom first used the term "border collie" to distinguish strong sheep working dogs registered by the ISDS from the Kennel Club's collie (or Scotch collie, including the rough collie and smooth collie) [1].

Similar to the history of many contemporary dog breeds, one or more individuals have contributed to contemporary Border Collie out of proportion to others in terms of historical and genetic contributions. Old Hemp (1893-1901) was a tricolor dog born in Northumberland in September 1893 out of a black and tan sire and black-coated Meg (Fig. 2). He was outstanding at sheep herding and his style of work became the breed standard. Many shepherds used him for stud, and he may have sired as many as 200 pups over the span of his life. All contemporary border can trace ancestry back to Old Hemp [1].

Fig. 2. Picture of Old Hemp (1893-1903) from the Border Collie museum. http://www.bordercolliemuseum.org/AuldHemp/AULDHEMP.html

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

Many Border Collie enthusiasts oppose the inclusion of the breed in bench and show ring activities, fearing that conformation breeding will lead to a decline in the breed's abilities as a world-class working dog and a change their appearance. Indeed, the genetic bases for these concerns have been well documented. Conformation traits have a higher heritability than performance traits and dogs selected for conformation traits will rapidly become more uniform in genotypic make-up and phenotypic appearance [30]. In spite of this resistance, an increasing number of Border Collie breeders have wholeheartedly embraced the show ring, albeit with a

nod towards maintaining performance attributes [29]. As a result, conformation bred Border Collie are seldom seen in strict performance events such as sheepdog field trials, which require a high degrees of specialty training and herding instinct. Therefore, Border Collie strictly bred for performance seldom grace show rings and *vice versa*. A similar debate has arisen in many breeds and frequently ends in schisms [30]. These schisms have been handled in different manners. Some breeds such as the English Shepherd and Irish Setter have created separate registries for each activity, while breeds such as the Brittany have created a single registry that accommodates both conformation and performance breeding with champions having to be certified in both endeavors [30]. Schisms in other breeds have not been so easily resolved. Breeders of Biewer-type Yorkshire terriers are deadlocked over whose dogs adhere closest to the original Biewer lineage. Still other organizations such as the Old Scottish Collie Association (OTSCA) and the Scottish Collie Preservation Society (SCPS) debate what constitutes the original working Collie [2-4].

C. Registries and Breed Standard [1]

The principal registry for border collies in the United States is the American Kennel Club [29]. The United States Border Collie Association (USBCA) [28], the American Border Collie Association (ABCA) [27], and the Border Collie Society of America (BCSA) [26] are dedicated to preserving performance traits and resist registration of conformation-bred dogs. The ABCA is strongly against conformation breeding and voted in 2003 to de-list any dog that wins a championship in the show ring regardless of performance ability [27]. Cross-registration of dogs in strictly performance-based registries is usually allowed, while the AKC accepts Border Collie from all registries.

There are two separate registries for border collies in the UK. The International Sheep Dog Society encourages breeding for herding ability, whereas the Kennel Club (UK) encourages breeding for a standard appearance. The ISDS registry is by far the older of the two, and ISDS dogs are eligible for registration as pedigree Border Collies with the Kennel Club (KC) — but not vice versa. The only way for a Border Collie without an ISDS pedigree to be added to the ISDS registry is by proving its worth as a herding dog so that it can be Registered on Merit (ROM).

Border Collie in Australia are registered either with the Australian National Kennel Council (ANKC) or in an associated working dog registry [1]. ANKC registration allows Border Collie to compete in conformation, obedience, agility, tracking, herding and other ANKC-sanctioned events held by affiliated clubs. Inclusion in this limited register prohibits entry into conformation events. The ANKC provides a breed standard that applies only to conformation events and not to dogs in performance events. Non-ANKC pedigreed dogs may also be eligible for inclusion on an ANKC associate or sporting register and be able to compete in ANKC sanctioned performance or herding events. Agility organizations such as the Agility Dog Association of Australia (ADAA) have their own registry which allows any Border Collie wishing to compete.

Agriculture Canada has recognized the Canadian Border Collie Association as the official registry under the Animal Pedigree Act for any purebred Border Collie in Canada. The criteria

used in the standard are based on herding ancestry rather than appearance. The registration of working sheepdogs in South Africa is the responsibility of the South African Sheepdog Association. ISDS registered dogs imported into the country can be transferred onto the SASDA register. Dogs not registered can become eligible for registration by being awarded a certificate of working ability by a registered judge.

D. Appearance [1]

Border Collie vary somewhat in appearance depending on whether they are bred purely for performance or for conformation. Dogs bred for show tend to be more homogeneous in appearance than working border collies, since to win in conformation showing they must conform closely to breed club standards that are specific on many points of the structure, coat, and color. Conformation bred Border Collie also tend to have thicker coats, a more harmonic movement, a wider skull, and heavier bones, while working lines tended to have thinner and shorter coat and were smaller in size [33]. Kennel clubs specify, for example, that the Border Collie must have a "keen and intelligent" expression, and that the preferred eye color is dark brown. In deference to the dog's working origin, scars and broken teeth received in the line of duty are not counted against a border collie in the show ring.

Border Collie are medium-sized dogs. The males' height from withers comes from 48 to 56 cm (19 to 22 in), females from 46 to 53 cm (18 to 21 in). The coat is double, medium length and prone to shedding, and varies from smooth to rough and is occasionally curled. The breed appears in the wide range of color and pattern known to exist in dogs, although black and white is the most commonly seen color pattern. Other common colors include black tricolor (black/tan/white), liver and white, and red tricolor (red/tan/white). Less common colors include blue, lilac, red merle, blue merle, brindle, and Australian red (blonde, gold). Some border collies are even solid colored. Eye color varies from brown to blue, and occasionally eyes of differing color occur as often seen with merles. The ears are also variable, some being fully erect, some fully dropped, and others semi-erect ears.

E. Temperament [1, 7-9]

Due to their working heritage, border collies are very demanding, playful, and energetic. Some of the more difficult behaviors require patience, as they are developmental and may disappear as the dog matures. Border Collie require a great deal of play and exercise, either with humans or other dogs. Due to compulsive personalities and excessive need for mental stimulation and exercise, many border collies develop problematic behaviors in households that are not able to provide for their needs. They can become distressed if left in isolation, ignored or inactive. Like many working breeds, border collies can be motion-sensitive and may chase moving vehicles and bicycles, but this behavior can be modified by training. They are infamous for chewing holes in walls and furniture, and destructive scraping and hole digging, due to boredom. Border collies may exhibit a strong desire to herd, a trait they may show with small children, chickens, cats, and other dogs. The breed's herding trait has been deliberately encouraged, as it was in the dogs from which the border collie was developed, by selective breeding for many generations. However, being eminently trainable, they can live amicably with other pets if given proper socialization training.

II. Preliminary genetic diversity studies of 57 registered Border Collie

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 57 Border Collie) are listed in Table 1. Link to Table 1.

Table 1. Identified allele and their frequencies for 33 autosomal STR loci in Border collie (n=57).

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
92 (0.036)	131 (0.263)	117 (0.009)	219 (0.009)	238 (0.139)	87 (0.098)
94 (0.089)	137 (0.061)	119 (0.018)	221 (0.009)	240 (0.231)	89 (0.107)
98 (0.054)	141 (0.009)	121 (0.316)	225 (0.152)	242 (0.185)	91 (0.741)
100 (0.089)	145 (0.395)	125 (0.035)	227 (0.018)	244 (0.009)	93 (0.027)
102 (0.295)	147 (0.061)	127 (0.114)	229 (0.071)	246 (0.361)	95 (0.018)
104 (0.018)	149 (0.114)	129 (0.070)	231 (0.009)	248 (0.056)	97 (0.009)
106 (0.250)	153 (0.096)	131 (0.246)	233 (0.482)	252 (0.009)	
108 (0.125)		133 (0.044)	235 (0.036)	254 (0.009)	
110 (0.009)		135 (0.149)	237 (0.205)		
112 (0.036)			241 (0.009)		

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
284 (0.080)	114 (0.054)	128 (0.054)	144 (0.009)	232 (0.009)	91 (0.123)
286 (0.107)	116 (0.313)	132 (0.366)	148 (0.107)	234 (0.148)	95 (0.711)
288 (0.348)	118 (0.536)	136 (0.018)	152 (0.446)	236 (0.028)	97 (0.053)
290 (0.018)	120 (0.018)	140 (0.018)	156 (0.107)	238 (0.102)	101 (0.105)
292 (0.446)	124 (0.054)	144 (0.473)	160 (0.214)	240 (0.231)	103 (0.009)
	126 (0.018)	148 (0.071)	164 (0.045)	242 (0.296)	
	128 (0.009)		168 (0.054)	244 (0.185)	
			172 (0.009)		
			176 (0.009)		

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.116)	144 (0.196)	208 (0.193)	85 (0.259)	227 (0.028)	200 (0.089)

120 (0.009)	148 (0.054)	210 (0.711)	95 (0.563)	231 (0.037)	202 (0.402)
122 (0.009)	150 (0.339)	212 (0.009)	107 (0.170)	233 (0.222)	204 (0.268)
124 (0.313)	152 (0.321)	214 (0.009)	111 (0.009)	235 (0.167)	206 (0.080)
126 (0.116)	154 (0.089)	216 (0.044)		237 (0.037)	208 (0.161)
128 (0.018)		218 (0.035)		239 (0.176)	
130 (0.009)				241 (0.324)	
132 (0.411)				245 (0.009)	

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.176)	160 (0.018)	266 (0.139)	222 (0.009)	139 (0.018)	12 (0.071)
210 (0.111)	162 (0.045)	268 (0.241)	226 (0.143)	143 (0.035)	14 (0.277)
212 (0.083)	164 (0.127)	270 (0.194)	228 (0.009)	145 (0.421)	15 (0.009)
214 (0.009)	166 (0.027)	272 (0.204)	232 (0.500)	147 (0.237)	18.2 (0.027)
216 (0.435)	168 (0.427)	276 (0.083)	234 (0.116)	149 (0.149)	20.2 (0.143)
218 (0.167)	170 (0.355)	278 (0.139)	236 (0.188)	153 (0.140)	21.2 (0.357)
220 (0.009)			238 (0.036)		22.2 (0.027)
224 (0.009)					23.2 (0.089)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
16.1 (0.027)	8 (0.045)	17 (0.018)	15 (0.125)	9 (0.429)	13 (0.027)
17.1 (0.473)	9 (0.598)	18 (0.009)	16 (0.063)	10 (0.045)	14 (0.384)
18.1 (0.170)	11 (0.009)	20 (0.339)	17 (0.339)	11 (0.009)	15 (0.054)
19.1 (0.179)	12 (0.036)	21 (0.161)	19 (0.071)	12 (0.018)	16 (0.036)
20.1 (0.080)	13 (0.036)	22 (0.018)	20 (0.018)	13 (0.214)	17 (0.330)
21.1 (0.018)	14 (0.107)	25 (0.009)	21 (0.205)	14 (0.080)	18 (0.125)
22.1 (0.054)	15 (0.089)	26 (0.143)	22 (0.179)	15 (0.196)	19 (0.036)
	17 (0.009)	27 (0.071)		16 (0.009)	20 (0.009)
	19 (0.009)	28 (0.080)			
	20 (0.045)	29 (0.036)			
	21 (0.009)	30 (0.107)			
	22 (0.009)	31 (0.009)			

VGL2918	VGL3008	VGL3235
12 (0.313)	10 (0.018)	12 (0.116)
13 (0.125)	14 (0.054)	13 (0.027)
14 (0.179)	15 (0.098)	14 (0.321)
15 (0.143)	16 (0.313)	15 (0.080)
16 (0.018)	17 (0.080)	16 (0.143)
17.3 (0.107)	18 (0.205)	17 (0.089)
18.3 (0.098)	19 (0.045)	18 (0.214)
19.3 (0.009)	20 (0.098)	19 (0.009)
20.3 (0.009)	22 (0.063)	
	23 (0.018)	

24 (0.009)

The number of possible alleles identified at each locus was higher than many other breeds usually only one allele occurred at frequencies greater than 30% of the population, while the remaining alleles at each locus were reasonably dispersed in frequency. The average number of alleles known to currently exist at these 33 loci for all dog is 15.4, but only an average of 7.52 of these have been inherited through descent at each locus by Border Collie. Therefore, the 57 Border Collie as a group possessed 7.52/15.4= 48.8% of all possible alleles for dogs. This means that only about one-half of known canid genetic diversity has been lost up to this point in the breed's evolution. The amount of retained canid 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 somewhat lower than popular breeds such as the Golden retriever (54.5%), toy poodle (55.6%) and (Standard poodle (58%). Taking all of this information together, it appears that Border Collie are among the more genetically diverse dog breeds by virtue of the available canid genetic diversity.

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 standard genetic parameters of heterozygosity (Table 2), such as the number of alleles found at each STR locus (Na); the number of effective alleles (Ne) per locus (i.e., the number of alleles that contribute most to genetic differences); the observed or actual heterozygosity (Ho) that was found; and the heterozygosity that would be expected (He) if the parents of the existing population were randomly selected. The value F is a coefficient of inbreeding derived from the Ho and He values. A value of +1.0 would occur only if every individual were genetically indistinguishable at each of the 33 STR loci, while a value of -1.0 would be seen when all of the dogs were completely different at each of the 33 loci. A value of 0.00 would be seen if the overall selection of sires and dams for the 57 individual dogs was as random as possible given the existing gene pool.

The observed (actual) heterozygosity in this group of 57 dogs was 0.68, while the expected heterozygosity (He) for a population in Hardy-Weinberg equilibrium (HWE) was 0.71. The principle behind HWE states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences and that Ho will equal He and the value for F will be zero. Although usually applied to spontaneous mutations in large random breeding natural populations, it is often used by breeders to define a contained population that is not being subjected to intense artificial selection for traits possessed by a small subset of the population. The observed or actual heterozygosity (Ho) was very close to the expectation (He) of a population in HEW and this yielded a coefficient of inbreeding (F) of -0.02. Therefore, this group of dogs was, on average, 2% more outbred or heterozygous than expected for HWE. Although the standard genetic assessment values indicate that these 57 Border Collie were as genetically different from each other as possible, this conclusion is based on the population as a whole and not on individual dogs making up the population. Interrelatedness (IR) scores will provide a better picture of heterozygosity among individual dogs (see section D-1).

Table 2. Standard Genetic Assessment values for 57 Border Collie based on 33 autosomal STR loci

	N	Na	Ne	Ho	He	F
Mean	57	7.52	3.76	0.68	0.71	0.04
SE		0.34	0.19	0.02	0.02	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 3). 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 Na values for an individual STR locus for this population of 57 Border Collie ranged from a low of 4 to a high of 12 alleles per locus, while the Ne ranged from 1.75 to 5.17 alleles per locus. It is important to remember that each STR locus can have from 7-27 different alleles (avg. 15.4 alleles/locus) when testing across all dogs. The observed heterozygosity (Ho) for an individual STR locus ranged from 0.32 to 0.85, while expected heterozygosity (He) ranged from 0.43 to 0.83 (Table 3). This meant that some loci were contributing more to heterozygosity than others. The relatively high observed heterozygosity scores for all loci with the exception of AHTk211 indicates a significant degree of genotypic diversity across the genome, which in turn suggests a greater than usual degree of phenotypic diversity. Twenty-four loci had positive F values and nine were negative. The low F values at each locus except for AHTk211 (F=0.25) also indicate that most these regions of the genome have not been under strong artificial positive selection during the period of breed development.

Table 3. Standard Genetic Assessment of individual autosomal STR loci for 57 Border Collie

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	57	10	5.36	0.77	0.81	0.06
2	**AHT137**	57	7	3.92	0.65	0.75	0.13
3	**AHTH130**	57	9	4.91	0.79	0.80	0.01
4	**AHTh171-A**	57	10	3.28	0.68	0.70	0.02
5	**AHTh260**	57	8	4.15	0.80	0.76	-0.05
6	**AHTk211**	57	6	1.75	0.32	0.43	0.25
7	**AHTk253**	57	5	2.95	0.66	0.66	0.00
8	**C22.279**	57	7	2.56	0.68	0.61	-0.11
9	**FH2001**	57	6	2.73	0.63	0.63	0.01
10	**FH2054**	57	9	3.66	0.66	0.73	0.09
11	**FH2848**	57	7	4.79	0.82	0.79	-0.03
12	**INRA21**	57	5	1.87	0.42	0.47	0.10
13	**INU005**	57	8	3.40	0.75	0.71	-0.06
14	**INU030**	57	5	3.73	0.84	0.73	-0.15
15	**INU055**	57	6	1.83	0.49	0.46	-0.08
16	**LEI004**	57	4	2.43	0.59	0.59	0.00

17	**REN105L03**	57	8	4.61	0.67	0.78	0.15
18	**REN162C04**	57	5	3.66	0.59	0.73	0.19
19	**REN169D01**	57	8	3.74	0.76	0.73	-0.04
20	**REN169O18**	57	6	3.05	0.62	0.67	0.08
21	**REN247M23**	57	6	5.47	0.85	0.82	-0.04
22	**REN54P11**	57	7	3.12	0.66	0.68	0.03
23	**REN64E19**	57	6	3.61	0.68	0.72	0.05
24	**VGL0760**	57	8	4.18	0.71	0.76	0.06
25	**VGL0910**	57	7	3.39	0.66	0.70	0.06
26	**VGL1063**	57	12	2.60	0.59	0.62	0.04
27	**VGL1165**	57	12	5.36	0.75	0.81	0.08
28	**VGL1828**	57	7	4.67	0.66	0.79	0.16
29	**VGL2009**	57	8	3.61	0.68	0.72	0.06
30	**VGL2409**	57	8	3.59	0.77	0.72	-0.06
31	**VGL2918**	57	9	5.34	0.75	0.81	0.08
32	**VGL3008**	57	11	5.71	0.82	0.83	0.00
33	**VGL3235**	57	8	5.04	0.75	0.80	0.06

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. Figure 3 is a PCoA graph comparing inter- and intra-relatedness of three breeds - Border Collie, Collie and Scottish Collie. Border Collie appeared as a distinct breed but with a significant amount of intra-breed diversity similar to Labrador and Golden retrievers. In contrast, the Collie and Scottish Collie clustered as a single breed, but one made up of distinct subpopulations similar to North American and European Italian greyhounds or performance and conformation types of Golden retrievers. However, this segregation has not yet reached the level of varieties within a breed, such as seen with American and Japanese Akita, or Black and Salt and Pepper Giant Schnauzers. Interestingly, Border Collie appear more closely related to Scottish Collie than Collie, an indication of how far conformation breeding of Collie has narrowed their genetic diversity from the more performance related Collie of the past.

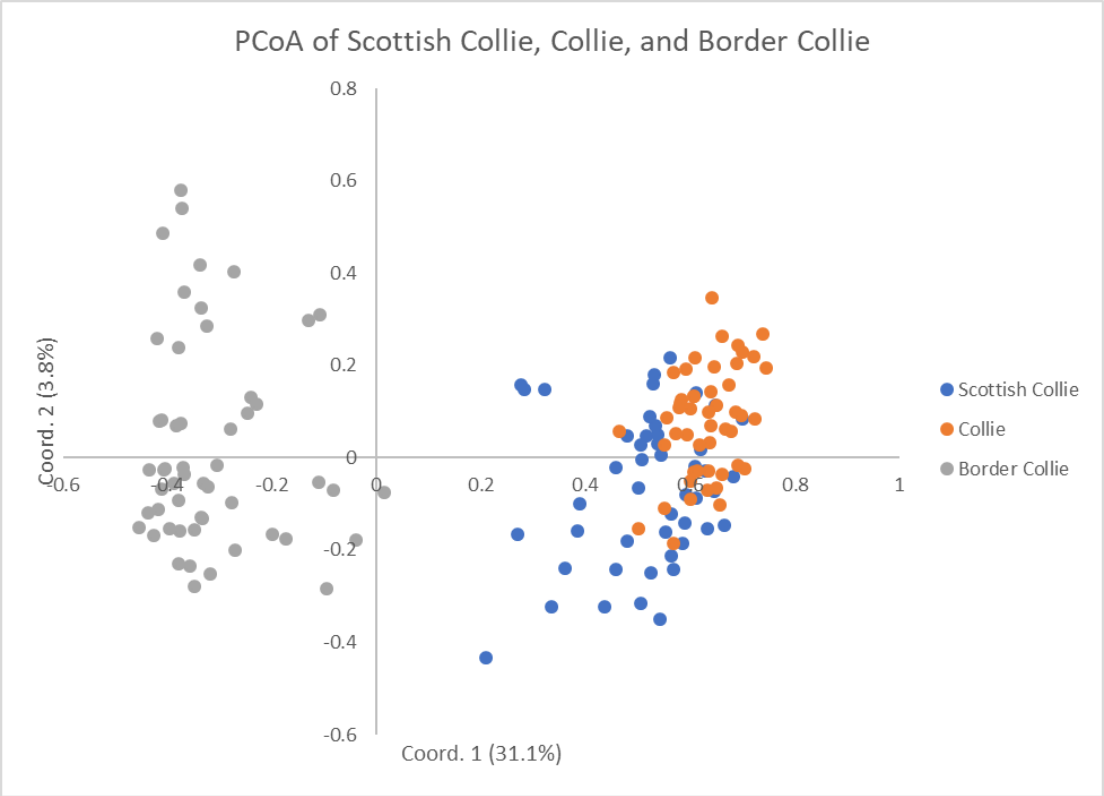


Fig. 3. PCoA graph comparing relatedness of Border Collie (n=57), Scotch Collie (n=43), and Collie (n=55).

A second 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, and individual Border Collie still demonstrated significant genetic differences. Scottish Collie and Collie still clustered closely together with the latter still appearing as a distinct subpopulation within the Scottish Collie. The Border Collie, Italian greyhound and Scottish Collie/Collie remained genetically distinct breeds, although a small number of individuals among both Scottish Collie and Border Collie were now more closely aligned with each other. These individuals shared more of the original Collie genes.

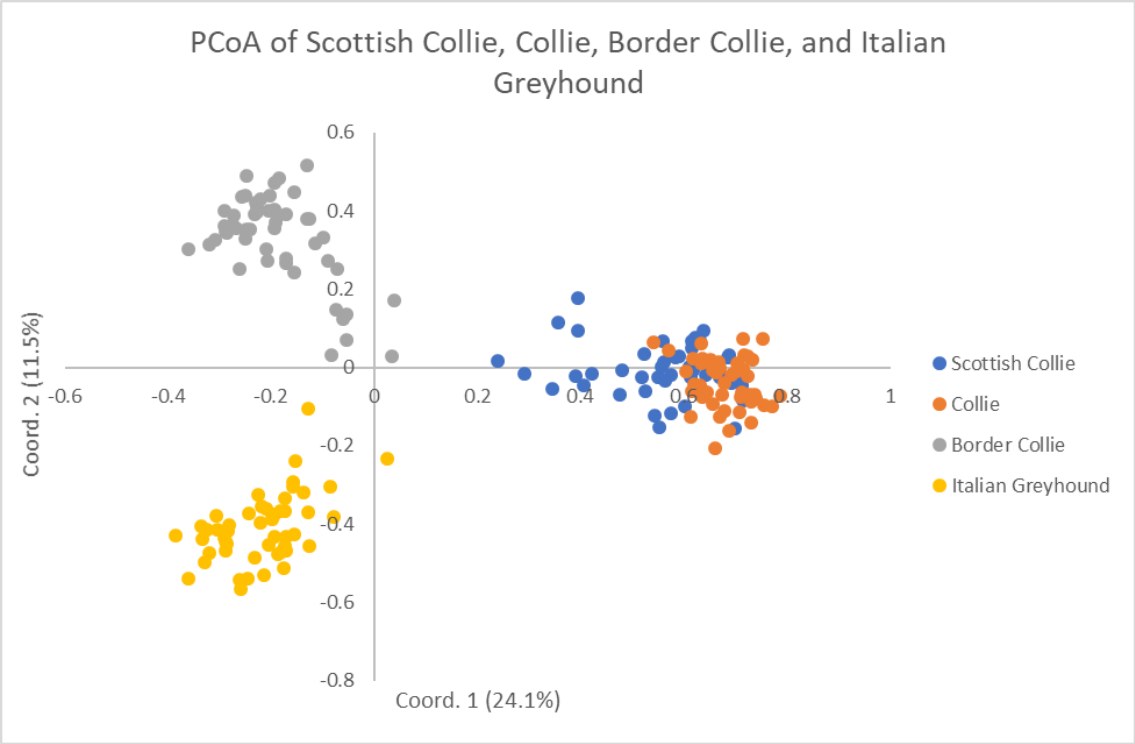


Fig. 4. PCoA comparison of the Italian greyhound, Scotch Collie, Collie and Border Collie.

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 4 lists the IR values for the 57 Border Collie that were initially tested. The most outbred dog in the population had an IR score of -0.206, while the most inbred dog in the group had an IR score of 0.281, while the mean (average) IR score for the group was 0.033. This means that one quarter of the dogs had IR values 0.107-0.281 making them inbred to the level of puppies from first cousin to 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 4. Internal relatedness (IR) values calculated using allele numbers and frequencies for 57 Border Collie. 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)

Quartile		IR	IRVD
Min	Most outbred	-0.206	-0.050
1st Qu		-0.100	0.200
Mean		0.033	0.250
3rd Qu		0.100	0.300
Max	Most inbred	0.281	0.475

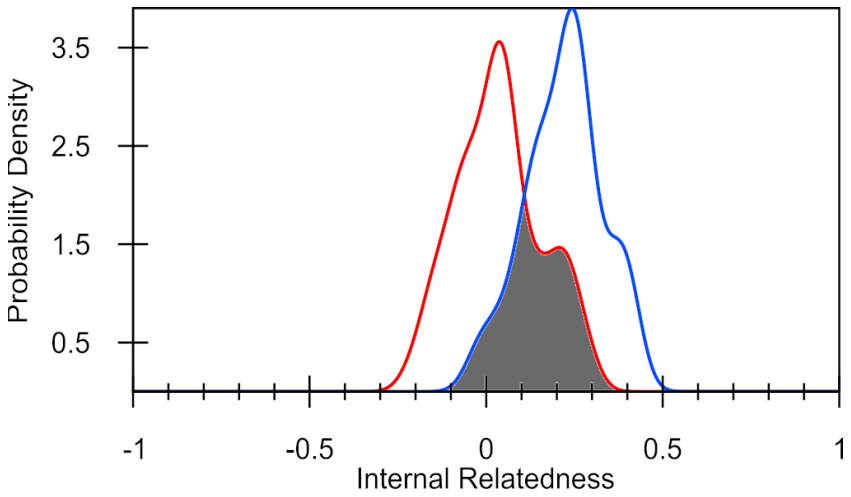


Fig. 5. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Border Collie (n=57). The area under the curve (black) represents the degree of allele sharing (43.4%) between Border Collie and village dogs.

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 collie 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 4) can then be graphed and the graphs overlaid (Fig. 5). One quarter of the most related (most inbred) dogs had IRVD scores

0.300 to 0.475 and one quarter of the least related (most outbred) dogs had scores 0.200 to -0.050 (Table 4). Therefore, if this group of dogs had been selected from among village dogs, over one-half of them would be considered more inbred than offspring of full sibling parents.

The IRVD curve for the Border Collie tested was shifted to the right of the IR curve, and the area of overlap was 43.4% (Fig. 5). This figure is comparable to the 48.8% 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% observed in the Miniature/toy poodle or 54% in Labrador Retriever, but higher than the 26.8% of Scottish Collie, 23% for Irish wolfhound, 15% in Doberman Pinchers and 7% in Swedish Vallhund. All pure breeds of dogs have come from relatively small founder populations 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 for Border Collie at each locus and their frequencies. Allele numbers and frequencies are used to do a standard genetic assessment of heterozygosity within the DLA region (Table 7).

#	Locus	N	Na	Ne	Ho	He	F
1	**DLA I-3CCA**	57	5	3.03	0.69	0.67	-0.03
2	**DLA I-4ACA**	57	6	3.15	0.71	0.68	-0.05
3	**DLA I-4BCT**	57	6	3.45	0.77	0.71	-0.08
4	**DLA1131**	57	7	3.06	0.63	0.67	0.072
5	**5ACA**	57	5	2.33	0.57	0.57	-0.01
6	**5ACT**	57	4	3	0.59	0.67	0.117
7	**5BCA**	57	5	3.12	0.74	0.68	-0.09

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 across many regions of the genome (Table 2) as well as in the DLA region (Table 7). This comparison indicates that the DLA region is equally heterozygous as the rest of the genome (Ho=0.67 vs. 0.68). The proportion of alleles (Ne) that are major determinants of heterozygosity was also similar than observed for the 33 autosomal STR loci (56% vs. 50%) and the observed heterozygosity at each locus is much lower (0.67 vs. 0.68). The F values for autosomal STRs was 0.04 and DLA STRs -0.01, or both essentially zero. These values indicate that heterozygosity within the DLA class I and II regions is in equilibrium with the rest of the genome.

Table 7. Standard genetic assessment of alleles and allele frequencies in the DLA class I and II regions for 57 Border Collie.

	N	Na	Ne	Ho	He	F
Mean	57	5.43	3.02	0.67	0.67	-0.01
SE		0.34	0.12	0.03	0.02	0.02

2. DLA class I and II haplotypes in Border 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 51 Border Collie **(Updated Dec 4, 2019)**

DLA Class	STR Alleles	Allele frequency
DLA-I		
1008	386 373 289 182	0.01
1011	376 365 281 180	0.08
1012	388 369 289 188	0.03
1014	375 373 287 178	0.09
1030	380 373 293 178	0.01
1045	376 371 277 186	0.16
1052	380 372 289 184	0.01
1068	380 373 287 181	0.25
1092	376 379 277 181	0.01
1104	386 373 289 186	0.12
1222	386 379 277 181	0.01
1236	376 379 289 181	0.21

1237	376 379 277 178	0.01
1238	376 371 277 184	0.01
1239	376 371 277 181	0.01
DLA-II		
2001	343 324 284	0.22
2002	343 327 280	0.13
2003	343 324 282	0.03
2005	339 322 280	0.01
2017	343 322 280	0.22
2022	339 327 282	0.01
2023	341 323 282	0.01
2031	339 322 282	0.01
2033	339 323 282	0.01
2037	341 327 280	0.09
2039	345 327 276	0.15
2053	343 324 280	0.03
2067	343 322 284	0.01
2075	341 327 282	0.01
2096	351 322 280	0.03
2125	351 322 286	0.05

The 51 Border Collie in this study possessed 15 DLA class I and 16 DLA class II haplotypes (Table 7). The numbers of DLA class I (n=15) and II (n=16) haplotypes found in Border Collie were higher than the Swedish Vallhund (6,4) and Shiloh shepherd (7, 6); similar to the Giant Schnauzer (14/15), Samoyed (13/12) and Shiba Inu (16/15); and lower than Golden Retriever (26/23) and Miniature Poodle (33/ 23).

Four minor class I (1236-1239)) and one minor class II (2125) have not been identified to date by the VGL in other breeds, while the majority of haplotypes were shared with a number of other breeds (Table 8). Four class I (1045, 1068,1104, 1236) and four class II (2001, 2002, 2017, 2039) haplotypes occurred in frequencies over 10% and were collectively found in around 70% of the dogs tested. All of the remaining haplotypes occurred at incidences ranging from 1-9%.

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. Although recombination occurs slowly between class I and II regions of the DLA, it would be very low during the last 200 years when most breeds were created. 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.

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

The Border shares DLA class I and II haplotypes with over 37 different breeds, and with the exception of minor sharing with Akita, all of them are from continental Europe and the UK (Table 8). The greatest number of shared haplotypes was with the Toy; Miniature and Standard Poodles, Golden and Labrador retrievers, Italian greyhound, Shiloh shepherd, Biewer terriers, Giant Schnauzer, Bulldog, and Havanese.

Table 8. DLA class I and II haplotype sharing between Border Collie and other breeds that have been tested by the VGL

DLA1 #	STR types	Black Russian Terrier (n=134)	Lakeland Terrier (n=75)	Labrador Retriever (n=181)	Irish Red and White Setter (n=60)	Doberman Pinscher (n=618)	Flat Coated Retriever (n=547)	Havanese (n=448)	Samoyed (n=189)	Saint Bernard (n=52)	Shiba Inu (n=107)	Giant Schnauzer (n=220)	Polish Lowland Sheepdog (n=19)	Borzoi (n=68)	English Bulldog (n=163)	Biewer (n=121)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=107)	Yorkshire Terrier (n=16)	Italian Greyhound (n=943)	Alaskan Klee Kai (n=548)	Shiloh Shepherd ISSA (n=183)	Magyar Agar (n=60)	English Mastiff (n=20)	Irish Setter (n=49)	Llewellin Setter (n=94)	American Akita (n=101)	Japanese Akita (n=366)	Berger Picard (n=67)	Golden Retriever (n=718)	Collie (n=49)	Irish Wolfhou nd (n=55)	Border Collie (n=51)	Miniatur e Poodle (n=288)	Scottish Collie (n=47)	Barbet (n=66)	Swedish Vallhund (n=225)	Poodle (n=2884)	Toy Poodle (n=142)
1008	386 373 289 182	--	--	--	0.064	--	0.583	--	--	--	--	0.045	--	--	0.006	0.006	--	--	0.06	0.1267	0.0611	--	--	--	0.11	0.069	--	--	--	0.0014	--	--	0.01	--	--	0.01	--	--	0.0168	0.018
1011	376 365 281 180	--	--	--	--	--	0.033	--	--	--	--	0.011	--	--	--	--	--	--	--	--	0.0584	--	--	--	0.1	--	--	--	--	0.0007	--	--	0.078	0.003	--	--	--	--	0.0186	0.021
1012	388 369 289 188	--	--	0.74	--	--	--	--	--	--	--	0.016	0.013	--	--	0.414	0.19	0.151	0.229	0.28	0.0074	--	--	--	--	--	--	--	--	0.0014	--	--	0.029	0.054	--	--	--	--	0.0094	0.028
1014	375 373 287 178	0.037	--	--	--	0.042	--	0.032	0.003	--	--	0.252	--	--	--	--	0.029	0.009	0.07	0.09	--	0.3741	--	--	--	0.37	--	--	--	0.0197	--	--	0.088	0.003	--	--	--	--	0.0095	0.049
1030	380 373 293 178	--	--	--	0.033	--	0.0914	--	--	--	--	--	--	--	--	--	0.459	0.349	0.472	0.31	0.026	--	--	--	0.13	--	0.027	--	--	0.0007	--	--	0.01	--	--	0.03	--	--	0.0026	--
1045	376 371 277 186	--	--	--	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.003	--	--	--	0.005	0.005	--	--	--	0.96	--	0.157	0.003	0.79	--	--	0.001	0.032
1052	380 372 289 184	--	--	0.02	--	--	0.0016	--	--	0.007	--	--	--	--	--	--	--	--	--	--	0.1888	--	0.363	0.05	--	--	--	--	--	0.06	--	--	0.355	0.01	--	--	--	--	--	--
1068	380 373 287 181	--	--	--	0.047	--	--	0.2706	0.018	0.042	0.163	--	--	--	0.041	--	--	0.009	--	--	--	--	0.249	--	--	0.01	--	--	--	0.0522	--	--	0.082	0.245	0.016	--	--	0.349	--	0.011
1092	376 379 277 181	0.243	--	--	--	--	--	--	0.086	--	--	0.245	--	--	0.006	--	0.038	--	0.06	--	--	--	--	--	--	--	--	0.311	--	--	--	--	0.01	--	--	--	0.038	--	0.0002	--
1104	386 373 289 186	--	--	0.033	--	--	--	--	--	--	--	--	--	--	0.003	0.012	0.019	0.005	--	--	0.0021	--	--	--	--	--	--	--	--	--	--	--	0.118	--	--	--	--	--	--	--
1222	386 379 277 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	0.02	--	--	--	--
1236	376 379 289 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.206	--	--	--	--	--	--	--
1237	376 379 277 178	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	--	--	--	--	--
1238	376 371 277 184	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	--	--	--	--	--
1239	376 371 277 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies (Updated Dec 6, 2019)

DLA2 #	STR types	Black Russian Terrier (n=134)	Lakeland Terrier (n=75)	Labrador Retriever (n=181)	Irish Red and White Setter (n=60)	Doberman Pinscher (n=618)	Flat Coated Retriever (n=547)	Havanese (n=448)	Samoyed (n=189)	Saint Bernard (n=52)	Shiba Inu (n=107)	Giant Schnauzer (n=220)	Polish Lowland Sheepdog (n=19)	Borzoi (n=68)	English Bulldog (n=163)	Biewer (n=121)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=107)	Yorkshire Terrier (n=16)	Italian Greyhound (n=943)	Alaskan Klee Kai (n=548)	Shiloh Shepherd ISSA (n=183)	Magyar Agar (n=60)	English Mastiff (n=20)	Irish Setter (n=49)	Llewellin Setter (n=94)	American Akita (n=101)	Japanese Akita (n=366)	Berger Picard (n=67)	Golden Retriever (n=718)	Collie (n=49)	Irish Wolfhou nd (n=55)	Border Collie (n=51)	Miniatur e Poodle (n=288)	Scottish Collie (n=47)	Barbet (n=66)	Swedish Vallhund (n=225)	Poodle (n=2884)	Toy Poodle (n=142)
2001	343 324 284	--	--	--	0.003	--	--	--	0.048	--	0.01	0.005	0.007	--	--	--	--	--	--	--	--	--	0.005	0.092	--	--	--	--	--	0.1386	--	--	0.216	0.014	--	0.189	--	0.612	0.007
2002	343 327 280	--	--	--	--	--	--	--	--	0.003	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.021	--	--	--	--	--	0.127	0.005	--	--	--	0.0883	--
2003	343 324 282	--	0.74	0.017	--	--	0.1444	0.217	0.013	--	--	0.036	--	--	0.598	0.231	0.208	0.252	0.38	0.0069	--	--	0.008	--	--	--	--	--	--	0.023	--	--	0.029	0.516	0.01	0.129	--	0.091	0.43
2005	339 322 280	0.015	0.007	0.03	0.117	--	0.4196	0.002	--	0.067	--	0.009	--	--	0.015	0.045	0.009	0.047	--	--	--	--	--	--	--	0.04	0.793	--	--	0.016	--	0.227	0.01	--	--	--	--	0.0203	0.004
2017	343 322 280	--	0.033	--	--	--	0.0009	0.008	--	--	--	--	0.32	0.007	0.215	--	--	--	--	0.2179	--	--	0.363	0.408	0.13	--	--	0.015	0.005	0.06	0.0418	--	--	0.216	--	--	0.03	--	0.0028	0.004
2022	339 327 282	--	0.173	0.075	--	0.0016	0.1252	0.119	0.108	--	--	0.005	--	0.596	0.015	--	--	--	--	--	--	--	0.063	--	--	0.18	0.09	--	--	0.0007	--	0.382	0.01	0.002	--	--	0.053	0.0002	0.011
2023	341 323 282	0.004	--	0.033	--	0.0914	--	0.003	--	0.404	--	--	--	--	--	0.459	0.349	0.472	0.31	0.026	--	--	--	--	0.13	--	0.018	--	--	0.0007	--	--	0.01	--	--	0.03	--	0.0026	--
2031	339 322 282	0.034	--	0.008	--	--	--	--	--	--	--	0.036	--	--	--	--	--	--	--	0.0583	--	--	--	--	--	--	--	--	--	--	--	0.018	0.01	--	--	0.02	--	--	--	--
2033	339 323 282	0.519	--	--	--	0.0008	--	0.002	--	--	--	0.045	--	--	--	--	--	--	--	0.0048	--	--	--	--	--	--	0.015	--	--	--	--	--	0.01	--	--	--	--	--	--	--
2037	341 327 280	0.276	--	--	--	--	--	0.032	--	--	--	0.341	--	--	--	0.029	0.009	0.07	0.09	0.008	0.3741	--	--	0.017	--	0.37	--	0.168	0.241	--	--	--	0.088	0.007	--	--	--	--	--	0.007
2039	345 327 276	--	--	--	0.008	--	0.0089	--	--	--	--	--	--	--	--	--	--	--	--	0.0901	--	--	0.003	--	--	--	0.099	0.094	--	--	--	0.95	--	0.147	--	--	0.78	--	0.001	--
2053	343 324 280	--	--	0.044	--	--	--	0.1344	0.039	0.558	0.144	--	0.048	--	--	--	0.009	--	--	--	--	--	0.309	--	--	0.01	--	--	--	0.0306	--	0.082	0.029	0.016	--	--	0.496	--	--	0.014
2067	343 322 284	--	--	0.02	--	--	--	--	--	--	0.21	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.94	--	0.01	0.009	--	--	--	--	--	--
2075	341 327 282	--	--	--	--	--	--	0.029	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	--	--	--	--	--	--
2096	351 322 280	--	--	--	--	--	--	--	0.114	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.029	--	--	--	--	--	--	--
2125	351 322 286	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.049	--	--	--	--	--	--	--

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

IV. Heritable disorders of Border Collie

A. General health and lifespan

The natural life span of the border collie was reported to be between 12 and 15 years in one study and an average of 12 years in another [10]. A second study listed the lifespan to be 10-14.5 years with an average of 12.5 years [11]. The median longevity for all pure breeds is 12 to 13 years [11]. Leading causes of death are cancer (23.6%), old age debilitation (17.9%) and cerebral vascular afflictions (9.4%) [11]. Collie eye anomaly (CEA) and epilepsy are considered the primary genetic diseases of concern in the breed at this time [12].

B. Heritable disease problems

1. Heritable disorders of Mendelian inheritance

a. ***Collie eye*** - Collie eye anomaly (CEA) is a congenital, inherited, eye disease of dogs affecting the retina, choroid, and sclera [13]. CEA is inherited as an autosomal recessive mutation in the NHEJ1gene with complete penetrance and localized to canine chromosome 37 [13]. 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 [13]. 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). 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 [15]. This malady renders the dog more sensitive to the negative effects of certain medications including ivermectin, a popular de-worming treatment. Vets have alternative medications they can use for MDR1 effected dogs. A DNA test can check for this issue.

c. ***Glaucoma -*** Glaucoma in Border Collie is associated with an autosomal recessive variant on the OLFML3 gene [15]. A DNA test has been developed.

d. ***Deafness-*** Two types of hearing loss occur in the breed, puppyhood and adulthood. The puppy form is often associated with the Merle coat color and is found in border collie puppies, although the puppies can have congenital sensorineural deafness from birth as well [16]. The second type is known as adult-onset hearing loss [17]. Dogs with the adult onset form of deafness have normal auditory brainstem responses puppies but gradually lose their hearing between one and eight years of age. The genetic cause is unknown at this time.

e. ***Neuronal ceroid lipofuscinosis*** (NCL) is a rare but serious disease that is limited to show border collies [18]. NCL results in severe neurological impairment and early death and afflicted dogs rarely survive beyond two years of age. There is no treatment or cure, but a DNA test is now available to detect carriers as well as affected dogs.

f. ***Trapped Neutrophil Syndrome (TNS)-*** TNS is a hereditary disease in which the bone marrow produces neutrophils (white cells) but is unable to effectively release them into the bloodstream [19]. Affected puppies have an impaired immune system and will eventually die from infections. The mutation responsible for TNS has been found in border collies in English working dogs, in show dogs that had originated in Australia and New Zealand, and in unrelated Australian working dogs [19]. This indicates that the gene is widespread and probably as old as the breed itself. There is no cure, but a DNA test is now available to detect carriers as well as affected dogs.

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

h. ***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.

*i. **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.

j. ***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 AP3B1 gene.

k. **Imerslund Grasbeck syndrome-** This is an uncommon autosomal **r**ecessive early onset disorder identified in Border Collie and Australian Shepherd that reduces the ability of the intestines to absorb Vitamin B12 resulting in failure to thrive [31, 32]. A DNA test for a mutation in the CUBN gene is available [31] and about 6% of Border Collie are carriers (heterozygous) of the mutation. The fact that the mutation exists also in Australian Shepherd indicates the it was not a recent occurrence.

2. Disorders of a heritable nature of unknown or complex (polygenic) nature

a. ***Elbow and hip dysplasia***- Elbow and hip dysplasia occur at low frequency in Border Collie and tend to be less progressive to osteoarthritis. Juvenile osteochondritis has also been recognized in the breed.

b. ***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.

c. ***Juvenile cataracts*** - Juvenile cataracts have been described in young Border Collie but the genetic basis, if any, is unknown.

c. ***Exercise induced collapse*** - A syndrome of exercise induced collapse (wobbles) similar to that seen in Labrador retrievers has been recognized in Border Collies in North America, Europe and Australia [1, 20]. The collapse is triggered by episodes of collapse associated with periods of intense exercise and is thought to be genetic and currently the subject of investigation. This disorder has been recognized in many herding/working breeds and the cause is currently unknown. Clinical signs include disorientation, mental dullness, loss of attention, unsteady hind legs, dragging of hind legs, and ultimately the need to sit or lay down. There is no current treatment recommended, and it is advised to limit the episodes by avoiding the activities that trigger the collapse.

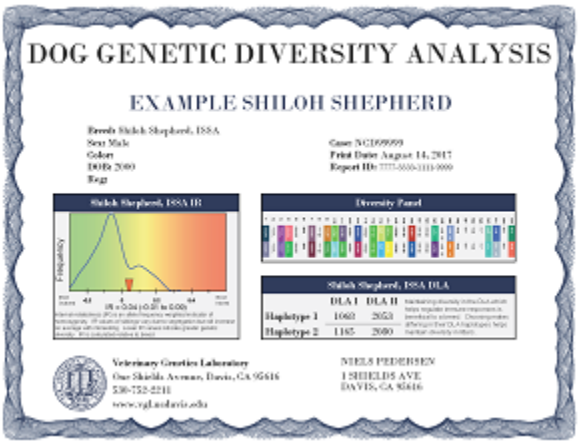
d. Autoimmune diseases- Autoimmune disorders are relatively uncommon in Border Collie and occur at variable incidence in many dog breeds as well as in mongrels [22]. Autoimmune disease is polygenic and involves the accumulation of risk factors that have evolved over a long period of dog evolution and segregating by descent among both pure- and mixed-breeds of dogs [23, 24]. Certain DLA class II haplotypes have been associated with increased risk of autoimmune disease in various breeds [25]. Thyroiditis is seen in Border Collies, as it has been documented in most pure breeds. It is usually the first type of autoimmune disease recognized and is therefore considered an indicator of autoimmune predisposition. Type I diabetes has also been recognized in Border Collie, as well as forms of systemic lupus erythematosus (SLE).

e. ***Cancer***- The incidence of cancer in Border Collie has been reported as 23.6% [10]. This is slightly lower than the 27% average incidence reported for purebred dogs in general. The major types of cancer in Border Collie are similar to all dogs - lymphoma, hemangiosarcoma, melanoma, mast cell sarcoma, osteosarcoma and soft tissue sarcomas [21]. Mammary carcinoma can be a problem in intact females, as it is in other pure breeds and mongrels.

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



B. What should you do with this information?

Based on this group of 57 Border Collie, it appears that the breed possesses above average genetic diversity in both genomic and DLA regions. There is also evidence of extensive sharing of founders with 37 or more breeds of UK and Continental European origin. However, four of these DLA class I and II haplotypes occurring in over 70% of Border Collie. This suggests that a great deal of outcrossing was done at the early stages of the breed, but that four founders or closely related founder lines contributed disproportionately to the breed. Both conclusions are supported by an analysis of 99.6% complete 15 generation pedigrees from 13,339 Border Collie born in Hungary from the late 1800's to 2016 [33]. The number of founders based on this study was 894, but only eight individuals were responsible for contributing 50% of the genetic variability.

The Border Collie appears to possess an above average amount of genetic diversity compared to other breeds. Therefore, it is important at this point for breeders to catalog this diversity and to identify any additional diversity that still exists throughout the breed. Such information will be useful in maintain the genetic health of the breed now and in the future. Although the breed as a whole appears to be in a state of HWE, DNA testing shows that many Border Collie are much more inbred than imagined and that re-establishing genetic diversity and heterozygosity in puppies should be an objective. This can be done by utilizing DNA testing 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 homozygous for the same haplotypes, and 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 four extended DLA class I/II haplotypes. However, it is fortunate that many Border Collie 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 v*s* common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.

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

Schisms within the breed pitting performance vs. conformation continue to plague the breed. However, there is no evidence that this is leading to genetic segregation of the breed into performance and conformation sub-populations as seen in other performance-type breeds [30]. Nonetheless, numerous competing performances or conformation registries may make it difficult to manage and maintain genetic diversity in a breed. It is well known that selection for conformation or performance will rapidly lead to genotypic and phenotypic differences such as seen between show and working English Shepherds [30]. Acceptance of both activities in registries such as the Brittany have definitely slowed such schisms [30]. Therefore, it would behoove the various Border Collie registries to compare DNA based genotypes as early as possible to determine the degree to which dogs in various registries and geographic regions have drifted from each other. The ultimate goal is to retain all of the known canid genetic diversity within the breed and to monitor how it is managed to assure that it survives for as long as possible.

Border Collie have accumulated a surprisingly large number of heritable deleterious diseases, mostly of a simple autosomal recessive nature. This problem occurs in even genetically diverse breeds used for both performance and conformation such as the Golden and Labrador retrievers. Although deleterious mutations tend to be blamed on selection for show traits, they can also occur in sub-populations of a breed under strong artificial selection for traits that might benefit a growing number of performance activities.

Heritable diseases usually involve autosomal recessive mutations and do not become of concern until the incidence of the encoded disease reaches levels of 1-5% or so. At this point, a quarter or more of healthy individuals will carriers. For instance, even if the carrier rate for an autosomal recessive mutation is 20%, only 0.2 x 0.2 at 0.25 =1% of randomly bred puppies will be diseased. If genetic diversity in a breed is high, and the number of such heritable disorders is low, the choice may be to use DNA testing to eliminate the mutation. However, if genetic diversity in a breed is low and the number of deleterious mutations great, it may be impossible to eliminate the mutant genes without losing a considerable amount of normal genetic diversity. Therefore, many some breeds with low genetic diversity may use DNA testing not for eliminating the trait, but rather to avoid producing affected puppies.

An effective use of this the genetic information is to contribute it to a web repository, hopefully under the control of the registry. 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 of the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters.

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