Genetic Diversity Testing for Llewellin Setter

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers to determine genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel is useful to dog breeders who wish to use DNA-based testing to track and distribute genetic diversity as a supplement to in-depth pedigrees. Information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions in order to improve the overall genetic health of a breed.

Genetic diversity testing in the Llewellin Setter has been established, and almost all existing alleles at the 33 STR loci and 7 DLA class I and II regions have potentially been identified. As of October of 2022, 135 Llewellin Setters from the United States were tested to assess genetic diversity in the breed. Allele and DLA haplotype frequencies will be updated as more dogs are tested.

Results reported as:

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

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

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

I. Introduction to the Llewellin Setter

A. Introduction to setter breeds [1-7]

Dogs known as setters were originally bred as far back as the 14th and 15th centuries in England, Wales, Scotland and Ireland, presumably from ancestors such as the Water Spaniel, Spanish Pointer and English Springer Spaniel. Setters were originally bred in the kennels of aristocracy for use in estate hunts for game-bird species such as grouse, partridge, woodcock, snipe, quail, and pheasants. These types of birds crouch silently in the grass and only flush when closely approached, thus requiring a special type of dog to locate them. The scent of game birds is airborne; therefore, a setter will carry its head high and range systematically and methodically over large areas. When the prey is detected, a setter will freeze in a distinct crouch or stance, which is known as a "set." The hunter will then follow the point and flush the bird so it can be either netted or shot in flight. Dogs are born with a natural proclivity to hunting, and setters evolved by continuous selective breeding over many generations for this particular ability.

There are four basic types of setters: English Setter, Gordon Setter, Irish Red and White Setter, and the Irish Setter. These four basic setter breeds are also subdivided into several distinct breeds and bloodlines based on show (conformation) or fieldwork (performance). The English Setter is thought to be the oldest of the setters (dating back to the 14th century) and is the smallest of the setter breeds. The Gordon Setter originated in Scotland around the 16th century, being named after the Fourth Duke of Gordon, who bred these dogs at his Gordon Castle. They are known as the heaviest and slowest of the setters. The Irish Red and White Setter originated in Ireland as early as the 16th century, and differs from the Irish setter mainly in its mottled white and red coat. The Irish Setter, developed in the 19th century, is a large gundog breed known for its solid chestnut/red coat.

B. History of the show (conformation) and performance (field) type English Setters [8-13]

Setters are versatile dogs, with different bloodlines selectively bred to meet a variety of needs, whether it is for hunting on horseback or foot, field hunts or trials, or shows. Each variety possesses the characteristics that the breeder most desires. In the case of the English Setter, that characteristic involves primarily hunting, and relatively lately during breed development, showing. Divergences in bloodlines favoring conformation over performance began to occur in the 19th century as large estates disappeared and, with them, the associated kennels and estate hunts. Regional hunting competitions and pay-to-hunt activities became more accessible to the non-aristocracy and the showing of dogs first in agricultural fairs and later in urban venues became increasingly popular. English Setters bred by Edward Laverack (1800-1877) were well known for both appearance and performance because of his attention to a pleasing and uniform appearance as well as hunting ability in the field. Therefore, Laverack-type dogs ultimately came to dominate the show circuit. In contrast, R. L. Purcell Llewellin (1840-1925) took the best field dogs from the Laverack kennel and concentrated his subsequent breeding efforts solely on outstanding fieldwork. Further selection for one or the other activity lead to subtle divergence of genotypes and, consequently, of phenotypes. However, it would be incorrect not to recognize the favorable show appearance of some field dogs and the hunting prowess of some show types. It is nonetheless uncommon for one variety of setter to excel in the other's domain; dual champions are somewhat rare in the breed.

C. History of Llewellin Setter [8-14]

The Llewellin Setter is a variety of English Setter that traces its bloodlines to the 19th century sportsman and breeder R. L. Purcell Llewellin, born in Pembrokeshire, South Wales. Llewellin began his breeding program in 1872, utilizing the best dogs from the kennel of Edward Laverack, an Englishman credited for developing show type English setters. Starting his breeding program in 1825 with four bitches (Countess, Nellie, Lill II and Petrel) and two setters (Old Moll and Ponto), Laverack claimed to have bred his dogs without an outcross for over 40 years. Llewellin's original dogs were also infused with new blood in the form of dogs that he obtained in northern England from the kennels of Mr. Statter and Sir Vincent Corbet and known as the Duke-Rhoebe line. Llewellin also continued to line breed for many generations back to what he referred to as the pure "old strain" of Laverack stud dogs.

After Llewellin's death in 1925, William Humphrey acquired all of his pure Dashing Bondhu Setters, and vowed to continue breeding, training, and campaign Llewellin's strain. Following the disruptive period of WWII, William Humphrey imported between thirty and forty setters back from the USA. Using these dogs, he bred the following strains: Wind'em, Horsford Dashing, and Dashing Bondhu. With the death of William Humphrey in 1963, most of his dogs went to Rev. Bannon in Ireland, Ms. M.T. Goes in Belgium and T. Wostenholm in South Africa. The three individuals, Laverack, Llewellin and Humphrey are associated to this day with the Llewellin strain.

Importations to America began in earnest in the 1870's. These dogs were initially referred to as the "Field Trial Breed" (later named Llewellins by the Americans) and imported in great numbers, including the "six pillars" of American lines: Gladstone, Count Noble, Druid, Leicester, Lincoln and Rake. The Llewellin Setter was officially recognized in 1902 and registered with the Field Dog Stud Book (FDSB), dominating American field trials though the early 20th century. Additionally, the separation of bench type (or show) Llewellin Setters took place during this period.

A small group of dedicated US breeders, committed to maintaining the high standard of the "old time Llewellins," was active for many decades into the 1970's. The group included names like Herb Anderson, Harold Shaw, Dr. John Holtz, Judge Miller, Dr. Ersig, Chester Doherty, Alex Boutacoff, William Brown and Al King. Several other US breeders were importing from Europe, Scotland and Wales, most notable E.L. Stephenson, Glen Roark and Everett Bickers.

Today, most of the big kennel Llewellin Setter breeders are gone and virtually no Llewellin Setters exist outside the United States. The legitimate (FDSB registered) effective population in the US is only a fraction of its original size, with numerous small hobby breeders maintaining the population.

There is always a question in some people's minds regarding what is a Llewellin Setter. The Field Dog Stud Book (FDSB) of Chicago recognizes Llewellins as those English Setters whose ancestry traces back to the original Duke-Rhoebe-Laverack crosses, and currently registered via the FDSB separately from English Setters. Outcrossing of Llewellin lines disqualifies a litter from registration as Llewellin with the FDSB. Therefore, it can be said that not all performance-bred English Setters are Llewellin Setters, but all Llewellin Setters are English Setters.

D. Appearance [11]

The field and show types of English Setter are phenotypically distinguishable to the knowledgeable eye, even though they belong to the same breed. Field-type setters are usually smaller and have thinner coats, less conspicuous feathering and more distinctive spotting than show-type setters. Show types tend to carry their tails straight off the back, while field types carry their tails at around 12 o'clock. Show types are more uniform in appearance and "prettier", while performance types have more variable appearance and can be considered more "rugged". Therefore, performance-type English Setters may not always conform to the AKC breed standard that is based on Laverack's vision of the English Setter.

English Setters are a medium to large sized dog, weighing 35 to 65 pounds and standing 20 to 26 inches tall. Males are larger than females. They have a fairly deep chest, level topline and a straight tail that is carried high. The head and muzzle are long and lean, the nose is black, and the eyes are dark brown. The ears are fairly long, set low and close to the head, and hang down. The single coat is soft and flat, and hair is straight and of medium length. Feathering of the hair is present on the tail, legs and ears. The standard tri-color coat has small distinct patches consisting of two colors (blue/black, orange, lemon, chestnut) in a background of white. An alternative coat pattern known as belton is equally common. A belton setter has ticking rather than patches. A tick is a mixture of white and colored hair, giving a bluish or orange tint. Pups are usually born all white unless they have an eye patch or mask, and gradually develop more and more patches or ticks over their bodies as they grow to adulthood.

E. Temperament [8-11]

The temperament of English-type Setters has been well documented and conformation- and performance-bred dogs are similar in that they love people and make good pets. English Setters tend to be the mildest-mannered of the setter breeds and conformation-bred dogs tend to be more laid-back and require less attention than performance-bred dogs. Llewellin Setters are said to be gentle and calm when indoors and energetic when outside. They need a daily quota of exercise and can develop separation anxiety if ignored or lonely for long periods. Any activity with people is appreciated. They are obedient, eager to please, and form strong bonds with owners and family and are good watchdogs.

F. Health

1. Lifespan

The Llewellin Setter is a healthy breed, with a lifespan of 10 to 12 years.

2. Diseases

2.1. Simple Mendelian genetic disorders [12-16]: An autosomal recessive canine form of neuronal ceroid lipofuscinoses (CNCL), or Batten disease, was identified in English setters, caused by a mutation in the *CLN8* gene. Congenital deafness was observed in 12.4% of 701 random English Setters tested by the Louisiana State University in 2010. The ESAA puts the figure at 10% deaf in one or both ears. There are no comparative studies on

deafness in performance breeds such as the Llewellin Setter and conformation English Setters. The exact genetic basis for deafness in English setters has not been determined, although it has been associated with a number of genes causing white pigmentation patterns in other species.

2.2. Complex (polygenic) genetic disorders [16-24]: *Autoimmune disease* - Autoimmune thyroiditis was shown to affect 26.2% of 747 English Setters reported to the Orthopedic Foundation for Animals (OFA) between 1974 and 2012. This was much higher than the 4.6% reported in the more randomized Kennel Club survey. It is possible that the OFA survey concentrated more on conformation-type English Setters from North America, while the Kennel Club survey contained a greater proportion of performance dogs from the UK. Autoimmune disorders in dogs are associated with increased inbreeding.

Skin allergies - reported in 10.2% of English Setters in the Kennel club survey. Signs of atopic disease usually appear between the ages of 6 and 18 months and nearly always begins with itchy feet. The itching then progresses to the abdomen and underneath the tail. Affected dogs will constantly scratch and bite at the affected sites, leaving areas of alopecia, redness and bleeding. The condition may be seasonal, suggesting allergies to pollens and grasses. Others seem to be more constant, indicating a reaction to certain foods. Bitches are often worse or occasionally better during estrus, a possible hormonal influence. Atopic dermatitis can range from occasional itchiness to a debilitating disease that may lead to euthanasia.

Intestinal disorders – diseases such as chronic inflammatory bowel disease (IBD) were reported in 12.6% of English Setters in the Kennel club survey. Many of these intestinal disorders are associated with dietary allergies or other types of immunological disorders.

Orthopedic disorders – the incidence of orthopedic problems such as hip and elbow dysplasia varies from one report to the other. About 24% of English Setters suffered from hip dysplasia in one study, but the percentage appeared to be decreasing due to OFA testing and improved breeding practices. Elbow dysplasia and osteochondritis dissecans (OCD), mainly in the shoulder, have also been recognized in the breed. The Kennel Club survey reported an incidence of combined hip/elbow/stifle/shoulder problems at 8.1%. The differences in incidence of these conditions between North America (OFA) and the UK (Kennel Club) might reflect differences in the proportion of performance and conformation English Setters.

Cancer – Cancer was the most common cause of death (32.8%) of English Setters in a Kennel Club survey. This incidence is not statistically different from the average 27% incidence reported across all pure breeds of dogs. Cancers were most common at or after 10 years of age and were often seen in mammary gland, testicles, brain, and liver. Lymphoma, hemangiosarcoma and osteogenic sarcoma, which are common in several breeds, are not common in English Setters. However, mast cell tumors starting in the skin are a problem in the breed, as they are in other breeds and must be treated aggressively upon discovery.

Epilepsy – the frequency of this disorder is increasing in the breed, as it is in many other pure breeds. The incidence in a 2007 study was 5.7% in English Setters, with an average of 8.2% across all breeds included in study. However, one report states that seizure disorders in Llewellin Setters is rare. The incidence of epilepsy in a dog breed may also be linked to the degree of inbreeding.

II. Results on Genetic Diversity of Llewellin Setters

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

STR markers are multiallelic, highly polymorphic, and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes [25,26]. The average number of alleles identified per locus across the dog breeds tested at the VGL to date is 15.4 alleles/locus. Dog breeds, having evolved from a small number of founders and having been exposed to artificial population bottlenecks, will end up with only a portion of the total available genetic diversity found in canids. Artificial genetic bottlenecks can include phenomena such as sire effects, geographic isolation, catastrophes, outbreaks of disease, and variation in popularity, which can lead to a decrease in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 135 Llewellin Setters used in this study are listed on **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Llewellin Setters (n=135). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
80 (0.089)	131 (0.022)	119 (0.241)	217 (0.015)	240 (0.107)	87 (0.163)
96 (0.330)	137 (0.044)	**121 (0.463)**	**219 (0.496)**	244 (0.041)	89 (0.230)
98 (0.041)	145 (0.070)	123 (0.007)	225 (0.115)	246 (0.333)	91 (0.163)
100 (0.037)	**147 (0.485)**	127 (0.267)	229 (0.004)	**248 (0.515)**	93 (0.037)
102 (0.270)	149 (0.367)	129 (0.011)	233 (0.141)	254 (0.004)	**95 (0.407)**
106 (0.137)	151 (0.011)	131 (0.011)	235 (0.074)		
108 (0.004)			239 (0.004)		
112 (0.030)			241 (0.152)		
114 (0.063)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
284 (0.370)	**116 (0.663)**	124 (0.141)	148 (0.063)	238 (0.133)	95 (0.007)
286 (0.174)	118 (0.015)	128 (0.067)	152 (0.189)	**240 (0.433)**	**97 (0.659)**
288 (0.156)	124 (0.019)	**132 (0.256)**	156 (0.078)	242 (0.407)	101 (0.074)
290 (0.111)	126 (0.241)	136 (0.070)	160 (0.163)	244 (0.022)	103 (0.178)
292 (0.189)	130 (0.063)	140 (0.111)	164 (0.015)	246 (0.004)	105 (0.081)
		144 (0.185)	168 (0.044)		
		148 (0.167)	172 (0.100)		
		152 (0.004)	**176 (0.259)**		
			180 (0.070)		
			188 (0.019)		

INU005	INU030	INU055	LEI004	REN105L03	REN162C04

110 (0.044)	**144 (0.622)**	**210 (0.437)**	**85 (0.681)**	227 (0.007)	198 (0.004)
124 (0.096)	146 (0.085)	212 (0.181)	95 (0.293)	231 (0.096)	**202 (0.852)**
126 (0.189)	148 (0.070)	214 (0.381)	97 (0.004)	233 (0.100)	204 (0.037)
128 (0.670)	150 (0.181)		105 (0.022)	235 (0.374)	206 (0.107)
	152 (0.037)			**241 (0.422)**	
	156 (0.004)				

REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.593)	156 (0.004)	268 (0.237)	222 (0.300)	145 (0.344)	12 (0.007)
206 (0.041)	160 (0.011)	270 (0.311)	**226 (0.307)**	147 (0.215)	18.2 (0.307)
210 (0.019)	162 (0.019)	**272 (0.444)**	232 (0.252)	149 (0.004)	19.2 (0.163)
212 (0.115)	164 (0.267)	274 (0.007)	234 (0.141)	**153 (0.437)**	**20.2 (0.341)**
216 (0.089)	166 (0.019)				21.2 (0.033)
218 (0.115)	**168 (0.637)**				22.2 (0.107)
220 (0.022)	170 (0.044)				23.2 (0.033)
224 (0.007)					24.2 (0.007)

VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
13 (0.030)	8 (0.148)	14 (0.093)	14 (0.033)	9 (0.004)	14 (0.004)
14 (0.233)	9 (0.096)	16 (0.007)	16 (0.119)	11 (0.115)	15 (0.030)
15 (0.104)	**13 (0.415)**	18 (0.015)	18 (0.004)	13 (0.144)	**16 (0.433)**
16 (0.007)	14 (0.115)	19 (0.044)	**19 (0.619)**	**14 (0.733)**	17 (0.422)
17.1 (0.019)	15 (0.015)	20 (0.007)	20 (0.148)	15 (0.004)	18 (0.078)
18.1 (0.026)	18 (0.059)	21 (0.152)	22 (0.078)		19 (0.033)
19.1 (0.107)	19 (0.152)	22 (0.148)			
20.1 (0.030)		**23 (0.304)**			
21.1 (0.263)		24 (0.007)			
22.1 (0.133)		27 (0.004)			
23.1 (0.007)		30 (0.204)			
24.1 (0.041)		31 (0.015)			

VGL2918	**VGL3008**	**VGL3235**
12 (0.004)	**14 (0.367)**	**12 (0.333)**
13 (0.556)	15 (0.089)	13 (0.330)
14 (0.211)	16 (0.019)	14 (0.159)
15 (0.026)	18 (0.256)	15 (0.107)
16 (0.015)	18.2 (0.004)	16 (0.004)
17.3 (0.007)	19 (0.159)	17 (0.041)
18.3 (0.022)	20 (0.100)	18 (0.022)
19.3 (0.159)	21 (0.007)	19 (0.004)

Table 1 shows that allele distribution within the 33 autosomal STR loci in the Llewellin Setter is typical of most pure dog breeds, i.e., one or two alleles are observed at higher frequency than others per locus (bold on **Table 1**). The number of alleles identified for each STR locus ranged from three (INU055) to 12 (VGL0910 and VGL1165), which corresponds to a fraction of alleles known to exist among all dogs. The average number of alleles across loci was 6.36 (**Table 2**). Loss of alleles is a common feature of pure dog breeds, and reflects the small number of founders existing at the time a breed registry is closed. Not only is the number of founders low, but some founders often contribute more of their genetics to the breed than others. This was demonstrated by the disproportionately high incidence of one or two alleles at each locus (**Table 1**). These high incidence alleles have been inherited by descent from founding dogs whose phenotypes (and consequently genotypes) were highly valued and therefore most conserved. A single allele occurred in 50% or more of the dogs tested at 12 of the 33 loci (**Table 1**). Additionally, one allele at locus REN162C04 occurred in 85.2% of the dogs tested. The dominance of these alleles was due to inheritance from founders (or founding lines) that contributed significantly to the development of the breed.

B. Assessment of population diversity using standard genetic parameters

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

Table 2. Genetic Assessment of 135 Llewellin Setters based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	6.4	3.24	0.63	0.65	0.02
SE	0.4	0.22	0.02	0.02	0.01

The average number alleles (Na) known to exist at the 33 STR loci across breeds, based on all dog breeds tested at the VGL so far, is 15.4 (see section IIA). In Llewellin Setters this number was estimated at 6.4 (**Table 2**). Therefore, approximately 41.5% of the known canid diversity at these 33 loci has been retained during breed evolution. Breed evolution not only includes the time period since the breed was officially registered, but also the much longer preceding period of human-directed selection. This proportion of retained canid-wide genetic diversity is slightly higher than that of other setter breeds, the Irish Red and White Setter (34.8%) and Irish Setter (30.3%). However, the average number of effective alleles (Ne) constitutes a more important metric for diversity, since these alleles have the greatest genetic influence on heterozygosity. This number was estimated at 3.24 for this cohort, indicating that genetic diversity for Llewellin Setters is determined by approximately half of the alleles segregating in the breed.

The mean observed heterozygosity (Ho = 0.63) was lower than the estimated expected heterozygosity (He = 0.65), thus yielding an inbreeding coefficient (F) of 0.02 (**Table 2)**. These

values indicate a small deficit in heterozygosity from that expected for a population in Hardy-Weinberg equilibrium (HWE); i.e., this cohort is approximately 2% more inbred than a random mating population.

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

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each STR locus (**Table 3**). This provides an estimate of genetic diversity in the genomic regions associated with each STR marker. In Llewellin Setters, the average number of effective alleles (Ne) per locus across individuals ranged from 1.35 (REN162C04) to 6.37 alleles (FH2054). The lowest average observed heterozygosity (Ho) for an individual STR locus was 0.26 (REN162C04), whereas the highest was estimated at 0.86 (FH2054). Average expected heterozygosity (He) values ranged from 0.26 (REN162C04) to 0.84 (FH2054) (**Table 3**).

Loci with the lowest Ho values contribute the least to heterozygosity levels across the breed; they are most likely associated with inherited traits that are important for the breed's phenotypic standard (and thus tend to vary less). Conversely, high Ho values for a particular locus means that it shows greater genetic diversity across the breed, and that these loci can be associated with phenotypic variation among individuals. The values for Ho and He are used to calculate what is known as inbreeding coefficient (or F), which is a measure of how near that locus is to Hardy-Weinberg equilibrium (HWE). An F value of zero signifies that a population is in HWE, or in other words, is randomly breeding (no artificial selection). Positive F values indicate non-random selection (inbreeding), while negative values indicate outbreeding (increased heterozygosity).

Table 3. Standard Genetic Assessment of individual STR loci for 135 Llewellin Setters. Individual STR loci with high inbreeding coefficients (F > 0.1) are bolded.

Locus	N	Na	Ne	Ho	He	F
AHT121	135	9	4.62	0.74	0.78	0.055
AHT137	135	6	2.65	0.59	0.62	0.048
AHTH130	135	6	2.91	0.69	0.66	-0.05
AHTh171-A	135	8	3.25	0.68	0.69	0.015
AHTh260	135	5	2.57	0.62	0.61	-0.01
AHTk211	135	5	3.66	0.7	0.73	0.042
AHTk253	135	5	4.17	0.8	0.76	-0.05
C22.279	135	5	1.99	0.55	0.5	-0.1
FH2001	135	8	5.92	0.76	0.83	0.091
FH2054	135	10	6.37	0.86	0.84	-0.02
FH2848	135	5	2.69	0.61	0.63	0.033
INRA21	135	5	2.09	0.47	0.52	**0.105**

INU005	135	4	2.02	0.5	0.5	0.015
INU030	135	6	2.31	0.54	0.57	0.045
INU055	135	3	2.71	0.66	0.63	-0.05
LEI004	135	4	1.82	0.39	0.45	**0.127**
REN105L03	135	5	2.96	0.6	0.66	0.094
REN162C04	135	4	1.35	0.26	0.26	0.008
REN169D01	135	8	2.58	0.57	0.61	0.068
REN169O18	135	7	2.09	0.55	0.52	-0.05
REN247M23	135	4	2.85	0.61	0.65	0.065
REN54P11	135	4	3.74	0.62	0.73	**0.15**
REN64E19	135	4	2.81	0.62	0.64	0.046
VGL0760	135	8	3.98	0.76	0.75	-0.01
VGL0910	135	12	5.95	0.76	0.83	0.083
VGL1063	135	7	4.11	0.73	0.76	0.041
VGL1165	135	12	5.27	0.82	0.81	-0.02
VGL1828	135	6	2.35	0.51	0.57	**0.11**
VGL2009	135	5	1.75	0.47	0.43	-0.09
VGL2409	135	6	2.67	0.64	0.63	-0.03
VGL2918	135	8	2.63	0.56	0.62	0.092
VGL3008	135	8	4.11	0.78	0.76	-0.03
VGL3235	135	8	3.86	0.79	0.74	-0.06

According to **Table 3**, high inbreeding coefficients ($F > 0.1$) were estimated for four of the 33 STR loci (INRA21, LEI004, REN54P11, and VGL1828). This suggests that these loci have been under strong positive selection since breed development and might be associated with breed-defining phenotypic traits. Conversely, F values around or below zero were estimated for the remaining 29 loci; these alleles were likely to be found among the more outbred dogs.

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

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (coordinates 1 and 2). The closer two individuals cluster together on the plot, the more closely related they are to each other. The 135 Llewellin Setters used in this study clustered as expected for a pure dog breed on the PCoA plot. However, they did not form a tight cluster around the X/Y axis as seen in most breeds, but rather were more spread across the graph. This indicates that this cohort is genotypically more diverse than many pure breeds of dogs tested so far. Interestingly, a tendency for the 135 dogs to segregate into subpopulations was also identified, which suggests that this cohort was sampled from among at least five genetically distinguishable bloodlines (**Figure 1**).

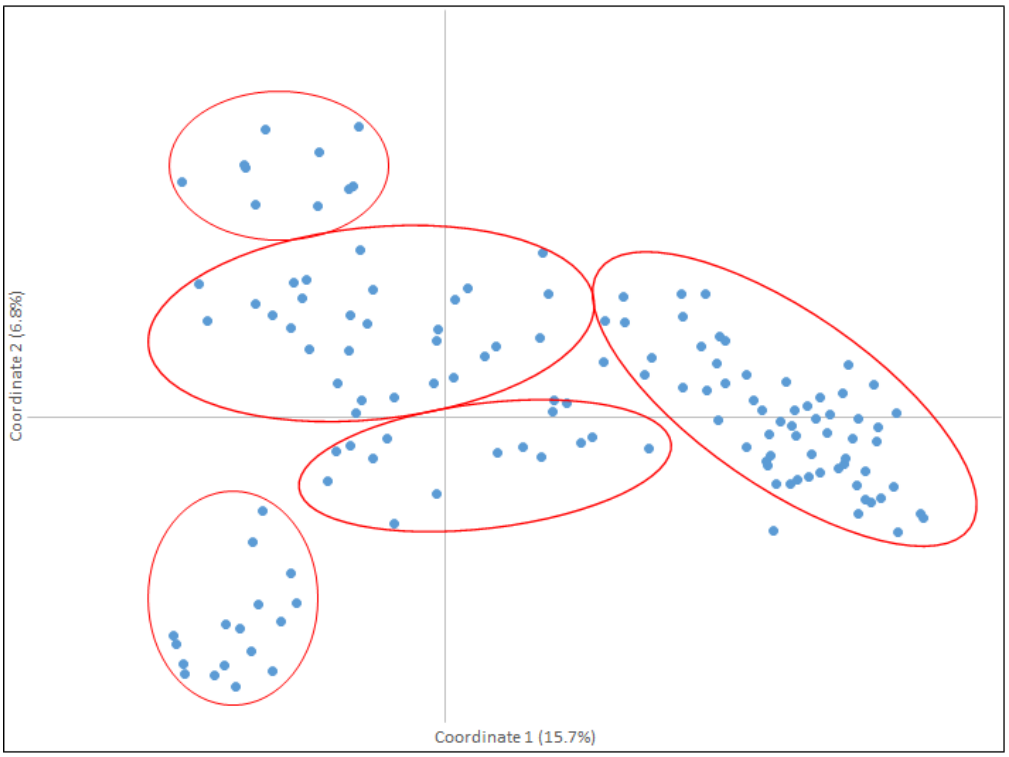


Figure 1. PCoA of Llewellin Setters (n = 135) based on alleles and allele frequencies at 33 autosomal STR loci. Five subpopulations were identified within the sampled individuals (red circles), indicating different potential bloodlines.

The degree of intra- and inter-breed relatedness can be further assessed by generating a PCoA of the 135 Llewellin Setters with closely related setter breeds (Irish Setter and Irish Red and White Setter) and a somewhat unrelated breed (Irish Wolfhound) [27] (**Figure 2**). Interestingly, the Irish Setter (orange dots) and Irish Red and White Setter (gray dots) cluster together on top of the graph whereas Llewellin Setters (blue dots) cluster together in the bottom right quadrant. This indicates that the Irish-type Setters are more closely related to each other than to the Llewellin Setters, being virtually indistinguishable at the breed level. Further, this analysis shows that Llewellin Setters are more diverse than the other breeds represented in the plot due to being more dispersed around the X/Y axes (**Figure 2**).

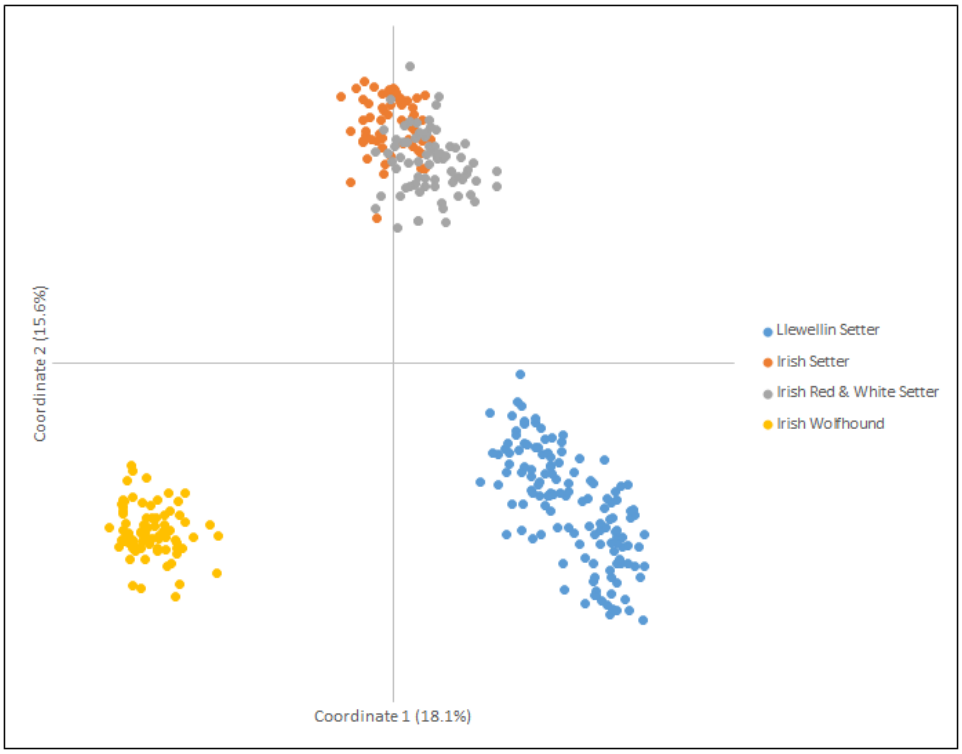


Figure 2. PCoA plot of Llewellin Setter (blue dots; n = 135), Irish Setter (orange dots), Irish Red and White Setter (gray dots), and Irish Wolfhound (yellow dots).

E. Internal relatedness (IR) scores for Llewellin Setters

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree of relatedness of parents of an individual dog. The IR calculation takes into consideration homozygosity at each of the 33 STR loci in this study and gives more weight to rare and uncommon alleles, which would presumably be identified in less related individuals. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with an IR value of -1.0 would have parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that are genetically identical at all loci. IR values above +0.25 occur when the parents of the full sibling parents are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 135 Llewellin Setters.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies for 33 STR loci in 135 Llewellin Setters.

	IR	IRVD
Min	-0.2914	-0.1072
1st Qu	-0.0721	0.1430
Mean	0.0266	0.2595
Median	0.0133	0.2585
3rd Qu	0.1174	0.3645
Max	0.4532	0.7382

The most outbred dog of the study cohort had an estimated IR score of -0.29, while the most inbred dog had an IR score of +0.45, with a mean IR of 0.02 for the cohort. **Table 4** shows that one-half of the dogs had IR values over +0.02, and one quarter over +0.11. *Therefore, only a small proportion of Llewellin Setters were equally or more inbred than theoretical offspring of full sibling parents.* Finally, the wide range of IR values indicate genetic heterogeneity in the cohort (typical for most pure breeds), and highlight the importance of assessing IR values for individual dogs in order to maintain within-breed diversity by selecting the least related individuals possible for mating purposes.

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

The IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to the present day. Village dogs that exist throughout the SE Asia, the Middle East and the Island Pacific region are randomly breeding descendants of dogs from which most modern breeds evolved. The known STR alleles and their frequencies of a given breed can be compared with the same alleles and their frequency in modern village dogs to yield an adjusted IR score (IR-village dog or IRVD) (**Table 4** and **Figure 3**, blue line).

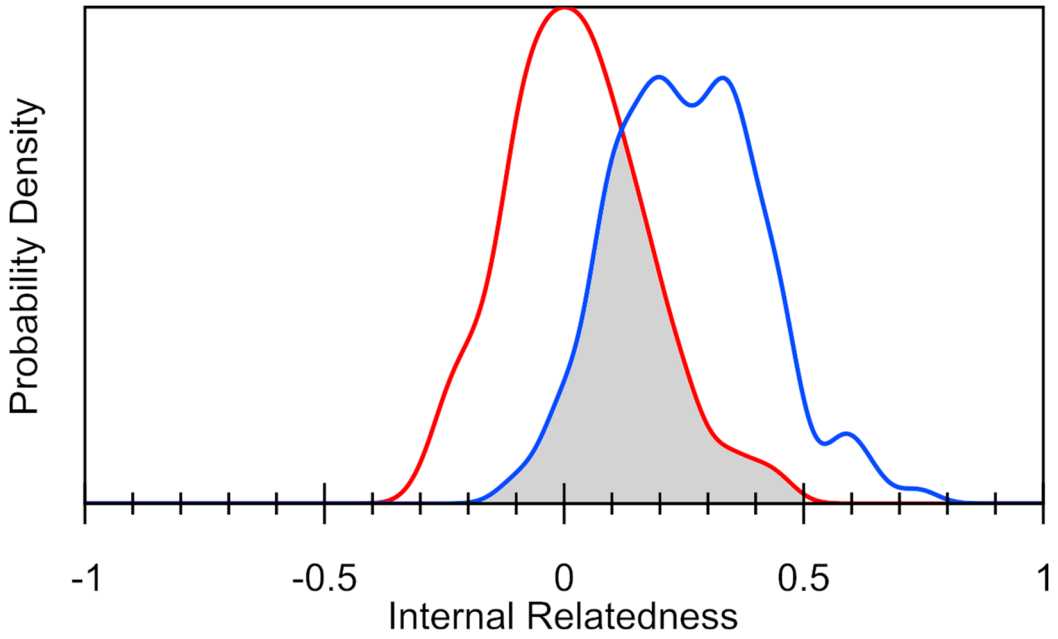


Figure 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Llewellin Setter (n=135). The overlap between the curves (gray area) shows that the breed retains 45.8% of the genetic diversity existing in randomly breeding village dogs.

The mean IRVD value was approximately 0.26 for the population, with at least one dog having an IRVD value as low as -0.1 (most outbred) and at least one dog with a value as high as +0.74 (most inbred) (**Table 4**). The IRVD curve (**Figure 3**, blue line) is shifted to the right when compared to the IR curve (red line), which is typical for all pure breeds of dogs. The overlapping area of the two curves is an estimate of how much genetic diversity was lost in the creation of a breed. The estimated retention of available canid genetic diversity in Llewellin setters was 45.8%, which is greater than the 30% retained genetic diversity calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL (**section IIB**).

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

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

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

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

1. DLA class I and II haplotypes existing in the Llewellin Setter

Twelve DLA class I and nine DLA class II haplotypes were identified in Llewellin Setters (**Table 5**). Most of the class I haplotypes were identified at a low frequency (<10%); however, one DLA class I haplotype (1077) was found in 60% of the individuals tested, whereas another (1134) was detected in 13% of Llewellin Setters. Similarly, all DLA class II haplotypes were found at low frequencies except for 2005 which was present in 80% of the individuals tested. This is the highest incidence of a single DLA class II haplotype in any breed tested at the VGL to date, and indicates that a single founder or founder line played an important role in the evolution of all contemporary Llewellin Setters which is corroborated by historical evidence.

Table 5. DLA class I and II haplotypes identified in Llewellin Setters (n = 135) and their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 Haplotype	STR types	Frequency
1008	386 373 289 182	0.056
1016	382 371 277 178	0.03
1026	390 369 289 186	0.041
1028	376 369 291 186	0.004
1030	380 373 293 178	0.022
1045	376 371 277 186	0.004
1054	382 379 277 184	0.085
1077	**376 369 291 178**	**0.6**
1134	**384 365 291 178**	**0.133**
1142	376 379 277 180	0.019
1201	382 381 277 181	0.004
1212	380 373 293 180	0.004
DLA2 Haplotype	**STR types**	**Frequency**
2002	343 327 280	0.03
2005	**339 322 280**	**0.793**
2012	345 322 280	0.044
2015	339 327 280	0.004
2018	339 324 284	0.019
2022	339 327 282	0.085
2023	341 323 282	0.015
2035	341 323 280	0.004
2104	341 323 284	0.007

DLA class I and II regions are in linkage (i.e., are inherited together), forming distinct extended haplotypes. This linkage is strong, but not so strong as to preclude a certain degree of recombination in some individuals, which causes a shuffling between existing class I and class II haplotypes. Such examples of recombination can be seen among Llewellin Setters. The DLA class II haplotype 2005, identified in 80% of Llewellin Setters used in this study, was also found in 22 other dog breeds tested at the VGL (**Table 6**). However, in Llewellin Setters, this haplotype was in linkage with two different class I haplotypes, forming 1077/2005 and 1134/2005 extended haplotypes.

Interestingly, one DLA class I haplotype (1212) appears to be unique to the breed, occurring at a frequency of 0.4% (**Table 6**). Two additional haplotypes made up of common dog class I and II types, 1016/2002 and 1030/2104, are also unique to the breed even though all of these haplotypes occur in other breeds, but in different combinations. The 1030 haplotype is commonly seen in linkage with the 2023 haplotype in other breeds, while 1016 has not been observed before in combination with 2002. DLA class I haplotype 1201, also found in 0.4% of Llewellin Setters, was found to be shared with another setter breed, the Irish Red and White Setter. Finally, many DLA class I and II haplotypes found in Llewellin Setters were shared with a number of common dog breeds, such as Poodle and Labrador Retriever (**Table 6**).

Table 6. Sharing of DLA class I and II haplotypes between Llewellin Setter (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=51).

DLA1 #	STR types	Llewellin Setter (n=135)	American Eskimo, Standard (n=63)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=84)	American Akita (n=158)	Japanese Akita (n=564)	Alaskan Klee Kai (n=634)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=140)	Black Russian Terrier (n=149)	Biewer (n=128)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=201)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Borzoi (n=147)	Collie (n=49)	Doberman Pinscher (n=1105)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=807)	Great Dane (n=55)	Golden Retriever (n=855)	Giant Schnauzer (n=320)
1008	386 373 289 182	0.056	--	--	--	0.208	--	--	0.0576	--	0.008	--	--	0.008	--	--	0.06	--	--	--	--	0.006	--	--	--	--	0.0012	0.036
1016	382 371 277 178	0.03	0.008	0.04	0.11	--	--	--	--	--	--	0.043	0.01	0.023	0.019	0.012	0.03	--	--	0.04	0.0213	0.095	0.15	0.14	--	0.191	0.0047	0.047
1026	390 369 289 186	0.041	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1028	376 369 291 186	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1030	380 373 293 178	0.022	0.008	0.03	--	--	--	--	--	0.029	0.008	0.011	--	0.457	0.349	0.495	0.31	0.5	--	--	0.095	--	0.08	--	--	0.236	0.0012	--
1045	376 371 277 186	0.004	--	--	--	--	0.003	--	--	--	0.164	--	--	--	--	--	--	--	--	0.96	0.0005	--	--	--	--	--	--	--
1054	382 379 277 184	0.085	0.008	0.21	0.32	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1121	--	--	0.003
1077	376 369 291 178	0.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.003	--	--	--	--	--	--
1134	384 365 291 178	0.133	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	--
1142	376 379 277 180	0.019	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1406	--	--	--
1201	382 381 277 181	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1212	380 373 293 180	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA1 # (cont'd)	STR types	Llewellin Setter (n=135)	Havana Silk (n=36)	Havanese (n=835)	Italian Greyhound (n=1217)	Irish Setter (n=59)	Irish Red and White Setter (n=74)	Irish Wolfhound (n=78)	Lakeland Terrier (n=130)	Labrador Retriever (n=278)	Magyar Agar (n=78)	Polish Lowland Sheepdog (n=71)	Toy Poodle (n=214)	Miniature Poodle (n=403)	Poodle (n=4780)	Rat Terrier (n=41)	Samoyed (n=191)	Saint Bernard (n=89)	Scottish Collie (n=115)	Shiba Inu (n=141)	Shikoku (n=84)	Greater Swiss Mountain Dog (n=57)	Shiloh Shepherd (n=13)	Shiloh Shepherd, ISSA (n=250)	Swedish Vallhund (n=421)	Cardigan Welsh Corgi (n=43)	Whippet (n=107)
1008	386 373 289 182	0.056	--	--	0.1233	0.093	0.601	--	--	0.097	--	--	0.021	--	0.0137	0.02	--	--	0.009	--	--	--	--	--	--	--	0.047
1016	382 371 277 178	0.03	0.08	0.1958	0.06	--	--	--	0.008	0.016	--	0.028	0.023	0.029	0.0224	0.05	--	--	0.096	--	--	0.07	--	--	--	--	0.107
1026	390 369 289 186	0.041	--	--	--	--	--	--	--	--	--	--	--	--	0.0035	--	--	--	--	--	--	--	--	--	--	--	--	--
1028	376 369 291 186	0.004	--	--	--	--	--	--	--	--	--	--	0.019	0.036	0.0009	--	--	--	--	--	--	--	--	--	--	--	--	--
1030	380 373 293 178	0.022	0.04	0.003	0.0288	--	--	--	--	0.038	--	--	--	--	0.0022	0.02	--	--	--	--	--	--	--	--	--	--	--	--
1045	376 371 277 186	0.004	--	--	--	--	--	--	--	0.005	--	--	0.021	0.005	0.0007	--	--	--	0.717	--	--	--	--	--	0.002	--	0.33	0.005
1054	382 379 277 184	0.085	0.07	0.1275	0.0148	0.153	--	--	--	0.054	0.141	--	0.002	0.001	0.0001	0.01	--	--	--	0.355	0.006	--	--	--	--	--	--	0.005
1077	376 369 291 178	0.6	--	--	--	--	0.007	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1134	384 365 291 178	0.133	--	0.0012	--	--	--	--	--	0.013	--	--	--	--	0.0002	--	--	--	--	--	--	--	--	--	--	--	--	--
1142	376 379 277 180	0.019	--	0.0042	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.005
1201	382 381 277 181	0.004	--	--	--	--	0.088	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1212	380 373 293 180	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Llewellin Setter (n=135)	American Eskimo, Standard (n=63)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Hairless Terrier (n=84)	American Akita (n=158)	Japanese Akita (n=564)	Alaskan Klee Kai (n=634)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=140)	Black Russian Terrier (n=149)	Biewer (n=128)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=201)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Borzoi (n=147)	Collie (n=49)	Doberman Pinscher (n=1105)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=807)	Great Dane (n=55)	Golden Retriever (n=855)	Giant Schnauzer (n=320)
2002	343 327 280	0.03	--	--	--	--	--	--	--	--	0.123	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2005	339 322 280	0.793	--	--	--	--	--	--	--	--	0.008	0.143	0.013	0.043	0.009	0.025	--	--	--	--	--	0.015	--	--	0.4195	0.055	0.014	0.008
2012	345 322 280	0.044	--	--	--	--	--	0.0009	0.0584	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	0.009
2015	339 327 280	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2018	339 324 284	0.019	--	--	--	0.006	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.1413	--	--	--
2022	339 327 282	0.085	0.024	0.21	0.32	0.048	--	--	--	--	0.008	--	--	--	--	--	--	--	0.592	--	0.0009	0.015	--	--	0.1171	0.082	0.0006	0.003
2023	341 323 282	0.015	0.008	0.03	--	--	--	--	--	0.029	0.008	--	0.003	0.457	0.349	0.498	0.31	0.5	--	--	0.095	--	0.08	--	--	0.236	0.0012	--
2035	341 323 280	0.004	--	--	--	--	--	0.3103	--	0.051	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2104	341 323 284	0.007	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Llewellin Setter (n=135)	Havana Silk (n=36)	Havanese (n=835)	Italian Greyhound (n=1217)	Irish Setter (n=59)	Irish Red and White Setter (n=74)	Irish Wolfhound (n=78)	Lakeland Terrier (n=130)	Labrador Retriever (n=278)	Magyar Agar (n=78)	Polish Lowland Sheepdog (n=71)	Toy Poodle (n=214)	Miniature Poodle (n=403)	Poodle (n=4780)	Rat Terrier (n=41)	Samoyed (n=191)	Saint Bernard (n=89)	Scottish Collie (n=115)	Shiba Inu (n=141)	Shikoku (n=84)	Greater Swiss Mountain Dog (n=57)	Shiloh Shepherd (n=13)	Shiloh Shepherd, ISSA (n=250)	Swedish Vallhund (n=421)	Cardigan Welsh Corgi (n=43)	Whippet (n=107)
2002	343 327 280	0.03	--	--	--	--	--	--	--	--	--	--	--	0.005	0.0869	0.01	0.003	--	0.004	--	--	--	--	--	--	--	--
2005	339 322 280	0.793	0.04	0.0024	--	0.085	0.101	0.199	0.004	0.056	--	--	0.002	--	0.0166	0.07	--	0.067	--	--	--	--	--	--	--	--	--	--
2012	345 322 280	0.044	--	0.0036	--	0.051	0.203	--	--	--	0.083	--	0.051	0.087	0.0068	0.01	--	--	0.004	--	0.018	--	--	--	--	--	--	--
2015	339 327 280	0.004	--	--	0.0086	0.144	--	--	0.008	--	--	--	0.044	0.038	0.0043	0.01	0.016	--	--	0.06	--	--	--	--	--	--	--	--
2018	339 324 284	0.019	--	0.0192	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.209	0.208	--	--	--	--	--	--	0.005
2022	339 327 282	0.085	0.07	0.1216	--	0.153	--	0.353	0.173	0.054	--	--	0.007	0.002	0.0005	0.15	0.107	--	--	--	--	--	0.04	0.046	0.051	--	--	--
2023	341 323 282	0.015	0.04	0.003	0.0288	--	--	--	--	0.038	--	--	--	--	0.0022	0.02	--	0.399	--	--	--	--	--	--	--	--	--	--
2035	341 323 280	0.004	--	0.003	0.0863	--	0.088	--	--	--	--	--	--	--	0.0001	0.01	--	0.006	--	--	--	--	--	--	--	--	0.08	--
2104	341 323 284	0.007	--	--	--	--	--	--	--	--	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

2. Heterozygosity in the DLA region

Due to their physical proximity in canine chromosome 12, the seven loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to other parts of the genome. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will be inherited randomly as well. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and averaged across all loci (**Table 8**).

The number of alleles (Na) identified at each DLA locus in Llewellin Setters ranged from 3 (5BCA) to 6 (DLA I-3CCA, DLA I-4ACA, and DLA1131). As observed in the 33 STR loci across the genome, the number of effective alleles (Ne) per DLA locus was lower, ranging from 1.23 (5ACA) to 2.33 (DLA I-3CCA) (**Table 7**). The average observed heterozygosity at each locus was somewhat lower than the expected heterozygosity, yielding F values that were all mildly positive (0.01-0.12). This finding indicates a 1-12% deficit of heterozygosity (or excess of homozygosity) at each locus. The average population-wide observed heterozygosity was 0.34 and the expected heterozygosity was estimated at 0.37, yielding an average F value of +0.08 across DLA loci. The population-wide F value for the DLA STRs was even more positive than the F value for the 33 autosomal STRs (F=0.02, **Table 2**). This suggests that the DLA region is not in equilibrium with the genome at large, and appears to be undergoing either deliberate or inadvertent positive selection for certain DLA genotypes.

Table 7. Standard genetic assessment for Llewellin Setters (n=135) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	6	2.33	0.53	0.57	0.08
DLA I-4ACA	6	2.21	0.5	0.55	0.1
DLA I-4BCT	4	1.75	0.38	0.43	0.12
DLA1131	6	1.59	0.33	0.37	0.12
5ACA	4	1.23	0.18	0.19	0.05
5ACT	4	1.4	0.26	0.28	0.09
5BCA	3	1.29	0.22	0.23	0.01

Table 8. Summary of standard genetic assessment for Llewellin Setters (n=135) using 7 STRs in the DLA class I and II regions. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	4.71	1.69	0.34	0.37	0.08
SE	0.44	0.15	0.05	0.05	0.01

III. What does this assessment of genetic diversity tell us about Llewellin Setters

Llewellin setters have retained an average amount of genetic diversity during their evolution compared to other pure breeds, and individual diversity levels appear to be more heterogeneous throughout the population when compared to other setter breeds (Irish Setter and Irish Red and White Setter). However, when compared to other more popular breeds, genetic diversity in Llewellin Setters is relatively low. This is not in itself harmful, providing the founder dogs (or bloodlines) were relatively free of genetic disorders, and that random breeding has been maintained from the time of registry closure to modern times. The term "relatively free" is used because many of the heritable predispositions to more complex disease traits of dogs have accumulated over centuries and perhaps millennia of domestication. The absence of normal selection pressures for "survival of the fittest" that accompanied domestication has allowed for the accumulation of numerous risk factors for undesired traits. These risk factors are inherited by descent and can be further concentrated by the intense human selection that yields a new breed. These ancient heritable predispositions are the best explanation for major polygenic health problems such as cancer, susceptibility to certain infections, autoimmune disorders, allergies, orthopedic problems such as hip and elbow dysplasia, etc.

Breeders should always monitor their breeding stock due to the potential occurrence of diseases caused by mutations inherited in a simple Mendelian fashion, usually of an autosomal recessive or autosomal dominant type. Breeds with low amounts of genetic diversity and subjected to intermittent periods of intense artificial selection are much more likely to suffer from simple genetic diseases. Simple genetic mutations associated with breed-specific diseases rarely come from the founders, but rather occur spontaneously or by inadvertent introgression.

So far, the only autosomal recessive disease reported in Llewellin Setters is canine neuronal ceroid lipofuscinosis (CNCL), caused by a mutation in the *CLN8* gene [17]. The incidence of this mutation in the breed has always been extremely low due to careful selection and genetic testing. Fortunately, most complex genetic traits also occur at a low incidence in Llewellin setters. Therefore, the breed has been remarkably free of heritable disease in spite of its relatively low genetic diversity.

This loss in genetic diversity in Llewellin Setters is much more pronounced in the DLA region than in other parts of the genome. This characteristic is shared by the Irish Red and White Setter and the Irish Setter. This imbalance is most likely a result of the strong tendency to tie Llewellin registration as far back as 1902 to a registry distinct from other English Setters. Llewellins also have to trace their ancestry to the original Duke-Rhoebe-Laverack cross for FDSB registration. This strong anchoring to these founders would best explain the dominance of a single DLA haplotype in modern Llewellin Setters. Indeed, the 1011/2005 and 1134/2005 haplotypes may be genetic markers for these original crosses.

The DLA region is maintained in what is known as strong linkage disequilibrium (LD) with less recombination than most other regions of the genome. Therefore, an old lineage of dogs that was inadvertently selected for the same DLA haplotypes and only occasionally infused with a small amount of new blood, would maintain the same diversity in the DLA region, while gaining diversity in other parts of the genome. Thus, ancestors of the Llewellin Setter most likely came

from a much more ancient inbred line of dogs that had a similar imbalance in genome-wide and DLA region diversity. This assumption is supported by the historic records of the evolution of the setter breeds.

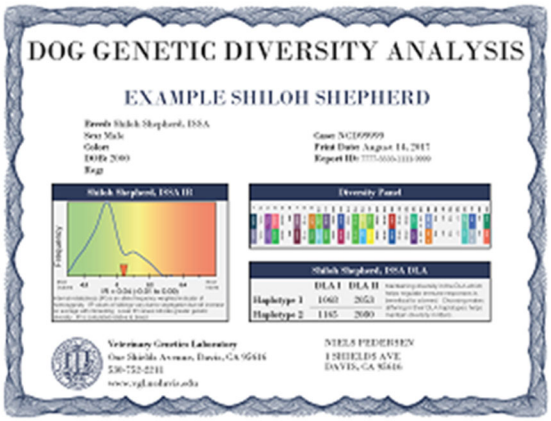
The need to maintain genetic diversity in the DLA region, and to avoid certain haplotypes, has a scientific and historical basis. However, it does not follow that a lack of diversity is always predisposing to such conditions. It really depends on the exact DLA types that are inherited and how genetic polymorphisms in the DLA and other parts of the genome interact. Health problems that might be associated with low genetic diversity in the DLA region include an autoimmune disorder, chronic thyroiditis and hypothyroidism, which is fortunately, the most treatable of autoimmune diseases. There is also a higher than expected incidence of allergic disorders of the skin and intestine. It is not known whether any of these immunologic disorders is associated with the peculiar make-up of the DLA region in the breed.

Without a need to improve health, there is little impetus to increase genetic diversity within the breed. This does not mean that a search for additional genetic diversity should be curtailed. It may exist in geographically different areas of the world, among non-Llewellin performance lines of English Setters, in show lines of English Setters, and in closely related breeds such as the Irish Red and White Setter. At the least, breeders should continue to maintain current genetic diversity. It is also possible by proper selection among existing dogs to correct specific genetic imbalances such as in the DLA region.

IV. Results of VGL Canine Diversity Testing

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

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


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE SHILOH SHEPHERD

Breed: Shiloh Shepherd, ISSA
Sex: Male
Color:
DOB: 2/00
Reg:

Case: VGL99999
Print Date: August 14, 2017
Report ID: 7777-5555-1111-3000

Shiloh Shepherd, ISSA IR

Diversity Panel

Shiloh Shepherd, ISSA DLA

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

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B. What should you do with this information?

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

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

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

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