Genetic Diversity Testing for the Irish 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 that will measure 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 will be useful to dog breeders who wish to use DNA-based testing as a supplement to in-depth pedigrees. DNA based information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions.

A DNA-based genetic assessment of the Irish setter breed is now in the preliminary results phase with the objective of creating a snap-shot of individual- and breed-wide genetic heterogeneity and diversity. This initial testing involved 50 conformation-class Irish setters from across the USA. This data base will be progressively expanded as more dogs are added with the goal of characterizing all the known alleles for the breed at 33 STR loci across the genome as well as all existing DLA class I and II haplotypes identified by seven STRs. We are accepting additional dogs from all parts of the world with a goal of 100 individuals tested to complete this preliminary phase.

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

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

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

I. Introduction to the Irish setter

A. History of the Irish setter *(1-8)*

The breed now known as the Irish setter evolved to a standard form over the nineteenth century in the UK from white and red coated hunting lines. An increasing popularity of largely chestnut red coated dogs led a group of enthusiasts to create an Irish Red Setter Club in 1882. The red colored dogs evolved after this time mainly for show (conformation), while the red and white dogs remained the preferred variety for field work (performance). However, some contemporary Irish setters are still used in the field and are listed as Irish red setters by the Field Dog Stud Book. Therefore, the AKC recognizes both show type Irish setters and performance type red setters as the same breed.

Show lines of Irish setters became increasingly popular up to the early 20th century, when they temporarily lost popularity, purportedly from excessive inbreeding. However, British breeders ultimately reversed the trend and dogs of both great beauty and health were bred. The Irish setter in the UK (and the USA) reached a second peak of popularity in the 1970s with 2400 registrations in 1982. This peak may have been stimulated by the internationally famed Disney movie, "Big Red "which showed in 1962 (20) and created a subsequent generation of devoted adults. The peak was followed by a sharp drop between 1982 and 1986 and a gradual decline to less than 800 dogs in 2015 (10). This decline was most likely due to their overly-active and exuberant nature, behavior problems when left alone, and need for lots of exercise, making them poorly suited to modern apartment and city living. The Irish setter was recognized by the AKC in 1878 in the sporting group and currently ranks 76 of 193 breeds in popularity.

B. Characteristics of the Irish setter

1. Physical appearance

Selective breeding for either conformation or performance has led to subtle differences in appearance of Irish red and white setter vs. Irish setter/Irish red setter (7). A similar dichotomy of appearance has also been described in English setters bred for performance or conformation (4). The Irish setter is said to have a more uniform and refined or racy look, while the contemporary Irish red and white setter is slightly shorter and stockier in body, broader head, and the back of the skull is less peaked. The eyes tend to be almond shaped in the Irish setter and round in the Irish red and white setter. The coat is in both breeds is non-curled and flat with feathering on the outside of the ears, neck, chest, down the back of the front legs, under the belly and back legs, and onto the tail. The hair is somewhat longer and finer, and the feathering more pronounced on the Irish setter than the Irish red and white setter. The classic differentiating feature of the Irish setter is its flashy chestnut red coat with no traces of black. The heights desired by the FCI and AKC for the Irish setter and Irish red and white setter are similar at 22.5 to 24 inches (57 to 61 cm) in females and 24.5 to 26 inches (62 to 66 cm) in males, while no specific height or weight is given in the Kennel Club breed standard and males can be up to 27 inches (69 cm). Males usually weigh no more than 70 lb (32 kg). However, Irish setters tend to be somewhat taller and more uniform in size, while the size of Irish red and white setters may be more variable. The overall impression is that Irish setters are "flashier" and the Irish red and white setter more "rugged."

2. Temperament

The Irish setter is a devoted and affectionate companion to owners and families and gets along well with children and other pets. They do best in active families with many outlets for their high energy and joyful exuberance and require space to run freely. They require more than usual attention and can become anxious when left alone for long periods of time. They may be stubborn at times and require a firmer hand. Their friendliness does not make them well suited as guard- or watch-dogs.

II. Genetic studies of contemporary Irish Setter

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, those 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 STR locus manifests several different genetic configurations known as alleles. Each dog inherits one of these alleles from the sire and the other from the dam.

Table 1a lists the alleles recognized at each STR locus among 50 Irish setters collected from among dogs participating in shows in the USA. The number of alleles per locus ranged from a low of 3 to a high of 9, which is only a fraction of alleles known to exist among all dogs (Table 1b). Loss of alleles is a common features of pure breed dogs and reflects the small number of founders existing at the time registries were 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 1a). These high incidence alleles have been inherited by descent from founding dogs whose genotypes/phenotypes were highly valued and therefore most conserved. A single allele at four loci (AHT137, AHTk253, FH2001, VGL760) occurred in 88%, 82%, 85% and 82% of the dogs, respectively. These alleles are virtually fixed in Irish setters and have been inherited by descent from a founder or founder line that strongly embodied the breed type.

Table 1a. Allele designation and frequency at 33 STR loci for 50 Irish setters. The allele occurring at the highest incidence is highlighted.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
100 (0.300)	131 (0.300)	117 (0.880)	219 (0.100)	240 (0.060)	87 (0.100)
102 (0.190)	137 (0.040)	119 (0.040)	225 (0.180)	244 (0.170)	89 (0.100)
108 (0.040)	141 (0.030)	123 (0.020)	227 (0.050)	246 (0.400)	91 (0.750)
110 (0.090)	143 (0.090)	127 (0.060)	233 (0.300)	250 (0.020)	93 (0.050)
112 (0.380)	145 (0.030)		237 (0.370)	252 (0.240)	
	147 (0.340)			254 (0.110)	
	149 (0.170)				

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
288 (0.130)	116 (0.050)	132 (0.060)	152 (0.360)	230 (0.010)	95 (0.410)
290 (0.050)	118 (0.320)	144 (0.070)	156 (0.050)	234 (0.030)	97 (0.310)
292 (0.820)	120 (0.570)	148 (0.850)	160 (0.010)	236 (0.380)	101 (0.280)
	126 (0.020)	152 (0.020)	164 (0.510)	238 (0.410)	
	130 (0.040)		168 (0.010)	240 (0.160)	
			172 (0.060)	242 (0.010)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
124 (0.710)	144 (0.480)	210 (0.330)	85 (0.220)	233 (0.050)	198 (0.410)
126 (0.050)	148 (0.020)	212 (0.070)	95 (0.570)	235 (0.200)	202 (0.190)
132 (0.240)	150 (0.500)	214 (0.360)	97 (0.210)	237 (0.010)	206 (0.180)
		216 (0.240)		239 (0.670)	208 (0.220)
				241 (0.070)	

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
212 (0.370)	162 (0.180)	268 (0.750)	222 (0.120)	139 (0.020)	13 (0.110)
216 (0.160)	164 (0.150)	270 (0.050)	226 (0.230)	145 (0.540)	14 (0.820)
218 (0.140)	166 (0.180)	274 (0.200)	228 (0.140)	147 (0.380)	19.2 (0.040)
220 (0.010)	168 (0.480)		232 (0.480)	153 (0.060)	20.2 (0.010)
222 (0.320)	172 (0.010)		238 (0.030)		23.2 (0.020)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
17.1 (0.010)	9 (0.190)	15 (0.010)	14 (0.090)	13 (0.270)	15 (0.190)
18.1 (0.140)	13 (0.290)	16 (0.010)	16 (0.050)	14 (0.730)	16 (0.620)
19.1 (0.030)	14 (0.030)	19 (0.030)	20 (0.070)		17 (0.050)
20 (0.080)	17 (0.150)	21 (0.020)	21 (0.390)		18 (0.060)
20.1 (0.030)	18 (0.340)	26 (0.390)	22 (0.400)		19 (0.080)
21.1 (0.660)		27 (0.260)			
22.1 (0.050)		28 (0.080)			
		29 (0.010)			
		30 (0.190)			

VGL2918	VGL3008	VGL3235
12 (0.020)	15 (0.290)	13 (0.210)
13 (0.640)	16 (0.060)	14 (0.700)
14 (0.030)	17 (0.280)	15 (0.030)
17.3 (0.230)	18 (0.260)	18 (0.040)
18.3 (0.040)	19 (0.020)	19 (0.020)
19.3 (0.040)	22 (0.040)	
	23 (0.030)	

The number of known alleles per locus for the 33 autosomal STRs for all dogs and wolves tested ranged from 7 to 28 (Table 1b). The number of alleles identified among the 50 Irish setters tested ranged from 2 to 9 per locus and the percent of known alleles occurring at each locus ranged from 13% to 57% (average 30.3%) (Table 1b). Therefore, about 30% of known genetic diversity for these 33 loci has been retained within the breed or 70% has been lost, during breed evolution. Breed evolution includes also includes the decades or centuries prior to actual registry creation during which their abilities as "setters" was subjected to positive human selection.

Table 1b. The number of known alleles for all dogs and wolves tested to date and the percent of those detected in the Irish setters tested.

Locus	**Known Alleles for all dogs**	**% known alleles in IS**
AHT121	24	20%
AHT137	17	41%
AHTH130	20	20%
AHTh171-A	14	36%
AHTh260	28	21%
AHTk211	7	57%
AHTk253	11	33%
C22.279	13	38%
FH2001	17	23%
FH2054	23	26%
FH2848	24	25%
INRA21	15	13%
INU005	14	21%
INU030	15	20%
INU055	11	36%
LEI004	15	20%
REN105L03	22	23%
REN162C04	14	21%
REN169D01	14	36%
REN169O18	14	36%
REN247M23	11	27%
AvREN54P11	14	36%
REN64E19	12	33%
VGL0760	26	19%
VGL0910	27	26%
VGL1063	17	29%
VGL1165	23	39%
VGL1828	22	23%
VGL2009	12	42%
VGL2409	13	38%

VGL2918	19	31%
VGL3008	18	44%
VGL3235	13	38%

Average retained diversity of all dogs - 30.3%

B. Assessment of population heterozygosity using standard genetic parameters

Allele frequencies across all 33 STR loci taken from Table 1a were used to calculate a mean (average) observed heterozygosity (*Ho*) and expected heterozygosity (*He*) for this group of Irish red setters (Table 2). The 50 dogs that were initially tested had a mean number of alleles *(Na*) per locus of 4.82 across all 33 genomic STR loci. The average number of alleles per locus was low compared to many larger breeds such as the Italian greyhound (*Na*=7.12), Labrador retriever (*Na*=7.27) and Golden Retriever (*Na*=8.23), slightly higher than the Swedish Vallhund (*Na*=4.67), and lower than the Flat coated retriever (*Na*=5.70) alleles/locus. The mean effective alleles (*Ne*) (i.e., alleles contributing most to heterogeneity) per locus was 2.64. The fact that a few alleles contribute to most of the heterogeneity of the breed is also characteristic for all pure breeds of dogs.

Table 2. Standard Genetic Assessment for 50 Irish setters using 33 STR loci. (Updated March 2, 2019)

	N	***Na***	***Ne***	***Ho***	***He***	***F***
Mean	50	4.818	2.635	0.563	0.571	0.007
SE		0.262	0.153	0.027	0.027	0.017

The mean observed heterozygosity (*Ho*) was 0.563, which was nearly identical to the expected heterozygosity (*He*) of 0.571. *He* is the heterozygosity that would be found in this group of dogs if their parents had been chosen in an entirely random manner (i.e. Hardy-Weinberg equilibrium or HWE). The fact that *Ho* and *He* were nearly identical indicates that the average dog in this group of 50 had parents that were as unrelated as possible given the population size and existing genetic diversity.

Ho and *He* can be used to calculate an inbreeding coefficient *F*. An *F* value of -1.0 would occur if the parents of all test dogs were totally unrelated to each other, while a value of +1.0 would indicate that the parents were genetically identical. The *F* value for these 50 Irish setters was 0.007, indicating and excess homozygosity (or deficiency of heterozygosity) of less than 1% over what would have been expected if parent selection was entirely random (i.e., at HWE).

Although the *Ho*, *He* and *F* values look very good for this group of dogs, these scores are averages for the group and thus do not represent the genetic heterogeneity of individual dogs. The genetic relatedness of a given dog's sire and dam are better reflected by internal relatedness (IR) scores (see below).

C. Standard genetic assessment values for individual STR loci

The allele frequencies (Table 1) can be used to do a standard genetic assessment of heterozygosity at each STR locus (Table 3). The *Na* values for individual STR loci for this population of 50 Irish setters ranged from a low of 2 to a high of 9 alleles/locus, while the *Ne* ranged from 1.28 to 4.22. The *F* value calculated from *Ho* and *He* was greater than +0.10 for 6/33 loci and lower than -0.10 for 3/33 loci (highlighted). Individuals with alleles that have positive *F* values are the most inbred in the group, and dogs with alleles that have negative *F* values are the more outbred dogs. The findings suggest that there are a few more highly inbred individuals in the population than strongly outbred dogs. This was confirmed with the internal relatedness (IR) values.

Table 3. Standard Genetic Assessment for 50 Irish setters using 33 STR loci; loci with *F* values < -0.10 (light highlight) and >+0.10 (dark highlight). (Updated January 14, 2019)

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	50	5	3.569	0.76	0.720	-0.056
2	**AHT137**	50	7	4.065	0.74	0.754	0.019
3	**AHTH130**	50	4	1.282	0.24	0.220	-0.091
4	**AHTh171-A**	50	5	3.679	0.66	0.728	0.094
5	**AHTh260**	50	6	3.808	0.62	0.737	0.159
6	**AHTk211**	50	4	1.709	0.42	0.415	-0.012
7	**AHTk253**	50	3	1.446	0.36	0.308	-0.168
8	**C22.279**	50	5	2.316	0.58	0.568	-0.021
9	**FH2001**	50	4	1.367	0.28	0.269	-0.042
10	**FH2054**	50	6	2.525	0.52	0.604	0.139
11	**FH2848**	50	6	2.948	0.7	0.661	-0.059
12	**INRA21**	50	3	2.919	0.54	0.657	0.179
13	**INU005**	50	3	1.772	0.4	0.436	0.082
14	**INU030**	50	3	2.080	0.48	0.519	0.076
15	**INU055**	50	4	3.322	0.84	0.699	-0.202
16	**LEI004**	50	3	2.396	0.6	0.583	-0.030
17	**REN105L03**	50	5	2.015	0.52	0.504	-0.033
18	**REN162C04**	50	4	3.509	0.76	0.715	-0.063
19	**REN169D01**	50	5	3.514	0.64	0.715	0.105
20	**REN169O18**	50	5	3.147	0.72	0.682	-0.055
21	**REN247M23**	50	3	1.653	0.46	0.395	-0.165
22	**REN54P11**	50	5	3.143	0.7	0.682	-0.027
23	**REN64E19**	50	4	2.273	0.56	0.560	0
24	**VGL0760**	50	5	1.456	0.32	0.313	-0.021
25	**VGL0910**	50	7	2.146	0.56	0.534	-0.049
26	**VGL1063**	50	5	3.858	0.62	0.741	0.163
27	**VGL1165**	50	9	3.791	0.72	0.736	0.022
28	**VGL1828**	50	5	3.053	0.72	0.672	-0.071
29	**VGL2009**	50	2	1.651	0.34	0.394	0.137
30	**VGL2409**	50	5	2.309	0.58	0.567	-0.023

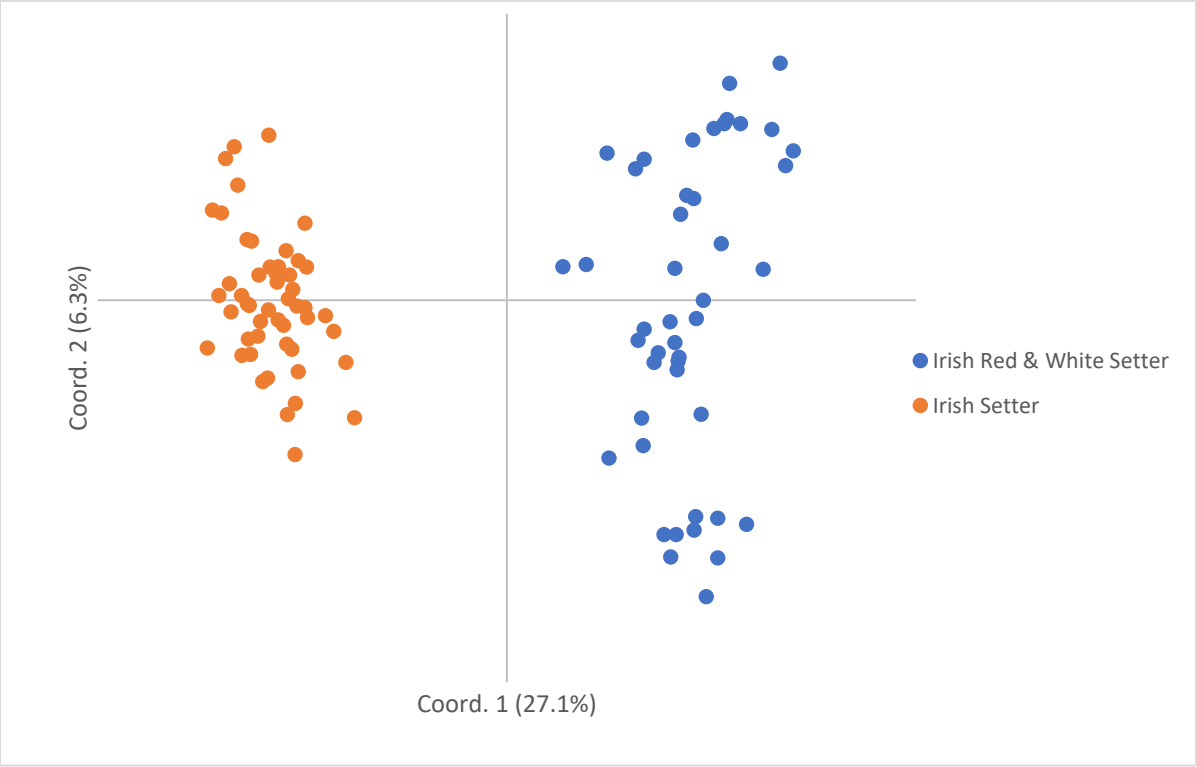
31	**VGL2918**	50	6	2.141	0.5	0.533	0.062
32	**VGL3008**	50	8	4.219	0.7	0.763	0.083
33	**VGL3235**	50	5	1.862	0.42	0.463	0.093

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

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to demonstrate genetic differentiation between individuals in related or unrelated populations. The resulting data is multi-dimensional (spherical) but can be accurately portrayed in a two-dimensional graph by selecting values from the two coordinates that represent the greatest proportion of individuals (coordinate 1 and 2 in this case).

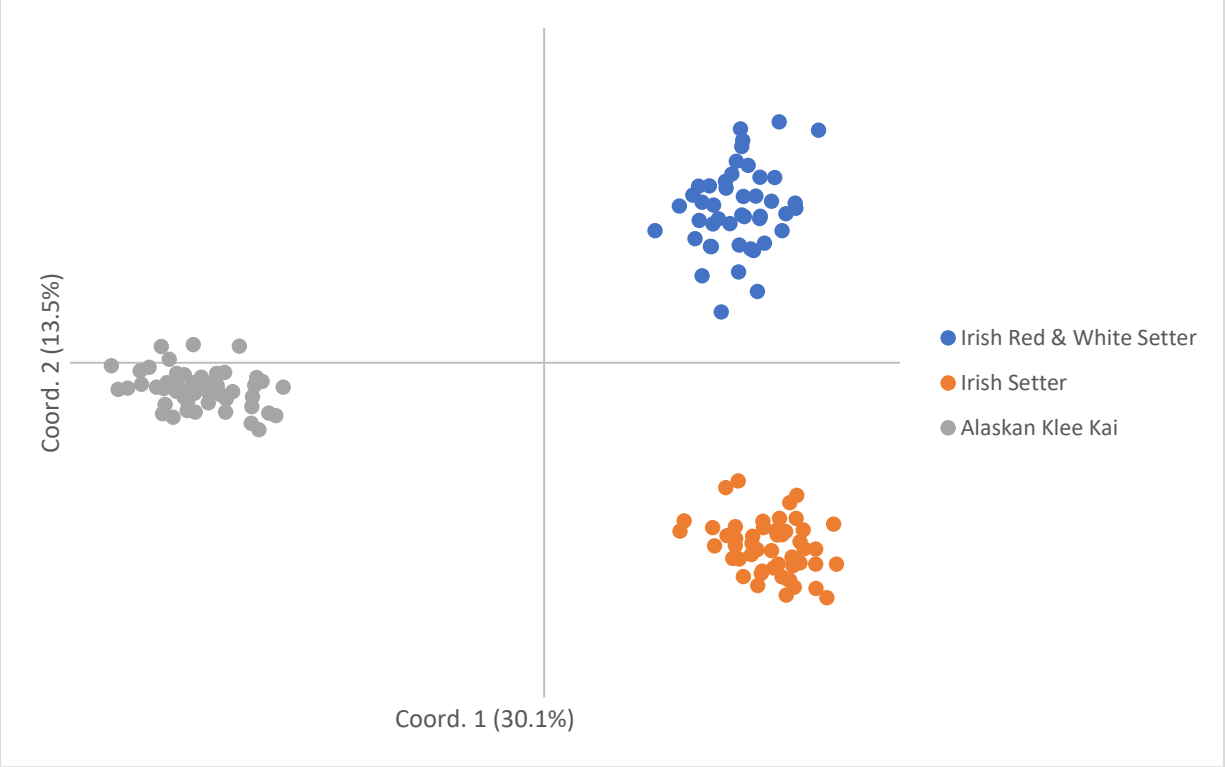
Figure 1 is a PCoA plot of the 50 Irish setters and 49 Irish red and white setters shows them clustering as a distinct breed, as might be expected given differences in founder lines and the many decades that have separated them. Individual Irish setters are more tightly clustered around the central X/Y axis than individual Irish red and white setters, indicating that Irish setters are more closely related to each other than Irish red and white setters.

Figure 1. PCoA plot showing the genetic relationship of 50 Irish setters to each other and to 41 Irish red and white setters.



In order to test the level of relationship between Irish setters and Irish red and white setters, a PCoA plot was made that compared the two Irish-type setter breeds with a breed that has no known relationship, the Alaskan klee kai (Fig. 2). This type of comparison will draw individuals within a breed closer. If the breed relationship is close, such as Japanese and American Akita, the related breeds (varieties) will begin to merge with each other. If they are related, but not to the level of a definite bloodline or variety, the breeds will come closer together but not touch or begin to merge. The PCoA plot of the three breeds shows a tight clustering of individuals in each breed, as expected. In addition, all three breeds remained genetically distinct but with the Irish setter and Irish red and white setters forming distinct clusters to the right of the Y-axis, but in different quadrants. In comparison, the Alaskan klee kai clusters on the left side of the Y-axis. Therefore, it can be concluded that the Irish Setter and Irish red and white setter or distantly related. This will be confirmed by DLA class I and II haplotypes.

Figure 2. PCoA plot showing the relatedness of two related breeds, Irish setters, Irish red and white setters, and an unrelated breed theAlaskan klee kai.



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

1. IR testing

Genetic assessments such as those presented in Table 2 are indicators of population-wide heterozygosity and do not reflect the heterozygosity of individual dogs. The genetic diversity of an individual dog is largely determined by the diversity inherited from each of its parents. Internal Relatedness (IR) is a calculation that has been used to determine the relative genetic contributions of both parents to an individual. The IR calculation evaluates homozygosity and

assigns greater importance to rare and uncommon alleles (Table 4). IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0 (Fig. 4). A dog with a value of -1.0 has parents that are 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 equivalent to 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.

Table 4. IR and IR-Village Dog values for Irish setter (n=50)

	IR	IRVD
Min	-0.210	0.019
1st Qu	-0.051	0.022
Mean	0.010	0.310
Median	-0.009	0.300
3rd Qu	0.081	0.388
Max	0.320	0.600

The value of IR over standard genetic assessments is apparent in this group of dogs. The entire population is on average slightly inbred with a mean IR score of 0.010 (Table 4, Fig. 3). However, one half of the dogs had IR values from +0.010 to +0.320 and one fourth from +0.081 to +0.320. An IR score of +0.250 would be equivalent to mating half siblings from a random bred population, while an IR score of +0.320 would be equivalent to breeding full siblings that also came from the same highly inbred population. Highly inbred individuals were countered by an equal number of individuals with IR scores ranging from +0.0010 to -0.210. This balancing effect of highly outbred and inbred dogs is why the average *Ho, He* and *F* values based on allele frequency data from the 33 genomic STR loci were so favorable. It is common in many pure breeds of dogs to have a mixture of highly inbred and outbred individuals in the population.

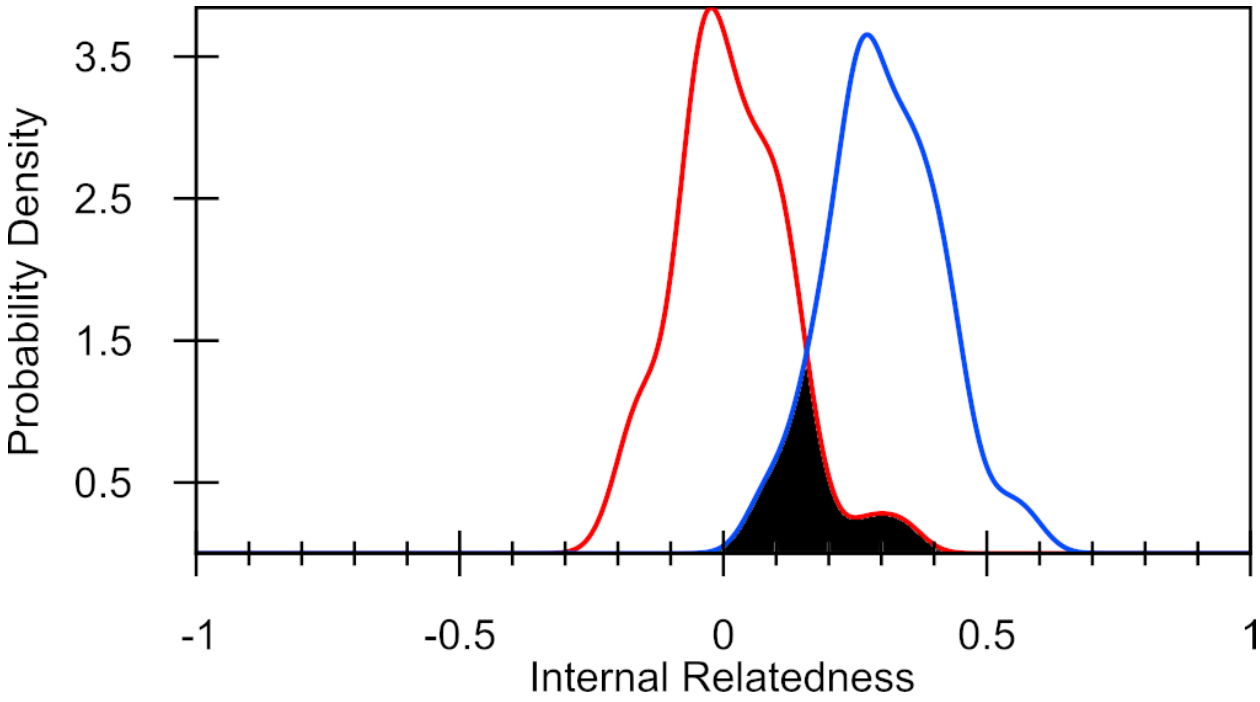


Fig. 3. IR vs IRVD graph for 50 Irish setters. The IR graph (redline) graphs the IR scores of individual dogs, while the IRVD adjusts the IR scores to indicate the IR scores of these dogs if they were to be compared against village dogs. The black area that is formed by the two overlapping curves approximates how much known canid genetic diversity still exists in the group Irish setters that were tested.

2. Adjusted IR values based on village dogs (IRVD) as a measure of lost or retained genetic diversity

The IR values can be evaluated in such a way as to provide one estimate of the amount of species-wide genetic diversity that still exists in a breed from the time of its creation (closure of registry to outside dogs) to current time. This amount of retained genetic diversity is measured by comparing breed associated alleles and allele frequencies with the frequency of those same alleles among present-day village dogs from the Middle East, SE Asia and Island Pacific nations. Village dogs are the most random bred and genetically diverse population that has been studied to date and are ancestral to most modern breeds such as the Irish red and white setter. The IR value adjusted to village dogs is known as IR-village dogs or IRVD.

The IRVD curve for Irish setters was shifted well to the right, reflecting an 83% loss (or 17% retention) of potential genetic diversity during breed development (Fig. 3). This 17% estimate of retained genetic diversity is lower than the 30% estimate of retained genetic diversity obtained from known vs. observed alleles and their frequencies presented in Table 1b. This dichotomy is atypical from other breeds and suggests that contemporary Irish setters are less related to village dogs from the middle east and SE Asia than other breeds. This level of 17-30% retained genetic diversity is lower than genetically diverse breeds such as Toy Poodle (60%), Labrador Retriever (54%), Golden Retrievers (50%), and Alaskan Klee Kai (50%); similar to the Samoyed (35%),

Flat Coated Retriever (35.2%), Shiba Inu (29.8%), and Shiloh shepherd (27%); and higher than Japanese Akita (24.4%), Doberman Pinscher (15%) and Swedish Vallhund (7%).

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

The DLA consists of four gene rich regions (classes I-IV) comprising part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. 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 class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Class I and II haplotypes can be determined by direct sequencing or by their association with linked STR loci. Sequencing is time consuming and expensive, while the use of linked STR markers is simpler and much less expensive.

There are four STR loci that are linked to the DLA class I region and three STR loci associated with the DLA class II region (Table 5). Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and inherited as a single block or haplotype. One haplotype comes from each of the parents. Specific class I and II haplotypes are often linked to each other and inherited as a genetic block with limited recombination over time. Therefore, DLA class I and II haplotypes can be viewed as reasonable surrogate markers for breed founders. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune and autoinflammatory diseases.

1. DLA class I and II haplotypes observed in Irish setters

The STR-based haplotype nomenclature used by the VGL in their breed diversity analyses 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 (Table 5). It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry. To date, the VGL has identified 205 unique DLA I and 112 DLA II haplotypes among all dogs. DLA class I and II regions are in looser linkage than each region alone, leading to some shuffling between class I and class II haplotypes and over 355 combinations of DLA class I and II haplotypes.

Table 5. A comparison of DLA class I and Class II haplotypes and their frequencies for Irish setters (n=49) and Irish red and white setters (n=41).

DLA class I #	STR types	Irish Setter (n=49)	Irish red and white setter (n=41)
1006	387 375 293 180	---	0.05
1008	386 373 289 182	0.11	0.60
1011	376 365 281 180	0.10	---
1014	375 373 287 178	0.37	0.05

1054	382 379 277 184	0.18	---
1068	380 373 287 181	0.01	---
1069	380 365 281 184	0.03	0.04
1175	380 375 293 180	---	0.10
1201	382 381 277 181	---	0.07
1202	390 373 289 183	---	0.10
1210	380 365 277 184	0.12	---
1211	386 369 277 183	0.07	---
DLA class II #			
2005	339 322 280	0.04	0.15
2007	351 327 280	---	0.15
2012	345 322 280	0.06	0.11
2015	339 327 280	0.12	---
2022	339 327 282	0.18	---
2035	341 323 280	---	0.07
2037	341 327 280	0.37	---
2045	339 325 284	0.03	0.04
2052	345 321 280	0.11	0.49
2053	343 324 280	0.01	---
2114	345 323 284	0.07	---

The Irish setters in this study possessed 8 DLA class I and 9 DLA class II haplotypes (Table 5). Two DLA class I haplotypes (1210, 1211) appear to be unique to Irish setters. The incidence of most of the haplotypes was from 3-18%. However, one DLA class I (1014) and one DLA class II (2037) are found in solely or predominantly in the Irish setter. These two haplotypes are in linkage, forming an even more extended 1014/2037 haplotype that was inherited by descent from an important founder or founder line of the breed. These higher incidence haplotypes are likely to remain the most common even after more dogs are tested, although several lower incidence haplotypes are likely to be identified as more dogs are tested.

Three DLA class I haplotypes and four class II haplotypes are shared between the two breeds (Table 5). However, a larger number of haplotypes were observed in only one breed or the other. It is noteworthy that a founder or founder line with the 1014 DLA class I haplotype played a major role in the evolution of the Irish setter breed, but not in the Irish red and white setter. Conversely, the 1008 DLA class I haplotype was an even more significant contributor to the Irish red and white setters but a minor contributor to the Irish setter.

The existing DLA class I and II haplotypes in Irish setters make-up 8/205=3.9% and 9/112=8.0%, respectively, of DLA haplotypes known to exist in all dogs tested at the VGL to date. These percentages may increase somewhat as more haplotypes are identified. However, they provide another estimate of canine genetic diversity that has been retained by contemporary Irish setters since the breed split from the original Irish red and white setters. The percentage of known DLA class I and II haplotypes retained by the Irish red and white setters is only a fraction

of the percentage of retained autosomal STR alleles calculated in Table 1b (30%) and from the retained genetic diversity calculated by the IR/IRVD (17%) comparison (Figure 3). This difference is due to a much higher number of haplotypes in the DLA class I and II regions than alleles in any of the 33 autosomal STR loci. The DLA region has been subjected to more positive selection than any other region of the genome, due to continuous exposure to new pathogens that have appeared during millions of years of canid evolution.

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

Several of the DLA class I and II haplotypes found in Irish setters were shared with other dog breeds (Table 6). DLA class I and II haplotype sharing were greatest with the Golden retriever, Labrador retriever, and Poodles, as might be expected, given their shared history as outstanding hunting dogs. Significant DLA sharing with the Giant Schnauzer, Havanese and Samoyed were less expected. With the exception of the Havanese, all related breeds had their origins in Europe at around the same period.

Table 6. DLA class I and II haplotype sharing between Irish setters and other breeds

DLA1 #	STR types	Black Russian Terrier (n=124)	Lakeland Terrier (n=48)	Labrador Retriever (n=164)	Irish Red and White Setter (n=44)	Irish Setter (n=49)	Doberman Pinscher (n=517)	Flat Coated Retriever (n=446)	Havanese (n=406)	Samoyed (n=189)	Shiba Inu (n=98)	Giant Schnauzer (n=190)	English Bulldog (n=163)	Biewer (n=119)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=93)	Yorkshire Terrier (n=16)	Italian Greyhound (n=773)	Alaskan Klee Kai (n=497)	Shiloh Shepherd, ISSA (n=151)	Magyar Agar (n=59)	American Akita (n=98)	Japanese Akita (n=332)	Golden Retriever (n=700)	Miniature Poodle (n=268)	Barbet (n=54)	Swedish Vallhund (n=181)	Poodle (n=2498)	Toy Poodle (n=129)
1006	387 375 293 180	0.04	--	0.043	0.05	--	--	--	0.048	0.005	--	0.05	0.003	--	--	--	--	--	--	--	--	0.056	--	0.0143	0.004	--	0.262	0.0458	0.004
1008	386 373 289 182	--	--	0.064	0.59	0.11	--	--	--	--	--	0.047	0.006	0.008	--	--	0.06	0.1332	0.059	--	--	--	--	0.0014	--	--	--	0.0184	0.019
1011	376 365 281 180	--	--	--	--	0.1	--	--	--	0.272	--	0.013	--	--	--	--	--	--	0.058	--	--	--	--	0.0007	0.004	--	--	0.0198	0.023
1014	375 373 287 178	0.036	--	--	0.05	0.37	--	--	0.033	0.003	--	0.25	--	0.025	0.009	0.081	0.09	--	0.367	--	--	--	--	0.0407	0.004	--	--	0.0092	0.05
1054	382 379 277 184	--	--	0.082	--	0.18	--	0.117	0.117	--	0.383	0.005	--	--	--	--	--	0.0155	--	--	0.144	--	--	--	0.002	--	--	--	--
1068	380 373 287 181	--	--	0.052	--	0.01	--	0.275	0.015	0.042	--	0.037	--	--	0.009	--	--	--	--	0.245	--	--	--	0.05	0.017	--	0.343	--	0.012
1069	380 365 281 184	--	--	--	0.03	0.03	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0393	--	--	--	--	--
1175	380 375 293 180	--	--	0.003	0.13	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1201	382 381 277 181	--	--	--	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1202	390 373 289 183	--	--	--	0.09	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1210	380 365 277 184	--	--	--	--	0.12	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1211	386 369 277 183	--	--	--	--	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies (Updated Mar 5, 2019)

DLA2 #	STR types	Black Russian Terrier (n=124)	Lakeland Terrier (n=48)	Labrador Retriever (n=164)	Irish Red and White Setter (n=44)	Irish Setter (n=49)	Doberman Pinscher (n=517)	Flat Coated Retriever (n=446)	Havanese (n=406)	Samoyed (n=189)	Shiba Inu (n=98)	Giant Schnauzer (n=190)	English Bulldog (n=163)	Biewer (n=119)	Biewer Yorshire Terrier (n=53)	Biewer Terrier (n=93)	Yorkshire Terrier (n=16)	Italian Greyhound (n=773)	Alaskan Klee Kai (n=497)	Shiloh Shepherd, ISSA (n=151)	Magyar Agar (n=59)	American Akita (n=98)	Japanese Akita (n=332)	Golden Retriever (n=700)	Miniature Poodle (n=268)	Barbet (n=54)	Swedish Vallhund (n=181)	Poodle (n=2498)	Toy Poodle (n=129)
2005	339 322 280	0.016	0.01	0.055	0.14	0.04	--	0.414	0.002	--	--	0.011	0.015	0.046	0.009	0.054	--	--	--	--	--	--	--	0.0157	--	--	--	0.022	0.004
2007	351 327 280	0.04	--	0.046	0.17	--	--	--	0.052	0.005	--	0.05	--	--	--	--	--	--	0.015	--	--	0.056	--	0.0143	0.002	--	0.268	0.0158	0.004
2012	345 322 280	--	--	--	0.13	0.06	--	--	0.005	--	--	0.013	--	--	--	--	--	--	0.062	--	0.102	--	0.002	0.0007	0.063	--	--	0.005	0.054
2015	339 327 280	--	0.02	--	--	0.12	--	--	--	0.016	0.046	--	--	--	--	--	--	0.0091	--	--	--	--	--	--	0.047	--	--	0.0072	0.039
2022	339 327 282	--	0.18	0.082	--	0.18	0.0019	0.124	0.116	0.108	--	0.005	0.015	--	--	--	--	--	--	0.046	--	--	--	0.0007	0.002	--	--	0.0002	0.012
2035	341 323 280	--	--	--	0.07	--	--	--	0.001	--	--	--	--	--	--	--	--	0.0893	--	--	--	--	0.295	--	--	0.037	--	--	--
2037	341 327 280	0.29	--	--	--	0.37	--	--	0.033	--	--	0.329	--	0.025	0.009	0.081	0.09	0.0097	0.367	--	0.008	0.168	0.256	--	0.007	--	--	--	0.008
2045	339 325 284	--	--	--	0.03	0.03	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.04	--	--	--	--	--
2052	345 321 280	--	--	0.055	0.47	0.11	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0021	--	--	--	--	--
2053	343 324 280	--	--	0.049	--	0.01	--	0.146	0.037	0.558	--	0.042	--	--	0.009	--	--	--	--	0.308	--	--	--	0.0293	0.017	--	0.481	--	0.016
2114	345 323 284	--	--	--	--	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

3. Heterozygosity in the DLA region

The DLA region encompassing type I and II genes is only a small part of chromosome 12 and an even smaller part of the whole genome. However, it is a very important region of the genome in regulating immunity and controlling not only resistance to pathogens but autoimmunity and allergies as well. Therefore, it is also important to see if this part of the genome is also undergoing random selection and is in HWE. Inbreeding within the DLA can lead to increased susceptibility to a number of autoimmune disorders (17, 18), increased allergies and decreased resistance to certain infections. Heterozygosity within the DLA region can be measured in the same manner as heterozygosity across the genome, i.e., by doing a standard genetic assessment of allele frequencies at individual STR loci that define the DLA class I and II haplotypes (Table 7) and across all seven STR loci (Table 8).

Table 7. Standard genetic assessment of heterozygosity based on allele frequencies at each of the seven STR loci that define the DLA class I and II haplotypes.

#	Locus	*N*	*Na*	*Ne*	*Ho*	*He*	*F*
1	**DLA I-3CCA**	**50**	6.0	4.31	0.66	0.77	0.14
2	**DLA I-4ACA**	**50**	5.0	2.98	0.48	0.67	0.28
3	**DLA I-4BCT**	**50**	4.0	3.2	0.60	0.69	0.13
4	**DLA I-1131**	**50**	6.0	3.7	0.64	0.73	0.12
5	**DLA II-5ACA**	**50**	4.0	3.04	0.60	0.67	0.11
6	**DLA II-5ACT**	**50**	6.0	2.14	0.48	0.53	0.10
7	**DLA II-5BCA**	**50**	4.0	1.87	0.30	0.46	0.35

Table 8. Standard genetic assessment of heterozygosity in the DLA class I and II regions of Irish setters (n=50)

	N	*Na*	*Ne*	*Ho*	*He*	*F*
Mean	50	5.0	3.03	0.54	0.65	0.18
SE		0.4	0.3	0.04	0.04	0.04

The number of alleles (*Na*) at each of the seven DLA loci varied from 4-6 and effective alleles (*Ne*) varied from 1.87 to 4.31 (Table 7). The expected heterozygosity (*He*) exceeded the observed heterozygosity (*Ho*) at each locus, yielding *F* values that were all positive, ranging from 0.10 to 0.35 (Table 7). This indicated an average deficiency of heterozygosity (or excess of homozygosity) of 18% across all loci. This deficiency of heterozygosity was not observed with the 33 autosomal STR markers (*He* 0.563, *Ho* 0.571, *F* 0.007) (Tables 1a, 2). These findings indicate either that dogs with certain DLA types are being preferentially bred at this time or that the DLA class I and II haplotypes among the 50 dogs studied are not representative of the population. This should be resolved upon the testing of more dogs.

III. What does DNA-based genetic testing tell us about contemporary Irish setters

It is not possible based on 50 individual dogs to make definitive conclusions, as more alleles of the 33 STR loci and DLA class I and II haplotypes will undoubtedly be found as additional dogs from a wider region of the world are tested. However, based on experience with other breeds, over 80-90% of known diversity is probably represented in the dogs reported herein and additional alleles and haplotypes will be relatively few and exist at a very low frequency.

This initial population of the 50 Irish setters appeared, on average, to be products of parents that were as unrelated as possible. However, IR scores that more accurately measure the relatedness of parents of individual dogs identified a significant sub-population of highly inbred dogs. This might be expected in a small breed with relatively low numbers. Irish setters appear to have below average genetic diversity compared to other breeds, having retained an estimated 30% of the autosomal STR alleles known to currently exist among all dogs. Based on DLA haplotypes, the breed has evolved around less than 10 founders or founder lines. The lack of genetic diversity

in the breed has been confirmed from pedigrees, with an effective population size of 27 distinct individuals (10). There is evidence from this study, as with other reports (4), that breeding for conformation traits leads to more inbreeding and potential loss of breed-wide genetic diversity than selection for performance.

The relative lack of genetic diversity in contemporary Irish setters most likely occurred over a long period of time, but stopped with the first registrations of the breed and closure of the breeding book to outside dogs. At that point, genetic diversity could only be lost and not gained. Loss of genetic diversity after closure of the breed is known to occur, although most breeds have retained on average of 87% of their original diversity to date (11). Artificial genetic bottlenecks may involve popular sires, popular dams, geographical isolation or separation, loss of popularity and decrease in number of breeding dogs, breed reconstitution, elimination of deleterious genetic mutations (e.g., CLAD, vWD), and cataclysms such as world wars. Irish red and white setters have been exposed to all of these bottlenecks during their documented history. Sudden increases in popularity can also lead to rapid changes in the genetics of a population (breed). The Irish setter in the UK reached a peak of popularity with 2400 registrations in 1982, followed by a sharp drop between 1982 and 1986 and a gradual decline to less than 800 dogs in 2015 (10). The COI based on pedigrees increased to an average of 0.14 during this period. The effective population size, based on this study, was estimated to be 27.3 individuals in 2015, which is lower than many other popular breeds and commiserate with findings reported herein.

Low genetic diversity is not in itself serious, providing founder dogs were relatively free of heritable disorders and random breeding has been strictly enforced. Any subsequent health problems would either be due to inadvertent or illegal introgressions or intra-breed mutations. However, a low degree of genetic diversity makes it much more difficult to manage heritable problems present in the founders or subsequent internal mutations (12). The deleterious effects of breeding to an extremely popular show line has been documented for the Standard Poodle (16), as has the effect of a popular sire for Italian greyhounds (17).

IV. Health problems of Irish setters

A. Overview

Irish setters are considered to be generally healthy, with an expected lifespan of around 10–14 years (8, 9). The Irish setter in the UK reached a peak of popularity in the 1970s with 2400 registrations in 1982, followed by a sharp drop between 1982 and 1986 and a gradual decline to less than 800 dogs in 2015 (10). The rise and decline in popularity were worldwide and has been attributed to the great popularity of the Disney movie "Big Red", which starred an Irish setter. A similar phenomenon have been seen with a number of other breeds that were highlighted in movies, including The Wizard of Oz (1939 - Cairn terrier), Lassie Come-Home (1951 - Collie) and 101 Dalmatians (1996 - Dalmatian). A rise and decline in popularity occur with many breeds, and there are many reasons why a breed loses popularity. However, the consensus reason is often referred to as "overbreeding." Overbreeding often leads to unwanted changes in temperament, intelligence, and health. All three of these changes have been put forward as reasons why Irish setters lost popularity.

B. Hereditary concerns

Irish setters suffer from several conditions that have a heritable basis. The first group of diseases are those caused by simple Mendelian traits. A second group have a heritable predisposition and are often common in other pure breeds and even random-bred dogs. These traits are predominantly of a polygenic nature and involve the interactions of numerous positive and negative risk factors.

1. Deleterious conditions inherited in a simple Mendelian fashion

a. Canine Leucocyte Adhesion Deficiency (CLAD) is a failure of the immune system to fight infection. The disorder is caused by a missense mutation in the ITGB2 gene and is inherited as an autosomal recessive (13). Young puppies do not thrive and continually develop infections. They can also have growth problems and may die well before they reach their first birthday. Breeders have made a concerted effort to reduce the incidence of CLAD by testing for the mutation and not registering, and therefore breeding, carriers.

b. von Willebrands Disease (vWD) is a blood clotting disorder that occurs in many dog breeds. It is often clinically silent, although in extreme cases, severe bleeding can occur. Various clubs will only register litters of Irish Setters if both parents are DNA tested clear of the von Willebrand Disease (vWD) mutation, or if the parents are known offspring of test negative parents.

c. Progressive retinal atrophy is an irreversible, inherited blinding disease, that occurs in many breeds and clinical forms. Irish Setters suffer from an "early onset" form known as rod-cone dysplasia type 1, or rcd1. Pups begin showing signs of night-blindness by 6 weeks of age, and by 1-2 years of age most affected dogs are completely blind. There is a diagnostic test for the autosomal recessive mutation.

2. Disorders presumed to have a heritable predisposition

a. **Hypertrophic osteodystrophy (HOD**) is a canine developmental disease that affects the metaphysis of long bones in large breed dogs between eight weeks and eight months of age. Sick dogs exhibit swelling and pain in their legs with reluctance to stand or walk. In addition to bone pain, there are variable general signs including fever, lethargy, depression, and loss of appetite. Evidence for a heritable nature includes its tendency to occur in most often in certain breeds (Weimaraner, Irish setter, Great Dane) and in multiple individuals within a litter and in close relatives. The disease is treated with corticosteroids and is usually self-limiting by 8-10 months of age. A recent study links HOD with high intrinsic serum levels of cytokines involved in innate immunity (IL-1beta, Il-18, IL-6, GM-CSF, CXCL-10 and TNF (14).

b. **Posterior Polar Cataract (PPC)** – sight is impaired by a cataract on the back of the lens of the eye. Developmental hereditary cataracts have been recognized in many dog breeds originating in the UK (15). The disorder is uncommon and can appear at any time up to eight years of age and does not cause blindness. The cause of PPC in Irish setters has eluded 16 years of scientific investigation and there is no evidence that it results in blindness or causes pain or inconvenience to affected dogs. Therefore, it is no longer necessary in some breed clubs to

withdraw affected dogs from breeding programs, although a mating of two affected dogs should be avoided.

c. **Bloat** tends to occur in larger, deep-chested dogs, and it is uncertain whether it a purely an anatomical problem or whether other predispositions are involved as well.

d. **Cancer** is responsible for an average 27% deaths among all pure breeds of dogs, and the Irish setter is slightly above this at 27.3% (16). The most common cancers of Irish setters are hemangiosarcoma, lymphoma, and osteosarcoma.

3. Disorders with a complex genetic basis

Complex (polygenic) disorders involve the collective effect of numerous positive and negative risk factors. These risk factors may also be affected by environmental factors. Risk factors are genetic polymorphisms that have occurred across the genome over tens of thousands of years of dog evolution. They were originally products of natural selection for survival. As natural selection pressures decreased and artificial (human-directed) selection pressures increased, certain risk factors become concentrated by inbreeding and were passed to future generations by descent.

a. **Hip and elbow dysplasia** are common problem in many dog breeds as well as Irish setters. The exact reasons why so many pure breeds suffer from disorders like hip dysplasia are unknown, but there is no doubt that risk factors for the disease are highly susceptible to human-directed selection for the various traits that people desire in their dogs. The only known way to decrease the incidence of disorders such as hip dysplasia is to select parents that are as free of the disorder as possible. The problem is that it may have taken hundreds of years to inadvertently select dogs for such disorders and hundreds of years of reverse selection to eliminate them. The more genetic diversity a breed has lost, the more difficult it will be to recreate the normal phenotype.

b. **Hypothyroidism** is the first autoimmune condition that is often recognized as a breed problem. Like other autoimmune conditions, it is most likely as a result of inbreeding and the accumulation of risk factors that go far back in dog's domestication. Other forms of autoimmunity often evolve over time as a breed or bloodline within a breed becomes more inbred (17, 18).

c. The cause of **epilepsy** is unknown, but it is believed to have a heritable basis (19). It occurs in many pure-breeds and the incidence increases with the degree of inbreeding.

C. Outcross program

The Irish Kennel Club (IKC) announced a plan in 2011 to increase genetic diversity in Irish red and white setters by allowing Irish Red and White Setters to be crossed with Irish Setters that can still perform in the field. Outcross mating's were to be carefully monitored. The reasons for such outcrossing are obvious - Irish Setters were presumed to possess genetic diversity no longer found in Irish red and white setter, while phenotypes are virtually identical except for coat color.

However, the plan was vigorously opposed by many other breed clubs worldwide. The major reason for these objections was the feeling that Irish setters had even more genetic problems than Irish red and white setters. Nonetheless, some Irish red and white breed clubs have embraced limited use of outcrossing but only to Irish setters with field abilities.

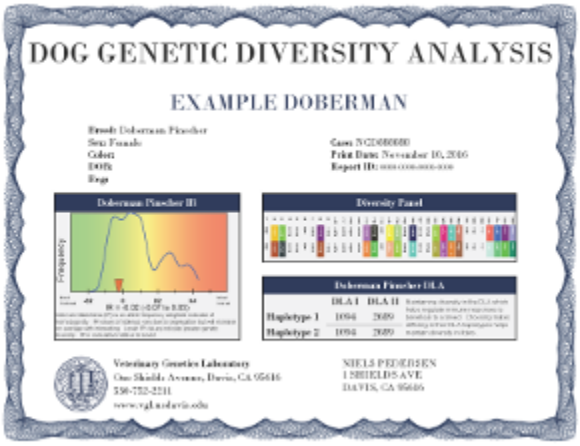
The genetic studies done on Irish red and white setter and Irish setter done at the VGL, confirm that founders for the two breeds were selected from among the same and larger pool of original dogs. Therefore, the sum total of genetic diversity in the two breeds provides an accurate picture of the genetic diversity in Irish-type setters before the split that started in the late nineteenth century. Some of the original founders or founder lines have been shared by both breeds, while some are unique to one breed or the other. This is identical to the evolution of American and Japanese Akita. The major difference is that Japanese and American Akita are related more to the level of varieties than breeds, reflecting their even more recent post-WWII separation and less time for geographic differentiation.

Based on the number of dogs tested to date, Irish setters have about 5% less genetic diversity in the 33 autosomal STR markers than Irish red and white setters that were tested. This is in line with previous studies comparing performance and conformation breeds (4). Although the Irish setters reported herein were somewhat less heterogeneous than Irish red and white setters across the genome, they possessed more DLA class I and II haplotypes (17 haplotypes) than the Irish red and white setter (13 haplotypes). This suggests that a greater number of original founder lines were used to create contemporary Irish setters than Irish red and white setters. The fact that only about one-third of the DLA class I and II haplotypes are shared between the two breeds is a positive finding, as it means that each of the two breeds has a lot of genetic diversity that can be added to the other. DNA testing would be essential for detecting the best individuals to be used for the introgression and in monitoring how new diversity is spread throughout the breed following its introduction.

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 entire population.


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE DOBERMAN

Doberman Pinscher III

Diversity Panel

Doberman Pinscher DLA

	DLA I	DLA II
Haplotype 1	1094	2689
Haplotype 2	1094	2689

Veterinary Genetics Laboratory
One Shields Avenue, Davis, CA 95616
530-752-2211
www.vgl.ucdavis.edu

NIELS PEDERSEN
1 SHIELDS AVE
DAVIS, CA 95616

B. What should you do with this information?

The goal for breeders should be to continue to produce puppies with IR scores less than 0. Although this initial population appeared to be outbred on average, there was a subpopulation of dogs that were much more inbred than the rest of the breed. Therefore, there is a possibility to better balance genetic diversity in the breed by DNA testing. 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, like what is being done by many Standard Poodle breeders. However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

The next step is to compare the DLA class I and II haplotypes. You want to avoid breeding pairs that will produce puppies that will be homozygous for the same haplotypes, and once again, less common haplotypes may offer more diversity than common ones.

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

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

VII. References

References

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