Genetic Diversity Testing for Irish red and white 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 red and white 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 41 dogs from the USA (n=31), Canada (n=1), Great Britain (n=1), The Netherlands (n= 6), Austria (n=1), and Belgium (n=1). 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-type setters (1-3)

The original Irish-type setters were predominantly white or white with variable red patches. This type of setter was thought to originate in the 1700s from the old Spanish pointer and early indigenous Scottish setters. The predominantly white color made them more visible over a distance to the hunters. However, the chestnut red color caught people's fancy and mostly red dogs became progressively dominant by the late 19th century in shows and as pets. Field-type Irish setters tended to remain red and white colored.

A. Irish red and white setter

The Irish Red and White Setter is an old breed originating in Ireland with documented descriptions as early as 1775. The breed was originally developed to hunt gamebirds such as grouse, pheasants, ptarmigan, snipe or woodcock. These are birds that prefer to hide in the grass or brush and only fly when seriously provoked (flushed). Therefore, the function of setters is to range widely and locate the birds by scent. Once located, the dog will freeze and point (set) at where the birds are hiding. The point or set is characteristic. The dog will stare intently at where the bird is hiding, and the stance will be rigid with head raised and tail held out and level with the back.

The numbers of largely red and white Irish setters engaged in field activities decreased during the 19th century with the decline of estates, estate-owned kennels, and estate hunting. In contrast, the number of mostly red Irish setters exhibited in agricultural festivals, shows and homes as pets increased. Fortunately, a small number of people actively preserved the original working Irish red and white setters during the 19th century. These breeders included the Rossmore family of County Monaghan in Ireland, who had a strain of red and white setters purportedly dating back to the mid-8th century. These and other early bloodlines were also sustained by the Reverend Mahon of Castlegar, Yelverton O'Keefe, Maurice Nugent O'Connor and Miss Lidwell (Ledwich). Dogs from these kennels were all known for their working abilities.

WWI was another time of great hardship and the numbers of working Irish white and red setters declined to the point of extinction. The Rev. Noble Huston of Ballynahinch, County Down and a small number of undocumented breeders in the distant countryside's were credited with once again saving the breed. Rev. Huston with the aid of his cousin Dr. Elliot were able to slowly bring back the breed. Rev. Huston did not keep official pedigrees but did document his litters in the parish register. The next important person in this breed restoration was Mrs. Will Cuddy in 1940, who nursed an Irish red and white setter female puppy back to health and subsequently became an influential supporter of the breed. This puppy became known as Judith Cunningham of Knockalla and is included in the pedigree of every contemporary Irish red and white setter. The Irish Red and White Setter Society was formed in Ireland 1944. Irish breeders slowly built up the numbers between the end of WWII and early 1980's and more and more dogs went to England and other countries. The Irish Kennel Club approached the Irish Red Setter Club during 1976 to ask if it was willing to help oversee the revival of the Irish Red and White Setter. With the help of the Irish Red and White Setter Field & Show Society, which was formed in 1981, and others, the breed became firmly re-established and has received national and international recognition. Irish Red and White Setters bred by the Gormleys under their kennel name of 'Meudon' proved influential worldwide as their Red and Whites were exported to the UK, Italy, America, and Holland (3). All present-day registered Irish Red and White Setters are the descendants of the dogs accepted by the Irish Kennel Club at the time of the revival of the breed in the 1970s.

Irish red and white setters came to the United States and Canada in the 1960's, mainly as pets, and active breeding occurred in the 1980's. It is noteworthy that their cousins, the Irish setter, came to North America a half century earlier. Around 500 Irish red and white setters are currently found in the USA and Canada and the Canadian Kennel club fully recognized the breed in 1999. The Irish red and white setter association was formed in America in 1997 and and the American Kennel Club recognized the Irish red and white setter 2009 as its 159th breed. The US Field Dog Stud Book also recognizes the breed.

B. Irish setter (Irish red setter)

The solid red and red and white Irish setters were considered the same breed until the end of the nineteenth century. However, a group of Irish enthusiasts created an Irish Red Setter Club in 1882 and a breed standard in 1886 to promote the "showier" red dogs in difference to the more "athletic" red and white type. The red type dogs were preferred in shows, which brought this particular type to the attention of the public and increased use as pets. Thereafter, solid red colored dogs predominated in shows (bench, conformation), while the red and white color remained the preferred variety for field trials and hunts. 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 lost popularity, purportedly from excessive inbreeding. However, British breeders ultimately reversed the trend and dogs of both great beauty and health were bred. Irish setters became popular in America in the nineteenth century and were one of the most popular companion and show breeds at one time. The Irish setter was recognized by the AKC in 1878 In the sporting group and currently ranks 76 of 193 breeds in popularity.

II. Characteristics of Irish-type setters

A. Physical characteristics

Artificial selection has changed the basic look of the show vs. work lines over the last century or more. Show lines became more uniform and refined (flashier, racier) in appearance (11). It is interesting to note parallels with the phenotypic evolution of show vs. performance English setters (4).

The differences that exist today between Irish setters and Irish red and white setters are mainly phenotypic and relatively minor (11). The contemporary performance type Red and White Setter remain much the same as its ancestors. The head is broader and more doomed with a prominent occiput and fairly square muzzle and longer, leaner and more oval shaped than in the Irish setter. The eyes tend to be round in the Irish red and white setter and almond shaped in the Irish setter. The coat is non-curled, short and flat with long silky fringes ('feathering) on the outside of the ears, neck, chest, down the back of the front legs, under the belly, on the back legs, and down the tail. The body coat of contemporary Irish red and white setters is pearly white with solid patches of chestnut red (not light-red or ginger). The face, feet, front and lower hind legs are white, but some red flecking not extending to other areas of the body is allowed. The FCI and AKC give desired heights that are similar in the Irish red and white setter and Irish setter at 22.5 to 24 inches (57 to 61 cm) in females and 24.5 to 26 inches (62 to 66 cm) in males, although the Irish setter tends to be slightly taller. 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).

B. Temperament of Irish-type setters

The Irish setter and Irish red and white setter both make devoted and affectionate companions to owners and families and make wonderful pets to those willing to give them the extra time required. They do best in active families with many outlets for their high energy and require space to run freely and to be walked. Setters are good natured and get along well with children and other dogs, although Irish red and white setters are often described as being calmer and less stubborn than Irish setters.

III. Genetic studies of contemporary Irish Red and White 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 41 Irish red and white setter tested to date. 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 two loci (AHTk253, REN64E19) occurred in 80% and 87% of the dogs, respectively. These alleles are virtually fixed in Irish red and white settlers and have been inherited by descent from a single founder or founder line that strongly embodied the breed standard.

Table 1a. Allele designation and frequency at 33 STR loci for 41 Irish red and white setters. The allele(s) that that contribute disproportionately to the breed are highlighted.

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
98 (0.28)	131 (0.01)	117 (0.40)	219 (0.09)	240 (0.37)	87 (0.05)
100 (0.12)	137 (0.13)	121 (0.02)	225 (0.32)	246 (0.15)	89 (0.38)
102 (0.17)	143 (0.37)	127 (0.30)	233 (0.13)	252 (0.22)	91 (0.20)
104 (0.15)	145 (0.06)	129 (0.05)	235 (0.05)	254 (0.26)	93 (0.17)
106 (0.15)	147 (0.34)	131 (0.22)	237 (0.16)	256 (0.01)	95 (0.21)
108 (0.01)	149 (0.09)		241 (0.26)		
110 (0.09)					
112 (0.04)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
286 (0.16)	116 (0.72)	132 (0.23)	148 (0.06)	230 (0.01)	95 (0.57)
288 (0.04)	118 (0.01)	136 (0.23)	152 (0.07)	234 (0.28)	97 (0.09)
292 (0.80)	120 (0.10)	144 (0.01)	156 (0.01)	236 (0.06)	99 (0.04)
	124 (0.16)	148 (0.20)	164 (0.09)	238 (0.38)	101 (0.30)
	126 (0.01)	152 (0.30)	168 (0.60)	240 (0.01)	
		156 (0.02)	172 (0.07)	242 (0.23)	
			176 (0.10)	246 (0.02)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
110 (0.06)	144 (0.24)	210 (0.35)	85 (0.17)	231 (0.02)	202 (0.68)
124 (0.30)	146 (0.30)	212 (0.05)	95 (0.66)	233 (0.04)	206 (0.13)
126 (0.39)	150 (0.44)	214 (0.56)	97 (0.17)	235 (0.44)	208 (0.18)
128 (0.24)	154 (0.01)	218 (0.04)		239 (0.09)	
				241 (0.41)	

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
212 (0.72)	162 (0.51)	268 (0.22)	222 (0.30)	145 (0.87)	13 (0.04)
216 (0.27)	164 (0.35)	270 (0.02)	232 (0.22)	149 (0.04)	14 (0.38)
218 (0.01)	168 (0.12)	272 (0.29)	234 (0.17)	153 (0.10)	18.2 (0.07)
	170 (0.01)	276 (0.12)	238 (0.30)		22.2 (0.10)
		278 (0.34)			23.2 (0.01)
					24.2 (0.11)
					25.2 (0.29)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
14 (0.13)	8 (0.01)	14 (0.02)	19 (0.32)	13 (0.18)	15 (0.34)
15 (0.38)	9 (0.51)	15 (0.28)	20 (0.21)	14 (0.82)	16 (0.37)
15.1 (0.01)	13 (0.11)	16 (0.07)	22 (0.48)		18 (0.18)
16 (0.01)	14 (0.07)	19 (0.09)			19 (0.11)

18.1 (0.01)	15 (0.18)	21 (0.05)
19.1 (0.22)	17 (0.07)	22 (0.20)
20.1 (0.04)	18 (0.02)	28 (0.02)
21.1 (0.13)	20 (0.01)	30 (0.26)
22.1 (0.06)		31 (0.01)

VGL2918	VGL3008	VGL3235
12 (0.22)	15 (0.20)	12 (0.04)
13 (0.02)	16 (0.06)	13 (0.04)
14 (0.06)	17 (0.50)	14 (0.48)
15 (0.54)	18 (0.16)	15 (0.45)
17.3 (0.12)	19 (0.01)	
23.3 (0.04)	20 (0.04)	
	21 (0.04)	

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 41 Irish red and white setters tested ranged from 2 to 9 per locus and the percent of known alleles occurring at each locus ranged from 14% to 71% (average 34.8%) (Table 1b). Therefore, approximately one-third of known genetic diversity for these 33 loci has been retained within the breed or two-thirds has been lost, during breed evolution. Breed evolution also includes the decades or centuries prior to actual registry creation during which their abilities as "setters" was subjected to increasing positive artificial 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 red and white setter tested.

Locus	**Known Alleles for all dogs**	**% known alleles in IRWS**
AHT121	24	33%
AHT137	17	35%
AHTH130	20	25%
AHTh171-A	14	42%
AHTh260	28	18%
AHTk211	7	71%
AHTk253	11	27%
C22.279	13	38%
FH2001	17	35%
FH2054	23	30%
FH2848	24	29%
INRA21	15	27%
INU005	14	21%
INU030	15	27%
INU055	11	45%

LEI004	15	20%
REN105L03	22	23%
REN162C04	14	21%
REN169D01	14	36%
REN169O18	14	29%
REN247M23	11	45%
AvREN54P11	14	29%
REN64E19	12	25%
VGL0760	26	27%
VGL0910	27	33%
VGL1063	17	47%
VGL1165	23	39%
VGL1828	22	14%
VGL2009	12	25%
VGL2409	13	38%
VGL2918	19	32%
VGL3008	18	39%
VGL3235	13	31%

Average 34.8%

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 and white setters (Table 2). The 41 dogs that were initially tested had a mean number of alleles (Na) per locus of 5.09 across all 33 genomic STR loci. The average number of alleles per locus was low compared to many larger breeds such as the Golden Retriever (Na=8.23), Labrador retriever (Na=7.27) and Italian greyhound (Na=7.12). The Flat coated retriever is similar with an Na=5.70 alleles/locus and higher than the Swedish Vallhund (Na=4.67). The mean effective alleles (Ne) (i.e., alleles contributing most to heterogeneity) per locus was 3.06. 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. Summary of Standard Genetic Assessment for 41 Irish red and white setters using 33 STR loci. (Updated January 14, 2019)

	N	Na	Ne	*Ho*	*He*	*F*
Mean	41	5.09	3.06	0.619	0.627	0.013
SE		0.32	0.18	0.027	0.025	0.015

The mean observed heterozygosity (*Ho*) was 0.62, which was nearly identical to the expected heterozygosity (*He*) of 0.63. *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 41 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 41 Irish red and white setters was 0.013, indicating 1.3% more homozygosity than what would have been expected if parent selection was entirely indeed random (i.e., at HWE). These findings for 41 individuals are encouraging, if they accurately reflect the breed as a whole, as they indicate an active attempt to seek out and use the most unrelated sires and dams.

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 41 Irish red and white setters ranged from a low of 2 to a high of 9 alleles/locus, while the *Ne* ranged from 1.48 to 5.77. The *F* value calculated from *Ho* and *He* was greater than +0.10 for 4/33 loci and lower than -0.10 for five (loci highlighted). Individuals with alleles that are 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 influence of more homozygous (i.e., inbred) alleles is balanced by the influence of more heterozygous (i.e., outbred) alleles.

Table 3. Standard Genetic Assessment for Irish red and white 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**	41	8	5.737	0.829	0.826	-0.004
2	**AHT137**	41	6	3.577	0.683	0.72	0.052
3	**AHTH130**	41	5	3.267	0.756	0.694	-0.09
4	**AHTh171-A**	41	6	4.568	0.756	0.781	0.032
5	**AHTh260**	41	5	3.715	0.805	0.731	-0.101
6	**AHTk211**	41	5	3.914	0.732	0.744	0.017
7	**AHTk253**	41	3	1.483	0.366	0.326	-0.123
8	**C22.279**	41	5	1.809	0.415	0.447	0.073
9	**FH2001**	41	6	4.182	0.756	0.761	0.006
10	**FH2054**	41	7	2.574	0.488	0.612	0.202
11	**FH2848**	41	7	3.573	0.707	0.72	0.018

12	**INRA21**	41	4	2.325	0.585	0.57	-0.027
13	**INU005**	41	4	3.242	0.683	0.692	0.012
14	**INU030**	41	4	2.896	0.634	0.655	0.031
15	**INU055**	41	4	2.255	0.512	0.557	0.08
16	**LEI004**	41	3	2.033	0.463	0.508	0.088
17	**REN105L03**	41	5	2.675	0.585	0.626	0.065
18	**REN162C04**	41	3	1.931	0.488	0.482	-0.012
19	**REN169D01**	41	3	1.695	0.415	0.41	-0.011
20	**REN169O18**	41	4	2.485	0.585	0.598	0.02
21	**REN247M23**	41	5	3.761	0.61	0.734	0.169
22	**REN54P11**	41	4	3.799	0.707	0.737	0.04
23	**REN64E19**	41	3	1.315	0.22	0.239	0.083
24	**VGL0760**	41	7	3.891	0.732	0.743	0.015
25	**VGL0910**	41	9	4.299	0.756	0.767	0.015
26	**VGL1063**	41	8	3.13	0.61	0.681	0.104
27	**VGL1165**	41	9	5.033	0.756	0.801	0.056
28	**VGL1828**	41	3	2.705	0.683	0.63	-0.084
29	**VGL2009**	41	2	1.426	0.268	0.299	0.102
30	**VGL2409**	41	4	3.379	0.805	0.704	-0.143
31	**VGL2918**	41	6	2.804	0.61	0.643	0.052
32	**VGL3008**	41	7	3.127	0.805	0.68	-0.183
33	**VGL3235**	41	4	2.312	0.634	0.568	-0.117

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 proportions of individuals (coordinate 1 and 2 in this case). Figure 1 is a PCoA plot of the 41 Irish red and white setters that shows them clustering as a single breed, as would be expected. However, individuals are not tightly clustered around the central X/Y axis but are spread at some distance from each other across the graph. This suggests that this group of dogs is genetically diverse, and this diversity may reflect phenotypic differences as well. A group of nine dogs is clustered as outliers in the upper right quadrant and probably represent closely related dogs from the same kennel or bloodline. The low level of relatedness shown by this plot is good because it indicates that these 41 dogs are likely to be representative of the entire breed.

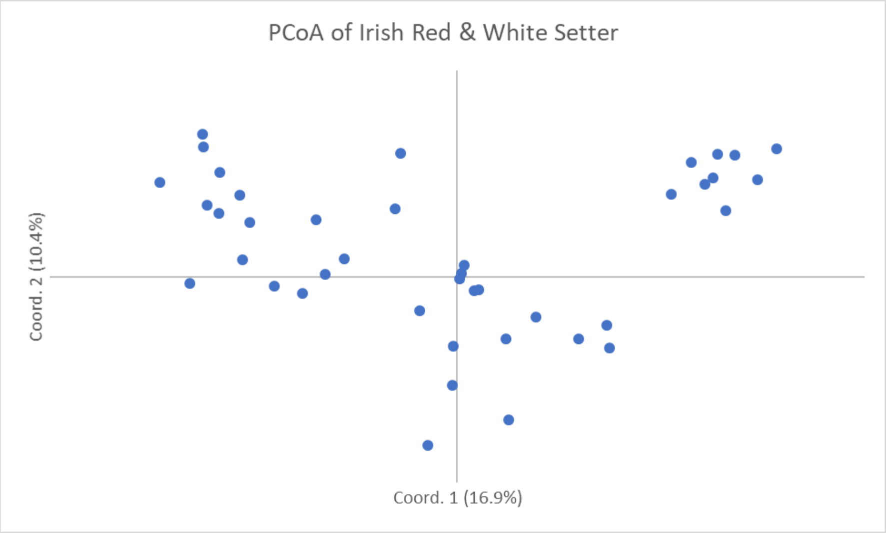


Figure 1. PCoA plot showing the genetic relationship of 41 Irish red and white setters

The genetic relatedness of Irish red, white setters with the Irish setter was compared in Fig. 2. A third breed, the Alaskan klee kai was added in order to enhance the relationship of the two Irish-type setter breeds. The three groups of dogs segregated in this plot as unrelated breeds even with an outlier breed to bring them together. However, both Irish-type setter breeds segregated closer to each other than to the Alaskan klee kai and on the same side of the Y-axis. As expected, the comparison with different breeds caused the relationship of individual Irish red and white setters to become closer than shown in Fig. 1.

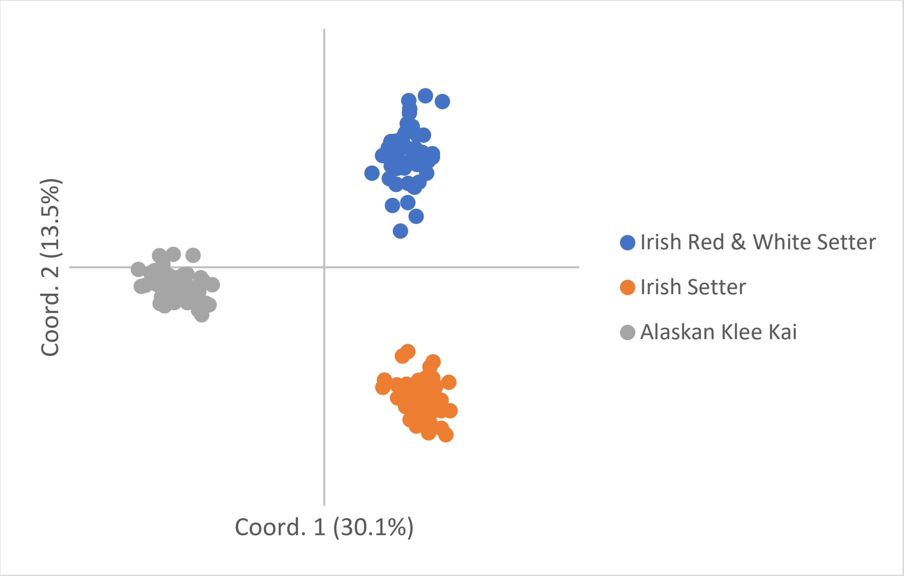


Figure 2. PCoA plot comparing the genetic relationship of the Irish red and white setter with the Irish setter.

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 genetic diversity of individuals within the population. 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.

The IR values can be broken down into mean (average), minimum (least related parents), maximum (most related parents) and quartiles (Table 4), or the values for individual dogs can be graphed to form a curve ranging from -1.0 to +1.0 (Fig. 3). 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 (IRVD) values for Irish setter (n=50) and Irish red and white setters (n=41)

	IR	IRVD
Min	-0.224	0.015
1st Qu	-0.084	0.019
Mean	0.014	0.275
Median	-0.009	0.204
3rd Qu	0.103	0.316
Max	0.338	0.542

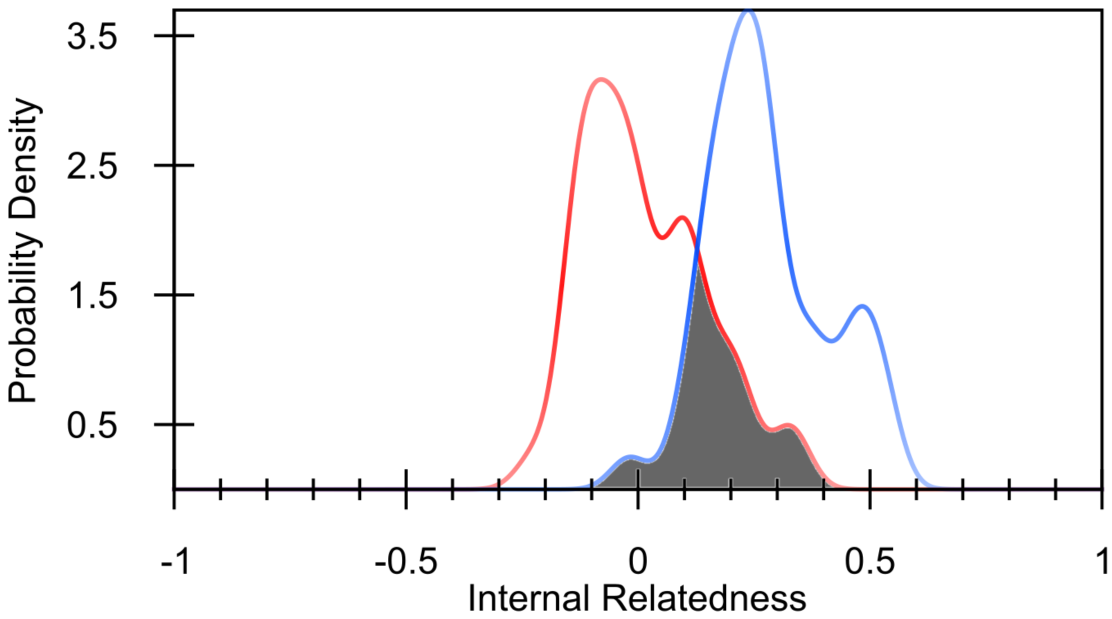


Fig. 3. IR vs IRVD graph for Irish red and white setters (n=41). The IR graph (redline) graphs the IR scores of individual dogs, while the IRVD adjusts the IR scores to indicate the IR scores of these dogs if they were to be compared against village dogs. The darkened area that is formed by the two overlapping curves approximates how much known canid genetic diversity still exists in the group of Irish red and white setters that were tested.

The mean IR score for all of the 41 Irish red and white setters tested was of +0.014 (Table 4, Fig. 3). This meant that one-half of the dogs had IR values from +0.014 to -0.224 (most heterogeneous, most outbred), and one half from +0.014 to +0.338 (least heterogeneous, most inbred dogs). One quarter of the population had IR scores that ranged from +0.103 to +0.339. Therefore, at least 10 dogs in the population tested were significantly inbred with some dogs being inbred to the level of offspring of full sibling parents (+0.250) or greater. This inbred population was balanced with an equal number of dogs with IR scores as low as -0.084 to -0.224. This balancing effect of highly outbred and inbred dogs is why the average *Ho, He* and *F* values based on breed-wide allele frequency data from the 33 genomic STR loci were misleading.

The IR curve for the Irish red and white setters was actually made up of two major overlapping peaks of decreasing size. This type of graph is a strong indication of what is called population substructure, i.e., smaller populations within the whole population that are more related to each other than to the population as a whole. Substructure is most often due to inadequate numbers of dogs tested or from testing groups of dogs from distinctly different bloodlines or geographic origin. Substructure in this group of 41 dogs can also be shown from the DLA class I and II haplotypes.

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 values are an estimate of how Irish red and white setters would rank in genetic diversity if they were compared to village dogs rather than to each other.

The IRVD curve for Irish red and white setters was shifted well to the right (Table 4, Fig. 3), and the area of overlap between IR and IRVD curves was 30.8%, thus reflecting a 69.2% loss (or 30.8% retention) of known canine genetic diversity during breed development (Fig. 3). This 30.8% % estimate of retained genetic diversity is comparable to the 35.8% estimate of retained genetic diversity obtained from known vs. observed alleles and their frequencies presented in Table 1a. This level of retained genetic diversity is lower than genetically diverse breeds such as the Toy Poodle (60%), Labrador Retriever (54%), Golden Retriever (50%), and Alaskan Klee Kai (50%); similar to the Samoyed (35%), Flat Coated Retriever (35.2%), and Shiba Inu (29.8%); and higher than the Shiloh shepherd (27%), 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 red and white 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 red and white setters in this study possessed 7 DLA class I and 6 DLA class II haplotypes (Table 5). Four DLA class I haplotypes (1006, 1175, 1201, 1202) appear to be unique to Irish red and white setters. The incidence of most of the haplotypes was ≤15%. However, one DLA class I (1008) and one DLA class II (2052) are found in 60% and 49% of individuals, respectively. These two haplotypes are in linkage, forming an even more extended 1008/2052 haplotype. More DLA class I and II haplotypes are likely to be identified as more dogs are tested, but their incidence will likely be low.

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 1008 DLA class I haplotype played a disproportionate role to the Irish red and white setters but a minor contributor to the Irish setter. Conversely, the 1014 DLA class I haplotype played a major role in the creation of the Irish setter breed, while this haplotype was present at a low level in the Irish red and white setter.

The existing DLA class I and II haplotypes in Irish red and white setters make-up 7/205=3.4% and 6/112=5.4%, 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 (35.8%) and from the retained genetic diversity calculated by the IR/IRVD (30.4%) 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

A number of DLA class I and II haplotypes found in Irish red and white setters were shared with a number of common dog breeds in addition to the Irish setter (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 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	--	0.061	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

It appears from the comparative incidence of various DLA class I and II haplotypes that certain types dominate over others, giving the impression that dogs with certain DLA types are under positive selection over others. This is true for the initial founding population for all dog breeds and perhaps for several generations afterward. However, this initial period of strong artificial selection for the founding population is almost always followed by a period of sustained random selection. During this secondary period of random selection, the genetic imbalances in the founding population come into balance. The only reason for a genetic imbalance to be maintained in the DLA region, would be positive selection (inbreeding) for a particular trait or traits that are inadvertently linked to certain DLA haplotypes. If such inadvertent positive selection is responsible for the perceived imbalance in DLA types, it should be apparent from allele frequencies at each of the seven STR loci that define the DLA class I and II types (Table 7). These allele frequencies can then be used to do a standard genetic assessment of heterozygosity (Table 8).

Table 7. Standard Genetic Assessment of individual STR loci within the DLA region of Irish red and white setters (n=41) based on alleles and their frequencies. The first four STR loci are linked to the DLA class I region and the last three STR markers to the DLA class II region.

#	Locus	N	Na	Ne	Ho	He	F
1	**DLA I-3CCA**	41	6	2.534	0.634	0.605	-0.048
2	**DLA I-4ACA**	41	4	1.72	0.341	0.419	0.184
3	**DLA I-4BCT**	41	5	1.947	0.439	0.486	0.097
4	**DLA1131**	41	6	2.518	0.61	0.603	-0.011
5	**5ACA**	41	4	2.396	0.585	0.583	-0.005
6	**5ACT**	41	5	3.015	0.732	0.668	-0.095
7	**5BCA**	41	2	1.076	0.073	0.07	-0.038

Table 8. Summary of Standard Genetic Assessment for Irish Red & White Setter using 7 STRs in the DLA region

	N	Na	Ne	Ho	He	F
Mean	41	4.571	2.172	0.488	0.491	0.012
SE		0.489	0.224	0.078	0.071	0.034

The average standard heterozygosity values for the seven DLA-associated STR loci (Table 7) parallel those from the 33 more genome-wide autosomal STR loci shown in Tables 1a and 2. This indicated that breeders have not deliberately or inadvertently selected certain dogs for breeding based on their DLA types. Therefore, the present imbalance in DLA types occurred at the time the breed was officially created, and the registry closed, accurately reflects the makeup of the founding dogs.

IV. What does DNA-based genetic testing tell us about contemporary Irish red and white setters

It is not possible based on 41 individual dogs to make definitive conclusions, but the results of initial testing do provide important insights. This initial population of dogs 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. It can be difficult to identify least-related mates that are accessible when needed.

The breed appears to have average genetic diversity compared to other breeds, having retained an estimated 35% of the autosomal STR alleles known to currently exist among all dogs. However, the number of founders or closely related founder lines present in contemporary Irish red and white setters is small based on the percentage of known canid DLA class I and II haplotypes (i.e., 3-5%). The lack of genetic diversity in the breed has been confirmed from pedigrees, with an effective population size of 39.4 individuals (10).

The lack of founders in contemporary Irish red and white setters may have occurred at the time the first registered dogs were selected or from artificial genetic bottlenecks that have occurred since the registry was closed. 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 its documented history. Sudden increases in popularity can also lead to rapid changes in the genetics of a population (breed). A large rise in registrations of Irish red and white setters by The Kennel Club occurred between 1980 and 1995 (25 to 220 dogs, respectively) (10). This was associated with an increased use of popular sires and a rise in COI from 0.05 to 0.15 between 1980 and 1995. The numbers of registrations fell to a stable level around 120 dogs from 2000-2014 with the average COI remaining around 0.15. The author of the study stated that genetic diversity had been lost during this period, although more correctly it was a decrease in heterogeneity and increase in homogeneity. The only way to determine whether this event decreased genetic diversity across the breed would be to have accurate pedigrees back to the founders of a representative number of dogs and/or through DNA testing.

A low genetic diversity, if confirmed, is not in itself serious. If, as it appears, founder dogs were relatively free of lifespan shortening deleterious genetic polymorphisms and random breeding was strictly adhered to during the breed's post-registration history, the health of the breed can be sustained with the present course. However, a low degree of genetic diversity makes it much more difficult to avoid the problems that might occur as a breed changes from performance to conformation and from relatively unknown to very popular. The problems that arise when breeds go from performance to conformation have been well documented (6). Performance breeds vary more in appearance (phenotypes) and variation in phenotypes reflects more different genotypes, and more genotypes relates to more genetic diversity. Performance traits are also less heritable than conformation traits, which helps to resist popular sire and popular bloodline effects. Booms and busts in breed popularity can also encourage poor breeding practices and bouts of inbreeding (7, 10). The deleterious effects of breeding to an extremely popular show line has been documented for the Standard Poodle (8), as has the effect of a popular sire for Italian greyhounds (9).

The lack of genetic diversity makes it easier to inbreed certain litters and harder to avoid the repercussions of artificial genetic bottlenecks. It will be more and not less difficult to safely and effectively introduce new genetic diversity, should that be a future objective, in a population that lacks genetic diversity than a population that has retained a reasonable amount of genetic diversity.

V. Health problems of Irish red and white setters

A. Overview

Irish red and white setters are generally healthy, and the expected lifespan is around 10–14 years, with some dogs living to age 16 years (5). Breeders have been proactive in maintaining health in the breed by putting strict requirements on registration. The UK Kennel Club established the Assured Breeders Scheme (ABS) in 2004. ABS members are required to adhere to additional criteria than those necessary for basic Kennel Club registration, starting with with a requirement for the parents of each litter to be identifiable by either Microchip, Tattoo or DNA profile. ABS breeders are currently required to physically screen for eye disorders and do DNA testing for von Willebrands disease (vWD) and canine leukocyte adhesion deficiency (CLAD). Screening for hip dysplasia is also encouraged.

An extensive survey of health problems in the Irish red and white setter was conducted in 2013 and published in 2014 (5). One hundred and two dogs were from the UK, 162 from the USA, and 136 from other countries mainly in Continental Europe. Reproductive problems, skeletal problems, intestinal disorders and cancer were highest on the list. Reproductive problems included false pregnancies, pyometra, need for Caesarean section, infertility, cryptorchidism, and abortion/stillbirths. Skeletal disorders included kinked tails in puppies, hip dysplasia, disc disease, elbow dysplasia, and anterior cruciate rupture. Gastrointestinal disorders included food allergies/intolerance and bloat. Cancers most often involved mammary glands, hemangiosarcoma, mast cell sarcoma, soft tissue sarcomas, osteosarcomas, gastric neoplasia and pituitary tumors. Immunologic disorders include thyroiditis; allergies to food, fleas, grasses; panosteitis; and autoimmune hemolytic anemia. Ocular disorders included posterior polar cataracts, distichiasis, progressive retinal atrophy, iris papilloma, iris cysts, and cherry eye. These types of disorders are common to dogs whether pure- or random-bred.

B. Hereditary concerns

There are four known diseases in the breed which are currently monitored by the breed clubs (5,6).

1. Canine Leucocyte Adhesion Deficiency (CLAD) – a failure of the immune system to fight infection. 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. The Kennel Club has only registered Irish Red and White Setters that are proven clear of the CLAD mutation, either by direct DNA testing or by virtue of having parents that are proven to be clear of the CLAD mutation (i.e. hereditarily clear). No carriers can be registered. Breeders have made a concerted effort to reduce the incidence of CLAD by testing for the mutation and not registering, and therefore breeding, carriers.

2. 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 Red and White 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.

3. 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 (6). The disorder in Irish red and white setters is uncommon and can appear at any time up to eight years of age and does not cause blindness (6). The cause of PPC in Irish red and white 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.

4. Hip and elbow dysplasia is a common problem in many dog breeds and the genetic basis is complex. The incidence in the breed is low but must be constantly monitored by certified radiographic studies of the elbows and hips.

C. Outcross program

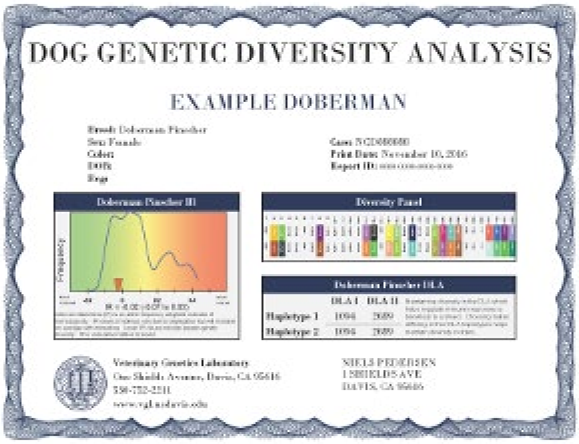
The Irish Kennel Club (IKC) announced a plan in 2011 to increase genetic diversity in Irish red and white setters by allowing Irish Red and White Setters to be crossed with Irish Setters that can still perform in the field. Outcross matings were to be carefully monitored. The reasons for such outcrossing are obvious - Irish Setters were presumed to possess genetic diversity no longer found in Irish red and white setter and phenotypes were 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 breed clubs have embraced limited use of such outcrossing. Genetic studies done on Irish red and white setter and Irish setter support such outcrossing, as the two types of dogs were obviously selected over the last century from an earlier and larger pool of original dogs, 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.

The benefit of using working-type Irish setters is that much less backcrossing would be required to re-establish the phenotype. However, little is known about the genetic diversity that exists in this small sub-population of working, red-colored Irish setters. The present study provides this type of information for show-type Irish setters found in the US. It appears that the show Irish setters have about 5% less genetic diversity in the 33 autosomal STR markers and about 10% less in the DLA region than Irish red and white setters that were tested. This is in line with previous studies comparing performance and conformation breeds (4). The fact that only about one-third of the DLA class I and II haplotypes are shared between the two breeds is a good finding, as it means that each of the two breeds has a lot of genetic diversity that can add to the other. This cross-infusion of genetic diversity across these breeds should be based on actual DNA-based genotypes. The introduction of new genotypes will also have to be closely monitored to assure that it is comingled as efficiently as possible with existing genotypes and so that the introduction of new genetic diversity is not done at the expense of existing genetic diversity.

VI. 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

Breed: Doberman Pinscher
Sex: Female
Color:
DOB:
Reg:

Print Date: November 10, 2016

Doberman Pinscher IR

Diversity Panel

Doberman Pinscher DLA

	DLA I	DLA II
Haplotype 1	1094	2089
Haplotype 2	1094	2089

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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 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. <u>However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates.</u> 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

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