

## Determining the Usefulness of Homozygosity Tools for Airedale Breeders

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A basic understanding of genetics and animal breeding tells us that, by definition, a “breed” is a group of related animals with similar genetic characteristics. Since these animals are genetically similar, they will have many genes in common. If any particular gene, at a particular location on one chromosome is the same as the gene on its sister chromosome, the gene is homozygous. If the gene on the sister chromosome is different, it is heterozygous. Over the course of the creation of a breed, the tendency is to increase homozygosity reducing the variation within the gene pool. This is great for creating similarity in type and the development of a breed. However, variation within the gene pool has substantial genetic advantages in areas unrelated to breed type. For example, many health issues are believed to have genetic components.

A simple example of health issues related to genetics is the classic Mendelian inheritance of certain recessive genes that lead to illness, like cerebellar ataxia. In this example, a dog that is homozygous for the recessive gene (aa), is affected by the illness, but one that is heterozygous (Aa) or homozygous for the dominant, disease-free gene (AA) is not affected by disease. These simple Mendelian patterns are fairly easy to select against as a breeder when we can identify who has a copy of the disease gene. Unfortunately, there are many genetic diseases for which we still have not identified the causative gene mutation and for many of those, they are likely to be complicated by involving multiple genes (polygenic) and/or other factors (environment, development, etc).

We don't know as much about the genetics of such polygenic diseases at this point. So as breeders, we must identify tools that allow us to select for the desired breed type, but still allow us to include as much genetic variation as we can in our breeding programs and our breed. Genetic research tools have advanced dramatically in recent years and the costs of such tools has become significantly more economical. One such tool is called a single nucleotide polymorphism (SNP), referred to as a “SNIP” in slang vernacular. SNPs are markers that at various locations (segments of DNA) within a genome, are known to vary. This is a very useful tool for genetic research and potentially for dog breeders. [[http://www.wisdompanel.com/why\\_test\\_your\\_dog/faqs/#33](http://www.wisdompanel.com/why_test_your_dog/faqs/#33) ; De La Vega et. al, *Nature Genetics* (2008), 40 (5), 491-492.]

The MARS Veterinary company has developed a panel of markers at locations all over the dog genome. They have found that breeds have specific identifying patterns of these markers, with each breed's pattern being unique. But there is still some variation in the markers that an individual dog within the breed can have compared to other individuals in the same breed. They have also found that some breeds have less variation (heterozygosity) within the breed than other breeds. [Rat terriers-Terriers](#) and [American Bulldogs](#) don't have as fixed a type as a breed, so not surprisingly they have more heterozygosity within their breeds.

Airedales have relatively little variation at these markers, with an average homozygosity of 78%, which is the 5<sup>th</sup> highest of all in over 180 breeds tested. Since we are dealing with so many genetic unknowns with associated unintended consequences, when breeding purebred dogs, it is best to minimize genetic constriction.

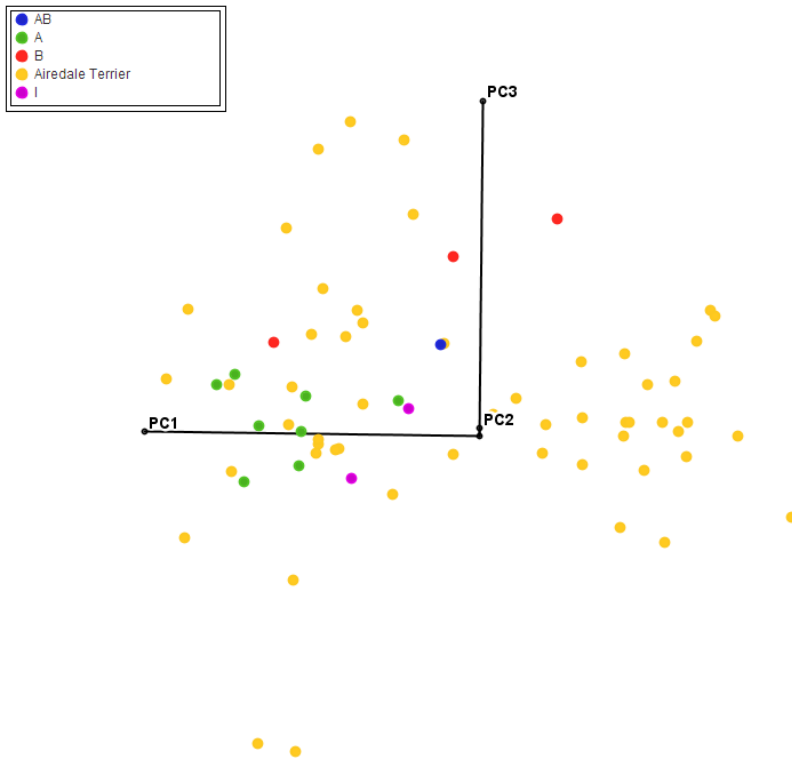
The Airedale Health Foundation initiated a test of the Mars Veterinary system in Airedales to determine the usefulness of this genetic tool for Airedale breeders. Fifteen dogs were selected from two

prominent American kennels, along with two imported dogs. -Kennel B has tightly line bred for nearly three decades. Kennel A has more variation of pedigrees with many prominent stud dogs represented. One import was Russian, with no dogs in common with any other tested dog in the first five generations of pedigree. The second import was half Russian pedigree, half American pedigree, with the American portion representing a third American kennel. Few dogs were common between the pedigrees of the three American kennels.

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## Results

**Graph 1. Principle Component Analysis of Airedales** from Kennel A, Kennel B, International Kennel I, and a breeding resulting from a mating between a Kennel A Airedale and a Kennel B Airedale (AB).



**Graph 2. COI versus %Homozygosity for all Airedales in study:**



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**Table 1. Data Points for Graph 2:**

	COI	% HZ
A1	10.88	82
A2	9.31	75
A3	9.31	75
A4	15.66	77
A5	14.43	76
A6	9.35	76
A7	9.49	71
A8	7.60	69
A9	15.66	79
B1	21.06	79
B2	19.09	78
B3	7.88	77
I1	6.57	76
I2	5.56	77
AB1	7.31	73

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Litter mates with the same pedigree can have quite a bit of variation in genetic content. -We had two examples of litter mates included in the samples. Sibling pair one, A2 and A3, did happen to have the same level of homozygosity at 75%, however, this will not always be the case. Note that their COIs are identical since they have the same pedigree. As each dog represents a different combination of their parents' DNA, there can be quite a bit of variation in litter mates at particular locations in the genome. Differences at the genetic level do occur between the two siblings in this case. The haplotypes represented in each dog varied quite a bit, and their principle component analysis had them at opposite sides of the central area of an Airedale cluster. Principle component analysis allows analysis of the genetic relatedness of dogs and how similar they are overall. The point with these two is that they are related pedigree-wise, but not genetically the same. This is a very important point. In the other sibling comparison, A9 and A4, one is 77% homozygous, the other is 79%, but their genetic similarity is quite high based on both the principle component analysis and the particular haplotypes they happen to have. These two dogs are from a breeding with a COI of 15.66.

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Line breeding does not necessarily mean different from the mainstream. The dog B1 that illustrates this point is from a line that has been tightly line bred in a textbook manner for 25 years. His COI is 21.06, quite high. However, his homozygosity is 79%, not dramatically different from the breed average of 78%. Principle component analysis shows his overall genetic relatedness is quite similar to the mainstream of Airedales. Two key lessons come from this example. *Firstly, line breeding does not automatically mean genetic restriction. Secondly, this genetic tool makes line breeding safer.* You can now tell if you are inadvertently selecting for a narrowing genetic pool with your breeding selections. The breeder of this dog could easily have been doing so, but was lucky in this case to have maintained typical breed homozygosity levels. Through selection and the use of this tool, the breeder may be able to increase heterozygosity while continuing to line breed for the phenotypic traits that have been pursued for 25 years.

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Low homozygosity does not mean different from the mainstream. Dog A8 illustrates this point. She is 69% homozygous, with a COI of 7.6. Yet her principle component analysis is fairly close to mainstream, meaning she is genetically similar to most of the dogs tested. This makes sense, she is the ancestor of quite a few of the dogs. Anecdotally, she is 14 years old and going strong, as are at least two of her litter mates.

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Imported dogs with different pedigree components do not mean different from the mainstream. Dogs I1 and I2 are imports to the United States. The pedigree of I2 is quite dissimilar to any pedigrees in the current analysis. -Dog I1 has an American sire, but that sire is not closely related to any other dogs in this study. The COI of both dogs are relatively low at 6.57 and 5.56, but their homozygosity levels are 76% and 77%, still not that far from the breed averages. Plus, their principle component analysis is not far from the central area of most of the other dogs in the study. *So these two dogs, imported to add genetic diversity, may in fact not be that diverse from the dogs of the kennel that imported them.* Ironically, Kennel A dogs in our analysis are more genetically different from the imported dogs, and for that kennel, these dogs might add genetic diversity.

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Coefficient of inbreeding and genetic homozygosity are not the same. COI is a pedigree analysis designed to predict genetic diversity. Since there is no information in a pedigree about specific genes or genetic combinations and any given breeding can produce a wide variety of combinations, COI is not of great predictive value for the level of homozygosity of a particular dog. Graph 2 illustrates this point. -Notice there isn't a strong correlation between COI and % homozygosity, which demonstrates

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that genetic relatedness between dogs depends entirely on what the offspring actually inherits. Genetic diversity is not revealed through COI study.

It is important as breeders to understand the value of the term “genetically different”. Dog B3 is an excellent demonstration of “genetically different”. Her pedigree starts with a line bred dog from Kennel B that was bred to an outside dog from a more popular strain. The resulting offspring was bred to a stud from yet another kennel that has maintained relatively isolated genetics. Dog B3 was the result. She has a relatively low COI of 7.88, but her homozygosity is 77%, not far from breed average. However, her principle component analysis shows her as far from the mainstream of the dogs studied in this analysis. This makes her particularly valuable as a breeding dog. This dog was bred to dog A3 (homozygosity of 75%) and produced dog AB, with a COI of 7.31. Dog AB has a homozygosity score of 73%, well below the breed average. Plus, the principle component analysis shows this dog outside the central cluster of dogs in the analysis. Effectively, this means that, as intended with the breeding, there has been an increase in genetic diversity for the involved lines of Airedales.

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## Conclusion

Pedigree analysis would have led to the conclusion that certain dogs would have the greatest heterozygosity and/or the greatest variation from the mainstream of Airedales. This was not the result. Dogs expected to be far from the mainstream of Airedale genetics, like the Russian import dogs, had homozygosity patterns right in the mainstream. A breeder using this dog would assume, based on pedigree analysis, that they were increasing the genetic variation of their line. In fact, little variation is added with this dog. In the counter example, a dog thought to be a “mainstream” American-bred dog had the greatest heterozygosity and the greatest variation from the mainstream.

This study illustrates that the real, hardcore data of true genetic information is far more valuable than theoretical information that pedigree analysis provides.

How do geneticists recommend this tool to be used?

- 1) Select for type just as you have always done.
- 2) Screen for health concerns, as you have always done.
- 3) Use genetic testing to determine carrier status of breeding dogs to avoid breeding carrier to carrier, as these tests become available.
- 4) Use homozygosity analysis to preserve genetic variation within your breeding program and within the breed.

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## Future Application Possibilities with Homozygosity Analysis

Breeds with considerably high average % homozygosity levels may take steps to maintain the best genetic diversity possible using this method of analysis. As an example, analysis of over 200 Dandie Dinmont Terriers in the U.S. demonstrated that within this breed, some chromosomes have a large degree of heterozygosity and others have lower diversity which may be related to traits that breeders have “fixed” in the breed (e.g. chondrodysplasia, coat texture/length). Additionally, the breed has an average observed SNP panel homozygosity of 74% (range of 60% - 86%). The breeders then had the option to utilize the Optimal Selection database to evaluate and compare potential breedings. In the first one and a half years, Optimal Selection was used in planning 7 litters with 30 puppies born (average 4.28/litter, range 1-6). All inseminations were properly timed based on progesterone levels

and used fresh (2), fresh chilled (2), and frozen (3) semen. It can be difficult to obtain historical birth rates, however puppy AKC registration rates are available for comparison. Twenty-six Optimal Selection puppies survived and were registered (average 3.71/litter) which compares favorably to the historical breed average of approximately 2.75 registered pups/litter and is statistically significantly higher than the 2010 average of only 2.11/litter (38 registered in 18 litters). Nineteen puppies from four Optimal Selection litters have also been genetically evaluated and have shown an overall decrease in their average homozygosity compared to their parents. Additionally, semen parameters are available for 9 collections of 7 males who have been evaluated with Optimal Selection. Decreasing individual homozygosity shows a moderately positive trend for post-thaw percent motility and a mild positive trend for percent morphologically normal sperm. Additional frozen semen samples will be collected to better evaluate these trends. These results suggest better male fertility may be associated with greater individual dog genetic diversity as has been observed in several other species.

Limited diversity within and across dogs may have an impact on the breed's overall health and reproductive well-being. Thus, Optimal Selection was created to help breeders capture, understand, and leverage the genetic diversity within their breed to maintain, and perhaps increase, allelic variety in future generations. Based on this preliminary data in the Dandie Dinmont Terriers, using Optimal Selection in litter planning is increasing the puppies/litter average compared to recent breed statistics, may positively impact the average puppy homozygosity, and lower levels of homozygosity may improve post-thaw semen motility.

While Optimal Selection should not be the only means of determining a desirable mating, the diversity of the individuals should be included as a factor to maintain the genetic health of the entire breed. Optimal Selection is now commercially available for over 150 breeds.