Genetic Diversity

Jerold S Bell DVM, Dept. of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University (This article was presented at the 2021 AKC Canine Health Foundation National Parent Club Canine Health Conference. Reprinted with kind permission)

Genetic diversity is a concept that is universally embraced as necessary in the evolution and maintenance of dog breeds. What is the meaning of genetic diversity? How is it measured or determined? What are the methods and consequences of gene pool manipulation to achieve and maintain genetic diversity?

Genetic diversity is important because it allows for variability within a breed's gene pool. **Genetic variability is important in selection because if there is no variation for a particular trait or disease, then there can be no improvement through selective breeding**. Genetic improvement requires genetic variability between dogs.

Some people concerned with genetic diversity recommend preventing homozygosity (the pairing of "like" genes). This recommendation derives from the Species Survival Plan (SSP) rescue programs designed for endangered species. The basis for this recommendation is to breed the least related individuals together to prevent the homozygosity of all disease-related genes. Commercial genetic testing companies can easily compute homozygosity measurements from DNA samples and promote them as genetic diversity panels; reported as inbreeding coefficients (ICs). These DNA derived ICs are correlated with deep pedigree-based ICs.

What does homozygosity indicate, and what does breeding for heterozygosity (the pairing of "unlike" genes) achieve in dog breeding? To understand these questions, we need to understand the genetic differences between species and dog breeds.

Genetic differences between species and dog breeds.

The obvious difference between species and breeds is natural versus artificial selection. Natural selection in a species always selects for fitness and reproductive traits in a natural environment. Natural species are maintained if they can thrive and reproduce. Artificial selection which is used to create breeds is toward any conformational, behavioral and health characteristics that are being selected for, and away from those being selected against. Artificial selection is hopefully positive towards genes for quality and health. However, artificial selection can also directly select for genes and traits that are detrimental to health and fitness. Selection for extreme conformation is an example.

The process of speciation, the continued evolution of a species, causes divergence in the population or subpopulation. This divergence causes a loss of genetic diversity and creates

unique population (gene pool) structure. These changes are not detrimental to the population if they continue to improve the fitness of the species. The same must be accepted for dog breed populations. They should be allowed to change and evolve if those changes allow for increased fitness (quality and health) and the ability to reproduce. There are plenty of undesirable traits and diseases that breeds strive to lose, and their loss causes a loss of genetic diversity.

There are many examples of natural species with very limited genetic diversity and high levels of homozygosity with no negative health or reproductive consequences. Some of these are common species, like the Northern Elephant Seal.1 Others are geographically isolated species, like Sable Island Horses2 or Channel Island Foxes.3 Population genetics calculations suggest that these populations have lost their genetic diversity due to homozygosity and will eventually go extinct. However in reality, these populations are robust and expanding because deleterious genes are not at a high frequency. This is not to say that homozygosity should be a goal of breeding**. It does show that homozygosity by itself does not cause disease and poor health, and is not necessarily deleterious to a population.** What is deleterious is the accumulation of disease-associated genes.

Natural selection requires large populations and genetic drift to improve species. With artificial selection, breeds do not require a large population size for genetic improvement. Few dog breeds fulfill the population thresholds determined for natural species to be able to survive**. However, few breeds exhibit inbreeding depression requiring SSP-like rescue programs**. Most dog breeds are robust, and only require continued reproduction and selection for quality and health. Breeds with small populations look like populous breeds did earlier and just need proper selection and population expansion.

What is homozygosity, and what does it tell you?

Homozygosity is the pairing of "like" genes in gene pairs. All genes come in pairs – one from the sire and one from the dam. If the sire and dam share a common ancestor, then the same genes can be passed down through both parents and pair up in the offspring. The effect of homozygosity is that it causes uniform expression (I.e., trait, characteristic, or disease) in all individuals inheriting the homozygous gene pair. There are positive genes that you want to select for (and create homozygosity), as well as deleterious or disease-causing genes that you want to select against.

To understand what homozygosity measurements represent, we must understand how homozygosity purposefully develops in a breed. Purebred dog breeds were created through artificial selection for specific tasks or traits. Through constant selection towards these breeding goals, breed characteristics reproduce uniformly through generations.

For a breed to reproduce uniformly, it requires homozygosity of genes. The genes that cause mammals to be mammals are homozygous, the genes that cause dogs to be dogs are homozygous, and the genes that cause a Gordon Setter to be a Gordon Setter are homozygous.

It does not take intense linebreeding to create homozygosity. Constant selection for certain traits will increase the frequency and homozygosity of their causative genes. Creating homozygosity of genes for desirable traits and against disease-associated genes is the measurable result of selective breeding. Mars Wisdom Panel computations show that mixed-breed dogs have on average 53% homozygosity and purebred dogs 63% homozygosity. This increase in homozygosity is not deleterious to breeds unless it causes increased expression of genetic disease.

Endangered species survival is based solely on producing viable offspring. This underscores the importance of SSP programs to prevent the homozygous expression of disease-associated recessive genes. Published metadata from Mars show that mixed-breed dogs carry statistically higher frequencies of 152 testable disease-associated genes than the combined tested purebred dog populations.4 It is the population diversity of mixed breed dogs that reduces the expression of these recessive diseases. Linebreeding in mixed breed dogs would be expected to produce more recessive genetic disease than it does in purebred dogs. (Common complexly inherited genetic diseases are seen routinely in mixed-breed dogs.) Selection for health occurs in purebred dog matings. Selection for health diminishes the frequencies of disease associated genes and increases the homozygosity of health related genes.

Diversity Breeding

Diversity breeding enthusiasts recommend SSP-type mating plans and only outbreeding (matings between dogs less related than the average in the population). What does outbreeding do to breed genetic diversity? If you take a group of dogs and only breed them to the least related in the group, you will have lower homozygosity. If you take the same group of dogs and do linebreedings (matings between dogs more related than the average in the population) you will have higher homozygosity. Have you changed the population or the genetic diversity of the breed? No. It is the same group of dogs with the same genes. Breeding for heterozygosity does not improve or change genetic diversity. It only masks the expression of recessive or additive genes; both positive and deleterious.

Does breeding for heterozygosity improve breed health? Embark studied data from the Morris Animal Foundation Golden Retriever Lifetime Study and found that on average, a 10% increase in inbreeding coefficient of the mother 2 (not the litter IC, which was not

studied) decreased litter size by 1 puppy.5 This puppy loss would be expected to be the result of homozygosity of embryologically fatal recessive genes.

Every breed and breed family has different frequencies of deleterious recessive and additive genes in their background. The effects of linebreeding are going to be different in each situation. If a breed or family shows higher frequency of genetic disease with linebreeding, then more intense outbreeding and purposeful selection against those specific diseases is necessary to diminish the causative gene frequencies. If deleterious genes causing breed-related disease are old and dispersed in the gene pool, then those diseases are just as likely to be expressed with outbreeding. Direct selection against those diseases is the only way to reduce their incidence.

Some advocate for heterozygosity of major histocompatibility complex (MHC) genes that regulate the immune system. However, all peer-reviewed published studies on immune-related, immune-mediated, and auto-immune diseases identify specific MHC liability genes, and not general MHC homozygosity or diversity.6-9

Breed genetic diversity involves selecting individuals for breeding from the breadth of the gene pool, not the types of matings that they are involved in. With an expanding breed population, the average relationship (IC based on a set number of generations) between individuals in one generation will be lower than in the previous generation. This is why (in the absence of popular sire effect or other diversity limiting parameters) generational inbreeding coefficients over time go down in well managed breeds. However, the breeders of these breeds are all doing different types of matings (outbreedings, linebreedings, etc.) based on their needs and their selection preferences to improve the health and quality of their dogs.

Diversity breeder enthusiasts look at the graph of a breed's average ICs over time and say, “Well if decreasing average ICs represent a healthy breed then why not just plan matings with lower ICs?” It sounds reasonable.

However, the impact of everyone outbreeding causes the homogenization of breeds, so differences between "lines" disappear. If outbreeding between the two most unrelated dogs, their offspring make those lines related. The next mating must be to a dog unrelated to the two original lines and now these three lines are related in the offspring. Continued matings in additional generations to unrelated dogs becomes more difficult as dogs become homogenized and related to each other. If everyone outbreeds, it disrupts the ancestral pedigree structure of breeds that was based on selection. It removes the genetic differences between dogs that are necessary for genetic improvement through selective breeding.

Outbreeding proponents state that molecularly identified low frequency gene variants and genetic markers should be selected for and increased in breeds (without knowing what the associated genes code for). It is more likely that those low frequency markers are the result of generations of selection against specific undesirable traits and diseases.

Heterozygosity should not be a selected goal. **Heterozygosity and homozygosity measurements are tools and not goals.** They can be utilized in different situations to bring in novel genes and traits, or to create uniformity of existing genes and traits. Increased homozygosity should also not be a breeding goal. Inbreeding coefficients should only increase due to purposeful linebreeding for quality and health.

Homozygosity measurements are not a measurement of individual or population health or vitality. The only way to measure breed health is through breed health surveys that document clinical disease and reproduction parameters. Homozygosity is not inherently correlated to impaired genetic health and does not need to be artificially controlled. Managing breeds requires breed conservation efforts, not species survival plans.10

Practical aspects of gene pool diversity

Based on AKC statistics, on average only 10.4% (for populous breeds) to 13.9% (for smaller population breeds) of dogs within a breed reproduce to create the next generation of dogs. This represents a genetic bottleneck with each generation in every purebred dog population. It emphasizes the fact that breeders must utilize the breadth of the gene pool background in selecting dogs for breeding, and judiciously select dogs with the best health and quality.

Genetic diversity also exists in dogs from the same breed on different continents. Molecular genetic studies show that breed subpopulations diverge and can be differentiated, even though all members of the breed descended from the same breed founders. While there may be subtle differences in selection for conformation between continents/kennel clubs, this genetic diversity can be utilized in matings.

Frozen semen from quality dogs several generations back are another source of genetic diversity. Many breed clubs have created club-owned frozen semen repositories for breeders who do not wish to retain semen or continue to pay for their storage. Knowledge of the dog's health and qualities are important in their use. DNA testing can be performed on a semen sample.

Having a stable or expanding breed population size is important to maintain genetic diversity. Diminishing breed population size can cause a loss of gene pool diversity. If a breeder is retiring from breeding, their line should be maintained. **New owners should be**

mentored to become health-conscious breeders to grow the population, especially in small population breeds.

Each breed has its own unique history, genetic makeup and gene pool structure that will require different efforts to improve its health and quality. **There is no simple solution (just outbreed) or one way of breeding (just linebreed) that maintains a healthy gene pool.** The most important aspect of gene pool diversity is maintaining the breadth of the breed's gene pool. Unique family lines should not be abandoned, and gene pool narrowing popular sire effects should not sideline other genetically unique male lines. The most robust breed gene pools have everyone doing something a little different. In each generation based on the particulars of the breed, if everyone practices health-conscious breeding, if some breeders are outbreeding, some linebreeding on one line, others linebreeding on another line, and there is no popular sire effect, then the health and genetic diversity of the breed is being maintained.

References 1. Abadía-Cardoso A, Freimer NB, Deiner K, Garza JC. Molecular Population Genetics of the Northern Elephant Seal Mirounga angustirostris. J Hered. 2017 Sep 1;108(6):618-627. https://pubmed.ncbi.nlm.nih.gov/28821186/ 2. Uzans AJ, Lucas Z, McLeod BA, Frasier TR. Small Ne of the Isolated and Unmanaged Horse Population on Sable Island. J Hered. 2015 Sep-Oct;106(5):660-5. https://pubmed.ncbi.nlm.nih.gov/26170253/ 3. Robinson JA, Ortega-Del Vecchyo D, Fan Z, et. al. Genomic Flatlining in the Endangered Island Fox. Curr Biol. 2016 May 9;26(9):1183-9. https://pubmed.ncbi.nlm.nih.gov/27112291/ 4. Donner J, Anderson H, Davison S, et. al. Frequency and distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs. PLoS Genet. 2018 Apr 30;14(4):e1007361. https://pubmed.ncbi.nlm.nih.gov/29708978/ 5. Chu ET, Simpson MJ, Diehl K, et. al. Inbreeding depression causes reduced fecundity in Golden Retrievers. Mamm Genome. 2019 Jun;30(5-6):166-172. https://pubmed.ncbi.nlm.nih.gov/31115595/ 6. Denyer AL, Massey JP, Davison LJ, et.al. Dog leucocyte antigen (DLA) class II haplotypes and risk of canine diabetes mellitus in specific dog breeds. Canine Med Genet. 2020 Oct 31;7(1):15. https://pubmed.ncbi.nlm.nih.gov/33292601/ (Read the discussion section) 7. Nakazawa M, Miyamae J, Okano M, et. al. Dog leukocyte antigen (DLA) class II genotypes associated with chronic enteropathy in French bulldogs and miniature dachshunds. Vet Immunol Immunopathol. 2021 Jul;237:110271. https://pubmed.ncbi.nlm.nih.gov/34044267/ 8. Gershony LC, Belanger JM, Short AD, et. al. DLA class II risk haplotypes for autoimmune diseases in the bearded collie offer insight to autoimmunity signatures across dog breeds. Canine Genet Epidemiol. 2019 Feb 15;6:2. https://pubmed.ncbi.nlm.nih.gov/30783534/ 9. Kennedy LJ, Barnes A, Short A, et. al. Canine DLA diversity: 3. Disease studies. Tissue Antigens. 2007 Apr;69 Suppl 1:292-6. https://pubmed.ncbi.nlm.nih.gov/17445220/ 10.

Sponenberg P, Beranger J & Martin A. Managing Breeds for a Secure Future: Strategies for Breeders and Breed Associations (Second Edition). 5m Publishing, Essex UK, 2017. https://www.amazon.com/Managing-Breeds-Secure-Futur