Using DNA panel testing to increase genetic fitness in dogs and cats by improving genetic diversity and limiting genetic disorders

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Abstract
Many purebred dog and cat populations have limited gene pools similar to endangered exotic species in which it is critical to consider the entire population and the individuals involved in each breeding. To make breeding decisions, breeders tend to incorporate family history, phenotype assessments, and limited genetic information such as inbreeding coefficients and specific disease or trait DNA mutation tests. Unfortunately, these resources may not provide a complete overview of an animal’s potential genetic contribution. We have used SNP marker sets to evaluate genomic diversity within individual dogs and breeds. In two separate studies, initial litters have shown decreased offspring homozygosity compared to their parents and in one intensively monitored breed, the Dandie Dinmont Terrier, mate selection incorporating genetic diversity resulted in increased litter sizes. Additionally, by creating species-specific panel-based genetic tests that can genotype over 175 different disorder and trait mutations in dogs and over 40 genetic disorders and traits in cats, breeders have new, easy to use, cost-effective tools to improve the health of their breeding programs. While genetic diversity or whether an animal carries a particular undesirable mutation should not be the only means of determining a desirable pairing, individual diversity and disease results should be included as factors in order to maintain the genetic health of the entire breeding population.

Introduction
The creation of a purebred dog breed, and to a lesser extent a pedigreed cat breed, by definition limits the gene pool by specifying which individuals may participate in breeding. A select group of individuals is chosen upon which to build the population. This closely resembles the situation faced in conservation genetics for endangered and threatened species where genetic diversity is extremely limited, and therefore closely monitored, as it is considered one of the fundamental levels of biodiversity. In general, genetic diversity, the variability of genes within a population, can impact both the health and long-term survival of that population since a decrease in diversity has been associated with reduced fitness. In dogs in particular, that reduction in fitness can be demonstrated as higher rates of puppy mortality (Van der Beek et al., 1999), decreased litter sizes (Van der Beek et al., 1999; Gresky et al., 2005; Calboli et al., 2008), increased incidence of inherited diseases (Janutta et al., 2008; Engelhardt et al., 2008; Donner et al., in preparation), and a decrease in life span (Long & Klei, 2014).

It is important to remember that the genetic diversity of a breed is constantly changing from generation to generation. Regardless of the number of genetic variants that are present in a breed at a point in time, the only ones that are important are the ones that are passed to the next generation and, as such, can affect future diversity. This is why genetic diversity is so easily lost and difficult to regain. Breed genetic diversity can be increased slowly through maintaining genetic diversity and allowing new mutations to develop over many generations or it can be increased quickly through an outcross to another breed.

Populations with low genetic diversity or a small population size are particularly at risk of suffering further losses in diversity. Small populations are more sensitive to the effects of selection and founder effects when only a few animals are used to start a new closed breeding group. These bottlenecks can prove to be particularly adverse to the genetic diversity of the population and without enough diversity or population size to overcome new challenges (e.g. emerging infectious diseases), a breed could be significantly impacted. Due to the nature of most companion animal populations which are spread
throughout the world, it is unlikely for a single environmental challenge to completely eliminate a breed, however there is the possibility to lose a breeding line or colony in a single event.

In the world of conservation genetics for endangered species, a species earns the “endangered” designation when there are fewer than 500 individuals which hinders efforts to avoid inbreeding. Furthermore, a “critically endangered” species is defined as a population with less than 50 genetically-unique individuals available to contribute to the next generation, also called the “effective population size” (N_e). This is the point where population genetic theory indicates that inbreeding depression will likely impact the health of the group. For comparison, a recent study estimating the inbreeding effective population size of ten breeds using pedigree information over approximately eight generations obtained from the United Kingdom Kennel Club found that eight of the breeds investigated – Akita Inu, Boxer, English bulldog, Chow chow, Rough collie, Golden retriever, German shepherd dog, and English springer spaniel – had effective population sizes of between 33 and 76 dogs which was much smaller than, but generally correlated to, the population sizes for each breed (Calboli et al., 2008). Thus, these eight populations would all be effectively considered critically endangered when evaluated by the parameters applied in conservation biology. It was also estimated that seven of the breeds whose pedigrees were studied lost over 90% of the founders genetic variants by the sixth generation demonstrating the severe effects of the breeding patterns used. The Golden retriever, in particular, showed a strong popular sire effect with 10% of the sires used producing more than 100 registered offspring; the next strongest popular sire effect was in the Labrador retriever with 5% (Calboli et al., 2008).

Based on data such as these, it is critical that we work with breeders and kennel clubs to assess their breeds and breeding animals to encourage the maintenance of genetic diversity and limit the impact of known genetic disorders. While pedigrees can be used to estimate inbreeding coefficients, there are limitations to consider. First, the inbreeding coefficient derived from a pedigree is specific to all of the offspring produced by a particular mating but cannot assess which genetic variants were inherited by individual offspring. Additionally, there are likely to be pedigree errors as estimates range up to approximately 10% of canine pedigrees contain an incorrect ancestor (Leroy et al., 2012) that could skew the results of an inbreeding coefficient calculation. Finally, the inbreeding coefficient calculation can be artificially under-estimated due to the “founder” generation in a pedigree being presumed to be genetically unrelated individuals as this is often not the case. Alternatively, using a genetic means of identifying the level of inbreeding within individual offspring eliminates the biases of pedigree analysis and provides information that can be compared equally across the breed population.

Modern molecular genetic tools can better assess the genetic diversity in any animal using cost-effective genome-wide genotyping of genetic markers (e.g. single nucleotide polymorphisms, SNPs) as shown in Figure 1. Such information is now no longer available only to researchers, but also to breeders and breed groups to be used in practical breeding programs. SNP-based genotype data can be used to:

- explore the typical level of genetic diversity/inbreeding in a breed by measuring heterozygosity (percentage of alleles that are different at each marker) or homozygosity (percentage of alleles that are the same at each marker)
- monitor the change in genetic diversity levels over generations
- identify which matings optimally increase genetic diversity in the offspring
- visualize genetic relationships and population substructure within the breed or population

In conjunction with the creation of genetic marker panels for diversity, it is also possible to incorporate known genetic disease and trait mutations so that potential breeding animals can be rapidly and efficiently screened to inform the breeder of any concerns prior to mate selection. This screening can be performed at any age so it can also be used to determine which offspring of a desirable mating can be retained for future inclusion in the breeding program.

Ultimately, lack of genetic diversity can risk a breed’s sustainability and therefore, we need to equip breeders with new genetic tools that can consider the entire breed population, as well as the
individuals involved in any given breeding, to preserve their breed. This study demonstrates, as a proof of concept, the use of two different SNP-based genetic panels used to calculate genetic diversity and inform breeders for mate selection in order to improve genetic diversity in the offspring and improve other measures of fitness.

**Materials and methods**

Blood, cheek swab, or semen samples were obtained from purebred dogs of various breeds and their DNA was typed on one of two custom SNP panels (Optimal Selection™ by Mars Veterinary, Vancouver, WA or MyDogDNA® Breeder by Genoscoper Laboratories, Helsinki, Finland) using either the Sequenom platform (Sequenom, Inc., San Diego, CA) or the Illumina HD Ultra platform (Illumina, Inc., San Diego, CA), respectively, following standard manufacturer protocols. Any low quality samples were discarded and retests offered. DNA data for each panel were analyzed for genetic diversity through either their percentage of homozygosity in Optimal Selection™ or heterozygosity (1-homozygosity) in MyDogDNA®. Breeder and prospective matings were scored based on the diversity that could be achieved in the expected litter. Results were then presented to breeders in the form of a Breeding Score or Genetic Health Index, respectively.

For the intensive study of the Dandie Dinmont Terrier (DDT) breed, over 250 DDTs in the U.S. (>90% of the potential breeding population) were analyzed using Optimal Selection. Breeders incorporated this genetic diversity data into their mate selection process and the resulting litters were evaluated for the number of puppies born and their genetic diversity through homozygosity levels. Statistical analyses were performed using a two-tail t-test assuming unequal variance. Their long-term health is also being monitored.

To date, a combined reference database has been built using a population of over 20,000 purebred dogs representing more than 300 breeds.

**Results**

Heterozygosity and homozygosity levels were determined for each breed analyzed, an example of which is shown in Figure 2a which illustrates the utility of and information gained by comprehensive SNP-based genotyping. As shown, on average, the evaluated Labrador retrievers are slightly more genetically diverse than purebred dogs as a whole, however, they are clearly less diverse than the mixed breed population. A particular dog’s heterozygosity can also be presented on the graph in comparison to the rest of the breed. Population substructure can also visualized on heterozygosity graphs in some cases. In Golden retrievers, a subgroup of dogs with a higher heterozygosity can be identified (Figure 2b). This group represents working line dogs, supporting the observed divergence between working and show/companion Golden retriever populations. Similar evaluation of genetic heterozygosity can be used to monitor the levels of diversity over time. As an example, the Finnish Kromfohrländer breed club has been actively outcrossing to other breeds – Tibetan Terrier, Parson Russell Terrier, and Poodle (Medium variety by the FCI size standard) – to revive the breed’s genetic diversity. The heterozygosity levels of the two subpopulations (original Kromfohrländer dogs in the left peak and the Kromfohrländer outcross population in the right peak) as shown in Figure 2c provides a concrete view of the progress that has been made in increasing the breed’s mean heterozygosity.

In the US DDT population, DNA from the resulting puppies was evaluated to determine if there was improvement in the genetic diversity. Nineteen puppies from four Optimal Selection DDT litters have been genetically evaluated and have shown an overall decrease in their average homozygosity compared to their parents, although it does not quite reach statistical significance (71.7% vs. 75.4%, P=0.053).

Specific litter data from the MyDogDNA Breeder evaluation show a similar trend towards an improvement in puppy heterozygosity compared to their dam and sire (Table). Importantly, knowledge of the litter variation and individual differences in heterozygosity between the puppies enables gene pool-maintaining selection of future breeding animals.
While it can be difficult to obtain historical birth rates for the DDT breed, the 2010 American Kennel Club registration rate for the DDT was only 2.11 pups/litter (38 registered puppies in 18 litters) and the DDT Club of America reports the historical breed average registration rate is approximately 2.75 puppies/litter (Miriam Couto, personal communication). By comparison, Optimal Selection data were available for 23 DDT matings through 2013 with 83 puppies born, indicating an average of 3.6 puppies/litter (range 1-6). The type of breeding/insemination performed varied among the matings. Natural breeding (4), artificial insemination (AI) with fresh semen (3), AI with fresh chilled semen (4), AI with frozen semen (2), surgical implantation of fresh chilled semen (1), and surgical implantation of frozen semen (9) (Figure 3) were all used to varying degrees which likely also had an impact on litter size.

Additionally, SNP-type genomic information can be used to better understand the breed’s ancestry, population substructure, and the genetic relationships of animals to one another. As an example, differences due to geography and breeding line divergence can be visualized in dog breeds as shown in Figure 4 for the Norwegian elkhound breed and in Figure 5 for the German shepherd dog breed group including the longhaired and White Swiss shepherd subpopulations.

Discussion

Limited diversity within and across breeds can have an impact on the breed’s overall health and reproductive well-being. This study has sought to demonstrate that in any litter, you will find variation in genetic diversity in the form of heterozygosity between the siblings. Thus, using these types of whole-genome assessment tools can help breeders capture, understand, and leverage the genetic diversity within their breed to maintain, and perhaps increase, allelic variety in future generations. Based on these data, using these tests in litter planning is:

- Increasing puppies/litter average compared to recent breed statistics
- Positively impacting the average puppy diversity
- Allowing breeders to observe substructure in their lines compared to the breed as a whole due to factors like geography, phenotype, and intended use (work vs show vs companion)

It is entirely possible to work with the genetic diversity present in any given population or breed to maintain the genetic diversity on any level, be it the entire breed, the breed within a certain geography, or within a single breeder’s family line and thereby have a positive impact on the fitness of the animals involved.

Additionally, the use of panel-based genetic testing for hundreds of disease and trait associated mutations, in combination with the diversity markers, can further assist breeders with appropriate mate selection. This method of testing has also been able to identify when disease mutations thought to affect only a narrow range of breeds, may in fact affect additional breeds that can be important information for breeders and pet parents to have. For example a recent study by Donner et al. (2016) examining nearly 100 disorders in approximately 7,000 purebred dogs found the presence of several disorders in dog breeds not previously known to carry the mutations. It is important to remember that not every disease mutation will be clinically relevant in every breed so additional clinical follow-up is required to establish a causal link which this study was able to do for several of the new breeds identified. This type of widespread mutation testing also allows for unbiased assessment of mutation allele frequencies in breeds and subpopulations that can be extremely informative to researchers and breeders. Similar type population studies in even larger cohorts of pure and mixed-breed dogs, as well as for cat populations, are currently underway.

Building on the increasing number of identified disease mutations that can clinically affect individual breeds, obtaining informed and understandable genetic information and counselling for veterinary professionals as well as breeders is increasingly important. Thus, it is imperative that clear information and tools are provided to assist in mate selection that makes use of all the data available or in disease diagnosis should an animal be affected by a genetic condition.
In conclusion, while genetic diversity and disease mutation results should not be the only means of determining a desirable mating, the genetic assessment of the individuals should be included as a factor to maintain the genetic health of the entire breeding population. Breed health and welfare is ultimately best promoted by a holistic view to breeding, taking all areas of information into consideration including health information from hip/eye examination, physical and behavioral characteristics, tests and trial scores if we are to ensure that our dog and cat breeds can survive and thrive for generations to come. Sustainable breeding should also involve thoughtful consideration of inherited disorders and genetic diversity and panel testing can be used to further these goals.

References


Figure 1. Transition of canine genetic testing from single mutation in the genome to genome-wide testing of thousands of mutations at once.
Figure 2. Measurement and visualization of heterozygosity levels. (a) Labrador retriever heterozygosity as compared to all pure and mixed breed dogs tested using a SNP-panel based measure of genetic diversity. A single Labrador retriever’s data are presented as an open circle on the graph. (b) Golden retrievers as compared to all pure and mixed breed dogs tested. Note the shoulder on the right side of the Golden Retriever curve representing working lines with more genetic diversity. (c) Heterozygosity of two subpopulations of Kromfohrländer dogs – purebred dogs in the left peak and the Kromfohrländer outcross population in the right peak – as compared to the entire purebred population tested (large middle peak) and the mixed breed population tested (large right peak).
Table. Average heterozygosity and range in two litters of Lagotto Romagnolo puppies compared to heterozygosity of their sire and dam.

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<td>Sire</td>
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<tbody>
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<tr>
<td>Sire</td>
<td>0.313</td>
<td></td>
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<tr>
<td>Litter average (5 pups)</td>
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Figure 3. Comparison of Optimal Selection breeding score (lower score predicts improved genetic diversity in the offspring) and litter size with insemination technique for 23 litters of Dandie Dinmont Terriers.
**Figure 4.** Principle component analysis (PCA) demonstrating geographical divergence in Norwegian Elkhounds in the Nordic countries (cluster on the left side) versus the United States (cluster on the right side). Principle component 1 (PC1) which accounts for the greatest variance between samples is plotted on the X-axis and PC2 which accounts for the second greatest variance is plotted on the Y-axis.

**Figure 5.** Principle component analysis (PCA) demonstrating the genetic relationships and substructure in the German Shepherd Dog breed group including the Longhaired variety and the White Swiss Shepherd. The German Shepherd samples are noted in light grey (left cluster) while the White Swiss Shepherd samples are noted in dark grey (right cluster) with samples from the Longhaired German Shepherd variety represented in medium grey and cover both clusters.