

The Canine Diversity Argument.

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George Carlin used to do a routine[1]: When you're driving, there are two kinds of people in the world (other than you, of course): "idiots" and "maniacs". Anyone going slower than you chose to go is an idiot, and anyone going faster is a maniac. Dog enthusiasts, from breeder to pet owner, if they are in the game for very long, will eventually reach their own comfort zone regarding health testing, inbreeding, etc. (i.e. they choose their own "speed"). They may decide to breed borderline hips, but no worse. They may decide that uncle/niece breedings are as close as they are going to breed. Unfortunately, many then decide that their own position has a moral imperative and that anyone who reaches a slightly different place along a continuum is unethical or otherwise irresponsible (i.e. a maniac or an idiot). Even if you, yourself, escape the self-indulgence of "knowing" the right weights to give the conflicting interests, there will be plenty of critics unwilling to forgo it. You are, to them, either an idiot or a maniac.

There are many incompatible demands on breeders. The focus of this article is on one of the most important dimensions—one that has only been discussed for the last 10-12 years: diversity vs. selection differential[2]. On one end of the continuum we are told to breed "only the best to the best", an attitude you see on most of the dog email lists, and usually directed toward "health". "If there is any hint of a genetic problem, I won't breed this dog". "People who breed dogs that have any suggestions of skin problems are irresponsible". "Once a dog sneezes, you are not to breed it, or any of its first order relatives". Some interpret the "best of the best" to refer to conformation and will only breed dogs that approach their idea of perfection relative to the standard. Others may breed only the very best working dogs. The more selective you are, the greater the selection differential (the difference between those you chose to breed and the overall average), and the fewer dogs you consider worthy of breeding.

On the other hand, we are also told to breed to maintain "genetic diversity", i.e. we must prevent losing alleles (differing forms of a gene) in the population. The population will be healthier, more robust, and more adaptable if we avoid the eventual increases in homozygosity that will certainly come with the loss of alleles. Further, there may come a time when we have critical need of those alleles.

Clearly, these two goals are contradictory. If we are to breed only the best to the best, we would breed only a very few females and even fewer males. Breeding only the best to the best would mean that a lot of dogs, representing considerable genetic diversity would not get bred. Clearly, if we breed for diversity, we will be breeding, not just the best, but also a lot of other dogs, just so we don't lose alleles that they may be carrying. These dogs are not the "best of the best," even with respect to health. No matter where a breeder is positioned, he or she will be open to complaints from both sides.

Diversity and homozygosity

There are actually two separate arguments usually brought by the diversity supporters, both having to do with homozygosity resulting from loss of alleles (or inbreeding). The greater the homozygosity, the greater the chance that two recessive alleles [3] will come together in an offspring, producing problems that neither of the parents show. Large, diverse, randomly bred, populations could have as many, or more, bad recessive alleles, but the probability of them meeting in the same dog would be much smaller. It is undoubtedly true that inbreeding will produce greater homozygosity and reveal recessive problems where they might otherwise be hidden.

A second potential bad result of increased homozygosity is usually referred to as “inbreeding depression”, meaning that the highly inbred (homozygous) individuals will be less robust, have weaker immune systems, have fewer litters, smaller litters, smaller, less robust puppies, and be less able to carry litters to term. Animals may carry a lot of sub-lethal (or maybe sub threshold) double recessive problems that cumulatively decrease reproductive and immune fitness. On the other hand, these traits may require particular combinations of genes (which cannot be passed on) and so suffer with increasing homozygosity. The low heritability of reproductive fitness is consistent with this view [4].

In spite of what you may read on the web, it is not true that inbreeding, per se, decreases the genetic diversity of the population [5] (i.e. causes a loss of alleles) and the two homozygosity problems above are only indirectly related to the main diversity argument. Different non-selective breeding strategies don't change the overall population frequency of alleles, they just change the relative frequency of genotypes.

For example, consider a gene with two alleles, *A* and *a*—and say, for arguments sake, that they each have a frequency of 50%. Suppose everyone starts doing littermate to littermate breedings. Eventually, all the inbred families will be homozygous, some with the *A* allele and some with the *a* allele. They will be roughly equal in number and we will not lose any alleles from the population or even change their overall frequency. So, even though all the dogs are now homozygous, in the population as a whole, both alleles are equally represented. Interestingly, this is exactly what has happened across dog breeds. There is just as much diversity now in dogs as there ever was [6] and as much as there was in the progenitor, the grey wolf [7]. Within breeds, however, there is much less diversity than there is between breeds [8]. Some breeds are fixed at some alleles and other breeds are fixed at others.

There is a way, however, that doing a lot of linebreeding or inbreeding will cause a loss of alleles. If everyone line breeds on the same dog, we will eventually be overloaded with the alleles he is carrying and lose a great many that he is not carrying. To generalize this a bit, one has to consider the main mechanism for losing alleles in small populations (and the Newfoundland is a small population—much smaller than we might think).

Genetic Drift.

Unfortunately, in a closed population (like the AKC breed studbooks) we will lose alleles over time even without inbreeding or linebreeding on anybody. Genetic drift [9] refers to the random alteration of allelic frequency by “sampling error”. The next generation results from a random draw of alleles from the current one. In extreme situations, it is always a possibility that an allele just doesn’t happen to be passed on at all in a generation and so is lost. Genetic drift is particularly significant in small populations, the smaller the population, the more rapidly alleles are lost [10]. Imagine a population, for example, with just 1 dog and 3 bitches and one of the bitches is Landseer recessive. If each bitch has 1 litter of 6, the probability that we lose the Landseer allele completely in one generation is a little over 1.5%. (It would be the probability that the Landseer recessive bitch would contribute the “solid” allele 6 times in a row). This probability seems pretty small, but don’t forget that this is happening simultaneously with many thousands of genes (almost 20,000 in the dog), some of which are rare and therefore especially vulnerable. Note-yes it is true, neither dogs (nor people) have millions and millions of genes. Humans have slightly more than 20,000, dogs slightly less [11].

There are two factors that make our population size even smaller than we might think, certainly much smaller than a simple count of all Newfoundlands. One of these, we can’t do anything about in the current AKC structure. The “effective” population size is restricted by the number of founders. Most breeds started out with a very small number of “founders” that had the characteristics being sought who were then line-bred very intensely to quickly “set type.” Even if we started breeding everybody as often as possible, we would still have a very modest effective population size because of the founder effect. We would have lost a great many alleles in those first few generations. The only way to get them back, would be to breed to something that was around prior to the bottleneck [12] or bring in a dog from a completely different population [13], both of which are impossible with a closed studbook (i.e. the only way a dog is registerable is if both its parents are).

The second factor is that the effective population size is limited by the number of males being bred [14]. Clearly, males can produce many more offspring than females and a small number of high quality males may be the only ones being bred. No matter how many females are used, a small number of males puts an absolute limit on the effective population size.

Popular Sire effect.

The popular sire effect refers to the result of one or a couple of dogs being used so much that their offspring dominate the next generation

There are two components of this effect. First, when one stud is used to a great extent, that means that others are not. Thus, even if there were no undesirable genes, you would decrease the effective population size and make the population more vulnerable to chance loss of alleles via genetic drift. Over-used stud dogs will also have the effect of

increasing homozygosity in the breed in subsequent generations as offspring are then bred to related dogs.

Second, is that in an effort to spread “good” genes throughout the population (remember-this used to be the responsible thing to do), we are also inadvertently spreading any “bad” genes around that the dog might carry. There was a great deal of concern when Cystinuria in the Newfoundland was characterized in that one of the apparent carriers was a heavily used dog [15]. Clearly, if the dog produces well, heavily used stud dogs will tend to have heavily used offspring who will in turn spread these genes further into the population. Particularly if no one linebreeds, the gene may saturate the population before anyone knows it’s there.

The dangers of homozygosity

There is actually very little explicit data regarding the harmful effects of homozygosity (usually inferred from the degree of inbreeding) on populations of purebred dogs—certainly none in Newfoundlands. A few studies suggest that more highly inbred dogs have more health problems [16], but to be honest, these are extraordinarily difficult studies to do and other characteristics of the more inbred population might account for any difference.

There is, however, one specific set of genes (and proteins) for which heterozygosity really does seem to confer an advantage and for which lack of diversity of alleles in a population is clearly dangerous. These genes and the proteins encoded by them are usually referred to as MHC, the major histocompatibility complex. They are remarkable loci, having the greatest extent of polymorphism than any other loci (e.g. as many as 50 different alleles at some loci rather than 2 or 3). Further, lack of diversity in these alleles can leave an entire population at risk to an epidemic from a novel pathogen, rather than just a subset. The bubonic plague (or possibly small pox) might have wiped out everybody in northern Europe rather than half, if it weren’t for a particular mutated allele which made some individuals resistant [16]. Lack of diversity in MHC genes may leave whole populations similarly susceptible to novel pathogens. They are absolutely central to the diversity argument and we will examine them in the future article.

The MHC complex.

The second half of this article begins by considering how the Major Histocompatibility Complex (MHC)¹ alleles are important to immune function and why homozygosity at these loci may put populations at high risk. The MHC were first identified in studies of tissue transplantation (mostly in mice). The fact that transplants within inbred strains did not undergo immune rejection while those between strains did, resulted from the fact that within an inbred strain all mice shared the same alleles, and resultant proteins, at the MHC loci.

These loci are “tightly linked”, i.e. they lie in close proximity along the chromosome and are almost always passed on to offspring as an intact group. (The combination of

alleles taken together is referred to as a haplotype). There are two main classes of MHC genes, class I and II (each of which with several genes) that have related, but distinct functions. The MHC genes are the most variable (polymorphic genes) that we know about. Rather than two or three alleles, some of these genes may have as many as 60 or 70. Together, they do two major things. First, they define “self”. They code for proteins that protrude from the cell surface. The highly complex shapes formed by the extracellular components are what determine whether the immune system recognizes something as self or “foreign”. The second function they have is to “present” antigens (molecules that evoke immune responses) to immune cells that participate in the immune response. In order to do this, they must bind to the antigen, which will usually be a molecule from a foreign cell like a bacteria, or something expressed within a self-cell, e.g. an aberrant protein formed by viral, or possibly cancer activity.

MHC homozygosity and immune response

It's the second function, binding to and presenting antigens to promote an active immune response that most directly puts a population with little diversity at risk. Some haplotypes are more favorable than others for binding particular antigens—thus a population might lack alleles that are the best at binding some antigens. Further, animals (and people) inherit one haplotype from their father and one from their mother—and both are expressed. A heterozygote, then, has a richer complement of antigen binding capability than a homozygote. High homozygosity in the MHC, in itself, does not necessarily appear to be detrimental². The fear, however, is that eventually a novel pathogen will appear to which the species will be unable to mount an immune response. The appearance of the Parvovirus in the late 70's threatened to be such a pathogen and some breeds; most notably Rottweilers³ were more susceptible than others and had a less favorable response to vaccination. The Rottweiler population had gone through a relatively recent and severe bottleneck, possibly leaving them with less population-wide MHC diversity than other breeds. This has been at least partially confirmed in studies looking specifically at MHC alleles across breeds.

Because of the apparent long-term importance of MHC diversity, it is tempting to suggest that we base our strategy to counter-act increasing homozygosity explicitly on preserving MHC alleles. This has been seriously suggested for species survival plans in conservation biology⁴, though it has not been universally accepted as focus on these alleles would allow others to go more quickly to homozygosity in small populations and most species, even highly inbred ones, maintain diversity at these loci⁵.

MHC “associations” with autoimmune disorders

To date, there has been much more information regarding specific associations between certain MHC alleles and different autoimmune-related disorders, than data on the effects of breed level homozygosity. This clearly focuses on the “self-defining” function of the MHC genes. These genes are obvious candidates for contributing to these disorders. Thus far, we have seen MHC associations with diabetes⁶, rheumatoid arthritis⁷, immune mediated hypothyroidism⁸ immune-mediated hemolytic anemia⁹, and VKH-like syndrome¹⁰. Interestingly there is strong evidence that these associations may differ in

different breeds¹¹. Apparently one thing hasn't changed. We cannot assume that a genetic test developed for one breed will be useful in a second, nor can we assume that inheritance between breeds is the same as within. Phenotypically identical diseases may involve a different gene or a different mutation in the same gene. These reports also point out some other aspects of future genetic considerations into breeding decisions. Thus far, these associations of MHC alleles with immune-related disease are all "associations". This usually means that the proportion of affected animals carrying the allele in question is "significantly greater" than that of similar controls. This will usually be expressed as an "odds ratio", a ratio meant to convey the increase in risk when the allele is present vs. when it is absent. This is a far cry from the one-to-one relationship we are used to seeing in the case of cystinuria. By and large, these odds-ratios range from around 1.4 to 2.5 (also typical in some newly discovered human breast-cancer susceptibility genes). This complicates both the task of finding the genes and of making practical use of results. The next generation of genetic tests will give us profiles of risk factors rather than simply the presence or absence of alleles of a small number of recessive disorders.

The future

Are we at the point of a "rising storm?"¹² Is the breed in desperate need of alleles from "founders" that were lost in the development of the breed. In some cases breeders have attempted to bring in genes from founding populations¹³. Bragg¹⁴, in his influential article, suggests doing the same with Siberian huskies, though he speaks of changing the entire closed studbook system. Dalmatian breeders, in an effort to recover the normal allele for uric acid metabolism, cross-bred to a pointer with the plan of repeatedly back-crossing the cross-bred offspring to registered Dalmatians—always selecting for normal uric acid levels. This was highly controversial and is a fascinating case study of breed club/AKC relations to read¹⁵.

The more conservative approaches advocated by others¹⁶ are actually things we ought to be doing anyway and are not responses to the imminent demise of the breed. We should guard against the popular sire effect, not double up on autoimmune problems, nor continue to heavily breed a dog that consistently produces offspring with such problems.

Some advocates suggest limiting the number of offspring a dog may have or mandating that dogs may not be bred with any common ancestors in 4 or 5 generations. Unfortunately, these strategies are no more than delaying tactics in a closed population that will ultimately exhaust the genetic diversity and continue to the inevitable homozygosity. A breeder's decision to outcross rather than do an uncle-niece breeding will not alter even the steepness of this path. We may be in the position of a holding action to maintain the breed until "technology" can save us. We already have techniques to insert or substitute genes even across species, e.g. "humanized" mouse strains¹⁷ in which a human form of a gene is expressed in the mouse. This might eventually be a tactic for the Dalmatian problem, i.e. a single gene that was inadvertently fixed in the formation of the breed, but it is difficult to see this as a solution for small population drift.

Meanwhile, the breed is probably at more risk from legislation, changing demographics, and increases in veterinary health-care costs than from genetic holocaust.

Questions for the future:

This article will not give definitive answers to any questions about what the NCA ought to do, if anything, to counter these threats. Instead it will pose questions that we might want to begin discussing.

1. Planning for disaster. First, should we be planning for catastrophe? Seed banks with upwards of a billion samples are stored against the time that plants may be extinct and we have need of them¹⁸. It may be reasonable to collect sperm and ova from current dogs to provide a method by which to re-establish the diversity we have now in the case of disaster. Selecting the dogs to be collected would be a daunting task, since the criteria would be preservation of current diversity rather than quality.

2. When to define our current status. Should we consider directly contracting someone to define the current diversity in our population? I suspect that we will see a decline in the use of coefficient of inbreeding in attempts to define the genetic diversity in breeds. The COI is a probabilistic statement that does not directly represent the resulting homozygosity. It will undoubtedly be supplanted by molecular techniques to assess actual, rather than theoretical, homozygosity. The state of current breeds is certainly as dependent upon the diversity of founders, bottlenecks, and distant breed history than on current coi's. The Hanoverian Hound, for example, has much greater current diversity than expected from the small population size and multiple bottlenecks¹⁹.

3. Defining our population

Third, regardless of our decision on the second question above, we should consider defining the population for which the NCA is responsible. Breed wide assessment of disease-causing genes, and more particularly any survey of genetic diversity will have to pay a great deal of attention to the population structure of the sample. NCA breeders are now breeding less than 25% of the registered Newfoundlands in this country. With few exceptions, non-NCA newfoundlands represent a separate population with only very rare immigration into the NCA show and working population. Were we to do something to assess our current diversity in MHC genes or with more general markers, do we want to include all dogs that are registered Newfoundland or only those that are in the population the NCA has some influence over? If we need alleles in the non-NCA population do we want to breed out to them and pick up alleles, which we have worked so hard, to eliminate?

4. Future genetic testing

The next generation of tests will provide much richer information, but will be a great challenge for the development of any breed-wide policy. We can hope that the club will

avoid draconian policies such as severe selection criteria on genes conferring modes elevation of risk. The well-accepted use of cystinuria carriers in breeding programs was welcome in this regard. The Danish Bedlington club was advised after a few years of banning copper toxicosis carriers that they were already losing rare alleles and would do well to reverse the decision and return to breeding carriers²⁰. By and large we should view our genetic testing as opportunities-- providing ways to confidently use dogs in ways that do not produce affecteds, rather than as further ways to eliminate dogs from the breeding pool.

Lastly—this second half of the article discusses almost exclusively avoiding disease, either in individual breeding programs or in population-wide catastrophes. Clearly one could spend their life breeding against disease and with either crude or sophisticated testing, make mating decisions by minimizing the chance of problems. What is missing is the goal of breeders to produce dogs that are uniquely Newfoundlands, who look and act and move like Newfoundlands. This is not to be lost in the drive for “health.” We do not want to lose our breed to a gradual decline in type any more than we want to lose it to disaster. The Newfoundland world could end in a “Bang or a whimper²¹”—neither is something we want to contemplate. What a tragedy it would be to lose such creatures, far more complex and awe-inspiring than any Picasso painting.

1. http://en.wikipedia.org/wiki/Major_histocompatibility_complex
2. Mikko, S., and Andersson, L. *PNAS*, 92:4239-4263, 1995.
Mikko, S., Roed, K., Schmutz, S., Andersson, L, *Immunological Review*, 167:169-178, 1999.
3. <http://www.medicine.manchester.ac.uk/cigmr/research/veterinary/caninemhc/breedimage.pdf>
4. Hughes, A.L., *Conservation Biology*, 5:249-251, 1991.
5. Sommer, S. *Frontiers in Zoology*, 2:16, 2005.
Aguillar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D., and Wayne, R.IK., *PNAS*, 101:3490-3494.
6. Kennedy, L.J., Davison, L.J., Barnes, A., Short, A.D., Short, A.D., Fretwell, N., Jones, C.A., Lee, A.C. *Tissue Antigens*, 68:467-476, 2006.
7. Ollier, W.E. Kennedy, L.J., Thomson, W., Barnes, A.N., Bell, S.C., Bennett, D., Angles, J.M., Innes, J.F., Carter, S.D. *Immunogenetics*, 53:669-673, 2001.
8. Kennedy, L.M. Quarmby, S., Happ, G.M., Barnes, A., Ramsey, I.K., Dixon, R.M., Catchpole, B., Rusbridge, C., Graham, P.A., Hillbertz, N.S., Roethel, C., Dodds, W.J., Carmichael, N.G., and Ollier, W.E.R., *Tissue Antigens*, 68:82-86, 2006.
Kennedy, L.J., Huson, H.J., Leonard, J., Angles, J.M., Fox, L.E., Wojciechowski, J.W., Yuncker, C., and Happ, G.M. *Tissue Antigens*, 67:53-56, 2006.
9. Kennedy, L.J., Barnes, A., Ollier, W.E.R., and Day, M.J. *Tissue Antigens*, 68:502-508, 2006.
10. Angles, J.M., Famula, T.R., and Pedersen, N.C., *Tissue Antigens*, 66:656-665, 2005.
11. different associations in different breeds
12. Sharp, C.A. http://www.ashgi.org/articles/immune_rising_storm.htm

- 13 <http://www.basenji.org/african/project.htm>
14. Bragg, J. <http://siriusdog.com/articles/genetic-health-dogs-bragg.htm>
15. <http://www.dalmatianheritage.com/about/links.htm>
Nash, J. http://users.nbn.net/jseltzer/dal_poin.html
- 16 . Sharp, C.A. http://www.ashgi.org/articles/immune_rising_storm.htm
17. <http://www.genoway.com/humanization.htm>
18. <http://en.wikipedia.org/wiki/Seedbank>
19. Lupke, L. and Disti, O. *Journal of Animal Breeding and Genetics*, 122:131-139, 2005.
20. Proschowsky, H.F., Olsen, J.B., Jepsen, B., and Fredholm, M. *Animal Genetics*, 34:142-145, 2003.
21. T.S. Eliot *The Hollow Men*. <http://www.cs.umbc.edu/~evans/hollow.html>