THE GENETIC CUL-DE-SAC

by Susan Thorpe-Vargas Ph.D., John Cargill MA, MBA, MS, D. Caroline Coile, Ph.D.

DOGS AS AN ENDANGERED SPECIES

Why the dog opted to share his fate with men, may never be known, we suspect it had something to do with filling his stomach, but when he did, mankind took on a moral and ethical obligation. When we started to selectively breed dogs for our own ends, our responsibility only increased. How have they done under our stewardship? We will let you and your conscience answer that, but from our perspective it seems we have "improved" Canis familaris into a genetic nightmare. We have created designer dogs which cannot whelp freely or even breathe correctly. Concern for cosmetic attributes have selected for dogs who get lost at the end of the leash. Every year billions of veterinary dollars are spent ameliorating the effects of our tampering. Is it too late? For some breeds it may indeed be too late. If they were a wild species certain breeds of dogs would be on the endangered list. That is why this series of genetic articles is so important. If you are a breeder, you need to pay your intellectual dues. Every breeder who professes to love his breed needs to know more than rudimentary genetics. At a recent genetics conference hosted by the Canine Health Foundation, author Susan Thorpe-Vargas cringed to hear "What you see is what you get" at the dinner table, from a parent club representative.

This is the first, in a reference series of six breeding-related articles by a special task force of four authors. The learning curve is apt to get steep at times and if your eyes start to glaze over then put the paper down for a bit, but it is your obligation to pick it up again. Under discussion will be such diverse subjects as the origin and domestication of the dog, a mini primer on Population Genetics, the techniques being used to discover the causes of genetic disease at the molecular level and tests currently available to breeders for genetic screening. We will be providing both general and technical information to a level one expects of a serious breeder. We hope to make this an exciting journey and if you are a breeder, a very necessary one. The authors will presume some knowledge of the subject as we will draw on previous articles published during 1996 and 1997 in Dog World. They start with A Genetic Primer for Breeders ; The Mapping of the Canine Genome ; Open Registries Promote Honesty in Breeding, , Canine Genetic Disease: is the situation changing? Part 1-4 , and Tipping the Genetic Scales , For those of you on-line, some sites will be mentioned and a glossary of genetic terminology will be available by e-mailing the authors. Words throughout this series in bold-faced type, other than headings, are included in the glossary.

Some of you may question the need for such a series and may ask yourself why it should concern you. This quote by Jay Russell Ph.D. perhaps explains that WHY far better than we can.

"Every breeder has the ability in a free society to "determine their own stopping point." But, a single breeder's actions may have consequences that are far-reaching. A breed is necessarily maintained by a society of breeders. As such, the actions of each breeder affects the actions of every breeder who dips their brush in the gene pool and every buyer -- present and future -- who buys one of these "works of art." Pragmatically (and ethically), a breeder loses his/her right to independence and his/her ability to be independent the minute he/she puts up a shingle that says "Puppies for Sale."

ORIGIN OF THE DOMESTIC DOG

About 60 million years ago a small weasel-like animal lived in the part of the world that is now called Asia. This ancestor of all modern day canids (dogs, jackals, wolves and foxes) was called Miacis, and although they did not leave any direct descendants, Cynodicis, the first true dog-like canid did

descend from them. Cynodictis appeared about 30 million years ago. This line eventually split off into two branches, one in Africa and the other in Eurasia. The Eurasian branch was called Tomarctus and is the progenitor of wolves, dogs, and foxes. Until recently, it was thought that wolves and jackals were both the ancestors of the domestic dog , but a recent paper appears to demonstrate that the wolf is the only ancestral species. This somewhat controversial paper also asserts that the first domestication of wolves, seems to have taken place about 100,000 years ago. Whether or not it happened that long ago is still in dispute as the fossil records do not support this, however, different domestication events did most likely occur from multiple populations. This makes sense as both wolves and humans coexisted over a wide geographical area and so multiple domestication opportunities would have arisen. As a hunter-gatherer, humans would have found these animals very useful, but then about 8,000 years ago humans turned to a more settled way of life. This is when severe selection for specific behaviors and traits became important and 'modern' breeding practices started. And so it begins.....

Evolution, by definition, is change and diversification over time in a species. However, if there is no genetic variability, there can be no evolution. Genetic variability is the result of naturally-occurring mutations and a genetic process called recombination.

GENE MUTATIONS

Mutations can be caused by a variety of mechanisms. Some of the most common are mistakes made when the organism's DNA is replicated prior to a cell dividing. Although there are body system safe-guards in place to prevent this from happening, nothing is fool-proof, and eventually over time, failure to replicate DNA accurately will occur. Likewise, errors can occur all along the pathway that leads to the translation of messenger RNA into a specific protein. These errors can occur spontaneously or be the result of exposure to natural and man-made mutagens. Certain chemicals can cause genetic changes or exposure to certain types of radiation. What is important to remember is that these mutations are random events with respect to their adaptive potential. In other words, they will happen independently of whether they have beneficial or harmful consequences. More often than not these mutations are harmful as they are changes to the make up of a living organism. Just how harmful depends upon the type of mutation that occurs and the environment in which they occur. Most mutations fail to thrive, reproduce or survive and thus are not passed on to successive generations.

There are several kinds of gene mutations, each having a unique range of potential effects. This is important to recognize because many genetically transmitted diseases result from a specific kind of mutation. Each of these forms of mutation is the result of the organism failing to reproduce its DNA accurately all of the time and subsequently passing these genetic changes to successive generations.

BASE-PAIR SUBSTITUTIONS

The result of this type of mutation can range from a null effect to one that has severe consequences to the affected organism. DNA is made up of four different nucleic acids: thymine (T), adenine (A), guanine (G) and cytosine (C). Thymine always pairs up with adenine and guanine always pairs up with cytosine. Hence the name base-pair. Sometimes when the DNA strand is being replicated the wrong base is inserted. This can result in a different amino acid being added to the protein being made. If the essential biological function of that protein is not changed then there is no detectable effect. However, if the substitution affects the active site of an important enzyme or changes its three dimensional shape, then it modifies the fundamental nature of the protein. If this occurs along an essential metabolic pathway the results can be disastrous.

The most unfortunate result of a base-pair substitution is when this mutation codes for a stop codon. A codon is that portion of the messenger RNA that codes for a specific amino acid. A start codon (AUG) serves rather like a capital letter indicating the start of a sentence. A stop codon is a codon that

does not specify an amino acid, and serves much as a comma or a period punctuating the genetic message. The Genetic Code is composed of sixty-four different arrangements of three nucleotides each (codons). This set of combinations codes for a total of twenty different amino acids and the stop codon. Some of the combinations code for the same amino acid and three of them signal for termination.

This redundancy is why some base-pair substitutions have no effect, because the change results in the same amino acid being produced. If, by chance, a mutation produces one of the stop codons, than the process of making the protein is terminated. This is not good.

"An example of this type of mutation is the one that leads to a form of progressive retinal atrophy (PRA) in the Irish setter. A substitution of an A for a G produces the stop codon (TAG) that replaces the normal codon for the amino acid tryptophan (TGG). This prevents a protein called PDEB (phosphodiesterase beta) from being produced in its full length form. The shortened protein is unstable and is degraded by the retinal cells in which it is needed. The lack of this protein causes the retina to degenerate, resulting in blindness in those Irish setters that have two copies of the mutant gene, and no normal copy."

FRAMESHIFT MUTATIONS

In the normal cell replication process, DNA is transcribed into messenger RNA, which in turn is translated into a series of amino acids. This always occurs in a specific manner, i.e., it always begins at a definite spot and it is 'read' in multiples of three (codon) and in a particular orientation along the length of the strand of DNA . This is called a reading frame. If there is an addition or deletion of one or two base-pairs, then the result is often a very altered sequence of amino acids in the final protein product. This is definitely not good.

"An example of this is the mutation that leads to an inherited form of anemia in Basenjis. A deletion of a single nucleotide in the 433rd codon of the gene encoding a protein called PK (pyruvate kinase) causes a shift in the reading frame. The misformed and shortened protein (a new stop codon is ultimately encountered) is unstable in the red blood cells that carry oxygen throughout the body. The lack of the PK causes the red blood cells to slowly be destroyed and results in the anemia."

SPLICE-SITE MUTATIONS

Molecular geneticists used to think that all of the DNA coding for a particular protein was continuous, that is, until they started to look at more complex organisms. What they found, in these types of cells, is that the DNA that makes up a gene is often distributed in discontinuous sections called exons, interspersed with long segments of non-coding DNA known as introns. These sections are transcribed into messenger RNA along with the exons, but before the RNA is translated into a protein they are 'edited' or 'spliced' out. A change of even a single nucleotide in one of the exons of the gene can cause a shift or alteration of the splice-site.

A genetic disease that affects Dobermans is a perfect illustration of this type of mutation. Von Willebrand disease is a bleeding disorder that effects the animals ability to form blood clots. Other breeds also have this disease, but what had perplexed those doing vWD research , was that Dobermans appeared to have a milder form of the disease. The discovery of a splice-site mutation that codes for von Willebrand factor has cleared up their mystery. George Brewer MD of the University of Michigan suggests that one use the following analogy in order to explain how the mutation functions.

Imagine that a freight train is supposed to go from point A to point B along a railroad track. Somewhere between A and B is a spot where a sidetrack goes to point C. Normally, the train never

goes to point C because the switch, that connects the two tracks, is never thrown. Then the switch is broken (the mutation) and the lock that prevents the track from connecting to point C is no longer effective. The switch can now toggle back and forth, sending some trains to point B and sometimes to point C. In affected Dobermans, the defective switch sends the train to the wrong destination and about 95% of the time, the train rumbles over the cliff and is never heard from again. (and the proper protein is never made) However, sometimes the switch jiggles the right way and the train ends up at the normal destination and the proper protein is made.

If both copies of the gene are mutated, then each gene can make the right protein about 5 to 10% of the time. Affected Dobermans are thus producing von Willebrand factor about 10 to 20 % of the time and so their symptoms are not as severe. A mystery explained...

DIVERSITY AND RECOMBINATION

In mammals, DNA is not just one continuous strand, but exists within the cell nucleus in a number of pieces of genetic material called chromatin. Before a cell divides, the chromatin collects itself up into a structure called chromosomes. Dogs have a total of 78 chromosomes, humans have 46. The total number of chromosomes is called the diploid or 2n number. The point of this division is so one member of each chromosome pair can become part of a gamete, or sex cell (egg and sperm). These gametes have half the number of total chromosomes (termed haploid, or n), so when they join together the resulting progeny will be 2n. The sire contributes 39 chromosomes and the dam another 39. They form into matching (homologous) pairs that have the same type of genes on them, but not necessarily in the same form. For instance, the gene that codes for albinism exists at the same position, on the same chromosome, in both parents.

However, one parent has the gene that produces pigmentation and the other carries the gene that produces no pigmentation. The same gene in a different form is called an allele. If the genes are of the same form then the dog is homozygous at that position. If the animal has different alleles at a certain location, then it is said to be heterozygous. In a diverse population, almost every gene has multiple forms of the same gene. This is known as genetic diversity. Another genetic process, called recombination, further adds to genetic diversity. This is how it works.

Prior to division a cell duplicates its DNA and in the process four chromosomes are produced: two sets of homologous (matching) pairs. Before the cell divides these homologous pairs line up and sometimes they swap DNA. This DNA swapping process is called recombination. If the original pair was heterozygous (not matching) at two genes, say A and A+ and B and B+, then the possible gametes formed would be AB, A+B+. A B+, and A+ B. Without recombination, if the A allele was on the same chromosome as the B allele, they would always be inherited together. In fact, such "linked" chromosomes more often than not are inherited together, because the chances of such a split and subsequent recombination decreases the less space there is between the two genes. When recombination does occur, two gametes would be parental types and two of them would be a combination of their parents. Without recombination, traits carried by genes on one chromosome would always be inherited as a group, and dogs would basically only have 39 different "gene-groups."

The take home message should be that recombination adds to the genetic diversity. This is especially important in a highly in-bred population, such as a specific dog breed.

WHAT HAPPENS WHEN WE LOSE DIVERSITY?

One of the purported purposes of breeding purebred dogs is to not only improve the breeder's own stock, but to ultimately improve the breed. The degree to which one breeder can influence the genetic direction of a breed is influenced by many factors; one of the most important is the size and diversity of the existing breed population. In the long scheme of things, individual dogs will live and die, but if

bred, their genes will live on through their progeny. Thus from an evolutionary viewpoint, a population, or breed, can be thought of as consisting of as the total number of alleles, rather than individuals, present at one time. This "gene pool" is equal in size to approximately twice the number of dogs in a population, because each dog carries two alleles per gene (except in the case of sex-linked genes). Evolution results when the relative proportions of alleles change with successive populations. The more variability that exists at one locus, the more room exists for evolutionary change. Goals of purebred dog breeders involve increasing, reducing, and preserving various gene frequencies within a population.

Although individual dogs making up the population change, total gene frequencies within the populations remain fairly constant unless four specific situations (mutation, migration, genetic drift, and non-random selection) apply. Mutation provides the foundation of genetic variability, but without the remaining three situations a single mutant allele will seldom become fixed in a population. Migration refers to the introduction of new alleles from another population, and was especially influential in early development of breeds through cross-breeding. Selection is the main tool of the breeder, who chooses which dogs will pass on their genes to the next population. Selection, plus drift, both play a part in the phenomena known as founder effect and inbreeding depression.

FOUNDER EFFECT

When a new population is established by a sample (founders) drawn from the parent population, as in the development of a new breed, the genetic make-up of the foundation stock will most likely be very different (simply by chance) from that of the original population from which it was drawn. The smaller the sample the greater the probability of difference in that the sample does not fairly represent the parent population. The genome of such a subpopulation with its limited number of founder individuals will carry the alleles of the new group rather than those of the source group. An allele that is quite rare normally in the original population might be very common is the new one, and visa a versa. This, in effect, abruptly changes the kinds of alleles represented and how often they appear. This founder effect is in essence a form of acute genetic drift (variation in gene frequency from one generation to another due to chance). The problem with losing genetic diversity is severalfold. Once lost, an allele cannot reenter the population except through mutation (unlikely) or migration (which, if a breed is considered a population, means either going back to its rootstock from its country of origin or crossing with another breed). Genetic diversity is the foundation of evolution; it may be acceptable to loose deleterious alleles due to selection, but the loss of other unknown alleles due to chance reduces the variability upon which selection can act, and thus the possibility of further evolution. Loss of genetic diversity also can result in inbreeding depression.

INBREEDING AND INBREEDING DEPRESSION:

You can't fool Mother Nature

Evolution is thought to be a gradual change in the kind and frequencies of alleles. Those mutants that are harmful are either eliminated or kept at low frequencies by natural selection. However; with artificial selection , especially when a breed is being developed, it is the individuals that exhibit the greatest expression of the desired traits that are chosen to breed the subsequent generations. When only a few dogs are used to produce the next generation, a high proportion of their genes will be in the next population of potential breeding animals. When these related dogs are then interbred, the chances of them passing on the same allele that they both received from their sire and dam is 25%. Thus, inbreeding increases the chance that subsequent offspring will carry identical copies of the same allele (be homozygous at that locus). Increasing inbreeding increases the chance of homozygosity and can lead to the loss of one of the alleles from the population.

Breeders walk a tightrope between needing to reduce genetic variation to maintain uniform breed type and needing to maintain genetic diversity to avoid inbreeding depression, which results from

homozygosity of deleterious alleles. The majority of alleles detrimental to life and reproduction tend to be recessive, for the simple reason that if they were dominant, they would have been expressed in the individual's phenotype, and that individual would have been less likely to reproduce. If recessive, only those individuals with homozygous recessive alleles would be reproductively compromised; heterozygotes would be unaffected. Every dog (and every human) carries deleterious recessive alleles, so the chances of the foundation stock carrying them is virtually 100%. If very few dogs were used as foundation stock, their progeny would have to be interbred, and in only a few generations all of the dogs would be closely related. Breeding closely related dogs is inbreeding. An inbred dog has a greater likelihood of receiving the same allele from both its sire and dam, and thus a greater likelihood of being homozygous for a deleterious trait. In an inbred population, as long as the animals can still reproduce, this homozygosity can become fixed in the population due to the chance loss of the other allele. What this means for the breeder is that too great a reliance on inbreeding will lead to loss of 'fitness', i.e., failure to reproduce. Fewer litters are produced, the number of whelps will decrease and those that are born will fail to thrive. Taken to extreme, the effective breeding population could be so diminished that the breed would face extinction.

BOTTLENECK

Modern dog breeds have all been subjected to a founder effect, and many of them have had their gene pools further reduced by subsequent genetic bottlenecks. The best-documented case of a canine bottleneck was created by World War II, when hardships in Europe made it impossible to keep many dogs, especially giant ones. The populations of giant breeds in Europe were practically decimated after the war, and some breeds had to rely upon only a few survivors or imports from less affected areas in effect, reducing the gene pool and creating a second, even more limited, foundation for the breed.

Other bottlenecks are created when a breed, for whatever reason, becomes extremely unpopular and rare, or when dogs from one country (or worse, one kennel) are used to found the breed in a new part of the world. Yet one of the most pervasive bottlenecks is brought on voluntarily by breeders: the rush to breed to only a few favored sires, the 'flavor of the month', while the majority of potential breeding males are never bred.

This bottleneck is made all the worse by the fact that the majority of breeding bitches are often sired by a handful of the last generation's "favored sons". In fact, the effective population size can never be greater than four times the number of males in a population, no matter how many breeding females exist. In certain rare breeds, their effective breeding population is thus so reduced, that they are in effect, in a genetic cul-de-sac.

CONCLUSION

We have considered the origin of the dog, how it evolved from precursors and how initially there was tremendous genetic diversity within the species. We then examined how mutations occur and contribute to that diversity. It was then necessary to introduce those factors that reduce diversity when new dog breeds are established, such as founder effect and inbreeding. Our goal was to inform breeders of the dangers inherent in common breeding practices that can exacerbate the problems to the point that viability is lost.

In Part II of this series, we will continue our discussion of some of the concepts involved with population genetics and suggest ways for preventing or correcting the problems associated with highly inbred populations. We will also introduce and clarify such molecular genetic terms as dominant, recessive, and co-dominant traits which are central and fundamental to the breeding selection process.

So fundamental are these concepts that no breeder can honestly claim to be an ethical breeder without a working understanding of the underlying principles. At a still higher level of complexity, but still of extreme importance to breeders in their selections for breeding, we will need to deal with such ideas as penetrance, overdominance and best of all, epistasis. This may seem a little daunting at first, but the health and future of our favorite companion may depend upon what we breeders do now...so hang on tight, as its apt to be a wild ride.