Can You I.D. Your Dog with DNA?

by Ray Coppinger Ph.D.

Editor's note;

The following article originally intended for owners of livestock guarding dogs, discusses a study involving several subspecies of gray wolves, coyotes, cape hunting dogs, dingos, and numerous domestic dog breeds.

The first puppies we raised were Siberian Huskies and our first customers asked that grand old question, "Aren't huskies closely related to wolves?" Being a novice instant expert I answered in the affirmative. About 12 years later when we began looking at livestock guarding dogs, we read in club newsletters that these guardian breeds are very old, and closely related to wolves. We were told the reason Komondors are so protective is because they are closely related to wolves. Konrad Lorenz once wrote a book called Man Meets Dog in which he said that the German Shepherd is closely related to wolves - and he got a Nobel Prize(no, not for that).

I have never been told by Beagle or Poodle breeders that their dogs are one step away from moose eaters, which is too bad because they are just as close to wolves as are Huskies and Komondors.

Recent studies have dealt with the relationship between various breeds and their ancestors. Interest is sparked by people who would like to be able to classify breeds into groups such as sheep dogs, hunting dogs, or working dogs of one kind or another. Others are interested in how breeds got their individual characteristics. Theories abound, such as the Mastiff being descended from large Mongolian wolves, the Husky from grey wolves, the bird dog from jackals, and the Indian dog from coyotes. Other theories suggest that dogs didn't descend from wolves at all, but rather came from an ancient wild dog, a Pariah Dog, or maybe a Dingo.

If someone could find an incontrovertible genetic marker for breeds of dog, he or she would find immediate employment in such controversies as the recent nationwide one about Pit Bull Terriers. When attacks on people by members of this breed made the news, some towns and counties in the U.S. passed laws banning Pit Bulls. But the question immediately arose, "What exactly is a Pit Bull?" Was there some characteristic, or a gene or set of genes, which would distinguish this breed from all others? When I visited the Smithsonian Institution this fall, all the "Pit Bull" skulls had been checked out as researchers measured lengths and angles in an attempt to find some diagnostic feature. If such features could be found, they would be interesting to people who work with dogs, if for nothing else than to prove ancestry. For example, in Europe, sled dog racers are required in most races to use only purebred dogs. Many racers have bought fast dogs in the U.S. and proceeded to dominate the sport in Europe. The losers cried foul, claiming that the imported dogs weren't pure but were Alaskan Huskies, a hybridized sled dog noted for its speed.

But as it turns out, so far the only way to determine if an animal is a Pit Bull or a Siberian Husky is to have papers from a registry like the A.K.C. However, relying on these data means trusting the breeder who filled out the form.

Recently, a new technique has been used to trac genealogies, which has revolutionized our ability to classify animals and decipher their relationships. Although the technique does not result in cells that are stamped "Great Pyrenees" or "Golden Retriever...pure for 10 generations", it does reveal a great deal of previously unknown information.

It is a fairly simple system to understand, although one needs a well-equipped laboratory to perform the procedure. All one has to do is get a tissue sample from an animal, extract the mitochondrial DNA (mtDNA), and then map the genes on that mtDNA molecule. If two animals have the same map, then they were related sometime in the past. The reason mtDNA is used is because it doesn't recombine with other DNA, the way nuclear DNA (the DNA in the nuclcus Of the cell) does. Remember the tall and short peas from high school biology? Those are the result of recombined, or nuclear, DNA.

Humans and other animals get their nuclear DNA from both parents; DNA from each parent combines to program the new organism. But you get mtDNA only from your mother. Your mtDNA is exactly like that of your mother and hers is just like her mother's and so on back through time. Males can not pass their mtDNA on to the next generation. It's maternal mtDNA all the way back.

To get into the biology of this a bit more, here is the story. Mitochondrial DNA is the genetic code that builds and maintains the mitochondria. Mitochondria contain enzymes that convert food into energy. They are inside the cell but outside the nucleus. In other words, they are in the cytoplasm. Female germ cells (eggs) have cytoplasm, but male germ cells (sperm) do not. Thus, you get that organ system- the mitochondria and its DNA - only from your mother.

When I said that your mtDNA is exactly like your mother's, that was probably a true statement, but once in a while - in every hundred, or thousand, or ten thousand years - a mutation occurs, probably by accident.

The longer two species have been separated, the more mutations have occurred. Therefore, if two strands of mtDNA have the same sequence with only a gene or two being different, than we are looking at the same species (or family or breed or race). But if we find lots of differences then the animals are much more distantly related.

The complicated part of mapping is the technique needed to do it. The mtDNA molecule must be extracted from the tissue and then cut into pieces, photographed, and measured, The concept is simple but takes careful work. The genes, which we refer to here by letters of the alphabet, are much too small to be seen even with the most advanced equipment. In order to look at their sequences, we need a chemical, called a cutter, to find them. The cutter is specific to a certain molecule. Whenever the cutter finds its specific gene, it breaks it. For example, if I have two animals, one with a gene sequence of ABCBAD and the other with ABCABCD, a C cutter would give AB BAD for the first and AB AB D for the second. For the first animal I'd have two pieces and for the second, three. I can further refine my map by now using a cutter for A, then B, then D. The pieces are then all photographed and measured and I get masses of data which I put in a computer programmed to tell me which samples are the same and which differ. Those animals with

A's or B's or C's in the same location on the map are the most closely related.

One of my students at Hampshire College, Paul Vrana, did this for 27 breeds of dog, plus wolves (from Mexico, Minnesota, the Northwest Territories, and the Lincoln Park zoo), western coyotes, eastern coyotes, Cape Hunting dogs from Africa, Singing Dogs from New Guinea, and an Australian Dingo. Working under the direction of Dr. Rodney Honeycutt at Harvard University, he used 15 different cutters and produced thousands of segments of mtDNA which he measured and entered the lengths into a computer. It took a year and a half. We criticized his thesis because he didn't have a very big sample size for each type of canid - nice of us after all his work, but valid nonetheless. Paul is now in graduate school at Columbia University, working at the Museum of Natural History, and gathering more data.

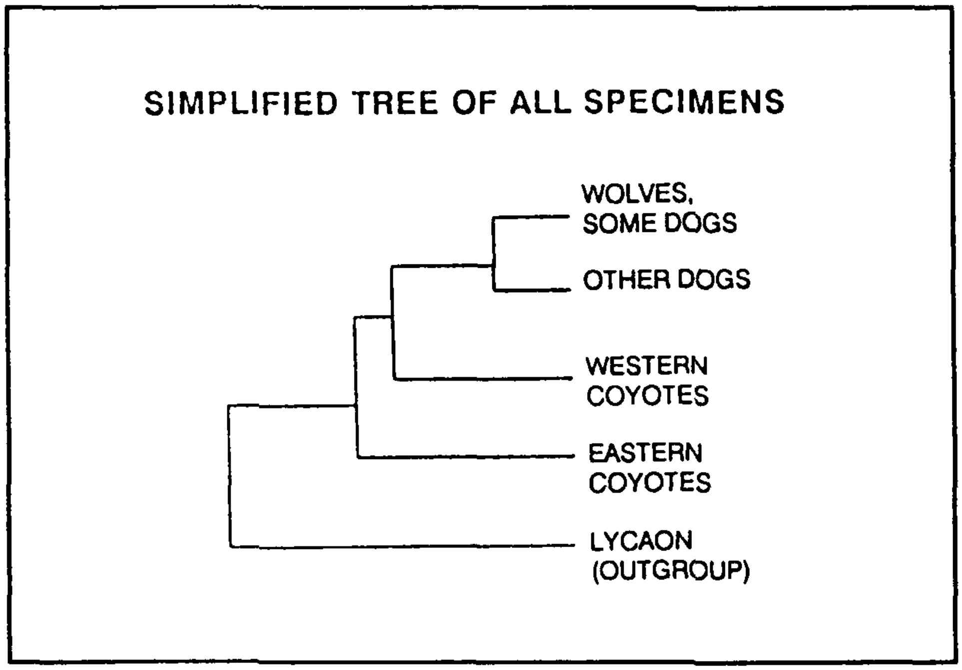
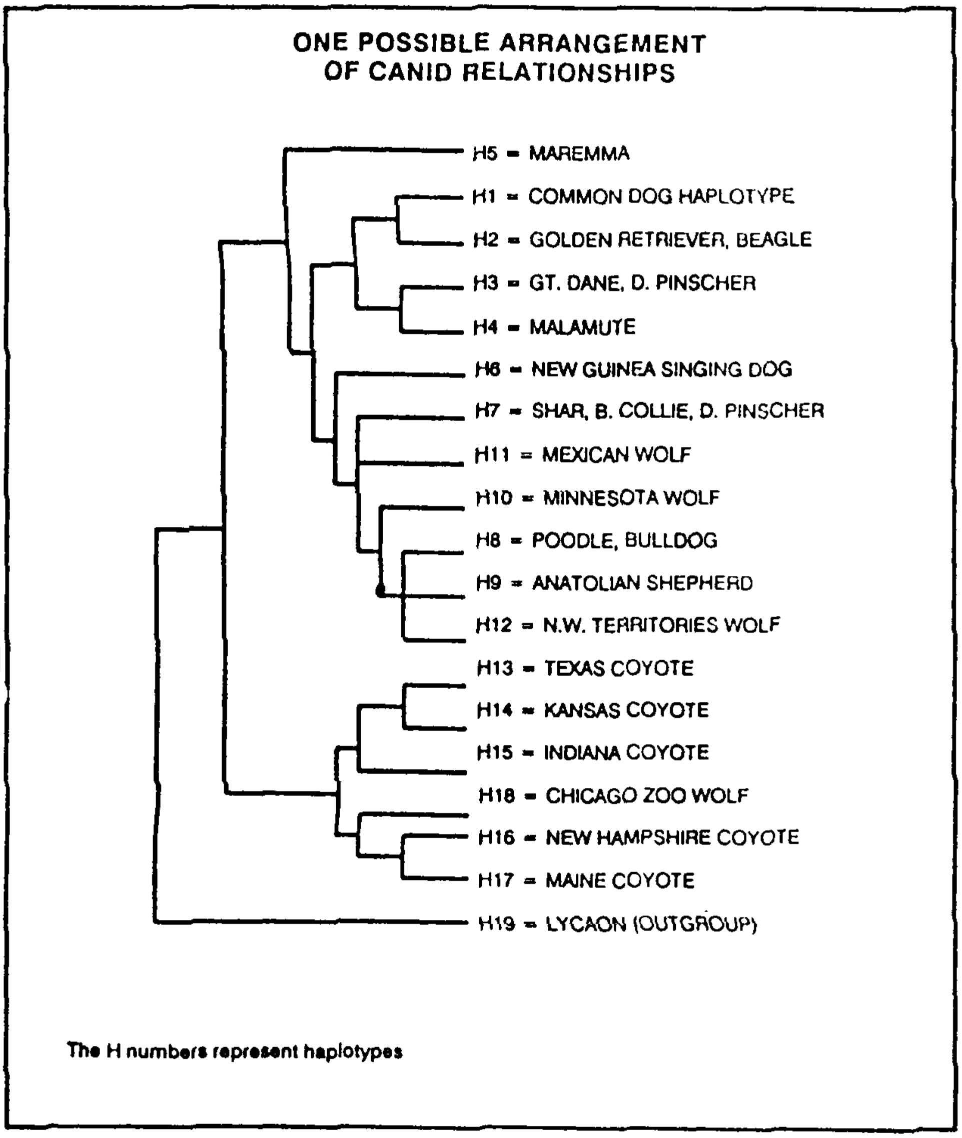


Chart 1

Chart 1 shows an overview of Paul's results about relationships of the canids in his study. That tree is "rooted", in that we know which groups, or taxa (that is, family, genus, or species), are more primitive or genetically derived than others. This is done by comparing data from animals You are interested in to an animal known to be distantly related Cape Hunting Dog (Lycaon pictus) serves as the outgroup. Lycaon is not in the genus Canis, but it is closely related, and so characters shared by the outgroup and the other taxa are primitive. The tree is constructed by mapping the fewest steps needed to get from the outgroup to the other taxa.

Most striking about the chart is that the different species of coyotes, wolves, and dogs are more closely related than is generally thought. Since these "species" are all interfertile, we are not really surprised at the results. Dr. Robert Wayne and his students at the University of california at Los Angeles have shown similar results with red and grey wolves sharing maternal ancestors with the coyote in the not too distant past. Paul says in his thesis that man's best friend is a wolf - but it looks to me like a wolf is just another breed of dog. It seems there is less difference between wolves and dogs, than dogs from each other. So far, says Vrana, there is less difference between dogs and wolves than between frogs in a single pond.


ONE POSSIBLE ARRANGEMENT OF CANID RELATIONSHIPS

The H numbers represent haplotypes

Chart 2 shows one version of the details of Chart I. The H numbers arbitrarily represent the 19 unique haplotypes (two animals have the same haplotype if they have identical patterns with every enzyme tested) found in the 59 specimens. Hl, the common dog haplotype, was shared by 21 individual dogs of 18 breeds. This means they all had a common mother a short time ago. Even the Austrailan I Dingo shares this common mtDNA genotype. You might ask if indeed we had a purebred Dingo or was it crossed with a dog. Of course, we don't know.

So which breed of dog is closest to the wolf? So far they are all in the same basket, even though Paul's map places the Maremma a little bit outside. Note that some breeds, for example the Doberman Pinscher, appear in two categories. This means they share an ancestral mother with Great Danes and also with Shar Planinetz and Border Collies. That is not hard to imagine, since Herr Doberman created this breed by crossbreeding. The chart also shows Shars and Border Collies sharing a great-grandmother sometime in the past, great Danes and Golden Retrievers with a common mother with other dogs, and all of them with an unrelated mother. Now that is news.

What this all means is that through time there has been an infusion of unrelated genes, a sharing of mothers, so to speak, between species and between breeds. The wild animals we think of as distinct from one another, the purebred dogs we appreciate for their special form and behavior are_not really unrelated. To a geneticist, the lines between the dog (Canis familiaris), the wolf (Cani lupus), or the coyote (Canis latrans) are indistinct because they are not true species, that is not reproductively isolated.

Consider for a minute the Maremma. This breed is classified in dog books with the Shar, Anatolian Shepherd, Komondor, and Great Pyrenees as a working livestock guarding dog. But this is a functional definition and has little to do with genetic ancestry. That separation of the Maremma could go back only a generation or two. The differences between the haplotypes in Chart 2 are so small that they are essentially meaningless. No real differences show.

It is the similarities that are striking. That domestic dogs and wild wolves share maternal ancestors, and that they are more closely related than even wolves and coyotes is the unexpected discovery in these data. Many people don't like these results. The people involved with the red wolf recovery program are dismayed because the animals tested show a large percentage of coyote genes. The same is true of some populations of grey wolf. If animals known as "red wolf" or "grey wolf" are actually, genetically, hybrids, then they may lose their status as endangered species. Many dog fanciers like to think their breed traces directly back to ancient originals. They see their breed on the Bayeux tapestry, created to celebrate William the Conqueror's successful invasion of England in 1066. But the data do not support these fantasies. Types or breeds of dogs are created, reshuffled, and recreated. Superficial characters like size or color or shape are not indicative of a pure genetic ancestry.

For those of us interested in working breeds the message is clear. Pedigrees do not tell us if we have a working dog. Working parents are the best indicators of puppy success. Even litters from working stock are going to show ancestral variation and not all are going to perform the guarding task equally well.

For those of us interested in the rare livestock guarding dogs the findings should be encouraging. Breeders have long been aware of the problems of inbreeding. The whole process of developing a breed is of course a kind of inbreeding. (isolation of a few genes from the, rest of the population, and using these genes over and over to produce dogs that look the same). This means that if you have isolated a few bad genes, they will show up at a higher frequency. Genes for retinal atrophy, hip dysplasia, kidney trouble, and many more are rampant in some pure breeds. But for the time being, most breeds will be healthy because they are not pure, and given the minutest chance, the neighbors mutt will maintain the genetic diversity, adding some genetic health to the loftiest blue-blood.

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