

Mapping the Alpaca Genome

By Patricia A. Craven, PhD

Why do We Need DNA Analysis?

One of the major goals of DNA analysis is to identify those alpacas which appear normal but are carrying an abnormal gene so that they can be removed from a breeding program. The focus of this article is on genes which cause disease and deformity. However, DNA analysis can also be used to track the inheritance of genes which determine fiber characteristics including those for suri and huacaya fiber. Genes are inherited in pairs, one from the sire and one from the dam. In the case of a recessive disease, the inheritance of one normal gene makes up for the presence of the abnormal gene. The individual appears normal but is still a carrier of the disease. Statistically speaking, 1 in 4 crias produced from the mating of 2 carriers will be affected by the disease, 2 of 4 crias will be carriers of the disease and only 1 of 4 crias will be completely free of the disease. Another way of looking at it is that if you have a male and a female which have produced several crias, one of which is affected by an inherited disease or abnormality, the sire, dam and all the siblings are potential carriers and should be removed from the breeding pool at a tremendous financial loss. On the other hand, if we could detect carriers of genetic diseases through DNA testing, only those carrying the abnormal gene would have to be removed.

How are Abnormal Genes Detected?

Up until the mid 1980's, identification of a gene which was responsible for causing disease could only be accomplished by first identifying the underlying cause of the disease, characterizing the deficient protein or enzyme and then isolating the gene. This was a long and laborious process. Only a small number of diseases were amenable to such an approach. With the advent of modern molecular genetics it is now possible to predict the presence of a gene responsible for a disease without having prior knowledge of the mechanism of the disease or the protein responsible. This is possible through a process called gene mapping.

What is Gene Mapping?

In the early 1990's scientists constructed a detailed map of human DNA by identifying and locating 10,000 markers which occur naturally in the structure of DNA. Like signs on a road map, a DNA marker occurs at a specific site or locus and is easy to detect. In the case of the alpaca, only approximately 80 markers have been identified to date (1-3). Our lack of knowledge about the alpaca genome is roughly equivalent to trying to find one's way from San Francisco to New York based on a single signpost as compared to one signpost every 30 miles for the human genome. A major goal for future research is to identify more markers to place on the alpaca gene map.

Each individual inherits a characteristic set of markers from their parents, just like they inherit a characteristic set of genes. For this reason, DNA markers are useful in forensics and for determining parentage. If you look at the DNA analysis that you receive from

UC Davis when you register a cria, you will find the markers listed which were used to verify its parentage. The profile of markers in blood samples from the presumed parents are screened and compared to that of the cria. If a marker is found in the cria sample which is not present in the DNA of one of the presumed parents, the sire is eliminated as a parent.

Use of Linkage to Track Heritable Defects

In addition to their use in forensics and in determining parentage, DNA markers are used to track the inheritance of abnormal genes in families. The closer a gene is to a marker the more likely it is that the gene and the marker will be passed on together to the offspring, a phenomenon scientists call linkage. Therefore genes of interest, such as those that cause disease, can be tracked by following the inheritance of the marker rather than the gene itself. The ability to track the inheritance of a disease gene by following the marker rather than having to identify the abnormal gene itself greatly simplifies the tracking process and allows rapid identification of animals which carry abnormal genes. It is particularly valuable in the alpaca because no genes of interest have yet been identified. A marker can be as big as an entire gene (several hundred nucleotides) or as small as 2 or 3 nucleotides. Today most markers are quite small and have names like microsatellites and SSRs (simple sequence repeats). The important properties of a marker are a) it is easy to detect; b) it is located very near the gene of interest and c) it occurs infrequently. The fact that a marker occurs infrequently means that when it is inherited along with the abnormal gene, the linkage of the marker with the abnormal gene will continue to occur more frequently than would be predicted by chance. This concept will become more clear in the next section when we discuss how linkage occurs.

The ability to predict the occurrence of an abnormal gene by linkage analysis forms the basis of many current tests for inheritable diseases. The first disease gene to be tracked by linkage analysis in humans was Huntington's disease. A linkage test was available which predicted the presence of the defective gene 10 years before the gene responsible for Huntington's was identified.

How Does Linkage Occur?

Abnormal genes are produced by mutation of normal genes. Mutations may occur once, multiple times in the same gene or in different genes all leading to the same disease condition. The simplest case is one in which a mutation occurs once and then is spread through the population over the course of time. In that case, if the individual in which the mutation occurred also carries a known marker near the locus at which the mutation occurred, it is highly likely that the individual will pass the marker on to his descendants along with the mutated gene. Linkage analysis could then be used to look for the presence of the marker gene and, by inference, the mutated gene in family members. It is important to remember that the marker gene does not cause the disease. Moreover, many people outside this family may carry the marker gene but do not carry the mutated gene. This situation leads to false positives in linkage analysis. Over time, as the family that possesses the mutant gene linked to the marker reproduces, the number of people in the population and the number of families who possess the mutant gene in association with the marker gene will increase. When this occurs it becomes possible to use linkage

analysis as a test for a disease gene in the general population. The situation becomes more complex if a particular mutation, leading to a particular defect, has occurred spontaneously in different individuals or if a mutation in a different gene results in the same disease process. For each new mutation, a different marker may have to be identified.

What Causes False Negatives in Linkage Analysis?

A defective gene sometimes gets separated from its marker during meiosis through a process called crossing over. Meiosis is a specific type of cell division which results in the production of eggs and sperm. The figure illustrates crossing over during meiosis in a germ cell with only one pair of chromosomes, one from the dam, shown in red and one from the sire, shown in blue. The position on the chromosomes of marker, "M"; normal gene, "A"; and abnormal gene, "a" are also shown. The first step in meiosis is for each pair of chromosomes to reproduce itself so that there are now two pairs or 4 chromosomes in a single cell. At the conclusion of meiosis, each of the 4 chromosomes ends up in a different egg. The same process occurs for sperm.

In the first example, no crossing over occurs and each of the four chromosomes ends up intact in one of the four eggs. The marker "M" and the abnormal gene "a" are passed on together.

In the second example, two of the chromosomes are shown breaking at the crossing over point and exchanging portions of their genetic material. Two of the eggs will receive chromosomes intact but two of the eggs will receive chromosomes which are hybrids of their sire's and dam's chromosomes. You will remember that chromosomes are made up of several hundred genes aligned in a specific order according to their location or locus on the chromosome. In the first example, where there is no crossing over, all of the genes on the same chromosome are inherited together. By contrast, in the second example, where crossing over occurred, genes above and below the crossing over point are not inherited together. In the second example, the marker "M" and the abnormal gene "a" are far apart and are separated by crossing over. The occurrence of crossing over during meiosis can result in false negatives in linkage analysis if the marker is separated from the mutated disease gene.

In the third example, the marker "M" and the abnormal gene "a" are close together. Even though crossing over occurred, the linkage was maintained and "M" and "a" were passed on together. The closer a marker is to the mutated gene of interest, the more likely the two genes will be inherited together. It follows that the more markers on DNA we can identify the more likely it is that we will be able to find markers close enough to a mutated gene so as to reduce the occurrence of false negatives to a minimum.

How are Markers Detected?

The procedures for marker detection are automated, and a large number of markers can be analysed very rapidly from a single blood sample. A detailed description of laboratory methods for the detection of markers is outside the scope of the present article. The reader may refer to a standard textbook (4,5). Suffice it to say that markers can be

detected with probes which are complementary to their unique sequence of nucleotides or by exploiting differences in the size of fragments produced when the marker site is cleaved by enzymes which cut up DNA into different size fragments depending on the nucleotide sequence. Typically, marker sites are amplified by PCR (polymerase chain reaction) so that a sufficient quantity of pure DNA marker is available for analysis.

What Sorts of Disorders are Amenable to Linkage Analysis?

Before attempting to develop a genetic test to predict the presence of a defective gene in alpacas, it is necessary to determine through breeding trials and pedigree analysis that the disease of interest is in fact a Mendelian disease. Mendelian diseases are the result of a mutation in a single gene. They are inherited in a predictable pattern like that described by Gregor Mendel for garden peas. In humans, over 4000 Mendelian diseases have been identified.

Non Mendelian diseases are inherited in more complex ways and their expression may vary. Even though an individual has inherited a pair of abnormal genes for a particular disease he or she may not be affected by disease due to environmental factors (diet, exercise) or the requirement for more than one gene at more than one locus to be inherited at the same time for expression of the disease to occur. Diseases such as hypertension, diabetes, common birth defects such as cleft palate are not inherited in a simple Mendelian fashion in humans. Inheritance of diseases which are multifactorial in their causation are far more difficult, if not impossible, to trace in families and far more difficult to develop DNA tests for.

As in other species, many defects in alpacas are probably inherited. However, the proof is lacking. Unlike in humans, there are no congenital disorders in alpacas which have thus far been determined to be inherited, much less found to be Mendelian in their inheritance pattern (6). Possibilities include choanal atresia, angular limb deformities, wryface, polydactyly, brachygnathia, cleft palate, deafness, ventricular septal defect, testicular hypoplasia, ovarian hypoplasia, juvenile lymphosarcoma and immunodeficiency syndrome. The reluctance of breeders to disclose the presence of disease in their herds is one factor which has retarded the identification of disorders which are amenable to DNA testing. If you have the misfortune of having a cria born with a congenital defect please remember to contact Dr. George Saperstein, DVM, Department of Environmental and Population Health, Tufts University School of Veterinary Medicine, 200 Westboro Rd. North Grafton, MA 01536 to obtain a Confidential Breeders' Survey Form. Dr. Saperstein is collecting information on an anonymous basis, concerning the frequency of occurrence of congenital defects. This information will help to identify defects which might be amenable to genetic analysis.

Where Do We Go From Here?

Linkage analysis spawned the development of tests to predict the presence of abnormal genes in human carriers. Linkage analysis enabled scientists to make a genetic map of human DNA, an essential first step to the development of a physical map based on the sequencing of the human genome. As a result of the Human Genome Project, scientists

will be able to identify disease genes directly. Once the disease gene is identified, the defective protein produced can be characterized, unequivocal tests for its presence can be developed and new pharmacological interventions can be developed.

Future studies of the alpaca genome which make use of technology developed for the human genome should rapidly result in the identification of many more markers in alpacas. Moreover, as more and more genes are identified on the human genome this knowledge can be utilized to help identify genes in the alpaca genome. As in the case of human disease, identification of genes which cause disease and deformity in alpacas will lead to definitive tests for the presence of the gene, an understanding of the disease process and the development of new therapies. However, mapping the alpaca genome is only one half of the equation. In order to link markers to genes which cause disease or deformity, we must first have evidence that a disease is inherited by a Mendelian pattern and secondly we must have DNA available from individuals who are affected by the disease and their families. In fact, the accumulation of a sufficient number of DNA samples for testing is usually the rate limiting step in any linkage analysis. This can be accomplished through breeding trials and through voluntary and anonymous contributions of blood or tissue from alpacas affected by an inherited disease and their families. Once a sufficient number of DNA samples have been collected, the distribution of markers on the chromosomes of alpacas which are affected with a disease can be determined. By selecting those marker(s) which always occur when the disease is present and eliminating those markers which do not always occur in affected individuals, we can develop tests which predict the presence of abnormal genes in phenotypically normal alpacas.

Further Reading

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Glossary:

Carrier: An individual who appears normal but carries a defective gene.

Chromosomes: Structures in the cell nuclei which contain DNA and protein. Alpacas have 36 pairs of chromosomes plus two sex chromosomes for a total of 74.

Crossing Over: Breaking and rejoining of chromosomes during meiosis which results in exchange of genetic material.

DNA (deoxyribonucleic acid): A polymer which is composed of four different units called nucleotides (abbreviated A,G,C and T). DNA contains genetic information encoded in the sequence of As, Gs, Cs, and Ts.

Gene: A segment of DNA which contains a unit of inherited information. A single gene encodes a single protein.

Genetic Map of DNA: The relative position of genes and markers along a chromosome as determined by linkage analysis.

Linkage: Coinheritance of two or more genes because their loci are in close proximity on the same chromosome, such that after meiosis they remain associated more often than the 50% expected for unlinked genes.

Locus: The location of a gene on a chromosome.

Marker Locus: A locus which is easily detectable. A marker locus may or may not be part of an expressed gene.

Meiosis: Special type of cell division occurring in germ cells which results in eggs or sperm that contain half the number of chromosomes present in mature cells.

Mendelian Disease: A disease which is caused by a mutation in a single gene and which is inherited in a simple pattern like that for Mendel's peas.

Mutation: Any permanent heritable change in the sequence of DNA.

Nucleotide: The building blocks of DNA abbreviated A,G,C and T.

PCR (polymerase chain reaction): A procedure for making many copies of a short segment of DNA.

Physical Map of DNA: The actual location of genes and markers along a chromosome as determined by direct physical methods.