

CAMELID GENETICISTS CHART COURSE FOR FUTURE RESEARCH

By Murray E. Fowler, DVM

AN HISTORIC EVENT IN THE CAMELID WORLD TOOK PLACE ON FEBRUARY 22-24, 2008, AS THE FIRST INTERNATIONAL WORKSHOP ON CAMELID GENETICS CONVENED IN SCOTTSDALE, ARIZONA.



Photos by Dave Belt

THE PURPOSE OF THE MEETING WAS TO BRING TOGETHER GENOMIC SCIENTISTS FROM THE National Institutes of Health, academic geneticists, clinical veterinarians, veterinary pathologists, and organizational administrators to share thoughts and opinions on camelid genetics. The workshop was truly international in scope with attendees from Australia, Canada, Peru, and the United States. Among the disciplines represented were geneticists, molecular biologists, genomic researchers, clinical veterinarians, pathologists, academicians, and fiber specialists.

Hosted by the Alpaca Research Foundation (ARF) and the Alpaca Registry Inc. (ARI) with financial underwriting by ARI, the timing of the meeting was apropos because the alpaca genome has just been sequenced and will be completed in early 2008. The alpaca joins a short list of other species such as yeast, several plants, mice, rats, dogs, cats, horses, cattle, and recently humans for which genomes have been sequenced.

The workshop began with an overview of genetics and its rise to prominence since the structure of DNA was reported by James Watson and Francis Crick in the scientific journal "Nature" in 1953. Molecular biologists have a language all their own, but slowly, all were brought to a basic understanding. Several attendees (myself included) had a hard time with the terminology initially.

The most significant factor in the success of the meeting was that everyone listened to each other. No egos burst forth. Did differences of opinion crop up? Yes indeed, but courtesy prevailed and both formal and informal discussions were highly fruitful. Genetic research has progressed from



Conference organizers (L-R), Drs. Abe Rosenbloom, Pat Craven, Shauna Brummet, and Warren Johnson pose for a group photo following the very successful International Genetics Conference

the laborious counting of a given trait resulting from a selected mating to the sophisticated chemical and physical technology and instrumentation that can analyze for proteins and other sub-molecular entities. Evaluation of the data collected (bioinformatics) could not be done without computer technology and the expertise of scientists who are able to interpret the data. Such computer specialists were present at the conference to answer questions.

The veterinary clinicians in attendance sought answers for questions such as, "How can we identify the heritability of congenital defects?" "Can we determine if the parent is a carrier of a hereditary defect?" "After the alpaca genome is completed will we be able to manufacture vaccines that are safe and effective against infectious diseases of camelids?" The answers were qualified yeses! Does more research need to be done? Yes! Will it cost money? Yes! Will it take time to solve the clinical problems? Yes, indeed!

In general, congenital defects are more common in camelids than in other livestock species. Although over 100 such defects have been reported in camelids, none have been subjected to enough scientific scrutiny to be certain that they are inherited. Many of these defects are known to be inherited in two or more other species of animals and may be presumed to be inherited in alpacas and llamas, but we don't know this for sure. Management of camelids requires knowledge of which defects may be inherited and how the process works. Is it important for the

camelid industry to know if a trait is inherited and how it is inherited? Absolutely!! It is economically important to the camelid industries and may obviate the heartache that goes with waiting for eleven months only to see a deformed, non-functioning cria.

After the basic principles of genetics were presented, attendees heard how the tools that are already known and used in cattle may be applied to alpacas and other camelids. Reproductive anatomic and physiological traits are known to be controlled by genetics. Unfortunately, there are several anatomic and physiologic (hormonal) defects that interfere with the reproductive process and prevent optimal birthing of healthy crias. Many of these traits were described and the discussions which followed indicated genomic research may be useful to identify and solve these problems.

A special congenital defect called choanal atresia was brought to the fore as a priority for genetic problem solving. Prior genetic research has been unsuccessful. The genomic era may enlighten us. At least one of the traits possessed by camelids may have relevance to human medicine. Camelids have a "normal" blood sugar level that in humans and other animals would be considered to be diabetic. Yet alpacas and llamas suffer no apparent ill-effects of this hyperglycemia. It might be important for researchers investigating human diabetes to know how camelids remain healthy while maintaining such high levels and why? Genetic technology is helping to solve these kinds of problems in other species and there is every reason to believe that it will do so in camelids.

Some of the research reports presented at the meeting were preliminary such as suri genetics using microsatellite markers, pedigree mapping, and parentage

GLOSSARY OF SELECTED GENETICS TERMS

ALLELE — One of several different forms of a gene. Slight differences may produce changes in the end product of gene function (eye color, hereditary diseases, resistance to a microorganism).

ANTIBODY — Specialized serum proteins produced when the body is exposed to specific antigens (infectious agents).

ANTIGEN — Any substance that is capable of inducing a specific immune response that produces antibodies when ingested or injected into the body.

BIODIVERSITY — The sum total of all life on earth.

BIOINFORMATICS — The process of using a computer to search through massive biological data bases.

CHOANAL ATRESIA — A membranous or bony partition in the nasal cavity. It may occur on one side only or both sides, and be complete or incomplete. If complete, the cria is unable to breathe and eat at the same time, because a camelid must breathe through its nose.

CHROMOSOME — A linear or circular strand of DNA that contains genes. Each animal has a specific number of paired chromosomes. In the case of camelids, there are 36 pairs of chromosomes plus one pair of sex chromosomes, or as usually written $2n=74$.

CONGENITAL DEFECT — Abnormalities of structure or function which are present at birth. Not all of these are genetic defects as other physical, chemical, and infectious agents may affect the fetus.

DNA — An acronym for deoxyribonucleic acid. It is the genetic material that comprises the genes, chromosomes, and the genome. DNA is in the form of a double helix (spiral) as reported by Watson and Crick in 1953.

DNA FINGERPRINTING — Much like the fingerprint used in human identification, but done with the unique DNA characters for each individual animal. Commonly used in forensic medicine (crime solving).

DNA SEQUENCING — Establishing the anatomy of DNA by chemical analysis

GENE — The fundamental unit of heredity. A specific section of DNA within a chromosome. The unit of information in DNA that specifies the translation of a particular protein. Mammals have 20,000 to 35,000 distinct genes in their genome.

GENETIC DEFECT — Sometime called a hereditary defect, certain genetic disorders may cause serious defects in a single individual, but the disorders will not be passed on to subsequent generations.

GENETIC DIVERSITY (VARIATION) — Variation that occurs in a group of interbreeding organisms (camelids) by the frequency of alleles appearing in a population or the frequency of genotypes.

GENETIC ENGINEERING — The direct manipulation of genes to alter the physical appearance of an animal.

GENOTYPE — The entire genetic makeup of an individual.

GENOME — A full-length copy of an individual's genetic endowment. A genome is the sum total of all the genes, DNA, and genetic information, neatly compiled in two distinct copies (one from each parent) in every cell of the body.

HEREDITARY DEFECT — A defect that is passed from one generation to the next by the parents.

HYPERGLYCEMIA — An excess of glucose (blood sugar) in the blood.

IMMUNOLOGICAL — Pertaining to immunology, the study of all aspects of immunity.

KARYOTYPE — A microscopic picture of the chromosomes.

MICROSATELLITE — A stretch of DNA that is repeated several times in a row. All mammals examined so far have 100,000 to 200,000 such repeats. These are located at random throughout a chromosome. The variation in these markers between individuals allows for parentage verification and is a tool in the forensic community for matching blood and semen left at a crime scene. These microsatellite markers are given names and numbers.

MENDELIAN GENETICS — Simple inheritance based on dominant and recessive traits that segregate according to mathematical ratios. Gregor Mendel was an Austrian monk who used plant breeding and direct observation to establish the ratios. He is considered the father of modern genetics.

MOLECULAR BIOLOGY — The study of the biochemical and biophysical aspects of the structure and function of genes and other sub-cellular entities.

PHENOTYPE — The observable expression of the genotype of an individual (structure, color).

RECOMBINANT DNA TECHNOLOGY — A process of finding a gene on a chromosome, snipping it out of its original location, and inserting it into a new location (another organism). Currently used in the production of safe and efficient vaccines for animals and humans.

TITER (*British spelling TITRE*) — The degree of reaction of an antibody when exposed to an antigen. Usually expressed as a dilution 1/50, 1/250 etc.

validation for pedigree studies. The completed alpaca genome will add more sophisticated tools for studying these practical challenges.

Genetic studies have been conducted for decades. It would be unwise and incorrect to suggest that such things as Mendelian genetics will be completely superseded by genomic studies. For instance, color inheritance may be studied by simple inheritance as reported at this meeting. Likewise, Mendelian principles have been utilized in camelids for selecting breeding animals and herd management.

Early in the development of the camelid industry in the private sector it was deemed necessary to develop a method of validating the parentage of a cria. In 1987, such a test was developed using the variation of proteins in the blood of each individual. For several years, serologic blood type testing was mandated for animals to be registered in either the llama or alpaca registries. In 1998, advances in DNA technology made it possible to validate parentage by that means.

Fiber is one of the easiest conformation traits that can be improved upon by genetic selection. Presentations were made on qualities that that can be measured. Fiber color is important and we were informed as to the state of current knowledge of color genetics, and how the genome project may help in the future. There is still much to be learned about fiber and knowing the location of genes on the chromosomes will further the industry's ability to produce a still higher quality of fiber. Suri genetics were also discussed.

Infectious diseases are an ever-present threat to all animals. While alpacas and llamas have few unique diseases, they seem to be somewhat susceptible to diseases that generally affect only cattle, sheep, or horses. Some of these diseases are transmitted by insects and ticks. Camelids may develop a titer to these organisms and may or may not develop clinical disease. Only in rare situations may a camelid become a source of infection for other livestock species. It would be important to know why

camelids allow these infectious agents into their bodies and why they react immunologically to the antigen and produce antibodies or develop the clinical disease.

Likewise, it would be desirable to show genetically that camelids are resistant to many of the diseases that are listed by regulatory agencies. It would make camelid owners' lives less complicated for going to shows and sales if camelids were not treated in the same category as ruminants. We still do not have the ability to determine scientifically whether or not an individual is a hybrid. That may change when the genome project is completed.

It is not necessary for camelid owner/breeders to know all the chemistry and physics associated with camelid genetics that has brought us to such a high state of the art. Hundreds of genetic scientists have devoted their entire careers to sorting out the most detailed secrets of DNA. The camelid industries are in a position to benefit from those studies and camelid owners should be grateful that the alpaca was chosen as worthy of study. Much of the information obtained will have direct application to all camelids.

It became evident during this meeting that teamwork is essential for solving problems. Researchers in the camelid community need to work closely with geneticists to identify conditions that need attention. Genetic research is expensive. For example, the human genome project cost about three billion dollars and was heralded as one of the great feats of modern science. The human genome project took 16 years to complete from the time it was first proposed until the first draft was reported in 2001. The development of automated systems has cut the time and expense of such research to a fraction of that necessary just a few years ago. As a result, the alpaca genome project will be finished in less than two years.

Part of the expense of completing the alpaca genome project and applying it to practical day to day problems must be supported by the industries that will

benefit from the research. Furthermore, associations must become knowledgeable about what has been done, what is being done, and what needs to be done in the future. To that end, I have appended the titles of two books I recommend for serious owner/breeders and clinical veterinarians. It may also be necessary for some politicking to help raise funds to support this vital research.

The industries should support the alpaca genome project philosophically and financially. In order to do that, individuals should be conversant with basic principles. We don't need to be geneticists, but we do need to be able to talk to them. We need to understand some of their terms. I have included a glossary of some terms that may be encountered when reading about genetic articles in our journals.

What of the future? Those in attendance agreed to prepare some documents that will outline the need for investigation. Task forces were assigned to people with an interest in camelids and who are willing to devote time and effort on their behalf. The first international workshop on camelid genetics must be followed by similar gatherings to hammer out specifics and to communicate with others who have similar goals, ideals, experience, and expertise.

Together, we can make a difference in the exciting world of camelid genetics.

Dr. Murray Fowler, DVM graduated from Iowa State University Veterinary School in 1955 and taught for many years at the University of California, Davis. He is the author of 18 books, including *Medicine and Surgery of South American Camelids*, *Restraint and Handling of Wild and Domestic Animals*, and an autobiography, *Murray - From Hummingbirds to Elephants and other Tales*. Dr. Fowler is a diplomate of three specialty boards: The American College of Zoological Medicine (ACZM), The American College of Veterinary Internal Medicine (ACVIM), and the American Board of Veterinary Toxicology. Now Professor Emeritus of the University of California, he devotes his time to writing and speaking at conferences and workshops around the world.

SUGGESTIONS FOR FURTHER READING

For camelid owners and others wishing to obtain a further understanding of genetics, I recommend the following books:

O'Brien, Stephen J. 2003.

Tears of the Cheetah - The Genetic Secrets of our Animal Ancestors.

St. Martins Press, 175 Fifth Ave, New York NY 10010. ISBN 0-312-27286-3 (hard cover), ISBN 0-312-33900-3 (paper back)

Robinson, Tara Rodden. 2005. *Genetics for Dummies.*

Wiley Publishing, 111 River St., Hoboken NJ 07030-5774. ISBN 13:978-0-7645-9554-7

Both of these books may be ordered through most book stores using the ISBN number. Amazon.com may also be a source.

LETTER FROM THE ARF-ARI GENETICS CONFERENCE

ALPACAS MAGAZINE'S CUB REPORTER, DR. RUTHANNE MCCASLIN, SHARES WITH US THIS LETTER FROM THE GENETICS CONFERENCE, POSTED ON ALPACASITE, FEB 22, 2008:

Hey, you guys!!!!

I am in Scottsdale at the 1st International Workshop on Camelid Genetics cosponsored by ARF and ARI. I have been sitting in the back of the room listening to some amazingly intelligent people flinging around phrases like "amplifying polymorphic tetranucleotide microsatellites" and "radiation hybrid mapping" of "conserved sequence blocks" using "chromosome painting" and other technical jargon as casually as most of us talk about fleece weights and crimp styles. I am in awe!

And my eyes are not glazing over. I am actually following most of it and learning so much – such as how traces are used to identify contigs, that are combined into scaffolds, that are then used to build chromosomes. I have also learned how the various sequences are annotated using computer programs such as GARField. And the information learned from the genome of one species can be used to illuminate and interpret the genomic information in another species. (The alpaca genome is actually one of about a dozen mammalian genomes that will be used in a comparative approach to shed light on the human genome).

There has been an explosion of genomic information in the last ten years and the pace is accelerating. It took three years to map the genome of a single alpaca. The genome of a second alpaca is underway now and is proceeding much more quickly. We are on the verge of being able to answer so many questions. The possibilities are mindboggling (in case you haven't already noticed my mind has been very definitely boggled!)

I am so pleased that ARF had the wisdom to propose this conference at this time and that ARI had the foresight to support it. They have brought together genetic researchers from all over the world to discuss and compare their work, to brainstorm, and to strategize about how best to proceed

from here. It's important we figure out how to utilize scarce resources and coordinate with one another for the benefit of all.

At least one major collaborative effort was identified during the conference. An Australian researcher is working on identifying the gene that codes for the suri trait. During this conference, she discovered that many of the markers she needed for her project had already been identified by the folks at the U.S. National Institutes of Health. Markers are important tools in this process that help figure out where the various genes are. Then we just have to figure out what each one does and how. Not as simple as it sounds, but absolutely fascinating.

In another presentation, a cytogeneticist from Texas A&M explained that we still need a better standardization of the nomenclature for the camelid chromosomes so that when one researcher talks about a gene on chromosome 6 the other researchers around the world will all know which chromosome (out of the 37 pairs) she is talking about. That problem wasn't solved here at the conference, but discussing it puts this matter one step closer to being solved.

Tomorrow, we are going to meet in small groups to make recommendations on the next steps to take. I feel very honored and blessed to be allowed to participate in this momentous undertaking. (I am here representing the ARF Development Committee). I had better get to bed now, but I was so excited I just had to tell SOMEONE before I could sleep, so now I have told all of you!! Well, just the tip of the iceberg. I'm sure I'll be telling you more in the days to come.

Smiles,

Dr. Ruthanne



Photo by Darby Vannier

CAMELID GENETICS CONFERENCE ABSTRACTS

ALPACA ENTERS THE GENOMIC ERA



Speaker photos by Dave Belt

Warren E. Johnson, PhD
Polina Perelman, PhD
Laboratory of Genomic Diversity
National Cancer Institute

Camelid species offer numerous opportunities for important discoveries as research animals in the genomics era. The alpaca 2x whole genome sequence, to be completed in 2008, will serve as an important impetus for taking advantage of these opportunities. Here, we will attempt to set the stage for what we envision will be a productive and informative workshop. We will provide an overview of some of the molecular genetic tools and resources that are being developed and improved at the Laboratory of Genomic Diversity that will facilitate and stimulate future work on camelids. These include an alpaca radiation hybrid (RH) panel for mapping of genetic markers, which is critical for the more efficient annotation

of low coverage genomes, a preliminary STS map depicting relative relationships between alpaca, human, and cow genomes, development of flow sorted chromosome probes for comparative ZOOFISH cytogenetic analyses and the initial plans for the assembly and annotation of the alpaca genome. In addition, we will propose for discussion several options or blueprints for maximizing the utility of these resources for basic research, gene discovery, advancing veterinary medicine, and contributing to comparative genomic research. This will include the need for developing additional resources (e.g. SNPs and coordinated medical surveillance) and future collaborative, multidisciplinary research.



USE OF ONLINE RESOURCES FOR THE ANNOTATION OF 2X GENOMES



Joan U. Pontius, PhD
Stephen J. O'Brien, PhD
Laboratory of Genomic Diversity
National Cancer Institute

A wide variety of publicly-available resources are useful in the annotation of mammalian genomic sequences. These resources were recently used in the annotation of the *Felis catus* 1.9x genome by the Laboratory of Genomic Diversity (LGD) and could prove equally useful in the annotation of the 2x alpaca genome.

The National Center of Biotechnology Information (NCBI), includes the genome annotations for 10 mammals. The annotations include ~20,000 genes for each mammal, chromosome assignments for the sequences, as well as the original trace sequences used to assemble the genome. For human, mouse, and rat genes, gene function is cross referenced with the Gene Ontology Database and, for human, Mendelian Inheritance in Man (OMIM). NCBI also provides

the software for Basic Local Alignment Search Tool (BLAST), which enables the alignment of two DNA sequences. Once a best match is found between a new sequence and the sequence of a well annotated genome, the function of the new sequence can be proposed using the annotations of the second genome.

The Generic Genome Browser (Gbrowse) from Lincoln Stein in Cold Spring Harbor is free software that provides an interactive display for the annotations of a genome project. The cat version of Gbrowse, Gene Annotation Resource Fields (GARField) is available online at lgd.abcc.ncifcrf.gov. The eventual alpaca genome annotations could be easily integrated with the existing software at the LGD for an alpaca genome browser.



APPLICATION OF BOVINE GENOMIC TOOLS TO ALPACA

Speaker photos by Dave Belt



Kent M Reed, PhD
Department of Veterinary and Biomedical Science
University of Minnesota

Camelids (*Camelus*, *Lama*, and *Vicugna*) are economically important species outside North America and are increasingly becoming more important within the United States. Unlike the more commonly studied Bovine, Ovine, and Caprine species, the genome of these genera have not been extensively studied. Comparative studies utilizing the recently-assembled bovine genome sequence hold promise for advancing our understanding of the camelid genome. In this study, two commonly used tools, polymerase chain reaction (PCR) and in silico mapping, were

employed to identify homologous regions between the species. Results show that although bovine microsatellite primer sets will amplify both alpaca and llama DNA, the necessity of lowering PCR annealing temperature to account for sequence variation at primer sites results in an unacceptable rate of false amplification. In silico approaches, however, show greater promise and will be invaluable as the alpaca genome sequence becomes available. Based on these findings, the bovine sequence has a high level of applicability to the studying of the alpaca and llama genomes.

CHOANAL ATRESIA AND WRY FACE IN CAMELIDS



LaRue W. Johnson, DVM, PhD
Colorado State University

Facial defects as congenital problems are of a relatively high occurrence in llamas and alpacas. Choanal atresia and wry face (campyloognathia) are reported wherever in the world that domestic camelids are being bred, suggesting environmental factors are not the explanation. Veterinarians who have worked extensively with camelids tend to be of the opinion that the cause of both these condi-

tions is likely based on genetics. While breeding trials for both of these conditions have been attempted, to date no conclusive mode of inheritance has emerged. This presentation will explain each of the defects, report results of a veterinarian survey and summarize the breeding trial research for each condition.

REPRODUCTIVE DISORDERS IN CAMELIDS AND THE ERA OF GENOMICS



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Department of Veterinary Clinical Sciences and Center for Reproductive Biology
College of Veterinary Medicine
Washington State University

During the last decade, the practice of theriogenology in camelids has improved greatly, due largely to better understanding of regulation of reproduction and diagnostic techniques. The objective of this presentation is to give an overview of the main congenital and acquired disorders leading to loss of fertility or complete sterility in the male and female camelids. Although our ability to diagnose these reproductive disorders and treat some of them has been improved, there are aspects of variation of fertility and especially reproductive-life performance that remain poorly

understood. Development of new molecular techniques has enabled scientists to better understand genomic regulation of reproductive phenomena. Many of the reproductive traits and disorders can be linked to gene expression in the embryo, fetus, or adult animals. The new era in reproductive medicine in camelids should focus on understanding the interaction between reproductive disorders and gene expression or fertility markers. A brief presentation on how these techniques may be applied to camelid reproduction will be given.

VIRAL DISEASES OF CAMELIDS CAUSED BY VIRUSES OF OPPORTUNITY OR VIRUSES NATIVE TO THE SPECIES

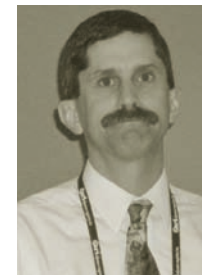


Edward J. Dubovi, PhD, Professor of Virology
Animal Health Diagnostic Center
College of Veterinary Medicine, Cornell University

As disease outbreaks occur in animals, the diagnostician seems to be more frequently faced with the issue of whether one is dealing with a pathogen that is naturally found (associated with the species over many years) in that species or with one that has come from another co-mingled species. Fortunately, many viruses show a strong species preference and jumping of the species barrier rarely produces a productive infection that

can be propagated in the contact species. Surprisingly, camelids seem to be able to borrow viruses from both cattle and horses. Somewhat more surprising is the lack of viruses that could be considered naturally infecting camelids. Examples of defined outbreaks of viral infections in camelids will be presented with emphasis on a recent problem of a bovine virus causing persistent infections in alpacas.

POSSIBLE GENETIC EFFECTS ON GLUCOSE HOMEOSTASIS IN CAMELIDS



Christopher K. Cebra, DVM, Bernadette V. Stang, Ling Jin, DVM
Oregon State University
College of Veterinary Medicine

Interesting characteristics of glucose homeostasis in camelids include high resting blood glucose concentrations, severe and prolonged stress hyperglycemia, poor glucose tolerance, low basal and stimulated blood insulin concentrations, and partial insulin resistance. Several of these features have been described in both Old and New World camelids, and in a variety of species of New World camelid, suggesting that they are characteristics of the entire family of Camelidae. Through a series of experiments, a reduction in insulin production

between birth and adulthood has been identified as the most likely causative event. The cause of this reduction has not been identified, but the timing and nature of the reduction resemble a monogenic, adolescent-onset type of diabetes in people, called Maturity Onset Diabetes of the Young (MODY). At least six individual genes have been identified as triggers for MODY in people. We are currently investigating the possible contributions of those genes to the unusual glucose and insulin homeostasis in camelids.

Abstracts continue on the next page

COMMON CONGENITAL DISORDERS IN ALPACAS

Speaker photos by Dave Beit



George Saperstein, DVM
Cummings School of Veterinary Medicine
Tufts University

Alpaca breeders were surveyed in 2001 for data on normal and abnormal births by year between 1991 and 2000. The survey was voluntary and reasons for carrying it out included the following:

1. Determine the approximate frequency of congenital defects in alpacas.
2. Identify the most common defects for further study as to etiology.
3. Investigate indicators for environmental causes of common defects.

Of 1,659 births, 92 resulted in crias with congenital defects (5.5%). The most common defects reported included choanal atresia, facial

defects, eye defects, and umbilical hernia. No evidence of teratogenicity for any defects was uncovered in this survey. When added to data in llamas from two previous surveys, the most common defects reported in camelids in the United States are choanal atresia, facial defects, female reproductive defects, immunodeficiency, heart defects, and cleft palate.

This survey indicates that further basic research is needed for the most common lethal and semi-lethal defects to determine which ones have a genetic basis and their mode of inheritance.

CYTOGENETICS OF REPRODUCTIVE DISORDERS IN LLAMAS AND ALPACAS



Terje Raudsepp, PhD, Keith Durkin, Bhanu Chowdhary, PhD
Department of Veterinary Integrative Biosciences, Texas A&M University
Michelle Kutzler, DVM, PhD, DACT
Department of Clinical Sciences, Oregon State University

Worldwide interest in alpaca and llama breeding shows steady increase, primarily due to the peculiar characteristics of their wool but also because these camelids are becoming valued pet and companion animals. Infertility or reduced fertility of breeding animals is therefore of considerable economic concern. Fertility is a complex trait, governed by multiple environmental and genetic factors and chromosome rearrangements count for part of the latter. Therefore, collaborative efforts by Texas A&M and Oregon State Universities have been undertaken to combine clinical diagnostic studies on llama/alpaca infertility with systematic chromosome analysis. Studied animals show a variety of abnormalities, ranging from complete male or female infertility to freemartin and intersex phenotypes. The majority of these animals have normal karyotype and the causes of their

abnormalities remain idiopathic. Some conditions, however, can be correlated with chromosomal rearrangements. Altogether we have identified four types of cytogenetic aberrations in seven individuals: three infertile females carrying a "minute" chromosome, one infertile male with an autosomal translocation, one XY SRY-neg sex reversal female and two freemartins. Due to high chromosome number ($2n=74$) and the complexity of camelid karyotypes analysis of chromosome abnormalities using traditional cytogenetic techniques has limitations. These can be overcome by complementing chromosome analysis with molecular tools like chromosome microdissection and fluorescent in situ hybridization with chromosome specific markers or painting probes. Approaches to apply these tools for revealing the origin of the "minute" chromosome in infertile females will be discussed.

OBJECTIVE MEASURES OF ALPACA FIBER QUALITY



Christopher J. Lupton, PhD
Texas A&M University

Most of the fiber characteristics that influence mechanical processing and properties of finished textile are measurable with instruments. Similarly, many of the fleece and fiber characteristics that are important to alpaca breeders are capable of being objectively measured. The important characteristics that cannot be objectively measured directly (e.g., handle, style, and loft) are closely correlated to one or more measurable properties. The product of an objective measurement is a function of the technique used to sample the fleece, live animal, consignment, etc., the manner in which the sample is sub-sampled at the testing lab, and also a function of the instrument or method used to measure a particular property. Measurements that will be considered in the presentation

include: greasy fleece weight, proportion of each fleece component, clean yield, vegetable matter content, content of other contaminants, mean fiber diameter (SD, CV, comfort factor, spinning fineness, staple profile), medullated fiber content (and mean fiber diameter, SD and CV), mean staple length (SD and CV), staple and fiber crimp, mean fiber curvature (SD and CV), resistance to compression, staple strength (SD, CV, and position of break), color, dark fiber content in white and light colored fleeces, fiber (follicle) density, and luster. Each of these measurable traits will be considered in the context of how genetics might be used to produce improvement (or inadvertently detriment) in alpaca fiber production and quality.

ALPACA GENETICS – MENDEL IS THE FOUNDATION



D. Phillip Sponenberg, DVM, PhD
Virginia Tech

The power of Mendelian genetics is easily overlooked when viewed in light of the advances in molecular genetics. The Mendelian approach brings to light many interesting and useful phenomena which can then be further explored with molecular techniques. Among these Mendelian phenomena is color, for which the Mendelian approach is especially suited. The existence of multiple loci each having an effect on color is well established in a number of species, and is a useful springboard for investigating alpaca color. The Agouti locus is important as a major determinant of color, and has multiple alleles coding for white, fawn, red with black trim, bay, mahogany, and black (somewhat in order from dominant to recessive). Unusual results are rare, and can be attributed to rare alleles including dominant black (likely at Extension) or recessive red (likewise probably at Extension). Ironclad proof of either of these is lacking, but anecdotal evidence is abundant. Modification of base color through dilution rarely occurs, likely through several

dominant dilution alleles. A recessive brown is tempting to propose, but phenotypic evidence is slim at this point. Anomalous color segregations or rare color phenotypes are the most likely places to spot new or rare phenomena, some of which can be very useful in producing new shades of color. These are more likely to be useful to hand spinners than to industrial markets, due to their subtlety and rarity. White spotting also occurs in alpacas, and the relationships of these patterns have yet to be determined. Certainly there are multiple patterns, each under control of a separate allele (piebald, tuxedo, grey, roan, speckled, others). It is likely that several are at a single locus, however. Mendelian genetics also helps to understand the suri story, especially as there are exceptions to the "single dominant gene" model. Exceptions to any of the common inheritance mechanisms, rather than being annoying perturbations, can instead lead to a more complete understanding of desirable phenotypes whether this be for fleece quality or color.

DISCOVERY OF ALPACA TETRANUCLEOTIDE MICROSATELLITE MARKERS

Speaker photos by Dave Belt



Kylie Munyard B.Sc. (Hons.) PhD
Curtin University of Technology

Tetranucleotide microsatellite markers were identified from alpaca through a modified hybridization capture method. Ten unique enriched libraries were made with the following motifs: TCCC, GACA, GCTT, GATA, GCAC, GGAT, TGCC, GAAA, AAGG, and GTTT. A total of 235 markers were found, with 134 having sufficient flanking sequence for further analysis. To date, analysis of 64 of these markers

is complete. Of these, 19 were polymorphic, 14 were monomorphic (minor allele frequency is <0.1), and 27 were unsuitable (i.e. either no amplification or non-specific amplification). Analysis is continuing, and it is expected that a selection of the most polymorphic markers will be combined into a multiplex PCR that will be suitable for individual ID and parentage testing.

SINGLE NUCLEOTIDE POLYMORPHISMS IN THE ALPACA MC1R GENE SEGREGATE WITH SKIN COLOUR



Kylie Munyard B.Sc. (Hons.) PhD
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Curtin University of Technology

The Australian Alpaca Herd Book details multiple occurrences of dark-coloured cria being born to two white parents, and, conversely, white cria being produced from two dark-coloured parents. It was hypothesised that this result could be explained if a white phenotype could be produced by either homozygosity of a recessive allele of the MC1R gene or a dominant allele of the Agouti gene. The MC1R gene was sequenced in a group of Australian alpacas and eight single nucleotide polymorphisms (SNPs) were identified in the coding region (A82G, T126C, G259A, T354C, G376A, G618A, C901T, and G933A). All but two of these SNPs (G259A, G376A) occurred as a haplotype. The haplotype segregated with skin colour, so that all animals born with pink skin were homozygous for one version of the haplotype, while black-skinned alpacas were either

homozygous for the other variant or were heterozygous. Inspection of the animals revealed that the pink-skinned group did not have any eumelanin fibres, whereas the fibre of the dark-skinned group contained both pheomelanin and eumelanin. Both of the two non-synonymous SNP in the haplotype (A82G & C901T) are potentially capable of altering MC1R function. It is possible, therefore, that we have discovered wild-type (dominant) and loss-of-function (recessive) alleles of the alpaca MC1R gene. Alpaca with white and fawn fibre were present in both of the skin colour groups. There was no difference in MC1R genotype between different coloured animals within a skin-colour group. It is likely, therefore, that other genes are responsible for the intensity of pigmentation.

SURI GENETICS - APPROACHES TO MAPPING THE SURI TRAIT



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While suris constitute 5% of the alpaca population worldwide, they represent nearly 10% of the Australian national herd. Approaches from interested Australian Suri breeders led to the inception of this project. At that time, the absence of a genome sequence and standard genetic tools provided incentive to select a variety of methods in order to map the suri trait.

The advantages and disadvantages of using a pedigree in our mapping design will be discussed as will the paternity testing to ensure pedigree

accuracy. These data will contribute to the beginnings of a genetic map and the investigation of the variation in the Australian population. We have begun to construct a map based on Amplified Fragment Length Polymorphism markers with a particular focus on searching for markers close to the gene of interest. We have also incorporated a candidate gene approach, investigating sequence changes and gene expression differences in suri and huacaya individuals.

GENETIC ANALYSIS, ORIGIN, AND CONSERVATION OF THE SOUTH AMERICAN CAMELIDS



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In 1994, Helen Stanley, Miranda Kadwell, and Jane Wheeler published the first DNA sequence for the South American camelids in Proceedings of the Royal Society London B. Their analysis of the mitochondrial cytochrome B gene confirmed the generic separation of the genera Vicugna and Lama and showed evidence of considerable bidirectional hybridization between the domestic alpaca and llama. In 2001, the results of subsequent research spearheaded by Jane Wheeler and Michael Bruford, were published in the same journal. Using a sample set of more than 700 vicuñas, guanacos, alpacas, and llamas from their

entire Andean distribution, and based on both mitochondrial and nuclear DNA, the results reconfirmed the generic separation of Vicugna and Lama; documented the vicuña ancestry of the domestic alpaca and the guanaco origin of the llama; and produced further evidence of hybridization between the domestic forms. Subsequent research has confirmed that between 6 and 20% of alpacas remain unhybridized, and efforts are underway to identify and insure survival of the original genome alpaca.