Proceedings of the 1st International Workshop on Camelid Genetics

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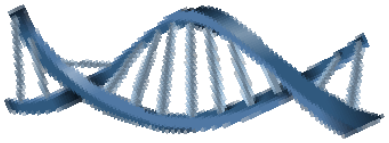

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Introduction

The 1st International Workshop on Camelid Genetics marked the start of a new era for camelid genomics and provided new opportunities for collaborations among geneticists, research scientists and veterinarians. The impetus for the workshop was the completion of the alpaca genome sequence in early 2008 and the availability of new molecular genetic tools and resources that are being developed at the laboratory of Genomic Diversity NCI, NIH and in other laboratories around the world.

The goals of the workshop were to facilitate the sharing of these tools and resources, stimulate future genetic work on camelids and develop options or blueprints for maximizing the utility of these resources for basic research, gene discovery, veterinary medicine and comparative genomic research.

We would like to thank the boards of directors of the Alpaca Registry, Inc and the Alpaca Research Foundation for agreeing to co-host this workshop and give special thanks to the Alpaca Registry, Inc. for funding it.

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The Alpaca Enters The Genomic Era

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The alpaca was nominated and funded by National Human Genome Research Institute for 2x whole genome sequence (WGS) assessment as one of the representatives of the diversity observed in the mammalian radiation, and because of the strategic position of the camelid lineage at the base of the Artiodactyla radiation, which is the most successful group of herbivores with approximately 240 very diverse members. This order contains the majority of domesticated mammal species, including cattle, pigs, goats, and sheep, reindeer, and camelids, which consist of the dromedary camel of northern Africa and south-west Asia, the Bactrian camel of eastern Asia and the South American (new world) camels, with the wild guanacos and vicuñas and the domesticated llamas and alpacas.

The camelids differ from other ruminants in several ways. They have a three-chambered instead of a four-chambered digestive tract, an upper lip split into two separately mobile parts, and an isolated incisor in the upper jaw. Also uniquely among mammals, they have elliptical red blood cells and in addition to the normal antibodies a type of antibody lacking the light chain. These species have also developed unique ecological adaptations including extensive adaptations to their life in harsh, near-waterless environments. Camels offer numerous opportunities for important discoveries as research animals in the genomics era, including as models for human congenital defects, as a third of the over eighty congenital defects identified in camelids have similar inherited conditions in humans. Comparative genomics has become one of the keys to decoding the rapidly growing amount of genomic sequence that is being obtained, and of linking genetic variation with functional variations.

The development of molecular tools for the camelids is also a crucial step in identifying genomic regions, and ultimately the genes and their functions, that are associated with phenotypic differences of economic interest, such as productivity traits. Identification of expressed genes and genetic markers that contribute to phenotypic variation in economically important traits would have a huge impact on improving the management of these species. In many of the poorest communities in the world, camelids are one of the few livestock species that can thrive in the regions harsh environmental conditions, and as such are among their most important assets and the mainstays of their economy. These animals provide income, food, and fertilizer and help sustain the community's health and environment. The benefits from the development of genetic tools to characterize camelids could eventually assist many of these communities.

The Laboratory of Genomic Diversity (LGD) and others have developed several molecular genetic tools for the alpaca. These tools have helped stimulate research on these species and will facilitate future work. These include development of an alpaca radiation hybrid (RH) panel for mapping of genetic markers, a critical tool for annotation of low coverage genomes, a preliminary STS map depicting relative relationships between alpaca, human, and cow genomes,

and development of flow sorted chromosome probes for comparative ZOOFISH cytogenetic analyses. Based on hybridization of human painting probes onto alpaca chromosomes, the karyotype of alpaca is relatively conserved compared with the human genome with differences between the alpaca karyotype and the ancestral mammalian karyotype appears to be mostly the result of fission rearrangements.

Genetic analysies are already an important component of our integrated efforts to preserve biodiversity. Through the investigation of the evolutionary processes that have acted on the species at different spatial and temporal scales, genetics helps us understand the natural experiments that have influence an individual's and species persistence and their evolutionary potential. SNP have become one of the most powerful tools to help incorporate genomic information in theoretical and applied research. First, the availability of single nucleotide polymorphism (SNP) markers widely distributed over the genome greatly enhance the power and resolution of the analysis of geographic patterns of variation and of the demographic and evolutionary processes that have shaped them. This is especially true when SNPs are tagged with positional and, potentially, functional information and they are arrayed on SNP chips that can be efficiently scored at large scales with high throughput technologies (e.g. microarrays). Second, genomic information allows for the expansion of genetic analysis beyond purely -or mostly- neutral variation to include genetic variation subjected to natural selection, both adaptive and deleterious, a pending and necessary step for comparative genomics. Regarding potentially deleterious variation, genomic information allows a better evaluation of genetic risks, including the identification of genes responsible for particular genetic disorders and diseases. On the other hand, direct assessment of adaptive variation improves our ability to detect local adaptations and to gauge levels of adaptive divergence among populations.
The estimated size of the alpaca genome size based on preliminary assembly of most of the sequence data by Dr. Jim Mulllikin is 2.7-3.0 Gb and we will have slightly more than the target 2X sequence coverage with high quality sequence. A preliminary non-assisted quick-pass Phusion assembly that was done to get a preview of how well this genome will go together was very encouraging. There were 11.2 million reads that resulted in 1.76 Gb of assembled base pairs placed on 145,000 scaffolds (total length of 2.07 Gb including gaps) that consisted of 500,000 contigs. This compares very favorably with (is better than) other genomes that have been sequenced at the 2X level, such as the domestic cat. The total number of single nucleotide polymorphisms (SNPs) is around 750,000, of which at least 90% are likely to be validated based on past experience. These data are already being extended and prepared for display as was described recently for the domestic cat.
The current version of the alpaca whole-genome RH map alpaca that was developed with support from the Alpaca community consists of 96 clones developed from a male, with sufficient DNA on reserve for typing of over 5,000 markers. An additional 450 clones were developed through a variety of methods designed to reduce marker retention rates, including different fusion ratios of hamster to alpaca cells (1:1, 1:2.5, and 1:4) and different amounts of radiation (5000 and 8000 rads) are available for future genotyping. As of March 2008, 568 total markers that have been genotyped with high confidence, and of these 423 have been mapped to specific linkage groups and chromosomes at a LOD score of 8 (a relatively high degree of confidence). Additional markers will be placed on the RH map in conjunction with the analysis and assembly of the Alpaca whole genome sequence as increasing the number of markers using an assembled sequence is much faster and more efficient.

To organize our results, including the position of the RH markers, and to provide an efficient means of accessing and updating our analyses, we are developing a web-based browser, similar to the one developed for the domestic cat, denominated the NCI-GARFIELD browser (http://lgd.abcc.ncifcrf.gov), with links to other pertinent public databases and web sites such those available at the National Center for Biotechnology Information (NCBI). The alpaca genome will be made available to the scientific community using the GARFIELD browser at the Laboratory of Genomic Diversity. The genome will be annotated as done for the 1.9x cat genome described in our paper (Pontius and O'Brien 2007) with several additions. The GARFIELD browser includes a display of cross-species sequence comparisons. For cat, this included human, chimp, mouse, rat, dog and cow. As the number of high resolution mammalian genome assemblies as provided by NCBI increases, additional genomes will be added to GARFIELD to enable further cross-species comparisons. The alpaca GARFIELD browser will allow a display of chromosomal rearrangements between alpaca and these other genomes. As part of these cross-species comparisons, there will also be access to the annotation of genes in the high-resolution genomes. The user will be able to query GARFIELD using information from the annotated genes, such as the gene symbol, title, and functional terms as provided from the Gene Ontology database. The GARFIELD browser will also include information on the alignment between the genome and mRNA sequences from NCBI's Genes database. For alpaca, this annotation track will include the mRNA sequences for Cetartiodactyla (currently 34,000 genes of which 30,000 are cow, and 3,000 pig, and 864 sheep), all of which will be mapped to alpaca using GMAP. GARFIELD will also include links to the UniSTS database from NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez?db=unists), which represents sets of short unique regions found in individual genomes. Upon the eventual completion of the alpaca 2x genome, the mapping of these STSs to alpaca chromosomes will allow a preliminary analysis of chromosome rearrangements between alpaca and those genomes that do not have a high-resolution genome, such as pig and sheep, which currently have 9,110 and 5,194 markers respectively. The GARFIELD database will be maintained by LGD and will continue to be modified and updated as technologies and other available databases of interest are created.

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Application of Bovine Genomic Tools to Alpaca

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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.

Summary Report of Choanal Atresia and Wry Face in Camelids

LaRue W. Johnson DVM, PhD

Facial defects in camelids are relatively common as compared to other species. The now deceased Professor Horst Leipold of Kansas State University was a recognized “guru” of congenital and genetic conditions. He is quoted as saying that “If the incidence of a condition seems unusually high as compared to other species, chances are it is genetic”.

The two most commonly recognized congenital facial defects in both llamas and alpacas are Choanal Atresia (CA) and Wry Face (WF). While possible causes of these defects would include chromosomal aberrations, trauma, en utero positioning and Teratogenic effects, genetics appears to be involved.

CA is defined as a failure of the internal nares (choanae) to develop the normal opening. It may be complete or partial, unilateral or bilateral as well as variable in structure ranging from membranous to boney. Consequently, affected individuals will likely have variable manifestations with the most severe being total inability to breathe through the nose at birth making it impossible to nurse without aspirating milk. The gold standard for making a diagnosis employs contrast media placed in the nose and diagnostic imaging to demonstrate blockage.

While heroic but ill advised surgical efforts can be employed to correct the problem, generally affected individuals are euthanatized soon after birth. Researchers at Oregon State University (Smith, Timm et al.) conducted extensive necropsy and breeding studies with goals to establish mode of heritability and identification of carrier llamas. Many affected individuals were found to also have facial distortions like WF, cardiac and limb alterations as well as Arhinencephalia. Breeding trial results were erratic and planned DNA studies were not completed. They concluded that their research supports the presumption that the problem is passed by both the male and female and is not a sex linked problem.

WF (Campylognathia) is by definition a maxillary jaw distortion that can occur in varying degrees and progress throughout life resulting in dental malocclusion. Most cases are present at birth and may have concomitant CA as well as cardiac abnormalities. Breeding trial research I performed at Colorado State University using “proven” carriers, presumed carriers as well as affected male and female llamas produced a 10% occurrence which is much greater than the natural occurrence of <1%. My conclusions from the study include that WF is a genetic problem, selective breeding will increase WF occurrence, the inheritance is to date not understood but likely involves multiple recessive genes with both parents contributing and that CA and WF have a genetic association.

From a survey I conducted of 34 respected camelid veterinary colleagues, I received 18 responses to questions related to CA and WF. These individuals had and average of 17.6 years working with llamas and or alpacas upon which to base their opinions and recollections. Their averaged response to % prevalence of congenital defects in llamas was 3.4% with alpacas 4.0%. A total of 174 llama and 153 alpaca cases of CA were recorded along with 46 llama and 69 alpaca WF cases. 18 examples of CA and WF occurring together were reported. Of all congenital defects, respondents expressed that CA represented approximately 15% and WF 5% in both llamas and alpacas. The prevalence of CA from all births was estimated to be .75% for llamas and .48% for alpacas while for WF, .48% for llamas and .35% for alpacas. When asked if camelid genetics are involved in the occurrence of CA, 16 responded yes and 2 probably. For WF, 13 responded yes, 2 probably, 2 not clear and 1 no.

There is no question that CA and WF need further research. With the reported progress of the alpaca genome research, it seems logical to utilize this tool to focus on these conditions. The camelid community will need to provide credibility, capital and cooperation to accomplish progress in the goal to further understand and hopefully eliminate these conditions.

Reproductive disorders in alpacas and llamas

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A review of 10 years of the author's clinical service on 2460 females showed that the main complaints are repeat breeding syndrome (75.6%), recurrent pregnancy loss (18.3%), abnormal external genitalia (4.9%) and abnormal behavior (2.4%). Diseases of the vulva and vagina represented 11.5% of the diagnosis with abnormal sexual or anatomical differentiation (ambiguous external genitalia, intersex, vaginal stricture, vulvar agenesis, aplasia) representing a major part followed by postpartum injuries. Diseases of the cervix represented 6.1% of diagnosis. Abnormal conformation or stenosis of the cervix may be congenital or acquired (primarily following obstetrical manipulation). Fluoroscopy proved a good method for diagnosis of cervical morphology abnormalities. The majority of reproductive disorders concerned the uterus (46.1%). The single major uterine disorders remain endometritis. However, caution should be exercised because of the lack of standardized method for diagnosis and lack of evidence-based information on treatment. Endometrial biopsy is still a relatively poorly studied technique. Endometrial cysts are rare but can be part of degenerative processes particularly after severe dystocia, repeated inflammation and aggressive intrauterine therapy. Uterine congenital abnormalities are dominated by segmental aplasia. Uterine neoplasia is rare but leiomyomas, adenocarcinoma and hemangiomas have been diagnosed. Diseases of the uterine tube (oviduct) represent 2.5% and include segmental aplasia, hydrosalpinx and salpingitis. Ovarian diseases are the second most common disorder in the female (37%). Congenital ovarian diseases are dominated by ovarian hypoplasia which has been linked to several chromosomal abnormalities (73XO, 75XXX, 74XX/XY and minute chromosome) in our laboratory. Cystic ovarian disease is seen in the form of hemorrhagic follicles and other presentations. Variations in the follicular dynamic patterns may have a genetic basis and need to be further investigated. Ovulation failure and acquired ovarian hypoplasia are primarily due to metabolic diseases and other endocrine problems resulting from obesity, weight loss, trace-minerals deficiency and possibility abnormal thyroid function.

Recurrent pregnancy loss is often presumed to be due to luteal insufficiency. Studies are underway to determine the role of luteal insufficiency and factors affecting progesterone level in alpacas. In our experience, recurrent pregnancy loss may also have a genetic component linked to twining, fiber selection and metabolic disorders.

In the male, reproductive disorders are dominated by cystic conditions of the testicles, testicular hypoplasia and testicular degeneration.

Some of the disorders described in this presentation may have a genetic basis. However without proper standardized diagnostic techniques and complete documentation of the genealogy of affected animals it would be hard to discover the genetic nature of these diseases. Client and veterinary education on documentation and proper diagnosis are a must in order to further our understandings of these phenomena. Treatment of reproductive disorders remains empirical at best and controlled studies are needed in this area.

Viral Diseases of Camelids Caused by Viruses of Opportunity or Viruses Native to the Species

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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 (associated with the species over many years) found 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. New World camelids evolved in the absence of contact with domesticated cattle and horses. 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 were presented with emphasis on a recent problem of a bovine virus causing persistent infections in alpacas.

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 vicuña, guanaco, alpaca and llama 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.

Possible Genetic Effects on Glucose Homeostasis in Camelids

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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 6 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.

Common Congenital Disorders in Alpacas

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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 2 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

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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. Since now we have cytogenetically analyzed over 30 alpacas/llamas. The studied animals all show a variety of abnormalities ranging from complete male or female infertility to freemartin and intersex phenotypes. 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 7 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 and painting probes. Overall, there is an urgent need to expand chromosome analysis in alpaca/llama populations and to combine traditional cytogenetics with the resources emerging from the Alpaca Genome Project.

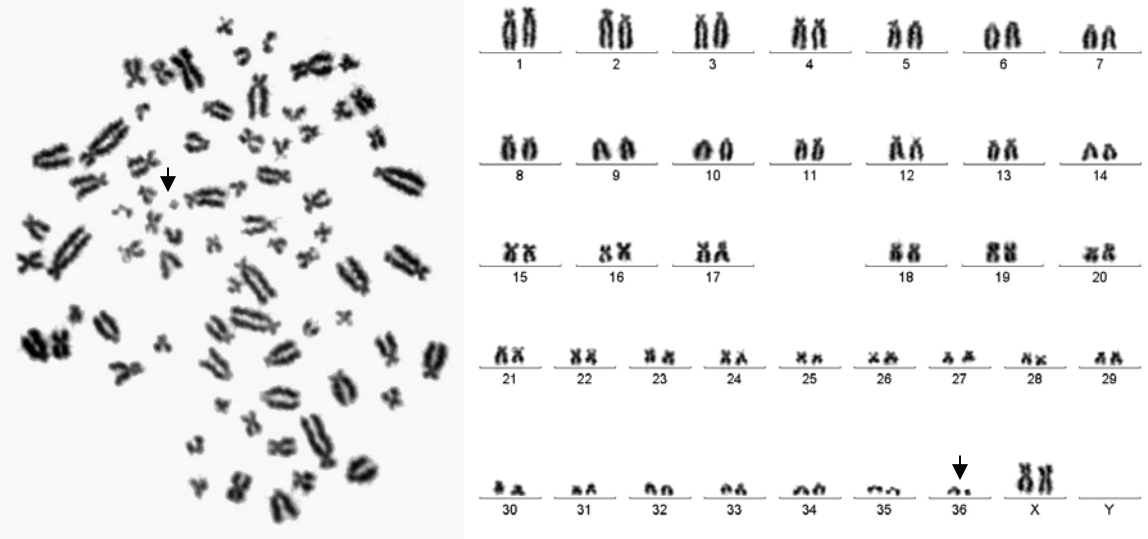

Figure. Left – metaphase spread of a female alpaca (2n=74,XX) containing a minute chromosome (arrow); right – the same metaphase arranged into a karyotype.

Objective Measures of Alpaca Fiber Quality

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Most of the fiber characteristics that are important to alpaca breeders and also influence mechanical processing and properties of the finished textile are measurable with instruments. The influence of various objective measures of fiber quality on the properties of fiber, yarn and textiles is shown in figure 1 below. 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 were discussed in the presentation include: grease 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. An understanding of the genetics of each of these measurable traits can be used in the future to produce improvement (or inadvertently detriment) in alpaca fiber production and quality. The heritability of some of these traits is illustrated in figure 2.

Figure 1: Heritability estimates for alpacas (Chavez, 1991; Ponzoni et al., 1999; and Wuliji et al., 2000)

Trait	h^2
Grease fleece weight	0.21 - 0.83
Clean yield	0.37 - 0.67
Clean fleece weight	0.68 - 0.79
Mean fiber diameter	0.67 - 0.73
CV fiber diameter	0.90
Mean staple length	0.43 - 0.63
Live weight	0.27 - 0.69
Staple strength	0.16
Resistance to compression	0.69

Figure 2: Relative commercial importance of raw specialty animal fiber traits (McGregor, 2006).

Trait	Scoured	Top/noil	Yarns	Cloth
Mean fiber diameter	****	****	****	****
Comfort factor	-	-	*	***
CV of fiber diameter	-	-	**	**
Clean yield	****	-	-	-
VM (amount and type)	***	***	**	**
Staple strength/ POB	**	*	-	-
Mean fiber length	**	***	**	**
CV of fiber length	**	**	*	*
Dark fibers	*	*	*	***

Discovery of Alpaca Tetranucleotide Microsatellite Markers

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Tetranucleotide microsatellite markers were identified from alpaca through a modified hybridization capture method. Twelve unique enriched libraries were made with the following motifs (Table 1). A total of 249 markers were found, with 142 (57%) being suitable for further analysis. Reasons for exclusion at this stage were lack of flanking sequence, inside a SINE, a repeat of an already identified marker or being flanked by a dinucleotide marker. Forty four (31%) of these amplified cleanly in PCR, producing no non-specific amplification. Of these, 24 (54%) were polymorphic when tested on 10 unrelated animals. These 10 animals had a maximum of eight and a minimum of two alleles for each marker. Given the probable founder effect in the Australian alpaca population, it is likely that many of the other markers will be polymorphic in more diverse alpaca populations.

Table 1: Summary of Tetranucleotide Marker Results

Motif	# Found	# Testable	# Specific	# Polymorphic
TCCC	0	0	0	0
GCTT	0	0	0	0
GCAC	**41**	**23**	**3**	**2**
TGCC	0	0	0	0
AAGG	0	0	0	0
GACA	**3**	**1**	**0**	**0**
GATA	0	0	0	0
GGAT	**167**	**96**	**37**	**22**
GAAA	0	0	0	0
GTTT	**27**	**16**	**4**	**0**
CATA	**9**	**5**	**0**	**0**
GCAT	**2**	**1**	**0**	**0**
Total	**249**	**142**	**44**	**24**

Fourteen of the most polymorphic markers were tested on two alpaca Families (sire half-sib and dam half-sib). Only two of the markers were not segregating in a Mendelian fashion.

Conclusions:

- Australian alpacas appear to have relatively low heterozygosity.
- Alpacas have lower frequency of tetranucleotide markers than other species. This is consistent with ruminants such as sheep and cattle.
- The markers discovered in this research will be suitable for pedigree testing.

Single Nucleotide Polymorphisms in the Alpaca MC1R Gene Segregate with Skin Colour

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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. In other mammals, the products of the genes MC1R and Agouti work together to control the type and distribution of pheomelanin (yellow/ red pigment) and eumelanin (black pigment). Therefore it was hypothesised that these alpaca breeding results 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 coding sequence was sequenced in 36 Australian alpacas and seven single nucleotide polymorphisms (SNPs) were identified, as well as a further 14 mutations that were too infrequent to be considered population polymorphisms. Only three of the SNPs caused a change in the amino acid sequence of the gene (T28A, G126S, R301C). When the animals were grouped by fibre colour, no correlation between fibre colour and SNPs was detected.

However, close inspection of the animals revealed two groups: those that did not have any eumelanic fibres or skin, and those that contained at least some eumelanin in their skin and/or fibre. When the genotypes were compared in this grouping is became apparent that the non-eumelanic animals were all homozygous for G82/ T901 or G82/ C901, and that all eumelanic animals were either homozygous or heterozygous for A82/ C901 (Table 1). This corresponds to the presence of a recessive (loss-of-function) MC1R allele “e” and a wild-type MC1R allele “E”. A82G is in the extracellular N-terminus of the MC1R protein. C901T is in the intracellular C-terminus of the protein. Both regions have high functional significance. Both SNPs have the potential to cause functional changes in the MC1R protein.

Table 1: SNP Genotypes in the MC1R Coding Region.

SNP		Fibre Colours	Eumelanin Present?	Proposed MC1R Alleles
A82G	C901T			
G	T	white, medium fawn, dark fawn, grey	N	ee
G	C/T	white, dark fawn	N	ee
A	C	white, fawn, dark fawn, dark brown, grey, black	Y	EE
A/G	C/T	white, medium fawn, dark fawn, bay, black	Y	Ee
A/G	C	bay, black	Y	Ee

There was no difference in MC1R genotype between different coloured animals within these groups. 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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Alpacas have two broad fleece types: huacaya and suri. Whereas huacayas carry wool with characteristics similar to sheep fleece, the suri fleece is prized for the extraordinary lustre and silkiness of the fleece. The suri trait is an ideal candidate for genetic testing for two reasons. First, pedigree analysis suggests the phenotype arises from a single locus and is dominant over the huacaya phenotype (Ponzoni *et al* 1997), thus the application of a genetic test would be simple and reliable.

Breeding from heterozygous suris results in some huacaya offspring. At present, breeders must progeny test suri individuals in order to determine their genotype at this locus –especially stud males. Due to the age of sexual maturity and length of gestation, this process can take up to 5 years of substantial effort, only to potentially result in production of unsuitable stock. Breeders often make extensive use of any suspected homozygous suri males which may reduce genetic gain in other traits and increase inbreeding, leading to numerous recessive abnormalities and inbreeding depression. A DNA test that distinguishes homozygous from heterozygous suris is the modern solution to these problems. Such a test would allow breeders to select homozygous suri males and females at birth. The involvement of the Partner Organisation in this project illustrates the importance that commercial suri alpaca breeders place on the availability of such a test.

We have approached the problem from a number of different angles.
1. We have obtained samples from a large family of alpacas that have descended from a heterozygous suri male. The suri allele from this male has been followed though the pedigree and we have chosen offspring that have arisen only from huacaya females. This simplifies mapping by ensuring that new suri alleles do not enter the analysis. We have also included unrelated suri and huacaya individuals in order to combat the possibility that markers we study are present only in the family we have chosen.

2. We have chosen to use our pedigree and Amplified Fragment Length Polymorphisms (AFLPs) as markers to map the location of the gene responsible for the suri trait. AFLPs are advantageous because a single PCR reaction can produce multiple markers throughout the genome, to be scored based on presence/absence in suri and huacaya individuals. Locating the position of the gene will allow us to produce a reliable test for use by breeders. We aim to eventually identify the gene responsible for the trait.

3. We have joined with CSIRO Australia to trial a new microarray technology to investigate the gene expression pattern in the skin of suri and huacaya individuals. This may give information on genes that are differentially expressed and allow us to focus research on those particular genes.

4. We are also using a candidiate gene approach where we are investigating those genes identified as fleece related genes in sheep.

Concluding Remarks

Murray E. Fowler, DVM

The workshop was a resounding success. Numerous disciplines were involved in sharing ideas, experiences and expertise. Basic Scientists with many years of experience with using molecular genetics to solve problems in other species reported on how the new alpaca genome research project may be expected to aid the camelid industries. They shared the podium with academic geneticists, clinical veterinarians, veterinary pathologist and other interested parties. The workshop was international in scope with representation from Australia, Canada, Peru and the United States.

My impressions as a clinical veterinarian may be summarized in several words.

1. **Listening** – It was great to see and hear 45 participants with radically different backgrounds and experiences listen to each other.

2. Participants came to understand that **teamwork** is essential for the alpaca genome project to be completed and the application of information gained.

3. **Communication** – is vital so that those in the camelid industries feel that they are part of the action and for fellow researchers to know what is being done and what needs to be done.

4. **Financial Issues –** must be addressed, recognizing that the camelid industries must help support research projects.

5. **Language and terminology** must be made available so that each of us may more fully understand each other.

6. A great debt of **gratitude** must be expressed to the molecular geneticists who have made it possible for the alpaca genome project to go forward so that the camelid industries may reap the benefits in the future.

What of the future – Priorities were set for individuals and task forces to continue discussions on important issues and decide who should do the investigations. Additional meetings were encouraged to continue dialog and keep all parties enthusiastic about camelid genetics.

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