Dr Belinda Appleton earned a PhD in genetics at Melbourne University in 2002. She has broad research experience in evolutionary biology and molecular biology and her research has included analyses of native fauna including bats, marine invertebrates and commercially important fisheries. Her interest in agriculture and approaches from the industry led to a change in direction to the study of alpacas and in particular fleece related traits.

A second lecture by Dr Andew Merriwether was an overview of ongoing and future research into the genetic mechanisms and inheritance patterns of the suri phenotype in camelids. Most research has pointed to a single gene or to two very tightly linked genes close together on the same chromosome that are almost always transmitted together. Current studies by Merriwether (Binghamton University), Appleton (University of Melbourne), Gutierrez (Universidad Complutense de Madrid) and Renieri (University of Pisa) were discussed. The outcome of the suri genetics research is that a PCR-based genetic test for suri homozygosity/heterozygosity will likely become available in the future.

a handle on

Houston Conference 2011

suri

By **Dr Belinda Appleton**

APPROACHES FROM A GROUP of Australian breeders led to the formation of Alpaca Genomics Australia and the inception of this project. These breeders were hoping we could find a test for suri so they could tell from birth whether a male was worth keeping. This would save both time and money. We have moved from a world without any alpaca specific genetic tools to a world with a radiation hybrid library and where a 10X public sequence is imminent (**10X public sequence** is the new improved version of the alpaca genome sequence that will be publically available).

Our early genetic mapping with traditional microsatellite markers has provided information on the genetic location of suri while the newer technologies of genome wide SNP genotyping and the whole genome sequencing of six animals have provided further focus on Suri,

There are many factors by which we may judge a fleece – factors such as density, diameter, lustre, crimp, rate of growth will be controlled by many genes

There are many factors by which we may judge a fleece. Factors such as density, diameter, lustre, crimp, rate of growth will be controlled by many genes. I have focused on the fundamental difference between huacaya and suri - the structure of the fibre. Work in other species has implicated many genes in the production of hair/wool (Purvis and Franklin 2005). The epidermal growth factor-EGF [Moore et al. 1985; Mann et al. 1993], Tumor necrosis factor-TNF (Srivastava et al. 1997; Laurikkala et al 2002), (wingless/int)-WNT (Millar et al. 1999; Kishiomto et al. 2000) , sonic hedgehog signaling-SHH (St-Jacques et al. 1998), and Bone morphgenetic protein-BMP (Kulessa et al. 2000; Botchkarev et al. 2001) pathways are all thought to assist in the regulation of hair follicle formation and activity. Each of these pathways can include many genes. In all of these pathways there are genes that turn on and off, genes that modulate expression of other genes and then there are the genes that create the structural proteins required to make the hair. As you can see there are many candidates for the gene that creates the suri trait and as such we began with optimism but not a conviction that we would actually find the gene.

For some time the suri trait has been thought to be inherited as a single gene, with the suri phenotype dominant. Every alpaca has two copies of all of their genetic information. A dominant trait means that only one copy is required to produce that trait.

Our analysis of the pedigree information in the Australian Alpaca Association database found very few unexplained examples of unexpected fleece type in small families. However there has been a recent publication (Presciuttini et al 2010) that suggests that Suri could be produced from the action of two genes that are very close together. This would provide the opportunity for some small number of unexpected phenotypes in offspring. However it is important to remember that in all livestock, low levels of unexpected results can often be explained by mis-identification of parents. Sometimes animals do not behave

The more homozygous suri females you have in the mix, the more matings it will take before you can be sure of the male's genotype

as we would like! This can only be ruled out by parentage testing. Unexpected results may also be due to the fact that many of the sires are not homozygous suri males. The removal of huacaya offspring alone is not enough to effectively fix the suri alleles- even in a single gene scenario. Without a test to determine whether they are heterozygous or homozygous, we are making assumptions. Regardless of the inheritance we want to find the genetic location. This would allow us to develop a test that could assist breeders.

Assuming a single gene dominant inheritance, then crosses with suri parents will result in a minimum of 50% suri offspring- but many of the offspring produced will be heterozygous. This is problematic for suri breeders as they are keen to know which of the males they have are homozygous for stud purposes.

The best way to progeny test a suri male is to mate with huacaya females. Calculations show that you would need to mate him with (and produce offspring) with 10 huacaya females- and all of these crosses/matings would have to produce suri progeny to be 99% sure that the male is homozygous suri.

Breeders may not want to breed to huacayas, but the alternative is to mate the suri male with suri females and would require 24-60 matings all resulting in suri progeny – to be 99% sure he is homozygous suri. The variation from 24-60 suri females depends on the ratio of homozygous or heterozygous females. Homozygous females should not be used for these tests as when crossed with a suri male (even a heterozygous suri male) all offspring will be suri. However it is difficult to know whether a female is homozygous or heterozygous as they do not produce offspring quickly enough or even enough offspring in their lifetime to be sure. So the more homozygous suri females you have in the mix, the more matings it will take before you can

be sure of the male's genotype. It is valuable to remember that as the proportion of suris in the population increase and as you get more homozygous suris- it becomes more and more difficult to remove the last few heterozygotes from the population, especially if they are female.

In order to find the genetic region that contained the suri gene we began with a pedigree design. We began with the largest possible family headed by a heterozygous suri sire. This heterozygous suri sire had over 500 descendents and about 250 individuals that were informative to our question. We wanted to trace the suri allele originating from the original heterozygous suri sire and to ensure that no other suri alleles crept into the family that would confuse the signal. Therefore we selected offspring from our heterozygous sire that were produced from huacaya females. On selecting this family, we then approached animal owners and requested a blood sample from each animal. We were very lucky that our suri breeders have been so accommodating to our requests for samples. Numbers in the family were reduced as some animals had died, while others had been sold off as pets or sheep guards and their breeders had lost track of them. After all of our searching we ended up with 140 individuals in our pedigree. All blood samples were collected from animals by a vet specializing in alpaca.

Considering alpacas have 37 chromosome pairs, we were not expecting to find anything quickly. We initially genotyped 56 microsats in our family and were lucky to find two markers that showed linkage to the trait within our family. So we focused in on the region. We used the alpaca, the human and bovine genome sequences to find and design more microsats and we have now found over 30 markers in the region – all of which are linked in the family and are positively associated with the trait.

We have recently sequenced the genomes of six Australian alpacas to assist in our research. We are currently searching these data for more information and we are looking at more microsatellites to help us narrow in on the correct location.

We hope that in the near future we will have a test that will assist suri breeders everywhere, and ideally have a better understanding of the genetic basis to the trait.

REFERENCES

Botchkarev et al. (2001) *Noggin is required for induction of the hair follicle growth phase in postnatal skin.* FASEB J. 15 2205 – 2214.

Kishiomto et al. (2000) *Wnt signaling maintains the hair-inducing activity of the dermal papilla.* Genes Dev. 14 1181-1185.

Kulessa et al. (2000) *Inhibition of Bmp signaling affects growth and differentiation in the anagen hair follicle.* EMBO J. 19 6664 – 6674.

Laurikkala et al. (2002) *Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar. Development* 129 2541 – 2553.

Mann et al. (1993) *Mice with a null mutation of the TGF-a gene have abnormal skin architecture, wavy hair and curly whiskers and often develop corneal inflammation*, Cell 73 249 – 261.

Millar et al. (1999) *WNT signaling in the control of hair growth and Structure.* Dev. Biol. 207 133 – 149.

Moore et al. (1985) *Pattern and morphogenesis in skin.* J. Theor. Biol. 191 87 – 94.

Presciuttini et al (2010) *Fleece variation in alpaca (Vicugna pacos): a two-locus model for the Suri/ Huacaya phenotype.* BMC Genetics 11 70.

St-Jacques et al. (1998) *Sonic hedgehog signaling is essential for hair development.* Curr. Biol. 8 1058 – 1068.

Srivastava et al. (1997) *The Tabby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin-A) with collagenous domains.* Proc. Natl. Acad. Sci. USA 94 13069 – 13074