Emilie Campbell, Ph.D.: An Investigation of Genes Controlling Pigmentation in Alpacas

Pigmentation in Alpaca Fleece Color: Red or Black?

"You may call me after 8:30 Mountain Time," the note read. After several rings, a toddler's squeaky voice answered and merrily jabbered away in rapid-fire sequence.

"Oh, I'm sorry," Dr. Campbell apologized. "That was my three-year-old son. I'll call you back after I've tucked the children in bed."


Emilie Campbell

Welcome to Emilie Campbell's incredibly busy life… as assistant professor at Brigham Young University, teacher of a General Genetics class, wife, mom of three children under the age of six, and the woman who is firmly in charge of an *Investigation of Genes Controlling Pigmentation in Alpacas.* The study is funded by the Alpaca Research Foundation for the period of 9/1/04 to 8/31/06.

With her four-month-old baby girl softly cooing in the background, Dr. Campbell started to answer my interview questions. Growing up on her family's cattle ranch in Florida sparked her interest in animal genetics. "As a matter of fact, my dad still ranches," she proudly told me. An undergraduate degree in Animal Science and Molecular Biology led to a Ph.D. in Genetics from Texas A&M University in 1998. Until now, most of Dr. Campbell's work has been concentrated on cattle and swine. She's presently still actively engaged in researching a genetic defect found in cattle called "rat-tail" syndrome. Calves expressing this trait have short, curly hair and a sparse tail. What is interesting is that in order for the rat-tail syndrome to be expressed, animals must be heterozygotes (hetero: different) at the rat-tail locus and must at least have one allele coding for black at the Extension locus.

So how did Dr. Campbell become involved in work for the Alpaca Research Foundation? Surprisingly, I found out that Brigham Young University, located in Provo, Utah, owns an alpaca herd of over one hundred gelded males. All were donated to the university and represent a wide assortment of colors and patterns. "We obtained roughly one hundred blood and fiber samples from these geldings," Dr. Campbell explained. "We've already started to sequence their DNA samples to identify alpaca genes at the Extension locus," she added. Collaborating researchers are Professor Beverly L. Roeder and Assistant Professor Todd F. Robinson. "I also have Aaron Powell, a graduate student, helping me in the lab with this project. He's quite enthusiastic about working on this research."

Of course, credit should also go to the pioneers of camelid color studies. Long before the Alpaca Research Foundation existed, llama breeders Dr. Julie Koenig and Dr. Dale Graham discussed and wrote extensively about the inheritance of camelid color genes.

More recently, Dr. D. Phillip Sponenberg, professor of pathology and genetics, proposed a theory in which "the Extension locus has an intricate, predictable, and complicated interaction with the Agouti locus," (*The Complete Alpaca Book,* 2003). This applies to other species, where inheritance of

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The Alpaca Research Foundation (ARF), in conjunction with Morris Animal Foundation (MAF) and other groups in the llama and alpaca communities, provides funding grants to veterinarians and scientists engaged in research that has the potential to improve the health and well-being of our animals. Alpacas Magazine is pleased to bring you another in a series of interviews with the researchers carrying on this important work.

Photo by Nancy Rondeau

color and pattern genes has been well documented. Dr. Campbell's research will "test this theory and determine if the extension and agouti loci control red versus black fleece color in the alpaca" (ARF Grant Proposal Request, March 29, 2004).

Basic Concepts

To understand the scope of her work, let's review some basic genetic concepts.

- Mammals only have two pigment types: eumelanin (black) and pheomelanin (red).
- All other "colors" are the result of the previously mentioned Agouti-Extension interaction as well as modifying and diluting genes at additional loci (genetic "addresses").
- White is not a "color" but the absence of pigment.
- Think of each alpaca (including a white one!) as either red or black.
- In scientific literature, an upper case letter denotes a dominant gene, a lower case letter a recessive one.
- The symbols used to identify color genes (alleles) can vary from scientist to scientist. You may see the recessive allele for black described as either **a** or $\mathbf{A^a}$.
  The dominant allele coding for gray at the G locus can either be written up as **G** or $\mathbf{G^G}$.
- Individual animals inherit two alleles (one from each parent) at each locus. A black alpaca, for example, could be $\mathbf{A^a\ A^a\ E^+\ E^+}$ or possibly $\mathbf{A^a\ A^a\ E^+\ E^e}$ at the Agouti locus (**A**) and the Extension locus (**E**) studied by Dr. Campbell.
- The complete color genome of every alpaca probably spans many loci. One example: $\mathbf{A^a\ A^a\ E^+\ E^e\ B^B\ B^B\ C^C\ C^C\ D^+\ D^+\ G^G\ G^+}$.
  This alpaca is a silver gray.
  Let's remember that the investigation at Brigham Young University will initially be limited to the Agouti locus and Extension locus, an ambitious undertaking in itself. Of course, the other loci, such as the B, C, D, and G loci shown here, also play an important role in determining final color and/or patterns.

Dr Campbell's Project

Table 1 in Dr. Campbell's grant proposal lists the alleles found in most mammalian species:

Table 1

Agouti alleles

$\mathbf{A^A}$	Red (dominant)
$\mathbf{A^a}$	black (recessive)

Extension alleles (in order of dominance)

E^D	dominant black
E^+	wild type (neutral), as determined by Agouti
E^e	loss of function mutation (recessive red or tan)

"The E^D allele is not found in all species," Dr. Campbell pointed out. "If it exists in alpacas, it will complicate matters quite a bit," she warned. We both laughed at that bit of understatement. Dominant and recessive blacks look identical, but their genomes differ. For example, $A^A\ A^A\ E^D\ E^+$ as well as $A^a\ A^a\ E^+\ E^+$ are phenotypically black. Breeding results, depending on which alleles are carried by their mates, will vary. According to Dr. Sponenberg, "intensity" of color can vary between recessive and dominant blacks found in several other species. Contrary to what many alpaca breeders believe the dominant blacks have a more "washed-out" coat/fleece. Whether this applies to alpacas remains to be seen.

It becomes obvious that, aside from locating and identifying alleles on alpaca DNA, it's the *interaction* of these two loci that makes Dr. Campbell's research so interesting and meaningful to alpaca breeders. Another good example of such genetic gamesmanship is how the E^e allele impacts expression at the Agouti locus.

Let's imagine that we breed two alpacas. Both are $A^a\ A^a\ E^D\ E^e$ (black). Remember that at *each* locus, both sire and dam only "pass on" *one* of their two alleles to their offspring. In our example, the only choice at the Agouti locus is A^a. Let's pretend that, at the Extension locus, both parents "pass on" E^e. The dominant E^D allele is therefore "lost" and not part of the cria's genome.

Our baby is $A^a\ A^a\ E^e\ E^e$. Normally, the $A^a\ A^a$ combination should produce a black alpaca. However, both alleles at the Extension locus now code for "loss of function." It has been proven in other species that the homozygous (homo: the same) $E^e\ E^e$ combination will not allow black pigment to express itself. Although the cria is, quite literally, a genetically black alpaca, its fleece will be red (cream/beige/yellow/fawn). "I am pretty sure we will find the E^e allele in alpacas," Dr, Campbell said. "Many species have it, even chickens. It also controls red hair color in humans." The example most readers will be familiar with is the "yellow" Labrador Retriever. The yellow Lab is… surprise… genetically black!

For the purpose of the ARF Grant Proposal Request, the listing of only two alleles (A^A and A^a) was sufficient. Alpaca breeders will be interested to know though that there are probably more alleles at the Agouti locus.

In 2003, Dr. Sponenberg proposed seven Agouti locus alleles based on his registry research, "on farm" observations, and educated guesses extrapolated from his considerable knowledge of color inheritance in other species. They are in the order of dominance:

A^T	tan
A^+	tan with minor trim
A^r	red with black trim
A^b	bay
A^t	black with light belly
A^m	mahogany
A^a	black

As a contributor to Eric Hoffman's *The Complete Alpaca Book,* Sponenberg reminds readers: "It is important to note that each animal can have only two of these variants, and generally only express the paler of the two."

"How long will it be until you are ready to present results to alpaca breeders?" I finally asked. "Possibly two years," Dr. Campbell replied. Throughout the interview, her enthusiasm for the project was palpable even over the telephone.

We briefly discussed the other numerous loci coding for color/pattern variations in alpacas. "Once your research has been completed on the Agouti and Extension loci, will you be interested to continue with other color genes?" I inquired. "Oh, yes, I'd be very interested!" Dr. Campbell responded without hesitation. "Of course, alpaca breeders will have to help out by making a sufficient number of alpacas with specific colors and patterns available to the study."

As I quickly scanned my notes, I clearly heard Dr. Campbell demand, "Go to bed!" This was said in a firm "don't argue with me" voice. It took a second before I realized the order was not addressed to me but rather was directed at one of the little

Alpaca breeders, unlike those of other species in a not-so-distant past, will not have to resort to numerous and time consuming test breedings to establish inheritance of color genes. We will soon know… with the competent help of one very busy, very knowledgeable woman and a few drops of blood… exactly why that alpaca is red or black. Imagine that!

Campbells. He was still frolicking around, no doubt taking advantage of mom's pre-occupation with a conversation about weird stuff. On the other hand, it's possible that the little guy simply needed to keep an eye on his mother. "My oldest is in Kindergarten and got very upset when I wrote your phone number in his school journal," Dr. Campbell confessed. Well, of course! Even at the Kindergarten level, a serious scholar should not have to tolerate another scientist doodling in his important notes.

In light of Dr. Campbell's awesome schedule and responsibilities, I felt positively silly asking my last question. "Do you have any hobbies?" Dr. Campbell's good natured laughter boomed from Utah clear across the country to New Jersey. "Hobbies?" she repeated with a chuckle. "Well, I'd have to say that right now, my only hobby is sleeping." Who can blame her?

Once Dr. Campbell's research has been completed, I hope to present her findings in a follow-up article. Her work will have practical applications for alpaca breeding programs. Alpaca breeders, unlike those of other species in a not-so-distant past, will not have to resort to numerous and time consuming test breedings to establish inheritance of color genes. We will soon know… with the competent help of one very busy, very knowledgeable woman and a few drops of blood… exactly why that alpaca is red or black. Imagine that!

For more information on the Alpaca Research Foundation, visit www.alpacaresearchfoundation.org.

References:

Alpaca Research Foundation Grant Proposal Request (2004), Emilie Campbell, Ph.D.
The Complete Alpaca Book (2003), Eric Hoffman (contributing author D. Phillip Sponenberg D.V.M., Ph.D.)
A Breeder's Guide to Genetics: Relax, It's Not Rocket Science (2004) Ingrid Wood and Denise Como.

Ingrid Wood has been breeding alpacas under her Stormwind prefix since 1997. A published author in the USA as well as abroad, her latest book, co-authored with Denise Como, is entitled *A Breeder's Guide to Genetics: Relax, It's Not Rocket Science.* Additionally, several of her articles are featured at www.StormwindAlpacas.com. Ingrid can be reached at (609) 261-0696.