

# Genetics of coat color and fiber production traits in llamas and alpacas

Melina Anello,<sup>†</sup> María Silvana Daverio,<sup>†,‡</sup> and Florencia Di Rocco<sup>†,\*</sup>

<sup>†</sup>Laboratorio de Genética Molecular, Instituto Multidisciplinario de Biología Celular (IMBICE), CONICET-UNLP-CIC, La Plata, Argentina

<sup>‡</sup>Cátedra de Biología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

## Implications

- The genetic mechanisms that regulate economically important fiber traits in South American camelids are not yet fully understood, resulting in low productivity.
- In recent years, mutations responsible for some monogenic or oligogenic traits have been identified, enabling molecular testing to assist breeding decisions.
- The recently developed 76K SNPs array for the alpaca, will facilitate the identification of genes affecting more complex traits through genome wide association studies.

**Key words:** color phenotypes, fiber traits, polymorphisms, South American Camelids

## Introduction

### Origin and uses of South American camelids

The use of South American camelids by man dates to more than 10,000 years ago, when the first human populations from the region began hunting the wild species, the guanaco (*Lama guanicoe*), and the vicuña (*Vicugna vicugna*) for food (Yacobaccio and Vilá, 2013). Archaeological and genetic evidence indicates that the llama (*Lama glama*) and the alpaca (*Vicugna pacos*) were domesticated more than 5000 years ago, from *L. guanicoe* and *V. vicugna* respectively, mainly in the Peruvian Andes (Kadwell et al., 2001) although independent domestication centers in northern Argentina and Chile could have existed (Wheeler, 2012).

Both the llama and the alpaca played a preponderant role in the economy and the culture of the Andean communities,

which persists in the present. It is believed that llamas were first selected for meat production and their ability to carry heavy loads, while alpacas were selected for their fiber (Kadwell et al., 2001; Marin et al., 2018). Thus, physical characteristics and their diversity in these species were shaped by domestication.

Nowadays, in Peru, Bolivia, and Chile, the alpaca is still primarily raised for fiber production, although its meat is also consumed. In Argentina, where alpaca population is small, llama farming is mainly intended for fiber production. However, in the northwest of that country, as in Bolivia and Peru, llamas are raised for multipurpose use providing other resources such as meat and leather. Additionally, (small populations of) alpacas and llamas can be found throughout the world, where they are bred mostly, but not only, for their fiber. Because of their intelligence and docile nature, domestic South American camelids are also good companion animals and they are used for other purposes too, like golf animal-caddy, tourist attraction or zootherapy (Marcoppido and Vila, 2013).

Among all domestic South American camelids uses, fiber production is one of the most important for breeders and for the industry. There are several traits that determine the value of the fiber, being fiber mean diameter the most important, followed by fiber length, fiber uniformity, and color (Mueller et al., 2015; Frank, 2017). Other characteristics such as fleece type and the amount of fiber produced (fleece weight) also impact on the producer's income. The elucidation of the genetic mechanisms underlying these commercially important fiber traits would help breeders to select and improve the productive characteristics, as well as to conserve and add diversity to the species.

In general, traits can be classified into two types: qualitative, such as color and fleece type, and quantitative, like the amount of fiber produced or fineness (diameter). Qualitative traits are under genetic control of one or several genes and are exposed to little or no environmental influence. Conversely, quantitative traits are encoded by many genes, each one contributing a little to the phenotype and their expression is modified by the environment (Mackay, 2003).

Advances have been made in the genetic understanding of fiber traits; however, this is a vast field, and a lot remains to

© Anello, Silvana Daverio, Di Rocco

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com <https://doi.org/10.1093/af/vfac050>

be studied. The purpose of this review is to update the reader on the current state of knowledge of fiber genetics in domestic South American camelids and to discuss how genomics and the emergence of modern technologies for sequencing and discovering genetic variants will contribute to the advancement in this field.

## Coat Color Genetics

Llamas and alpacas have more than 22 natural colors ranging from black and brown through gray and fawn to white, including all intermediate shades. Llamas present greater color variation compared to alpacas; tricolor phenotypes may be observed and the presence of white spots is common in llamas. Additionally, this variety of colors and patterns normally occurs in the same herd, unlike alpaca's herds that tend to be more homogeneous. The difference can be attributed to the selection process during the domestication of each species. The llama, as a multipurpose animal, was selected for greater body size and fiber weight rather than other characters, such as color uniformity or fiber fineness (Mueller et al., 2015).

Nowadays, the value of a particular color phenotype depends on the production goal. For example, animals with nonuniform coat are of lesser value for fiber production but they are more in demand as pets or for animal exhibitions. White color has traditionally been preferred by the industry because it can be dyed without bleaching. Because of the pressure in favor of white alpacas, there has been a significant decrease in colored animals, especially in Peru (Hick et al., 2009), with subsequent losses in genetic diversity. Fortunately, in the last years, the demand for natural colors by the textile industry has increased along with the worldwide trend towards consumption of eco-friendly products. In this context, the vast diversity of natural colors offered by domestic South American camelids results very attractive for the current market (Kozłowski and Mackiewicz-Talarczyk, 2020).

However, color inheritance in domestic South American camelids is complex and frequently leads to unexpected results for breeders. For example, the mating between two white animals, may produce a black offspring and vice versa, two black parents can have a white descendant. As color prediction is quite difficult based only on the parents' appearance, it is necessary to better understand how color is inherited and which genes are involved.

Based on homology with other species, Frank (2001) described color phenotypes and their segregation in the llama. Using a scheme of test crosses and backcrosses, he concluded that white (absence of pigmentation) is dominantly inherited with incomplete penetrance and that black is recessive with respect to the other pigmentary patterns. Similar conclusions were reached by Valbonesi et al. (2011) when analyzing color segregation in white and pigmented alpacas. However, the molecular bases of color inheritance remained to be proved. It was not until recent years that some of the genes and alleles that govern coat color in alpacas and llamas were identified.

Color variation occurs when there are alterations in the process of pigmentation. Briefly, this process comprehends the development, migration, and survival of the pigment-producer cells (melanocytes) as well as the synthesis of pigments (melanogenesis) and its transport to the skin and fibers (Cieslak et al., 2011). During mammalian melanogenesis, only two types of pigments can be produced: eumelanin (black) and pheomelanin (yellow or reddish-brown). Basic coat colors are defined by the relative proportion between these two types of melanin. At molecular level, one enzyme synthesizes both pigments, the tyrosinase (TYR), but the eumelanin/pheomelanin ratio is regulated primarily by the interaction of two different proteins with a receptor. This receptor is the melanocortin 1-receptor (MC1R), which is in the cellular membrane of melanocytes and has the ability to signal the cell so that melanogenesis begins. If the protein that bind to MC1R is alpha-melanocyte stimulating hormone ( $\alpha$ -MSH), eumelanin will be produced. However, if the agouti signaling protein (ASIP) is present, it will preferably bind to MC1R, switching melanin production to pheomelanin (Figure 1) (Lu et al., 1994).

It is thought that *ASIP* presents four alleles for the alpaca (Munyard, 2011), in the following order of dominance:

A = white to fawn

A<sup>b</sup> = brown with dark trims

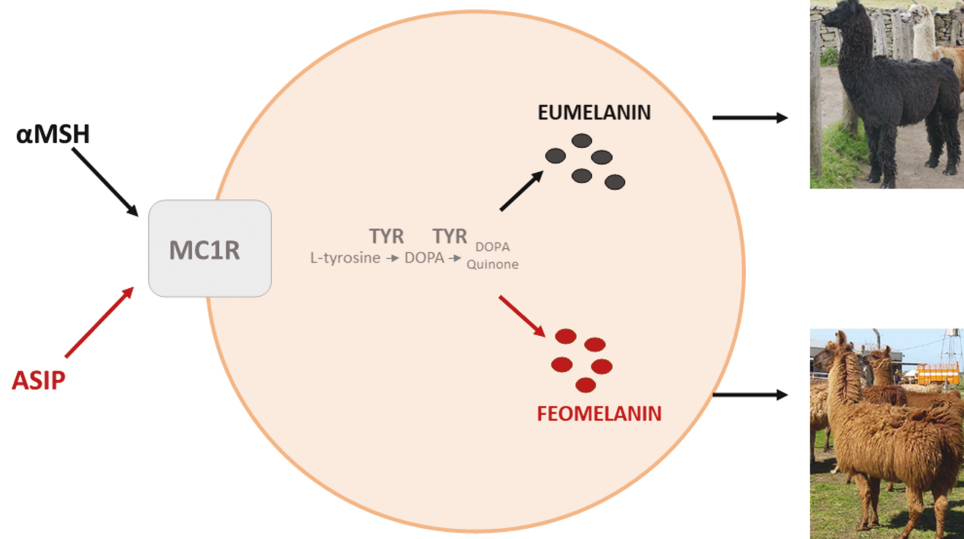
a<sup>t</sup> = black and tan

a = black

The existence of dominant black, as occurs in other species, has been discussed in South American camelids, although there is no genetic evidence to support it. Instead, three *ASIP* mutations have been identified in the alpaca, c.292C>T (allele a<sup>1</sup>), c.325\_381del57 (allele a<sup>2</sup>), and c.353G>A (allele a<sup>3</sup>). Black animals carry two copies of one or another of these mutations (here referred as the a allele) (Feeley et al., 2011). The first two mutations are also responsible for the same phenotype in the llama, while the third one is quite infrequent in this species (Daverio et al., 2016; Marín et al., 2018). Considering all these studies, only the a allele has been molecularly identified so far, supporting a recessive inheritance pattern for black, as originally proposed by Frank (2001) and Valbonesi et al. (2011). Black animals can be homozygous or compound heterozygous for two of the mutations, but the effect is the same: due to the mutations, ASIP protein is nonfunctional (unable to bind to MC1R), thus there is no signal for switching from eumelanin to pheomelanin synthesis, and eumelanin continues to be produced.

The *MC1R* gene is highly variable in llamas and alpacas. Among all reported polymorphisms, a combination of them (called "haplotype"), has been associated with the ability to produce or not to produce eumelanin. In the alpaca, animals with at least one allele of *MC1R* with the combination c.82A/c.901C (allele E) are eumelaninic, whereas animals homozygous for the combination c.82G/c.901T (e/e) are unable to produce this pigment and express pheomelanin instead (Feeley and Munyard, 2009).

In the llama, there are three alleles of *MC1R* E, E<sup>+</sup> and e. A particular *MC1R* haplotype (c.259A/c.376A/c.383T),



**Figure 1.** Scheme of melanogenesis simplified within a melanocyte. Both types of melanin are synthesized from the amino acid L-tyrosine in a series of reactions catalyzed by TYR enzyme. Photographs at the right show animals with each kind of pigment.

different from that of alpacas, determines the ability to produce eumelanin. One variant of this allele (E) is sufficient to produce a colored coat, which can be black if ASIP is nonfunctional (aa) or reddish brown if ASIP is functional (AA or Aa). In contrast, if the animal is homozygous for the *MC1R* haplotype c.259G/c.376G/c.383C (ee), its coat will be white, regardless of the alleles present in ASIP (Daverio et al., 2016). So, for instance, a black dam and a black sire (aa), both heterozygous E/e for *MC1R*, are able to produce white or light fawn offspring (ee aa) when they mate.

Moreover, the whole coding sequence of *MC1R* was studied in guanacos and the ancestral allele (wild allele, E<sup>+</sup>) was identified (Daverio et al., 2016). White llamas frequently have E<sup>+</sup>/e or E<sup>+</sup>/E<sup>+</sup>. Although not yet molecularly confirmed, we can hypothesize that those animals carry at least one copy of the dominant *ASIP* allele, A.

Table 1 Summarizes *ASIP* and *MC1R* allele combinations and the resulting phenotypes for the llama and the alpaca.

It can be observed from Table 1 that in some cases different phenotypes share *MC1R* and *ASIP* genotypes. This suggests other unidentified alleles (probably of *ASIP*) are involved.

## Expression of Color Related Genes

Besides variation within genes coding regions, their expression plays a role in modifying hair pigmentation in camelids.

*ASIP* has a complex structure with noncoding exons which are alternatively transcribed into different mRNAs isoforms. Differential expression of those transcripts in distinct parts of the body results in the diverse pigmentation patterns observed in mammals. Although the expression of *ASIP* in relation to coat color patterns has not been analyzed in domestic South American camelids, it was studied for the solid phenotypes. *ASIP* expression resulted significantly higher in white llamas and alpacas compared to black ones. A chimeric transcript of

**Table 1. Brown refers to pheomelanin reddish-brown (See Fig. 1)**

Genotypes	Alpaca	Llama
E/-	a/a Black	Black
	A/- Brown/ white	Brown
e/e	a/a Brown	White
	A/- Brown/ white	White
E <sup>+</sup> /E <sup>+</sup>	A/- -	White/ brown (rarely)
	a/a -	Black
E <sup>+</sup> /e	A/- -	White/ brown (rarely)
	a/a -	Black

*ASIP* containing part of the *NCOA6* gene was found in the skin of white animals while it was not present in black animals, accounting for the *ASIP* expression differences observed between these two phenotypes. *NCOA6-ASIP* transcript was also highly expressed in brown llamas, at similar levels to those observed in white animals (Chandramohan et al., 2013; Anello et al., 2022).

Other genes, such as *KIT* and *MITF*, which are involved in the development, migration and survival of melanocytes are obvious candidates for white color. *KIT* and *MITF* genes have been sequenced in white llamas but no mutations associated with this phenotype were identified (Anello et al., 2019a). Nonetheless, analysis of gene expression profiles in different studies showed that *KIT* and *MITF* are downregulated in white llamas, as well as other important genes of melanogenesis such as *TYR* and *SLC7A11* (Anello et al. 2019b, 2019c).

The expression of pigment-related genes has also been associated with diluted phenotypes in alpacas and llamas (Figure 2A and B). Eight genes (*RAB38*, *SLC24A5*, *TYRP1*, *SILV*, *MATP*, *KRT4*, *OCA2*, and *TYR*) were observed to be expressed in a common pattern in alpacas: high in black, moderate in bay, and low in white (Munyard, 2011). Similar results

were observed for *TYR* and *SLC7A11* in llamas (Anello et al. 2019b, 2019c) where white, diluted and nondiluted pheomelanin animals were compared. It was observed that the expression levels were the highest for reddish brown animals (nondiluted) and the lowest for the white group, while fawn (diluted) llamas presented an intermediate gene expression. Though these studies do not explain how diluted phenotypes are produced, they show that gene expression is correlated with color dilution.

## Spotted Phenotypes

Domestic South American camelids also present a range of white-spotting phenotypes (Figure 2C and D), including regular and irregular spots, exemplified by phenotypes like the appaloosa (or tajlio), the tuxedo, and piebald (Frank, 2001; Munyard, 2011). There are discrepancies regarding spots classification, and spotted phenotypes are usually named arbitrarily, which complicates its study. White-spotting is quite frequent and suspected to present a recessive inheritance. This was confirmed by segregation studies conducted by Frank et al., (2006) in llamas, who observed that full white is dominant over irregular white-spotting. This implies that white-spotting patterns are unable to be detected in animals whose base coat color is white (Munyard, 2011), complicating the identification of the spotting locus. Moreover, it is not clear if the different spotted phenotypes are product of separate genes, or different alleles of the same gene. *KIT* has been proposed a candidate gene for this feature since it is responsible for similar white spotting phenotypes in other species (Munyard, 2011). However, no molecular studies have addressed this issue in South American camelids and the locus responsible for spotting remains unclear.

## Classic Gray and Blue-eyed White Alpacas

Another phenotype relevant for alpacas' breeders is classic gray. These alpacas present gray body with whiter areas, especially the head, and differ from gray llamas which present a uniform body color and a usually darker head (Figure 2E and F). The causal mutation of the classic gray phenotype in alpacas has recently been identified in exon 3 of the *KIT* gene (Jones et al., 2019). Gray alpacas are heterozygous for the c.376G>A mutation (p.Gly126Arg), as are BEW alpacas, supporting the hypothesis held by breeders that the latter phenotype is caused by a combination of the allele causing classic gray and a white spotting allele (not identified yet) (Jackling et al., 2014). On the other hand, no homozygous individuals have been found for the classic gray mutation, confirming the existing belief that this condition causes early embryonic lethality (Jones et al., 2019).

On another note, congenital deafness is a common defect in domestic South American camelids that present the BEW phenotype, which is white hair coat and solid blue eye color, as opposed to white animals which have pigmented eyes (Figure 2H and G). No data is available in South American populations, but Jost et al., (2020) reported that more than 50% of BEW animals are suspected to be deaf, based on breeders' observations in domestic South American camelids from European countries. This number may be higher, as suggested by a previous study carried out by Gaulty et al. (2005) who objectively assessed the auditory function of llamas and alpacas and found that near 80% of BEW animals were deaf. Two *KIT*-microsatellite markers, named bew1 and bew2, have been associated to the BEW phenotype. These markers are in linkage disequilibrium with the causal mutation, which could be in either the *KIT* gene itself or its regulatory region (Jackling et al., 2014).



**Figure 2.** Photographs of llamas and alpacas illustrating color phenotypes. A. reddish-brown (nondiluted) llama. B. fawn (diluted) llama C. appaloosa or tajlio spotted llama. D. spotted llama. E. gray llama. F. gray alpaca. G. white alpaca. H. BEW alpaca.

## Advances in Fiber Genetics

South American camelids produce diverse types of fibers, but not all of them present the adequate characteristic to reach the market. The traits that determine fiber quality are mean fiber diameter, color, type of fleeces, fiber length, and uniformity of diameter (Mueller et al., 2015; Frank, 2017). Alpaca fiber is recognized worldwide for its fineness and softness. Llama fiber does not enjoy as much prestige, although different studies showed that most of llamas have fiber diameters < 24  $\mu\text{m}$ , equivalent to those of the alpaca fiber belonging to the superfine category (Laime Huarcaya et al., 2016; Hick et al., 2009). However, there is a low level of awareness among consumers and clothing industry about llama fiber quality, added to inadequate marketing strategies.

The structure of the fleece of domestic camelids is given by the combination of fiber length, thickness, luster (or shininess), and the presence and type of curls (Frank, 2001). Variations of these components determine different types of fleeces. In this way, a double-coat fleece is characterized by two types of fibers: cover fibers (thick, long, and straight) and “down” fibers (short, fine, and curly). On the other hand, single-coat animals have fibers with a certain degree of undulation and luster do not present undulations (Frank, 2001) (Figure 3).

Luster fleece presents less volume and less prickling effect, double-coat is the one with the most puncture (if primary fibers are not separated or dehaired) while single-coat has the less. In general, the finer and less prickling the fiber is, the higher its value.

Unlike the alpaca which is single-coated, the llama has been traditionally considered a double-coat species. However, studies in Argentinian llama herds found that there is a predominance of single-coat, with lower frequencies of double-coat and luster fleeces (Frank et al., 2006).

According to fleece characteristics, llamas are classified in: K'ara, with very short fleece and no fibers in the head and extremities, and T'hampulli, with dense fleece around the body (Figure 4A and B) (Frank et al., 2006). With regard to alpacas, two different types of fleeces are described, Suri (luster) with long fiber and Huacaya with fluffy fleece of shorter fibers (Figure 4C and D) (Renieri et al., 2009).

### Fiber Structure and Composition

In mammals, the fiber is a highly organized structure, composed mainly of the cuticle, the cortex, and the medulla (Plowman et al., 2009) (Figure 5). The cortex represents 90% of the fiber and it consists of keratin intermediate filaments (KIFs) embedded in a matrix of keratin-associated proteins (KAPs) (Kuczek and Rogers, 1987). KAPs alongside KIFs provide the major structural support for the fiber and define its physical-mechanical properties, such as strength, inertia, and stiffness (Powell and Beltrame, 1994). The large number of KIFs and KAPs that conform the fiber suggests that their relative composition and interactions are key determinants of fiber characteristics (Rogers, 2006).



Figure 3. Type of fleece (reproduced and modified from Frank, 2001).

Fiber growth in mammals is a cyclical process with rapid growth phases followed by rest phases. This cycle is under genetic, nutritional, and hormonal control. Fiber formation consists of three defined phases: anagen (follicles grow and produce fiber), catagen (follicles regress and fiber elongation ceases since matrix cells stop proliferating) and telogen (fiber is anchored in a keratin matrix and the hair follicle enters a period of relative quiescence in growth) (Paus and Cotsarelis, 1999). Control of follicle cycling seems to reside at the follicle level and it is believed that growth factors may be the regulatory agents that control the expression of fiber genes. The proteins that form the fiber are encoded by keratin genes (*KRT*) and keratin-associated proteins (*KRTAP*) which are expressed in a highly regulated manner during hair follicle growth (Yu et al. 2009).

### Molecular Studies of Fibers Traits

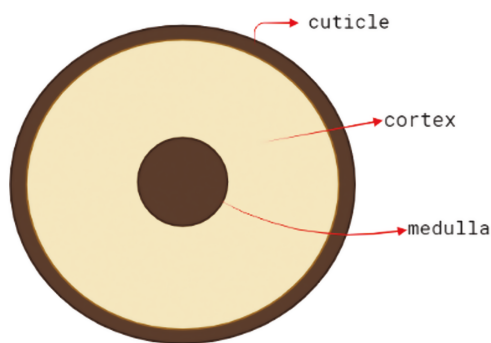
A few genetic selection programs have been implemented in domestic camelids to improve fleece characteristics, among them the PROMECAD (Frank, 1999), the PROMEGE (Renieri et al., 2007), and the genetic and genomic improvement program carried out by PACOMARCA (Inca Tops S.A.). Nevertheless, genetics mechanisms controlling fiber traits in llamas and alpacas are not fully understood.

It is known through segregation studies that luster phenotype is dominant over no-luster with a penetrance effect that allows some heterozygotes to show an intermediate fiber type. Instead, double-coat is governed by an additive genetic mechanism (Frank et al., 2006). The Suri and Huacaya inheritance in alpacas is described by a single autosomic gene with two alleles, or alternatively by a two linked loci model, being Suri dominant over Huacaya (Presciuttini et al., 2010)

The presence of major genes affecting quantitative fiber traits such as fiber diameter, standard deviation of fiber diameter, variation coefficient, and comfort factor in both Huacaya and Suri alpacas has been proposed by Perez-Cabal et al., (2010) based on segregation analysis. Thus, molecular identification



**Figure 4.** Morphotypes of llamas and alpacas: A) llama K'ara B) llama T'hampulli C) alpaca Huacaya D) alpaca Suri (photo courtesy of Alan Cruz Camacho).



**Figure 5.** Scheme of the structural organization of the fiber, cross section.

of the genes involved in the mentioned traits would facilitate genetic improvement.

### Fiber Length

There are many genes that regulates hair follicle's growth, among them the fibroblast growth factor (FGF5) controls hair

cycle and affects hair length. Alternative splicing of the *FGF5* gene results in two transcripts: a full-length form a short form, lacking exon 2 (Hattori et al., 1996). Full-length *FGF5* transcript induces the catagen phase, a function that is antagonized by the short form during the anagen phase (Suzuki et al., 2000). Loss-of-function mutations in *FGF5* gene have been associated with long-hair phenotypes in some species, including camelids. In alpaca, fiber growth in different regions of the body is controlled by differences in the expression of *FGF5* (Liu et al., 2011). Moreover, Fan et al., (2020) that *FGF5* has higher expression in the skin of the Suri alpaca compared to the Huacaya. The same was observed for *FGF5* protein expression.

On the other hand, a point mutation (c.499C> T) that generates a premature stop codon was identified in the long isoform of alpacas (Pallotti et al., 2018). However, western blot analysis did not reveal any effect on *FGF5* protein, suggesting that a stop read-through event occurs.

This gene was also studied in T'hampulli llamas for which the two transcript variants were identified. Besides four

polymorphisms in the coding region were found: a synonymous SNP (c.210A>G), a single base deletion (c.348delA), a 12-bp insertion (c.351\_352insCATATAACATAG) and the same non-sense mutation found in alpacas (c.499C>T). The haplotype c.348delA/c.351\_352insCATATAACATAG codifies for a truncated protein. All llamas analyzed were homozygous for one of the deleterious mutations or compound heterozygous for both. Contrarily, in the guanaco, who presents short hair, no mutations were observed. So, we proposed that FGF5 might not be functional in long hair llamas while guanacos have a functional protein (Daverio et al., 2017).

## Fiber Diameter and Other Related Traits

The fiber diameter or the fineness represents the most important feature for the textile industry. It is affected by the presence of the medulla (medullation): the diameter increases when the medulla (broken or complete) is present and decreases in its absence (Guillén and Leyva, 2020). At the same time, fiber uniformity is related to the average fiber diameter (Aguilar et al., 2019). The heritability and genetic correlation of these characteristics were analyzed for both llamas and alpacas and it was found that fiber diameter heritability was moderate to low and there was a negative correlation between variation coefficient and fiber diameter (Frank et al., 2009; Aguilar et al., 2019).

Variation in *KRTAP7-1* and *KRTAP8-1* genes has been associated with fiber diameter and fleece weight in sheep and goat (Parsons et al., 1994; Liu et al., 2011). We studied these genes in llamas and found they were highly polymorphic. Most of the polymorphisms produce nonconservative amino acid changes, thus they might modify the fiber characteristics, but it remains to be investigated (Daverio et al., 2019). In alpacas, Mendoza et al., (2019) cytogenetically mapped several candidate genes related to fiber growth and bioinformatically inspected all VicPac2.0.2 scaffolds for additional genes. They found a tandemly arranged cluster of five more keratin genes (*KRT31*, *KRT13*, *KRT9*, *KRT14*, and *KRT16*) in chromosome 16 around *KRT15* which makes this chromosome a good target to identify sequence variants potentially associated with hair texture and growth. In another related study, 35 markers were mapped, four of which corresponded to candidate genes for fiber traits (*BMP4*, *COL1A2*, *GLII*, *SFRP4*) (Mendoza et al., 2020). Even if these studies represent significant advances, it still remains to be investigated how these genes affect fiber characteristics.

## Conclusions

In the last decade, significant progress has been made in identifying the genes that control coat color in South American camelids. The detection of causal mutations of relevant phenotypes for breeders, such as the classic gray in alpacas, has allowed the development of a commercial test by Neogen (Jones et al., 2019). This is the first test for color genes in camelids launched on the market, but it is expected that others will appear very soon.

## About the Author(s)



Florencia Di Rocco received her PhD degree in Biological Sciences from the University of La Plata in 2008. She is currently the head of the Molecular Genetics Laboratory at the Instituto Multidisciplinario de Biología Celular (IMBICE), La Plata, Argentina. Her research focuses on llama coat color genetics as well as wild camelid conservation genetics and genomics. Animal and human forensic genetics are among her other scientific interests. Corresponding author: [fdirocco@imbice.gov.ar](mailto:fdirocco@imbice.gov.ar)

Maria Silvana Daverio has obtained PhD from the National University of La Plata (UNLP). Currently, she is assistant researcher of the National Council of Investigation (CONICET), professor of Biology at Faculty of Exact Sciences of UNLP. Her fields of interest are related to molecular genetic and animal production and improvement. Recently, she has been working on the study and characterization of candidate genes, in the identification and association of polymorphisms with characters of productive interest in domestic camelids, mainly in traits associated with fiber quality. She also has been involved in projects concerning with coat color in Argentine llamas and vicuña conservation.



Melina Anello is a post-doctoral researcher at the “Instituto Multidisciplinario de Biología Celular (IMBICE) UNLP-CONICET-CIC”, Argentina. She has a degree in Biotechnology and Molecular Biology a PhD in Biological Sciences (National University of La Plata, Argentina). During her doctoral thesis she studied genes related to pigmentation in the llama. Currently, she continues researching in various topics related to the genetics and genomics of South American camelids. Among her scientific works, stand out: “TYR Gene in Llamas: Polymorphisms and Expression Study in Different

Color Phenotypes” (Anello et al., 2019a); “Characterization and expression analysis of KIT and MITF-M genes in llamas and their relation to white coat color” (Anello et al., 2019b); “The ASIP gene in the llama (Lama glama): transcripts, expression and relation with color phenotypes”, (Anello et al., 2022).

Molecular testing presents diverse applications, from optimizing the chances of obtaining a desired phenotype to preserve color allele diversity. For example, to obtain a black alpaca a pair of the three different alleles is necessary, thus mating could be designed between black animals with different genotypes (e.g.,  $a^1a^2xa^2a^3$ ,  $a^1a^3xa^1a^2$ , etc.) to conserve the existing color allele pool. Additionally, genetic testing can be useful to prevent some health conditions associated to coat color, like lethal gray. By performing a DNA test on sires and dams, alpaca breeders could identify animals carrying classic gray alleles and avoid risky mating.

Some advances have also been made in understanding the inheritance mechanisms of other fiber traits in llamas and alpacas, ranging from simple single gene models to more complex additive multigene systems, depending on the characteristic. Moreover, significant genetic variability has been found in genes encoding structural protein of the fiber, suggesting that genetic improvement would be possible when the variants associated to desirable traits are discovered.

Despite the progress achieved, there is still a lack of large-scale studies linking phenotypic traits to candidate genes in South American camelids. In this sense, the release of the chromosome-level Alpaca Reference Genome (VicPac3.1) in 2019 marked a turn point in camelid research, allowing the mapping of candidate genes for fiber traits, the identification of thousands of SNPs and the development of a high-density SNP array. The application of this array in genome wide association studies (GWAS) in both the alpaca and the llama, will contribute to accelerate the discovery of new genes and pathways related to pigmentation and will allow to advance towards the identification of genetic variation influencing more complex traits, like the ones that affect fiber quality.

## Acknowledgment

We would like to thank Eduardo N Frank, Alan Cruz Camacho, and Rubén Pinares Huamani by providing us with photos of llama fleece and alpacas color phenotypes. F. Di Rocco is a researcher from the Commission of Scientific Research of the Province of Buenos Aires (CIC).

*Conflict of interest statement.* The authors declare that they have no conflict of interest.

## Literature Cited

Aguilar, H., G. Gutiérrez, and M. Wurzinger. 2019. Parámetros genéticos de caracteres asociados a la uniformidad del diámetro de fibra en alpacas Huacaya en Puno, Perú. *Rev Inv Vet Perú* 30(3):1150–1157. doi:10.15381/rivep.v30i3.15370

Anello, M., M.S. Daverio, S.S. Rodríguez, S.R. Romero, C. Renieri, L. Vidal Rioja, F. Di Rocco. 2022. The ASIP gene in the llama (*Lama glama*): Alternative transcripts, expression and relation with color phenotypes. *Gene* 809:146018. doi:10.1016/j.gene.2021.146

Anello, M., M.S. Daverio, M.B. Silbestro, L. Vidal-Rioja, and F. Di Rocco. 2019a. Characterization and expression analysis of KIT and MITF-M genes in llamas and their relation to white coat color. 1–7. doi:10.1111/age.12769

Anello, M., E. Fernández, M.S. Daverio, L. Vidal Rioja, and F. Di Rocco. 2019b. TYR Gene in Llamas: Polymorphisms and Expression Study in Different Color Phenotypes. *Front. Genet.* 10:1–9. doi:10.3389/fgene.2019.00568

Anello, M., E. Fernandez, M.B. Silbestro, F. Veiga, L. Vidal-Rioja and F. Di Rocco. 2019c. Characterization and expression analysis of SLC7A11 in llamas. In: Gerken M, Renieri C, Allain D, et al. (eds) *Advances in Fibre Production Science in South American Camelids and other Fibre Animals*. Universitätsverlag Göttingen. Göttingen 2019. pp. 61–69.

Cieslak, M., M. Reissmann, M. Hofreiter, and A. Ludwig. 2011. Colours of domestication. *Biol. Rev.* 86:885–899. doi:10.1111/j.1469-185X.2011.00177.x

Chandramohan, B., C. Renieri, V. La Manna, and A. La Terza. 2013. The alpaca agouti gene: Genomic locus, transcripts and causative mutations of eumelanic and pheomelanic coat color. *Gene* 521:303303310. doi:10.1016/j.gene.2013.03.060 310

Daverio, M.S., M. Anello, V. Alcolea Ersinger, S. Alvarez, E. Frank, L. Vidal-Rioja, and F. Di Rocco. 2019. Identification of llama KRTAP7-1 and KRTAP8-1 fiber genes and polymorphism screening. *Small Rumin Res* 175:149–154.

Daverio, M.S., F. Rigalt, S. Romero, L. Vidal Rioja, F. Di Rocco. 2016. Polymorphisms in MC1R and ASIP genes and their association with coat color phenotypes in llamas (*Lama glama*). *Small Rumin Res* 144:83–89. doi:10.1016/j.smallrumres.2016.08.003

Daverio, M.S., L. Vidal-Rioja, E.N. Frank, and F. Di Rocco. 2017. Molecular characterization of the llama FGF5 gene and identification of putative loss of function mutations. *Anim. Genet.* 48(6):716–719.

Fan, R., Z. Gu, X. Guang, J.C. Marín, V. Varas, B.A. González, J.C. Wheeler, Y. Hu, E. Li, X. Sun, et al. 2020. Genomic analysis of the domestication and post-Spanish conquest evolution of the llama and alpaca. *Genome Biol.* 2:21(1):159.

Feeley, N.L., S. Bottomley, and K.A. Munyaerd. 2011. Three novel mutations in ASIP associated with black fibre in alpacas (*Vicugna pacos*). *J Agric Sci* 149:529–538. doi:10.1017/S0021859610001231

Feeley, N.L., and K.A. Munyard. 2009. Characterisation of the melanocortin-1 receptor gene in alpaca and identification of possible markers associated with phenotypic variations in colour. *Anim Prod Sci* 49:675. doi:10.1071/AN09005

Frank, E.N. 1999. Mejoramiento genético en camélidos sudamericanos domésticos. Una propuesta para la población argentina. [https://www.produccion-animal.com.ar/libros\\_on\\_line/23-curso\\_camelidos\\_1999/04-mejoramiento\\_genetico.pdf](https://www.produccion-animal.com.ar/libros_on_line/23-curso_camelidos_1999/04-mejoramiento_genetico.pdf)

Frank, E.N. 2001. Descripción y análisis de segregación de fenotipos de color y tipos de vellón en Llamas argentinas. Tesis Doctoral UBA, 204pp

Frank, E.N. 2017. Comercialización de fibras de camélidos sudamericanos. Serie Documentos Internos SUPPRAD N° 5, Red SUPPRAD 2016. Versión electrónica en [http://pa.bibdigital.uccor.edu.ar/1332/1/DT\\_Frank.pdf](http://pa.bibdigital.uccor.edu.ar/1332/1/DT_Frank.pdf)

Frank, E.N., M.V.H. Hick, C.D. Gauna, H.E. Lamas, C. Renieri, and M. Antonini. 2006. Phenotypic and genetic description of fibre traits in South American domestic camelids (*Llamas* and *alpacas*). *Small Rumin Res* 61:113–129.

Frank, E.N., M.V.H. Hick, M. Molina, and L. Caruso. 2009. Heredabilidades, correlaciones fenotípicas, correlaciones genéticas y repetibilidades de variables productivas en llamas argentinas. *Archivos Latinoamericanos de Producción Animal.* 17(3-4):103–107. ISSN-e 1022-1301, ISSN 1022-1301.

Gauly, M., J. Vaughan, S.K. Hogueve, and G. Erhardt. 2005. Brainstem auditory evoked potential assessment of auditory function and congenital deafness in llamas (*Lama glama*) and alpacas (*L. pacos*). *J Vet Intern Med.* 19:756–760. doi:10.1892/0891-6640(2005)19[756:bapaoa]2.0.co;2.

Guillén, P.A., and V.V. Leyva. 2020. Variación en el diámetro de fibra por efecto de la medulación en vellones finos de alpacas Huacaya de tres grupos etarios. *Rev Inv Vet Perú* 31(4):e19026. doi:10.15381/rivep.v31i4.19026

Hattori, Y., M. Yamasaki, and N. Itoh. 1996. The rat FGF-5 mRNA variant generated by alternative splicing encodes a novel truncated form of FGF-5. *Biochim. Biophys. Acta* 1306:31–33. doi:10.1016/0167-4781(19)60001-1

Hick, M.V.H., H.E. Lamas, J. Echenique, P. Alejandro, M.F. Castillo, and E.N. Frank. 2009. Estudio demográfico de los atributos morfológicos y productivos en poblaciones de Llamas (*Lama glama*) de la provincia de Jujuy, Argentina. *Anim Genet Res Information* 45:71–78.

Jackling, F.C., W.E. Johnson, and B.R. Appleton. 2014. The genetic inheritance of the blue-eyed white phenotype in alpacas (*Vicugna pacos*). *J. Hered.* 105:847–857. doi:10.1093/jhered/ess093



- Jones M., C. Sergeant, M. Richardson, D. Groth, S. Brooks, K. Munyard. 2019. A non-synonymous SNP in exon 3 of the KIT gene is responsible for the classic grey phenotype in alpacas (*Vicugna pacos*). *Anim Genet.* 50(5):493–500. doi:[10.1111/age.12814](https://doi.org/10.1111/age.12814)
- Jost, S.M., A. Knoll, G. Lühken, C. Drögemüller, P. Zanolari. 2020. Prevalence of coat colour traits and congenital disorders of South American camelids in Austria, Germany and Switzerland. *Acta Vet Scand* 62:56. doi:[10.1186/s13028-020-00554-y](https://doi.org/10.1186/s13028-020-00554-y)
- Kadwell, M., M. Fernandez, H.F. Stanley, R. Baldi, J.C. Wheeler, R. Rosadio, and M.W. Bruford. 2001. Genetic analysis reveals the wild ancestors of the llama and the alpaca. *Proc Biol Sci.* 268(1485):2575–2584. doi:[10.1098/rspb.2001.1774](https://doi.org/10.1098/rspb.2001.1774)
- Kozłowski, R.M., and M. Mackiewicz-Talarczyk. 2020. Introduction to natural textile fibres. *Handb Nat Fibres Second Ed* 1:1–13. doi:[10.1016/B978-0-12-818398-4.00001-3](https://doi.org/10.1016/B978-0-12-818398-4.00001-3)
- Kuczek, E.S., and G.E. Rogers. 1987. Sheep wool (glycine + tyrosine)-rich keratin genes family of low sequence homology. *Eur. J. Biochem.* 166: 79–85
- Laime Huarcaya, F.M., R. Pinares Huamani, V. Paucara Ocsa, V. Machaca Machaca, and E.C. Quispe Peña. 2016. Características Tecnológicas de la Fibra de Llama (*Lama glama*) Chaku antes y después de Descender. *Rev Inv Vet Perú* 27(2):209–217. doi:[10.15381/rivep.v27i2.11643](https://doi.org/10.15381/rivep.v27i2.11643)
- Lu, D., D. Willard, I.R. Patel, S. Kadwell, L. Overton, T. Kost, M. Luther, W. Chen, R.P. Woychik, W.O. Wilkison, et al. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature* 371(6500):799–802. doi:[10.1038/371799a0](https://doi.org/10.1038/371799a0)
- Liu, H., C. Yue, W. Zhang, X. Zhu, G. Yang, and Z. Jia. 2011. Association of the KAP 8.1 Gene polymorphisms with fibre traits in inner mongolian cashmere goats. *Anim Biosci* 24(10):1341–1347.
- Liu, D.D., Z.Y. Zhang, X.Y. He, Y.J. Dong, Z.W. Zhu, R. Bai, J. Zhang, H.Q. Hao, and H.Q. Xing. 2011. FGF5 Expression and immunolocalization in back and ear skin of young alpaca (*Lama pacos*). *Chin J Biochem Mol Biol.* 27(5):473–479.
- Mackay, T.F.C. 2003. The genetic architecture of quantitative traits. *Annu. Rev. Genet.* 35:303–339. doi:[10.1146/ANNUREV.GENET.35.102401.090633](https://doi.org/10.1146/ANNUREV.GENET.35.102401.090633)
- Marcoppido, G.A., and B.L. Vila. 2013. Manejo de Llamas extra-andinas: Observaciones que contribuyen a su bienestar en un contexto no originario. *Rev Argentina Prod Anim* 33(2):139–154.
- Marín J.C., R. Rivera, V. Varas, J.Cortés, A. Agapito, A. Chero, A. Chávez, W.E. Johnson and P. Orozco-terWengel. 2018. Genetic variation in coat colour genes MC1R and ASIP provides insights into domestication and management of South American Camelids. *Front. Genet.* 9:487. doi:[10.3389/fgene.2018.00487](https://doi.org/10.3389/fgene.2018.00487)
- Mendoza M.N., T. Raudsepp, F. Alshanbari, G. Gutiérrez and F.A. Ponce de León. 2019. Chromosomal localization of candidate gGenes for fiber growth and Color in Alpaca (*Vicugna pacos*). *Front. Genet.* 10:583. doi:[10.3389/fgene.2019.00583](https://doi.org/10.3389/fgene.2019.00583)
- Mendoza M.N., T. Raudsepp, M.J. More, G.A. Gutiérrez and F.A. Ponce de León. 2020. Cytogenetic mapping of 35 new markers in the alpaca (*Vicugna pacos*). *Genes (Basel)*. 11(5):522. doi:[10.3390/genes11050522](https://doi.org/10.3390/genes11050522)
- Mueller, J., F. Rigalt, H. Lamas, D. Sacchero, A. Cancino, and M. Wurzing. 2015. Fibre quality of South American camelids in Argentina: a review. *Anim Genet Resour/Ressour Génétiques Animales/Recursos Genéticos Animales* 56:97–109. doi:[10.1017/S2078633614000496](https://doi.org/10.1017/S2078633614000496)
- Munyard, K. 2011. Inheritance of White Colour in Alpacas (RIRDC Publication No. 11/074). Rural Industries Research and Development Corporation, Barton, Australia.
- Pallotti, S., D. Pediconi, D. Subramanian, M.G. Molina, M. Antonini, M.B. Morelli, C. Renieri, and A. La Terza. 2018. Evidence of post-transcriptional readthrough regulation in FGF5 gene of alpaca. *Gene* 647:121–128.
- Parsons, Y.M., D.W. Cooper, and L.R. Piper. 1994. Evidence of linkage between high-glycine-tyrosine keratin gene loci and wool fibre diameter in a Merino half-sib family. *Anim. Genet.* 25:105–108.
- Paus, R., and G. Cotsarelis. 1999. The biology of hair follicles. *N Engl J Med.* 341(7):491–7. doi:[10.1056/NEJM199908123410706](https://doi.org/10.1056/NEJM199908123410706)
- Pérez-Cabal, M.A., I. Cervantes, R. Morante, A. Burgos, F. Goyache, and J.P. Gutiérrez. 2010. Analysis of the existence of major genes affecting alpaca fiber traits. *J. Anim. Sci.* 88(12):3783–3788.
- Plowman, J.E., S. Deb-Choudhury, W.G. Bryson, S. Clerens, and J.M. Dyer. 2009. Protein expression in orthocortical and paracortical cells of merino wool fibers. *J. Agric. Food Chem.* 57:2174–2180.
- Powell, B.C., and J.S. Beltrame. 1994. Characterization of a hair (wool) keratin intermediate filament gene domain. *J. Invest. Dermatol.* 102:171–177.
- Presciuttini S., A. Valbonesi, N. Apaza, M. Antonini, T. Huanca, and C. Renieri. 2010. Fleece variation in alpaca (*Vicugna pacos*): a two-locus model for the Suri/Huacaya phenotype. *BMC Genet.* 11:70. doi:[10.1186/1471-2156-11-70](https://doi.org/10.1186/1471-2156-11-70)
- Renieri, C., C. Pacheco, A. Valbonesi, E. Frank, and M. Antonini. 2007. Selection program in south American domestic camelids. *Arch. Latinoam. Prod. Anim.* 15(Supl. 1):205–210.
- Renieri, C, A. Valbonesi, V. La Manna, M. Antonini, and M. Asparrin. 2009. Inheritance of Suri and Huacaya type of fleece in Alpaca. *Italian J. Anim. Sci.* 8(1)83–91. doi:[10.4081/ijas.2009.83](https://doi.org/10.4081/ijas.2009.83)
- Rogers, G.E. 2006. Biology of the wool follicle: an excursion into a unique tissue interaction system waiting to be re-discovered. *Exp. Dermatol.* 15(12):931–949.
- Suzuki, S., Y. Ota, K. Ozawa, and T. Imamura. 2000. Dual-mode regulation of hair growth cycle by two Fgf-5 gene products. *J. Invest. Dermatol.* 114:456–463. doi:[10.1046/j.1523-1747.2000.00912.x](https://doi.org/10.1046/j.1523-1747.2000.00912.x)
- Valbonesi, A., N. Apaza, V. La Manna, M.L. Gonzales, T. Huanca, and C. Renieri. 2011. Inheritance of white, black and brown coat colours in alpaca (*Vicugna pacos* L.). *Small Rumin Res* 99:16–19. doi:[10.1016/j.smallrumres.2011.04.003](https://doi.org/10.1016/j.smallrumres.2011.04.003)
- Wheeler, J.C. 2012. South American camelids-past, present and future. *J Camelid Sci* 5:1–24.
- Yacobaccio, H.D., and B. Vilá. 2013. La domesticación de los camélidos andinos como proceso de interacción humana y animal. *Intersecc en Antropol* 14:227–238.
- Yu, Z., S.W. Gordon, A.J. Nixon, C.S. Bawden, M.A. Rogers, J. E. Wildermoth, N. J. Maqbool, and A. J. Pearson. 2009. Expression patterns of keratin intermediate filament and keratin associated protein genes in wool follicles. *Differentiation* 77(3):307–316. doi:[10.1016/j.diff.2008.10.009](https://doi.org/10.1016/j.diff.2008.10.009)