

ALPACA COLOUR GENETICS: Mendel Meets Molecular

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Over the past seven years the team at the Alpaca Molecular Research group at Curtin University has been researching the inheritance patterns and molecular causes of colour in alpacas. Using a combination of Mendelian genetics principles, molecular genetic techniques, objective chemical analysis of the fibre and observation of skin and nail colour we have been able to arrive at a model that, we think, describes most of the colour variation in alpacas. The current nomenclature for alpaca colours contributes to the confusion. One person's fawn is another's light brown and one person's mid brown is another's red brown. We therefore propose a new set of names for base colour varieties that reflects the genetic basis of the colour.

Alpaca colour genetics can be broken down into two parts, base colour and pattern. There are only two genes that control the base colour of the animal, MC1R and agouti. However, there are several genes that control the many patterns that are possible, e.g. classic grey, roan, greying, tuxedo, piebald, appaloosa, vicuna, dilution. Any base colour can co-exist with any pattern, more than one pattern, or none of the patterns.

BASE COLOUR

The base colour in alpacas ranges from white to black, through fawn and brown, with or without black on the extremities. The base colour arises because the genes MC1R and agouti work together in the pigment producing cells to tell the cells what colour pigment to produce. Mammals can only produce two types of pigment, yellow and black, and all the variety of colour is produced by differing amounts and locations of these two pigments.

Agouti variants are probably responsible for most colours in alpacas. Our data suggests that agouti has four variants in alpacas, each of which leads to a different colour outcome. The difficulty in assigning an accurate colour classification, (that is, determining which agouti variant is present) is that there is a range of colour intensity for each variant. The most dominant agouti variant "A" produces white through to fawn fibre. The next most dominant variant "A^b" which we propose to call 'bay' is characterised by a tan/brown body with black on the extremities (the same as bay in horses). Next in the hierarchy is "a", which we propose to call 'black & tan'. This one produces a

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black body with tan on the undersides, similar to a Doberman dog, and could be considered to be the reverse of bay. Finally, the most recessive agouti variant is "a". An alpaca with only "a" present will be black, and should more correctly, from a scientific point of view, be called recessive black. All animals with an Agouti base colour have black skin regardless of the colour of their fibre. Because each individual has two copies of its genome, each gene can have up to two variants in a single animal. Therefore, these four variants can occur in ten different combinations, leading to the huge range of different shades of base colour. The use of the term 'brown' to describe any alpaca colour is very misleading. From a scientific/genetic point of view, 'brown' describes a colour that is caused by a defect in black pigment, which makes the black pigment look brown. Our research has shown that the majority of alpacas described as brown are actually different shades of yellow. In fact we have not found ANY true brown alpacas, but we haven't tested them all, so we are being cautious in saying 'most'. The darker 'brown' alpacas are actually yellow with differing amounts of black mixed in. We have shown that:

- White and fawn alpacas have only small amounts of yellow pigment, and negligible amounts of black pigment;
- Brown, dark brown and black brown alpacas have mixed yellow and black pigment in different proportions, and
- Black alpacas have about the same amount of yellow pigment as fawns, but have much more black pigment, so that the black pigment masks the presence of the yellow pigment.

MC1R is a relatively simple gene, it either allows (via the dominant wild-type variant "E") or prevents (via the recessive variant "e") the

production of black pigment. So, agouti sets the base colour, then MC1R variants determine if the black part of the agouti colour will be allowed to occur or not. With white (AA) and fawn (AA^b), the fibre contains only negligible traces of black, so the only visible effect of the preventative MC1R variant is on the skin, this is how you get a pink skinned white alpaca. Preventative MC1R variants have a greater effect on bay (A^bA^b), black-bay (A^ba or A^ba^b), black & tan (a^ba^b or A^ba^b) and black (aa) alpacas. Bay coloured alpacas become chestnut (A^bA^b ee), just like in horses. Black-bay (A^ba ee or A^ba^b ee) could be anything from chestnut to fawn in colour. Black, when accompanied by preventative MC1R variants (aa ee), becomes chestnut through to white, depending on how much yellow pigment was hidden by the black pigment. All of these dark base outcomes are determined by how much of the yellow pigment is present, the more yellow pigment, the darker the fibre colour. These darker 'ee' alpacas have dark skin, but it will only be as dark as the fibre, and will not be black. Nature has added a twist to this story. Some animals with pink skin (genetically) will develop black pigment as they age, in response to sun exposure. So, it can be hard to tell if an older animal has: a) skin the same colour as its fibre, b) pink skin, or c) black skin.

If we add the two MC1R variants, which can occur in three different combinations, to the ten agouti variant combinations, we get 30 different colour outcomes possible from just these two genes. That is more than enough to explain all of the normal base colours in alpacas. Our research has identified the DNA signatures of the two different MC1R variants, and two of the four agouti variants.

A line up of colours!



PATTERNS

All of the patterns in alpacas are caused by genes creating a variation to the base colour. All of the pattern genes have a wild-type variant that does nothing, plus one or more variants that cause the pattern. Each alpaca will have two copies of every pattern gene, this could be two wild-type (do nothing) variants, or one of each of wild-type and pattern-causing, or two pattern-causing copies. The patterns classic grey, roan, greying, appaloosa, vicuna, tuxedo, piebald, blue-eyed white and dilution will be discussed here.

CLASSIC GREY (M)

Silver grey and rose grey are the result of a single pattern variant acting on different base colours. In our lab we call this pattern classic grey and the gene symbol is 'M' (named after the pattern 'merle' that it resembles) until the gene identity is confirmed. Classic grey is used as a term as opposed to simply 'grey' to differentiate it from the well known greying characteristic found in many other species and possibly in alpacas too. Silver grey (aa E- Mm) is a classic grey variant on a black base colour. Rose grey is a classic grey variant on any other base colour. This explains the huge variety of different rose greys that are seen. The typical signs of a classic grey are that the neck and legs are paler than the body, the body is a diluted version of the base colour, and that the overall depth of colour of the animal often increases with age. They also commonly have a pale face, and a non-diluted bonnet of colour on the head. When you examine the fibre under a microscope they are not a mixture of black and white fibres, instead the fibres are diluted to different degrees from white to the fully intense base colour.

Some classic greys have spots or patches of



undiluted fibre in random places. The classic grey variant is easy to see on a dark background, but can be very hard to see on a pale background, and impossible to see on a white. Genetically one copy of the classic grey variant is required to be present to cause the classic grey pattern (Mm). In genetic terms it is an incomplete dominant. Pedigree analysis by Elizabeth Paul has shown that the presence of two copies of classic grey (MM) is lethal at the embryo stage of development. Hence, you don't get any 'true breeding' classic greys. Our analysis of pedigrees, combined with work done by Dr Belinda Appleton suggest that there may be at least three different classic grey variants, each of which produces a different version of the pattern.

ROAN (Rn)

Roan is in many ways the reverse of the classic grey pattern. Roan animals have a diluted body with undiluted neck and legs. Microscopic examination of the fibre shows that the roan pattern is caused by a mixture of fully pigmented and white fibres. In contrast to classic grey, a roan will get paler with age, and the body may end up almost completely white. Roan animals are

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Top: Appaloosa. Above and right: Tuxedo/Piebald

Roan is not the same as progressive greying, although it can be hard to tell these two patterns apart



rarely born with the pattern showing, that is, a black roan will be born black, and will develop the typical dilution effect over time. The roan pattern can occur on top of any base colour. Similar to classic grey, the roan pattern is most easily seen on a dark background, and in our experience, white and fawn roans may not even be recognised as such. Roan is not the same as progressive greying, although it can be hard to tell these two patterns apart. Roan is also an incomplete dominant. Only one roan variant is needed to cause the pattern (RnRn). However, preliminary data suggests that when two roan variants are present the pattern progresses more quickly, and is not lethal (in some species homozygous roan is embryonic lethal). Breeders wishing to get 'whiter than white' fibre could introduce the roan pattern into the herd to remove any traces of pigment from the fibre.

GREYING (G)

Age related greying occurs in most species, and is caused by the premature death of stem cells in hair follicles. It is not clear whether this kind of grey occurs in alpacas as a distinct separate pattern, or if the milder forms of greying are a third variant of the roan pattern. The physical attributes are similar to roan, except for the differentiation of effect between the body and the legs. The inheritance pattern is unknown.

APPALOOSA (Lp)

Appaloosa is not, as most people think, a pale background with coloured spots. It is a dark

background (what is perceived as the spots) with pale spots (what is perceived as the background). Appaloosa can also occur on top of any base colour, and you can clearly see the bay base colour distribution of black and yellow pigment in some appaloosas. The pattern of inheritance has not been proven, but it is probably dominant or incomplete dominant. That is, one copy of the appaloosa variant is enough to cause the pattern (LpLp), and two copies will cause the same pattern (LpLp). Similar to all other patterns, appaloosa can't be seen on a white alpaca.

VICUNA

Vicuna is an intriguing pattern. This is the pattern where a fawn alpaca has white undersides with white extending on to the body behind the front legs. We have not completed a thorough analysis of this colour, and are hoping to do so as soon as possible. Two hypotheses to explain this pattern are currently under consideration. The first is that vicuna is a separate pattern in its own right, and the second is that it is simply a manifestation of the black & tan agouti base colour with non-permissive MC1R variants.

TUXEDO/PIEBALD

Animals with white patches are tuxedo or piebald. This is the only pattern that is localised to specific regions of the body. In effect the presence of a tuxedo or piebald variant leads to white spots of varying number, size and shape. It is not definitively known if tuxedo and piebald

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are caused by different genes, or by different variants of the same gene. It is also assumed that these two are distinct patterns. However, the evidence suggests that the tuxedo pattern is restricted to the head, neck and legs, while the piebald pattern occurs on the body as well, and tends to cross the dorsal mid line. Both tuxedo and piebald are dominant, that is, only one copy of the pattern variant needs to be present to cause the white pattern. Therefore, in any mating where one parent is white and the other is a solid colour and the cria is tuxedo or piebald, it is probably the white parent that has contributed the white spotting pattern. However, even a tiny amount of white on a solid animal is an indication that it is actually tuxedo or piebald.

BLUE-EYED WHITE

Blue-eyed white (BEW) is the most controversial of all alpaca patterns. The evidence indicates that classic grey is strongly implicated in this pattern. If a BEW is mated to a solid dark colour, the most common outcomes are classic grey or tuxedo, which suggests that the BEW is a combination of two pattern variants that leaches all the colour from the animal.

DILUTION

The final pattern being discussed is not really a pattern at all, but it does affect the base colour of the animal, so it fits into this section. Our research has shown that animals with the exact same gene variants present at A and E can be different colours. For example, fawn versus dark fawn. Therefore, there must be other genes acting to dilute out the colour in a uniform way. These genes are known to occur in other species, "D" in dogs causes black to appear steel grey, and brown

(real genetic brown) to appear milk chocolate coloured and red to appear champagne. In horses you see the very striking palomino and silver dilutions. Each of these dog and horse dilutions is caused by a variant of a single gene. We are currently analysing gigabases (i.e. billions of bases) of alpaca RNA sequence to try to find a gene or genes that have a similar effect in alpacas.

SUMMARY

The colour of an alpaca is controlled by its genes. If you evaluate colour in an objective way, you can usually work out which genes, and which variant of those genes, are creating the colour you see. If you also include information about an animal's parents and offspring, the success in predicting colour will increase markedly. DNA tests for these genes can be used to determine the genetic potential (in terms of colour) of an animal, and therefore allow a breeder to plan matings to produce, or not produce, a particular colour. We suggest that the names used to describe alpaca colours should reflect the genetics of these colours, so that there is more effective communication and more precise records, leading to higher predictability in breeding outcomes. 🐾

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Whites and fawns

