

AAA Colour Intensity Project October 2022 Update

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AAA has kindly granted us with funding to research the genetic cause for different in pigment intensity in alpacas. With the funding provided by AAA we aimed to do a combination of whole genome sequencing and objective colour measurements to try to find the cause(s) of different intensity in alpaca colour.

Thus far we have collected DNA and fibre samples, and received the raw sequence data generated from the DNA. Analysis of the sequence data is underway. We have also used the funds to assist with the purchase of a portable colorimeter, and have used this machine to objectively measure the fibre samples. We also trialled the use of the colorimeter on live animals at the Perth Royal Show (Figure 1).



Figure 1: Naomi using the colorimeter on live animals at the Perth Royal Show

A colorimeter is an instrument that measures colour in a way that matches the human perception of colour, and we explored the use of this instrument to classify colour of fibre more precisely & consistently than is possible with the human eye. The colorimeter measures colour using the $L^*a^*b^*$ colour space system. L^* (L-star) is a measurement of lightness, where 0 is black and 100 is white. The green-red spectrum is represented by a^* (a-star; negative values are green, positive values are red), and b^* represents the blue to yellow spectrum (b-star; negative values are blue, positive values are yellow).

We found that the colorimeter can be used to reproducibly and objectively measure the colour of alpaca fibre, both on and off the animal. There is even the possibility that it could be used at shows to provide information to help with classification of animals into different colour classes, in cases where there is doubt.

We used the colorimeter to measure the colour of warm black vs. true black (genotyped as $EEaa$) and white vs. light fawn fibre (genotyped as $eeAA$) samples. We found that true black fibre was darker (lower L^*), less red (lower a^*) and bluer (lower b^*) compared to warm black fibre (Figure 2). White and light fawn fibre both have relatively equal yellow measurements (b^*), however light fawn fibre has higher red measurements (a^*) compared to white fibre.



Figure 2: Comparison of true black and warm black colour as measured using CIEL*a*b*. All values are the average of six measurements per animal.

After confirming the utility of the colorimeter for this type of analysis, we expanded the study to over 30 fibre samples, many of which were unable to be differentiated by eye. This data confirmed that there is a continuum of intensity for both black and white fibre (Figure 3).

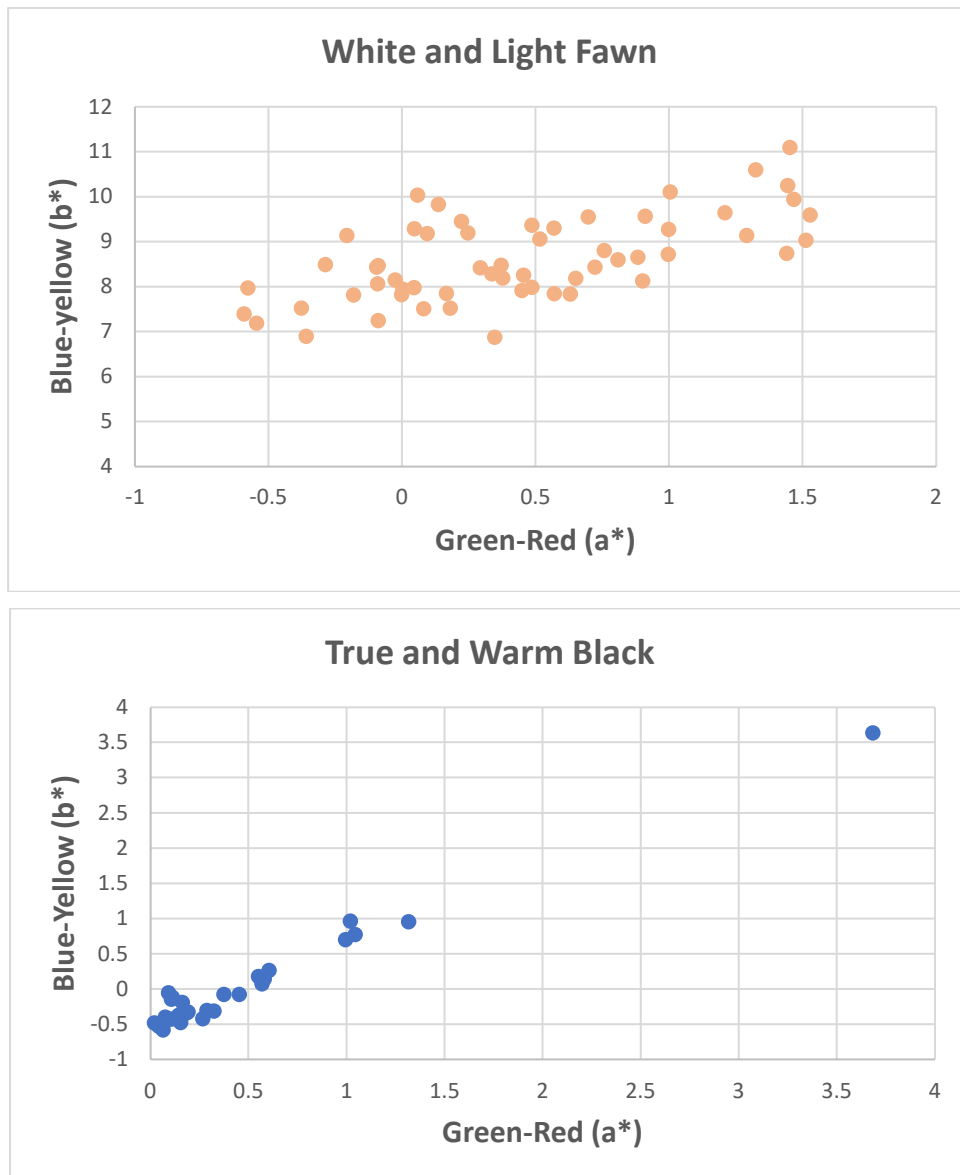


Figure 3: CIEL*a*b* measurement of alpaca fibre. Top: White/ Light Fawn, bottom: True black/ warm black. All values are the average of six measurements per animal.

Given the large range of colour measured in the true blacks and warm blacks, we then categorised the alpacas based on the specific ASIP genotype of the animals (a1, a2 or a3; Figure 4). Alpacas with a1a1 genotype tend to have true black colour measurements while alpacas with a3a3 genotype tend to have warm black colour measurements. This matches our understanding of the ASIP alleles as the a1 allele is thought to be the most disruptive mutation while the a3 allele has the weakest effect. Other allele combinations such as a2a2 or a1a3 are varied and tend to measure in between warm black and true black.

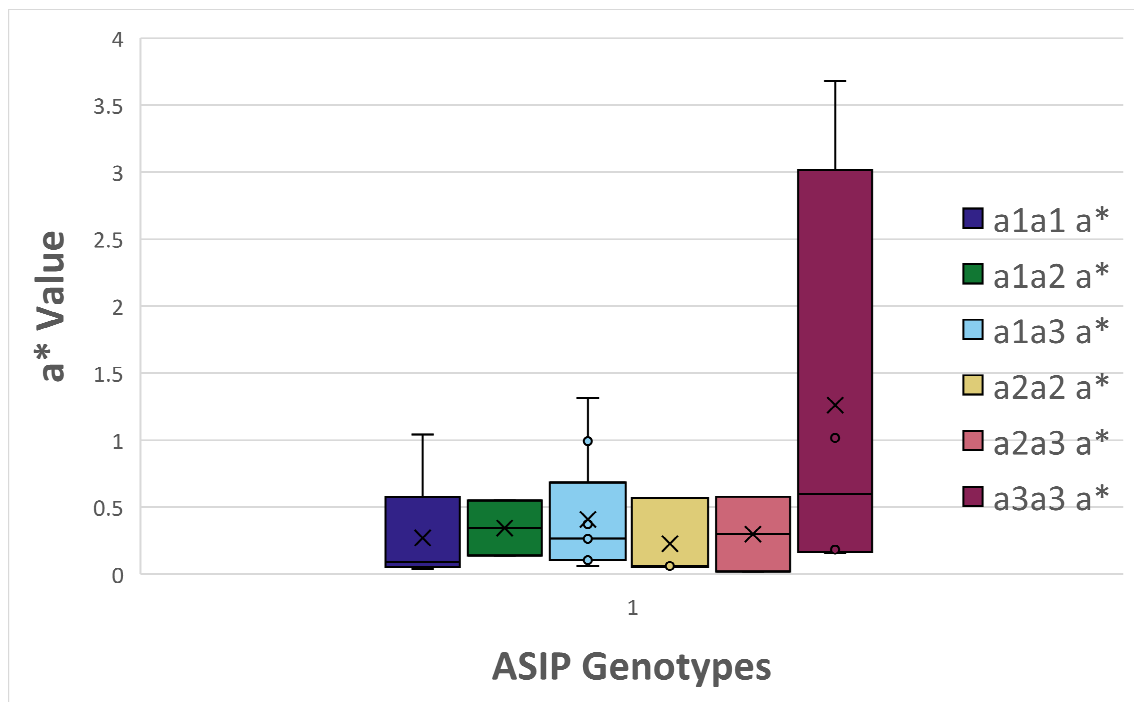


Figure 4: The fibre of alpacas genotyped as EEaa was measured using a colorimeter, and both a* and b* (not shown) values were compared between aa genotypes. Values shown are average and standard deviation a* value. The a* axis is representative of the green–red spectrum, with negative values toward green and positive values toward red. All values are the average of six measurements per animal.

However, there are warm black alpacas with a1a1 alleles and true black alpacas with a3a3 alleles. This indicates that the intensity of the black pigment is not solely linked to the ASIP allele. It is likely that there are additional variants that are either intensifying or diluting the pigment in these alpacas. For example, a warm black alpaca may have a1a1 alleles with a dilution variant or a true black alpaca may have a3a3 alleles with an intensity variant. Finding the genetic cause of this variation is the focus of the ongoing research.