



Color Genotyping: What We Are Learning

*Parade of Champions Auction
June 5, 2021*

Why We Decided To Learn Our Animals' Base Coat Color Genotypes

- Individual phenotypic colors are produced by more than one genotype
- Economic results are linked to color outcomes
- Better knowledge about the probability of various color outcomes allows selection pressure to shift more in favor of fleece traits
- Knowing color genotypes improves the value of our animals to other breeders



All Alpaca Base Coat Colors Are Produced By Varying Quantities of Yellow/Red and Black Pigment

The **ASIP** gene encodes a protein that regulates the production of yellow pigment, pheomelanin, by melanocytes



The **MC1R** gene encodes a protein that determines whether black pigment, eumelanin, will be produced by melanocytes

Here Is Why Knowing Our Animals' Genotypes For These Two Genes Is Valuable



**Snowmass
Standing Royalty**



**Snowmass
Rock Solid**



**Snowmass
Enchanted Wave**



**Snowmass
Smokin Waves**

Does he cover black?

Yes

No

Yes

Yes

Is he likely to produce
white or light
offspring when bred
to light females?

Yes

No

Yes

No

Will we breed the
male differently
knowing this
information?

Yes

Yes

Yes

Yes

Before We Tested Our Animals We Thought:

- There were three different “color genes”: Black, brown and white or light. Light was partially dominant over brown and fully dominant over black
- An animal could also be dilute and not express any black pigment in its fleece. This dilution was a recessive genetic trait





But Our Data Suggest:

- There may be no white or light ASIP gene mutation. White phenotypes are produced by dilution of an ASIP genotype that otherwise would result in a brown or black animal.
- Dilution is more complex and plays a greater role in the variation of color we see than we previously thought



Our First Sample Group

- We received results for 255 animals
- We tested mostly dark animals in the first group because we thought results for those animals would pay for themselves first
- The tests results revealed:
 - Whether or not the animal covered black (ASIP gene)
 - Whether it carried zero, one or two alleles for dilution (MC1R gene)
 - Whether or not our animals carried a homozygous-lethal mutation for tuxedo grey (KIT gene)



Interpreting the Test Results

- **ASIP Gene**
 - "A" indicates the presence of a non-black allele
 - "a" indicates the presence of one or more recessive mutations that result in reduced production of pheomelanin relative to eumelanin, with the outcome of a darker or black fleece
- **MC1R Gene**
 - "E" indicates the presence of an allele that allows for the expression of eumelanin, the black pigment
 - "e" indicates the presence of one or more mutations that result in reduced or eliminated production of eumelanin.
- **"Grey/Non-Grey"**
 - "Grey" indicates the presence of a homozygous-lethal mutation in the KIT gene that, when a single copy is present, produces a tuxedo grey phenotype visible in animals with darker coats. It does not capture the genotype of a modern grey, or roan. It is also possible that mutations other than that tested for can produce a tuxedo grey phenotype.



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Alpaca Coat Colour Test Results

Customer Name: LITTLE CREEK FARM ALPACAS

Date: 18/04/2021

Order Number: 620105

Here Is What The
Test Report Looks
Like

Sample Barcode	Animal ID	Registration Number	MC1R + ASIP Genotype	Base Coat Phenotype	Grey/Non-Grey Status^
31819038238	2871		EE aa	B	Non-Grey
31819038260	2756		ee AA	PSW CF CH	Non-Grey
31819038268	2345		EE Aa	W F BB	Non-Grey
31819038278	2144		Ee aa	B	Non-Grey
31819038249	2714		EE aa	B	Non-Grey



Our First Observations

- Almost all whites and lights carried two dilution mutations (“ee”), which made us wonder whether there was a light or white coat allele for ASIP (or any other gene) prevalent in our herd
- Some animals that were genotypically black were not phenotypically black. Most were brown and a few were lighter. WTH? (this is a technical term in the field of amateur genetics)
- The lighter our brown and fawn animals were, the more likely they were to carry one dilution mutation

Wild Color

The vicuna ancestor of alpacas was probably the same color as these modern vicuna – brown with a white ventral pattern. This color is presumably the “wild type” color, represented in our test results by “EE AA”. Over time, breeders have selected for gene mutations that produce other colors.

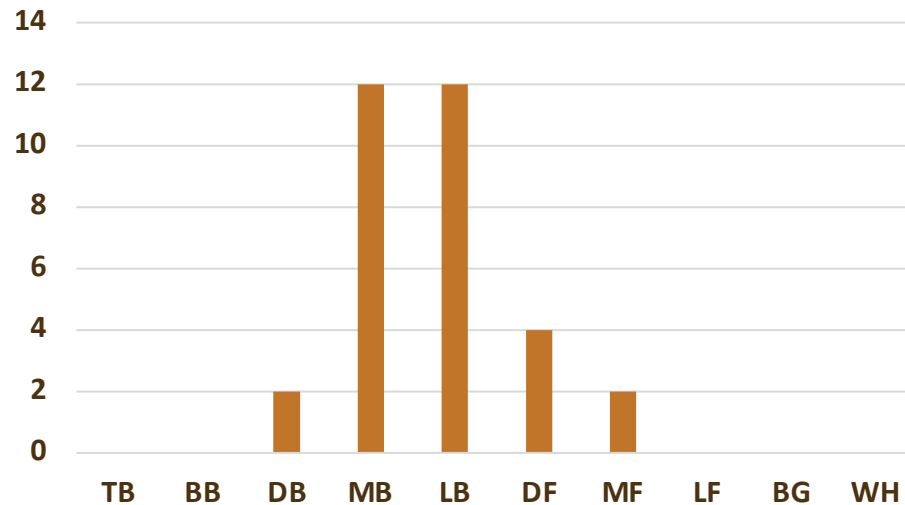




If the non-black ASIP "A" test result was capturing both a brown and a lighter type ASIP mutation, we would expect see some evidence of both in the chart below. But we only see evidence that "A" represents a wild-type color. This makes it harder to argue that there is a light-type ASIP allele present in our sample group.



**Number of EE AA animals by phenotypic color
in our first test group**



This May Be A Better Representation of ASIP Genotypes



"Wild Type" Brown AA



Dark Brown Aa



Black aa

And This May Better Represent the Complex Role That The MC1R Genotype Plays



**No dilution mutations
One black mutation
EE Aa**



**One dilution mutation
One black mutation
Ee Aa**



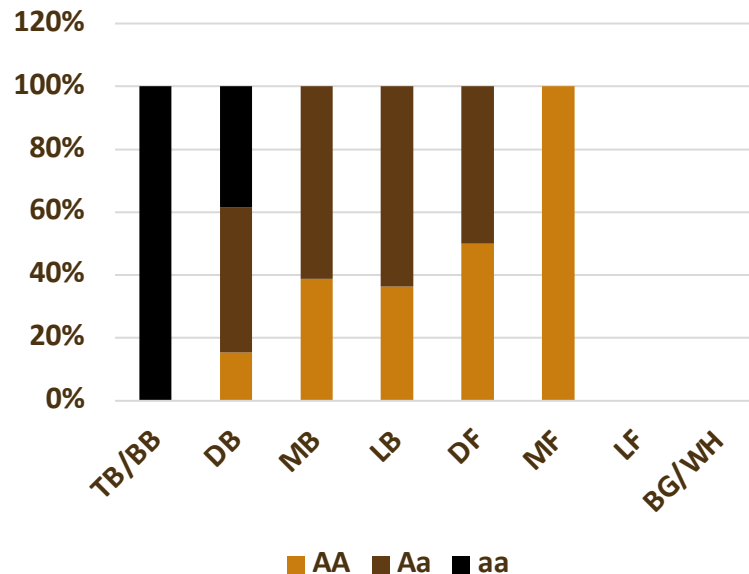
**Two dilution mutations
One black mutation
ee Aa**

These are tested genotypes.

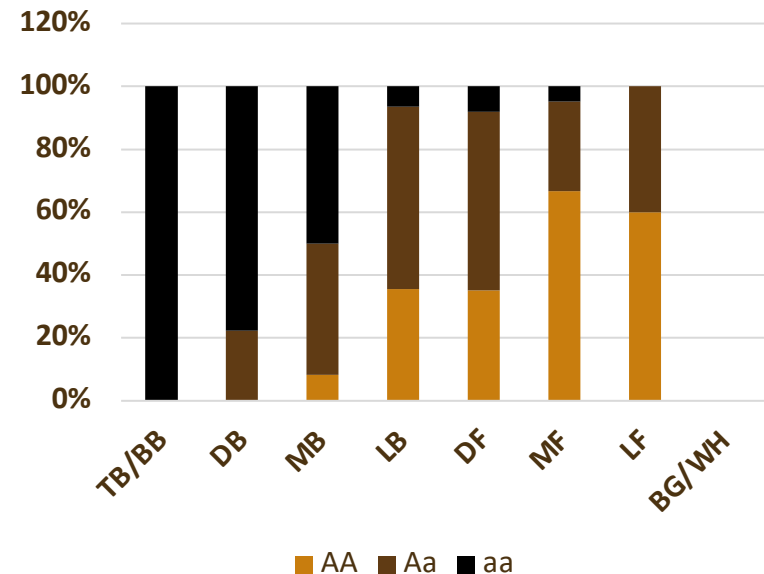
(if you don't like graphs cover your eyes!)

We See A Relationship Between Depth of Color and MC1R Genotypes

Of the Animals With Zero Dilution Mutations (EE), Percentage With Different ASIP Genomes



Of the Animals With One Dilution Mutation (Ee), Percentage with Different ASIP Genomes



For most phenotypic colors, the ASIP genotypes are on average lighter for animals with no MC1R dilution mutation present than for those with one. This association was statistically significant for the phenotypic colors for which we had sufficient data in the first group.

Genetic Researchers Have Found A Similar Relationship

The MC1R Genotypes And Phenotypes of Peruvian Alpaca

c.A82G (p.T28A)	c.A259G (p.V87M)	c.A376G (p.G126S)	c.C901T (p.R301C)	Color	Number of animals	Proposed MC1R alleles
A/A	A/A	A/A	C/C	Black	15	EE
G/G	G/G	G/G	T/T	White	14	ee
A/G	A/G	A/G	C/T	Brown	15	Ee
A/G	A/G	A/G	C/T	Black	01	Ee
A/G	A/G	A/G	C/C	Black	01	Ee
G/G	G/G	G/G	T/C	White	01	Ee

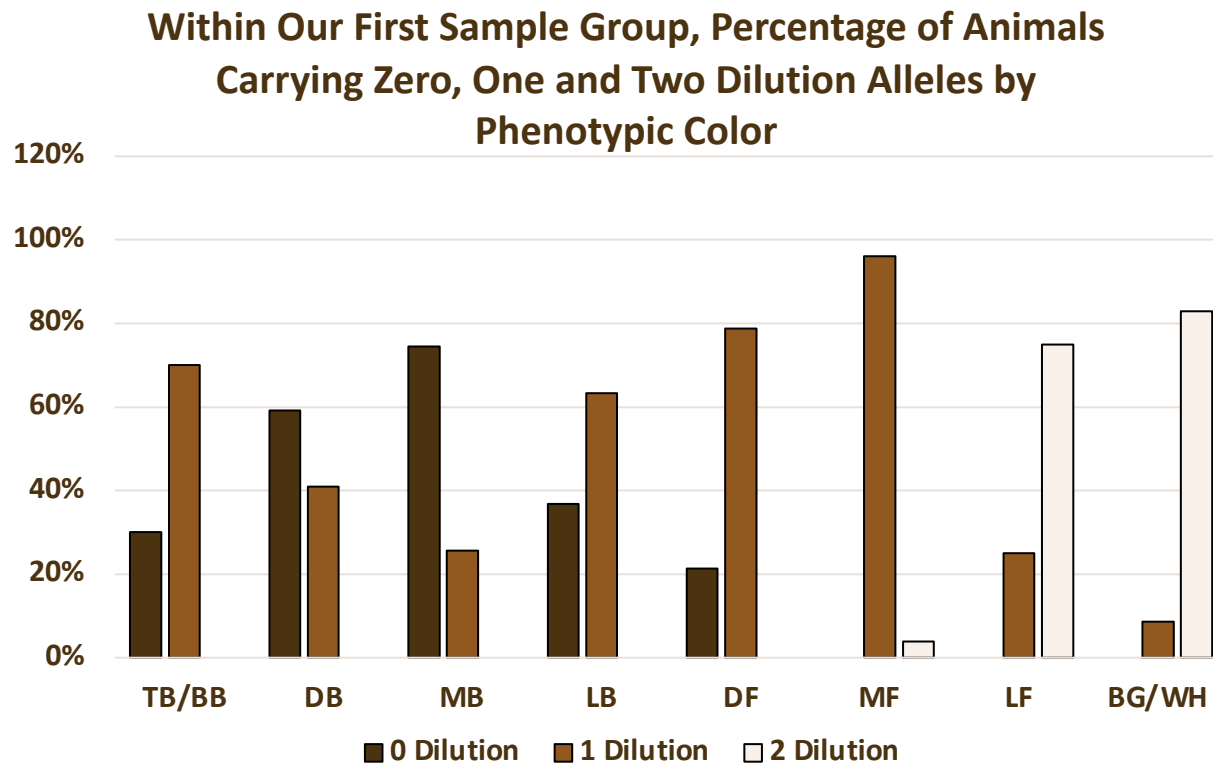
The Alpaca *Melanocortin 1 Receptor*: Gene Mutations, Transcripts, and Relative Levels of Expression in Ventral Skin Biopsies

Bathrachalam Chandramohan, Carlo Renieri, Vincenzo La Manna, Antonietta La Terza

ScientificWorldJournal. 2015; 2015: 265751. Published online 2015 Jan 5. doi: 10.1155/2015/265751

PMCID: PMC4313674

One Other Way To Look At It



A Rough Guide To
Expected *Average* Depth
Of Color For Different
Genotypes, Based On Our
First Round Of Testing

	0 Black AA	1 Black Aa	2 Black aa
0 Dilution EE			
1 Dilution Ee			
2 Dilution ee			

Blacks

- We tested 20 true black or bay black animals. All were genotypically black (aa)
- We also found 29 animals that are genotypically black at ASIP but not phenotypically black. Most were dark or medium brown
- The most common true black and bay black genotype was Ee aa (69% of true blacks and 83% of bay blacks)
- Is there more than one type of “black” genotype?



Dark And Medium Browns

- 67 animals tested with results
- The majority did not carry a MC1R dilution mutation. Of the ones that did, most were genetically black at ASIP ("aa")
- All but one of the dark browns covered black
- The most common genotypes in our sample group were:
 - Dark brown: Ee aa (44%)
 - Medium brown: EE AA (22%)





Light Browns

- We received results for 48 animals
- The most common genotype was Ee Aa.
- But along with medium browns, this phenotypic color was the most likely to be produced by wild/wild EE AA genotype (25% of our sample)

Fawns

- We have results for 79 animals
- Over 80% carried one MC1R dilution mutation
- The EE AA genotype (no dilution, no black) appears in less than 10% of fawn animals
- 55% of dark fawns carried one black allele, versus 24% of medium fawns



Light Fawns

- We only tested eight in the first round
- Six carried two MC1R dilution mutations and two carried one
- Five of the eight covered black
- While a very small test group, they looked more like whites than fawns



Whites and Beiges

- We tested 33 in the first round (far more are coming in round 2)
- 90% carried two dilution alleles, and the ones that did not carried a single dilution allele and were genotypically black at ASIP (Ee aa)
- The fact that all almost all light animals carry two dilution alleles (ee) also suggests that in our herd the light/white phenotype may be produced by dilution, not by a “white” mutation in another gene.



SNOWMASS BEST ORACLE

2672

AOA#: 36073184 MALE WHITE DOB: 8/22/2019

SNOWMASS ALPACAS

Shearing: 5/2021

No Prevalent White/Light Gene Mutation?

- We can see no indirect evidence for it in our first round of testing. If it was there, we would expect white MC1R genotypes to vary more. Instead, we see that the great majority of whites and lights have two copies of the MC1R dilution mutation
- A dilute “ee” animal may already be so light that it would be hard to independently select for a white or light genotype produced by the function of another gene or genes, making it less likely one would be prevalent

*Snowmass
Crystal Code's
color genotype
is ee AA.*



*Snowmass
Oblivion's
color genotype
is ee Aa.*





How We Will Start To Use This Information In The Current Breeding Season

- We will breed whites to dark animals that do not carry a copy of the MC1R dilution mutation
- We will use our whites from dark color lines more extensively in our white program
- We will provide color genotypes in the Snowmass/Accoyo America Community Database for you to use



Still To Learn From Our Color Genotyping Effort

- Do further results confirm or contradict our hypotheses?
- Can we improve our ability to accurately guess what genotype is producing a given phenotype?
- Can we integrate the results with our pedigree analytics to help see if there is more than one type of black ASIP mutation circulating in our herd?
- Can we effectively use this information to improve our breeding results, both in terms of fleece quality and financial value?
- Can we use our data and animals to attract interested researchers to further study alpaca color genotypes?