Color In Depth:
The Black ASIP Alleles

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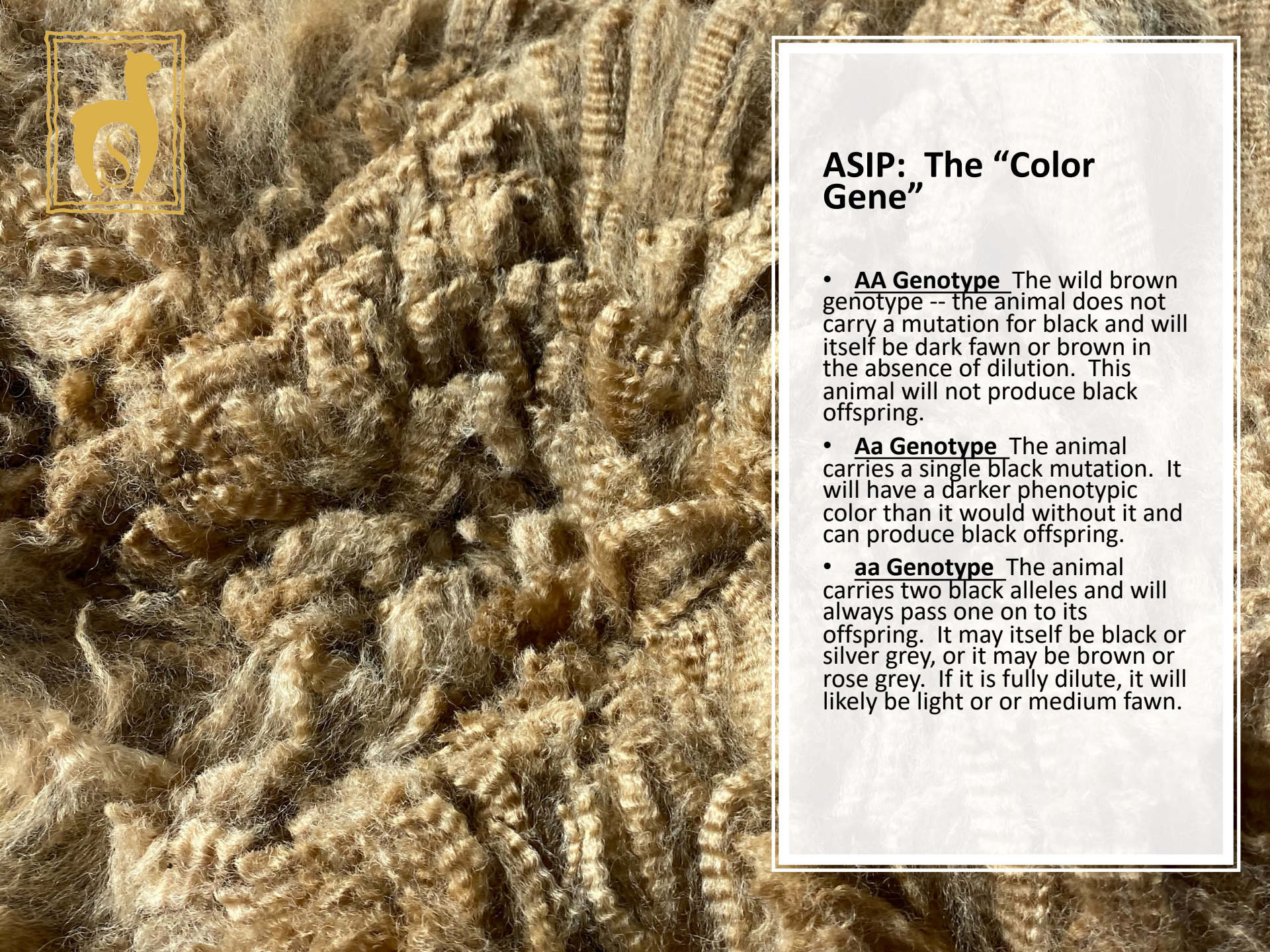
Black Alleles Influence All Color Outcomes

- Fully dilute animals with “ee” MC1R genotypes animals are less likely to be white and more likely to be beige if they have one black allele or light/medium fawn if they have two.
- Partially dilute animals with “Ee” MC1R genotypes are more likely to be brown if they have one black allele or black if they have two.
- Rose grey animals virtually always carry one or two black alleles, suggesting this is a requirement for the expression of the color
- Silver grey animals always carry two black alleles



ASIP: The "Color Gene"

- **AA Genotype** The wild brown genotype -- the animal does not carry a mutation for black and will itself be dark fawn or brown in the absence of dilution. This animal will not produce black offspring.
- **Aa Genotype** The animal carries a single black mutation. It will have a darker phenotypic color than it would without it and can produce black offspring.
- **aa Genotype** The animal carries two black alleles and will always pass one on to its offspring. It may itself be black or silver grey, or it may be brown or rose grey. If it is fully dilute, it will likely be light or or medium fawn.

MC1R: The "Dilution" Gene

- **EE Genotype** The animal does not carry a dilution mutation and will not produce white or light offspring
- **Ee Genotype** The animal carries one dilution allele and can make white/light offspring as well as black if it covers it.
- **ee Genotype** The animal carries two dilution mutations and will always pass one to its offspring. This animal is itself always white or light but depending on its ASIP genotype can produce any color from white to black.

The animal whose fleece is shown at left has an ee AA base coat color genotype.

Many Animals With Black ASIP Genotypes Are Not Phenotypically Black

Of the 206 ”EE aa” or “Ee aa” animals for which (thanks to input from multiple farms) we had data:

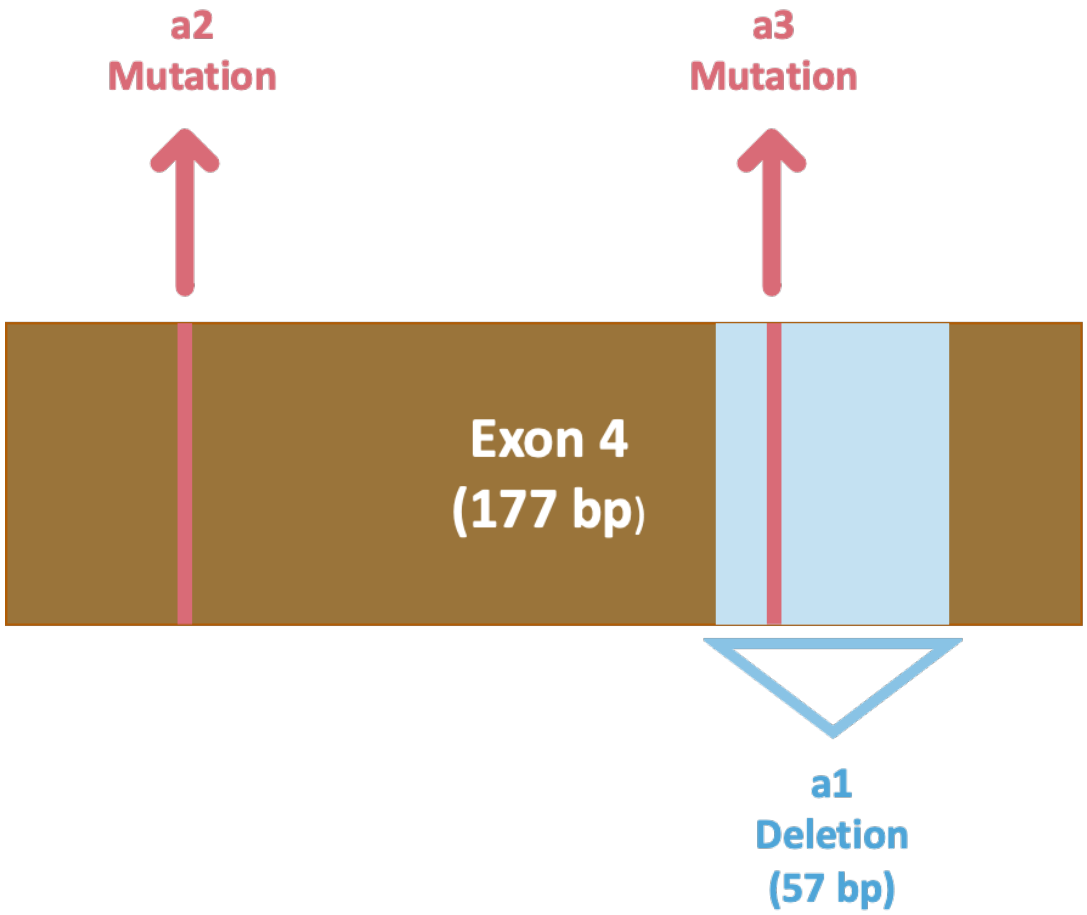
- 95 were true black or silver grey
- 17 were bay black
- 39 were rose grey
- 55 were brown

We knew we had more to learn.

The animal whose fleece is shown here has an EE aa genotype

Three Altered Versions Of ASIP: The Black Alleles

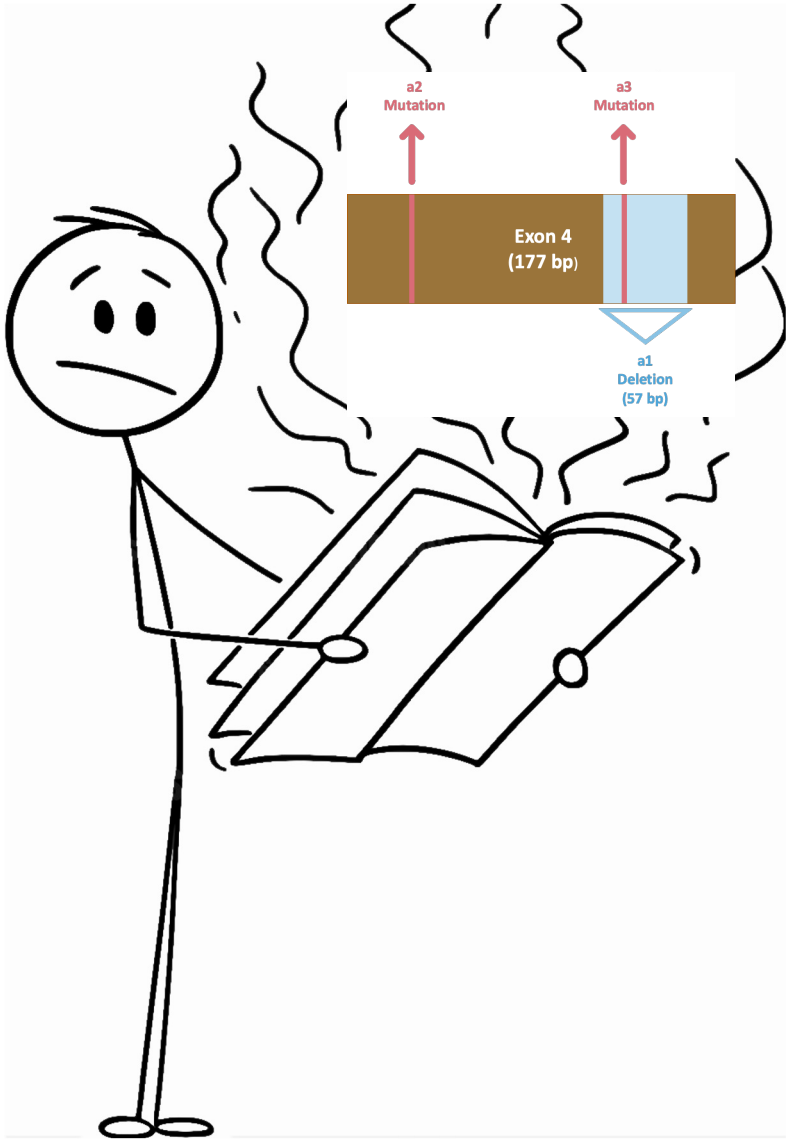
- Each occurs in the region of the gene described as Exon 4, where instructions for coding the agouti-signaling protein reside
- Each results in a change that reduces the functionality of the protein produced
- This is why they are referred to as “loss of function” mutations/deletions



Source: Feeley et al, ”Three novel mutations in ASIP associated with black fibre in alpacas, 2011.

Some Assembly Required!

- It doesn't necessarily take a "big change" to the protein coding instructions to affect the resulting protein
- The "a2" and "a3" mutations result in a change to just one chemical base of one of the 177 base pairs (bp) in the Exon 4 region
- The "a1" deletion, on the other hand, involves the absence of 57 bases of the coding instructions



We've Learned The Three Black Alleles Are Not All Alike With Respect To Expected Phenotypic Color Outcomes

EE a1a1

EE a2a2

Ee a3a3

An animal with one or two "a3" ASIP alleles is less likely to be true black and more likely to be bay black or brown than an animal without an a3 allele.

Behind The Scenes

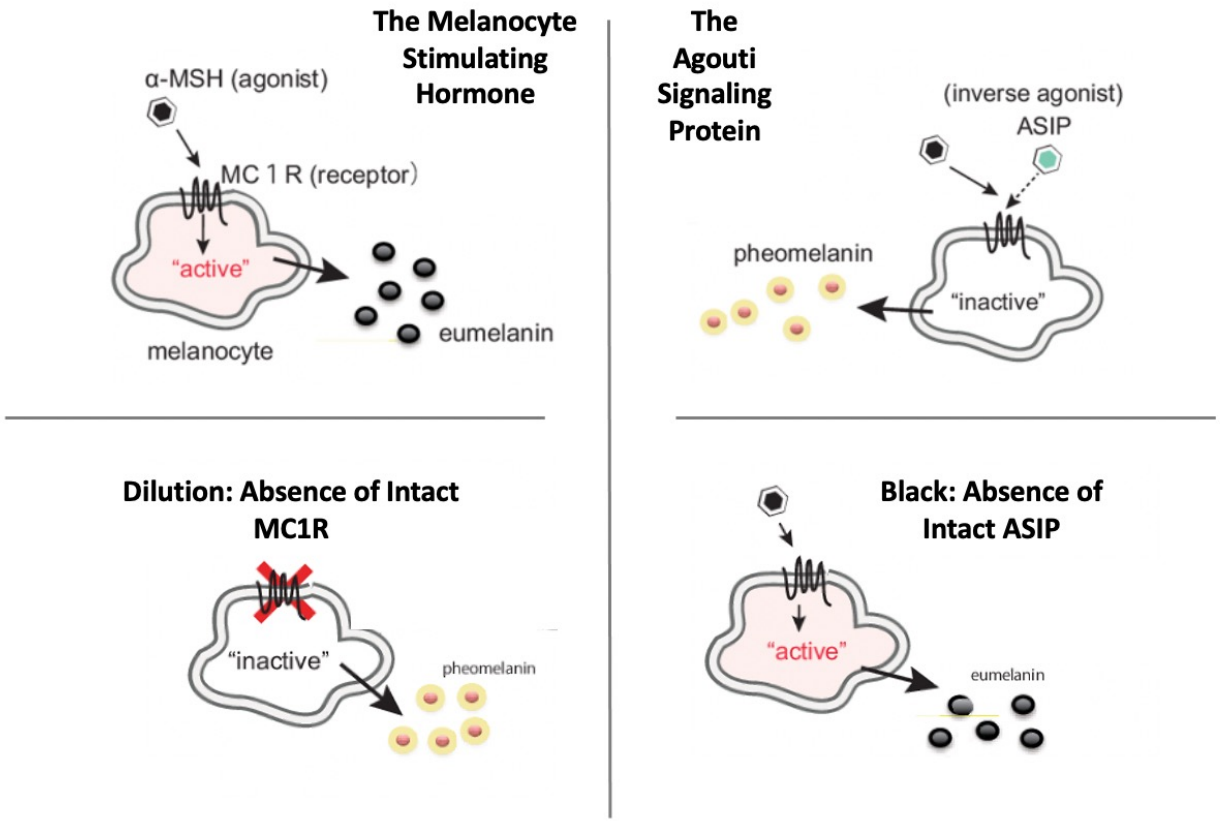


Image credit: Suzuki, H. Evolutionary and phylogeographic views on MC1R and ASIP variation in mammals, 2013.

Phenotypic Color Outcomes By Genotype

ASIP	MC1R	Percent Registered True Black or Silver Grey	Percent Registered Bay Black, Rose Grey or Brown	Number of Animals In Sample
a1a1	EE or Ee	80%	20%	15
a2a2	EE or Ee	59%	41%	37
a3a3	EE or Ee	26%	74%	27
a1a2	EE or Ee	61%	39%	33
a1a3	EE or Ee	30%	70%	37
a2a3	EE or Ee	40%	60%	57

What's Up With a3?

- Based on the relationships between genotypes and phenotypes in our sample we have a high degree of confidence that the a3 allele is less likely to produce a true black base coat color.

- There may be more pheomelanin, less total pigment, or both.

- Animals which have an a1a3 or a2a3 genotype are more likely than those with an a3a3 genotype to be registered true black or silver grey, but less likely than those without any a3 allele to be registered as these colors.

- Still, some a3a3 animals are registered true black or silver grey. There are likely other genes also influencing depth of color that we do not yet know about.

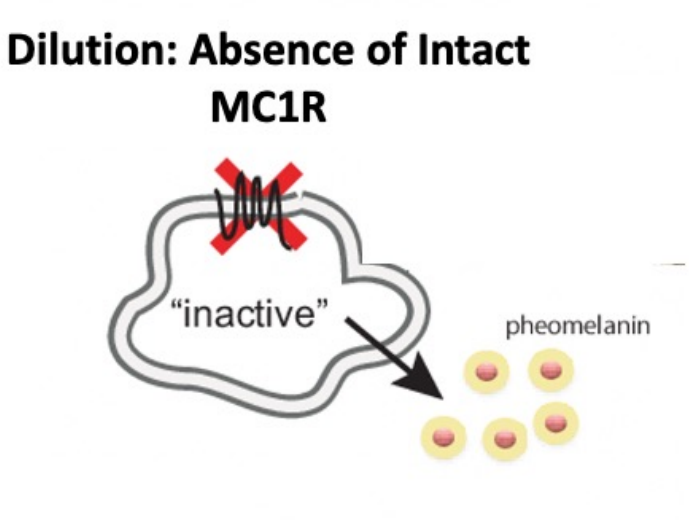
Are There Differences Between the Phenotypic Outcomes of a1 And a2 Alleles?

- The pattern of results for animals in our current dataset suggest that a1 produces true black base coat color animals more frequently than does a2.
- However, we have too few observations to have a high degree of confidence in this conclusion.
- Stay tuned….
- That said, our current speculation is that blue black animals have an EE a1a1 genotype, perhaps with further color boost from other as yet unidentified genes or mutations. Just a guess though!

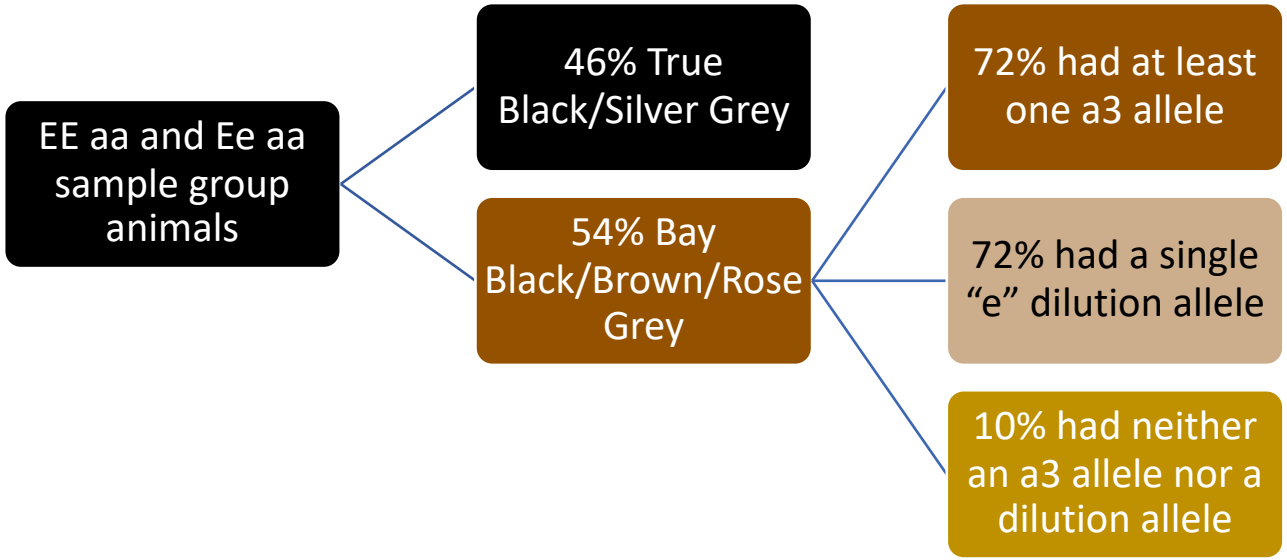
Expect a bold crimp and silkier style in a heavily pigmented animal like this EE a1a1!

What About ”Partial Dilution”?

- The results for our sample group provide a high degree of confidence that “Ee” animals are less likely to be registered as true black/silver grey animals than are EE animals.
- Nonetheless, many ”Ee” animals have true black phenotypes.
- We believe with more data we may be able to discern differences between the a1, a2, and a3 genotypes with respect to dilution.
- Again, there may also be other genes at work.



The a3 ASIP Allele and Partial Dilution Explain Most Of Our Non-Black “aa” Sample Group Animals

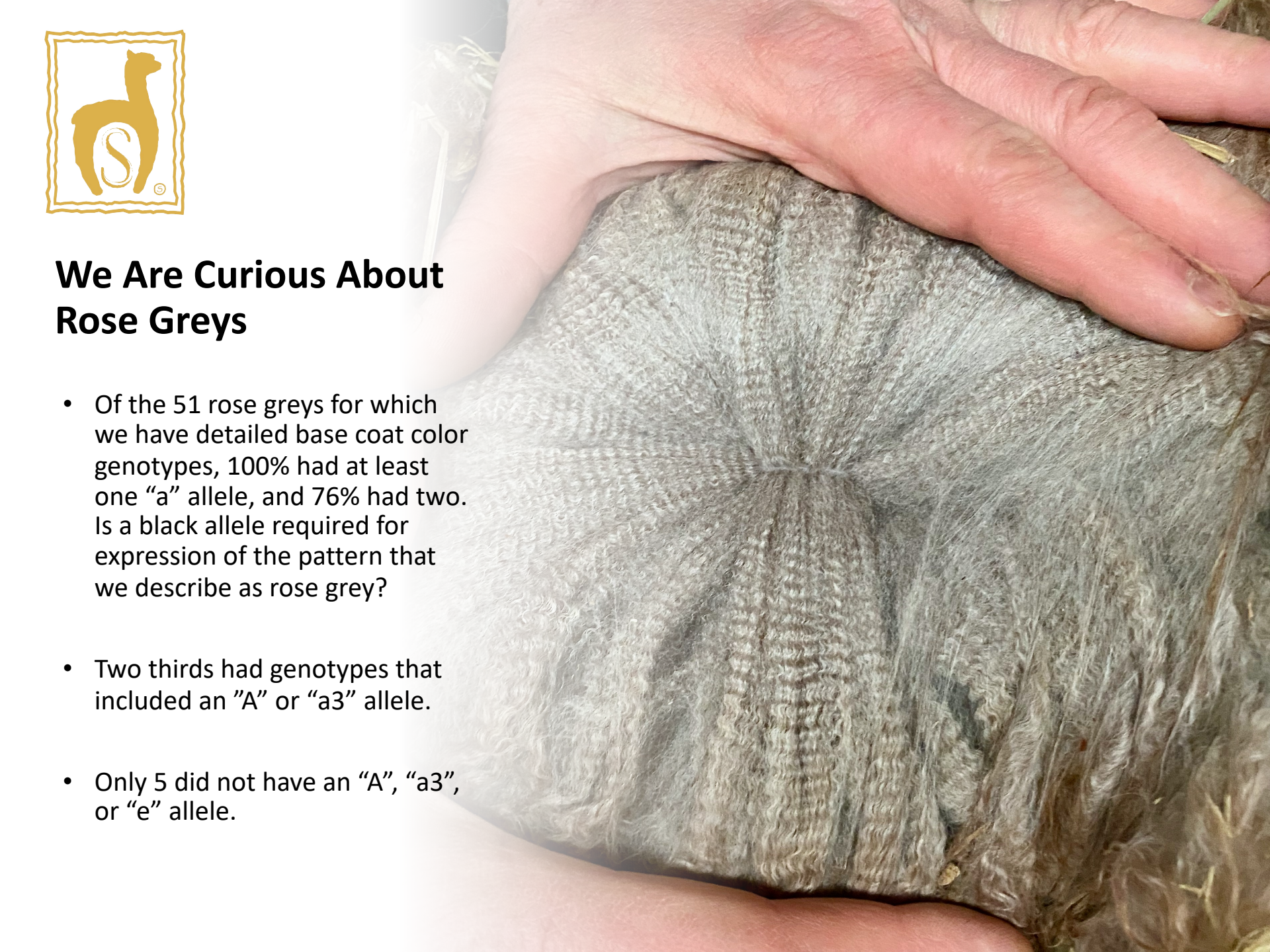


We Are Curious About Rose Greys

- Of the 51 rose greys for which we have detailed base coat color genotypes, 100% had at least one “a” allele, and 76% had two. Is a black allele required for expression of the pattern that we describe as rose grey?

- Two thirds had genotypes that included an ”A” or “a3” allele.

- Only 5 did not have an “A”, “a3”, or “e” allele.



Using This Information

- Because a1 and a2 ASIP alleles are associated with a higher likelihood of true black phenotypes, breeders focused on black and silver grey should prefer these, all else constant.

- But animals with a3 ASIP alleles can be true black and also produce it, even if less frequently. Don't exclude them from true black breeding programs, but perhaps set a higher bar for other phenotypic quality and genetic diversity value.

- Animals with "aa" ASIP genotypes and no dilution alleles will produce true black more frequently than those with one or two dilution alleles, all else constant.

How will this affect the price you wish to ask or pay for an animals?

Snowmass Moonlight Meadow, EE a1a1.

We Did This Together

Farms Contributing Data:

- Black Barn Alpacas, TX
- Cinco C's Alpacas, PA
- Claddaugh Farm Alpacas, NY
- Cotton Creek Farms, MI
- Fun In The Country Alpacas, MI
- Holdfast Hilty Family Farm, OH
- Paragon Alpacas, MD
- Seven Acres Alpaca Farm, NY
- Snowmass Alpacas, NY

Tables To Interpret Detailed Results From Neogen

ASIP	ASIP_325-381del57_b	ASIP_C292T	ASIP_G353A
AA	T	C	G
Aa1	GT	C	G
Aa2	T	TC	G
Aa3	T	C	GA
a1a1	G	C	G
a1a2	GT	TC	G
a1a3	GT	C	A
a2a2	T	T	G
a2a3	T	TC	GA
a3a3	T	C	A

MC1R	MC1R_G82A	MC1R_C901T	Rdel_224-227
EE	A	C	ACTT
Ee	GA	CT	ACTT
Ee	A	C	DEL.ACTT
ee	G	T	ACTT
ee	GA	CT	ACTT.DEL