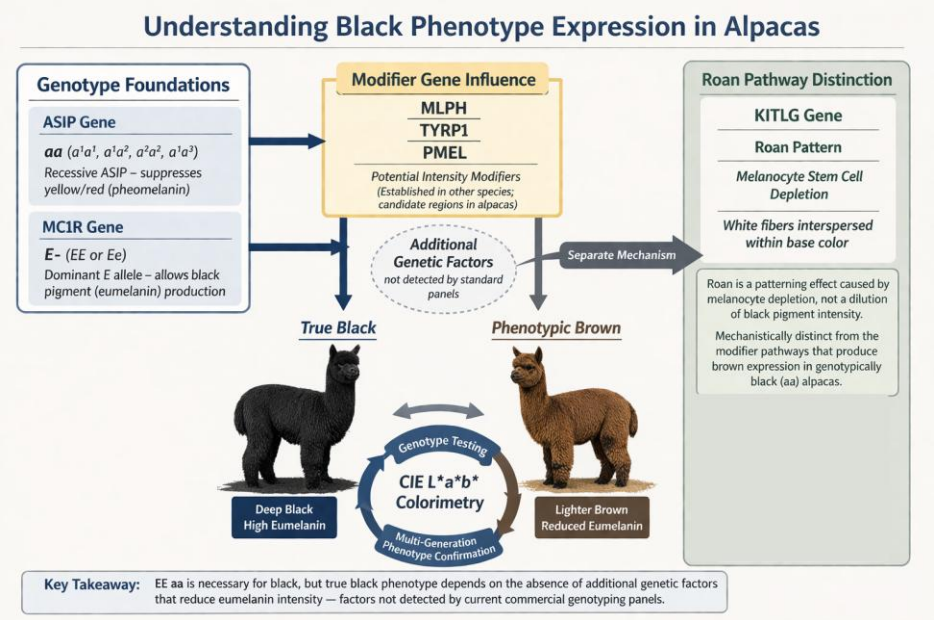
A Generationally Confirmed Phenotype Model™

The Case for Phenotype-Confirmed Selection in Black Alpaca Breeding Programs

Aimée Matheny-Skyfiber Ranch Alpacas-Marshall, VA

Executive Summary

Recent peer-reviewed research has confirmed what field breeders have observed: animals with the "blackest genotype possible" (EE aa) can appear brown in phenotype, due to intensity-modifying genetic factors not currently detected by standard genotyping panels. This finding has direct implications for black breeding programs. Animals that test as genotypically black but express as brown or fawn may carry hidden modifiers that compromise fiber consistency across multiple generations. The Generationally Confirmed Phenotype Model™ (GCPM™) addresses this gap by integrating genotype testing, objective CIE L*a*b* colorimetry, and multi-generational phenotype confirmation — providing a selection framework that goes beyond what standard ASIP and MC1R panels can reveal.



Understanding Basic Alpaca Color Genetics

The Two Primary Genes

MC1R (Melanocortin-1 Receptor Gene)

- **E allele**: Functional receptor, allows black pigment (eumelanin) production
- **e allele**: Non-functional receptor, results in dilution to white/fawn
- **EE genotype**: Enables consistent eumelanin production and eliminates risk of light offspring from MC1R dilution, though it does not determine pigment intensity on its own.
- **Ee genotype**: Can produce light offspring when paired with another Ee

ASIP (Agouti Signaling Protein Gene)

- **A allele**: Functional protein, promotes yellow/red pigment (pheomelanin)
- **a alleles (a1, a2, a3)**: Three loss-of-function mutations that suppress pheomelanin

Black alpacas require two "a" alleles (aa genotype) to suppress pheomelanin production. The combination of **EE aa** is traditionally considered the "blackest genotype possible."

The Critical Discovery: Genotype Does Not Guarantee Phenotype

Supporting Research Findings

Both genotypes — EE aa and Ee aa — can produce phenotypically light animals despite carrying black ASIP alleles, confirming that the complexities of black pigment expression are not fully captured by current genotyping panels.

2026 Confirmation: The ASIP–MC1R Interaction Defines Black Color

A subsequent study by Pinares et al. (2026), published in *Small Ruminant Research* (Vol. 258), directly examined ASIP and MC1R genotypes in 89 Huacaya alpacas and 9 vicuñas using CIE L*a*b* colorimetry. Its findings provide important independent corroboration for the GCPM framework:

The study confirmed that black/brown-black color is not determined by a single gene but by the **interaction** between recessive ASIP loss-of-function alleles (a^1, a^2, a^3) and at least one dominant E allele at MC1R. All black/brown-black animals in the study carried at least one E allele. The specific genotype combinations identified as reliable selection markers for black/brown-black animals were: a^1a^1/E-, a^1a^2/E-, a^3a^3/E-, and a^1a^3/E-. Critically, the study also confirmed that lower L* (lightness) and chromaticity values in CIE L*a*b* colorspace correspond to higher eumelanin concentration in black fiber—directly validating the use of objective colorimetry to verify true black phenotype.

Importantly, the 2026 paper notes that even with both genotyping and colorimetry, it was *not possible to identify a clear cut-off* between black and dark brown groups. This residual phenotypic ambiguity is precisely the gap that the GCPM™'s multi-generational confirmation requirement is designed to resolve. Where Pinares et al. (2026) establish the genotypic markers associated with black fiber, GCPM™ operationalizes those findings into a breeding selection protocol that requires both genotypic and phenotypic confirmation across multiple generations—removing animals that carry the right alleles but fail to consistently express true black. This positions GCPM™ as an advancement built directly on top of current peer-reviewed science, not merely consistent with it.

The Modifier Gene Evidence

Gray et al. (2023) identified **at least six genomic regions** associated with pigment intensity through a genome-wide association study. Their primary confirmed candidate in alpacas was a region upstream of KITLG. The remaining regions were interpreted using candidate genes established in other species, including:

- **MLPH (Melanophilin)**: Controls pigment transport — confirmed in dogs and chickens
- **TYRP1**: Stabilizes eumelanin production — confirmed in dogs, sheep, and bears
- **PMEL**: Affects pigment density and structure — confirmed in horses

Critical implication: MLPH, TYRP1, and PMEL are established intensity regulators in other species and were identified as candidate regions in alpacas by Gray et al. (2023); however, their specific functional roles in alpaca pigmentation remain to be confirmed. These genomic regions indicate areas of association rather than established causal pathways. Nonetheless, the broader implication remains: standard ASIP/MC1R genotyping does not capture the full genetic architecture influencing pigment intensity, and phenotypic expression remains the most practical indicator of that complete architecture.

A Note on KITLG and Pigment Pathway Distinctions

Not all modifier genes affect black expression through the same mechanism, and this distinction matters for breeding strategy. Gray et al. (2023)—whose work identifying six genomic regions associated with pigment intensity is central to this paper—published a companion study (Shah, Gray et al., 2023) in the same journal issue that directly associates KITLG with the roan pattern in alpacas, not with black pigment intensity. Their research describes roan as causing "contamination of coloured fibre with white fibres" and identifies the biological mechanism as premature depletion of melanocyte stem cells within hair follicles—leading to gradual and

permanent depigmentation of individual fibres. This is mechanistically distinct from the eumelanin suppression pathway that produces brown or fawn dilution in genotypically black animals. An animal appearing light due to KITLG disruption is losing melanocytes altogether; an animal appearing light due to ASIP/MC1R modifier interference is producing the wrong pigment type. Both result in phenotypic lightness, but they arise from different biological mechanisms and therefore represent distinct genetic challenges requiring different selection strategies. This is precisely why multi-generational phenotype confirmation—the foundation of GCPM™—cannot be replaced by single-gene or even two-gene genotyping: the pathways to dilution are multiple, mechanistically distinct, and not fully captured by any current standard genotyping panel.

The Risks of Breeding Phenotypically Light "aa" Animals

When a brown or fawn alpaca tests as “aa**”**, some breeders conclude the animal is valuable for a black program because it carries black ASIP alleles. This reasoning is incomplete — it ignores the more important question: *why does this animal appear light if it has the black genotype?*

Phenotypically light “aa” animals pose a specific risk because:

- They pass black ASIP alleles to offspring along with heritable genetic factors likely contributing to their lighter expression
- Those modifier alleles are not detected by standard ASIP/MC1R genotyping
- Offspring may test as aa yet produce inconsistent fiber colors within the same fleece
- Once introduced, restoring consistent pigmentation may require multiple generations of targeted selection and repeated colorimetry verification — consistent with established principles of recessive allele elimination in livestock breeding programs

Breeding Objectives: Genotype, Phenotype, and the Importance of EE

Serious black breeding programs are not just selecting for ASIP and MC1R genotypes. They are selecting for a complete genetic architecture that includes:

- **Correct ASIP genotypes** (aa)
- **Correct MC1R genotypes** (preferably EE)
- **Absence of detectable dilution modifiers** at those same genes and across the broader genetic background
- **Stable pigment production** that doesn't fade with age or environmental stress

Pinares et al. (2025, 2026) and Gray et al. (2023) — whose colorimetric classification of black alpacas underpins the six-region GWAS — both support the use of objective color measurement to reveal what genotyping alone cannot: the complete phenotypic expression of the genetic architecture. Introducing a phenotypically brown “aa” animal risks undermining that architecture regardless of genotype panel results.

The Importance of EE Genotype

The Peruvian research makes an important distinction that many breeders overlook: EE aa animals offer greater breeding predictability than Ee aa animals for black breeding programs. Pinares (2025) confirms that EE aa × EE aa matings will never produce light fawn offspring, while pairing two Ee aa animals carries a 25% risk of light fawn offspring when both parents pass the recessive e allele.

The Generationally Confirmed Phenotype Model™

To address the limitations of genotype-only selection and the risks of undetected modifier gene expression, this paper proposes the **Generationally Confirmed Phenotype Model™ (GCPM™)**—a comprehensive breeding framework designed specifically for black-focused alpaca breeding programs.

Framework Components

The GCPM™ integrates five essential elements:

1. EE Genotype Prioritization

Prioritize EE (homozygous functional MC1R) animals to eliminate dilution risk at the MC1R locus. As demonstrated by the Peruvian research, EE aa × EE aa matings will never produce light fawn offspring, providing breeding predictability that Ee aa combinations cannot offer.

2. Phenotype-Genotype Alignment Requirement

Select only animals where aa genotype combinations are expressed as true black phenotype. Animals testing as "aa" but appearing brown, fawn, or dilute black are excluded regardless of ASIP genotype, as phenotypic lightness strongly suggests the presence of additional genetic factors influencing pigment expression beyond what standard ASIP/MC1R panels can detect-consistent with the multiple genomic regions identified by Gray et al. (2023).

3. Multi-Generational Phenotype Documentation

Require minimum 3–4 generation pedigree documentation demonstrating consistent true black phenotype expression. This temporal validation confirms absence of recessively inherited dilution modifiers that may not express in every generation. Single-generation phenotypic assessment is insufficient to identify carriers of hidden modifier alleles.

4. Objective Colorimetry Integration

CIE L*a*b* colorimetry provides objective phenotypic verification that genotyping alone cannot. Pinares et al. (2025, 2026) establish that true black alpacas exhibit lightness values of 16.0–22.0 and chromaticity values of 0.9–3.2. Animals exceeding these thresholds (L* >25, C* >5) are strong candidates for modifier-based dilution and should be carefully evaluated before inclusion in black breeding programs.

5. Production Predictability as Commercial Objective

Define breeding success not merely as "producing some black offspring" but as achieving uniform, non-fading, commercially viable black fiber with minimal within-fleece color variation across successive generations. This shifts evaluation criteria from possibility ("can produce black") to probability and consistency ("reliably produces uniform black").

Application to Commercial Breeding

The GCPM™ framework aligns contemporary genomic research with applied breeding strategy and commercial fiber realities. Pinares (2025) documents a significant price differential between white fiber ($5.92/lb) and colored fiber ($2.26/lb) in Peruvian markets, attributing this gap in part to fiber inconsistency from undetected modifier gene expression.

Programs implementing GCPM™ principles aim to produce uniformly pigmented, stable black fiber — positioning breeders to meet growing demand for natural-color textiles with consistent, commercially viable product.

Breeding Strategy Recommendations

1. Select for phenotype-genotype alignment — Animals testing as "aa" but expressing brown or fawn phenotype should be carefully evaluated before inclusion in a black breeding program, as phenotypic lightness may indicate the presence of additional genetic modifiers influencing pigment expression beyond what current panels detect

2. Use colorimetry to verify true black phenotype — CIE L*a*b* measurements (L* 16–22, C* under 3.2) provide objective confirmation. Animals above these thresholds warrant further evaluation regardless of genotype.

3. Prioritize EE aa genotypes — EE aa × EE aa matings eliminate the 25% light fawn risk associated with Ee aa pairings (Pinares, 2025).

4. Select from multi-generational black lines — Phenotypic consistency across 3–4 generations is the most reliable indicator that the complete genetic architecture supports stable black expression.

5. Recognize that selection decisions compound over time — Modifier alleles are recessive and may not express immediately, making proactive phenotypic screening more effective than corrective selection after the fact.

Common Questions About GCPM™

"This animal tests as 'aa' — doesn't that mean it has the black genes?"

Carrying black ASIP alleles is necessary but not sufficient. Phenotypic lightness in an "aa" animal indicates that additional intensity-modifying factors are present beyond what ASIP/MC1R panels detect. Genotype and phenotype need to be considered together.

"The research says brown 'aa' animals can produce black offspring."

They can — but the research does not quantify what proportion of offspring express as true black, or whether that expression remains stable across generations. Producing some black offspring is a different standard than producing reliably consistent black fiber.

"I need genetic diversity in my program."

Diversity is valuable and can be maintained among animals that meet both genotypic and phenotypic criteria. GCPM™ does not restrict diversity — it defines the selection threshold within which diversity is sought.

"This seems complicated — can't I just breed blacks to blacks?"

That is precisely what GCPM™ recommends — with the added step of confirming true black expression through colorimetry. CIE L*a*b* measurement makes this objective and straightforward.

Conclusion

Converging peer-reviewed research — including Pinares et al. (2025, 2026), Gray et al. (2023), and Shah, Gray et al. (2023) supports a clear conclusion: standard ASIP/MC1R genotyping provides an incomplete picture of the genetic architecture controlling black fiber expression in Huacaya alpacas. Both EE aa and Ee aa animals can express phenotypic lightness consistent with

the influence of additional genetic modifiers affecting pigment intensity that are not captured by current commercial genotyping panels.

The Generationally Confirmed Phenotype Model™ responds to this gap by integrating genotype testing, CIE L*a*b* colorimetry, and multi-generational phenotype confirmation into a single selection framework. Phenotypic expression across multiple generations remains the most reliable indicator that the complete genetic architecture — beyond ASIP and MC1R alone — is aligned with the breeding objective.

Animals that test genotypically black but express as brown or fawn warrant careful evaluation before inclusion in a black breeding program. The combined use of genotyping and objective colorimetry provides the tools to make that evaluation systematic, consistent, and scientifically grounded.

References

Pinares, R. (2025). "New Insights into the Color Phenotypes and Genotypes of Huacaya Alpaca and Their Fiber's Economic and Commercial Value." In *Animal Breeding – Recent Advances and Applications*. IntechOpen. DOI: http://dx.doi.org/10.5772/intechopen.1013114

Pinares, R., Daverio, M.S., Cruz, A., Gutiérrez-Reynoso, G.A., & Munyard, K.A. (2026). "Association of ASIP and MC1R genotypes with the quantitative color variation in Huacaya alpaca." *Small Ruminant Research* 258:107704. https://doi.org/10.1016/j.smallrumres.2026.107704

Gray, N., et al. (2023). "Identification of six genomic regions associated with pigment intensity in alpacas." *Animal Production Science* 63(11):1052-1062.

Shah, I., Gray, N., Groth, D., Brooks, S., & Munyard, K. (2023). “A copy number variant near KITLG is associated with the roan pattern in alpacas.” *Animal Production Science* 63(11):1008–1016. https://doi.org/10.1071/AN22463