ANALYSIS OF COAT COLOR TRANSMISSION IN HORSES

Description

The three genes that control the four basic coat colors of horses: grey, bay, chestnut, and black, interact with each other and affect the type of basic pigment as well as its location on the animal's body. The main pigment in horses, as in all mammals, is melanin, which can take two forms: eumelanin, which is black, and pheomelanin, which is reddish in color.

These genes are:

- The Grey gene (*STX17*), which has two alternatives or alleles: G and g, with G being dominant and g being recessive. The G variant prevents pigment retention in the hair but does not affect the skin or the rest of the body, which is normally pigmented. Foals are born dark and lighten with age in a process similar to human graying, eventually becoming completely white. For a horse to display any coat color other than gray, it must be gg. All horses with GG or Gg are gray, regardless of their other coat color genes. As the grey mutation consists in a large duplication, it is difficult to diagnose since the DNA in samples collected in the field is usually fragmented. Therefore, we have resorted to detecting a single nucleotide polymorphism (SNP) in a neighboring gene, *INVS*, linked to the mutation, which, along with pedigree analysis, provides a reasonable reliability in its diagnosis.
- The Extension gene (*MC1R*) affects the type of pigment: eumelanin or pheomelanin. It has two variants: E and e, with E being dominant and e being recessive. A horse with at least one dose of the E variant (EE or Ee) will have black pigment, while a horse that is ee will have only red pigment and will be chestnut if it is also gg.
- The *ASIP* gene, with two alternatives: A and a, with A being dominant and a being recessive, influences the location of black pigment on the animal's body, without affecting red pigment. Thus, horses that have at least one dose of A (AA or Aa) only exhibit black color on the points and will be bay if they are also gg and have at least one dose of E. Horses with aa have black pigment uniformly distributed throughout the body and will be black in color if they are also gg and have at least one dose of E.

In addition to these three genes, there are other important genes that determine coat colors commonly found in many breeds, including a group of genes that lighten coat color and are called "dilution genes." Some of these genes include:

- The Cream gene (*SLC45A2*), which has two mutations, one of which, with the phenotype properly called Cream, has two variants: C and C^{Cr}, with one variant not dominating over the other. In a single dose, CC^{Cr} lightens the bay coat on the body but not on the points, resulting in a buckskin color. It also lightens the chestnut coat, resulting in a palomino, and the black coat, resulting in a smoky black color. In a double dose, $C^{Cr}C^{Cr}$, it dilutes all coat colors, producing very light horses generically called "albinos," although they are not true albinos. Double doses of the $C^{Cr}C^{Cr}$ allele transform bay to perlino, chestnut to cremello, and black to smokey cream. There are other less common color dilution genes that produce similar light coat colors. Another mutation of the same gene is called Pearl (Prl), which has little or no effect in a single dose but produces a type of dilution phenotype called "isabela" when homozygous and, in heterozygosity with the C^{Cr} allele, generates a different type of "albino" horse.
- The Champagne gene (*SLC36A1*), whose dominant mutation also produces another type of dilution that transforms all coat colors, turning black pigment into chestnut and red pigment into gold, also affecting the mane and tail.
- Other dilution genes that produce relatively less common coat colors include the Dun gene (*TBX3*) and the Silver gene (*PMEL17*), although they are becoming increasingly known and appreciated and have appeared in the Purebred Spanish Horse breeding, providing great economic value to the individuals that possess them.

Another set of coat color genes that are involved in the migration of melanocytes during embryonic development have mutations that produce spotted coats, such as Tobiano (*KIT*) and "Appaloosa" (*TRMP1*), which appear in individuals of multiple breeds.

In select horse breeds, such as the Purebred Spanish Horse (PRE), the value of an individual varies greatly depending on its coat color and, consequently, the coat colors it can pass on to its offspring. Therefore, it is interesting to know the genotype for the genes that determine color. The genetic makeup or genotype of an animal does not change throughout its life, so once it is known; further analysis of that individual is no longer necessary.

How does it work?

The genotype of horses can be determined through two complementary methods:

1. Pedigree analysis: By knowing the coat color of the ancestors, descendants, and siblings of a horse, it is sometimes possible to deduce its complete genetic makeup and predict the probability of coat color transmission to its offspring, provided that the genetic makeup of the mating partner is also known.

2. Molecular analysis: Sometimes, especially when dealing with grey horses as often found in the "Pura Raza Española" (Purebred Spanish Horse, PRE) breed, pedigree analysis is not sufficient, and it becomes necessary to perform molecular tests that can distinguish genetic variants. Specific analysis of each gene is conducted using the multiplex PCR technique, followed by a single nucleotide extension reaction using specific internal probes for each amplified fragment (SNaPshot®). The probes for each gene are designed to have different sizes so that they can be differentiated by their migration in the electropherogram. For example, it can be determined whether a grey horse is EE, Ee, or ee, with the latter two being capable of producing chestnut offspring. It can also be determined if a bay horse can produce black offspring if it is Aa and not AA. Similarly, it can be determined if a grey horse is a carrier of the C^{Cr} variant, which could result in bay offspring or even cremellos (Figure 1).

The equine genetics team at the Department of Genetics, Faculty of Biology, Complutense University of Madrid, conducts pedigree analysis and has developed molecular techniques to detect specific variants of these genes carried by a horse. The combination of pedigree analysis and molecular testing allows for determining the genetic formula of individuals for coat color-controlling genes and predicting, based on the knowledge of the horse's genome, the coat color of the offspring in different mating scenarios.

Pedigree analysis is conducted based on the pedigree information of the horses, including data on their ancestors and descendants if available. Molecular analysis is performed using samples of about 20-30 hairs with their roots from the mane or tail (simple instructions are provided to interested parties).

If an owner already knows the genotype of their horses and wants to assess the probability of coat color transmission in one or multiple crosses, they can simply provide the genetic formulas of the animals. A probability of transmission report can be generated at a reduced cost, as it does not require molecular analysis.

Advantages

In Spain, the majority of studies are conducted only at the pedigree level. The equine genetics group at the Department of Genetics, Faculty of Biology, UCM has developed molecular protocols that accurately determine the genetic formula of horses. This set of techniques greatly enhances the reliability of predicting coat color transmission probability and enables the design of specific matings when a breeder is interested in obtaining offspring with a particular coat color.

The sampling protocol has been optimized to the maximum to make it user-friendly. For this reason, a minimally invasive sample such as hair is used, which does not require refrigeration and can be sent by regular mail.

Where has it been developed?

The technology has been developed in the Genetics laboratory of the Faculty of Biological Sciences, optimizing protocols to achieve maximum effectiveness and reliability in the results.

The Equine Genetics Group at the Department of Genetics, Faculty of Biology, Complutense University of Madrid, is composed of a university professor and a PAD professor, as well as several students. The group has extensive experience in this field, having participated in specific funded projects on horse genetics and published several scientific articles related to the topic in international journals such as Theriogenology and Animal Genetics. Additionally, since 2008, they have successfully applied their diagnostic technique as an external service of UCM to numerous clients and institutions, including ANCCE (National Association of Spanish Horse Breeders), which is responsible for the management of the Stud Book of the PRE breed.

And moreover

The results of the analyses are communicated to clients through a technical report that provides detailed information on the following aspects:

- Genetic formulas of the individuals analyzed.
- For each individual: probability of coat color transmission based on each possible type of mating. As soon as the genotype of the other parent is known, the probability of each coat color in the offspring can be determined.

In the video "CABALLOS COLOR DE CAPA: Diagnóstico genético y transmisión del color de capa" (Horses Coat Color: Genetic Diagnosis and Coat Color Transmission), the basics of coat color genetics and the usefulness of applying this technology are explained.

Link: https://www.youtube.com/watch?v=S9AsHiCbFz8

An interview on the TVE program "La Aventura del Saber" (The Adventure of Knowledge) is also available at the following web address:

Link: https://www.rtve.es/play/videos/la-aventura-del-saber/aventura-del-saber-13-02-13/1689516/

Researcher in charge

Name and surname: mparana@ucm.es
Department: Genética, Fisiología y Microbiología
Faculty: Ciencias Biológicas