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BIO 305

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Equine color genetics and associated pleiotropic conditions

Color genetics in equines has long been researched and a major topic of discussion in the world of equine genetics; after all, horses come in a wide variety of colors and many breed registries have limitations on color. But what if I told you a purebred Friesian, a historically black breed of horse, could be chestnut? Genetics and modes of inheritance answer questions many breeders have, but also pose new questions such as is variation in color inherited? Understanding equine color genetics is not only important when breeding for a certain color, but also to avoid several lethal pleiotropic conditions that can result from improper breeding of certain phenotypes.

To understand the foundations of a horse's color, one must start at their base color. There are 3 base colors in horses: bay, black, and chestnut (red). Chestnut has historically also been referred to as "sorrel" and some breeders (especially those of stock breeds such as Quarter Horses and Paint Horses) still use the term "sorrel" to refer to a "yellow-based" chestnut horse. Brown was also previously used to describe a dark bay horse or sun-bleached black horse, although the term has gone out of style since it cannot be traced to a particular genotype. Two genes have been identified that control the base color of a horse: agouti and extension (or red factor) (Thiruvenkadan et. al., 2008).

A bay horse can be described as one with a brown or red-brown body and black mane, tail, and points. Black points aren't always visible in bay horses but can be described as black pigment concentrated on the lower legs on and below the knees, ear tips, and muzzle.

Figure 1 - An image of bay Thoroughbred, American Pharoah – 2015 Triple Crown Winner (https://coolmore.com/farms/america/stallions/american-pharoah)

A black horse is black all-over. Occasionally, black horses will become sun-bleached and may appear to have brown hair, especially concentrated around the barrel (belly) and mane/tail. These horses are often confused with mealy bay or dark bay horses.

Figure 2 - A BLACK KWPN (DUTCH WARMBLOOD) STALLION CURRENTLY STANDING AT STUD - HTTPS://WWW.STALLIONSONLINE.CO.UK/HOMOZYGOUS-BLACK-KWPN-STALLION/

Chestnut horses are reddish-brown in color all over. Sometimes, mane and tail color vary from the body color, but this often is the result of sun-bleaching or inconsistent exposure to the elements.

FIGURE 3 - CALIFORNIA CHROME – 2011 CHESTNUT THOROUGHBRED STALLION (HTTPS://PAULICKREPORT.COM/NEWS/THE-BIZ/TAYLOR-MADE-CALIFORNIA-CHROME-WEBSITE/)

The agouti and extension genes are the foundation for all equine colors (Thiruvenkadan et. al., 2008). These two genes and the proteins they code for control the "relative amounts of melanin pigments in mammals" (Rieder et. al., 2001). The dominant (E) extension gene encodes for the melanocortin-1-receptor (MC1R) and the dominant (A) agouti gene codes for MC1R's peptide antagonist agouti-signaling-protein (ASIP) (Rieder et. al., 2001). This recessive epistatic interaction supports the phenotypic trend seen in equines. "ASIP acts as an antagonist of MC1R by nullifying the action of a-melanocyte-stimulating hormone (a-MSH). Loss-of-function of MC1R results in yellow pigment (pheomelanin), whereas gain-of-function of MC1R or loss-of-function of ASIP seems to result in the production of black pigment—eumelanin" (Rieder et. al., 2001). To simplify this, a horse that is dominant at both loci (extension and agouti) will be bay, a horse dominant for extension and recessive for agouti will be black, and a horse recessive for extension will be chestnut (regardless of the presence of agouti) (Thiruvenkaden et. al., 2008).

Bay, black, and chestnut are just the foundation of all equine colors; additional "dilute" and "white pattern" genes have been identified that alter the coat and mane/tail color of the horse.

UC Davis' Veterinary Genetics Laboratory has provided extensive information and research into equine genetics, making understanding it far easier to those who don't have an extensive genetic background. The identified and researched dilutes provided by UC Davis include champagne, cream, dun, mushroom, pearl, and silver (Cotton, 2020). For conciseness, I will only touch on cream, silver, and mushroom dilutions. Additional coat modifiers can be seen in the equine world, yet research into their inheritance patterns and molecular interactions is not as complete; these include sooty, flaxen, and mealy (pangare). These coat modifiers certainly do exist, but related genes have either not been identified or have not been investigated thoroughly.

"Cream is expressed in an incomplete dominant fashion" (Holl et. al., 2019). Some may also view cream as an "additive" gene as it seems to cause more effect when 2 copies are present. One cream gene affects only red pigment, resulting in a palomino when applied to a chestnut base or a buckskin when applied to a bay base (Holl et. al., 2019). Two copies of cream affect both red and black pigment, lightening the entire coat, mane, and tail (Holl et. al., 2019). A "double-dilute" or "double-cream" as these horses are sometimes called results in a cremello (chestnut base) or perlino (bay base) phenotype.

FIGURE 4 - PALOMINO HORSE (HTTPS://WALLPAPERTAG.COM/QUARTER-HORSE-WALLPAPER)

FIGURE 5 - BUCKSKIN HORSE – THIS HORSE ALSO EXPRESSES SOOTY (DARK DAPPLED PATCHES EXTENDING UP FROM THE LEGS) - HTTPS://WWW.PINTEREST.COM/PIN/337981147014297295/

FIGURE 6 - CREMELLO HORSE – NOTICE HOW HE LOOKS LIKE A LIGHTER PALOMINO (HTTPS://WWW.HORSESPIRIT.SITE/2020/05/05/UNIQUE-AND-BEAUTIFUL-HERE-IS-EVERYTHING-YOU-NEED-TO-KNOW-ABOUT-CREMELLO-HORSES/)

Figure 7 - Perlino horse – even with 2 cream genes, the mane and tail still retain more pigment than the body (https://www.pinterest.com/pin/2111131050010705/)

Black horses are still affected by one and two copies of the cream dilute, however these horses can sometimes be difficult to phenotypically distinguish from other phenotypes. Black horses with one copy of cream are called “smoky black” and with two copies are called “smoky cream” (Holl et. al., 2019).

Figure 8 - Smoky black horse – this horse could very easily be confused with a dark bay horse or even a sun-bleached black (https://www.pinterest.com.mx/pin/277886239496229482/)

FIGURE 9 - A GENETICALLY TESTED SMOKY CREAM QUARTER HORSE STALLION – AT FIRST GLANCE, MANY WOULD CONFUSE THIS STALLION AS A PERLINO OR MAYBE EVEN CREMELLO - HTTP://WWW.DOUBLELACRES

Silver is another dilute that seems to "selectively" affect pigment. Silver is a pigment dilution that affects only black pigment (eumelanin) (Reissmann et. al., 2007). Even more strangely, silver seems to affect the mane and tail of the horse more than it does the body, making silver black and silver bay almost identical in some scenarios (Reissmann et. al., 2007). Silver is an autosomal dominant gene with no phenotypic distinction between heterozygotes and homozygotes (Cotton, 2020).

FIGURE 10 - SILVER BAY MORGAN STALLION (HTTP://WWW.MORGANCOLORS.COM/)

Figure 11 - Silver black Morgan mare – one example of a silver black looking very similar to a silver bay (http://www.brookridgemorgans.com/2007filly.htm)

Figure 12 - Another example of silver black – this horse has a much darker coat than the mare pictured above (https://beautyofourlives.proboards.com/thread/35)

The mushroom dilution is one only seen in Shetland Ponies and results in a gray-brown coat and often a flaxen (lightened, almost white) mane and tail (Cotton, 2020). Since Shetland Ponies are often used to increase genetic diversity in Miniature Horses, the mushroom dilution is occasionally seen in Miniature Horses as well (Cotton, 2020). While not fully understood, it is hypothesized that this dilute is the result of a recessive mode of inheritance (Tanaka et. al., 2019). One study "uncovered a frameshift variant, p.Asp201fs, in the MFSD12 gene encoding

the major facilitator superfamily domain containing 12 protein" (Tanaka et. al., 2019). This study analyzed this mutation in one mushroom-dilute Shetland Pony and compared it with 87 other genomes from horses of different breeds; the phenotype was found to be absent in 252 individuals but identified in Miniature Horses (Tanaka et. al., 2019). "MFSD12 is highly expressed in melanocytes and variants in this gene in humans, mice, and dogs impact pigmentation" (Tanaka et. al., 2019). The results of this study proposed that this frameshift mutation is the cause for the mushroom dilution observed in Shetland Ponies (Tanaka et. al., 2019).

FIGURE 13 - MUSHROOM DILUTE SHETLAND PONY (HTTPS://VGL.UCDAVIS.EDU/TEST/MUSHROOM)

All of the dilutes described above cause changes in pigmentation. Whether it be red pigmentation or black pigmentation, a change in pigmentation can cause deleterious effects in other systems of the horse (Bellone, 2010). As one might expect, horses with dilution genes can be more susceptible to a form of horse-specific melanoma, however other conditions have been linked to various pigmentation genes such as lavender foal syndrome, hearing defects, and multiple congenital ocular anomalies (Bellone, 2010). Far more pleiotropic effects can be seen when we begin to explore horse color patterns.

Lethal white overo (LWO) is a homozygous lethal condition that is the possible result when breeding two heterozygous frame overo horses (Genetic cause of overo lethal white foal

syndrome, 2001). Frame overo is one of many different white patterning genes in horses, characterized by white pigment concentrated on the “middle” of the horse.

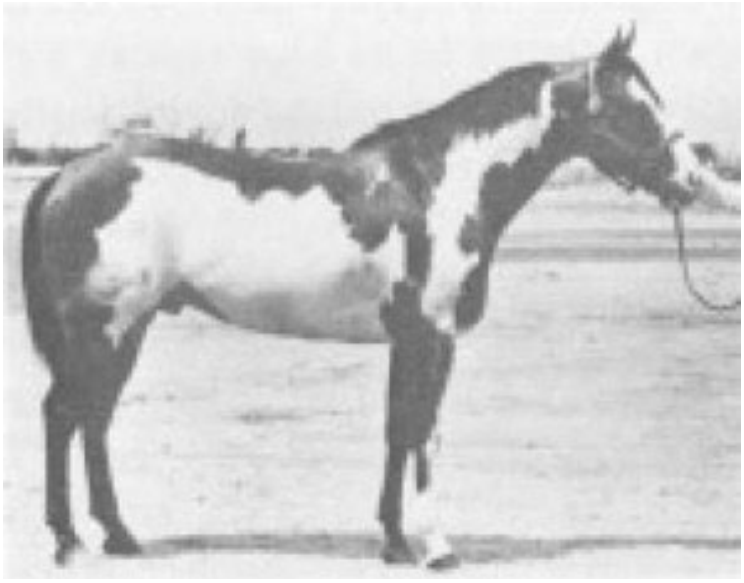

FIGURE 14 - A FRAME OVERO PAINT HORSE (GENETIC CAUSE OF OVERO LETHAL WHITE FOAL SYNDROME, 2001)

Lethal white overo is most commonly seen when a horse who does not display extensive frame overo markings is bred to another frame overo horse. One case from 2002 talks of a registered AQHA mare that had been bred to a Paint Horse stallion (Lightbody, 2002). The mare was pregnant when she was purchased at auction in 2000 and was registered as a solid chestnut with only a star, stripe, and snip (small facial markings), and a small white spot in one of her nostrils (Lightbody, 2002). Genetically, the mare was overo, and the small white spot in her nostril is the only phenotypic sign of overo (white markings that are so minimal can occur in any solid colored horse); her sire was a registered American Paint Horse and also frame overo (Lightbody, 2002). Lethal white overo causes ileocolonic agangliosis, or colic as it is more commonly referred to; unfortunately, this foal was euthanized after genetic testing proved the root cause of the colic being lethal white overo (Lightbody, 2002). This case, as well as many more cases of lethal white overo, show the importance of genetic testing, regardless of phenotype.

Another homozygous condition that is linked to white spotting is Splashed White, specifically SW2 and SW3. There are 6 variants of the splashed white gene that have been identified, and only homozygous SW1 and SW2 have been identified, suggesting that any other

homozygous variant is embryonic lethal (Cotton, 2020). SW2 and SW4 are both mutations in the PAX3 gene and is caused by a missense mutation in PAX3 (p.Cys70Tyr) (Cotton, 2020). A horse that is homozygous SW2 may be deaf or at least hard of hearing (Cotton, 2020). Homozygous SW4 is rare and thought to be homozygous lethal; this variant has only been identified in a family of Appaloosa horses (Cotton, 2020). All other splashed white variants are variants in the microphthalmia-associated transcription factor (MITF) gene (Cotton, 2020). "MITF is an important protein for normal pigment cell function" (Cotton, 2020). The SW1 variant is not considered to be homozygous lethal, as some SW1/SW1 horses have been identified; this variant is a 10 base-pair insertion in the MITF promoter region (chr16:g.20,117,302Tdelins11) (Cotton, 2020). SW3 has only been identified in a few lines of Quarter Horses and Paint Horses and is thought to be homozygous lethal as no homozygous SW3 horses have been identified (Cotton, 2020). SW3 is caused by a frameshift mutation of MITF (p.C280Sfs*20) (Cotton, 2020). "SW5 is a large deletion of 63 thousand nucleotides in the MITF gene (chr16:21,503,211-21,566,617)" (Cotton, 2020). SW6 was recently identified in a single family and is a large deletion of the MITF gene, much like SW5; this large deletion likely inhibits the proper function of the MITF gene and it is currently unknown if a homozygous SW6 horse would be viable (Cotton, 2020).

One final pleiotropic condition is one that deals with a very unique type of white spotting – leopard complex. "Appaloosa" has long been a term used to describe any horse with sporadic spotting that was popularized by the Appaloosa breed of horse. This type of spotting can be seen in many different breeds, however. In more recent years, the leopard complex gene has been identified as the source of this type of spotting and even more recently, an additional gene – PATN1 – has been identified as the possible cause for "leopard" patterns. Appaloosa spotting can be broken down into 3 main categories – varnish roan, leopard and few spot, and blanket and snowcap.

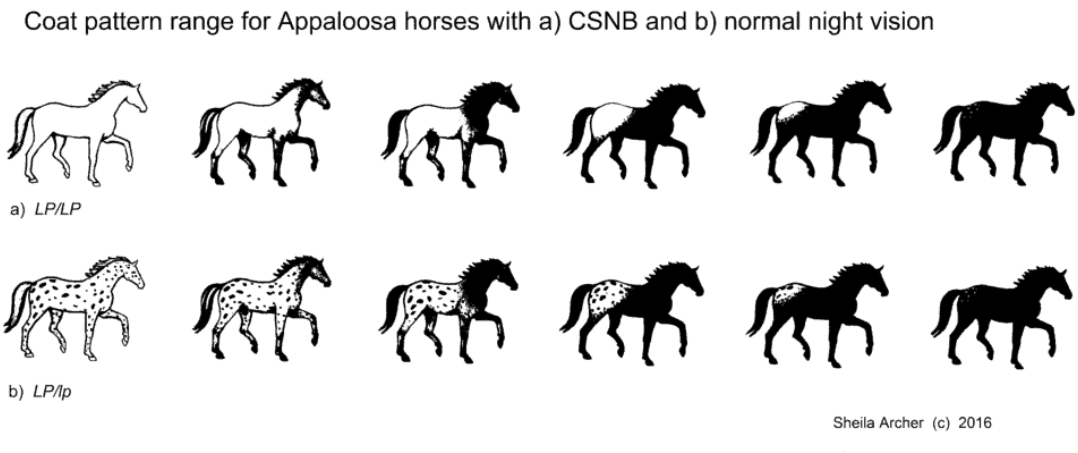

FIGURE 15 - RANGE OF APALOOSA SPOTTING (HTTPS://IHEARTHORSES.COM/WHATS-IN-A-SPOT-APPALOOSA-COAT-GENETICS/)

Leopard complex (Lp) is the result of an incompletely dominant mutation in TRPM1, however leopard complex is not the only gene responsible for this unique collection of depigmentation patterns in horses, additional modifiers of this gene are responsible for controlling its effect across the body of the horse with PATN1 being the most notable in recent years (Holl et. al., 2016). PATN1 seems to be responsible for making horses with high levels of white on the body (see left-most side of Figure 15), such as full varnish-roans and full leopards; it is theorized that an additional modifier may be responsible for restricting the amount of white seen on the body (see right-most side of Figure 15) such as small blankets and snowcaps (Holl et. al., 2016).

Even though it is responsible for some beautiful patterns in horses, leopard complex can be harmful when in its homozygous form. Leopard complex has been associated with decreased expression of TRPM1, however this has also been implicated as the cause for congenital stationary night blindness (CSNB) in horses (Bellone et. al., 2010). Simply put, CSNB causes the horse to be unable to see still objects when in a dark environment – the horse can see objects moving, but their overall sight in a dark environment is greatly impaired (Bellone et. al., 2010). While this is not proven by any means, I have also noticed that many varnish-roan appaloosas I've interacted with seem to have poor vision in general, especially when it comes to depth perception.

One final key player in coat depigmentation in horses is the KIT gene. “Mutations in the KIT gene have previously been shown to cause white coat color phenotypes in pigs, mice, and humans” so researching these mutations further could certainly lead to new information on how to treat those who are affected negatively by such a mutation (Haase et. al., 2009). The KIT receptor (type III receptor protein tyrosine kinase) plays an integral role in melanocyte development and KIT receptor signaling is crucial for the development of various stem cells (Haase et. al., 2009). Not only do mutations on the KIT gene cause pigmentation disorders (like those that are discussed below), but many pleiotropic effects such as anemia and male sterility in mice have also been linked to KIT mutations (Haase et. al., 2009). The most prominent depigmentation phenotypes caused by KIT mutations in horses include Sabino-1 (Sb1), tobiano, roan, and dominant white (Haase et. al., 2009). It should be noted that there have been 2 different sources of roan identified – one that is an inherited KIT mutation (regular roan or “true” roan) and one that seems to randomly pop up in various lines of horses (“spontaneous” roan). These 2 roan variants do vary phenotypically slightly (true roan has dark points and a dark head while spontaneous roan has white ticking all over the horse, including the face) so it is believed that they result from separate mutations, but no conclusions have been made as to the origins of “spontaneous” roan.

The Sb1 KIT mutation is an intronic mutation causing a partial skipping of exon 17 and resulting in large distinct white patches on the face and legs, often with smaller spots on the belly as well (Haase et. al., 2009). Sb1 can also cause “roaning” (often referred to as sabino roan) around the white patches – roaning is classified as scattered white specs throughout the body (Haase et. al., 2009). Homozygous Sb1 horses are almost entirely white (Haase et. al., 2009).

Figure 16 - A black "sabino roan" mare (https://www.deviantart.com/venomxbaby/art/Gray-roan-sabino-mare-1-296225771)

A large chromosomal inversion is the cause of the tobiano spotting pattern; this inversion disrupts the regulatory element of the KIT gene (Haase et. al., 2009). Heterozygous and homozygous tobiano horses are almost indistinguishable, with white lower legs and white body patches that cross over the horse's topline (Haase et. al., 2009). As a kid, I was always told that if the white touches and/or crosses over the spine, the horse was tobiano; I now understand it isn't that simple, but it is one easy way to distinguish tobiano from other white spotting patterns.

Figure 17 - A bay tobiano horse (https://s-media-cache-ak0.pinimg.com/736x/99/d4/42/99d442f949f4e77c642a511765724161.jpg)

The next prominent KIT mutation is roan, or "true" roan; the exact mutation that causes roan is still unknown (Haase et. al., 2009). Roan is characterized by a mixture of white and pigmented hair on the body and "true" roan horses have very little "roaning" on the face and lower legs (Haase et. al., 2009).

Figure 18 - A bay roan horse (https://th.bing.com/th/id/R.3ab29c56bad42dd595d43f693de4004c?rik=I%2bhkC3%2ffCNVglg&riu=http%3a%2f%2fcdn0.wideopenpets.com%2fwp-content%2fuploads%2f2015%2f12%2fBAY-ROAN_reitpony_napoleon41136_LR.jpg&ehk=GCEtYRKQw%2bLHGfLzwv80NurBu3uJ)

Dominant white is phenotypically similar to homozygous Sb1 horses, however it is a separate KIT mutation; dominant white horses are almost completely unpigmented, if not entirely (Haase et. al., 2009). "Dominant white is inherited as a monogenic autosomal dominant trait. It is hypothesized to be embryonic lethal in the homozygous state. To date, no homozygous horse could be identified" (Haase et. al., 2009). Prior to the study performed by Haase et. al., 4 KIT mutations were identified in dominant white horses (W1-W4), however this 2009 study identified an additional 7 KIT mutations in various "founder" horses (horses who were the first to be identified with a certain mutation) – there was little to no phenotypic difference among these horses, however a new mutation can provide vital information for future studies (Haase et. al., 2009). Two of these mutations are frameshift mutations, three are splice site mutations, and the last two are missense mutations – it is hard to predict whether the missense mutations affect the KIT gene function, however it is likely the other 5 identified mutations do affect the KIT protein function (Haase et. al., 2009).

Figure 19 - 'The Opera House' - Dominant white thoroughbred (http://www.whitehorseproductions.com/pintotbs.html)

One question remains – how are there so many different shades of bay and chestnut? Are these the result of a dilute or modifier that has yet to be identified? Surprisingly, pedigree analysis doesn't point towards such a clear answer.

Figure 20 - A few of the variations of bay horses (https://www.horsesandus.com/what-are-the-3-base-horse-coat-colours/)

One study suggests that the number of copies of extension could have an impact on the relative darkness of the body color in bay horses – homozygous extension bays were darker on

average than those heterozygous for extension (Corbin et. al., 2020). This relationship was not observed for agouti, however, this could vary from breed to breed, as does the average shade of bay (many warmblood breeds tend to be dark bay while Quarter Horses often lean more towards a sandy bay), which would likely skew observational data (Corbin et. al., 2020). This study identified a region upstream of the ASIP gene (agouti) that was associated with shade variations in bay horses – the region includes the distal 5' end of the ASIP transcript, as well as the RALY gene (encodes a heterogeneous nuclear ribonucleoprotein gene), which may affect pre-mRNA splicing and embryonic development (Corbin et. al., 2020). Because of the amount of variation that already seems to occur in this region, it is also likely that additional mutations and modifications occur in the region, affecting the coat shade with little to no adverse effects (Corbin et. al., 2020).

Another study of Kiso horse coat color variation done by Nakamura et. al. evaluated variation in MC1R, ASIP, and MATP genes at the extension, agouti, and cream dilute loci (2019). The variation in coat color that is seen in many riding horses is not seen in wild horses, suggesting the domestication of horses had some effect on these genes resulting in small variations (Nakamura et. al., 2019). While this study did not provide conclusive evidence on the effects of small variations on these loci (it's focus was on preserving a population of wild horses), it does suggest a correlation and provides information on how SNaPshot could be used in future studies to identify the cause of this color variation (Nakamura et. al., 2019).

Supplemental Figures

Figure 21 - The horse I lease (Chester) - this is his summer coat, he's more of a yellow-based chestnut in the summer

Figure 22 - Chester with his winter coat (top, reddish chestnut color) - He's clipped so you can see some of his summer coat peeking through on the bottom

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