integrity Research Journals

Journal of Animal Science and Veterinary Medicine
Volume 7(4), pages 127-149, August 2022
Article Number: 0E3842CC3
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2022.319
https://integrityresjournals.org/journal/JASVM

Review Article

Genes affecting coat colour and the resulting variation in horses (*Equus caballus*) – A Review

Marvellous O. Oyebanjo*, Etseoghena A. Obi and Adebowale E. Salako

Animal Breeding and Genetics Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

*Corresponding author. Email: ooyebanjo488@stu.ui.edu.ng

Received 7th April 2022; Accepted 25th May 2022

ABSTRACT: Horse colour is important. People chose horses for their colour pattern diversity, according to archaeological investigations and biological evidence. Horses were generally dark coloured before domestication, according to molecular testing. After domestication, the genes for white patterns and dilutions appeared. The appearance of their horses, as well as their competence and willingness to work, must have been important to early people. In many cases, the genes that impact coat colour patterns in one species also influence coat colour patterns in another. In horses, humans, cattle, dogs, and other species, the *MC1R* gene encodes the alleles for red and black of the *Extension* locus. White spotting is caused by mutations in the *KIT* gene in horses (tobiano, roan, sabino, dominant white), mice, and humans. Because of its similarity to a white hair gene seen in humans with Hirschprung's illness, the gene for overo in horses was discovered. The genes that impact coat colour patterns in one species generally play a similar role in another, according to molecular studies. It came as no surprise. Earlier geneticists claimed that coat colours in horses were caused by the action of genes similar to those observed in other species based solely on phenotype. This is a crucial lesson for genetic studies: we can apply what we have learned from other species to our own. All in all, the study of coat colour variations in the horse is a study of the horse's nature.

Keywords: Allele, coat colour, gene, horses, trait.

INTRODUCTION

The effects of genes that influence coat colour in animals, especially mammals, have long been a subject of great curiosity and investigated for years. This is partly because among domesticated animal species, coat colour may have significantly influenced events of domestication and initial choice of selection of these species (Henner *et al*., 2002a; Bruford *et al*., 2003; Andersson and Georges, 2004). Another reason is that coat colour has been the foremost means of recognition and also confirmation of parenthood (Bowling, 1996; Sponenberg, 1996; Stachurska *et al*., 2007). Coat colour phenotype seems relatively simple to be inherited from generation to generation as this inheritance appears to follow the Mendelian mode of inheritance (Hurst, 1906; Wright, 1917). Therefore, coat colour has become a unique model for studying gene effects and interactions.

Although it is believed that the wild coat colour phenotype, a black-based pattern, provided sufficient camouflage against predators and survival in nature, under domestication, the horse has sufficiently and rapidly evolved into an animal with high variability in coat colouration with many modern colours and spotting patterns (Fang *et al*., 2009; Ludwig *et al*., 2009). The gradual appearances of different coat colour phenotypes were controlled and promoted by genetic differences occurring at first as arbitrary mutations and have since been selected for during domestication. The most important colour pigment in mammals is known as melanin which is the pigment granules that occur in the skin, hair, iris, and some tissues in the body of an animal (Searle,

1968). Woolf and Swafford (1988) discovered that pigment production in the horse is similar to that of other species of animals and variation in coat colour is produced by genes by altering the switch between the two forms of melanin (eumelanin and pheomelanin) production in melanocytes which are the pigment cells in the body of the horse, or by the shape, the number, arrangement and or presence of pigment granules. Therefore, coat colour variation is associated with either pigment synthesis or the melanocytes, the pigment-producing cells (Nordlund *et al*., 1998).

Few genes are seen to influence coat colour, resulting in an epistatic mode of inheritance in the horse (Rieder *et al*., 2001; Henner *et al*., 2002a), it is also discovered been that these genes have some pleitropic effects with more complex traits (Rieder *et al*., 2000; Dobney and Larson 2006; Gilbert, 2006). These traits include temperament, diseases such as grey horse melanoma, or even developmental disorders, such as the lethal dominant white trait. Furthermore, breeders have often related performance qualities with specific coat colours, but this is highly controversial as Bowling (1996) and Stachurska *et al*. (2006) suggested that there is little involvement of the horse's coat colour with its performance.

In recent times, names for different colours in the horse are easy to designate just by appearance and are of practical relevance in the identification and registration of individuals and breeds of horse, thereby, distinguishing horses of particular breeds. The initial information of allelic segregation in parents-offspring is suggested by the coat colour phenotype and this practical example is seen in the grey horse and chestnuts, where these colour phenotypes result in at least one of the parents having these colour phenotypes (Bowling 2000; Sponenberg, 2009). However, Ludwig *et al.* (2009) were convinced that no colour is restricted or specific to a single breed and certain colour variants across breeds are a result of the same mutation, supporting the idea that during the domestication event, mutations producing the colour variants occurred preceding the development of a breed.

Therefore, the objective of this review is to describe, step by step, the major phenotypic groups of coat colours in horses and to give a comprehensive picture of gene effects on the coat colour variation by integrating recent studies in molecular genetics.

BLACK, BAY, AND CHESTNUT COAT COLOURS IN HORSES

It is believed that the horse's primordial color was a black-based pattern that served as camouflage against predators. The coat of the modern-day Przewalski's horse, the surviving wild species most closely related to domestic horses, is described as dun. Regardless of the exact original hue, the horse has certainly evolved into an animal with a wide range of coat diversity as a result of domestication. In reality, ancient DNA samples have been utilized to estimate the colour of prehistoric horses using DNA-based coat colour allele assays. This research showed that many of the contemporary colours and spotting patterns that we see today were acquired by the horse rather quickly after domestication, roughly 5000 years ago (Ludwig *et al*., 2009).

The various colour patterns are determined by genetic variations that first appeared as random mutations and have later been selected for through domestication. Melanin is the most important pigment for coat colour in mammals. Hair, skin, and iris pigment granules, as well as various interior tissues, contain it. Eumelanin (black or brown) and pheomelanin (red or yellow) are two types of melanin pigment (Searle, 1968). Pigment biosynthesis in horses has biochemistry that is similar to that of other animals (Woolf and Swafford, 1988). The existence, shape, amount, or arrangement of pigment granules, as well as the switch between eumelanin and pheomelanin production in pigment cells (melanocytes), are all factors that influence coat colour diversity viz a viz the biological information encoded in genes. Many phenotypes have colour names that are easy to identify by looking at them. Colours are frequently recorded by owners and breed registries as a means of distinguishing individuals. This word description of the colour phenotype might indicate which pigment synthesis and distribution alleles a given animal may have. The word description, albeit useful, has some limits – as, often, qualitative descriptions of abstract entities are unable to provide a holistic delineation of an abstract entity such as alleles or its following variation.

Horses' base coat colour is determined by the *Extension* locus (*E*). Other genes, such as *Agouti* (*A*), act to modulate or obscure the *Extension* locus' effects. The most frequent colour patterns of horses are black, bay, and chestnut, which are caused by *Extension* and *Agouti* working together. Hurst (1906) demonstrated that Thoroughbred stud book color records may be explained using Mendelian inheritance principles.

Extension locus

The *Extension* locus is responsible for the production of eumelanin (black pigment) detected in blacks, browns, bays, buckskins, duns, and grullas, as well as pheomelanin (red pigment) in chestnuts, sorrels, palominos, and red duns. This locus was originally known as *Brown* (*B*), but the *Extension* locus terminology was adopted because it was the same as the Extension genes found in other animals; for example, cats, and dogs.

The black/chestnut colour variation in horses is attributed to two extension alleles. The alleles either increase (*E*) or decrease (*e*) the quantity of eumelanin (black) in the coat, affecting the visible extent of

Table 1. Genotypes and phenotypes for the *Extension* (*E*) allele.

Colour	Genotype
Black, brown, or bay	*EE* or *Ee*
Chestnut (red)	*ee*

pheomelanin in the opposite direction (red). The extension gene affects whether the black pigment is found in the hair and skin (*EE* or *Ee*) or only in the skin (*ee*) (see Table 1).

The distribution of black hair pigment in horses is a second and crucial element. Black hair can be equally distributed across the body, as in a black horse, or it can be restricted to the mane, tail, and legs, with little or no hair on the body, as in a bay horse. A second gene – precisely the *Agouti signaling peptide* (*ASIP*) gene – is responsible for the black pattern. Because black pigment is inherited as a trait dominant (*E*) to its absence (*e*), breeding two chestnut-coloured (*ee*) horses will result in no black/brown/bay progeny. Parentage exclusion in extreme instances has frequently confirmed this "chestnut rule" (Trommershausen-Smith *et al*., 1976).

Both chestnut and black horses have different levels of colouring. Some chestnut horses have light red coats and are referred to as "light sorrel," whereas others have a considerably darker colour and are referred to as "liver chestnut". Similarly, genetically black horses can exhibit graded colour variation, causing some black horses to seem brown in appearance. This type of variation is not caused by the *Extension* locus. This difference in intensity is most likely caused by other, as yet undiscovered, modifying loci. “Bend D'Or” spots, which are small, irregular dark hair patches found on chestnut horses and resemble black smudges on the red coat, are another variety that can emerge. Small white patches on the coat are also referred to as "Birdcatcher" markings. In these *ee* horses, eumelanin production in the hair appears to break through, but not to the point that they may be confused with *E*– horses.

The *MC1R* (*Melanocortin 1 Receptor*) gene is also known as the *Extension* gene, E. The *MC1R* gene was first discovered on ECA3 alongside another essential colour gene, *KIT* (Raudsepp *et al*., 1999). *MC1R* is located at chr3:36259305-36260257, according to the second assembly of the horse genome sequence (EquCab2). A missense mutation of a single nucleotide in the *MCIR* gene was responsible for the mutation of the *E* allele to the *e* allele (Marklund *et al*., 1996). Consequently, a change in the open reading frame (ORF) of the mRNA leads to a substitution of phenylalanine for serine in a part of the protein that crosses the cell membrane. This mutation disables the *MC1R* molecule's receptor function, which leads to the formation of eumelanin. Except for Bend D'Or patches, horses homozygous for this mutation are unable to develop black pigment.

According to Wagner and Reissmann (2000), or chestnut colour, a highly unusual allele in the *MC1R* allele series has been discovered. The *ea* allele is functionally equal to the *e* allele; nevertheless, it interferes with the identification of the *e* allele in many routine DNA assays. Horses carrying this gene have the *e* mutation as well as a second mutation close by that makes it difficult to detect *e* using some testing methods. While this mutation affects DNA, it does not affect the protein. As a result, breeders will only become aware of this gene when they receive test results that are incongruous with the horse's colour, such as chestnut animals reported to have the *E* allele from molecular testing. The genetic basis for “dominant black” has been hypothesized for the fourth allele of *MC1R* in horses (named E^D); nevertheless, the genetic basis for this allele has yet to be established (Rieder *et al*., 2001).

Because it was known that *MC1R* had multiple alleles that control black hair distribution in mice, it was a promising candidate for *Extension* in horses. In reality, many mammalian species' hair colours show a eumelanin/pheomelanin transition, which is a common cause of colour diversity in domestic animals (cats are a quintessential example). Labrador Retrievers come in a variety of colours, including black and yellow. Although Holstein and Angus' cattle are usually black-pigmented, red cows do exist, and some owners seek red animals. Alleles of the *MC1R* gene have been identified as influencing red/black base coat colours in both cases.

MC1R genes for a receptor protein that is part of the membrane of melanocytes, the cells that make pigment, in mice. The cell produces eumelanin (black pigment) after this receptor interacts to its specific signalling hormone (melanocyte-stimulating hormone). In mice, recessive variants of the *MC1R* gene fail to bind the melanocyte-stimulating hormone, resulting in the production of only pheomelanin rather than eumelanin (Robbins *et al*., 1993). Owing to the similar mutation type, the same impact may exist in chestnut horses, though further research is needed.

The majority of horse breeds have red- and black-pigmented variants, however, a few breeds have little or no diversity in this gene. Only the dominant allele of *Extension*, E, is found in Friesians and Cleveland Bays. Rare recessives may exist, but without the use of a DNA-based test, or detection of minor allele frequency (MAF) from DNA pooling, they are extremely difficult to discover. Suffolks and Haflingers, on the other hand, only have the recessive allele of *Extension*, e (Bailey and Brooks, 2013).

Agouti locus

Black horse breeders should be aware of the unique combination of the two genes *Extension* and *Agouti* required to achieve the desired colour. The uncertainty about which *Agouti* alleles are present in chestnut (*ee*)

Table 2. Phenotypic combinatorics of the *E* and *A* alleles.

Phenotype	Genotype
Bay/Brown	*EEAA*, *EeAA*, *EEAa*, or *EeAa*
Black	*EEaa* or *Eeaa*
Chestnut (red)	*eeAA*, *eeAa*, or *eeaa*

horses makes predicting whether a given mating will produce black difficult. The *Agouti* gene's dominant allele *A* causes eumelanin dispersion in hair to be confined to a "points" pattern (e.g. mane, tail, ear rims, lower legs). When homozygous in the presence of *E*, the recessive gene, *a*, does not restrict the distribution of black hair and produces a uniformly black horse (see Table 2). Because the *a* allele is uncommon in many breeds (black horses are uncommon), most bays and chestnuts are *AA*. The *a* allele must be carried by any red or bay horse that sires or produces black offspring. A chestnut horse must be *eeaa* if it has two black (*Eeaa*) parents.

The hue of black coats ranges from blue-black to sun-fading black, but the genetic differences between them are currently unknown. Other *agouti* alleles (identified as *A+* or *A'* in previous investigations) have been proposed to determine the inheritance of wild patterns (as in Przewalski's horse) and brown horses, but they have not been thoroughly studied. Some of the variance in pigment distribution of colour hues of bays, seal browns, and blacks could be attributed to *Agouti* alternative alleles.

Agouti (*A*) in horses has been traced back to the *Agouti signaling peptide* gene (*ASIP*) (Rieder *et al*., 2001). *ASIP* regulates the pattern of eumelanin dispersion, therefore its effects are only visible when *E* is present (an example of epistatic gene interaction). In the EquCab2 genome assembly, the *Agouti* gene is found on ECA22 at chr22:25,167,080-25,171,073. According to Rieder *et al*. (2001), the *a* allele is caused by the deletion of 11 nucleotides from the *ASIP* gene sequence. The loss of these nucleotides is thought to render the peptide useless. Additional *ASIP* alleles – *At*, responsible for seal brown or black and tan; and *A+*, wild bay – have been hypothesized, however, the findings of these investigations have yet to be published, and the genetic basis for these alleles is unknown. Several laboratories offer testing for the"*a*" allele, which is frequently combined with testing for the *MC1R* alleles to predict offspring's base colour.

In terms of etymology, a South American rat with black-banded hairs is responsible for this gene's name. In dogs, there are four *Agouti* alleles: recessive black, black and tan, wild type, and fawn (Schmutz and Berryere, 2007). Cattle have several alleles, including recessive black, lighter points, and a regulatory mutation that causes a brindle pattern (Girardot *et al*., 2006; Seo *et al*., 2007). The *ASIP* controls the start-and-stop phases of eumelanin and pheomelanin during hair growth stages (black-banded yellow hairs) or across space (black hairs on the back but not on the abdomen). It competes for *MC1R* binding with melanocyte-stimulating hormone, affecting the cellular "switch" for pigment type. The modulation of fat storage in adipocytes is also influenced by this signaling pathway. Several mouse variants of *ASIP* cause obesity as well as coat colour variation (Bailey and Brooks, 2013).

Only the *A* allele is found in the consistently bay Cleveland Bay breed – black horses are never formed. Because Friesians are entirely black, they exclusively carry the *a* allele for *Agouti*. The frequency of alleles for this gene determines the distribution of black against bay horses in a population. Most light horse breeds appear to have a larger frequency of *A* than *a* (and are consequently bay); however, the frequency relationship may be flipped in the pony and draft breeds with a high predominance of chestnut horses, with a rise in black horses.

COAT COLOUR DILUTING GENES IN HORSES

Silver (*Z*), *Dun* (*D*), *Champagne* (*CH*), and *Cream* (*C*), are four genes that cause coat colour dilution in horses. The black (eumelanin) and red (pheomelanin) pigments produced as a result of gene action by the Extension locus (*E* or *MC1R*) modified by the *Agouti* (*A*) locus are diluted by these genes. As the function of the dilution genes is to affect the activity of another locus (*E*), the dilution genes are described as having an epistatic influence on coat colour. These genes are well known to horse owners and popular for many breeds. Molecular genetic investigations have led to the findings of the genes and the DNA mutations responsible for three of these four dilution genes.

Cream dilution

The gene that causes the golden body colour seen in palominos and buckskins is the most well-known of the colour dilution genes. The mane and tail of a palomino horse are white (flaxen), whereas the mane, tail, and legs of a buckskin horse are black. The *Cream Dilution* gene reduces the red pigment (pheomelanin) intensity while just slightly reducing the black pigment intensity. Palomino is created by diluting the red hair of chestnuts, whereas buckskin is created by diluting the red hair of bays.

Perlinos are "double dilutes" of bays with two copies of the *Cream Dilution* gene, whereas cremellos are "double dilutes" of chestnuts with two copies of the *Cream Dilution* gene. Cremellos are characterized by their pink skin, blue eyes, and ivory hair. Perlinos have the same characteristics as perlinos, with the exception that the mane and tail are a shade darker than the body. As previously stated, black horses (*aaE–*) do not appear to have red pigment. However, horses with one or two copies

of the *Cream Dilution* gene show modest dilution effects, resulting in a phenotype known as "smoky".

For the *Cream Dilution* gene, a third allele known as the *Pearl Dilution* has been discovered. This allele interacts with the *Cream Dilution* gene, resulting in coat colour dilutions that are quite similar to the Champagne Dilution phenotypes, which will be discussed later.

The *Cream Dilution* locus has the gene symbol *C*. This gene symbol was first used to denote the locus for albinism in other mammals, and it was then used to describe horses with white or nearly white coats (Castle, 1948). Even though albino horses are extremely unusual, this nomenclature has been utilized for Cream Dilution (Bowling, 1996; Sponenberg, 2009). Because this locus has nothing to do with albinism, a group of scientists chose to rename the locus symbol to "*CR*" for *Cream Dilution*. However, we shall continue to refer to it as the C locus for the remainder of this article.

There are three known alleles at the *C* gene. We use the symbol "*CR*" to identify the allele that causes the dilution effect because it is an incomplete dominant gene. The absence of dilution is recessive; therefore, we use the sign "*cr*" to represent this allele. A third allele, termed Pearl, has also been reported for this location, as previously mentioned. We use the sign "*prl*" to identify this allele, which is said to have a recessive mode of inheritance.

CR and cr alleles in bay, chestnut, and black horses

When heterozygous, *CR* dilutes pheomelanin (red) to yellow while having no effect on eumelanin (black). When the dilution allele is homozygous (*CRCR*), both eumelanin and pheomelanin are diluted to pale ivory (Adalsteinsson, 1974) – see Table 3. Palominos are diluted reds (*CRcr ee*) and buckskins are diluted bays (*A–E– CRcr*) when the dilution gene symbols are combined with those for the coat colour genes involved in the basic development of colour. Because they lack apparent red pigment to reveal the single dosage effects of the dilution gene, blacks (*aaE–CRcr*) can carry the dilution gene without expressing it. Breeders may be shocked when a palomino or buckskin progeny is generated from breeding a non-diluted horse (*crcr*) to a black horse since the presence of the dilution gene in black horses is not always noticed. The situation can be confirmed by molecular testing.

It is not clear why the palomino's mane and tail are white rather than gold like the body, but it could be due to changes in gene action between the melanocytes (pigment-producing cells) of the permanent hair (mane and tail) and those of the seasonally shed hair. The beauty of the palomino and buckskin hues entices novice breeders, but they are disheartened to realize that neither will breed true since the appealing colours are produced by heterozygosity for a dilution gene. If a breeder wants to recreate the colour of a favourite palomino, for example, by breeding that palomino to another palomino, the projected colours and frequencies among the progeny will be 50% palomino, 25% chestnut, and 25% cremello (see Table 4).

Table 3. The genotypes and phenotypes of the *CR* allele of the *Cream Dilution* (*C*) locus.

Phenotype	Genotype
Absence of dilution	*Crcr*[1]
Palomino/Buckskin	*CRcr*
Cremello/Perlino	*CRCR*

[1]*cr* represents the absence of dilution and is recessive.

Table 4. Punnett square for mating[2] a palomino mare (*ee CRcr*) and stallion (*ee CRcr*).

Sire/Stallion/Sperm (*ee CRcr*)		Dam/Mare/Ovum (*ee CRcr*)
e CR	***e cr***	
eeCRCR	*ee CRcr*	*e CR*
Cremello	Palomino	Color of cross
ee CRcr	*ee crcr*	*e cr*
Palomino	Chestnut	Color of cross

[2]Palominos will be produced 2 out of every 4 times, cremellos 1 out of every 4 times, and chestnuts 1 out of every 4 times.

A cremello (or perlino) is unattractive or perhaps unregistrable in some breeds. In this situation, palomino chestnut would be the preferable mating choice; the expected proportion of palominos for this hybrid is the same as for a palomino-palomino mating (50 percent), but no cremellos are expected. A homozygous diluted horse can be a significant component of a breeding program focusing on palominos and buckskins in breeds that enable cremello and perlino registration - hues such as Icelandic, Miniature, Peruvian Paso, or Paso Fino. When mated to non-diluted individuals, the cremello or perlino will pass on a color dilution gene to all children, resulting in the desired heterozygous genotype.

The Pearl (prl) allele

Sponenberg (2009) has written a review of the *Pearl Dilution* allele (*prl*). Horses with a single copy of the *Pearl Dilution* allele and the non-diluted allele (genotype: *crprl*) do not show dilution and look as bay, chestnut, or black. However, in the presence of *CR*, such as in the genotype *CRprl*, the dilution effect resembles that of horses with a copy of the *Cream Dilution* gene and a copy of the *Champagne Dilution* gene (*CRcr* and *CHch*), which produce greater pheomelanin dilution and a modest eumelanin dilution. The effect is virtually identical to the colours generated by heterozygote s for *Champagne* in

Table 5. Genotypes and phenotypes for the allelic variants of the *Cream Dilution* (*C*) locus with varying base color phenotypes.

Base color phenotype and genotype (A, E loci)	C genotype	Phenotypic effect
Chestnute (- - *ee*)	*crcr*	Chestnut
	CRcr	Palomino
	CRCR	Cremello
	crprl	Chestnut
	CRprl	Nearly white
	prlprl	Gold pear
Bay (*A*- *E*-)	*crcr*	Bay
	CRcr	Buckskin
	CRCR	Perlino
	crprl	Bay
	CRprl	Beige, brown points
	prlprl	Amber pearl
Black (*aa E*-)	*crcr*	Black
	CRcr	Black (smokey)
	CRCR	Smoky cream
	crprl	Black
	CRprl	Tan, tan points
	prlprl	Classic pear

horses with two copies of *prl*, but specified to reflect the different genetic origin: chestnut becomes pearl gold, the bay becomes an amber or sable pearl, and black becomes classic pearl in horses with two copies of *prl*. Shown in Table 5 are the effects of each conceivable genotype for the *Cream Dilution* locus on the base coat colours.

Microsatellites and family studies were used to locate the CR allele's locus to ECA21 (Locke *et al*., 2001). *SLC45A2* (solute carrier family 45, member 2 gene, formerly known as *MATP*, the gene for the membrane-associated transport protein) was later discovered to be an allele of *CR* (Mariat *et al*., 2003). In the EquCab2 genome assembly, *SLC45A2* is found at chr21:30,664,390-30,693,166. A missense mutation in *SLC45A2*'s second exon causes an aspartic acid to be replaced by asparagine in the amino acid sequence. This polymorphism is easily detectable, and there is a genetic test for it.

A wide range of breeds carries the CR allele. It is most commonly found in ponies and stock horses, but it can also be found in Paso Finos, Peruvian Pasos, American Saddlebreds, Morgans, and Tennessee Walking horses. Palominos and buckskins are found in Thoroughbreds in the United States, but they are extremely uncommon. Arabians are likely lacking in this dilution gene. While palomino societies accept Arabian iridescent light chestnuts with extremely flaxen manes and tails, the buckskin and cremello counterpart colours are not seen in this breed. Lusitano, Gypsy Cob/Vanner, and Paint horses, as well as American Quarter Horses, have been found to carry the *Pearl* allele (Sponenberg, 2009). It was formerly known as the "BarLink Factor" in Quarter Horses and Paints, but it was discovered to be genetically similar to the factor formerly known as *Pearl* (Bailey and Brooks, 2013).

The blue eyes of cremellos and perlinos are sun-sensitive, and owners claim that these horses seek out protective cover in the summer; that is., they are photophobic. According to Knottenbelt and Pascoe (1994), sunburn neoplastic disorders such as squamous cell and basal cell carcinomas around the eyes are more common in pink-skinned horses.

The *Dun* allele

The Dun gene causes a pinkish-red horse with darker red tips and a complicated pattern of dorsal stripe, shoulder stripe, and leg bars in an otherwise red horse (these three patterns are often referred to together as "primitive markings"). Red dun, often known as claybank dun, is the name given to this colour. Dun generates a more or less yellow-red animal with black points and primitive markings in a horse with a bay base coat, which is known simply as dun. Grulla is the name given to a black animal, in this

case, a horse, with the Dun gene that has a mouse-gray colouration and rudimentary patterns. The Dun gene has three known alleles: dun (D), non-dun1 (d1), and non-dun2 (d2) (Imsland *et al*., 2016). Because the gene is dominant, a horse with one or two copies of the gene will have the dun phenotype. A dun foal cannot be produced by two non-dun parents. Non-dun1 d1/d1 or d1/d2 horses may exhibit some asymmetry in pigment distribution, resulting in primitive markings, although not to the same extent as dun horses. Horses with homozygous non-dun1/non-dun1 markings have more primitive markings than horses with heterozygous d1/d2 markings. On a bay or chestnut horse, non-primitive dun1's markings are more evident; on a black horse, they fade in. Non-dun2 horses do not have primitive markings because they have two copies of the gene (Imsland *et al*., 2016).

The dominantly inherited *Dun* allele "*D*" dilutes both eumelanin and pheomelanin in body hair, but not in point hair. "*d*" stands for the recessive alternative, not to be confused with the actual *dun*. The black body hair is diluted to mouse gray (grulla), while the red body colour is diluted to light red (claybank or red dun) or yellow-red (buckskin dun) (Van Vleck and Davitt, 1977). *D* produces a coat pattern that comprises a dark head, dark points, dorsal stripe, shoulder stripes, and leg bars, in addition to pigment dilution. These markings can be modest and easily go missing. The dun markings on the shoulder and upper leg of some Przewalski's horses and Mongolian ponies may be in the shape of a characteristic network of webbing patterns. Homozygotes for *D* are phenotypically similar to heterozygotes and do not exhibit the dramatic colour dilution effects seen in homozygotes for the palomino/buckskin gene. Gremmel (1939) discovered that pigment granules in the hair shafts of duns are significantly concentrated on one side rather than being evenly distributed throughout the core. In contrast to the *CR* allele, which controls pigment amount, the *D* gene may impact the clumping of pigment granules, resulting in an optical dilution effect. Essentially, the *dun* gene is the strongest dilution gene, acting on any of the existing coat colours.

Although there are some key differences, the allelic effects of *D* can be confounded with those of *CR*. To begin with, unlike *CR*, *D* dilutes both the black and red pigments on the body while leaving neither pigment on the points alone. Black body colour is diluted to mouse gray; red body colour is diluted to a pinkish-red, yellowish red, or yellow. Second, in addition to pigment depletion, the striping pattern is a distinguishing feature of *D*. Finally, homozygosity for *D* does not result in an extreme colour dilution(see Table 6). Both the *CR* and *D* dilution alleles can be found in a horse. A red horse with both dilution genes resembles a dun-marked palomino. Extreme colour dilution is not caused by heterozygosity for dilution genes at both loci. On otherwise "regular" unadulterated chestnuts and bays, more or less faint primitive marks can be detected, and on juvenile grays, they can be conspicuous. These dun marks without colour dilution are most likely the result of another gene, but they can be mistaken for those caused by the *D* gene.

Table 6. Phenotypes and Genotypes for the *D* locus.

Genotype	Phenotype
Dun	*DD* or *Dd*
Non-dun	*dd*

The T-box 3 (TBX3) transcription factor has been linked to the dun colour (Imsland *et al*., 2016). *Non-dun1* and *non-dun2* are both present in an equine chromosome 8 region where the only gene is *TBX3*. At chromosome 8 base pair 18, 226, 905, *non-dun1* has guanine where as *dun* has adenine, which appears to be enough to generate *non-dun1* colouring. In addition, compared to the most frequent variant of *dun* in domestic horses, *non-dun1* has another single nucleotide polymorphism at Chr. 8: 18, 227, 267, where a guanine in *dun* is substituted with thymine in non-dun1. That SNP, however, was also detected in certain dun Estonian native horses, indicating that it is not required for *dun*. Non-dun2 has a 1,609-bp deletion and another deletion that is extremely close to 8-bp. The *non-dun2* deletion is a more derived allele when compared to *TBX3* in other animals. The *non-dun2* mutation most likely happened on a chromosome with *non-dun1* already present, based on nucleotide variability in the flanking areas of chromosome 8 for the multiple alleles (Imsland *et al*., 2016).

There are no obvious parallels in other animals for *Dun*. *d* is the sign for dilute, a recessively inherited colour gene that causes a distinctive clumping of pigment granules that produces the optical impression of colour dilution in other mammals (Searle, 1968). The colour dun in Dexter cattle, like brown in dogs, is an allele of the *TYRP1* gene, but because mutations in this gene shift the colour of eumelanin from black to brown, this is not a possibility for dun in horses. The *D* allele is found in stock horses, ponies, and wild horses in North America. The Norwegian Fjord is a popular dun breed. Because the dun phenotype is commonly present in breeds with *CR*, it is critical to be able to discern between the variants of these genes alone and in combination.

The *Silver Dilutionor* Dapple gene

Silver Dilution, sometimes known as "*Z*," is a colour diluting gene that works in tandem with *Cream Dilution*. The *Silver Dilution* gene dilutes black pigment in the same way that the *Cream Dilution* gene dilutes red pigment with little effect on black pigment. Castle and Smith (1953) were the first to describe *Silver Dilution*, which is thought to have started in the late 1800s among Shetland ponies. Although

this origin was initially recognized, the presence of *Silver Dilution* in horses and ponies shows that it may have a more ancient origin. The colour variety is sometimes known as taffy or silver dapple. The *Silver Dilution* gene's effects are most noticeable on black horses (*aa E*–), whose coat colour is diluted to chocolate or black chocolate colour, often with dapples, and whose mane and tail are diluted to silver-gray or flaxen. The gene causes colour dilution in genetically bay horses, resulting in a horse that is commonly described as a silver-maned chestnut with darker pigment in the legs. The “silver bay” is a possible colour name for a bay with silver dapple dilution. Beyond producing a slightly lighter mane and tail, the gene does not affect the chestnut (pheomelanin) coat colour. This colour is frequently referred to as silver sorrel, but it is difficult to tell apart from sorrel visually.

It is sad that the term "dapple" has been added to the name of this dilution gene. The name suggests that dapples are always linked with thiscolourr, which is not the case. Some owners mistake this colour for gray because of the word "dapple." Many silver dapple horses are registered as dapple gray in miniature horses. Classic silver foals are born with a light reddish-brown coat with a mane and tail of the same colour. The mane and tail expand in brightness as the foal coat is lost, and the body colour darkens to rich chocolate. Silver dapple interacts with gray, resulting in foals with the gray allele (*G*) having a birth colour similar to a mature gray.

Although the silver feature is inherited as a dominant trait, nothing is known about how the gene interacts with polymorphisms in other coat colour genes. *S* was offered by Castle and Smith (1953) for the gene symbol, based on the name given to the colour silver in horses, however, this symbol was not suited because it is used for spotting features in other species. The gene symbol for silver dapple is now *Z*, with *Z* and *z* indicating the dominant and recessive alleles for the presence and lack of the silver dilution characteristic, respectively.

As previously stated, this gene has no or minor effects on chestnut horses who lack black pigment. The dilution effects, on the other hand, are caused by a single copy of *Z*. Horses who are homozygous for *Z* may show more dilution, but the effect is minor. The effects of *Z* genotypes on base coat colours are shown in Table 7.

The *Silver Dilution* gene may be the cause of an apparent exception to the "chestnut coat colour rule" (Trommershausen-Smith *et al*., 1976). A chestnut horse (*A*–*E*–) with a dilution of the black pigment could be mistaken for a bay horse (*A*–*E*–). When this silver bay (registered as a chestnut) is bred to a chestnut, offspring with the E gene but not the dilution gene may legitimately be bay or black.

A missense mutation in the *PMEL17* gene (otherwise acronymised *SILV*; silver homolog gene) on horse chromosome 6 causes the silver dapple trait. It is inherited in an autosomal dominant manner (simple dominance).

Table 7. Alleles of the *Silver Dilution* gene (*Z*) with various base color phenotypes and their different genotypes and phenotypes.

Base color phenotype (*A*, *E* loci)	*Z* Genotype	Phenotypic effect
Chestnut (– –, *ee*)	*zz*	Chestnut
	Zz	Chestnut
	ZZ	Chestnut
Bay (*A–, E–)*	*zz*	Bay
	Zz	Silver mane
	ZZ	Silver mane
Black (*aa, E*–)	*zz*	Black
	Zz	Silver body
	ZZ	Silver body

PMEL17 is involved in the production of the black pigment eumelanin and is active from early embryonic development to the mature cell's melanosome (Brunberg *et al*., 2006). The missense mutation occurs in exon 11 and results in the substitution of arginine for cysteine as an amino acid. *SILV* is a virus that encodes a protein located on the surface of the melanosome, a pigment-producing organelle in melanocytes. Its function is unknown; however, it could be involved in the organization of melanosomes. In the EquCab2 genome assembly, *SILV* is found at chr6:73,665,135-73,672,980.

SILV mutations that are identical to the ones in horses have not been found in other species, while other *SILV* mutations in other species have changed coat colours. Mutations in the *SILV* gene cause a variety of faded hues and roan-like traits in mice. A variation in *SILV* has also been linked to the diluted coat of Charolais cattle (Gutiérrez-Gil *et al*., 2007). Merle dogs, on the other hand, have a conspicuously speckled coat as a result of the *SILV* locus, however, the polymorphism is the insertion of a repeat element rather than a mutation in the coding sequence (Clark *et al*., 2006).

Shetland, American Miniature, and Rocky Mountain horses, as well as Icelandic breeds, have the silver colour dilution gene, but it is also found in Quarter Horses, Paints, Morgans, American Saddlebreds, and Peruvian Pasos.

Multiple Congenital Ocular Anomalies (MCOA) syndrome is characterized by a bulbous bulging of the eye, cornea globosa, severe iridal hypoplasia, uveal cysts, cataracts, and, in a few cases, retinal detachment (Ségard *et al*. 2013). An ultrasonography examination of the eye can reveal these disorders. MCOA is inherited in a codominant manner and has been linked to the same genomic area as *SILV* (Andersson *et al*., 2008). As a result, homozygotes are afflicted more severely than heterozygotes. While many silver horses have MCOA, it is not a complete association. Some silver horses do not

have MCOA, and MCOA can also be found in horses that are not silver. Nonetheless, there has been a strong relationship, which could be owing to the proximity (linkage) of two different mutations.

The silver dapple colour in Rocky Mountain Horses is occasionally linked to Anterior Segment Dysgenesis (ASD), a condition that affects the structures of the face and the front of the eye. The syndrome is most commonly manifested as benign lesions, while homozygotes may experience vision impairment. Because ASD is observed in non-silver breed members, it is assumed to be related to the silver gene and to have originated from a silver dapple, ASD-affected foundation ancestor, demonstrating the Founder effect (Brunberg *et al*., 2006).

Champagne Dilution (*CH*)Gene

CH is mostly present in American Saddlebred, American Quarter Horse, Tennessee Walking Horse, and Appaloosa breeds developed in North America. Because it only appears in horse breeds produced in North America, the *Champagne Dilution* gene is assumed to be the consequence of a recent mutation. In contrast to the *Cream Dilution* and *Silver Dilution* genes, the *Champagne Dilution* gene dilutes both eumelanin and pheomelanin. The *Champagne Dilution* produces a gold body colour with a delicate flaxen mane and tail on a chestnut (*ee*) foundation coat. Gold champagnes, in particular, but also other champagne hues from time to time, can have an appealing metallic sheen. Champagne produces a somewhat darker golden body with a dark chocolate mane and tail when used in conjunction with a bay base coat. Hairs along the mane and tail's edges may be dilated even further to a colour that matches the body.

The term "amber" refers to the mix of champagne and bay. The "traditional" champagne is created by diluting champagne over a black base coat. The body coat colour of classic champagnes is a darker bronze, with a dark chocolate mane and tail. The gold, amber, and classic champagne colours look similar to, and can be readily confused with, palomino, buckskin, and dun and grulla due to *D*. The presence of lightly pigmented "pumpkin" coloured skin, mottled freckling of the hairless skin, and lightened eye colour, which are not frequently observed in horses with *cr*, can be used to distinguish the colours. Interactions with several other colour loci can make identifying the champagne dilution even more difficult. *CH* foals are typically born with blue eyes and pink skin, which darkens slightly with age.

Champagne Dilution is transmitted dominantly, with alleles *CH* (dominant) and *ch* (recessive); therefore, heterozygous and homozygous individuals have similar phenotypic characteristics. It can occur in conjunction with the other coat dilution genes, resulting in a two-locus additive effect. The *CH* and *CR* alleles produce a horse that is particularly light-coloured, similar to a cremello or perlino. The colour is muted in people who have both a *CH* and a *D* gene, but the Dun traits of a dorsal stripe and leg bands are still discernible. Horses homozygous for the *prl* allele of *Cream Dilution* will look quite similar to heterozygotes for *CH*, as previously stated. DNA tests may be required to determine the champagne colour's genetic source. Shown in Table 8 are the effects of each potential genotype for the *Champagne Dilution* gene on base coat colours.

Table 8. Different base colour phenotypes for alleles of the *Champagne Dilution* (*CH*) gene.

Base colour phenotype (*E*, *A* loci)	*CH* genotype	Phenotypic effect
Chestnut	*chch*	Chestnut
	CHch	Gold champagne
	CHCH	Gold champagne
Bay	*chch*	Bay
	CHch	Amber champagne
	CHCH	Amber champagne
Black	*chch*	Black
	CHch	Classic champagne
	CHCH	Classic champagne

ECA14 has been assigned to *CH* (Cook *et al*., 2008). The *SLC36A1* gene was sequenced, and a missense mutation was discovered that was entirely linked to *CH*. *SLC36A1* is found at EquCab2 chr14:26,678,645-26,701,135. *CH* is caused by a missense mutation in *SLC36A1*'s second exon, which results in the substitution of an amino acid (Bailey and Brooks, 2013).

CH is the first trait in any species to be linked to the *SLC36A1* gene. However, because the gene responsible for *C* (*SLC45A2*) has a similar structure, it is not surprising that the two genes have similar impacts on pigmentation. *SLC36A1*'s exact function is unknown at this time. It may be critical for the maturation of melanosomes, according to evidence from rats (Cook *et al*., 2008). *SLC36A*'s function will be better understood if the *CH* phenotype is studied further. Genetic testing is required to identify homozygotes with certainty due to the dominant mode of inheritance. A test for the *CH* allele is currently available from several commercial labs.

THE *KIT* GENE (TOBIANO, WHITE, SABINO, AND ROAN)

White hair is produced by the set of genes for tobiano, sabino, white, and roan against the fundamental colour

patterns of the chestnut, bay, or black, as indicated by the *Extension* (*E*) locus modified by the *Agouti* (*A*) locus. While other genes can cause white hair patterns, as explained previously, the four tobiano, sabino, white, and roan hair patterns are all caused by the same gene, *KIT*. Before the identification of the common genetic factor, the genetics of each pattern was well understood, thus they were given unique genetic names: *Tobiano* (*TO*), *Sabino* (*SB1*), *Dominant White* (*W*), and *Roan* (*RN*). Mutations in the *KIT* gene cause dominantly inherited white spotting patterns in numerous species, including the mouse and pig (Geissler *et al*., 1988; Besmer *et al*., 1993; Moller *et al*., 1996). Using different genetic languages for the four qualities remains relevant, although these diverse coat colour patterns have a single molecular source.

The *Tobiano* Allele

It is commonly known that the tobiano pattern of white spotting is inherited as a dominant feature. A tobiano foal must be born to a tobiano mother and father. Tobiano is inherited by fillies and colts from either their sire or dam or both. The tobiano gene is absent in the most popular North American breeds of Quarter Horse, Thoroughbred, Standardbred, and Arabian, but it is present in a wide range of other breeds, including Paint, Pinto, Dutch Warmblood, American Saddlebred, Tennessee Walking Horse, Missouri Fox Trotter, Paso Fino, Icelandic, Shetland, and Miniature horses.

Tobiano can be seen in a variety of coat colours (sorrel tobiano, bay tobiano, palomino tobiano, dun tobiano, black tobiano, and so on), as well as in a mix with other spotting patterns. A combination of tobiano and overo ("tovero" or "medicine hat"), for example, results in a horse with more overall white than either separate spotting gene could normally produce: this is known as an additive genetic impact. At its most extreme, the interplay of *Tobiano* and *Overo* genes can result in a white horse. *Tobiano* interacts with genes that create leg and face marks, affecting the level of whiteness (sabino, for instance). A tobiano with a modest white pattern may be missing genes for common white marks that are inherited separately.

Tobiano spotting patterns can coexist with roan (*RN*) and appaloosa (*LP*) spotting patterns. Because neither the Paint nor the Appaloosa breeds allow tobiano/appaloosa pattern blends to be registered, the "pintaloosa" is likely the most well-known colour variety now in American Miniature Horses and other breeds with no pattern registration limitations. Mule breeders have proven that using a tobiano mare is the best technique to get beautiful stockings on a mule. The degree of body spots in mules with the *Tobiano* gene may be limited compared to the expression of *Tobiano* in the horse parent, but the leg markings element of the pattern is always present.

TO is the *Tobiano* gene's locus symbol, and we also use *TO* for the dominant allele of the pattern; *to* is the recessive allele, which causes tobiano spotting. *TOTO* (homozygous) or *TOto* (heterozygous) is the genotype for a horse with the tobiano pattern (see Table 9). *Tobiano* homozygotes are often indistinguishable from heterozygotes. Homozygotes, on the other hand, will always produce offspring with tobiano spotting patterns, which breeders prize. Previously, researchers looked for genetic variants of two genes associated with the pattern (*Albumin* and *Vitamin-D binding protein* (GC)) and used pedigree information to tell a breeder whether their horse was homozygous for Tobiano. A commercially available definitive genetic test is now available (Brooks *et al*., 2007). *Toto* refers to horses that lack the tobiano pattern gene. The tobiano pattern is determined by a single dominant gene; if a horse does not have the *TO* gene, it will not pass the trait on to its children, even if both parents have the tobiano pattern.

Table 9. *Tobiano* (*TO*) allele's phenotypes and genotypes.

Phenotype	Genotype
Tobiano	*TOTO* or *Toto*
Non-tobiano	*toto*

In white areas, Tobianos frequently have a few little colourful dots. Tobiano horses occasionally display a spectacular proliferation of small, clustered spots, typically with roan margins – "halos" – or complete roaning. "Inkspots" or "paw prints" are clustered coloured dots that appear to be breaking through otherwise enormous regions of body white. These spots are frequent in horses that are homozygous for tobiano and have a moderate amount of white region. Although the link between ink spots and homozygosity appears to be strong at times, it does not appear to be absolute. As a result, DNA testing is the most accurate way to determine zygosity. For this pattern, only a horse with two copies of *Tobiano* (homozygous) will be a true breed (see Table 9).

Tobiano is caused by a unique chromosome rearrangement on ECA3, not by a nucleotide mutation in the *KIT* gene (Brooks *et al*., 2007). A piece of the ECA3 "flipped" during a recombination event, causing the rearrangement. This reversal resulted in a region of the chromosome with gene order that is inverted compared to that of ECA3. The inversion, which covers about a third of the chromosome, is thought to cause spots by isolating the *KIT* gene from key regulatory regions. *KIT* signalling, which controls melanocyte migration across the embryo, may be disrupted if certain regulatory areas lose control. As previously stated, a genetic test to discover the mutation that causes the tobiano pattern is commercially accessible from multiple laboratories.

The *Tobiano* DNA test has been used to prove that this pattern has an ancient origin (Brooks *et al*., 2007). The

DNA inversion linked with *KIT* is present in all horses with the tobiano pattern. The mutation's widespread prevalence throughout breeds from all over the world, especially those that do not select positively for spotting patterns, strongly shows that it was present early in the evolution of these breeds. *Tobiano* is indeed quite old, as it exists in a variety of animals that lived 3500 years before the present, according to recent research that analysed DNA samples from ancient horses (Ludwig *et al*., 2009).

The tobiano pattern is found in breeds all over the world, though "pied" is the English term that is perhaps more commonly used outside of the Americas. The term "Tobiano" is believed to have originated in South America, where it was once applied to distinctively marked horses thought to have been introduced by a Dutch emigrant named Tobias. Tobiano horses are referred to as "painted," "coloured," "piebald" (white and black), "skewbald" (white and any single colour except black), or "odd-coloured" (white and two or more colours) in the United Kingdom. Other international horse breeds with Tobiano spotting include East Prussian Trakehners and native ponies, such as the Pottok from Spain's Basque region and the Mongolian pony, from Central Asia, in addition to the North American horse breeds listed earlier.

The (Dominant) *White* Allele

Throughout history, the "white" horse has been prized; it has a ceremonial status and is frequently depicted in art and mythology. White horses, on the other hand, are frequently *Gray* or homozygotes for the *Cream Dilution* gene (cremello). The name "*White*" is solely restricted in genetics for the dominant hereditary characteristic that results in horses with substantial white colouring from birth. However, while the presence of the *White* gene dictates the existence of white colour, the amount of white colour varies greatly. In practice, the level of white colouration can range from being completely white to being primarily white but with pigment in the tips of the ears, to being completely white with pigment patches all over the back and tail. As a result, *White* is frequently said to as having "sabino-style spotting" (Mau *et al*., 2004; Sponenberg, 2009).

White colouration induced by the *Gray* or *Cream Dilution* genes is distinct from white colouration caused by the *White* gene. Gray horses are born with coloured hair that fades to white with age, but their skin remains black; white horses are born with white hair and pink skin. Because they have both the cream phenotypic and the pink skin from birth, Cremello horses (*CRCR*) are more difficult to identify from the influence of the *White* gene. Their eyes, on the other hand, are frequently blue, as opposed to the brown eyes found in white horses; slight changes in the hue of cream or white hair at transition zones around the head or on the legs can also be detected.

Extension, *Agouti*, the dilution genes, and *Tobiano* all have genetics that are more complicated than *White*. While *Extension*, *Agouti*, *Cream*, *Silver*, *Champagne*, and *Tobiano* are caused by unique, distinct mutations in the corresponding genes, White can be caused by many different mutations at different places within the *KIT* gene (see Table 10). In general, *KIT* mutations cause white hair, however, the extent and distribution of the white hair depend on the location and nature of the mutation (Bailey and Brooks, 2013).

According to the review by Castle (1948), *Dominant White* in horses was first represented by the symbol *W*. As mentioned by the name, the gene that causes white colouration is dominant, hence W stands for the allele that causes white, while w stands for the allele that causes white to be absent. The genotype *ww* is found in all non-white horses. Molecular investigations of *KIT* and white pigmentation resulted in the identification of *W1*–*W17*, a set of alleles that code for white hair colour (Table 10; Haase *et al*., 2007, 2009, 2011). However, because the distinct alleles can only be distinguished by DNA sequencing, breeders continue to utilize the simple terms *W* or *w*.

There has been no report of a living *WW* (homozygous) horse. Due to the lack of true-breeding, homozygous *White* horses, several breeders believed *Dominant White* was a homozygous embryonic fatal trait, according to Castle (1948). He speculated that homozygous white foals might die in the womb. Depending on how early the mare lost the foal, breeders may not even be aware that their white mare had been pregnant. Pulos and Hutt (1969) conducted a large-scale breeding study in which they mated white horses to white horses and examined the white-to-non-white progeny ratio. They should have seen a 3:1 ratio of white to non-white, according to Mendel, but instead saw a 2:1 ratio, implying that the homozygous children were lost as embryos. *Dominant White* is an example of dominant deadly genes. See Table 11 for the phenotypes and genotypes of the *W* alleles.

The homozygous lethality of *W* may be explained by the DNA sequencing results (mutation types) presented in Table 10. The majority of *W* mutations are nonsense mutations, frameshift mutations, or DNA deletions, all of which would prevent the production of a functioning *KIT* protein. Because they would have at least one copy of functioning protein, horses with just one *KIT* mutation might be viable, albeit with some white hair. Two malfunctioning copies, on the other hand, could result in an embryo's failure to develop, resulting in embryonic death. On the other hand, some of these mutations may not stop *KIT* from working. For example, missense and splice site mutations have a minor impact on gene function. They could keep the ability to generate a functional *KIT* protein intact. As a result, homozygotes for these white alleles may be viable, but we would not know for sure until two white horses with those mutations have mated.

Table 10. Dominant *White* (*W*) alleles for the *KIT* gene, along with its associated breeds, mutation types, and KIT gene location (adapted from Haase *et al*., 2011).

Designation	Breed	Mutation Type	Location on *KIT* gene
W1	Franches-Montagnes	Nonsense	Exon15
W2	Thoroughbred	Missense	Exon17
W3	Arabian	Nonsense	Exon4
W4	Camarillo White	Missense	Exon12
W5	Thoroughbred	Frameshift	Exon15
W6	Thoroughbred	Missense	Exon5
W7	Thoroughbred	Splice site	Intron2
W8	Icelandic	Splice site	Intron15
W9	Holstein	Missense	Exon12
W10	Quarter Horse	Frameshift	Exon7
W11	German Draft	Splice site	Intron20
W12	Thoroughbred	Deletion	Exon3
W13	Quarter Horse	Splice site	Intron17
W14	Thoroughbred	Deletion	Exon17
W15	Arabian	Missense	Exon10
W16	Oldenburger	Missense	Exon18
W17	Japanese Draft	Missense	Exon1
Other *KIT* Variants			
SB1	Many	Splice variant	Intron16
TO	Many	Inversion	Intergenic

Table 11. Phenotypes and genotypes for the Dominant *White* (*W*) series of alleles.

Phenotype	Genotype
White	*Ww*
Non-white	*ww*
Embryonic lethal	*WW*

W is encoded by the *KIT* gene, which is located on ECA3 with the *Tobiano* gene. As demonstrated in Table 10, the trait-causing mutations might arise anywhere in the gene. The majority of *W* alleles in horses have been discovered spontaneously, that is, in only one founder individual and their descendants. As a result, the *KIT* gene has a considerable number of distinct alleles (17 to date; see Table 10) (Haase *et al*., 2007, 2009, 2011; Holl *et al*., 2010). Although there is great heterogeneity in the spectrum of phenotypes within the *W* series, each allele is uniquely responsible for a *W*– type phenotype. Because many W alleles are specific to a breed or a family of horses, prognostic DNA-based testing is difficult without screening for all alleles or guessing which test is appropriate based on the target breed. While a DNA test can be used to test for *W*, the variety of mutations makes this commercially impractical.

W alleles are uncommon in practically all horse breeds. The colour has been seen in Thoroughbreds, Arabians, American Quarter Horses, Hanoverians, Icelandics, and a variety of other breeds, and it is the defining feature of the Camarillo White horse (Haase *et al*., 2011). The *KIT* gene has a wide range of mutations, indicating that it is susceptible to mutation. In that circumstance, there is a chance that novel *W* series alleles could develop in any horse breed. Individuals carrying the *W* gene, like any depigmented horse, will be more susceptible to sunlight. Although problems in other systems, such as the blood, testes, and ovaries, are common in mice with *W* alleles, no similar difficulties have yet been reported in horses (Haase *et al*., 2010).

The *Sabino* Alleles

A white spotting pattern with towering uneven stockings and a blaze on the face is referred to as sabino. A patch of white on the belly or flank is common on horses with this pattern. Sabino horses frequently have a variety of white hairs interwoven with the basic coat colour, giving them a roan or gray appearance. The boundaries of sabino white markings are frequently more jagged than those of a regular white marking gene's sock or blaze. Sabino is frequently mistaken for other designs, particularly splashed white and overo. Many foundation horses registered as "roan" in the Tennessee Walking horse registry, for example, have the traits of sabino patterning

in images. Anecdotally, some of these horses were described as "lit-up roans," owing to the bright appearance of their white socks.

The focus will be on a specific sabino pattern that can be found in a variety of horse breeds, many of which have Spanish ancestors. A genetic mutation in the KIT gene has been discovered to be responsible for the characteristic. This mutation is found in all horses with a sabino pattern, but it is not seen in Clydesdale horses or horses with another sabino pattern, indicating that different genetic paths might lead to this phenotype.

As mentioned in the previous paragraph, some sabino breeds have diverse DNA. As a result, *Sabino1* (*SB1*) was given to the gene for the phenotype reported here as the first gene discovered for the sabino pattern. *SB1* is an imperfect dominant gene that lacks sabino, while sb1 is the absence of sabino. In a horse, a single copy of the gene causes the sabino pattern. These people wear the flashy socks and blazing that they are known for. Homozygotes (*SB1SB1*), on the other hand, are white or nearly white, with some coloring along the dorsal midline. In contrast to similar phenotypes generated by the *W* series of alleles, sabino alleles do not produce a homozygous lethal (*WW*) as *W* does. As a result, rather than adopting the W sign as recommended by a previous mice study, sabino was given its symbol, *SB1* (Besmer *et al*., 1993). Table 12 lists the traits and genotypes linked with *SB1*. Listed in Table 12 are the traits and genotypes linked with *SB1*.

Within the *KIT* gene on ECA3, the allele responsible for the one form of sabino pattern (*SB1*) described here was discovered (Brooks and Bailey, 2005). The regulation of exon splicing is altered by a single nucleotide change in the 16th intron, resulting in a fraction of gene transcripts lacking the 17th exon (see the bottom of Table 10). Health problems have not been documented in *SB1* horses because splicing is not totally interrupted, and even homozygotes maintain some transcripts with the typical sequence.

SB1 can be found in a variety of horse breeds, including miniature horses and Shetland ponies, as well as mustangs and gaited breeds. This wide range of breeds suggests that the mutation is old. Ludwig *et al*. (2009) investigation established the very old roots of this sabino pattern. These researchers used ancient horse DNA samples to show that the *SB1* pattern was present in a horse that lived on the Siberian steppe around 5000 years ago. Although Clydesdales have a distinctive sabino-type pattern, *SB1* has yet to be discovered in this or any other draft breed. Sabino in these breeds has a different genetic basis, either due to another *KIT* mutation or a mutation at a different locus.

Table 12. Genotype and Phenotypes for the *Sabino* (*SB1*) locus.

Phenotype	Genotype
White Sabino	*SB1SB1*
Sabino	*SB1sb1*
Not sabino	*Sb1sb1*

The *Roan* Gene

The *Roan* gene causes silvering by combining white and coloured hairs, which is more noticeable on the body than on the head and lower legs. Although the summer coat generally appears lighter than the winter coat, the roan appearance does not whiten with age as gray does. Regrowth of hair in areas of skin wounds may not exhibit the white hair mixture, emphasizing scars (and branding) in the roan coat.

The colour variations produced by combining roan with the basic colours can be given a wide range of names, but most breed registries limit the possibilities. In some colour schemes, "blue roan" refers to black, brown, or bay with roan, whereas "red roan" refers to sorrel or chestnut with roan. Bay with roan is sometimes referred to as "strawberry roan". Other breeds just mark the horse as "roan", obliterating the basic coat colour information. The presence of the roan allele may be seen in the silvering impact on the coat colour, and it is passed on in a dominant pattern. Other genes, on the other hand, can produce a roan-like effect, which can lead to misunderstanding in colour designations for registration and genotype assignment. The gene that causes leopard (*LP*, appaloosa) spotting, for example, may also cause a mottled roaning effect known as varnished roan.

The letter "*RN*" is used to symbolize *Roan*. The roan trait has a dominant mode of inheritance, with the dominant allele *RN* representing the presence of roan and the recessive allele *rn* representing the absence of roan. Despite the fact that *RN* is linked to *KIT*, no mutations have been discovered (Marklund *et al*., 1999). There are no commercial tests available to detect the *RN* gene. After looking over the registry records for American-bred Belgian Draft horses, Hintz and Van Vleck (1979) concluded that *RN* is a homozygous fatal gene. They noticed that roan patterned offspring of roan parents were in short supply. Mendelian expectations suggest a 3:1 ratio of roan to non-roan offspring; the authors discovered a ratio closer to 2:1, which is consistent with roan homozygotes losing their homozygotes throughout embryonic development. However, there have been numerous anecdotal reports of homozygous, true-breeding roan stallions in the United States, Germany, and Japan since then; published reports of homozygous roan stallions, particularly in the Quarter Horse, include those of Guerts (1977), Bowling (2000), and Sponenberg (2009). If more than one *RN* allele is discovered, these contradictory statements may be resolved. See Table 13 for the phenotypes and genotypes of the *RN* allele (showing the

Table 13. Phenotypes and genotypes for the *Roan* (*RN*) alleles.

Phenotype	Genotype
Roan	*RNrn*
Not roan	*rnrn*
Lethal Roan	*RNRN*

possibility of homozygous lethals, as well as the absence of homozygous lethals).

Rabicano – A Roan Variant

Many horses have a few scattered white hairs, which could be mistaken for *RN*'s activities on occasion. A horse may have a significant amount of roaning without a roan parent to contribute an *RN* gene on rare occasions. For example, 290 Arabian horses are classified as roans out of over 500,000 historical registrations in the Arabian Horse Registry of America (AHRA) Stud Book. Some of the roans, especially in early records, are most likely misidentified grays (*G*). There are still 73 horses with the roan designation after excluding those having a *G* or an *RN* parent. Based on the traditional definition of roan as a dominant gene, these findings could be interpreted as indicating either a high rate of pedigree mistakes among Arab roans or a major underreporting of the roan pattern (Bailey and Brooks, 2013). The recent stud book records are validated by parentage verification through genetic marker testing, and there is no compelling evidence to support the notion that parentage assignment would be grossly inaccurate among the older records. If there is underreporting of the roan colour, it is unlikely to be intentional, as most breeders value the pattern's traditional position and admire its uniqueness. As a result, the action of a recessive allele may be causing the roan-like pattern. Rabicano is a term used to describe this "roaning" pattern.

When compared to the traditional roan gene, the rabicano characteristic has an uneven pattern, with the sides and barrel being heavier than the forehand. White flecking (irregularly formed white patches) on the flanks and belly, between the front and rear legs, and on the sides of the neck near where it joins the head are other distinguishing features. Pink skin may be underlain by particularly big flecked regions. A diffuse vertical white striping pattern, similar to brindling in dogs or cattle, may appear on the mixed coat. The hair on the top of the tail is frequently white, with numerous rows of noticeable stripes running across the dock. The rabicano characteristic is not unique to Arabian horses; it can also be found in Thoroughbreds and Quarter Horses. The rabicano inheritance has yet to be determined.

The *RN* gene appears to be located on ECA3, either close or within the *KIT* gene. Although sequencing *KIT* exons did not uncover the mutation that causes *RN*, the investigations did show that *RN* could be caused by a mutation within or near the *KIT* gene (Marklund *et al*., 1999). This finding implies that *RN* is a member of the *Tobiano*, *White*, and *Sabino* genetic series, which might be found in Table 10. As previously stated, there are no commercial tests available to detect the *RN* gene. However, the Veterinary Genetics Laboratory (VGL) at UC Davis has discovered DNA markers related to *Roan* in Quarter Horses and Paints that can be used to assess if a horse has the roan gene and how many copies it has.

Quarter horses, Peruvian Pasos, Paso Finos, Welsh Ponies, Miniatures, and Belgians are all carriers of the *RN* allele. Some Thoroughbreds with bay and grey are described as "roans" in stud books and racetrack descriptions to distinguish them from those with chestnut and grey, contributing to confusion over the term's definition. Although roan is listed as a colour in the Arabian studbook, a wide interspersed white hair pattern in this breed could be attributed to another gene or genes.

It is not far-fetched to submit that *RN* is caused by a combination of mutations in distinct breeds, such as the *W* allele series, rather than a single mutation in a single gene. This would fit with the nature of *KIT* mutations and explain the small phenotypic abnormalities observed, such as rabicano. It may also explain why some *RN* alleles are homozygous fatal while others are viable as homozygotes: deletion mutations would prevent *KIT* protein production, whereas missense mutations would generate the roan phenotype while simultaneously allowing *KIT* protein production. In the first situation, the embryo would die, however, in the second case, the pregnancy would be viable.

THE *GRAY* GENE

Everyone is aware of how human hair colour varies over time, with the hue of youth being replaced by gray or white. Horses exhibit a similar hair silvering phenomenon; however, it occurs at a much younger age than in humans. A young horse with the progressive graying gene can be any colour when it is born. Grays are frequently described as having a black birth colour in popular literature. However, this is not true for all breeds. A gray horse's birth colour is determined by the alleles of the individual's other coat colour genes, particularly Agouti and Extension. Most gray Arabians, for example, are born bay or chestnut, not black.

A graying foal will start to show intermixed white hairs soon after birth, and their number will proportionally grow with age. The rate at which this change occurs is determined by some factors, the most important of which is the base coat colour. Horses with a chestnut (*ee*) base colour will develop white hairs at a significantly faster pace than horses with a darker base colour (Pielberg *et al*.,

2008). Other unknown heritable characteristics that are particular to some breeds influence the rate of pigmentation loss. For example, Arabian horse stud book records based on foal colours at around 6 months of age frequently properly represent the adult horse's gray hue. In some breeds, such as the Thoroughbred, this judgment is not always possible at 6 months of age.

Many gray horses in their intermediate stages have a dappling pattern of light gray hair splotches bordered by dark gray rings. The knees, hocks, and fetlocks might be a dark gray colour, which usually lasts longer than dappling. The hair coat will be clear gray or gray with coloured speckling ("flea-bitten" or "mosquito-bitten") at maturity (it appears as a "pure" white horse with dark skin). Some graying chestnuts go through a period described as "rose gray." In intermediately gray foals, "dun-like" patterns, which are frequently subtle in other hues, can be fairly noticeable. White spotting patterns like tobiano, overo, and appaloosa may not be evident as a coat pattern on an adult gray horse, but they can be seen as pink skin patterns when the hair is wet.

The dominant allele for Gray is designated *G*, while the recessive allele for non-gray is designated *g*. The locus symbol for the *Gray* gene is *G*. *GG* or *Gg* will be the colour of a gray horse (see Table 14). It is impossible to identify whether a horse is homozygous for *G* just by looking at it. A horse without the *Gray* gene is denoted by the letter *gg*. Gray interacts epistatically with all other coat colour genes except white, masking their activities and making it difficult or impossible to tell what other coat colour genes a gray horse has just by looking at it. A close examination of the head of a young foal, particularly around the eyes, can reveal the first signs of the existence of the *G* allele. Later, the horse's body will be covered in a mixture of white and dark hairs, a stage that is frequently confused with roan. Foals carrying the *G* gene have a substantially darker base colour than their non-gray relatives. For example, a chestnut's foal coat is frequently a light fawn colour, which is especially noticeable on the legs, but it will eventually shed to reveal the darker adult shade. Newborn chestnut foals with the Gray gene have the dark chestnut colour of an adult, but this colour will fade as they mature into gray. The darkening impact of *G*– on the foal coat appears to influence all base colours. On their legs, bay foals often have fawn-coloured hair that sheds to reveal black. The legs of bay foals that will go gray are black at birth. Instead of the more common mouse-gray foal type, black horses with *G* are born a lustrous black.

Because gray colour is caused by the activity of a dominant gene, a gray horse must have at least one gray parent. If a gray horse does not have a gray parent, the claimed parentage is most likely false (Trommershausen-Smith *et al*., 1976). A foal born to two gray parents has a 25% chance of becoming homozygous for gray. Gray offspring should only be produced by homozygous grays, as with other dominant alleles. Individuals and breeds with the gray gene have a wide range of speckling ("flea-bites") occurrences. The speckling feature in grays has not been studied genetically for independent inheritance. The gray genotype, on the other hand, appears to have a significant impact on the appearance of speckles (Bailey and Brooks, 2013).

Table 14. Genotypes and phenotypes for the *Gray* gene.

Genotype	Phenotype
GG, *Gg*	Gray
gg	Not gray

Once the graying process is complete, *GG* homozygous horses normally do not have speckles. *Gg* heterozygous horses account for the majority of speckled horses. Alleles found solely in certain breeds, such as breeds where gray is uncommon (thus, most horses are heterozygous), and these horses frequently lack speckles, may also have a role. Similarly, if a non-flecked heterozygous gray is bred to a *gg* (not-gray), flecked gray children may result - showing that speckling is a distinct feature from gray, but that it interacts with the gray allele. Gg heterozygous horses account for the majority of speckled horses. Alleles found solely in certain breeds, such as breeds where gray is uncommon (thus most horses are heterozygous), and these horses frequently lack speckles, may also have a role. Similarly, if a non-flecked heterozygous gray is bred to a gg (not-gray), flecked gray children may result. This shows that speckling is a distinct feature from gray, but that it interacts with the gray allele.

Gray horses are also prone to vitiligo, a progressive loss of colour in the skin. This depigmentation occurs around the eyes, lips, and anus, and while it is unsightly, it does not pose a health risk. Owners may be frustrated when the disease disappears in some horses but persists in others. Vitiligo depigmentation can occur in a variety of hues, similar to melanomas, however, it is most commonly linked with gray. Vitiligo may be an inherited condition that is genetically unrelated to *Gray* yet interacts (epistatically) with it (the *Gray* gene).

Henner *et al*. (2002b), Locke *et al*. (2002), and Swinburne *et al*. (2002) independently linked the *Gray* gene to ECA25. *G* was later discovered to be caused by a duplication of 4200 bp of sequence within an intron of the *STX17* gene (Pielberg *et al*., 2008). As a result, a DNA test for *Gray* is commercially accessible.

STX17 belongs to the syntaxin protein family, which plays a crucial role in vesicle targeting and membrane trafficking. Although the presence of the *G* duplication increases the expression of *STX17* and an adjacent gene, *NR4A3*, both genes do not provide a straight forward explanation for how this modification resulted in the gray phenotype (Pielberg *et al*., 2008). Successive analyses of the duplication's sequencing revealed that it contained

regulatory regions specific to melanocytes that acted on the nearby *NR4A3* gene (Sundström *et al*., 2012). More research is needed, but because *NR4A3* is involved in cell cycle regulation, it now appears that upregulation due to the *G* duplication could result in melanocyte exhaustion in the hair follicle (leading to pigment loss with age) and overgrowth of some melanocyte populations in the skin (resulting in tumors). This mutation can be found in all gray horses. It is still not known why horses turn gray at different ages or exhibit the variety of secondary features indicated above, despite having the DNA sequence. These other traits could be the consequence of other genes working in tandem with *Gray*.

Gray can be seen in a variety of breeds throughout the world, including ponies, riding horses, and draft horses. In a few breeds, it is the dominating, but not exclusive, colour (e.g. Andalusian, Kladruber, and Lipizzaner). According to Bailey and Brooks (2013), the gray colour has a pretty old provenance due to its vast breed distribution.

The discovery of the gray mutation is of tremendous interest in medical studies since it increases the risk of melanoma in horses: according to some studies, up to 80% of grays over the age of 15 have some kind of melanoma (Elaine, 2021). Gray horses are susceptible to a type of tumor that, while originating in pigment-producing melanocyte cells, is not generated by sun exposure like most human melanomas are. In this situation, the gray colour duplication may also affect the regulation of the cell cycle in cutaneous pigment cells, leading to tumor development and expansion. Non-gray horses with equine melanomas are quite unusual. Tumors are most typically detected around the tail or head; however, they can also be discovered inside any organ system. Gray horse melanomas are benign, although they can be unsightly and cause loss of use of the affected body part if the tumor becomes inflamed or ulcerated (Seltenhammer *et al*., 2003). Internal tumors that interfere with crucial organ function can be life-threatening. Gray horses with the *Agouti* (aka *ASIP*) genotype *aa* (black) are more prone than those with *Aa* or *AA* to develop melanoma (Pielberg *et al*., 2008). Due to the loss of *MC1R* function (and black pigmentation) imparted by the *e* (*Extension*) allele, the *ASIP* genotype has no effect on the rate of melanoma formation in gray horses with the chestnut (*ee*) genotype.

COAT SPOTTING PATTERNS

The Frame Overo

Frame Overo has the overall appearance of a colourful horse with white patches. The regions of white skin are hidden beneath a layer of pink skin. Normally, the eyes are brown, although one or both of them may be blue or partially blue. The frame overo pattern is visible at birth and does not alter much throughout the horse's life. The frame overo pattern is the most noticeable and unique overo pattern, with dark colour throughout the topline, chest, legs, and tail, and white in a horizontal motif across the body, as well as considerable white face markings.

Table 15. Genotypes and phenotypes for the *Frame Overo* (*O*) allele.

Phenotype	Genotype
Frame overo	*Oo*
Non-overo	*oo*
OLWS	*OO*

Overo can appear in any coat color or pattern (sorrel overo, bay overo, palomino overo, dun overo, black overo, and so forth). “Tovero” is a term used to describe tobiano and overo composites, but it is also used to describe tobiano composites with a variety of white patterns, such as sabino, frame overo, and splashed white. A horse having both pattern genes will typically have more white area than a colourful region. Overo can be found in combination with a variety of appaloosa spotting patterns.

Tobianos are thought to be white horses with dark patches, whereas overos are thought to be the polar opposite. The white body markings in poorly marked tobianos usually appear as vertical stripes, but the white patches in overos tend to spread horizontally. Exceptions to these generalizations are common, especially in horses with numerous spotting genes. Individuals with the frame overo allele, for example, do not always show the spotting pattern. This is known as the phenomenon called “Reduced Penetrance” – a rare situation in which the allele is present but the phenotype is not always exhibited. The action of epistatic and modifying loci may influence the degree of phenotypic penetrance. In the instance of the frame overo, insufficient penetrance can result in individuals being misclassified as solid or breeding stock, as well as the emergence of an overo deadly white syndrome-affected foal from a "solid" coloured parent (Lightbody, 2002).

The *Frame Overo* gene's locus symbol is "*O*", while other texts have used "*Ov*," "*OV*," "*FR*," and "*FrO*." In Table 15 are the traits and genotypes linked with the *Frame Overo* gene. The dominant allele responsible for the frame overo pattern is denoted by the letter "*O*", while the allele’s absence is denoted by the letter "*o*". Because there is a clear difference between homozygotes and heterozygotes for *O*, the gene action is more correctly defined as incomplete dominance. Individuals who only have one copy of *O* have the frame overo pattern mentioned above. Overo Lethal White Syndrome (OLWS) is a fatal illness that affects people who have two copies of the *O* gene. Foals homozygous for *O* are alive throughout pregnancy, but appear virtually totally white at birth and have multiple developmental defects. These foals usually perish soon after birth. Because of the ambiguous use of the term

"overo," white foals born to parents with overo-type or overo-like patterns may be euthanized without need. For example, *Sabino1* is commonly described as an overo-type pattern, yet homozygotes for *SB1* are healthy white foals. While homozygotes for *SB1* and *CR* (cremello) exhibit comparable symptoms but are viable and healthy, *O* is a homozygous fatal gene.

The works of Metallinos *et al*. (1998), Santschi *et al*. (1998), and Yan *et al*. (1998) linked the *Frame Overo* gene to ECA17 and discovered a missense mutation in the endothelin receptor type B gene (*EDNRB*) gene that causes both the frame overo patterns and OLWS. The *O* mutation causes a dinucleotide alteration in the genetic code of the *EDNRB* gene, which affects its function by switching one amino acid from isoleucine to lysine. In the EquCab2 genome assembly, *EDNRB* is found at chr17:50,604,167-50,625,930. Because frame overo patterns might resemble other inherited patterns, testing for the *EDNRB* mutation is the most reliable technique to ensure that the colour pattern seen is frame overo (Bailey and Brooks, 2013). The *O* allele is easily identifiable, and a genetic test for it is commonly available. This test can be used to unambiguously identify frame overo allele carriers as well as diagnose probable cases of OLWS.

As previously stated, the *EDNRB* gene's function is disrupted by the switch from isoleucine to lysine produced by the dinucleotide mutation in *O*, and the ensuing altered endothelin receptor type B protein is unable to perform its normal function. This function is critical in the signaling of various types of neural crest-derived cells, including melanocytes and a particular subset of nerve cells in the digestive tract, under normal settings (intestinal ganglia). Melanocytes are pigment cells that start in the neural crest region of the early embryo and move to specific regions throughout the body during later developmental stages. The *EDNRB* system's signaling is merely one of the numerous biological pathways that contribute to this movement. When certain *EDNRB* signaling is lost in *Oo* animals, melanocytes are unable to properly migrate and reach some parts of the body, resulting in the obvious overo pattern. Melanocytes are completely absent in the skin of foals with OLWS (Lightbody, 2002). Although *EDNRB* signaling is required for intestinal ganglia precursor cells to migrate, they appear to complete their migration in *Oo* horses and provide normal gut function. The lack of *EDNRB* signaling, on the other hand, entirely blocks migration in *OO* horses, resulting in the loss of ganglion cells and gastrointestinal function.

Although not as widespread as sabino patterns, the frame overo pattern appears in breeds all over the world. It is common in American Paint Horses, Pinto Horses, American Mustangs, and American Miniature Horses. The *O* allele can also be found in Thoroughbreds, albeit it is extremely unusual.

Frame overos with blue eyes and significant white spots on the face are sun-sensitive, and these horses actively seek protective shade in the summer (photophobia). As with any pattern of depigmentation, pink-skinned parts of the face are sensitive to sunburn and may require additional protection when exposed to the sun.

For decades, overo-type parents have occasionally produced blue-eyed white foals (or virtually white foals with a few colourful spots around the nose, ears, or tail) (Trommershausen-Smith, 1977; Hultgren, 1982; Vonderfecht *et al*., 1983). Within a few hours of birth, nearly all show signs of digestive pain, similar to those seen in a foal with retained meconium. Neither medicine nor surgery is effective in removing the obstruction. These foals are unable to transfer food through the digestive tract because of a loss of intestinal ganglia, which control the peristaltic muscle activities of the gut, or, less commonly, missing parts of the intestinal tract (ileocolonic aganglionosis). The condition is known as overo deadly white syndrome (OLWS). Almost all affected foals are the result of phenotypically frame overo parents mating, but there have been a few exceptions, particularly in cases of incomplete penetrance. In several other species, there is a link between neurological impairment and a prominent and distinctive pigment pattern. This is due to the similar signaling pathways of migratory cells that arise from the same neural crest location during embryological development, such as nerve cells (ganglia) and pigment cells in this example (melanocytes).

The likelihood of producing a frame overo or OLWS foal is simple to estimate based on our understanding of *O*'s inheritance (check the genotypic possibilities shown in Table 15). When an *oo*, non-overo, horse is crossed with an *Oo*, frame overo, mating will result in 50% of the having the *Oo* genotype (and the frame overo pattern) and 50% will have the *oo* genotype (non-overo). A Punnett square can be used to depict the consequence of two *Oo* parents (see Table 16). The three genotypic classes suit a dominant gene paradigm for *O*, with deadly effects in homozygous individuals. OLWS is expected to impact 25% of foals, according to this model. It is worth noting that crossing "like to like" does not pay off in the case of frame overo. In each case, a maximum of 50% of foals will carry the desirable frame overo pattern, but one out of every four offspring will be lost to OLWS when two frame overo parents are mated.

The Splashed White

"Splashed white" is a spotting phenotype in which the horse's mode of appearance is that of a horse whose underside has been splashed with white paint. White patches across the extremities, generally all four lower limbs, the abdomen, and across the face, characterize the pattern (Klemola, 1933; Sponenberg, 2009). A broad blaze or bonnet, as well as blue or part-coloured eyes, are common face marks. These white markings are generally

Table 16. Genotypic predictions for a cross between 2 heterozygous *Frame Overo* (*Oo*) horses.

Genetic contribution from Stallion/Sire (*Oo*)		Offspring features	Genetic contribution from Mare/Dam (*Oo*)
O	***o***		
OO	*Oo*	Offspring genotype	
OLWS	Frame Overo	Offspring phenotype	*O*
25%	25%	Proportion of offspring	
Oo	*oo*	Offspring genotype	
Frame Overo	Non-overo	Offspring phenotype	*o*
25%	25%	Proportion of offspring	

Table 17. Genotypes and phenotypes for the *SW* (splashed white) *MITF* locus.

Phenotype	Genotype
No splash white	*MITF+/MITF+*
Splashed white (*SW1*)	*MITF-prom1/MITF+*
Splashed white (*SW3*)	*MITF-C280Sfs*20/MITF+*
Splashed white (Macchiato)	*MITF-N310S/MITF+*
Extensive splashed white	*MITF-prom1/MITF-prom1*
Not observed (possibly lethal)	*MITF-C280Sfs*20/MITF-C280Sfs*20*
Not observed	*MITF-N310S/MITF-N310S*

restricted within crisp borders, unlike the sabino and dominant white alleles of the *KIT* locus, and leave the remaining portions coloured without speckling or roaning. In the splashed white pattern, the amount of depigmentation varies. Some splashed white individuals have relatively few markings, which can be readily mistaken for ordinary socks/blaze markings. This disorder is thought to have a hereditary component (Bailey and Brooks, 2013).

The letters *SW* and *Spl* stand for a potential gene that causes splashed white patterns. However, observations of additional horses with splashed white phenotypes led to the finding of two distinct genes responsible for some of the splashed white phenotypes (Hauswirth *et al*., 2012). As a result, trait genetic designations must be made in conjunction with molecular genetic studies.

MITF, whose product is the microphthalmia-associated transcription factor (MITF) protein, was one of the genes linked to splashing white (Hauswirth *et al*., 2012). *MITF+* refers to the *MITF* allele that does not induce spotting. The mutations that cause splashed white phenotypes are dominant over the *MITF* allele in the wild type. Three *MITF* variants have been linked to splashed white: *MITF-prom1* (spotted, splashed white), *MITF-C280Sfs*20* (spotted, splashed white), and *MITF-N310S* (a spotting pattern termed“macchiato”). The allele designations pertain to molecular alterations and represent a mutation in promoter 1 (insertion), a frameshift (deletion), and a missense mutation in the MITF protein, respectively. *MITF-prom1* and *MITF-C280Sfs*20* are referred to as *SW1* and *SW3* for commercial testing reasons. Because the Macchiato pattern was unique and resulted from a spontaneous mutation in a single, sterile stallion, it is unlikely to be seen in other horses, thus a shortened name for this allele has not been assigned (*Macchiato*). *SW3* and *Macchiato* alleles are both extremely rare, and homozygotes have never been found (see Table 17 for the genotypes and phenotypes related to *MITF*).

The gene *PAX3*, whose product is the Paired Box 3 Transcription Factor, was identified to be a second locus responsible for the splashing white phenotype (Hauswirth *et al*., 2012). *PAX+* is a recessive allele that does not induce spotting and is recessive to *PAX3-C70Y*, the dominant allele that causes the splashing white pattern. Hauswirth *et al*. (2012) named this allele *SW2* for commercial testing reasons (see Table 18 for the genotypes and phenotypes of *PAX3*).

Horses with the *SW1* allele have been seen in good health, as well as compound heterozygotes with the *SW2* allele. These people show more white regions than heterozygotes, indicating that they have an additive mode of inheritance. The *SW3* allele is extremely rare, if not unique, and nothing is known about its interactions with other alleles. Based on the effect of comparable mutations in mice, Hauswirth *et al*. (2012) identified homozygotes for *SW1* that were rather healthy but hypothesized that homozygotes for *SW2* and *SW3* would be embryonic fatal combinations.

In the EquCab2 genome assembly, *MITF* is found at ECA16:20,089,347-20,170,130, while *PAX3* is found at

Table 18. Genotypes and phenotypes for the *SW2* (splashed white) *PAX3* locus.

Phenotype	Genotype
No splashed white	*PAX3+/PAX3+*
Splashed white (*SW2*)	*PAX3-C70Y/PAX3+*
Not observed (possibly lethal)	*PAX3-C70Y/PAX3-C70Y*

ECA6:11,340,602-11,431,275 (Hauswirth *et al*., 2012; Bailey and Brooks, 2013). Genes in these areas of ECA6 and ECA16 were found during a genome-wide scan in a Quarter Horse family that was segregating for splashed white. *MITF* and *PAX3* were obvious candidate genes in these two locations because they generated similar symptoms in mice and people. The discovery of the multiple mutations implicated in horses with splashed white came from sequencing animals of various breeds with splashed white.

The MITF gene makes the microphthalmia-associated transcription factor, creating a protein signal that triggers the transcription of genomic DNA into RNA in a variety of genes in response to a developmental or environmental trigger. *KIT* is one of *MITF*'s many targets, and it is vital for melanocyte growth. As a result, it appears likely that these genes, like those seen in other species, affect the development of *KIT* receptors, and hence the migration and maturation of melanocytes. *PAX3* is a member of a family of transcription factors that are essential for the development of diverse tissue types. By interacting with distinct proteins, it has been demonstrated to operate as both a transcriptional activator and a repressor. *PAX3* also controls the expression of genes involved in melanocyte formation, including *MITF*, as well as pigment-producing genes.

Splashed white patterns can be found on the American Quarter Horse, Paint horse, Icelandic horse, Shetland pony, Miniature horse, Shire, Clydesdale, Gypsy Vanner, and Welsh pony. The *MITF-prom1* allele appears to be found in a variety of breeds and may have a long history. This gene was discovered in 58 American Quarter Horses and Paint horses, as well as 11 Icelandic horses, a Shetland pony, and a Miniature horse (Bailey and Brooks, 2013). The last three mutations appear to be more recent. *PAX3-C70Y* (*SW2*) was discovered in a Quarter Horse mare born in 1987, according to pedigree investigations. As a result, it is improbable that the allele will be found in breeds that are not linked to the Quarter Horse. *MITF-C280Sfs*20* (*SW3*) is a relatively unusual mutation that appears to be limited to Quarter Horses. *MITF-Macchiato* was discovered as a spontaneous mutation in a Franche-Montagne horse, and it is likely unique to that individual in the world, especially since the mutation may have resulted in the horse's sterility.

Deafness, which is uncommon in horses, may be associated with splashed white on occasion, especially in mostly white horses, but more research is needed to verify anecdotal reports of deafness in overos, as well as to determine the incidence of hearing loss and whether it is linked to a specific white pattern. Another human condition related to deafness is Waardenburg's syndrome, which has a unique pigment distribution (white forelock), and one version of this syndrome has been localized to the human homolog on chromosome 2 of the rodent *PAX3* gene (Baldwin *et al*., 1992). Splotch, a mouse spotting pattern feature, is similarly linked to the *PAX3* gene.

The Appaloosa or Leopard Spotting

The spotted pattern of Appaloosa horses in the United States, Knabstruppers in Denmark, Norikers in Austria, and many pony breeds from across the world is known as leopard complex spotting. The pattern is named after one of the distinct patterns created by the Leopard Complex gene (*LP*), known as "leopard". The leopard pattern is made up of dark, oval dots on a white background that covers the majority of the horse's body. The leopard complex is named after the wide range of symptoms associated with the *LP* gene. The spotting patterns might range from a few white hairs on the rump to a coat that is almost all white. White specks on the rump, lace blanket, spotted blanket, snowcap blanket, leopard, and "fewspot leopard" are some of the patterns' names (Bellone *et al*., 2008).

Generally, the patterns are connected with white areas that are uniformly dispersed and centred over the hips, and black pigment patches might appear in these white areas. Leopard spots are the black markings on the skin. Furthermore, horses with the leopard complex trait will exhibit progressive roaning, also known as varnish roaning, as they grow older, with the level of roaning varying from horse to horse. Some of the patterns, particularly sabino and roan, may be mistaken for others; however, roaning caused by the leopard complex does not impact pigment on the bony surfaces of the face, hips, and lower legs. In addition, horses with the trait have striped feet, unpigmented sclera surrounding the eye, and mottled pigmentation around the anus, genitalia, and muzzle, which are referred to as "characteristics" (Sponenberg, 2009).

In contrast to the recessive allele (*lp*), which is responsible for the absence of Leopard Complex spotting, the Leopard Complex spotting gene (*LP*) possesses an incompletely dominant allele (*LP*) that is responsible for the existence of the spotting pattern. A coat spotting pattern, gradual roaning, and other *LP*-related traits can be seen in all horses with a single copy of the gene. Horses with two copies of the *LP* allele (*LPLP*) are distinguished by two characteristics: they have few to no pigment spots (leopard spots) in the white regions, resulting in patterns known as "snowcap blanket" or "fewspot leopard"; and

Table 19. Genotypes and phenotype for the *LP* gene

Phenotype	Genotype
Leopard complex and resulting features	*LPlp*
Leopard complex with few to no pigmentation and resulting features and congenital stationary night blindness (CSNB)	*LPLP*
No leopard complex	*lplp*

homozygotes for the *LP* allele are affected by congenital stationary night blindness (*CSNB*), which is described further down. Check Table 19 for the phenotypes and genotypes for the Leopard Complex gene.

The diversity of patterns associated with the Appaloosa or leopard breeds initially led to theories that invoked multiple genes to account for pattern variation, until Sponenberg *et al*. (1990) convincingly demonstrated that a single major gene with minor gene modifiers was sufficient to explain studbook and family data from a variety of breeds. While *LP* is the direct cause of the leopard complex phenotype, other, as yet undiscovered genes may be in charge of the extent of white and the pattern's nature (Sponenberg, 2009).

Terry *et al*. (2004) studied the co-segregation of microsatellite DNA markers and genes for appaloosa patterns in multiple kindred horses (two paternal half-sib families) and discovered that the genes involved were on ECA1. The authors identified two genes that may alter pigmentation within the region implicated on ECA1. The DNA of both genes' protein-coding domains was sequenced, but no probable *LP* mutation was found (Bellone *et al*., 2010a). However, when the expression of the two genes was examined in the skin and retina of *LP* and *lp* horses, the gene encoding the transient receptor potential cation channel, subfamily M, member 1 (*TRPM1*) was dramatically reduced in the skin and retina of horses homozygous for *LP* and affected with CSNB (Bellone *et al*., 2008). This revealed that *TRPM1* was responsible for leopard complex spotting, despite the fact that the *LP* mutation was located outside of the gene's protein-coding domain, in a region that regulates gene expression rate. Although a genetic marker was discovered to be beneficial in predicting whether or not horses possessed the *LP* gene, the marker was not the mutation itself (Bellone *et al*., 2010b; Pruvost *et al*., 2011). A 1378-bp long terminal repeat insertion of retroviral DNA in the leopard complex allele impairs *TRMP1* transcription (Bellone *et al*., 2013).

TRPM1's product is part of a family of proteins that regulate calcium ions and are known to be vital for cell motility and signaling. *TRPM1*, formerly known as *Melastatin1*, was chosen as a potential gene because it was found to be inversely expressed in malignant melanoma, implying a role in normal melanogenesis (Duncan *et al*., 1998). While its involvement in human melanogenesis is unknown, it has been postulated that it is important in melanin storage (Oancea *et al*., 2009). *TRPM1* mutations cause CSNB in mice and humans; however, no pigmentation abnormalities have been observed in these animals so far.

CSNB is a hereditary condition in horses that have been demonstrated to be entirely related to homozygosity for the *LP* allele in Appaloosa and American Miniature Horses (Sandmeyer *et al*., 2007, 2012; Bellone *et al*., 2008). The disease shows as a seemingly normal vision in bright light but substantially reduced vision in dim light. When going near items in low light, such horses may appear ungainly or nervous. Even under microscopic analysis, the eyes do not show any aberrant morphology. Electroretinography evaluation by veterinary ophthalmologists is used to make a clinical diagnosis (Sandmeyer *et al*., 2007; 2012). *TRPM1* regulates the polarization of the on-bipolar cell, which is in charge of transferring the signal from the rod photoreceptor cell in the eye under low light. *TRPM1* signaling is thought to be affected by the causal mutation in leopard pattern horses (Bellone *et al*., 2013). Horses carrying only one copy of the *LP* allele do not appear to be affected. The leopard complex pattern can be found in a variety of various breeds around the world, including Danish Knabstruppers, Austrian Norikers, and a variety of pony breeds.

CONCLUSION

The identification of the genes that cause the various coat colours will aid in the identification of other genes that affect those colours. It is now known that dominant white is the result of several mutations, not just one. It is now evident also that sabino, tobiano, dominant white, and roan are all related because they are all mutations of the same gene (*KIT*). Because the *SB1* locus is not responsible for the sabino pattern seen in Clydesdale horses, knowing that sabino has more than one genetic source (*SB1*). It is also clear that the Overo, Splashed White, Leopard, and Gray loci all produce depigmentation as a result of other genes.

Lastly, the emergence of new colours, particularly those coupled with white spotting, is a sign of domestication. This is undoubtedly due to natural selection acting against obvious, poorly disguised patterns linked to pleiotropic health problems. The parallel acquisition of tame behaviour and novel coat hues may not be coincidental, as pathways regulating both behavior and colour involve

cells that start at the embryonic neural crest.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

Adalsteinsson, S. (1974). Inheritance of the palomino colour in Icelandic horses. *Journal of Heredity,* 65, 15-20.

Andersson, L. S., Juras, R., Ramsey, D. T., Eason-Butler, J., Ewart, S., Cothran, G., & Lindgren, G. (2008). Equine multiple congenital ocular anomalies maps to a 4.9 megabase interval on horse chromosome 6. *BMC genetics*, 9, Article number 88.

Andersson, L., & Georges, M. (2004). Domestic-animal genomics: deciphering the genetics of complex traits. *Nature Reviews Genetics*, *5*(3), 202-212.

Bailey, E., & Brooks, S. A. (2013). *Horse genetics*, 2nd Edition. Gutenberg Press Ltd. ISBN-13: 978 1 84593 675 4 (pbk); 978 1 78064 329 8 (hbk).

Baldwin, C. T., Hoth, C. F., Amos, J. A., da-Silva, E. O., & Milunsky, A. (1992). An exonic mutation in the HuP2 paired domain gene causes Waardenburg's syndrome. *Nature*, *355*(6361), 637-638.

Bellone, R. R., Archer, S., Wade, C. M., Cuka-Lawson, C., Haase, B., Leeb, T., Forsyth, G., Sandmeyer, L., & Grahn, B. (2010b). Association analysis of candidate SNPs in TRPM1 with leopard complex spotting (LP) and congenital stationary night blindness (CSNB) in horses. *Animal Genetics*, *41*(Supplement 2), 207-207.

Bellone, R. R., Brooks, S. A., Sandmeyer, L., Murphy, B. A., Forsyth, G., Archer, S., Bailey, E., & Grahn, B. (2008). Differential gene expression of *TRPM1*, the potential cause of congenital stationary night blindness and coat spotting patterns (LP) in the Appaloosa horse (Equus caballus). *Genetics*, *179*(4), 1861-1870.

Bellone, R. R., Forsyth, G., Leeb, T., Archer, S., Sigurdsson, S., Imsland, F., Mauceli, E., Engensteiner, M., Bailey, E., Sandmeyer, L., & Wade, C. M. (2010a). Fine-mapping and mutation analysis of TRPM1: a candidate gene for leopard complex (LP) spotting and congenital stationary night blindness in horses. *Briefings in functional genomics*, *9*(3), 193-207.

Bellone, R. R., Holl, H., Setaluri, V., Devi, S., Maddodi, N., Archer, S., Sandmeyer, L., Ludwig, A., Foerster, D., Pruvost, M., & Brooks, S. A. (2013). Evidence for a retroviral insertion in TRPM1 as the cause of congenital stationary night blindness and leopard complex spotting in the horse. *PloS One*, *8*(10), e78280.

Besmer, P., Manova, K., Duttlinger, R., Huang, E. J., Packer, A., Gyssler, C., & Bachvarova, R. F. (1993). The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. *Development*, *119*(Supplement), 125-137.

Bowling, A. T. (1996). *Horse genetics*. CAB International, Wallingford, UK. Pp. 146-147.

Bowling, A. T. (2000). Genetics of colour variation. In: Bowling, A. T., & Ruvinsky, A. (eds.). *The genetics of the horse*. CAB International, Wallingford, UK. Pp. 53-70.

Brooks, S. A., & Bailey, E. (2005). Exon skipping in the KIT gene causes a Sabino spotting pattern in horses. *Mammalian genome*, *16*(11), 893-902.

Brooks, S. A., Lear, T. L., Adelson, D. L., & Bailey, E. (2007). A chromosome inversion near the KIT gene and the Tobiano spotting pattern in horses. *Cytogenetic and genome research*, *119*(3-4), 225-230.

Bruford, M. W., Bradley, D. G., & Luikart, G. (2003). DNA markers reveal the complexity of livestock domestication. *Nature Reviews Genetics*, *4*(11), 900-910.

Brunberg, E., Andersson, L., Cothran, G., Sandberg, K., Mikko, S., & Lindgren, G. (2006). A missense mutation in PMEL17 is associated with the Silver coat colour in the horse. *BMC genetics*, *7,* Article number 46.

Castle, W. E. (1948). The ABC of colour inheritance in horses. *Genetics*, *33*(1), 22-25.

Castle, W. E., & Smith, F. H. (1953). Silver dapple, a unique colour variety among Shetland ponies. *Journal of Heredity*, *44*(4), 139-145.

Clark, L. A., Wahl, J. M., Rees, C. A., & Murphy, K. E. (2006). Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog. *Proceedings of the National Academy of Sciences*, *103*(5), 1376-1381.

Cook, D., Brooks, S., Bellone, R., & Bailey, E. (2008). Missense mutation in exon 2 of SLC36A1 responsible for champagne dilution in horses. *PLoS Genetics*, *4*(9), e1000195.

Dobney, K., & Larson, G. (2006). Genetics and animal domestication: new windows on an elusive process. *Journal of Zoology*, *269*(2), 261-271.

Duncan, L. M., Deeds, J., Hunter, J., Shao, J., Holmgren, L. M., Woolf, E. A., Tepper, R.I., & Shyjan, A. W. (1998). Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer research*, *58*(7), 1515-1520.

Elaine, P. (2021) Equine Melanoma: Harmless Bump or Time Bomb. Practical Horseman. Retrieved 24th December 2021 from https://practicalhorsemanmag.com/health-archive/equine-melanoma-harmless-bump-or-time-bomb/.

Fang, M., Larson, G., Soares Ribeiro, H., Li, N., & Andersson, L. (2009). Contrasting mode of evolution at a coat color locus in wild and domestic pigs. *PLoS Genetics*, *5*(1), e1000341.

Geissler, E. N., Ryan, M. A., & Housman, D. E. (1988). The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell*, *55*(1), 185-192.

Gilbert, S. F. (2006). *Developmental Biology*, 8th edition. Sinauer Associates, Inc., Sunderland, MA.

Girardot, M., Guibert, S., Laforet, M. P., Gallard, Y., Larroque, H., & Oulmouden, A. (2006). The insertion of a full-length Bos taurus LINE element is responsible for a transcriptional deregulation of the Normande Agouti gene. *Pigment Cell Research*, *19*(4), 346-355.

Gremmel, F. (1939). Coat colors in horses. *Journal of Heredity*, *30*(10), 437-445.

Guerts, R. (1977) *Hair Colour in the Horse*. J.A. Allen, London.

Gutiérrez-Gil, B., Wiener, P., & Williams, J. L. (2007). Genetic effects on coat colour in cattle: dilution of eumelanin and phaeomelanin pigments in an F2-Backcross Charolais × Holstein population. *BMC Genetics*, *8,* Article number 56.

Haase, B., Brooks, S. A., Schlumbaum, A., Azor, P. J., Bailey, E., Alaeddine, F., Mevissen, M., Burger, D., Poncet, P. A., Rieder, S., & Leeb, T. (2007). Allelic heterogeneity at the equine KIT. locus in dominant white (W) horses. *PLoS Genetics*, 3(11),

e195.
Haase, B., Brooks, S. A., Tozaki, T., Burger, D., Poncet, P. A., Rieder, S., Hasegawa, T., Penedo, C., & Leeb, T. (2009). Seven novel *KIT* mutations in horses with white coat colour phenotypes. *Animal Genetics*, *40*(5), 623-629.
Haase, B., Obexer-Ruff, G., Dolf, G., Rieder, S., Burger, D., Poncet, P. A., Gerber, V., Howard, J., & Leeb, T. (2010). Haematological parameters are normal in dominant white Franches–Montagnes horses carrying a *KIT* mutation. *The Veterinary Journal*, *184*(3), 315-317.
Haase, B., Rieder, S., Tozaki, T., Hasegawa, T., Penedo, M. C. T., Jude, R., & Leeb, T. (2011). Five novel *KIT* mutations in horses with white coat colour phenotypes. *Animal Genetics*, *42*(3), 337-339.
Hauswirth, R., Haase, B., Blatter, M., Brooks, S. A., Burger, D., Drögemüller, C., Gerber, V., Henke, D., Janda, J., Jude, R., & Leeb, T. (2012). Mutations in MITF and PAX3 cause "splashed white" and other white spotting phenotypes in horses. *PLoS genetics*, *8*(4), e1002653.
Henner, J., Poncet, P. A., Aebi, L., Hagger, C., Stranzinger, G., & Rieder, S. (2002a). Horse breeding: genetic tests for the coat colors chestnut, bay and black. Results from a preliminary study in the Swiss Freiberger horse breed. *Schweizer Archiv fur Tierheilkunde*, *144*(8), 405-412.
Henner, J., Poncet, P. A., Guérin, G., Hagger, C., Stranzinger, G., & Rieder, S. (2002b). Genetic mapping of the (G)-locus, responsible for the coat color phenotype" progressive greying with age" in horses (*Equus caballus*). *Mammalian Genome*, *13*(9), 535-537.
Hintz, R. L., & Van Vleck, L. D. (1979) Lethal dominant roam in horses. *Journal of Heredity, 70*, 145-146.
Holl, H., Brooks, S., & Bailey, E. (2010). De novo mutation of KIT discovered as a result of a non-hereditary white coat colour pattern. *Animal Genetics*, *41*, 196-198.
Hultgren, B. D. (1982). Ileocolonic aganglionosis in white progeny of overo spotted horses. *Journal of the American Veterinary Medical Association*, *180*(3), 289-292.
Hurst, C. C. (1906). On the inheritance of coat colour in horses. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *77*(519), 388-394.
Imsland, F., McGowan, K., Rubin, C. J., Henegar, C., Sundström, E., Berglund, J., Schwochow, D., Gustafson, U., Imsland, P., Lindblad-Toh, K., & Andersson, L. (2016). Regulatory mutations in TBX3 disrupt asymmetric hair pigmentation that underlies Dun camouflage color in horses. *Nature Genetics*, *48*(2), 152-158.
Klemola, V. (1933). The "pied" and "splashed white" patterns in horses and ponies. *Journal of Heredity*, *24*(2), 65-69.
Knottenbelt, D. C., & Pascoe, R. R. (1994) *Colour Atlas of Diseases and Disorders of the Horse*. Mosby-Year Book Europe Limited (now of Elsevier Science), London.
Lightbody, T. (2002). Foal with Overo lethal white syndrome born to a registered quarter horse mare. *The Canadian Veterinary Journal*, *43*(9), 715-717.
Locke, M. M., Penedo, M. C. T., Bricker, S. J., Millon, L. V., & Murray, J. D. (2002). Linkage of the grey coat colour locus to microsatellites on horse chromosome 25. *Animal Genetics*, *33*(5), 329-337.
Locke, M. M., Ruth, L. S., Millon, L. V., Penedo, M. C. T., Murray, J. D., & Bowling, A. T. (2001). The cream dilution gene, responsible for the palomino and buckskin coat colours, maps to horse chromosome 21. *Animal Genetics*, *32*(6), 340-343.
Ludwig, A., Pruvost, M., Reissmann, M., Benecke, N., Brockmann, G. A., Castaños, P., Cieslak, M., Lippold, S., Llorente, L., Malaspinas, A. S., & Hofreiter, M. (2009). Coat color variation at the beginning of horse domestication. *Science*, *324*(5926), 485-485.
Mariat, D., Taourit, S., & Guérin, G. (2003). A mutation in the MATP gene causes the cream coat colour in the horse. *Genetics Selection Evolution*, *35*(1), 119-133.
Marklund, L., Moller, M. J., Sandberg, K., & Andersson, L. (1996). A missense mutation in the gene for melanocyte-stimulating hormone receptor (MCIR) is associated with the chestnut coat color in horses. *Mammalian Genome*, *7*(12), 895-899.
Marklund, S., Moller, M., Sandberg, K., & Andersson, L. (1999). Close association between sequence polymorphism in the KIT gene and the roan coat color in horses. *Mammalian Genome*, *10*(3), 283-288.
Mau, C., Poncet, P. A., Bucher, B., Stranzinger, G., & Rieder, S. (2004). Genetic mapping of dominant white (W), a homozygous lethal condition in the horse (Equus caballus). *Journal of Animal Breeding and Genetics*, *121*(6), 374-383.
Metallinos, D. L., Bowling, A. T., & Rine, J. (1998). A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschsprung disease. *Mammalian Genome*, *9*(6), 426-431.
Moller, M. J., Chaudhary, R., Hellmen, E., Höyheim, B., Chowdhary, B., & Andersson, L. (1996). Pigs with the dominant white coat color phenotype carry a duplication of the *KIT* gene encoding the mast/stem cell growth factor receptor. *Mammalian Genome*, *7*(11), 822-830.
Nordlund, J. J., Boissy, R. E., Hearing, V. J., King, R. A., & Ortonne, J. P. (1998). *The Pigmentary System – Physiology and Pathophysiology*. Oxford University Press, New York.
Oancea, E., Vriens, J., Brauchi, S., Jun, J., Splawski, I., & Clapham, D. E. (2009). TRPM1 forms ion channels associated with melanin content in melanocytes. *Science signaling*, *2*(70), ra21-ra21.
Pielberg, G. R., Golovko, A., Sundström, E., Curik, I., Lennartsson, J., Seltenhammer, M. H., Druml, T., Binns, M., Fitzsimmons, C., Lindgren, G., & Andersson, L. (2008). A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nature genetics*, *40*(8), 1004-1009.
Pruvost, M., Bellone, R., Benecke, N., Sandoval-Castellanos, E., Cieslak, M., Kuznetsova, T., Morales-Muñiz, A., O'Connor, T., Reissmann, M., Hofreiter, M., & Ludwig, A. (2011). Genotypes of predomestic horses match phenotypes painted in Paleolithic works of cave art. *Proceedings of the National Academy of Sciences*, *108*(46), 18626-18630.
Pulos, W. L., & Hutt, F. B. (1969). Lethal dominant white in horses. *Journal of Heredity*, *60*(2), 59-63.
Raudsepp, T., Kijas, J., Godard, S., Guérin, G., Andersson, L., & Chowdhary, B. P. (1999). Comparison of horse chromosome 3 with donkey and human chromosomes by cross-species painting and heterologous FISH mapping. *Mammalian Genome*, *10*(3), 277-282.
Rieder, S., Stricker, C. H., Joerg, H., Dummer, R., & Stranzinger, G. (2000). A comparative genetic approach for the investigation of ageing grey horse melanoma. *Journal of Animal Breeding and Genetics*, *117*(2), 73-82.
Rieder, S., Taourit, S., Mariat, D., Langlois, B., & Guérin, G.

(2001). Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat colour phenotypes in horses (*Equus caballus*). *Mammalian Genome*, *12*(6), 450-455.

Robbins, L. S., Nadeau, J. H., Johnson, K. R., Kelly, M. A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K. G., & Cone, R. D. (1993). Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell*, *72*(6), 827-834.

Sandmeyer, L. S., Bellone, R. R., Archer, S., Bauer, B. S., Nelson, J., Forsyth, G., & Grahn, B. H. (2012). Congenital stationary night blindness is associated with the leopard complex in the miniature horse. *Veterinary Ophthalmology*, *15*(1), 18-22.

Sandmeyer, L. S., Breaux, C. B., Archer, S., & Grahn, B. H. (2007). Clinical and electroretinographic characteristics of congenital stationary night blindness in the Appaloosa and the association with the leopard complex. *Veterinary Ophthalmology*, *10*(6), 368-375.

Santschi, E. M., Purdy, A. K., Valberg, S. J., Vrotsos, P. D., Kaese, H., & Mickelson, J. R. (1998). Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. *Mammalian Genome*, *9*(4), 306-309.

Schmutz, S. M., & Berryere, T. G. (2007). Genes affecting coat colour and pattern in domestic dogs: A review. *Animal Genetics*, *38*(6), 539-549.

Searle, A. G. (1968). *Comparative genetics of coat colour in mammals*. Logos Press, London/New York.

Ségard, E. M., Depecker, M. C., Lang, J., Gemperli, A., & Cadoré, J. L. (2013). Ultrasonographic features of PMEL 17 (Silver) mutant gene–associated multiple congenital ocular anomalies (MCOA) in Comtois and Rocky Mountain horses. *Veterinary Ophthalmology*, *16*(6), 429-435.

Seltenhammer, M. H., Simhofer, H., Scherzer, S., Zechner, P., Curik, I., Sölkner, J., Brandt, S.M., Jansen, B., Pehamberger, H., & Eisenmenger, E. (2003). Equine melanoma in a population of 296 grey Lipizzaner horses. *Equine Veterinary Journal*, *35*(2), 153-157.

Seo, K., Mohanty, T. R., Choi, T., & Hwang, I. (2007). Biology of epidermal and hair pigmentation in cattle: a mini-review. *Veterinary Dermatology*, *18*(6), 392-400.

Sponenberg, D. P. (2009). *Equine colour genetics*, 3rd edition. Wiley-Blackwell, Ames, Iowa.

Sponenberg, D. P., Carr, G., Simak, E., & Schwink, K. (1990). The inheritance of the leopard complex of spotting patterns in horses. *The Journal of heredity*, *81*(4), 323-331.

Stachurska, A., Pięta, M., Łojek, J., & Szulowska, J. (2007). Performance in racehorses of various colours. *Livestock Science*, *106*(2-3), 282-286.

Sundström, E., Komisarczuk, A. Z., Jiang, L., Golovko, A., Navratilova, P., Rinkwitz, S., Becker, T. S., & Andersson, L. (2012). Identification of a melanocyte-specific, microphthalmia-associated transcription factor-dependent regulatory element in the intronic duplication causing hair greying and melanoma in horses. *Pigment Cell And Melanoma Research*, *25*(1), 28-36.

Swinburne, J. E., Hopkins, A., & Binns, M. M. (2002). Assignment of the horse grey coat colour gene to ECA25 using whole genome scanning. *Animal Genetics*, *33*(5), 338-342.

Terry, R. B., Archer, S., Brooks, S., Bernoco, D., & Bailey, E. (2004). Assignment of the appaloosa coat colour gene (LP) to equine chromosome 1. *Animal Genetics*, *35*(2), 134-137.

Trommershausen-Smith, A. (1977). Lethal white foals in matings of overo spotted horses. *Theriogenology*, *8*(5), 303-311.

Trommershausen-Smith, A., Suzuki, Y., & Stormont, C. (1976). Use of blood typing to confirm principles of coat-color genetics in horses. *The Journal of heredity*, *67*(1), 6-10.

Van Vleck, L. D., & Davitt, M. (1977). Confirmation of a gene for dominant dilution of horse colours. *Journal of Heredity*, *68*(5), 280-282.

Vonderfecht, S. L., Bowling, A. T., & Cohen, M. (1983). Congenital intestinal aganglionosis in white foals. *Veterinary Pathology*, *20*(1), 65-70.

Wagner, H. J., & Reissmann, M. (2000). New polymorphism detected in the horse MC1R gene. *Animal Genetics*, *31*(4), 289-290.

Woolf, C. M., & Swafford, J. R. (1988). Evidence for eumelanin and pheomelanin producing genotypes in the Arabian horse. *Journal of Heredity*, *79*(2), 100-106.

Wright, S. (1917). Colour inheritance in mammals. *Journal of Heredity*, *8*(10), 473-475.

Yan, G. C., Croaker, D., Zhang, A. L., Manglick, P., Cartmill, T., & Cass, D. (1998). A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS); a horse variant of Hirschsprung disease (HSCR). *Human molecular genetics*, *7*(6), 1047-1052.