Molecular tests for coat colours in horses

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General introduction and relevance of the topic

Colour phenotypes may have played a major role during early domestication events and initial selection among domestic animal species (Henner et al. 2002a; Bruford et al. 2003; Andersson & Georges 2004). Domestication is a process resulting in numerous morphological, physiological and behavioural changes. Among these, coat colour seems to be a particularly obvious one (Grandin 1998). While many wild animal species are coloured relatively uniformly, domestic stock like, e.g. horses (Figure 1) show a broad variety of coat colour patterns. This seems to be at least partly due to differences in selection criteria for animals in the wild as opposed to captive breeding populations. Very recently, Ludwig et al. (2009) published a study on the occurrence and frequency of coat colour alleles found in ancient horse DNA samples from the Pleistocene Period to Roman times. They found less colour variation in ancient wild horse populations than in early and later domesticated horse populations. Although a particular coat colour might be essential for survival in nature (e.g. camouflage, mating behaviour, pathogen tolerance and environmental adaptation) (Gilbert 2006), human preferences and demand might have favoured rare alleles or led to previously unknown phenotypes due to selective breeding (Klungland & Vage 2000). Recently, Fang et al. (2009) presented convincing results on how human-mediated selection acted on colour phenotypes in the pig.

As coat colours appear to follow relatively simple modes of Mendelian inheritance most of the time (Table 1), they were among the first traits to be systematically analysed at the molecular level. Furthermore, they provide unique models for studying gene function and regulation, i.e. models for investigating the relations between phenotypic variation, particular genotypes and physiological processes.

Genes affecting mammalian coat and skin colour can be classified into two main groups: those acting on pigment synthesis and those acting on the pigment-producing cells, the melanocytes (Nordlund et al. 1998). Variation in coat and skin colours is therefore likely to be understood as the effect of modified genes causing changes to either pigment synthesis or to the melanocyte or its combinations (constitutive pigmentation – intrinsic), supplemented by environmental factors and hormones (facultative pigmentation –
inducible) (Graphodatskaya 2002). Generally, mutations in the external or surface environment of the melanocyte result in changes in the amount ofpheomelanin (red/yellow pigment) versus eumelanin (black/brown pigment), thus addressing the question of what kind of pigment are produced. Moreover, changes in the amount of pigment produced are caused by mutations affecting the internal machinery of the melanocyte, an effect which may lead to coat colour dilution. Last but not the least, other
### Table 1  An overview of the genetics of horse coat colours

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Locus name, symbol and gene abbreviation</th>
<th>Alleles and mutation types</th>
<th>Mode of inheritance, interaction, association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic coat colours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chestnut/Sorrel</td>
<td>Extension (E) MC1R</td>
<td>e/e C901T missense mutation; loss-of-function mutation</td>
<td>Autosomal recessive Epistatic over black</td>
</tr>
<tr>
<td>Bay</td>
<td>Extension (E) Agouti (A)</td>
<td>E/e and EE A/a and AA</td>
<td>Autosomal dominant Result of allele combinations other than e/e at MC1R and a/a at ASIP</td>
</tr>
<tr>
<td>Black</td>
<td>Agouti (A) ASIP</td>
<td>a/a 11bp deletion at position 2174–2184; frameshift loss-of-function mutation</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td><strong>Dilution coat colours</strong></td>
<td></td>
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</tr>
<tr>
<td>Palomino/Buckskin</td>
<td>Cream (CR) MATP</td>
<td>CR/cr G457A missense mutation</td>
<td>Autosomal codominant Epistatic to chestnut/sorrel and bay; no or poor epistatic effect on black Autosomal dominant Epistatic effect on black in homozygous horses</td>
</tr>
<tr>
<td>Cremello/Perlino – creamy white coat colour with blue eyes</td>
<td>Cream (CR) MATP</td>
<td>CR/CR</td>
<td></td>
</tr>
<tr>
<td>Dun – diluted basic colours and primitive markings [e.g. dorsal stripe, zebra stripes on legs]</td>
<td>Dun (D) Not known</td>
<td>QTL on ECA8 close to microsatellite UCDEQ46</td>
<td></td>
</tr>
<tr>
<td>Silver dapple – chocolate-to-reddish body with white/grey mane and tail</td>
<td>Silver (Z) PMEL17</td>
<td>Z/z and ZZ C1457T missense mutation</td>
<td></td>
</tr>
<tr>
<td>Champagne – metallic sheen (eye and skin colour may change with age)</td>
<td>Champagne (CH) SLC36A1</td>
<td>CH/ch and CH/CH C188G missense mutation</td>
<td>Autosomal dominant Epistatic to all basic colours</td>
</tr>
<tr>
<td><strong>Greying</strong></td>
<td></td>
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<tr>
<td>Progressive greying with age; associated with melanoma</td>
<td>Grey (G) STX17</td>
<td>G/G and G/G Intron 6 duplication with regulatory consequences</td>
<td>Autosomal dominant Epistatic to all colours; higher incidence of melanoma in carriers of loss-of-function mutation in ASIP Progression of greying accelerated in homozygous G/G horses</td>
</tr>
<tr>
<td><strong>Depigmentation</strong></td>
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<tr>
<td>Roan – interspersed white hairs in basic colour</td>
<td>Roan (RN) Associated to KIT</td>
<td>RN/rn and RN/RN C2613T silent mutation</td>
<td>Autosomal dominant Epistatic to all colours The homozygous RN/RN genotype is thought to be lethal</td>
</tr>
<tr>
<td>Overo-spotting – irregular white spotting, often horizontal distribution</td>
<td>Overo (O) EDNRB</td>
<td>O/o and O/O TC353-354AG dinucleotide missense mutation</td>
<td>Autosomal dominant Epistatic to all colours Homozygous O/O carriers are so-called lethal whites (OLWS) OLWS follows a recessive mode of inheritance</td>
</tr>
<tr>
<td>Tobiano-spotting – regular white spotting, often vertical distribution</td>
<td>Tobiano (TO) KIT</td>
<td>TO/to and TO/TO Inversion on ECA3q near KIT</td>
<td>Autosomal dominant Epistatic to all colours</td>
</tr>
</tbody>
</table>
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Table 1 (Continued)

<table>
<thead>
<tr>
<th>Phenotype</th>
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<th>Alleles and mutation types</th>
<th>Mode of inheritance, interaction, association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appaloosa-spotting or Leopard-spotting;</td>
<td>Leopard spotting</td>
<td>LP/Lp and LP/LP</td>
<td>Autosomal – incompletely dominant</td>
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<tr>
<td>associated with congenital stationary night</td>
<td>[LP]</td>
<td>Downregulation of TRPM1</td>
<td>Epistatic to all colours</td>
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<tr>
<td>blindness (CSNB)</td>
<td>Associated to TRPM1 and yet</td>
<td></td>
<td>Homozygous LP/LP carriers tend to show fewer</td>
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<td></td>
<td>unknown modifiers</td>
<td></td>
<td>pigmentation than heterozygous horses;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>homozygous horses show CSNB</td>
</tr>
<tr>
<td>White – heterogenous amount of depigmentation</td>
<td>White (W)</td>
<td>W/w and W/W</td>
<td>Autosomal dominant</td>
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<tr>
<td></td>
<td>KIT</td>
<td>Various independent, breed-specific</td>
<td>Epistatic to all colours</td>
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<tr>
<td></td>
<td></td>
<td>mutations in KIT</td>
<td>The homozygous W/W genotype is thought to be</td>
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<td></td>
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<td>lethal</td>
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<tr>
<td>Sabino-spotting</td>
<td>Sabino (SB)</td>
<td>5B/sb and 5B/5B</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td></td>
<td>KIT</td>
<td>K16 + 1037A base substitution T</td>
<td>Epistatic to all colours</td>
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<tr>
<td></td>
<td></td>
<td>with A – skipping of exon 17</td>
<td>Homozygosity for SB1 results in a complete or</td>
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<td></td>
<td></td>
<td></td>
<td>nearly completely white phenotype</td>
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<tr>
<td>White facial and leg markings</td>
<td>White markings</td>
<td>WM/wm and wm/wm</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>(WM)</td>
<td>1. QTL on ECA3q close to microsatellite AHT101 and SNP MSKIT17;</td>
<td>Epistatic to chestnut/sorrel</td>
</tr>
<tr>
<td></td>
<td>Associated to MCTR, KIT, MITF</td>
<td>2. QTL on ECA16q</td>
<td>Depending on basic colours, either</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>KIT or MITF primarily act on white</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>markings</td>
</tr>
</tbody>
</table>

For all listed alleles commercial genotyping is possible, except, so far, for Leopard-spotting and White markings. For Dun and Roan zygosy genotyping is possible for some breeds. Definition of mutation types: 1 = missense mutation (non-synonymous mutation) – a nucleotide change (transition, transversion/reversion) resulting in a codon that codes for a different amino acid. A silent mutation (synonymous mutation) – a nucleotide change not affecting the amino acid code of a protein. 2 = frameshift mutation – result of insertions or deletions (indels) of nucleotides that are not evenly divisible by three. Due to the triplet nature of gene expression by codons, the insertion or deletion disrupts the reading frame. 3 = inversion – an inversion is a chromosome rearrangement in which a segment of a chromosome is reversed. 4 = alternative splicing – alternative splicing is the RNA splicing variation mechanism in which the exons of the primary gene transcript, the pre-mRNA, are separated and reconnected to produce alternative ribonucleotide arrangements. Different modes of alternative splicing are distinguished, inter alia exon skipping.

loci, controlling differentiation, proliferation and migration of melanocytes determine the amount and form of white spotting (Sponenberg 2009).

Melanocytes belong to the group of neural crest-derived cells. It is known that genes acting on melanocyte development and distribution also act on cells such as primordial germ cells, haematopoietic cells or neurones. Furthermore, enzymes and hormones acting on pigment synthesis and regulation (e.g. tyrosinase and melanocortins) are involved in a number of physiological processes (Graphodatskaya 2002). These fascinating coherences explain some of the pleiotropic effects between coat colour variation and more complex traits such as developmental disorders (e.g. lethal dominant white), diseases (e.g. lethal white foal disease and grey horse melanoma) or even temperament (Trut 1999; Dobney & Larson 2006; Gilbert 2006). They might also be a key to the question why breeders in different parts of the world have often associated specific coat colours with particular performance characteristics in horses (fast and hot chestnuts, Hardy bays, gentle greys, etc.). However, this latter facet is discussed controversially (Stachurska et al. 2007).

Today, coat colour phenotypes (especially colour markings) are of practical relevance concerning the identification of individual horses. Coat colours give a first hint whether alleles between parents and offspring segregated correctly or not (e.g. grey rule – a grey horse has at least one grey parent; chestnut rule – breeding chestnuts always results in chestnuts – Bowling 2000; Sponenberg 2009), and they may enable us to distinguish among horses of particular breeds (Camargue all grey, Friesian all black, Haflinger all chestnut with flaxen mane and tail, etc.).

Genes controlling horse coat colours have been known for a long time. However, it was only recently that functional alleles or marker alleles have been detected at the DNA level. Among them, the chestnut allele $E'$, a single nucleotide mutation (C901T; AF288357) within the melanocortin receptor...
I (MC1R), was the first to be published in the horse (Marklund et al. 1996).

The following paragraphs describe, step by step, the major phenotypic coat colour groups of the horse and their molecular genetic background, as far as it is known and has been published to date. Table 1 summarizes the content of the paragraphs. Some of the phenotypes are presented in Figure 1. First, the genetics of the so-called basic coat colours – chestnut, bay and black – are presented. Thereafter, dilution phenotypes are discussed, followed by a paragraph on greying and a final paragraph on depigmentation phenotypes.

The Extension and Agouti loci – basic coat colours, chestnut, bay and black

Apart from white markings, chestnut horses are characterized by eumelanin pigment (black/brown pigment) in the skin and pheomelanin pigment (red/yellow pigment) in the hair (including mane and tail). By contrast, black horses have uniformly distributed black pigmented skin and hair, and bay horses show pheomelanin (body) and eumelanin (mane, tale and lower legs) patterns. The Melanocortin-1-receptor (MC1R), encoded by the Extension (E) locus (ECA3p12), and its peptide antagonist, the agouti signalling protein (ASIP), encoded by the Agouti (A) locus (ECA22q15–16), control the relative amounts of melanin pigments in mammals. ASIP acts as an indirect antagonist of MC1R by nullifying the action of the α-melanocyte-stimulating hormone (α-MSH). Loss-of-function mutation of MC1R results in red/yellow pigment (pheomelanin), whereas gain-of-function mutation of MC1R or loss-of-function mutation of ASIP results in the production of black pigment, i.e. eumelanin (Rieder et al. 2001). In the horse, the basic colours, chestnut, bay and black, are so far determined by four alleles, two of them encoded by the Extension (E) locus (E<sup>e</sup> and E<sup>d</sup>) and two encoded by the Agouti (A) locus (A<sup>A</sup> and A<sup>a</sup>). Chestnut and black follow a recessive mode of inheritance (E<sup>e</sup>/E<sup>e</sup> and A<sup>a</sup>/A<sup>a</sup>), chestnut being epistatic over black. Black is therefore only expressed when the genotype at the Extension locus differs from E<sup>e</sup>/E<sup>e</sup>. Bay is the result of allele combinations other than E<sup>e</sup>/E<sup>e</sup> at the Extension locus and A<sup>=</sup>/A<sup>=</sup> at the Agouti locus. So far, in the horse, no gain-of-function mutation (i.e. dominant black) at the Extension locus is known at the DNA level. Therefore, black coat colour results from a recessive allele at the Agouti locus only (11-bp deletion in exon2; AF288358) (Rieder et al. 2001). Shades of these basic colour patterns might be due to epistatic effects of the presented alleles as well as to so far unknown genetic variation (Henner et al. 2002a). A third allele segregating at the Extension locus, also completely associated with the chestnut phenotype, was published by Wagner & Reissmann (2000) – AF252541.

The Cream, Dun, Silver Dapple and Champagne loci – coat colour dilution

There are four confirmed loci in the horse which are responsible for dilution of the basic coat colours: Cream (C), Dun (D), Silver Dapple (Z) and Champagne (CH). They all act basically in dominant or codominant manner. However, the effects on the phenotypes are somehow different, depending on the characteristics of either the dominant or codominant alleles among heterozygote and homozygote carriers.

To start with the C<sup>CR</sup> and D<sup>d</sup> alleles, in a heterozygote state the incomplete dominant cream allele dilutes a chestnut background to palomino and a bay background to buckskin. However, one C<sup>CR</sup> allele usually has no phenotypic effect on black. In homozygote state C<sup>CR</sup> dilutes all basic colours to what is called a cremello/perlin phenotype, also featuring blue eyes. The cream locus was mapped to ECA21q (Locke et al. 2001). Mariat et al. (2003) showed that a single base substitution in exon 2 of the membrane-associated transporter protein (MATP) – G457A and AY187093 – appears to be fully associated with the phenotypes segregating with C<sup>CR</sup>. The mutation seems to disrupt the function of this transporter protein, leading to the known colour dilutions.

Compared with C<sup>CR</sup>, the dun allele D<sup>d</sup> shows complete dominance on all basic coat colours; heterozygotes cannot be distinguished from homozygotes. Furthermore, the dun coat colour (red dun, yellow dun, grullo or mouse dun, depending on the basic colour background) appears along with a dorsal stripe, zebra stripes and other primitive markings. This is, in particular not the case for the phenotypes resulting from all other horse coat colour dilution genes mentioned. The dun coat colour has been linkage mapped to a group of microsatellite markers on ECA 8 (Bricker et al. 2003). However, the protein encoded by the Dun locus is not yet known. Based on comparative mapping data, candidate genes would most probably be located on HSA 18q, MMU 18q and MMU 1q.

A flaxen mane and tail as well as a diluted chocolate-to-reddish body on a eumelanin background is
known as Silver Dapple (Z). Brunberg et al. (2006) showed that the silver phenotype segregates in an autosomal, completely dominant manner, and that it is fully associated with a C>T transition (DQ855465) at position five within the exon 11 of premelanosomal protein 17 (Pmel17), also known as the Silver locus. The mutation changes the second amino acid in the cytoplasmic region from arginine to cysteine (Arg618Cys). Another research group (Reissmann et al. 2007) detected a haplotype (DQ665301:g.697A > T/DQ665301:g.1457C > T) in Pmel17 which again was completely associated with the Silver Dapple phenotype. This haplotype also included the C>T mutation mentioned above. Pmel17 was originally mapped to ECA6q23 by Rieder (1999). In some breeds, the Silver locus was recently found to be part of a linkage group harbouring a quantitative trait locus (QTL) associated with multiple congenital ocular anomalies (Anderson et al. 2008; Grahm et al. 2008).

To complete the series of dilution coat colour loci in horses, a fourth locus, champagne (CH), is described. Champagne is a phenotype known for its metallic sheen, and may be confused with cream and silver. It is described as following an autosomal dominant mode of inheritance. Cook et al. (2008) mapped the locus to a region on ECA 14 between microsatellites UM010 and TKY329. The same authors found a missense mutation at position 76 in exon 2 of SLC36A1 (also known as PAT1 or LYAAT1) among several candidate genes. The cysteine to guanine base change (c188C > G) is predicted to cause a transition from a threonine to arginine, at amino acid 63 of the protein (T63R). The mutation is thought to affect a putative transmembrane domain. According to Cook et al. (2008), the protein coded by the SLC36A1 gene might play a role in melanosome development, resulting in a reduction in pigmentation induced by the mutation mentioned above.

The grey locus – progressive greying with age

Progressive greying with age (G) is a coat colour phenotype in the horse with a dominant mode of inheritance (GG versus Gg). A grey horse is always born with pigmented skin and coloured hair. As it gets older, its hair greys, while its skin remains pigmented (except for white markings and vitiligo). Grey is epistatic to all other colours. Grey horses are known to be particularly susceptible to skin melanoma (Rieder et al. 2000). The Grey locus has been mapped to ECA 25q by three independent groups, and has been fine mapped by a fourth group; all four groups worked with different horse breeds and resource families (Henner et al. 2002b; Locke et al. 2002; Swinburne et al. 2002; Pielberg et al. 2005). Depending on the particular resource family, the Grey locus (G) was found very close to the microsatellite marker COR080. Thus, the overall confirmative mapping data indicate that Grey is a rather old mutation. Candidate genes for the Grey locus were not available from comparative mapping data until recently, neither was the type of correlation with melanoma development (Sölkner et al. 2004). Rosengren Pielberg et al. (2008) demonstrated, that the grey phenotype is caused by a 4.6-kb duplication in intron 6 of STX17 (syntaxin-17) that constitutes a cis-acting regulatory mutation. Both STX17 and the neighbouring NR4A3 gene are overexpressed in melanomas of grey horses. Grey horses carrying a loss-of-function mutation in the ASIP (agouti signalling protein) have a higher incidence of melanoma, implying that increased melanocortin-1 receptor signalling promotes melanoma development in grey horses. Another interesting finding of the study concerned the interpretation of how a mutation causing loss of hair pigmentation could also cause melanomas with a massive production of melanin. The authors suggested that this could be due to differences in the life cycle of hair follicles and dermal melanocytes. The STX17 duplication leads to proliferation of dermal melanocytes, thus predisposing grey horses to melanoma development. By contrast, hyperproliferation of hair follicle melanocytes may cause premature depletion of stem cells, and thus progressive loss of pigmentation in the coat.

The White and White Spotting loci – coat and skin colour depigmentation

White and White Spotting loci are responsible for a group of phenotypes encompassing a mixture of white and coloured hairs (roan; head usually not affected), discrete white spots of varying size, extent and location (tobiano, overo, sabino, leopard spotting) to nearly complete depigmentation (dominant white). True albinos (normal structure and number of melanocytes, but complete depigmentation of the eyes, skin and hair), however, are not known in the horse so far. Phenotypic and genetic definition and distinction of these somehow heterogeneous characters may present substantial problems.

First of all, Marklund et al. (1999) described several mutations in the Tyrosine kinase receptor (KIT)
located on ECA3q21–22. A KIT exon19 substitution (C2613T – AJ224642–45) appeared to be strongly associated with the roan phenotype (interspersed white and coloured hair all over the body except the head) in most studied breeds. However, the causative mutation for roan is not yet known. Roan is assumed to be a lethal condition in homozygous state and segregates in a dominant manner (\(RN^{R}\)) (Hintz & Van Vleck 1979).

Yang et al. (1998), Santschi et al. (1998) and Metallinos et al. (1998) demonstrated independently that a dinucleotide mutation (TC353–354AG – AF019072; AF038900) in the Endothelin-B receptor gene (EDNRB; ECA17q23–24) is associated with lethal white foal syndrome (OLWS) and the overo coat colour pattern. The mutation leads to an amino acid change from lysine to isoleucine in the first transmembrane domain of this G-protein-coupled receptor, with profound impact on the normal development of enteric ganglia and melanocytes (similar to Hirschsprung’s disease in humans). Lethal white foals were found homozygous for the mutation, with all their parents showing a heterozygous state, carrying mostly the frame overo coat colour pattern. Some carriers showed tobiano and sabino markings (Santschi et al. 2001), indicating epistatic effects and variable penetrance of the mutant allele (\(O^\circ\)). In overo colour patterns, white appears irregularly and will rarely cross the dorsal line of the horse. Legs are often solid coloured, and head markings extensively white. OLWS has a recessive mode of inheritance, while the overo coat colour pattern follows a dominant segregation pattern.

Brooks et al. (2002) published a PCR–RFLP detected in the KIT gene (intron13; G693G – AY048669) which is very closely associated with a white spotting colour known as Tobiano (\(TO^{TO}\)). In 2007, the same group showed a chromosomal inversion, beginning approximately 100 kb downstream of the KIT gene on ECA3q21–22, to be the causative mutation for the (\(TO^{TO}\)) phenotype. The inversion does not interrupt any annotated genes but may disrupt regulatory sequences for the KIT gene and cause the white spotting pattern (Brooks et al. 2007; Haase et al. 2008). Tobiano is characterized by white, depigmented areas with distinct borders that cross the spine and usually include all four legs. Head markings appear to be like those in common solid-coloured horses.

Another spotting pattern is referred to as Leopard spotting (\(LP\)), also known as Appaloosa spotting. Horses that inherit the \(LP^{LP}\) allele will display one of several different patterns of white, \(LP^{LP}\) being incompletely dominant to the solid (non-spotted) allele \(LP^{p}\). Modifiers are likely to be responsible for the multiplicity of patterns associated with Appaloosa. KIT, its ligand the Mast cell growth factor (MGF) and the Microphthalmia-associated transcription factor (MITF) have been excluded as candidates for this coat colour by linkage analysis (Terry et al. 2001, 2002). The Leopard spotting locus was, however, assigned to ECA1q (Terry et al. 2004), a chromosomal region containing potential candidate genes that based on comparative mapping data are most probably responsible for LP in the horse. Among these are the Pink eye dilution (\(p\)) and the Transient receptor potential cation channel subfamily M, member 1 (TRPM1), also known as Melastatin (MLSN1). Homozygosity for LP seems to be directly associated with congenital stationary night blindness (CSNB) in Appaloosa horses. Bellone et al. (2008) demonstrated recently that LP/CSNB is the result of a downregulation of TRPM1 expression in the skin and retina of LP/lp and LP/LP horses. TRP proteins are thought to play a role in controlling intracellular Ca\(^{2+}\) concentration. Decreased expression of TRPM1 in the eye and skin may alter bipolar cell signalling as well as melanocyte function, thus causing both CSNB and LP in horses.

A final series of white pattern phenotypes concern the Dominant white (\(W\)) and Dominant Sabino loci (\(S\)). Both loci have recently been mapped to ECA3q22 and association was found with mutations in the KIT gene. A single nucleotide polymorphism (SNP) detected in KIT intron3 (G258A – AY232413) revealed linkage with microsatellite ASB23 and \(W^{W}\) in a pedigree of Swiss Franches Montagnes horses (Mau et al. 2004). The dominant white phenotype was traced back to one particular founder mare in this pedigree, indicating a spontaneous mutation event. Dominant white is thought to be lethal in its homozygous state, maybe as a consequence of a disordered development within the haematopoietic system (Pulos & Hutt 1969). Not all dominant white horses in the pedigree were of an entirely white phenotype; some of them appeared spotted, as Sabino lookalikes. So far, it is not known to what extent these two heterogeneous phenotypes share a common genetic and phenotypic background, apart from their association with the KIT gene. Haase et al. (2007, 2009a) published a study on allelic heterogeneity at the equine KIT locus among dominant white horses of different breeds. The authors demonstrated that independent mutation events within the equine KIT gene among non-related horse breeds.
resulted in similar dominant white/sabino phenotypes.

An SNP in the KIT intron16 (K16 + 1037A; AY910688) was found to be responsible for skipping exon17 and to be causative for the Sabino spotting pattern (called Sabino 1) in the Tennessee Walking Horse and in some other breeds (Brooks & Bailey 2005). All horses carrying the Sabino1 KIT intron16 SNP exhibited Sabino phenotypes, including homozygous all-white animals. However, not all Sabino lookalike horses carried the Sabino1 SNP. Thus, further research is needed to unravel the relations among those coat colour patterns and their impact on more complex traits.

Apart from the depigmentation phenotypes mentioned above, a pattern known as White markings (WM) is well known in many species, including the horse. Rieder et al. (2008) demonstrated that white markings are highly heritable ($h^2 > 50\%$), and that a first QTL on ECA3q, the chromosomal region harbouring the KIT gene, could explain up to 80\% of the total heritability of the trait. A strong positive correlation was found between the chestnut allele at MC1R and the extent of white markings, confirming data presented by, e.g. Woolf (1992) in several publications during the 1990s. Furthermore, the authors revealed that the trait-increasing allele seemed to follow a recessive mode of inheritance. Recent SNP data revealed an additional QTL on ECA16q acting on white markings – MITF. The data indicate that KIT influences in particular white markings on a chestnut background, whereas MITF seems to act primarily on white markings on a non-chestnut background (Haase et al. 2009b).

It is worth noting that all depigmentation phenotypes discussed in this paragraph, except overo and leopard spotting, share an association with the KIT gene. Thus, it would be interesting to study whether there is a pattern evolving about the location of the mutation and the type of colour seen for KIT mutations.

**Conclusion**

In 1995, a workshop was formed to foster collaboration and to share resources among scientists interested in the horse genome. Despite this relatively late start, an impressive number of tools have been developed during the past decade, enabling us today to analyse the basis of horse phenotypic variation at the genetic level (Chowdhary & Bailey 2003; Chowdhary & Raudsepp 2008). Coat colours were among the first traits to be studied, and as we can see, genetic tests, now commercially available, have been designed for some of them (e.g. Royo et al. 2008). These tests allow breeders to verify segregation within particular pedigrees, to select specific colour phenotypes according to market demand or studbook policies, and to avoid inherited diseases associated with some of the colour patterns. However, more research is still needed to fully differentiate and precisely define the heterogeneity of horse colour phenotypes (e.g. shades such as darker chestnut or darker bay, flaxen mane and tail, seasonal coat colour change, the problem of phenotypes that resemble one another but are genetically different, etc.), their underlying genetics, and, as supposed to some of them, the correlation to more complex traits as described above. Additionally, it would be interesting to compare and study the insights obtained from horse coat colour genetics with other members of the larger equid family. For example, little is known about the molecular basis of coat colour in the donkey or the genetics behind zebra stripes. The results of such research might give us new insights into the genetics of various physiological processes, i.e. related to early embryonic development, adaptation to particular environments, pathogen tolerance and even performance. As colour phenotypes played an important role during the development of modern livestock breeds, including the horse, the genetics of coat colour might also be helpful to better understand the process of domestication (e.g. the impact of white colour markings on behaviour, such as temperament). To achieve these goals, a focus should be given on the precise description of phenotypes and on the setting up of segregation and/or case/control resource families.

**Acknowledgements**

I thank numerous colleagues for fruitful discussions and continuous scientific exchange on horse and mammalian coat colour genetics over the years. Many thanks are due to two anonymous reviewers for helpful comments and for carefully reading and improving this manuscript.

**References**


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