

# Genotypes of predomestic horses match phenotypes painted in Paleolithic works of cave art

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Archaeologists often argue whether Paleolithic works of art, cave paintings in particular, constitute reflections of the natural environment of humans at the time. They also debate the extent to which these paintings actually contain creative artistic expression, reflect the phenotypic variation of the surrounding environment, or focus on rare phenotypes. The famous paintings “The Dappled Horses of Pech-Merle,” depicting spotted horses on the walls of a cave in Pech-Merle, France, date back ~25,000 y, but the coat pattern portrayed in these paintings is remarkably similar to a pattern known as “leopard” in modern horses. We have genotyped nine coat-color loci in 31 predomestic horses from Siberia, Eastern and Western Europe, and the Iberian Peninsula. Eighteen horses had bay coat color, seven were black, and six shared an allele associated with the leopard complex spotting (*LP*), representing the only spotted phenotype that has been discovered in wild, predomestic horses thus far. *LP* was detected in four Pleistocene and two Copper Age samples from Western and Eastern Europe, respectively. In contrast, this phenotype was absent from predomestic Siberian horses. Thus, all horse color phenotypes that seem to be distinguishable in cave paintings have now been found to exist in prehistoric horse populations, suggesting that cave paintings of this species represent remarkably realistic depictions of the animals shown. This finding lends support to hypotheses arguing that cave paintings might have contained less of a symbolic or transcendental connotation than often assumed.

ancient DNA | transient receptor potential cation channel subfamily M1 | single nucleotide polymorphism | leopard complex spotting | Franco-Cantabrian region

Prehistoric representations of animals have the potential to provide first-hand insights into the physical environment that humans encountered thousands of years ago and the phenotypic appearance of the animals depicted. However, the motivation behind, and therefore the degree of realism in, these depictions is hotly debated and it has yet to be shown to what extent they have been executed in a naturalistic manner. Neuropsychological explanations include “hyperimagery,” in which an internally generated image is perceived in external space (1), whereas others have argued for shamanistic significance (2) or simply art for art’s sake (3). Some paleontologists argue that cave paintings are reflections of the natural environment of humans at the time (4), but not all researchers agree with this opinion (5).

Exact numbers of Upper Paleolithic sites with animal depictions are uncertain because of ongoing debates regarding the taxonomic identification of some images and the dating of some (e.g., ref. 6). However, art of this period has been identified in at least 40 sites in the Dordogne–Périgord region, a similar number in coastal Cantabria [although Bicho and coauthors (7) argue for many more sites], and around a dozen sites in each of the Ardèche

and Ariège regions. Although it can be concluded that naturalistic depictions of animals in cave art constitute a restricted phenomenon, with more than 80% of the examples being found in two of the regions mentioned above (Ariège and Périgord in France and the Cantabrian coast in Spain), four important sites (i.e., Ignatieva and Kapova in Russia, Cuculiat in Romania, and Badanj in Bosnia) are located outside Western Europe (Fig. 1) (8). Chronologically, most of the evidence dates to the Magdalenian period (16–11 kyBP) although the earliest testimonies go back to the Aurignacian of Chauvet Cave in France (i.e., 31 kyBP) (9, 10). Post-Paleolithic art, shifting to more abstract and stylized forms, is of much less relevance for the discussion about possible naturalistic animal depictions (4, 5).

Where animal species can be confidently identified, horses are depicted at the majority of these sites. With more than 1,250 documented depictions (~30% of all animal illustrations) ranging from the Early Aurignacian of Chauvet to the Late Magdalenian (several post-12-kyBP sites in France and Spain) (11), and from the Iberian Peninsula to the Ural mountains, horses are the most frequent of the more than 30 mammal species depicted in European Upper Paleolithic cave art (5, 12). Depictions are commonly in a caricature form that slightly exaggerates the most typical “horsey” features (13). Although taken as a whole, images of horses are often quite rudimentary in their execution, some detailed representations, from both Western Europe and the Ural mountains, are realistic enough to at least potentially represent the actual appearance of the animals when alive. In these cases, attributes of coat color may also have been depicted with deliberate naturalism, emphasizing colors or patterns that characterized contemporary horses. For example, the brown and black horses dominant at Lascaux and Chauvet, France, phenotypically match the extant coat colors bay and black. However, the depictions in the cave of Pech-Merle, France, dated to 24.7 kyBP (14), featuring spotted horses in a frieze that includes hand outlines and abstract patterns of spots, have led prehistorians to argue for more complex explanations for several reasons. First, the juxtaposition of elements in this depiction raises the question of whether the spotted pattern is in some way symbolic or abstract, and second, a spotted coat phenotype is, at least by many researchers, considered unlikely for Paleolithic horses.

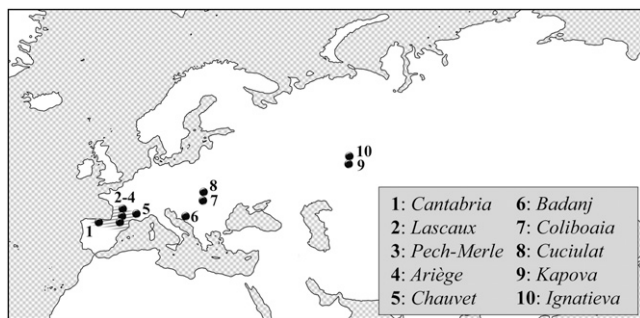
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**Fig. 1.** Map of key locations of Paleolithic cave art containing horse paintings. The Franco–Cantabrian region containing most of the Paleolithic cave paintings is highlighted.

However, the spotted horses depicted at Pech-Merle closely resemble the leopard complex spotting (*LP*) seen in some modern horse breeds. Leopard complex spotting is characterized by white spotting patterns that range from horses having a few white spots on the rump to horses that are almost completely white. The white area of these horses can also have pigmented oval spots known as “leopard spots” (15) after which one of the specific phenotypes (“leopard”) was named. Today, leopard is a popular phenotype in several horse breeds, including Knabstrupper, Appaloosa, and Noriker. Leopard complex spotting is caused by an incompletely dominant locus (*LP*) located on horse chromosome 1 (15, 16). Modifier genes are thought to be responsible for the variation in the amount of white patterning observed (15, 17). In the Appaloosa and Miniature horse breeds, homozygosity for *LP* has been associated with congenital stationary night blindness (CSNB). Horses with this disorder have problems with seeing at low-light conditions and the retinal rod pathway of vision is disrupted as shown by the diagnostic negative electroretinograph (ERG) (18, 19). Therefore, CSNB should have been under negative selection in wild horses. Recently, a single SNP in the *TRPM1* gene (ECA1:108,249,293C > T) was found to be linked to both *LP* and CSNB in Appaloosa horses (20).

So far, ancient DNA studies have produced evidence for bay and black horses only, whereas no evidence for white spotted phenotypes in predomestic horses has been found (21). Here we test the possibility that the leopard complex spotting phenotype was already present in horses and accurately depicted by their human contemporaries, nearly 25,000 y ago. To investigate whether *LP* spotting was present in ancient horses, we genotyped the associated SNP in predomestic horses from Siberia, Western and Eastern Europe, and the Iberian Peninsula.

## Results

The samples investigated for the SNP associated with leopard complex spotting have previously (21) been genotyped for eight coat-color SNPs in six genes (*MC1R*, *ASIP*, *SILV*, *MATP*, *EDNRB*, and *KIT*) including basic coat colors (bay, black, and chestnut), dilution phenotypes (cream and silver), and white spottings (tobiano, sabino, and overo) (Table 1). All samples that had previously been successfully typed could also be typed for the *TRPM1* SNP. We were able to type 31 predomestic samples for the *TRPM1* SNP and identified the *LP*-associated allele in 6 samples, all of which were heterozygous and thus not affected by CSNB. The tobiano and sabino alleles were previously identified in 8 and 3 ancient domestic samples, respectively (21), but never in predomestic or wild horses. Thus, leopard spotting is the only spotted phenotype found in predomestic horses to date.

Four of 10 of the Western European horses from the Pleistocene had a genotype indicative of the leopard complex phenotype (Fig. 2), suggesting that this phenotype was not rare in Western Europe during the Pleistocene. For our sample size and

assuming a large panmictic population, the 95% confidence interval (C.I.) for the actual allele frequency in the ancient horse population is 0.082–0.418. Therefore, the true allele frequency was most likely above 0.1. In contrast, we did not detect *LP* in five predomestic Asian samples from the Pleistocene. In postglacial times (i.e., after 11,700 y ago), our sample set of predomestic horses is geographically patchy because some periods are characterized by an absence of horse remains for some regions. The leopard complex spotted allele was not detected in the six Iberian remains dating to the Mesolithic, but 2 of 10 of the predomesticated, postglacial horses from Eastern Europe carried the allele associated with leopard complex spotting (Table 1).

## Discussion

The results of this study bear directly on debates concerning the nature of Paleolithic representations of animals, specifically whether these depictions constitute literal representations of phenotypic variation in the contemporary animal populations or not. We found evidence for a long-term existence of the leopard complex spotted phenotype in the European horse population. So far, *LP* is the only spotted phenotype that has been found in both predomestic and domestic horses. In addition to the *LP* horses and in striking agreement with Paleolithic cave paintings, only bay (e.g., Lascaux, France) and black (e.g., Chauvet, France) genotypes were discovered in predomestic wild horse remains (21), whereby bay seems to be the most common color phenotype in predomestic times (18 of 31 typed samples so far) and is also the most commonly painted phenotype. So far, no evidence has been found for horses with chestnut, white, diluted, or other spotted phenotypes in predomestic times (this study and ref. 21). Only a single chestnut allele of *MC1R* was discovered in a sample from Pietrele, Romania (6,300 yBP) (Table 1). It is likely that dun dilution was present in predomestic horses as it is for example in modern Przewalski horses. However, because the dun mutation has not yet been identified, we cannot distinguish between dun and nondun horses at the moment.

Our previous ancient DNA study of coat coloration in predomestic horses produced evidence that the only phenotypes present in ancestral, predomestic horse populations were bay and black (21). Today, bay–dun is still found in the Przewalski horse (*Equus ferus przewalskii*), which is listed as the last remaining wild horse by the International Union for Conservation of Nature (IUCN) and often discussed as a close relative of domestic horses (22), although its taxonomic status is controversial and there is genetic evidence for admixture between Przewalski and domesticated horses (e.g., ref. 23). Recently, studies of both maternal (mtDNA) (24–25) and paternal lineages (Y chromosomal DNA) (26) found that the Przewalski horse displays DNA haplotypes not present in modern or ancient domestic horses, suggesting that Przewalski horses are not directly ancestral to modern domestic horses. However, independently of its taxonomic status, several lines of evidence suggest that the bay phenotype of the Przewalski horse represents an ancestral character. Firstly, several wild ass species, which undoubtedly represent wild equids, also show a bay–dun phenotype; and secondly, horses of this phenotype are depicted in remarkable detail in Paleolithic cave paintings (e.g., in Chauvet). Whereas black or black–dun and leopard spotted phenotypes also occurred at measurable frequencies in Pleistocene and Copper Age wild horses, as shown by both contemporary depictions and our genotyping results, their absence in modern Przewalski’s horses is probably explained by the severe population bottleneck that they have undergone (27), possibly in combination with the Asian origin of these horses, where *LP* seems to have been rarer, if not entirely absent.

Most modern populations of wild animals display uniform coloration, whereas domesticated species show a remarkable variation in coat color (28). Most scientists believe that changes

**Table 1. Genotyping results for nine coat-color loci in the 31 ancient DNA samples**

| Age                                    | Region  | Sample  | Basic color | Spotting        | Dilution | ASIP    | EDNRB   | KIT13   | KIT16   | MATP | MC1R | SILV9 | SILV11 | TRPM1 |
|--|---------|---------|-------------|-----------------|----------|---------|---------|---------|---------|------|------|-------|--------|-------|
| Pleistocene                            | Siberia | SP1181A | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | SP1181B | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | SP1181C | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | SP1181E | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | SP1181F | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  | Europe  | PET6    | Bay         | Leopard complex | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | PET5    | Bay         | Leopard complex | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | PET3    | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | PET2    | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | PET1    | Bay         | Leopard complex | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | KG5     | Bay         | Leopard complex | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | KG4     | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | KG3     | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | KG2     | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | KG1     | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
| Mesolithic-Neolithic Iberian Peninsula | 44      | Bay     | No          | No              | A/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
|  | 3       | Black   | No          | No              | a/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
|  | 31      | Bay     | No          | No              | A/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
|  | 32      | Black   | No          | No              | a/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
|  | 34      | Black   | No          | No              | a/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
|  | 37      | Bay     | No          | No              | A/a      | ov/ov   | KM0/KM0 | sb1/sb1 | C/C     | E/E  | z/z  | z/z   | lp/lp  |       |
| Copper Age                             | Europe  | Spa1    | Bay         | No              | No       | A/A     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | PIE9    | Black       | No              | No       | a/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/e  | z/z   | z/z    | lp/lp |
|  |         | VIT4    | Black       | No              | No       | a/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | MAY3    | Bay         | No              | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | MAY5    | Bay         | No              | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | MAY6    | Bay         | No              | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
|  |         | MAY7    | Bay         | Leopard complex | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | MAY10   | Bay         | Leopard complex | No       | A/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | LP/lp |
|  |         | CAS1    | Black       | No              | No       | a/a     | ov/ov   | KM0/KM0 | sb1/sb1 | C/C  | E/E  | z/z   | z/z    | lp/lp |
| MOL5                                   | Black   | No      | No          | a/a             | ov/ov    | KM0/KM0 | sb1/sb1 | C/C     | E/E     | z/z  | z/z  | lp/lp |        |       |

The described phenotype is proposed on the basis of the genotypes at each of the nine loci tested, including basic coat-color (bay, black, or chestnut) dilution (cream or silver), and spotting pattern (leopard complex, tobiano, sabino, or overo). Gray shading indicates that at least one allele differs from the proposed wild type of the Przewalski horse.

in coat color and specifically an increase in coat-color variability are a direct consequence of the domestication (28, 29). Previous work by us supports this notion by demonstrating a comparative lack of coat-color variation in predomestic horses and an explosion of color patterns during and following the Iron Age (21). Although our results presented here may, at first glance, seem to contradict this pattern, the general picture of increased phenotypic variability in early domestic horses compared with their wild ancestors holds up, also in light of our recent results. Including the results in the current study, we have so far found 3 coat-color phenotypes in predomestic horses and 11 in early domestic horses. Predomestic horses inhabited, in vast numbers, large areas of Eurasia, and some extant species that still occupy a similarly large area, such as gray wolves, are also found in different color morphs. It is therefore not entirely surprising that not all wild horses shared the bay–dun or black–dun phenotypes. Moreover, previous studies suggested that morphological—and genetic—variability was much larger in Pleistocene animal pop-

ulations compared with their modern counterparts (30–32), and it is likely that this increased variability extended to color phenotypes as well. However, the overall picture still supports the notion that artificial selection was the driving force behind the rapid increase of coat-color variation in domestic animals and resulting in their remarkable modern variability.

Recently it was discovered that homozygosity for the leopard spotted SNP typed in this study is associated with congenital stationary night blindness in leopard spotted horses (18–20), which should have caused strong purifying selection against homozygote *LP* individuals in predomestic times. Nevertheless, we found several occurrences of the *LP* allele in Pleistocene and Copper Age samples. Although we can only speculate about potential processes that resulted in the Pleistocene frequency of the leopard phenotype, such as selective advantage due to camouflage in the snowy Pleistocene environment, sexual selection, or simply genetic drift, the reason why it did not disappear due to the CSNB after it had been established seems to be less





**Fig. 2.** Horse phenotypes found in Paleolithic artwork from caves in Lascaux (bay) (photo from N. Aujoulat from the Ministère de la Culture et de la Communication, France. The animal corresponds to the second horse from the “Panel of the Chinese horses.”); Chauvet (black) [The picture is showing a panel of horses (detail L., about 1.10 m). The photo (slide no. 12) is used with permission from the French Ministry of Culture and Communication, Regional Direction for Cultural Affairs, Rhône-Alpes region, Regional Department of Archeology], and Pech-Merle (“leopard” spotted) (photo from P. Cabrol ©, Centre de Préhistoire du Pech Merle. The picture shows the panel of the dappled horses—“Le panneau des Chevaux ponctués”, Cabrerets, Lot France), all France, and their genetic counterparts in modern horses. (Left to Right) Bay–dun Przewalski’s horse (genotype:  $A^a/A^a E^j/E^j C^c/C^c CH^{ch}/CH^{ch} D^p/D^p LP^p/ILP^p Z^z/Z^z$ ); black–dun Konik with winter coat (genotype:  $A^a/A^a E^j/E^j C^c/C^c CH^{ch}/CH^{ch} D^d/D^d LP^p/ILP^p Z^z/Z^z$ ); black–dun Konik with summer coat (same genotype); and leopard complex spotted Knabstrupper (genotype:  $A^a/A^a E^j/E^j C^c/C^c CH^{ch}/CH^{ch} D^d/D^d LP^p/ILP^p Z^z/Z^z$ ).

obscure. Considering that deleterious alleles may stay for a long time in a population at low frequency despite purifying selection, the fact that the frequency of the *LP* allele was comparatively low in the Holocene and the Copper Age samples could indicate that its low frequency protected the allele from being purged from the population.

Our results suggest that, at least for wild horses, Paleolithic cave paintings, including the remarkable depictions of spotted horses, were closely rooted in the real-life appearance of the animals. Therefore, any interpretation of those depictions from a symbolic or transcendental standpoint will necessarily need to draw upon data other than the coat pattern itself to back up its argument. This point has been made previously (33), as it has been shown that spot motifs on reindeer (*Rangifer tarandus*) depictions from Upper Paleolithic art from France are a naturalistic representation of a specific coat pattern found only in females, and thus, perhaps, a deliberate indicator of the sex of individual animals. Here, we are able to go one step further by confirming the prehistoric occurrence of the genotype that underlies a distinctive phenotype in Paleolithic cave art. Our results suggest that, at least in some cases, prehistoric paintings were closely rooted in the real-life appearance of the animals depicted and that any symbolic or transcendental connotation, if present at all, was not necessarily signaled by the color or pattern of these depictions.

## Materials and Methods

**Samples.** We genotyped successfully 31 (of 69) horse (*Equus caballus*) bone and teeth specimens from 14 different localities from Siberia, Eastern and Western Europe, and the Iberian Peninsula (Table S1). The specimens cover a period from the Late Pleistocene to the Copper Age and are all dated either by the archaeological context or with  $^{14}C$  dates (Table S2). All samples were previously genotyped for eight coat-color loci in six genes (Table 1) (21).

**Ancient DNA Extraction and Amplification.** Approximately 250 mg of bone material was used for each extraction. External surfaces of bones were removed by abrasion to minimize environmental contaminations. Each sample was ground to powder with a freezer mill and incubated in 0.45 M EDTA (pH 8.0) and 0.25 mg/ml proteinase K overnight at room temperature under rotation. After centrifugation, DNA was purified from the supernatant using a silica-based method as previously described (34, 35). Leopard complex spotting primers were designed on the basis of the associated SNP previously reported (20) and added to our primer set detecting coat-color SNPs. Amplifications were performed in two steps using multiplex PCR combined

with a singleplex PCR as previously described (21). PCR products varied in length between 52 and 78 bp (including primers) (Table S3). Four microliters of extract was used for each multiplex PCR. Negative extraction controls and negative PCR controls were used in each PCR. Amplification products were visualized on agarose gels.

**Authentication.** DNA sampling, extractions, and pre-PCR preparations were carried out in a laboratory dedicated to ancient DNA analyses following the standard procedures to avoid contamination. The multiplex and singleplex PCRs were set up in the laboratory dedicated to ancient DNA analyses, but the dilution of PCR products following the multiplex step and their addition to the singleplex reactions were done in a dedicated room in an annexed building, separate from the post-PCR laboratory, where all post-PCR analyses were carried out. All results were replicated at least four times. Two different primer pairs were used to detect the point mutation in the *TRPM1* gene associated with the leopard spotting phenotype (Table S4). Both primer pairs are designed for the pyrosequencing technology. Two negative PCR controls and a blank extraction were performed for every sample. Due to the small size of the amplified fragments, distinction between primer dimers and positive products is sometimes difficult. Therefore, all negative controls were systematically sequenced when a product was detected on the agarose gel. All of these products found in negative controls turned out to be primer dimers. Finally, each sample was confirmed at least once in a second laboratory also dedicated to ancient DNA analyses (Table S1).

**Pyrosequencing.** Biotinylated PCR products were prepared on the PyroMark Vacuum Prep Workstation according to the manufacturer’s instructions. Amplicons for each SNP were sequenced using pyrosequencing technology on a PSQTM 96MA (Biotage). The SNPs were identified using the PSQTM 96MA system and automatically edited by the PSQTM 96MA SNP software. The results for the color determination, including the previous determination of other color phenotypes (21), are summarized in Table 1 and Table S4.

**Allelic Dropout.** The probability ( $P$ ) of a false heterozygote individual is calculated after  $n$  replicates:  $P = K \times (K/2)^n - 1$ , where  $K$  is the observed number of allelic dropouts divided by all heterozygous individuals. For all genes we did a minimum of four replications that reduced the risk of nondetection of a heterozygote individual to an average of 0.3%.

**Estimating the Allele Frequency of Missed Alleles.** We computed the upper bound of the *LP* allele frequency having been present but not observed in our samples assuming a binomial sampling distribution (Table S5; for details, see ref. 21).

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