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Supporting Online Material for

Coat Color Variation at the Beginning of Horse Domestication

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Materials and Methods

Samples

Samples were obtained from Siberia, Middle and Eastern Europe, China and the Iberian Peninsula from different time points and cultural horizons (Table S1).

Ancient DNA Extraction and Amplification

DNA was extracted from 152 samples using between 250 mg and 400 mg bone material. 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 for 5 min at 4,000 rpm in a Universal 320 centrifuge (Hettich), DNA was purified from the supernatant using a silica based method as previously described (*S1*).

SNP amplifications were performed using multiplex PCR (*S2, S3*). PCR products for SNPs varied in length between 52 bp and 78 bp (including primers; see Table S3). Four microliters of extract were used for each multiplex PCR. The initial multiplex PCR was performed in a 20 µl reaction volume containing 1x AmpliTaq Gold PCR buffer II (ABI), 4 mM $MgCl_2$, 1 mg/ml Bovine Serum Albumin (BSA), 250 µM of dATP, dCTP and dGTP, 500µM of dUTP, 150 nM of each primer and 2 U of AmpliTaq Gold (ABI). For each sample, one PCR was performed by adding 1 U of heat-labile Uracil-DNA Glycosylase (USB) and an initial incubation step of 15 min at 37 °C to control for carry-over contamination. PCR products were diluted 1/30 and 5 µl (total reaction volume 20 µl) were used for the next step. Singleplex PCRs contained 1x AmpliTaq Gold PCR Buffer II, 4 mM $MgCl_2$, 1 mg/ml bovine serum albumin (BSA), 250 µM of dATP, dCTP and dGTP, 500µM of dUTP, 1.5 µM of each primer and 0.5 U of AmpliTaq Gold DNA polymerase. Both times, PCR was run under the following conditions: denaturation and Taq activation at 94 °C for 9 min; 30 up to 35 cycles at 95 °C 20 sec; annealing temperature depending on the primer pair (see Table S3) 30 sec; 72 °C 30 sec and final extension 4 min 72 °C. Negative extraction controls and negative PCR controls were used in each PCR. Amplification products were visualized on agarose gels.

Mutation analysis

Modern horses show a high variability in coat coloration. However, no color is confined to a single breed and the same mutation is responsible for a certain color variant across breeds, supporting the idea that mutations producing the color variants occurred prior to breed formation during the domestication process. Unfortunately, it is currently not possible to describe the coat color phenotype of ancient horses completely as until today not all genes associated with coat coloration were identified. Thus for some color phenotypes of interest (e.g. dun), there is as yet no genetic information available. We used a set of eight SNPs in six genes (Table S2) for detecting basic coat colors (bay, black and chestnut), two kinds of dilution (silver and cream) and three spotted or painted colorations (overo, tobiano and sabino). SNPs were chosen according to previous studies on modern horse populations (*S4-S9*); primers are listed in Table S3. Biotinylated PCR products were prepared at the PyroMark Vacuum Prep Workstation according to the manufacturer's instructions. Amplicons for each SNP were sequenced using pyrosequencing TM technology on a PSQTM 96MA (Biotage). The SNPs were identified using PSQTM 96MA and automatically edited by the PSQTM 96MA SNP software. Due to the large deletion (11bp) of the mutated *non–black* allele (*A*) of *ASIP*, the risk to lose the mutated allele is relatively high for ancient DNA. For this reason, the number of replications (n=6) was increased in order to reduce the risk of false homozygote individuals below 1‰. Additionally, allelic separation of *ASIP* was verified on page gels. The results for the color determination are summarized in Tables S4 and S5.

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 (*S10*). For all genes excepting *ASIP* we did a minimum of four replications which reduced the risk of non-detection of a heterozygote individual to an average of 0.3 % (P = 0.0078 for *KIT13*, 0.0015 for *KIT16* and *MATP*, and 0.00012 for *MC1R*).

Estimating the allele frequency of missed alleles

We computed the upper bound of the allele frequency of a coat color allele having been present in the pre-domestication population but not observed in our samples assuming a binomial sampling. Given *n* samples, we computed the maximum frequency $f_{5\%}$ of a color allele in the population so that the probability of sampling none of them is above 5% (Table S7).

Estimation of selection coefficient

We estimated the selection coefficient for each locus using Bollback et al.'s method (*S11*). This method has been developed to jointly estimate the selection coefficient and the effective population size from time-series data of allele frequencies. We binned the Siberian East/Central European and Chinese samples into five time periods with means about 13,100, 3,700, 2,800, 600 and 200 BC respectively. We assumed a generation time of five years.. We used default values for all parameters of the program except for the grid size (Ngrid) of the allelic frequencies that was set to 2000. The likelihood was computed for selection coefficient ranging from -0.5 to 0.5 and effective population size ranging from 10,000 to 100,000. All the loci considered are assumed to be independent, in particular we considered only *SILV9* because *SILV11* seems to be in complete linkage disequilibrium with *SILV9*. See Figure S3 for the change in allelic frequency for each locus and Table S8 for the estimates of the selection parameter.

Note that, for our dataset, the maximum likelihood values are sensitive to the grid size but that the confidence intervals seem to be robust to this parameter. Therefore we used a bigger grid than the one suggested in (*S11*).

Like the above estimate of maximum allele frequencies of missed alleles, these analyses are limited by the available sample size. This is certainly also true for our sampling of color variations for early domesticated horses. Thus, it is possible that we missed extremely rare variants at a certain point in time. However, detection of additional color variants at the beginning of domestication would only strengthen our conclusions but not change the overall picture.

Supplementary figures

Figure S1: Map of Eurasia showing the archaeological sites (black dots) from which the horse samples that were successfully analyzed for SNPs originate. The diagrams show the proportion of the different coat color phenotypes observed. Numbers show the total number of samples showing the indicated phenotype. From top to bottom: samples from the Pleistocene; the Neolithic/Copper Age; the Bronze Age; the Iron Age.

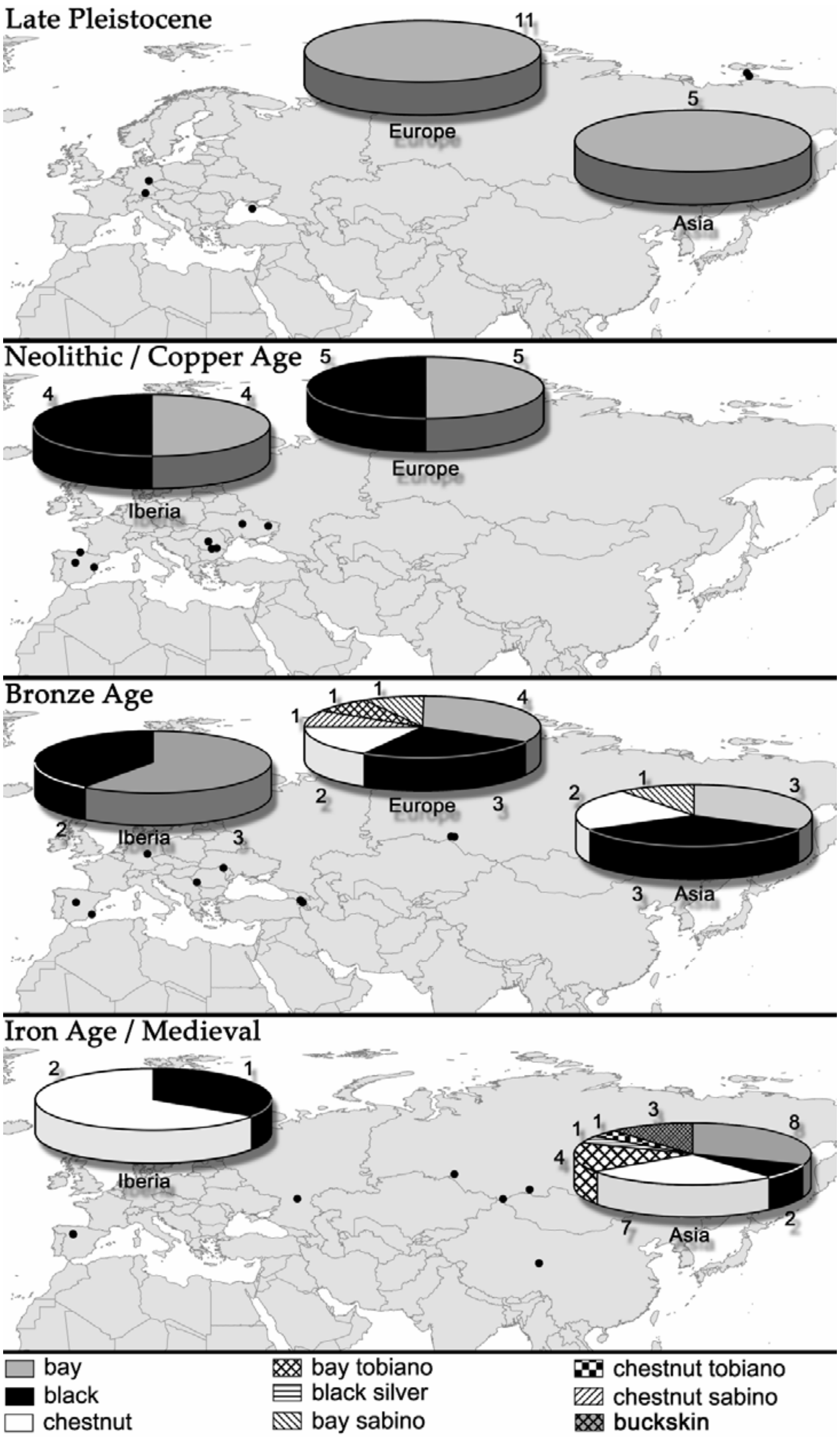

Late Pleistocene
11
Europe
5
Asia
Neolithic / Copper Age
4
4
Iberia
5
5
Europe
Bronze Age
2
Iberia
3
1
1
1
4
2
Europe
3
1
2
3
3
Asia
Iron Age / Medieval
2
1
Iberia
1
1
3
8
4
7
Asia
2
bay
black
chestnut
bay tobiano
black silver
bay sabino
chestnut tobiano
chestnut sabino
buckskin

Fig. S2: Timeline for the first occurrence of the observed coat color phenotypes in Siberia, Europe and the Iberian Peninsula, respectively. Above the timescale, the timing of the first depictions of various horse-related implements is shown. Note that the timeline is interrupted between 12,000 and 5,000 BC.

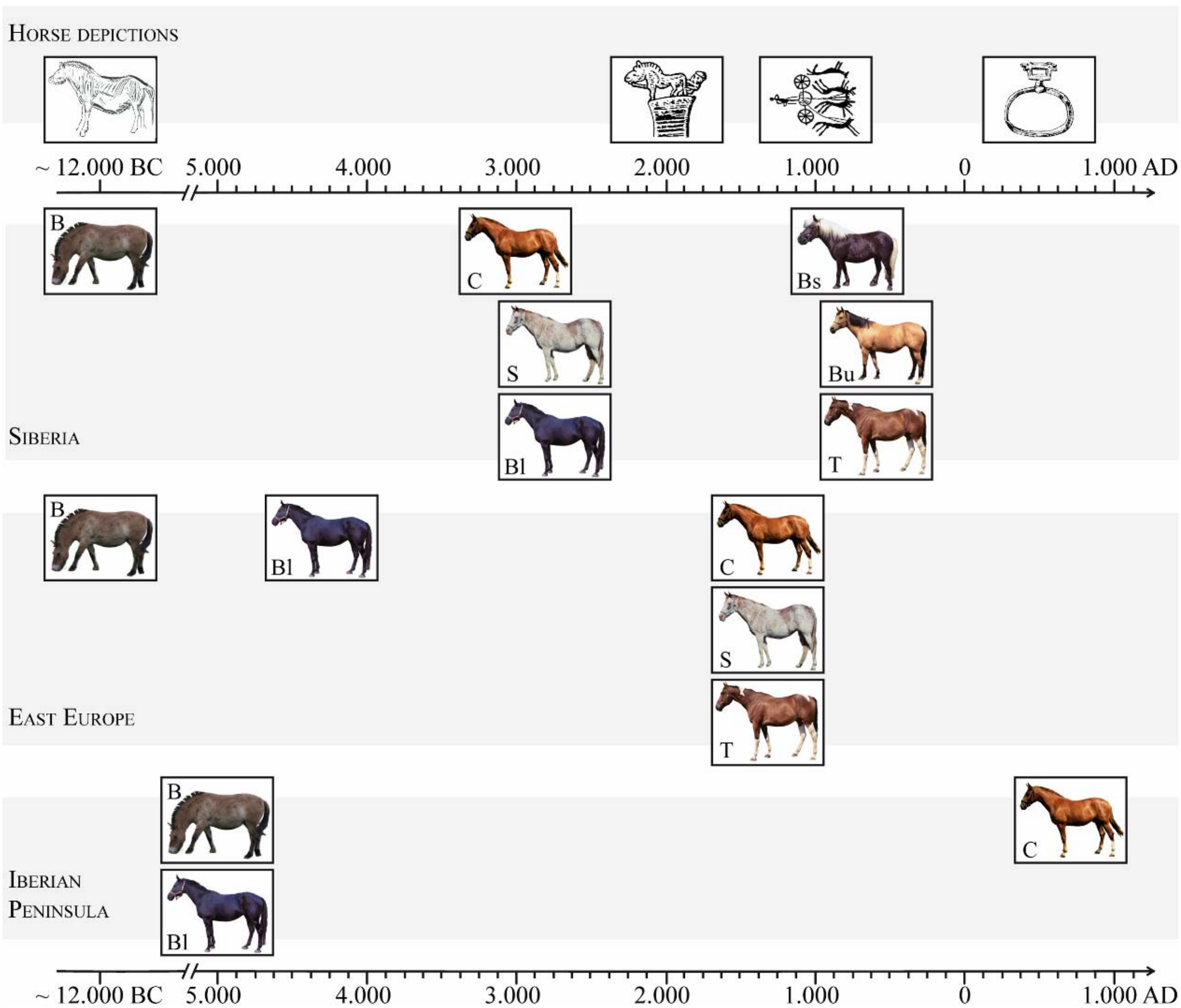
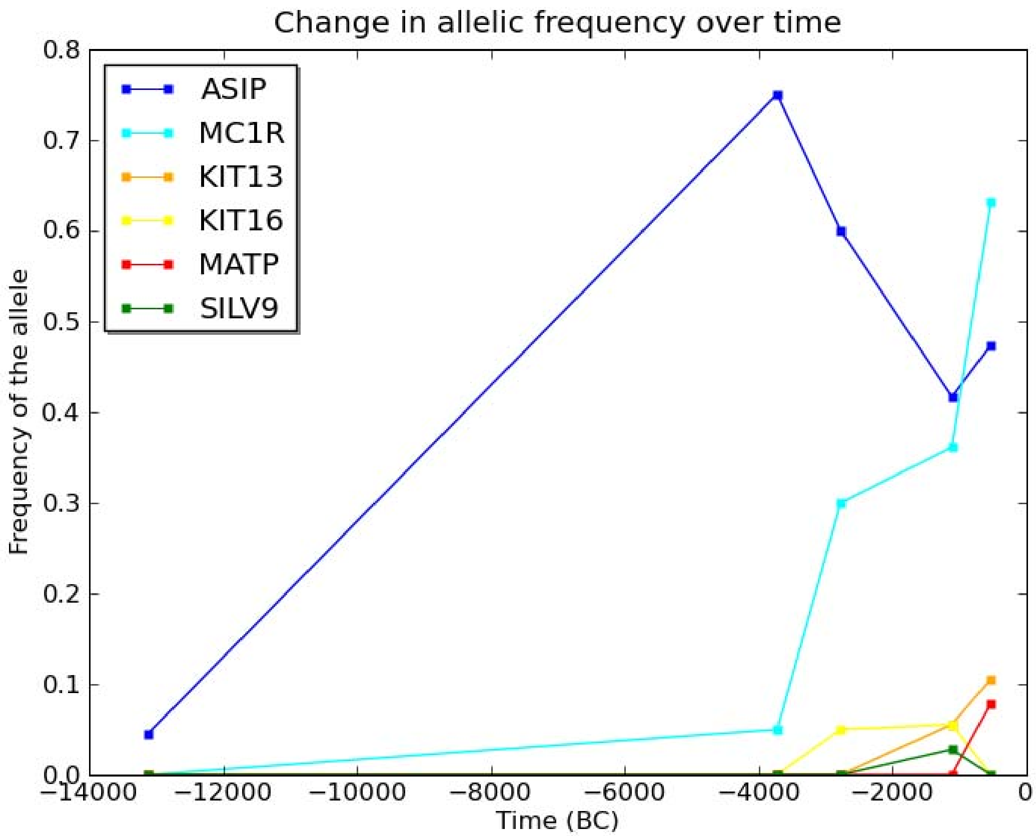


Figure S3: Change in allelic frequency for each locus. The number of chromosomes for each time point is 22, 20, 20, 36, 38 from left to right (see Table S5). The earliest Pleistocene samples were excluded. Note that for two genes (*ASIP* and *MC1R*), the allele frequencies change drastically.



Supplementary tables

Table S1: Samples analyzed for this study. The samples highlighted in grey are the samples that gave a complete and reproducible genotype for the coat coloration (Typing). The extraction and amplification of the samples (Ext/Amp) and the reproduction (Rep) were performed in two different institutes by Melanie Pruvost (MP) and Michael Cieslack (MC) at the Humboldt University in Berlin and Sebastian Lippold (SL) and Melanie Pruvost (MP) at the Max Planck Institute in Leipzig. The Pleistocene samples are not directly dated, but estimated from context should be around 20,000 years old. The remaining dates are either calibrated carbon dates or derived from archaeological context.

Siberia

	Sample	Ext/Amp	Rep.	Det.	Excavation	Geographical location	Date	Typing
PLEISTOCENE	SP1181A	MP/MP		W(ild)	Maliy Lyakhovsky Isl.	North Siberia	Pleistocene	Yes
	SP1181B	MP/MP		W	Bol'shoy Lyakhovsky Isl.	North Siberia	Pleistocene	Yes
	SP1181C	MP/MP		W	Bol'shoy Lyakhovsky Isl.	North Siberia	Pleistocene	Yes
	SP1181D	MP/MP		W	Bol'shoy Lyakhovsky Isl.	North Siberia	Pleistocene	No
	SP1181E	MP/MP		W	Oyagosskiy Yar, Kondrat'evo R., mouth	Siberia	Pleistocene	Yes
	SP1181F	MP/MP		W	Kotel'niy Isl., Anisiy Cape	Siberia	Pleistocene	Yes
EARLY BRONZE AGE	BER 001	MP/MP		D(om)	Denisova-Pescera	Siberia (Altai)	3000BC	Yes
	TAR 001	MP/MP	SL	D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 002	MP/MP	SL	D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 004	MP/MP		D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 005	MP/MP	SL	D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 006	MP/MP		D	Tartas1	West Siberia	2500-3000BC	No
	TAR 007	MP/MP	SL	D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 008	MP/MP		D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 009	MP/MP		D	Tartas1	West Siberia	2500-3000BC	No
	TAR 010	MP/MP		D	Tartas1	West Siberia	2500-3000BC	Yes
	TAR 011	MP/MP		D	Tartas1	West Siberia	2500-3000BC	Yes
	BAR 002	MP/MP		D	Preobrazhenka--/2005	Siberia	2000-2500BC	No
IRON AGE	BER 002	MP/MP	SL	D	Om-1	Siberia (Altai)	900BC	Yes
	Arz 1-2	CW/MP		D	Arzan1	South Siberia (Tuva)	800BC	Yes
	Arz 1-3	CW/MP		D	Arzan1	South Siberia (Tuva)	800BC	Yes
	Arz 2-1	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-2	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-3	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-4	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-5	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-6	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-7	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-8	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
	Arz 2-9	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes

Arz 2-10	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
Arz 2-11	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
Arz 2-12	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
Arz 2-13	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
Arz 2-14	CW/MP	MP	D	Arzan2	South Siberia (Tuva)	619-608 BC	Yes
Bars1A	CW/MP		D	Barsucij Log	South Siberia (Tuva)	370-150 BC	Yes
Bars1B	CW/MP		D	Barsucij Log	South Siberia (Tuva)	400-200 BC	Yes
OKG 001	MP/MP		D	Olon-Kurin-Gol 10	Siberia (Mongolia)	400-300 BC	Yes
OKG 002	MP/MP		D	Olon-Kurin-Gol 10	Siberia (Mongolia)	400-300 BC	Yes
OKG 003	MP/MP	SL	D	Olon-Kurin-Gol 10	Siberia (Mongolia)	400-300 BC	Yes

East European steppe

	Sample	Ext/Amp	Rep.	Det.	Excavation	Geographical location	Date	Typing
LATE GLACIATION-MESOLITHIC	PET1	SL/MP		W	Petersfels	South Germany	14000-11000 BC	Yes
	PET2	SL/MP		W	Petersfels	South Germany	14000-11000 BC	Yes
	PET3	SL/MP		W	Petersfels	South Germany	14000-11000 BC	Yes
	PET5	SL/MP	MP	W	Petersfels	South Germany	14000-11000 BC	Yes
	PET6	SL/MP		W	Petersfels	South Germany	14000-11000 BC	Yes
	Kg1	SL/MP		W	Kniegrotte	Germany (Thuringia)	15000-14000 BC	Yes
	Kg2	SL/MP	MP	W	Kniegrotte	Germany (Thuringia)	15000-14000 BC	Yes
	Kg3	SL/MP		W	Kniegrotte	Germany (Thuringia)	15000-14000 BC	Yes
	Kg4	SL/MP		W	Kniegrotte	Germany (Thuringia)	15000-14000 BC	Yes
	Kg5	SL/MP		W	Kniegrotte	Germany (Thuringia)	15000-14000 BC	Yes
	Spa 1	CW/MP	SL	W	Span-Koba	Ukraine (Peninsula Crimea)	9390-9210 BC	Yes
ENEOLITHIC	TRE1	MP/MP		W	Trestiana	Romania	5700-5600 BC	No
	TRE2	MP/MP		W	Trestiana	Romania	5700-5600 BC	No
	TRE3	MP/MP		W	Trestiana	Romania	5700-5600 BC	No
	BUP1	MP/MP		W	Bucsani Pod	Romania	5500-5000 BC	No
	ISA1	MP/MP		W	Isaia	Romania	5500 BC	No
	ISA2	MP/MP		W	Isaia	Romania	5500 BC	No
	HAR1	MP/MP		W	Harsova	Romania	5000-5500 BC	No
	HAR2	MP/MP		W	Harsova	Romania	4500-2000 BC	No
	HAR3	MP/MP		W	Harsova	Romania	4500-2000 BC	No
	Pie7	MP/MP		W	Pietrele	Romania	4300 BC	No
	Pie9	MP/MP		W	Pietrele	Romania	4300 BC	Yes
	Pie11	MP/MP		W	Pietrele	Romania	4300 BC	No
	VIT1	MP/MP		W	Vitanesti	Romania	4300-4220 BC	No
	VIT2	MP/MP		W	Vitanesti	Romania	4350-4220 BC	Yes
	VIT3	MP/MP		W	Vitanesti	Romania	4300-4220 BC	No
	VIT4	MP/MP		W	Vitanesti	Romania	4360-4220 BC	Yes
	ORL1	MP/MP		W	Orlovka	Moldova	4000 BC	No

	ORL2	MP/MP		W	Orlovka	Moldova	4000 BC	No
	ORL3	MP/MP		W	Orlovka	Moldova	4000 BC	No
	ORL4	MP/MP		W	Orlovka	Moldova	4000 BC	No
COPPER AGE	CAS1	MP/MP		W	Cascioarele	Romania	3700-3380 BC	Yes
	MAY1	MP/MP		W	Mayaki	Ukraine	3600-3100 BC	No
	MAY2	MP/MP		W	Mayaki	Ukraine	3600-3100 BC	No
	MAY3	MP/MP		W	Mayaki	Ukraine	3640-3490 BC	Yes
	MAY4	MP/MP		W	Mayaki	Ukraine	3600-3100 BC	No
	MAY5	MP/MP		W	Mayaki	Ukraine	3250-3100 BC	Yes
	MAY6	MP/MP		W	Mayaki	Ukraine	3520-3330 BC	Yes
	MAY7	MP/MP		W	Mayaki	Ukraine	3520-3380 BC	Yes
	MAY8	MP/MP		W	Mayaki	Ukraine	3600-3100 BC	No
	MAY9	MP/MP		W	Mayaki	Ukraine	3600-3100 BC	No
	MAY10	MP/MP		W	Mayaki	Ukraine	3650-3500 BC	Yes
	MOL5	MP/MP		W?	Molyukhov Bugor	Ukraine	3720-3630 BC	Yes
	MOL7	MP/MP		W?	Molyukhov Bugor	Ukraine	3720-3630 BC	No
	MOL8	MP/MP		W?	Molyukhov Bugor	Ukraine	3720-3630 BC	No
BRONZE AGE	GRO8	MP/MP		D	Großobringen	Germany	3000-2500 BC	No
	GRO9	MP/MP		D	Großobringen	Germany	3000-2500 BC	No
	GRO10	MP/MP		D	Großobringen	Germany	3000-2500 BC	No
	GRO11	MP/MP		D	Großobringen	Germany	3000-2500 BC	Yes
	Gar1	MP/MP		D	Garbovat	Romania	1500-1000 BC	No
	Gar2	MP/MP		D	Garbovat	Romania	1500-1000 BC	Yes
	Gar3	MP/MP		D	Garbovat	Romania	1500-1000 BC	Yes
	Gar4	MP/MP		D	Garbovat	Romania	1500-1000 BC	Yes
	Bar1	MP/MP		D	Garbovat	Romania	1500-1000 BC	No
	Bar3	MP/MP		D	Garbovat	Romania	1500-1000 BC	No
	Lch 1	CW/MP		D	Lchashen	Armenia	1410-1250 BC	Yes
	Lor 1	CW/MP		D	Lori-Berd	North Armenia	1950-1750 BC	Yes
	Mic1	MP/MP		D	Miciurin	Moldova	1500-1000 BC	Yes
	Mic2	MP/MP		D	Miciurin	Moldova	1500-1000 BC	Yes
	Mic3	MP/MP		D	Miciurin	Moldova	1500-1000 BC	Yes
	Mic4	MP/MP		D	Miciurin	Moldova	1500-1000 BC	Yes
	Mic5	MP/MP		D	Miciurin	Moldova	1500-1000 BC	Yes
	Shi 1	CW/MP	SL	D	Shirakavan	Armenia	895-795 BC	Yes

China

	Sample	Ext/Amp	Rep.	Det.	Excavation	Geographical location	Date	Typing
IRON AGE	Fen 1	CW/MP		D	Fengtai	China (Qinghai)	905-800 BC	Yes
	Fen 2	CW/MP		D	Fengtai	China (Qinghai)	1000-800 BC	Yes

Fen 3	CW/MP		D	Fengtai	China (Qinghai)	1000-800 BC	Yes
Fen 4	CW/MP		D	Fengtai	China (Qinghai)	1000-800 BC	Yes

Spain

Period	Sample	Extraction	Rep.	Det.	Excavation	Geographical location	Date	Typing
MESOLITHIC-NEOLITHIC	41	MC/MC	SL	W	Atxoste	Iberian Peninsula (Spain)	5500-4950 BC	No
	42	MC/MC	SL	W	Atxoste	Iberian Peninsula (Spain)	5500-4950 BC	No
	43	MC/MC	SL	W	Atxoste	Iberian Peninsula (Spain)	5500-4950 BC	No
	44	MC/MC	SL	W	Atxoste	Iberian Peninsula (Spain)	5500-4950 BC	Yes
	45	MC/MC	SL	W	Atxoste	Iberian Peninsula (Spain)	5500-4950 BC	Yes
	1	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5200 -4900BC	No
	2	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5200 -4900BC	No
	3	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5200-4900 BC	Yes
	31	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5210-4910 BC	Yes
	32	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5220-4980 BC	Yes
	33	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5220-4900 BC	No
	34	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5220-4900 BC	Yes
	35	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5380-5210BC	No
	36	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5070-4840BC	No
	37	MC/MC		W	Cueva Fosca -Valencia-Cartellon	Iberian Peninsula (Spain)	5210-4910 BC	Yes
	17	MC/MC	SL	W	Cueva De La Vaquera-Segovia	Iberian Peninsula (Spain)	5210-4940 BC	Yes
COPPER AGE	27	MC/MC		D	El Caprichio-Madrid	Iberian Peninsula (Spain)	4300-2200 BC	No
	28	MC/MC		D	Carmona-Sevillia/Andalusia	Iberian Peninsula (Spain)	4300-2200 BC	No
	19	MC/MC	SL	D	Las Pozas -Zamora	Iberian Peninsula (Spain)	4300 -2200 BC	No
	20	MC/MC	SL	D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	2880-2570 BC	No
	21	MC/MC	SL	D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	2900- 2500 BC	No
BRONZE AGE	22	MC/MC	SL	D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	1350 BC	Yes
	23	MC/MC		D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	1350 BC	No
	24	MC/MC		D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	1350 BC	Yes
	25	MC/MC		D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	1350 BC	Yes
	26	MC/MC		D	Cueva Rubia-Valmayor/Madrid	Iberian Peninsula (Spain)	1350 BC	No
	38	MC/MC		D	El Acequion	Iberian Peninsula (Spain)	2200-800 BC	No
	39	MC/MC		D	El Acequion	Iberian Peninsula (Spain)	2200-800 BC	Yes
	40	MC/MC		D	El Acequion	Iberian Peninsula (Spain)	2200-800 BC	Yes
	8	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	9	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	10	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	11	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	12	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	13	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No

	14	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	15	MC/MC	SL	D	Peñalosa-Jaén -Andalucia	Iberian Peninsula (Spain)	2200 - 800 BC	No
	18	MC/MC	SL	D	Morra Del Quintanar- Albacete	Iberian Peninsula (Spain)	2200 - 800 BC	No
IRON AGE	16	MC/MC	SL	D	La Mota -Medina Del Campo	Iberian Peninsula (Spain)	800 BC - 6 AD	No
	4	MC/MC	SL	W?	Soto de Medinilla -Valladolid	Iberian Peninsula (Spain)	800 BC - 6 AD	Yes
	5	MC/MC	SL	W?	Soto de Medinilla -Valladolid	Iberian Peninsula (Spain)	800 BC - 6 AD	No
	6	MC/MC	SL	W?	Soto de Medinilla -Valladolid	Iberian Peninsula (Spain)	800 BC - 6 AD	No
	7	MC/MC	SL	W?	Soto de Medinilla -Valladolid	Iberian Peninsula (Spain)	800 BC - 6 AD	No
MEDIEVAL	29	MC/MC		D	Mucientes-Valladolid	Iberian Peninsula (Spain)	660-780 AD	Yes
	30	MC/MC		D	Mucientes-Valladolid	Iberian Peninsula (Spain)	680-890 AD	Yes

Table S2. Genes associated with coat color variation typed in this study. Shown are gene name, GenBank accession number, position and type of mutation, the associated color of both the wildtype and the derived state and the reference in which the mutation was first described.

Gene	AccNo	Mutation	Associated color	Reference
MC1R	X98012	g.201C>T	C: non-chestnut (*E*) T: chestnut (*e*)	S8
ASIP	AF288358	g.2183_2193del	-: non-black (*A*) Deletion 11 bp: black (*a*)	S9
MATP (SLC45A2)	AY187093	g.72G>A	G: non-cream (*C*) A: cream (*cr*)	S7
KIT	AY048669	g.786G>C	G: tobiano pattern (*KM1*) C: non-tobiano pattern (*KM0*)	S5
	AY874542	g.1120T>A	T: non-sabino spotted (*sb1*) A: sabino spotted (*SB1*)	S4
SILV	DQ665301	g.1457C>T	C: non-silver T: silver	S12
		g.697A>T	A: non-silver, linked with g.1457C T: silver, coupled with g.1457T	
EDNRB	AF038900	c.323_333TC>AG	TC: non-overo pattern AG: overo pattern	S13

Table S3: Primers used for the coat color SNPs amplification. An annealing temperature of 57 °C was used for all of the primer pairs.

Gene	Name	Sequence 5' to 3'	bp	Modif.	Length
KIT	Pk-KIT-I13 F	CGT CAT GAC TCA TTC GTG AGA A	22		63 bp
	Pk-KIT-I13 R	GCT CTG AAG GTA ACA AGC AAC TAA	24	5'-Biotin	
	Pk-KIT-I13 S	TGA GAA ATT TCC GCC	15		
EDNRB	P-EDNRB-E1 F	CAG TAG TGT CCT GCC TAG TGT TCG	24	5'-Biotin	63bp
	P-EDNRB-E1 S	TCA GCA GTG TGG AGT TT	17		
	Pk-EDNRB-E1 R	TGA TTC TCA GCA GTG TGG AGT TT	23		
KIT	Pk-KIT-I16 F	TTT AAA TGG CTT TCT TTT CTC C	22	5'-Biotin	59 bp
	Pk-KIT-I16 R	TGC CAA GTC CCT ATG AAT ACA C	22		
	Pk-KIT-I16 S	CTA TGA ATA CAC TAT TAG GA	20		
MATP	P-MATP-E2F	GCC ATA ACC ATC ACC ATG ATA G	22	5'-Biotin	65bp
	Pk-MATP-E2 R	GGC CCA TCA ATG AAG TCA G	19		
	P-MATP-E2 S	GAA GTC AGC AGC AAA A	16		
MC1R	Pk-MC1R-E1 F	GCA CTC ACC CAT GTA CTA CTT CAT	24		71 bp
	Pk-MC1R-E1 R	GCA CGT TGC TCA TGC TCA C	19	5'-Biotin	
	P-MC1R-E1 S	CTG CTG CCT GGC CGT	15		
SILV	Pk-SILV-E11 F	TCC TTC TTC TTC TCC CAA ATC A	22	5'-Biotin	52 bp
	P-SILV-E11 R	GAG CTG AGC CCT GCT TCA TAA	21		
	P-SILV-E11 S	GCC CTG CTT CAT AAG TC	17		
SILV	Pk-SILV-I9 F	AGG ATG AAG GGG AGT GGG	18		62 bp
	Pk-SILV-I9 R	GGC ACA GCT TCA GTC AGT GTC T	22	5'-Biotin	

	Pk-SILV-I9 S	GGG GAG TGG GCA GAG	15		
ASIP	Pk-ASIP-E2 F	CAA GAA ATC CAA AAA GAT CAG C	22	5’-Biotin	67/78 bp
	Pk-ASIP-E2 R	ATG AGA AGT CCA AGG CCT ACC T	22		
	P-ASIP-E2 S	CCT ACC TTG GAA GAT CTC	18		

Table S4. Sample information for the horse fossils successfully typed for the coat color SNPs. The table contains information on geographic location, excavation, dating, and color phenotype of the samples. Grey shaded dates indicate samples directly dated by radiocarbon dating (see also Table S6). All radiocarbon ages are given in calibrated years (cal.). The Late Pleistocene samples were estimated at around 20,000 years based on context.

Siberia

	Sample	**Designation**	**Location**	**Excavation**	**Date**	**Phenotype**
PLEISTOCENE	SP1181A	W(ild)	North Siberia	Maliy Lyakhovsky Isl.	Late Pleistocene	**Bay**
	SP1181B	W	North Siberia	Bol'shoy Lyakhovsky Isl.	Late Pleistocene	**Bay**
	SP1181C	W	North Siberia	Bol'shoy Lyakhovsky Isl.	Late Pleistocene	**Bay**
	SP1181E	W	Siberia	Oyagosskiy Yar, Kondrat'evo River mouth	Late Pleistocene	**Bay**
	SP1181F	W	Siberia	Kotel'niy Isl., Anisiy Cape	Late Pleistocene	**Bay**
EARLY BRONZE AGE	BER 001	D(omestic)	Siberia (Altai)	Denisova-Pescera	3000BC	**Chestnut**
	TAR 001	D	West Siberia	Tartas1	2500-3000BC	**Bay**
	TAR 002	D	West Siberia	Tartas1	2500-3000BC	**Chestnut**
	TAR 004	D	West Siberia	Tartas1	2500-3000BC	**Bay**
	TAR 005	D	West Siberia	Tartas1	2500-3000BC	**Bay Sabino**
	TAR 007	D	West Siberia	Tartas1	2500-3000BC	**Black**
	TAR 008	D	West Siberia	Tartas1	2500-3000BC	**Bay**
	TAR 010	D	West Siberia	Tartas1	2500-3000BC	**Black**
	TAR 011	D	West Siberia	Tartas1	2500-3000BC	**Black**
IRON AGE	BER 002	D	Siberia (Altai)	Om-1	900BC	**Bay**
	Arz 1-2	D	South Siberia (Tuva)	Arzan1	800BC	**Chestnut**
	Arz 1-3	D	South Siberia (Tuva)	Arzan1	800BC	**Black Silver**
	Arz 2-1	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Bay**
	Arz 2-2	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Black**
	Arz 2-3	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Chestnut**
	Arz 2-4	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Bay**
	Arz 2-5	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Chestnut**
	Arz 2-6	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Bay**
	Arz 2-7	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Black**

	Arz 2-8	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Buckskin**
	Arz 2-9	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Buckskin**
	Arz 2-10	D	South Siberia (Tuva)	Arzan2	619-608 BC	Chestnut Tobiano
	Arz 2-11	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Chestnut**
	Arz 2-12	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Chestnut**
	Arz 2-13	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Chestnut**
	Arz 2-14	D	South Siberia (Tuva)	Arzan2	619-608 BC	**Bay**
	Bars1A	D	South Siberia (Tuva)	Barsucij Log	cal. 370-150 BC	**Bay Tobiano**
	Bars1B	D	South Siberia (Tuva)	Barsucij Log	cal. 400-200 BC	**Bay Tobiano**
	OKG 001	D	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC	**Chestnut**
	OKG 002	D	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC	**Bay Tobiano**
	OKG 003	D	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC	**Buckskin**
Eastern Europe						
GLACIATION - MESOLITHIC	PET1	W	South Germany	Petersfels	14000-11000 BC	**Bay**
	PET2	W	South Germany	Petersfels	14000-11000 BC	**Bay**
	PET3	W	South Germany	Petersfels	14000-11000 BC	**Bay**
	PET5	W	South Germany	Petersfels	14000-11000 BC	**Bay**
	PET6	W	South Germany	Petersfels	14000-11000 BC	**Bay**
	Kg1	W	Germany (Thuringia)	Kniegrotte	15000-14000 BC	**Bay**
	Kg2	W	Germany (Thuringia)	Kniegrotte	15000-14000 BC	**Bay**
	Kg3	W	Germany (Thuringia)	Kniegrotte	15000-14000 BC	**Bay**
	Kg4	W	Germany (Thuringia)	Kniegrotte	15000-14000 BC	**Bay**
	Kg5	W	Germany (Thuringia)	Kniegrotte	15000-14000 BC	**Bay**
	Spa 1	W	Ukraine (Peninsula Crimea)	Span-Koba	cal. 9390-9210 BC	**Bay**

ENEOLITHIC-COPPER AGE	Pie9	W	Romania	Pietrele	4300 BC	**Black**
	CAS1	W	Romania	Cascioarele	cal. 3700-3380 BC	**Black**
	VIT2	W	Romania	Vitanesti	cal. 4350-4220 BC	**Black**
	VIT4	W	Romania	Vitanesti	cal. 4360-4220 BC	**Black**
	MAY3	W	Ukraine	Mayaki	cal. 3640-3490 BC	**Bay**
	MAY5	W	Ukraine	Mayaki	cal. 3250-3100 BC	**Bay**
	MAY6	W	Ukraine	Mayaki	cal. 3520-3330 BC	**Bay**
	MAY7	W	Ukraine	Mayaki	cal. 3520-3380 BC	**Bay**
	MAY10	W	Ukraine	Mayaki	cal. 3650-3500 BC	**Bay**
	MOL5	W?	Ukraine	Molyukhov Bugor	cal. 3720-3630 BC	**Black**
BRONZE AGE	Gar2	D	Romania	Garbovat	1500-1000 BC	**Black**
	Gar3	D	Romania	Garbovat	1500-1000 BC	**Bay**
	Gar4	D	Romania	Garbovat	1500-1000 BC	**Bay**
	GRO11	D	Germany	Großobringen	3000-2500 BC	**Black**
	Shi 1	D	Armenia	Shirakavan	cal. 895-795 BC	**Chestnut**
	Lch 1	D	Armenia	Lchashen	cal. 1410-1250 BC	**Chestnut Sabino**
	Lor 1	D	North Armenia	Lori-Berd	cal. 1950-1750 BC	**Bay**
	Mic1	D	Moldova	Miciurin	1500-1000 BC	**Bay Tobiano**
	Mic2	D	Moldova	Miciurin	1500-1000 BC	**Chestnut**
	Mic3	D	Moldova	Miciurin	1500-1000 BC	**Black**
	Mic4	D	Moldova	Miciurin	1500-1000 BC	**Bay**
	Mic5	D	Moldova	Miciurin	1500-1000 BC	**Bay Sabino**

China

IRON AGE	Fen 1	D	China (Qinghai)	Fengtai	cal. 905-800 BC	**Bay Tobiano**
	Fen 2	D	China (Qinghai)	Fengtai	cal. 1000-800 BC	**Bay**
	Fen 3	D	China (Qinghai)	Fengtai	cal. 1000-800 BC	**Bay**

	Fen 4	D	China (Qinghai)	Fengtai	cal. 1000-800 BC	**Bay**
Iberian Peninsula						
MESOLITHIC-NEOLITHIC	44	W	Spain	Atxoste	5500- 4950 BC	**Bay**
	45	W	Spain	Atxoste	5500- 4950 BC	**Bay**
	3	W	Spain	Cueva Fosca - Valencia-Cartellon	cal. 5200-4900 BC	**Black**
	31	W	Spain	Cueva Fosca - Valencia-Cartellon	cal. 5210-4910 BC	**Bay**
	32	W	Spain	Cueva Fosca - Valencia-Cartellon	cal. 5220-4980 BC	**Black**
	34	W	Spain	Cueva Fosca - Valencia-Cartellon	cal. 5220-4900 BC	**Black**
	37	W	Spain	Cueva Fosca - Valencia-Cartellon	cal. 5210-4910 BC	**Bay**
	17	W	Spain	Cueva De La Vaquera - Segovia	cal. 5210-4940 BC	**Black**
BRONZE AGE	39	D	Spain	El Acequion	2200 BC - 800 AD	**Bay**
	40	D	Spain	El Acequion	2200 BC - 800 AD	**Bay**
	24	D	Spain	Cueva Rubia - Valmayor/Madrid	1350 BC	**Bay**
	22	D	Spain	Cueva Rubia - Valmayor/Madrid	1350 BC	**Black**
	25	D	Spain	Cueva Rubia - Valmayor/Madrid	1350 BC	**Black**
IRON AGE	4	W?	Spain	Soto de Medinilla - Valladolid	800 BC - 6 AD	**Black**
MEDIEVAL	29	D	Spain	Mucientes - Valladolid	cal. 660-780 AD	**Chestnut**
	30	D	Spain	Mucientes - Valladolid	cal. 680-890AD	**Chestnut**

Table S5. Results for SNP typing. In addition to the phenotype, the genotypes for all eight SNPs investigated are shown. Grey shading indicates that at least one allele differs from the wildtype. We did not detect any variation at the *EDNRB* locus.

	Sample	Designation	Phenotype	nuclear Genes							
Siberia				*ASIP*	*EDNRB*	*KIT13*	*KIT16*	*MATP*	*MC1R*	*SILV9*	*SILV11*
PLEISTOCENE	SP1181A	W(ild)	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	SP1181B	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	SP1181C	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	SP1181E	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	SP1181F	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
EARLY BRONZE AGE	BER 001	D(omestic)	**Chestnut**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	TAR 001	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	TAR 002	D	**Chestnut**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	TAR 004	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	TAR 005	D	**Bay Sabino**	A/A	ov/ov	KM0/KM0	SB1/sb1	C/C	E/e	z/z	z/z
	TAR 007	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	TAR 008	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	TAR 010	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	TAR 011	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
IRON AGE	BER 002	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Arz 1-2	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	Arz 1-3	D	**Black Silver**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	Z/z	Z/z
	Arz 2-1	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Arz 2-2	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Arz 2-3	D	**Chestnut**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	Arz 2-4	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Arz 2-5	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	Arz 2-6	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Arz 2-7	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Arz 2-8	D	**Buckskin**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/cr	E/e	z/z	z/z

Arz 2-9	D	**Buckskin**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/cr	E/e	z/z	z/z
Arz 2-10	D	Chestnut Tobiano	A/a	ov/ov	KM0/KM1	sb1/sb1	C/C	e/e	z/z	z/z
Arz 2-11	D	**Chestnut**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
Arz 2-12	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
Arz 2-13	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
Arz 2-14	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
Bars1A	D	**Bay Tobiano**	A/a	ov/ov	KM0/KM1	sb1/sb1	C/C	E/e	z/z	z/z
Bars1B	D	**Bay Tobiano**	A/a	ov/ov	KM0/KM1	sb1/sb1	C/C	E/e	z/z	z/z
OKG 001	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
OKG 002	D	**Bay Tobiano**	A/a	ov/ov	KM0/KM1	sb1/sb1	C/C	E/E	z/z	z/z
OKG 003	D	**Buckskin**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/cr	E/e	z/z	z/z

Eastern Europe

	Sample	Designation	Phenotype	nuclear Genes							
				ASIP	*EDNRB*	*KIT13*	*KIT16*	*MATP*	*MC1R*	*SILV9*	*SILV11*
GLACIATION - MESOLITHIC	PET1	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	PET2	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	PET3	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	PET5	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	PET6	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Kg1	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Kg2	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Kg3	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Kg4	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Kg5	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Spa 1	W	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Pie9	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	CAS1	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	VIT2	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z

ENEOLITHIC-COPPER AGE	VIT4	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MAY3	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MAY5	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MAY6	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MAY7	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MAY10	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	MOL5	W?	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
BRONZE AGE	Gar2	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Gar3	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Gar4	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	GRO11	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Shi 1	D	**Chestnut**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	Lch 1	D	**Chestnut Sabino**	A/a	ov/ov	KM0/KM0	SB1/sb1	C/C	e/e	z/z	z/z
	Lor 1	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Mic1	D	**Bay Tobiano**	A/a	ov/ov	KM0/KM1	sb1/sb1	C/C	E/E	z/z	z/z
	Mic2	D	**Chestnut**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	Mic3	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Mic4	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Mic5	D	**Bay Sabino**	A/a	ov/ov	KM0/KM0	SB1/sb1	C/C	E/e	z/z	z/z

China

	Sample	Designation	Phenotype	nuclear Genes							
				ASIP	*EDNRB*	*KIT13*	*KIT16*	*MATP*	*MC1R*	*SILV9*	*SILV11*
IRON AGE	Fen 1	D	**Bay Tobiano**	A/A	ov/ov	KM0/KM1	sb1/sb1	C/C	E/E	z/z	z/z
	Fen 2	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	Fen 3	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	Fen 4	D	**Bay**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z

Iberian Peninsula											
MESOLITHIC-NEOLITHIC	44	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	45	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	3	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	31	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	32	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	34	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	37	W	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	17	W	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
BRONZE AGE	39	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	40	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	24	D	**Bay**	A/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/e	z/z	z/z
	22	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
	25	D	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
IRON AGE	4	W?*	**Black**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	E/E	z/z	z/z
MEDIEVAL	29	D	**Chestnut**	a/a	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z
	30	D	**Chestnut**	A/A	ov/ov	KM0/KM0	sb1/sb1	C/C	e/e	z/z	z/z

* Based on archaeological context.

Table S6. Detailed information on the ages of the samples investigated.

Siberia

	Sample	Geographical location	Excavation	Stratigraphic date	Age ^{14}C	Calibrated date	Lab. No.	Details
PLEISTOCENE	SP1181A	North Siberia	Maliy Lyakhovsky Isl.	Pleistocene				
	SP1181B	North Siberia	Bol'shoy Lyakhovsky Isl.	Pleistocene				
	SP1181C	North Siberia	Bol'shoy Lyakhovsky Isl.	Pleistocene				
	SP1181E	Siberia	Oyagosskiy Yar, Kondrat'evo R., mouth	Pleistocene				
	SP1181F	Siberia	Kotel'niy Isl., Anisiy Cape	Pleistocene				
EARLY BRONZE AGE	BER 001	Siberia (Altai)	Denisova-Pescera	3000BC				
	TAR 001	West Siberia	Tartas1	2500-3000BC				
	TAR 002	West Siberia	Tartas1	2500-3000BC				
	TAR 004	West Siberia	Tartas1	2500-3000BC				
	TAR 005	West Siberia	Tartas1	2500-3000BC				
	TAR 007	West Siberia	Tartas1	2500-3000BC				
	TAR 008	West Siberia	Tartas1	2500-3000BC				
	TAR 010	West Siberia	Tartas1	2500-3000BC				
	TAR 011	West Siberia	Tartas1	2500-3000BC				
IRON AGE	BER 002	Siberia (Altai)	Om-1	900BC				
	Arz 1-2	South Siberia (Tuva)	Arzan1	800BC				
	Arz 1-3	South Siberia (Tuva)	Arzan1	800BC				
	Arz 2-1	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-2	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-3	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-4	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-5	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-6	South Siberia (Tuva)	Arzan2	619-608 BC				

	Arz 2-7	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-8	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-9	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-10	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-11	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-12	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-13	South Siberia (Tuva)	Arzan2	619-608 BC				
	Arz 2-14	South Siberia (Tuva)	Arzan2	619-608 BC				
	Bars1A	South Siberia (Tuva)	Barsucij Log		2170 ± 30 BP	cal. 370-150 BC	Poz-22611	3.3%N 11.5%C
	Bars1B	South Siberia (Tuva)	Barsucij Log	cal. 400-200 BC				
	OKG 001	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC				
	OKG 002	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC				
	OKG 003	Siberia (Mongolia)	Olon-Kurin-Gol 10	400-300 BC				

East Europe

	Sample	Geographical location	Excavation	Stratigraphic date	Age ^{14}C	Calibrated date	Lab. No.	Details
LATE GLACIATION	PET1	South Germany	Petersfels	14000-11000 BC				
	PET2	South Germany	Petersfels	14000-11000 BC				
	PET3	South Germany	Petersfels	14000-11000 BC				
	PET5	South Germany	Petersfels	14000-11000 BC				
	PET6	South Germany	Petersfels	14000-11000 BC				
	Kg1	Germany (Thuringia)	Kniegrotte	15000-14000 BC				
	Kg2	Germany (Thuringia)	Kniegrotte	15000-14000 BC				
	Kg3	Germany (Thuringia)	Kniegrotte	15000-14000 BC				
	Kg4	Germany (Thuringia)	Kniegrotte	15000-14000 BC				
	Kg5	Germany (Thuringia)	Kniegrotte	15000-14000 BC				

ENEOLITHIC-COPPER AGE	Spa 1	Ukraine (Peninsula Crimea)	Span-Koba			cal. 9390-9210 BC		
	Pie9	Romania	Pietrele	4300 BC				
	CAS1	Romania	Cascioarele		4820 ± 40 BP	cal. 3700-3380 BC	Poz-24925	1.0%N 7.6%C carbonate
	VIT2	Romania	Vitanesti		5400 ± 40 BP	cal. 4350-4220 BC	Poz-24899	1.5%N 6.0%C
	VIT4	Romania	Vitanesti		5430 ± 40 BP	cal. 4360-4220 BC	Poz-24900	1.7%N 6.5%C
	MAY3	Ukraine	Mayaki		4745 ± 35 BP	cal. 3640-3490 BC	Poz-24926	0.6%N 2.8%C carbonate
	MAY5	Ukraine	Mayaki		4550 ± 35 BP	cal. 3250-3100 BC	Poz-24826	2.4%N 9.8%C
	MAY6	Ukraine	Mayaki		4605 ± 35 BP	cal. 3520-3330 BC	Poz-24849	1.6%N 6.7%C
	MAY7	Ukraine	Mayaki		4640 ± 35 BP	cal. 3520-3380 BC	Poz-24850	2.6%N 10.4%C
	MAY10	Ukraine	Mayaki		4770 ± 40 BP	cal. 3650-3500 BC	Poz-24927	0.5%N 3.4%C carbonate
	MOL5	Ukraine	Molyukhov Bugor	cal. 3720-3630 BC				
BRONZE AGE	Gar2	Romania	Garbovat	1500-1000 BC				
	Gar3	Romania	Garbovat	1500-1000 BC				
	Gar4	Romania	Garbovat	1500-1000 BC				
	GRO11	Germany	Großobringen	3000-2500 BC				
	Shi 1	Armenia	Shirakavan		2670 ± 30 BP	cal. 895-795 BC	Poz-22615	1.5%N 5.5%C
	Lch 1	Armenia	Lchashen		3050 ± 30 BP	cal. 1410-1250 BC	Poz-22613	3.7%N 12.4%C
	Lor 1	North Armenia	Lori-Berd		3525 ± 35 BP	cal. 1950-1750 BC	Poz-22701	1.1%N 4.6%C
	Mic1	Moldova	Miciurin	1500-1000 BC				
	Mic2	Moldova	Miciurin	1500-1000 BC				
	Mic3	Moldova	Miciurin	1500-1000 BC				
	Mic4	Moldova	Miciurin	1500-1000 BC				
	Mic5	Moldova	Miciurin	1500-1000 BC				

China

	Sample	Geographical location	Excavation	Stratigraphic date	Age ^{14}C	Calibrated date	Lab. No.	Detail
IRON AGE	Fen 1	China (Qinghai)	Fengtai		2695 ± 30 BP	cal. 905-800 BC	Poz-22612	3.4%N 11.0%C
	Fen 2	China (Qinghai)	Fengtai	905-800 BC				
	Fen 3	China (Qinghai)	Fengtai	905-800 BC				
	Fen 4	China (Qinghai)	Fengtai	905-800 BC				

Iberian Peninsula

	Sample	Geographical location	Excavation	Stratigraphic date	Age ^{14}C	Calibrated date	Lab. No.	Detail
MESOLITHIC-NEOLITHIC	44	Iberian Peninsula (Spain)	Atxoste	5500- 4950 BC				
	45	Iberian Peninsula (Spain)	Atxoste	5500- 4950 BC				
	3	Iberian Peninsula (Spain)	Cueva Fosca - Valencia-Cartellon	cal. 5200-4900 BC				
	31	Iberian Peninsula (Spain)	Cueva Fosca - Valencia-Cartellon		6100 ± 40 BP	cal. 5210-4910 BC	Poz-24720	3.5%N 15.4%C
	32	Iberian Peninsula (Spain)	Cueva Fosca - Valencia-Cartellon		6135 ± 35 BP	cal. 5220-4980 BC	Poz-24743	3.4%N 17.2%C
	34	Iberian Peninsula (Spain)	Cueva Fosca - Valencia-Cartellon	cal. 5200-4900 BC				
	37	Iberian Peninsula (Spain)	Cueva Fosca - Valencia-Cartellon		6100 ± 40 BP	cal. 5210-4910 BC	Poz-24745	2.6%N 11.9%C
	17	Iberian Peninsula (Spain)	Cueva De La Vaquera - Segovia		6110 ± 40 BP	cal. 5210-4940 BC	Poz-24721	2.7%N 13.9%C
BRONZE AGE	39	Iberian Peninsula (Spain)	El Acequion	2200 BC - 800 AD				
	40	Iberian Peninsula (Spain)	El Acequion	2200 BC - 800 AD				
	24	Iberian Peninsula (Spain)	Cueva Rubia - Valmayor/Madrid	cal. 2880-2570BC				
	22	Iberian Peninsula (Spain)	Cueva Rubia - Valmayor/Madrid	cal. 2880-2570BC				
	25	Iberian Peninsula (Spain)	Cueva Rubia - Valmayor/Madrid	cal. 2880-2570BC				

IRON AGE	4	Iberian Peninsula (Spain)	Soto de Medinilla – Valladolid	800 BC - 6 AD				
MEDIEVAL	29	Iberian Peninsula (Spain)	Mucientes - Valladolid		1275 ± 25 BP	cal. 660-780 AD	Poz-24740	1.2%N 6.9%C
	30	Iberian Peninsula (Spain)	Mucientes - Valladolid		1230 ± 30 BP	cal. 680-890AD	Poz-24739	3.4%N 15%C

Table S7. Upper bound of the frequency $f_{5\%}$ a colour allele could have had in the horse population prior to domestication having a chance to be missed of $p \leq 0.05$ (n = number of alleles).

Population	**n**	$f_{5\%}$
Siberia	10	0.26
East Europe	42	0.07
Iberia	16	0.15
Siberia + East Europe	52	0.06
Siberia + East Europe + Iberia	**68**	**0.04**

Table S8. Maximum likelihood estimates of the selection coefficient for each of the loci. The results were obtained using Bollback et al's method (*S11*), excluding the Pleistocene samples. To get the 95% confidence interval we used the profile likelihood. For ASIP and MC1R loci the selection coefficient is significantly different than 0. For the other loci, the data is compatible with s=0. Therefore, the observed data are difficult to explain by genetic drift alone under a standard neutral model (for review see *S14*).

Note that we had no power to estimate the effective population size. Indeed, the confidence interval for the effective population size spans all the values of the grid we used to compute the likelihood, that is 10,000 to 100,000. This is not surprising since Bollback et al. (*S11*) noted that their method has little power to co-estimate the effective population size and the selection coefficient.

Gene	*Selection coefficient, maximum likelihood estimate and 95% confidence interval*
ASIP	0.0007 (0.0001, 0.0015)
KIT13	0.0003 (-0.0007, 0.007)
KIT16	-0.0003 (-0.0013, 0.0011)
MATP	0.000 (-0.001, 0.001)
MC1R	0.0019 (0.0007, 0.0035)
SILV19	-0.0003 (-0.0015, 0.0013)

Supplementary references

S1. N. Rohland, M. Hofreiter, *Nature Protocols* **2**, 1756 (2007).
S2. J. Krause *et al.*, *Nature* **439**, 724 (Feb 9, 2006).
S3. H. Römpler *et al.*, *Nat. Protocols* **1**, 720 (2006).
S4. S. A. Brooks, E. Bailey, *Mamm Genome* **16**, 893 (Nov, 2005).
S5. S. A. Brooks, R. B. Terry, E. Bailey, *Anim Genet* **33**, 301 (Aug, 2002).
S6. E. Brunberg *et al.*, *BMC Genet* **7**, 46 (2006).
S7. D. Mariat, S. Taourit, G. Guerin, *Genet Sel Evol* **35**, 119 (Jan-Feb, 2003).
S8. L. Marklund, M. J. Moller, K. Sandberg, L. Andersson, *Mamm Genome* **7**, 895 (Dec, 1996).

S9. S. Rieder, S. Taourit, D. Mariat, B. Langlois, G. Guerin, *Mamm Genome* **12**, 450 (Jun, 2001).
S10. P. Gagneux, C. Boesch, D. S. Woodruff, *Mol Ecol* **6**, 861 (Sep, 1997).
S11. J. P. Bollback, T. L. York, R. Nielsen, *Genetics* **179**, 497 (May, 2008).
S12. M. Reissmann, J. Bierwolf, G. Brockmann, *Anim Genet* **38**, 1 (2007).
S13. E. M. Santschi, A. K. Purdy, S. J.Valberg, P. D. Vrotsos, H. Kaese, J. R. Mickelson *Mamm Genome* **9**, 306 (1998).
S14. N. A. Rosenberg, M. Nordborg *Nature Reviews Genetics* **3**, 380 (May 2002).