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Limited number of patrilines in horse domestication

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Genetic studies using mitochondrial DNA (mtDNA) have identified extensive matrilinear diversity among domestic horses. Here, we show that this high degree of polymorphism is not matched by a corresponding patrilinear diversity of the male-specific Y chromosome. In fact, a screening for single-nucleotide polymorphisms (SNPs) in 14.3 kb of noncoding Y chromosome sequence among 52 male horses of 15 different breeds did not identify a single segregation site. These observations are consistent with a strong sex-bias in the domestication process, with few stallions contributing genetically to the domestic horse.

The finding of >90 mtDNA haplotypes among domestic horses indicates the incorporation of numerous genetic lineages into the breeding stock[1–3]. These data support domestication from geographically separate areas, which may have occurred through diffusion of the required human expertise or through truly independent domestication processes. But genetic diversity in mtDNA only reflects the maternal contribution to the gene pool. Recently, genetic markers from the male-specific Y chromosome have unveiled the patterns of evolution and migration among modern humans, as manifested by paternal genetic architectures[4]. Y-chromosome markers will also be informative for addressing the genetic and anthropological processes associated with animal domestication[5], as males and females may have been treated differently by early human societies[6].

To study the genetic contribution of stallions in horse domestication, we screened for SNPs in 14.3 kb of equine Y-chromosome sequence, divided into 37 fragments (**Table 1** and **Supplementary Methods** online). Because many breeds may derive from a small number of founders or from a limited number of registries in the studbook, bottleneck and founder effects may have reduced the genetic diversity within each single breed. Consequently, we used 52 male horses from 15 different breeds for screening, including divergent European (Ardennais, Connemara, Exmoor, Fjord, Gotland, Icelandic, Shetland, North-Swedish and Thoroughbred) and Asian (Akhal Teké, Arab, Caspian Pony, Khuzestan Arab, Malwari and Thai Pony) breeds. These breeds were selected to represent a wide variety of horses and ponies to cover as much as possible of the gene pool of the domestic horse; most of the breeds have old histories and substantial phenotypic variation.

Table 1 Description of horse Y-chromosome markers

Locus	Length (bp)	GenBank accession number		
		Equus caballus	*Equus caballus przewalskii*	*Equus asinus*
Y-chromosome introns				
AMELY1	394	AB091794	AY532824	AY532815
AMELY2	358	AB091794	AY532825	AY532816
AMELY3	481	AB091794	AY532826	AY532817
AMELY4	488	AB091794	AY532827	AY532818
AMELY6	470	AB091794	AY532828	AY532819
AMELY7	215	AB091794	AY532829	AY532820
AMELY8	478	AB091794	AY532830	AY532821
AMELY9	203	AB091794	AY532831	AY532822
AMELY11	245	AB091794	AY532832	AY532823
DBY7	259	AY532880	AY532884	AY532882
DBY8	85	AY532881	AY532885	AY532883
SMCY2	251	AY532886	AY532894	AY532890
SMCY3	848	AY532887	AY532895	AY532891
SMCY7	341	AY532888	AY532896	AY532892
SMCY17	80	AY532889	AY532897	AY532893
*SRY**	452	AB004572	AY532879	AY532878
Subclones from *ZFY*-positive BAC				
ZFYD	710	AY532845	AY532860	AY532833
ZFYG	539	AY532846	AY532861	AY532834
ZFYH	579	AY532847	AY532862	AY532835
ZFY27A	323	AY532848	AY532863	AY532836
ZFY43A	435	AY532849	AY532864	AY532837
ZFY43B2	452	AY532875	AY532877	AY532876
ZFY44A	391	AY532850	AY532865	ND
ZFY46A	341	AY532851	AY532866	AY532838
ZFY50A	252	AY532852	AY532867	ND
ZFY50B	314	AY532853	AY532868	ND
ZFY51A	353	AY532854	AY532869	AY532839
ZFY52A	381	AY532855	AY532870	AY532840
ZFY53A	414	AY532856	AY532871	AY532841
ZFY53B	358	AY532857	AY532872	AY532842
ZFY55A	342	AY532858	AY532873	AY532843
ZFY55B	426	AY532859	AY532874	AY532844
Anonymous Y-linked fragments				
Eca-Y2B17	438	G72335	AY532806	AY532805
Eca-Y3B1	468	G72336	AY532811	AY532807
Eca-Y3B8	445	G72337	AY532812	AY532808
Eca-Y3B12	392	G72338	AY532813	AY532809
Eca-Y3B19	215	G72339	AY532814	AY532810

All fragments were sequenced in 52 male horses from 15 divergent breeds. ND, not determined. *Untranslated region.

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All stallions carried the same Y-chromosome haplotype. This contrasts sharply with the extensive mtDNA diversity and indicates very low levels of Y-chromosome variability in domestic horses. Limited polymorphism is not a general feature of equine nuclear DNA, as a screening of 2.3 kb of X-chromosome intron sequence in a subset of the individuals identified 17 SNPs and a nucleotide diversity (π) of $1.4 \times 10^{-3} \pm 0.1 \times 10^{-3}$ (**Supplementary Table 1** online). Nor is it due to selective constraints on the Y chromosome, because, by determining the orthologous X- and Y-chromosome sequence in donkey, we found strong statistical support for lower levels of variability on Y than on X (HKA test $P < 0.00001$).

The ancestors to domestic horses have gone extinct in the wild, but a population of Mongolian wild horses, or Przewalski's horses (*Equus caballus przewalskii*), is kept in captivity. We sequenced one male Przewalski's horse for all Y-chromosome fragments and found that it differed from all domestic horses at six nucleotide positions over the 14.3 kb and also had a 7-bp deletion. Based on Y-chromosome data, the split between Przewalski's horse and domestic horses is estimated to have occurred 120,000–240,000 years ago[7]. As this is long before wild horses were domesticated (~6,000 years ago[8]), wild horses may have had Y-chromosome variability before domestication.

How low is Y-chromosome variability of domestic horses compared with that of, for instance, humans? Several large-scale studies uniformly estimated π in noncoding sequences of the human Y chromosome at $1.0–1.5 \times 10^{-4}$ (refs. 9–12). One of these studies[9] was similar to ours: 50 chromosomes from geographically diverse human samples were screened in 35.3 kb of *SMCY* intron sequence, identifying 37 segregating sites or one every 950 bp (larger studies found one every 576–840 bp; refs. 9,11,12). A randomization test provided strong statistical support for a significant difference in Y-chromosome polymorphism levels between horses and humans ($P < 0.0001$). Quantification of this difference is not straightforward, as our domestic horse sample was monomorphic for Y chromosome sequences. But we can conservatively assess the difference by assigning a variable site to the horse data set. With one rare allele at 2% frequency, π would be 3×10^{-6}; at 10 % frequency, π would be 1×10^{-5}. This implies that π estimates for the Y chromosome are at least 10–30 times lower for domestic horses than for humans.

Our observations are compatible with a scenario of strong sex bias in breeding with only a limited number of sires contributing genetically to the domestic horse (low male effective population size). Modern breeding practice selects stallions and lets them cover many mares each, a breeding scheme that reduces the number of patrilines in the population[13]. Our data suggest that using a limited number of stallions in breeding may be traditional breeding practice[14] and thus date back to the initial phase of horse domestication. If a strong sex bias in breeding were only a modern, breed-specific phenomenon, we would expect to see some Y-chromosome variability among breeds. Moreover, a bias in the early exploitation of each sex could also have contributed to a limited male gene pool. During the Paleolithic age, horses were an important part of the human diet[15], and this could have been the case during the early stages of horse domestication as well. In general, food production is maximized if most males are consumed and females are left for reproduction; such a sex bias in exploitation of several domestic animals is suggested from archaeological records[6].

Our observations do not exclude the possibility that the equine Y chromosome was low in variability before the time of domestication. The social structure of the wild horses from which domestic breeds evolved probably featured a single stallion holding a harem of multiple mares, implying skewed reproductive success of males. As mtDNA data point at domestication from geographically widespread areas, however, divergent Y-chromosome lineages might have been incorporated into the breeding stock even if local Y-chromosome variability was low. An alternative scenario would imply a single domestication event in a restricted geographical region, resulting in the incorporation of only a limited number of Y-chromosome haplotypes into the breeding stock. When domestic animals, or pastoralism in itself, then spread from one locality to another, the maternal gene pool may have been diversified by the capture of only wild females from local populations (while backcrosses with wild stallions were prevented). Under this scenario, the contrasting levels of variability in mtDNA and the Y chromosome seen in modern horses reflect how the practice of horse domestication spread among early human societies.

Note: Supplementary information is available on the Nature Genetics website.

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1. Lister, A.M. *et al. Ancient Biomol.* **2**, 267–280 (1998).
2. Vilá, C. *et al. Science* **291**, 474–477 (2001).
3. Jansen, T. *et al. Proc. Natl. Acad. Sci. USA* **99**, 10905–10910 (2002).
4. Goldstein, D.B. *Science* **291**, 1738–1742 (2001).
5. MacHugh, D.E. & Bradley, D.G. *Proc. Natl. Acad. Sci. USA* **98**, 5382–5384 (2001).
6. Zeder, M.A. & Hesse, B. *Science* **287**, 2254–2257 (2000).
7. Wallner, B. *et al. Anim. Genet.* **34**, 453–456 (2003).
8. Clutton-Brock, J. *A Natural History of Domesticated Mammals.* (Cambridge University Press, Cambridge, Massachusetts, 1999).
9. Shen, P. *et al. Proc. Natl. Acad. Sci. USA* **97**, 7354–7359 (2000).
10. The International SNP Map Working Group. *Nature* **409**, 928 (2001).
11. Hammer, M.F. *et al. Mol. Biol. Evol.* **18**, 1189–1203 (2001).
12. Hammer, M.F. *et al. Genetics* **164**, 1495–1509 (2003).
13. Cunningham, E.P. *et al. Anim. Genet.* **32**, 360 (2001).
14. Levine, M.A. *J. Anthropol. Archaeol.* **18**, 29–78 (1999).
15. Olsen, S.L. *Horses Through Time.* (Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, 1996).