ANCIENT GENOMES

Ancient genomes revisit the ancestry of domestic and Przewalski’s horses

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The Eneolithic Botai culture of the Central Asian steppes provides the earliest archaeological evidence for horse husbandry, ~5500 years ago, but the exact nature of early horse domestication remains controversial. We generated 42 ancient-horse genomes, including 20 from Botai. Compared to 46 published ancient and modern-horse genomes, our data indicate that Przewalski’s horses are the feral descendants of horses herded at Botai and not truly wild horses. All domestic horses dated from ~4000 years ago to present only show ~2.7% of Botai-related ancestry. This indicates that a massive genomic turnover underpins the expansion of the horse stock that gave rise to modern domesticates, which coincides with large-scale human population expansions during the Early Bronze Age.

Horses revolutionized human mobility, economy, and warfare (1). They are also associated with the spread of Indo-European languages (2) and new forms of metallurgy (3) and provided the fastest land transport until modern times. Together with the lack of diachronic changes in horse morphology (4) and herd structure (5, 6), the scarce archaeological record hampered the study of early domestication. With their preponderance of horse remains, Eneolithic sites (fifth and fourth millennia BCE) of the Pontic-Caspian steppe (2, 7) and the northern steppe of Kazakhstan (6, 8) have attracted the most attention.

We reconstructed the phylogenetic origins of the Eneolithic horses associated with the Botai culture of northern Kazakhstan, representing the earliest domestic horses (6, 8). This culture was characterized by a sudden shift from mixed hunting and gathering to an extreme focus on horses and larger, more sedentary settlements (5). Horse dung on site (6), as well as evidence for poleaxing and against selective body-part transportation, suggests controlled slaughter at settlements rather than hunting (9). Tools associated with leather thong production, bit-related dental pathologies (7, 10), and equine milk fats within ceramics support pastoral husbandry, involving milking and harnessing (8).

Geological surveys at the Botai culture site of Krasnyi Yar, Kazakhstan, described a polygonal enclosure of ~20 m by 15 m with increased phosphorus and sodium concentrations (6), likely corresponding to a horse corral. We revealed a similar enclosure at the eponymous Botai site, ~100 km west of Krasnyi Yar (Fig. 1A), that shows close-set post molds, merging to form a palisade trench, and a line of smaller parallel postholes inside (Fig. 1B). Radiocarbon dates on horse bones from these postholes are consistent with the Botai culture (11). The presence of enclosures at Krasnyi Yar and Botai builds on the evidence supporting horse husbandry.

We sequenced the genomes of 20 horses from Botai and 22 from across Eurasia and spanning the past ~5000 years (table S1). With the published genomes of 18 ancient and 28 modern horses, this provided a comparative panel of 3 wild archaic horses (~42,800 to 5100 years ago), 7 Przewalski’s horses (PH, 6 modern and 1 from the 19th century), and 78 domesticates (25 Eneolithic, including 5 from Boryl, Kazakhstan, ~5000 years ago; 7 Bronze Age, ~4100 to 3000 years ago; 18 Iron Age, ~2800 to 2200 years ago; 1 Parthian and 2 Roman, ~2000 to 1600 years ago; 3 post-Roman, ~1200 to 100 years ago; and 22 modern from 18 breeds).

The 42 ancient-horse genomes, belonging to 31 horse stallions and 11 mares, were sequenced to an average depth of coverage of ~1.1 to 9.3X.

Fig. 1. Sample location and corral enclosure at Botai. (A) Archaeological sites. The age (years ago) of the genomes considered is reported to the right of each site name. The number of genomes sequenced per site is reported between parentheses if greater than one. Triangles refer to the ancient genomes characterized here, whereas diamonds indicate those previously published. Blue refers to wild ancient individuals, light and dark green to the first domestic clade (Botai and Boryl4), and yellow to individuals of the second domestic clade (DOM2). The Botai culture site of Krasnyi Yar is indicated with an asterisk, although no samples were analyzed from this site. (B) Magnetic gradient survey and excavation at Botai, with interpretation. The enclosure and its excavated boundary are indicated by red and yellow squares, respectively. Round black circles correspond to pit houses.
Phylogenetic reconstruction confirmed that domestic horses do not form a single monophyletic group as expected if descending from Botai (Fig. 2B). Instead, PH form a slightly drifted, monophyletic group (median = 3.0X). Damage patterns indicative of ancient DNA were recovered (Figs. S8 and S9). Base-quality rescaling and termini trimming resulted in average error rates of 0.07 to 0.14% per site (tables S13 and S14).

Principal component analysis (PCA) revealed PH and the archaic horses as two independent clusters (Fig. 2A). Within domesticates, all 25 Botai-Borly4 Eneolithic specimens grouped together to the exclusion of all remaining horses.

Fig. 2. Horse genetic affinities. (A) PCA of the genome variation present in 88 ancient- and modern-horse relationships. The tree was reconstructed on the basis of pairwise distances calculated with ~14.1 million transversion sites. Node supports derive from 100 bootstrap pseudoreplicates. The archaeological site and age (years ago) of ancient specimens are indicated in the first and last fields of the sample name. (C) Outgroup f3-statistics showing the pairwise genetic affinities.

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Domestic genes (Botai horses as the direct ancestors of Borly4) are genetically closer to Botai-Borly4 individuals unambiguously nested within Botai-Borly4. Gaunitz et al. (12) established this tree topology (fig. S23).

Limited unpigmented areas and leopard spots, (Fig. 4A). One Botai individual likely showed 112 modern horses indicate that PH have been domesticated in the feral descendants of horses first herded at Botai. It appears that their feralization likely occurred until 1000 years ago. This gene introgression of Botai ancestry into multiple DOM2 (figs. S28) suggests that sporadic introgression between ~4977 and ~118 years ago, it drastically increased in modern animals (Fig. 4C). This accumulation of deleterious variants was thus not associated with PH feralization but with the recent introgression of deleterious variants from modern domesticates and demographic collapse, which hampered purifying selection.

That none of the domesticates sampled in the past ~4000 years descend from the horses first herded at Botai entails another major implication. It suggests that during the third millennium BCE, at the latest, another unrelated group of horses became the source of all domestic populations that expanded thereafter. This is compatible with two scenarios. First, Botai-type horses experienced massive introgression capture (22) from a population of wild horses until the Botai ancestry was almost completely replaced. Alternatively, horses were successfully domesticated in a second domestication center and incorporated minute amounts of Botai ancestry during their expansion.

**Fig. 3. Admixture graphs.** (A to F) The six scenarios tested. The scenario in panel (A) received decisive Bayes factor support. As indicated below each corresponding alternative scenario tested. Domestic-Ancient and Domestic-A or -B refer to three phylogenetic clusters identified within DOM2 (excluding Duk2): ancient individuals; modern Mongolian, Yakutian (including Tumeski_CGG101397), and Jeju horses; and all remaining modern breeds. (G) Posterior distributions of admixture proportions. p1 and p2 represent admixture proportions along the dotted branches in the best-supported scenario.
We cannot identify the locus of this hypothetical center because of a temporal gap in our data set throughout the third millennium BCE. However, that the earliest DOM2 member was excavated in Hungary adds Eastern Europe to other candidates already suggested, including the Pontic-Caspian steppe (2), Eastern Anatolia (23), Iberia (24), Western Iran, and the Levant (25). Notwithstanding the process underlying the genomic turnover observed, the clustering of ~4023–3574-year-old specimens from Russia, Romania, and Georgia within DOM2 suggests that this clade already expanded throughout the steppes and Europe at the transition between the third and second millennia BCE, in line with the demographic expansion at ~4500 years ago recovered in mitochondrial Bayesian Skylines (fig. S14). This study shows that the horses exploited by the Botai people later became the feral PH. Early domestication most likely followed the “prey pathway,” whereby a hunting relationship was intensified until reaching concern for future progeny through husbandry, exploitation of milk, and harnessing (7). Other horses, however, were the main source of domestic stock over the past ~4000 years or more. Ancient human genomics (26) has revealed considerable human migrations ~5000 years ago involving Yamnaya culture pastoralists of the Pontic-Caspian steppe. This expansion might be associated with the genomic turnover identified in horses, especially if Botai horses were better suited to localized pastoral activity than to long distance travel and warfare. Future work must focus on identifying the main source of the domestic horse stock and investigating how the multiple human cultures managed the available genetic variation to forge the many horse types known in history.

REFERENCES AND NOTES

11. See supplementary materials.

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Competing interests: The authors declare no competing interests. Data and materials availability: The archaeological material from Iran analyzed in this study was part of the collections of the Osteology Department of the National Museum of Iran. A subset of the morphological data described in this study was collected at the UMR 7041 ArScAn CNRS. Individual genome sequence data are available at the European Nucleotide Archive (accession no. PRJEB23390). Alignments underlying analyses are available on DRYAD at https://doi.org/10.5061/dryad.f9r95pm1.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S34

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References (27–174)

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Revisiting the origins of modern horses

The domestication of horses was very important in the history of humankind. However, the ancestry of modern horses and the location and timing of their emergence remain unclear. Gaunitz et al. generated 42 ancient-horse genomes. Their source samples included the Botai archaeological site in Central Asia, considered to include the earliest domesticated horses. Unexpectedly, Botai horses were the ancestors not of modern domestic horses, but rather of modern Przewalski’s horses. Thus, in contrast to current thinking on horse domestication, modern horses may have been domesticated in other, more Western, centers of origin.

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