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Spotlight

Horse Paleogenomes and Human–Animal Interactions in Prehistory

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A new analysis of paleogenomic data from 278 ancient horses (Fages *et al. Cell* http://doi.org/10.1016/j.cell.2019.03.049) finds that this animal – crucially important to many ancient and contemporary human societies for subsistence, transportation, conflict, and more – was domesticated in at least two different regions, but with the geographic and cultural origins of the modern domestic horse lineage remaining unknown. By tracing ancient horse population movements and inferring the spatiotemporal trajectories of phenotypic adaptations, this study provides fresh perspectives on past human group interactions and activities.

Our understanding of the human past can be enhanced substantially by reconstructing the histories of the many other species with which we have been closely associated. The horse (*Equus caballus*) is a uniquely charismatic animal that has shaped the subsistence, social, and symbolic lives of people around the globe, fostered rapid communication among far-flung groups, and had outsized importance in forging the rises and falls of political landscapes throughout many parts of the ancient world. Nonetheless, tracing precisely how horses were brought under human control, where and when horses were domesticated, and the translocation pathways of domesticated stock has remained a central challenge to archaeologists.

Analysis of ancient DNA from dated samples brings important new perspectives to prehistory as a powerful tool for studying past population dynamics and reconstructing the evolutionary trajectories of trait-associated alleles. Although population-scale paleogenomics has otherwise been headlined principally by analyses of human ancient DNA, a new horse ancient DNA study [1] demonstrates how insightful this approach can be when extended to non-human taxa [2], contributing to our understanding of how human–animal relationships have helped to define the social and political worlds of ancient communities.

Specifically, Fages *et al.* [1] screened the remains of hundreds of European and Asian horses from time-periods spanning the past 6000 years to identify 87 samples with ancient DNA quantities sufficient for generating quality paleogenome sequences. They also generated lower-coverage genome sequence data from another 149 individuals, and additionally included 42 previously published horse paleogenomes in their analyses.

This study provides insight into three different time-periods of horse domestication. First, the origins of horse domestication >5000 years ago. Decades of zooarchaeological research across Europe and Asia have documented shifts in the geographic distribution of horses, herd demographic structure, and skeletal morphology, and also identified the appearance of pathologies associated with riding, together leading to several different proposed 'centers' of horse domestication. Geographic contenders include the vast Eurasian steppe, where horses were intensively harvested and milked in Kazakhstan (Botai) by the mid fourth millennium BC [3], Anatolia, where equids were also intensively exploited by this time-period [4], and potentially Iberia [5].

A previous, smaller-scale horse ancient DNA study [6] revealed that living Przewalski's horses are actually feral descendants of early managed horses of the Central Asian Botai culture rather than being representatives of a surviving wild horse lineage. The Botai–Przewalski ¡ineage is genetically distinct from modern domestic horses, leading to inference of at least two independent centers of horse domestication.

Fages *et al.* [1] now report that ancient Iberian horses are largely genetically distinct from both the Botai–Przewalski lineage and the entire clade of modern domestic horses. It is not yet certain whether the Iberian horses sampled in this study were wild animals or if they represent a third independent center of domestication. Regardless, this result means that the geographic and cultural origins of the modern domestic horse lineage are still unknown. Given the ultimate spread and predominance of this lineage, and the transformative role of horses in human subsistence, movement, and knowledge transfer, the outcomes of further efforts to ¡dentify this center will be highly anticipated.

Second, population movements of the modern domestic horse lineage across the past 1500 years may track associated human cultural processes in this same time-frame. For example, Fages *et al.* [1] report that modern Shetland and Iceland ponies are re-ated both to pre-Viking Pictish horses from present-day Great Britain and to ancient Viking horses, which may reflect the legacy of Viking cultural expansions. Fages *et al.* [1] also identified a seventh to ninth centuries CE spread into Croatia of a horse clade with genetic affinities to earlier horses in Iran, a finding temporally consistent with documented timings of Arabian raids into Mediterranean Europe. The paleogenomic data also revealed a similar spread of this same clade of horses into Asia.

Time-stamped ancient DNA can also provide a powerful window into the evolutionary trajectories of genetic variants known to be associated with phenotypic traits [7]. Even in the absence of any trait association knowledge, paleogenomic data can still help to identify novel genomic signatures of past positive selection. Then, with availability of at least some

understanding of the general functional roles of genes located within or nearby the detected signatures (e.g., from human and model organism studies), researchers can develop rough evolutionary hypotheses.

With their paleogenomic database, Fages *et al.* [1] show that the frequencies of alleles known to be associated with faster speed and improved racing performance have increased over the past 1000 years of modern domestic horse lineage evolution. They also identified strong signatures of positive selection – but with unknown phenotypic effects – in multiple different clusters of *HOX* genes that have crucial roles in skeletal and body patterning.

Previous horse ancient DNA studies have presented similar mixes of evolutionary inference. Although explicit trait tracking has been feasible for coat coloration phenotypes [8–10], the phenotypic consequences of genetic variants located within most detected signatures of past positive selection remain uncertain. This presents an outstanding opportunity for new genotype–phenotype investigations that would simultaneously advance our understanding of modern horse biology and help to develop our picture of past human behavior and the relationships between people and their horses.

Third, the broad ancient DNA sample in this new study also provides insights into the consequences of horse-breeding practices on genetic diversity. Although reductions in paternally inherited Y-chromosome diversity are observed beginning ~2000 years ago, overall (autosomal nuclear genome) genetic diversity has markedly reduced only over the past 200 years (–16.4% heterozygosity). Otherwise, autosomal genetic diversity was largely steady across the previous 4000 years, suggesting major intensification of directed breeding in the recent past [1]. As a consequence, modern horses have an average 4.6% higher overall 'load' of mutations with potential negative health and fitness consequences.

Looking forward, as both horse and human paleogenomic databases continue to expand, it will become increasingly feasible to compare spatiotemporal genetic patterns between our two species on a region-by-region and ultimately archaeological site-by-site basis. Similar analyses can also be extended to other domesticated and otherwise human-associated animals and plants to help to develop an increasingly rich picture of the processes by which people intensified their exploitation and management of food, transport, and material resources.

Simultaneously, we encourage the development of deeper investigations into within-archaeological site patterns of horse genetic diversity. For example, many (and likely a disproportionate number of) horse burials excavated by archaeologists (and thus far included in the paleogenomic database) are associated with human individuals of inferred elite status (Figure 1). Through concerted excavation and sampling it may become

Figure 1. Variable Processes of Ancient Horse Burial and Skeletal Assemblage Accumulation. (A) Elaborate burial 2500 years before present (BP) of two Thracian horses with an associated chariot. Recovered from the Hellenistic royal necropolis of the former Northern Thracian capital in present-day northeast Bulgaria (Sboryanovo Historical and Archaeological Reserve) by an excavation led by Professor Diana Gergova of the National Archaeology Institute at the Bulgarian Academy of Sciences (photo: Diana Gergova). (B) In the foreground are comingled horse remains dated to ~5500 years BP in a midden (archaeological refuse mound) at the Botai site in present-day northern Kazhakstan. Adjacent to the midden is an excavated house. The locations of unexcavated houses are visible in the background as relatively darker green patches of grass (photo: Alan K. Outram, University of Exeter).

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increasingly possible to test whether any within-site horse genetic ancestry variation can be explained by human social organization. This type of analysis would also be interesting to perform on known trait-associated alleles. That is, at what point(s) do we begin to observe evidence suggesting distinct roles for different types (i.e., phenotypes) of horses within the same spatiotemporal horizon?

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Spotlight

Beneficial Noncancerous Mutations in Liver Disease

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Chronic liver disease results in fibrosis and cancer. While injury is associated with mutational burden, a recent study (Zhu *et al.* *Cell* 2019;177:608–621) highlights that not all positively selected mutations in the liver are precancerous. Indeed, some may be beneficial to the ability of the liver to not only withstand injury , but also to regenerate.

Genetic mutations are a hallmark of cancer. Throughout biological systems, from cells to organisms, intrinsic and extrinsic factors drive population dynamics. A nonneutral drift in cell populations is seen in health and disease, including cancer. The general consensus is that mutations that are clonally selected within a tissue are inevitable stepping stones towards the development of cancer, and not beneficial to the host.

The role of the liver in detoxification and bile synthesis exposes it to frequent damage, which it copes with due to its great regenerative potential. However, chronic damage leads to the accumulation of mutations that can lead to hepatocellular carcinoma (HCC) [1,2]. If the liver is chronically damaged, the liver lobules become separated by dense fibrosis and nodules of hepatocytes form, which are believed to be clonal in origin [3]. During nodule formation, it is unclear which mutations drive clonal 'fitness'. Understanding whether mutations driving such clonal expansion always carry the risk of malignant transformation, or whether they may instead be beneficial to liver regeneration, has importance not only scientifically, but also medically in regenerative medicine, and early cancer detection and treatment.

In a recent landmark study, Zhu *et al*. dissected the malignant potential of mutations found in nondysplastic lesions of patients with chronic liver disease by using whole-exome sequencing, followed by targeted deep sequencing [4]. After carefully excluding malignant tissue and using a highly stringent methodology for calling mutations, they were able to identify recurrent mutations occurring in clonal outgrowths in multiple patients. Intriguingly, it appeared that some of these recurring mutations were not drivers of HCC because they have not been found in previous large-scale cancer genomic studies. Using an unbiased *in vivo* CRISPR screen in mice, the authors then confirmed their observational findings, showing that many of these recurrent mutations provided a selective survival advantage to hepatocytes and, thus, represent genuine driver mutations. This screen, targeting 147 mutated genes identified in the human samples, was performed using a genetic mouse model of metabolic deficiency. This allowed the selection of clones rescued from the metabolic deficiency and compared the regeneration fitness of these clones based on the additional targeted gene mutations. Finally, Zhu *et al*. tested the regenerative capability and resistance to both acute and chronic injury after hepatic knockdown of three of their top hits, *Arid1a*, *Kmt2d*, and *Pkd1*. They showed that each was capable of either improved regeneration, reduced susceptibility to damage, or both. In summary, the authors observed clear clonal dominance from these 'fitness-promoting mutations', which did not appear to be associated with cancer.