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Widespread horse-based mobility arose around 2,200 BCE in Eurasia

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Abstract: Horses revolutionized human history with fast mobility¹. However, the timeline between their domestication and widespread integration as a means of transportation remains contentious²⁻⁴. Here we assemble a large collection of 475 ancient horse genomes to assess the period when these animals were first reshaped by human agency in Eurasia. We find that reproductive control of the modern domestic lineage emerged ~2,200 BCE (Before Common Era), through close kin mating and shortened generation times. Reproductive control emerged following a severe domestication bottleneck starting no earlier than ~2,700 BCE, and coincided with a sudden expansion across Eurasia that ultimately resulted in the replacement of nearly every local horse lineage. This expansion marked the rise of widespread horse-based mobility in human history, which refutes the commonly-held narrative of large horse herds accompanying the massive migration of steppe peoples across Europe ~3,000 BCE and earlier^{3,5}. Finally, we detect significantly shortened generation times at Botai ~3,500 BCE, a settlement from Central Asia associated with corrals and a subsistence economy centered on horses^{6,7}. This supports local horse husbandry before the rise of modern domestic bloodlines.

One-Sentence Summary: Accelerated production of horses promoted rapid and long-distance human mobility from ~2,200 BCE.

Main Text: The genetic makeup of modern domestic horses (hereafter, DOM2) emerged in the Western Eurasian steppes during the third millennium BCE². The spread of DOM2 horses, alongside the development of Sintashta spoke-wheeled chariots in Asia (~2,200-1,800 BCE), and the apparently limited DOM2 genetic influence in Europe before that time, have indicated that long-distance horse-based mobility developed no earlier than the late third millennium BCE. This chronology implies that the spread of steppe-related ancestry that reshaped the human genetic landscape of nearly all regions of central and western Europe over the course of the 3rd millennium BCE^{8,9} was not driven by DOM2 horseback riding.

However, recent population models have claimed significant DOM2 genetic ancestry into European horses affiliated to the Corded Ware Complex (CWC), a culture that developed from ~3,000 BCE against the backdrop of the Yamnaya steppe migration⁴. Bone pathologies potentially resulting from regular horseback riding also occur in ~5% of the human skeletons from the Carpathian Basin, mainly in steppe-related⁸ Yamnaya individuals, but also in pre-Yamnaya, up to the fifth millennium BCE⁵. Moreover, horse-related terminology commonly shared across Indo-European (IE) languages is often considered indicative of established equestrianism in the steppes, amongst Yamnaya-related proto-IE speakers³. These findings have revived theories associating horseback riding with the Yamnaya expansion³, and possibly with earlier human steppe migrations into the Carpathian basin after ~4,500 BCE¹⁰.

Also controversial is whether rapid mobility was the only incentive for horse domestication. Equine milk peptides were reported in Yamnaya human dental calculus ~3,300-2,600 BCE¹¹, but further work has

206 revealed that western steppe pastoral practices shifted from sheep and cattle dairying to horse milking no
207 earlier than ~1,000 BCE¹². Archaeological evidence for pre-Yamnaya horse milking and harnessing^{6,7} exists
208 further east in Central Asia, within the 5,500 year-old Botai culture, which developed a subsistence economy
209 almost entirely focused on horses¹³. At this site, evidence for horse milk consumption is supported by
210 residue analysis of fatty acids absorbed into pottery sherds (N=5), but this is not corroborated by the
211 palaeoproteomic analysis of human dental calculus (N=2)^{6,11,14}.

212 Furthermore, the unusual pattern of dental attrition on the Botai horse teeth initially identified as
213 bit wear¹⁵ has been challenged¹⁶. Unchanged sex-ratios in pre-Botai and Botai bone assemblages have also
214 advocated against the emergence of new horse management practices at Botai^{17,18}. Considering that DOM2
215 and Botai horses originate from two genetically distinct lineages⁷, new lines of evidence are needed to assess
216 the exact role played by horses in the Botai society, and more generally, how domestic horses contributed
217 to the steppe migrations and the possibly concurrent spread of IE languages (although see¹⁹).

218 **Datasets and experimental design**

219 To address the context into which horse husbandry developed in the fourth and third millennia BCE, we
220 analysed a total of 475 ancient horse genomes (Fig. 1a), combined with 77 publicly available modern horse
221 genomes, including 40 worldwide domestic breeds and six endangered Przewalski's horses (Table S1;
222 Extended Data Figs. 1 and 2). The 124 newly generated genomes show a median coverage of 1.40-fold (min
223 = 0.29; max = 10.92) and span Eurasian archaeological contexts dating over ~50,000 years ago, including
224 in the Carpathian basin, where bioanthropological evidence for horseback riding was reported^{5,20}. Together
225 with 401 radiocarbon dates, 140 of which are new, our dataset provides an unprecedented genome time-
226 series spanning the whole domestication process.

227 In this study, we investigate three possible markers of horse husbandry. First, we examine changes
228 in the genomic makeup of horses across Central and Eastern Europe to test whether they accompanied the
229 humans that moved from the steppe. Second, we reconstruct horse demographic trajectories to evaluate the
230 existence, timing and severity of domestication bottlenecks. This reveals when horses were bred in
231 significant numbers to sustain large-scale mobility. Third, we track evidence for controlled reproduction of
232 horses, in the form of close kin mating and accelerated generation times.

233 **Spread of DOM2 horses across Europe**

234 Assuming that steppe humans and horses moved together implies parallel shifts of genetic ancestry in both
235 species. Such concurrent shifts were supported by the population graphs presented by Maier and colleagues⁴,
236 who identified horses excavated from a CWC context in Germany with ~20% DOM2 ancestry, somehow
237 mirroring the ~70% Yamnaya-related steppe ancestry observed in humans⁸. However, Locator²¹ analyses
238 predict that the geographic origins of CWC horses is exclusively within Central Europe (Extended Data Fig.
239 3cd). We also identify population graphs fitting published data significantly better than those previously
240 proposed^{2,4} (p -value $<10^{-5}$; Extended Data Fig. 3b), and refining our understanding of the connectivity
241 between the steppes and the rest of Europe by including four additional population groups (Extended Data
242 Fig. 4). No such graphs support DOM2 genetic contribution into CWC horses (Extended Data Figs. 3ab
243 and 4), with the most comprehensive placing CWC horses close to pre-Yamnaya populations from Central
244 Europe (ENEOCZE, ~3,364-3,102 BCE, and NEOPOL, ~5,210-5,006 BCE). That a Central European
245 horse lineage remained isolated from the steppe is also supported by adjacent positioning in Multiple
246 Dimension Scaling analysis (Extended Data Fig. 5), distinctive ancestry profiles sharing the major genetic
247 component of CWC horses (Fig. 1bc, Extended Data Fig. 6), and qpAdm modeling (Table S2). qpAdm
248 models including two population sources indeed depict CWC horses as a mixture between ENEOCZE
249 (32.4%) and Northern European horses (FBPWC, ~3,050-2,950 BCE; 67.6%), while allowing for a third
250 source only returns negligible steppe contribution ($\leq 1.7\%$). Combined, these analyses uncover a distinct
251 cline of genetic ancestry peaking in CWC horses and declining both westwards (LPNFR, ~13,969-12,090
252 BCE), and eastwards across Central Europe (ENEOCZE and NEOPOL), the Carpathian and
253 Transylvanian Basins (HUNG, ~3,364-1,971 BCE, and; ENEOROM, ~4,494-3,658 BCE), and Anatolia
254 (NEOANA, ~6,396-4,456 BCE) (Fig. 1bc).

255 The CWC-related ancestry survives within wild European horses called "tarpan" (~45.1%) until
256 ~1868 CE in our dataset (and possibly later in the last surviving captive or free-ranging tarpans²²), but is at
257 best residual in the genetic makeup of modern domestic horses (Fig. 1b). In fact, it vanishes with the
258 expansion of the typical DOM2 ancestry profile outside of the steppe (Fig. 1c). Our extended time-stamped
259 panel of ancient genomes from the Carpathian Basin provided increased temporal resolution regarding the
260
261

262 arrival of DOM2 horses and the replacement of the local lineage found there (HUNG). This is pivotal for
263 clarifying the role of horses in the human migrations from the steppe. The date for the first typical DOM2
264 horse in the Carpathian Basin is ~1,822 BCE (1,895-1,749 BCE), while that for the last horse with a typical
265 local HUNG genetic profile is ~2,033 BCE (2,120-1,945 BCE). Considering individual archaeological sites,
266 rather than the whole region, indicates similar chronologies (at Budapest-Királyok Útja: ~1,822 BCE (1,895-
267 1,749 BCE) vs ~2,211 BCE (2,284-2,138 BCE), and; Százhalombatta-Földvár: ~1,822 BCE (1,893-1,751
268 BCE) vs ~2,033 BCE (2,120-,1945 BCE)) (Table S1). Combined, these findings narrow down the time for
269 the genomic turnover accompanying the arrival of DOM2 horses in the Carpathian basin to ~2,033-1,945
270 BCE. This timeline is consistent with the first evidence of DOM2 horses outside of the steppe reported by
271 Librado and colleagues (2021)² in Moldavia ~2,063 BCE (2,140-1,985 BCE), Anatolia ~2,125 BCE (2,205-
272 2,044 BCE), and Czechia ~2,037 BCE (2,137-1,936 BCE), post-dating the arrival of human steppe-related
273 ancestry in the respective regions by at least 600 years^{10,23}. Yamnaya-related steppe migrations and the spread
274 of DOM2 horses are, thus, chronologically incompatible.

275 However, humans may have migrated from the steppe using horses other than DOM2. To
276 investigate this, we mapped the genetic ancestry identified by Struct-f4²⁴ as characteristic of horse
277 populations living across the steppe before the expansion of DOM2 (C-PONT, TURG, and NEONCAS;
278 ~5,616-2,636 BCE; Fig. 1b). Around ~17.2% of this ancestry was present in the Carpathian Basin during
279 the fourth and third millennia BCE (~3,364-1,971 BCE). However, we find it also in Austria ~3,300 BCE
280 (28.9%, KT46), and in the Transylvanian Basin ~4,200 BCE (54.5%, ENEOROM), at the Pietrele site where
281 the genomic makeup of human populations suggests no steppe contact¹⁰. In fact, the steppe-related genetic
282 ancestry is found in even earlier horse populations spanning a broad geographic range, including Poland
283 (NEOPOL, ~5,210-5,006 BCE), Anatolia (NEOANA, ~6,396-4,456 BCE), Iberia (IBE, ~5,299-1,900
284 BCE), and as far back in time as in the Upper Paleolithic of France (LPNFR, ~13,969-12,090 BCE, and;
285 LPSFR, ~21,909-14,646 BCE). This is consistent with the best-fitting population graph showing
286 ENEOROM horses receiving steppe genetic material from an ancestor that also contributed to LPSFR
287 populations (Extended Data Fig. 4). Therefore, the spread of steppe-related horse genetic ancestry into
288 Europe must predate ~14,646 BCE, which is considerably earlier than any claimed evidence for horse
289 husbandry³ and, thus, occurred through natural contacts between wild populations, most likely dispersing
290 in the aftermath of the Last Glacial Maximum (~24,000-17,500 BCE)²⁵. Combined, the genomic makeup
291 of ancient European horses does not endorse widespread horse-driven mobility before the end of the third
292 millennium BCE. It, thus, dismisses any significant involvement of horses in the Yamnaya-related or earlier
293 human migrations from the steppe.

294 295 **DOM2 demographic history**

296 To time precisely the rise of widespread horse-based mobility, we next estimated the period when DOM2
297 horses were bred in sufficiently large numbers to sustain their global spread. Specifically, we tracked changes
298 in the DOM2 effective population size (N_e) during the 200 generations preceding ~1,864 BCE, which is the
299 average date of the earliest 24 DOM2 horses in our dataset with sufficient sequence data (Fig. 2a). Crucially,
300 LD-based demographic reconstructions²⁶ indicate a sharp demographic burst of ~13.7-fold increase within
301 the 30 generations preceding that period. Matching those 30 generations with the Yamnaya-related steppe
302 expansion, which had already reached Central Europe by ~2,750 BCE at the latest⁸, would require unrealistic
303 average generation times of ~27 years, largely exceeding horse life expectancy under modern intensive
304 veterinarian care^{27,28}. Assuming instead the commonly accepted generation time of 8 (12-7) years²⁹⁻³²
305 provides ~2,190 (2,310-2,160) BCE for the rise of widespread horse-based mobility. Restricting analyses to
306 horses from Sintashta contexts, which are associated with the spread of spoke-wheeled chariots in Asia,
307 returns similar demographic profiles and time estimates (~2,100 BCE; 2,200-2,075 BCE); Extended Data
308 Fig. 7a). These timelines not only coincide with the radiocarbon dating of the earliest DOM2 horses outside
309 of the steppe, but also with the earliest horse images in Akkadian art^{33,34}, and with major evidence of
310 conflicts, crises and political disruption, from the Balkans to Egypt and the Indus valley^{35,36}.

311 Our demographic reconstructions also provide the first evidence for a strong domestication
312 bottleneck in horses during the 75 generations preceding the DOM2 expansion (Fig. 2a). The interval
313 associated with minimal effective sizes (N_e ~500 diploid individuals) starts ~2,664 (3,064-2,564) BCE.
314 Therefore, the time when steppe people migrated did not coincide with expanding but plummeting
315 availability of DOM2 reproductive horses, which aligns with horses not driving Yamnaya-related steppe
316 migrations. Interestingly, the first evidence for horses carrying long runs of homozygosity only (≥ 15 cM),
317 which is indicative of close kin mating, is found in some of the earliest DOM2 sequenced (Fig. 2c), including

318 in the steppes of Central Asia and Anatolia. This indicates that the reproductive control underlying early
319 DOM2 spread involved some levels of inbreeding, which is avoided in the wild but represents a common
320 practice for breeding animals with desirable traits³⁷.

321 322 **DOM2 generation time contracted 2200 BCE**

323 In addition to the practice of close kin mating, early DOM2 breeders may have aimed to produce more
324 animals every year to meet the explosive demand for horses in the late third millennium BCE. To test
325 whether breeders used younger animals for reproduction, we developed two complementary proxies
326 measuring generation times from single pseudo-haploid time-stamped genomes. The first quantifies the
327 number of generations required for a genome to accumulate an observed number of mutations post
328 divergence from outgroup(s) (mutation clock; Supplementary Methods; Extended Data Fig. 8a). The second
329 leverages recombination patterns to estimate the number of generations elapsed since the most recent
330 common ancestor of the sampled specimens (recombination clock; Supplementary Methods; Extended
331 Data Fig. 9ab). We validate the performance of our methodology through coalescent simulations across
332 various inbreeding levels and demographic trajectories (Extended Data Fig. 10), and apply it to all of our
333 radiocarbon dated horse genomes to estimate ~7.4 years as the average time between two consecutive
334 generations in the last 15,000 years (Fig. 3b; Supplementary Information).

335 Our analyses also reveal that horse generation times did not remain constant, but accelerated ~1.8-
336 fold (~4.1 years) during the last ~200 years, as could be expected given the development of modern breeding
337 practices, optimized for animal production (Fig. 3a). Racing Quarter Horses and Thoroughbreds exemplify
338 breeds with the least accelerated generation time, possibly due to the extended reproductive lifespan
339 imposed on sport champions (Fig. 3a). No equivalent changes were detected backwards in time until
340 ~2,200-2,100 BCE, which coincides with a ~2.1-fold acceleration of the generation time, relative to the
341 ~7.4 average (~3.5 years; Fig. 3b). This acceleration did not affect any of the DOM2 relatives, including
342 those with individuals affiliated to Yamnaya, Turganik and Steppe-Maykop contexts (CPONT and TURG;
343 Fig. 3 and Extended Data Fig. 7c), and the older horses living in the steppe (NEONCAS), or in the
344 Carpathian and Transylvanian Basins (HUNG and ENEOROM; Extended Data Fig. 7c). This reveals that
345 new practices of DOM2 reproductive control, aimed at faster productivity, emerged by the late third
346 millennium BCE, and were a prerequisite to early DOM2 breeding and adoption of widespread horse-based
347 mobility.

348 349 **New evidence of horse husbandry at Botai**

350 Earlier research established minimal connectivity between horse populations during the fourth millennium
351 BCE². As this encompasses the timeline of the Botai settlement (~3,500 BCE), where controversial evidence
352 for horse domestication was found, the incentive for domestication at Botai, if any, could not be long-
353 distance horseback riding. In the 36 horses from the Botai site analysed, we found no evidence for close kin
354 mating, but shortened generation times, an acceleration comparable in magnitude to that accompanying
355 DOM2 breeding (Fig. 3). This trend is specific to Botai and a group directly descending from Botai (Borly4,
356 ~3,000 BCE; Fig. 3 and Extended Data Fig. 7d)⁷, and remains unprecedented in scale throughout the Ice
357 Age to the Eneolithic. Importantly, the Botai horse population experienced a 2.4-fold demographic
358 expansion starting ~80 generations before settlement (Fig. 2b), i.e. ~4,140 (4,460-4,060) BCE assuming
359 average generation times of 8 (12-7) years. This largely concurs with paleoclimatic data suggesting more
360 humid conditions, and pollen records indicating no forest encroachment on the steppes³⁸. These favorable
361 conditions for horses may have encouraged humans to settle and develop a subsistence economy almost
362 entirely focused on horses³⁹, hypothesized as primarily obtained through hunting⁴⁰. However, our
363 demographic reconstructions indicate that this once thriving resource progressively declined during the last
364 20 generations of Botai (i.e. within 140-240 years; Fig. 2b). In response to declining food resources, Botai
365 peoples may, thus, have exercised husbandry practices involving corralling and horse reproductive control
366 through shortened generation times, in line with the prey domestication pathway^{6,41}.

367 368 **Discussion**

369 This study tackles crucial debates regarding horse domestication with major implications for both horse and
370 human history. It reveals that the horse genomic makeup remained entirely local in Central Europe as well
371 as the Carpathian and Transylvanian Basins until the end of the third millennium BCE. This timeline
372 postdates the period of steppe contact in the Carpathian and Transylvanian Basins starting ~4,500 BCE¹⁰,
373 as well as the migrations potentially spreading proto-IE languages into Europe with the Yamnaya

374 phenomenon ~3,000 BCE. The dramatic spread of DOM2 horses immediately followed the foundation of
375 this new bloodline, and marked a new era of widespread horse-based mobility from ~2,200 BCE, ushering
376 in a monumental increase in connectivity and trade. It mirrors the archaeological record, which witnesses a
377 massive spread of horses in the Near East and Asia during the transition between the third and second
378 millennium BCE^{2,42,43}. Intensified herding practices¹², growing aridity (the so-called “4.2 ka BP aridification
379 event”⁴⁴), and/or increased exploitation of the steppe may have heightened the demand for expanding
380 grazing areas, potentially facilitated by horse-mediated mobility. Domestic horses and spoke-wheeled
381 chariots^{3,42} may also have aided the conquest and defense of larger geographic areas in the face of uprising
382 violence and social conflicts^{35,36}.

383 Our work does not reject the possibility of equestrianism developing in the Pontic steppe or the
384 Carpathian Basin before ~2,200 BCE. However, in such a scenario, the associated breeding practices would
385 not have involved close kin mating or accelerated generation times. The phenomenon would also have
386 remained confined in scale, both demographically and geographically, excluding long-distance fast mobility
387 as the primary domestication incentive. Our research strengthens the case for recognizing Botai as one such
388 location in the Central Asian steppe where horse husbandry developed before large-scale horse-based
389 mobility. There, the domestication process did not aim at global production but remained regional. It is
390 aligned with the expectations of the prey pathway⁴¹, in which a settled group of humans developed
391 husbandry through corralling and reproductive control, in the form of shortened generation times, but not
392 close kin mating, to ensure access to an otherwise depleting meat resource¹³.

393 Manipulating the animal life cycle by forcing earlier reproduction offers breeders enhanced
394 productivity, especially for species with long gestational periods and/or small litter sizes. Our research
395 demonstrates that this practice was integral to the array of breeding techniques developed to sustain the
396 massive global demand for horses from the Early Bronze Age. The pressure for accelerated production
397 relaxed quickly after ~1,000 BCE, as a large-enough horse breeding pool became available across extensive
398 geographic areas. However, the development of modern breeds required the fast production of specific
399 bloodlines from limited foundational stocks, which shortened again the horse generation time over the last
400 few centuries. Apparently, this process affected Asian breeds more than racehorses (Fig. 3a), especially
401 Thoroughbreds, for which artificial insemination is forbidden. These findings align with studbook pedigrees
402 recording increasingly faster generation times during the last three centuries, especially in coldblood horses⁴⁵.

403 Our methodological framework for measuring generation times expands the bioarchaeological
404 toolkit to detect molecular evidence of reproductive control. Together with close kin mating, it may prove
405 instrumental in clarifying the timing and context(s) into which past human groups first developed animal
406 husbandry, not only in horses, especially as early domestication processes may not always leave obvious
407 skeletal modifications and marked foundational bottlenecks. Beyond domestic animals, our approach could
408 be applied to measure the long-term generation times of ancient hominin groups, including Neanderthals
409 and Denisovans, and their potential shifts in the face of major lifestyle transitions, such as following the
410 Out-of-Africa dispersal, during the Ice Age⁴⁶ as well as during the Neolithic revolution^{47,48}. For now, our
411 analyses suggest that the last Ice Age may have impacted horse generation times, though to a lesser extent
412 than domestication (Fig. 3). Our work, thus, opens for a new line of research investigating the possible
413 consequences of past and present environmental and epidemiological crises on the reproduction of both
414 human groups and other species.

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511 Figure Legends

512 **Figure 1. Geographic distribution and genetic profiles of the 475 ancient horse genomes analysed**
513 **in this study.** **a)** Geographic location of the archaeological sites. The size of each location is proportional
514 to the number of horse genomes sequenced. The black dot points to the location of *E. ovodovi* outgroups.
515 **b)** Struct-f4 genetic ancestry profiles considering K=9 components. The top panel provides the color legend
516 for panel a). **c)** and **d)** Genetic ancestry profiles (K=9) across Central Europe, the Carpathian and
517 Transylvanian Basins before c) and after d) 2,150 BCE. The midpoint of the radiocarbon dating range
518 obtained for each site is indicated between parentheses.
519

520
521 **Figure 2. Horse demographic trajectory and inbreeding profiles.** **a)** GONE²⁶ demographic
522 reconstruction based on 24 early DOM2 horse genomes, where the thicker line depicts the most likely
523 effective population size up to 200 generations preceding ~1,864 BCE, and the thinner lines are 500
524 bootstrap pseudo-replicates. Conversions to calendar years BCE assume either average generation times of
525 8 (7-12) years, or our refined estimate for the time periods considered. **b)** Same as a) but for a set of 28
526 Botai horse genomes. **c)** Total fraction of the genome encompassing Runs-of-Homozygosity (ROH) of
527 various sizes, in which each dot represents a horse genome. For example, the category [1-2Mb) indicates
528 the fraction of a genome within ROH) that are longer than or equal to 1Mb, but shorter than 2Mb.
529

530 **Figure 3. Horse generation times.** **a)** Number of generations evolved since the Most Recent Common
531 Ancestor (MRCA) of all samples, as estimated from the recombination clock (y-axis) for each radiocarbon-
532 dated horse specimen (x-axis, age of the specimen; n=483). Samples are color-coded according to Fig. 1a.
533 The top inner panel breaks down the number of generations evolved for modern breeds. Each boxplot
534 summarizes the estimates per breed (Table S1), including its corresponding centre (median), box boundaries
535 (interquartile range), and whiskers (1.5 times the interquartile range). **b)** Time periods associated with
536 significant changes in horse generation times. The graph represents the slope (δ_{time}) of a General Additive
537 Model (GAM) regressing radiocarbon dates and number of generations evolved since MRCA, while
538 controlling for sequencing depth and population structure. This slope is, thus, proportional to the generation
539 time at a particular time period. The double-sided arrow reports the average generation time in the last

540 15,000 years (Supplementary Information). The error band represents the 95% confidence interval for the
541 GAM regressions. **c)** Same as b), but excluding BOTAI and BURL population groups.

542

543 **Methods**

544 **Archeological samples and radiocarbon dating**

545 We have gathered an extensive collection of 475 ancient horse remains spread across 230 sites in 41
546 countries. Sampling of archaeological horse remains was undertaken in collaboration with co-authors
547 responsible for the curation and description of underlying contexts, and with the approval of the relevant
548 institutions responsible for the archaeological remains, as detailed in the Reporting Summary. A total of 105
549 out of the 124 newly sequenced specimens originate from archaeological sites for which no ancient horse
550 genomes were characterized previously. Their underlying archaeological contexts are described in the
551 Supplementary Information. A total of 140 new radiocarbon dates were obtained in this study, all of which
552 at the Keck Carbon Cycle AMS Laboratory, UC Irvine (Table S1). Collagen was extracted and ultra-filtered
553 following mechanical cleaning of ~200 mg of cortical bone. Radiocarbon dates were calibrated using
554 OxCalOnline⁴⁹ and the IntCal20 calibration curve⁵⁰. Samples were named with reference to their original
555 internal label, followed by a 3-letter country code and their associated age in calendar years (Before
556 Common Era ((B)CE), all separated by underscore signs and appending the age with the 'm' prefix if BCE
557 (e.g. KT46_Aus_m3240 refers to sample KT46, originating from the Kittsee site from Austria, which
558 showed a midpoint radiocarbon date of 3240 BCE).

559

560 **Genome sequencing**

561 Osseous samples were processed for DNA extraction, library construction and shallow sequencing in the
562 ancient DNA facilities of the Centre for Anthropobiology and Genomics of Toulouse (CNRS and
563 University Paul Sabatier), France. The overall methodology followed the work from Librado and colleagues
564 (2021)², including: (1) powdering with the Mixel Mill MM200 (Retsch) Micro-dismembrator; (2) DNA
565 extraction according to the procedure Y2 from Gamba and colleagues (2016)⁵¹; (3) USER (NEB) enzymatic
566 treatment³⁰; (4) DNA library construction from double-stranded DNA templates DNA libraries in which
567 two internal indexes are added during adapter ligation and one external index is added during PCR
568 amplification; and (5) PCR amplification, purification and quantification on the TapeStation 4200 (D1000
569 HS) instrument before pooling for Illumina DNA sequencing on MiniSeq, NovaSeq and/or HiSeq4000
570 instruments (paired-end mode). Sequencing pools were prepared to represent each of the three individual
571 indexes only once.

572 FASTQ sequencing reads demultiplexing, trimming and collapsing was carried out using
573 AdapterRemoval2 (version 2.3.0⁵²) disregarding reads shorter than 25 bp. The resulting collapsed and
574 uncollapsed read pairs were processed through the Paleomix bam_pipeline (version 1.2.13.2)⁵³ for Bowtie2⁵⁴
575 alignment against the nuclear and mitochondrial horse reference genomes^{55,56}, appended with the 751 Y-
576 chromosome contigs from Felkel et al. (2019)⁴⁵, using the parameters recommended by Poulet and Orlando
577 (2020)⁵⁷, and removing PCR duplicates and requiring minimal mapping quality scores of 25. The presence
578 of DNA fragmentation and nucleotide misincorporation patterns indicative of post-mortem DNA damage
579 was assessed on the basis of 100,000 random mapped reads using mapDamage2 (version 2.0.8⁵⁸). Overall,
580 we obtained sequence data from 390 DNA libraries for a total of 124 ancient horse specimens, resulting in
581 genome characterization at an average depth-of-coverage of 0.288-10.925-fold (median 1.40-fold; Table S1),
582 as estimated using Paleomix coverage (--ignore-readgroups). The sequence data from a total of 352 ancient
583 and 81 modern genomes were processed following the same procedures to provide a comparative genome
584 panel including four donkeys⁵⁹, two *E. ovodovi*⁶⁰ and two Late Pleistocene North American horses⁶¹ that
585 were used as outgroups, plus 550 horses representing all lineages previously characterized at the genome
586 level (Table S1).

587

588 **Genome rescaling and trimming, error rates and SNP variation**

589 Sequencing errors and nucleotide mis-incorporations resulting from post-mortem DNA damage were
590 reduced subjecting alignments to a five-step procedure, including: (1) PMDtools (version 0.60⁶²)
591 identification and separation of those reads affected (--threshold 1; DAM) or not (--upperthreshold 1;
592 NODAM) by post-mortem DNA damage; (2) 5-bp end-trimming of NODAM-aligned reads; (3) rescaling
593 of DAM read alignments using mapDamage2 with default parameters (version 2.0.8⁵⁸); (4) 10-bp trimming
594 of rescaled read alignments, and; (5) merging of processed NODAM and DAM categories to obtain final
595 BAM sequence alignments. Error rates were estimated following Librado and colleagues (2021)² as the

596 excess of private mutations, relative to a high-quality modern genome considered as error-free
597 (P5782_Ice_Modern; Table S1). Single Nucleotide Polymorphisms were identified following the procedures
598 from Librado and colleagues (2021)², entailing data pseudo-haploidization with ANGSD (v0.917⁶³) for
599 those sites covered by two reads or more (base quality scores ≥ 30), and disregarding sites uncovered in
600 30% or more of the samples. An additional filter included the random selection of one transversion SNP
601 only, in cases where two successive transversion occurred in adjacent genomic positions. Overall, our final
602 data set retained a total of 9,099,487 high-quality nucleotide transversions spread across the 31 horse
603 autosomes. Alleles were polarized considering the allele common to the three outgroup lineages as ancestral.
604 A second data set of 7,092,366 variants was generated to mitigate for possible bias introduced by uneven
605 sequencing depths by repeating the procedure described above, but following the down-sampling of BAM
606 alignment files to the median value of the average depth-of-coverage values found across all specimens (i.e.
607 2.02-fold). Subsequent analyses were replicated on both variant data sets.

609 **Population graph modelling and population structure**

610 Population graph modeling was carried out using the MCMC framework implemented in AdmixtureBayes⁶⁴,
611 and in Admixtools2⁴, considering a pre-selection of 14 and 10 genetically-homogeneous population groups,
612 respectively, all represented by a minimum of two specimens. This was key for Admixtools2 analyses⁴, to
613 avoid biasing f_3 -statistics⁴ in the presence of population groups comprised of a single pseudohaploid
614 genome. AdmixtureBayes analyses involved three independent runs, each containing 163 MCMC chains
615 recording 200 million iterations. The final space of population graphs was obtained using a burnin of 90%
616 and thinning one every 40 iterations. The genomic makeup of CWC horses was further investigated through
617 the qpAdm rotating scheme⁶⁵ (Table S2), and using a threshold of 0.01 for statistical significance. The
618 geographic origins of CWC horses were also predicted using the Locator methodological framework based
619 on deep neural networks²¹. To achieve this, we considered genomic window sizes of 10 Mb and the panel
620 of 148 ancient horses pre-dating the radiocarbon date of CWC horses. Genetic ancestries decomposition
621 and multi-dimensional scaling were carried out using the Struct-f4 package²⁴, grouping together 272 ancient
622 and modern DOM2 horses to decrease computational costs. The first analytical step (assuming no
623 admixture) consisted of 100 million MCMC iterations, while the second one (assuming admixture) involved
624 500 million iterations, until strict convergence. Default parameters were used otherwise, and the analyses
625 were repeated assuming $K=8$ to $K=10$ admixture edges.

627 **Inbreeding**

628 Per genome inbreeding levels were estimated applying the methodology from Todd and colleagues (2022)⁵⁹
629 to individual BAM alignment files. This methodology does not require prior knowledge of population allele
630 frequencies; it involves instead the random sampling of two reads per nucleotide transversion position and
631 considering the density of sites within 1cM-long genomic windows where the same allele was sampled twice
632 (pseudo-homozygosity), versus two different alleles (pseudo-heterozygosity). Physical distances were
633 converted into genetic distances using the recombination map from Beeson and colleagues (2020)⁶⁶,
634 interpolating recombination rates linearly between two successive positions on the map. Windows showing
635 pseudo-heterozygosity rates lower than 0.005 were considered to represent Runs of Homozygosity (ROHs),
636 with their cumulative span providing an inbreeding proxy. Close-kin mating was assessed through the total
637 genome span encompassing long ROHs (i.e. ≥ 15 Mb).

639 **Demographic trajectories**

640 A total of 28 genomes from unrelated Botai horses were pseudo-haploidized for transversion sites, all with
641 a maximum missingness of 10%. The demographic dynamics was reconstructed using GONE²⁶ and
642 patterns of linkage disequilibrium along all autosomes, excepting chromosomes 7, 11, 12 and 20. The
643 parameter PHASE was turned to 0 to account for pseudo-haploid data, and default parameters were applied
644 otherwise. Confidence intervals for effective size variation were estimated from 500 bootstrap pseudo-
645 replicates. The same procedure was repeated considering a selection of 24 ancient horse genomes dating
646 back to an average of $\sim 1,850$ years BCE, which represents the earliest high-quality set of DOM2 genomes
647 characterized.

649 **Generation times**

650 Generation times and their potential variation were measured from the temporal accumulation of mutations
651 present in a given genome relative to an ancestral sequence (reconstructed based on three outgroup species;

652 i.e. mutation clock) as well as from the linkage disequilibrium between pairs of derived mutations (i.e.
653 recombination clock). The proportion of derived mutations present in a given genome provided a direct
654 proxy for the distance separating the sample considered from the ancestral sequence. This proportion was
655 converted into an estimate of number of generations, assuming the mutation rate from Orlando and
656 colleagues (2013)²⁹, rescaled for transversions, which provided our mutation clock estimate of generations
657 elapsed from the ancestral sequence.

658 Our ‘recombination clock’ estimate is based on the average probability to find in a given genome a
659 pair of SNPs separated by m Morgans, and both carrying a derived allele. This probability was normalized
660 by the proportion of derived mutations detected in the genome considered to mitigate potential bias
661 resulting from depth-of-coverage and/or error rates variation across individuals, providing a direct
662 measurement of the number of generations from the Most Recent Common Ancestor (MRCA) to all
663 Eurasian horses present in our data set. While the ‘mutation clock’- based estimate was derived from all 31
664 autosomes, chromosomes 7, 11, 12 and 20 were masked to obtain the ‘recombination clock’ estimate, due
665 to limitations in the recombination map currently available for horses in relation to unaccounted structural
666 variation, local mis-assemblies and the presence of neo-centromeres. The ‘recombination clock’ estimate
667 depends on three unknown parameters that were optimised through least square optimization (T : the total
668 genealogical length in the whole sample set averaged across loci; t_i : the genealogical length from the MRCA
669 to horse specimen i averaged across its loci, and a constant p_i capturing sample-specific variation in
670 demography and haplotype sizes).

671 Our methodology was validated using the serial coalescent simulation framework provided by
672 fastsimcoal v2.702⁶⁷ and considering 10 demographic scenarios, consisting of constant population sizes,
673 population contractions and population expansion of various magnitude and times, followed or not by
674 population recovery (Extended Data Fig. 10). Individual genomes were simulated as 31 autosomes of 75
675 Mb each, using 10^{-8} recombination events and 2.3×10^{-8} mutation events per bp and generation, respectively.
676 A total of 20 simulated chromosomes were sampled along the genealogy every 100 generations, starting 900
677 generations ago, to cover the entire temporal range of horse domestication. The 20 simulated chromosomes
678 sampled in each time bin, except the most recent, were then randomly paired to simulate diploid data under
679 random mating, and were further subjected to pseudo-haploidization to mimic the data processing carried
680 out on real data. The simulated chromosomes sampled for the most recent time period were paired with
681 themselves before pseudo-haploidization to account for the increased inbreeding levels found in modern
682 horse populations⁶⁸.

683 The real genome dataset was filtered to exclude the IBE, LPSFR, ELEN and Vert311 population
684 groups, which contain significant ancestry affinities with Late Pleistocene specimens from North America
685 (LPNAMR). This prevented biasing the generation time estimates due to DNA introgression from divergent
686 population groups, related to lineages used to polarize alleles as ancestral or derived. Ancient specimens not
687 associated with direct radiocarbon dating were also disregarded, except at Botai where the archaeological
688 context is similar across all samples. This left a total of 483 specimens delivering both “mutation clock” and
689 “recombination clock” estimates for the number of generations elapsed from the ancestral sequence and
690 since the tMRCA of Eurasian horses, respectively. Temporal shifts in generation times were identified based
691 on the downsampled dataset (Fig. 3), and using Generalized Additive regression Modeling (GAM), as
692 implemented in the R *mgvc* package. Radiocarbon dates, the first five coordinates of the Struct-f₄
693 MultiDimensional Scaling (MDS) analysis to capture the underlying population structure, and a parameter,
694 P_i , controlling for the depth-of-coverage of each individual genome, represented the model covariates.
695 Standard errors for the dependent variable were calculated by jackknifing, leaving one chromosome out at
696 a time, and the inverse of the resulting variances were used as regression weights. Regression models in
697 which radiocarbon dates were linearly related to the number of generations received significantly lower
698 support than those allowing relaxing linearity through cubic spline transformation of radiocarbon dates
699 (adjusted $R^2 = 0.803$ for the linear vs. 0.894 for the GAM regression; ANOVA $P < 2.2e^{-16}$). Finally, we used
700 the *derivative* function of the R *gratia* package and time bins of 1,000 years to measure temporal changes in
701 generation times.

702 703 **Data availability**

704 All collapsed and paired-end sequence data for samples sequenced in this study are available in compressed
705 FASTQ format through the European Nucleotide Archive under accession number PRJEB71445, together
706 with rescaled and trimmed bam sequence alignments against the nuclear horse reference genomes.
707 Previously published ancient data used in this study are available under accession numbers PRJEB7537,

708 PRJEB10098, PRJEB10854, PRJEB22390, PRJEB31613, and PRJEB44430, and detailed in Supplementary
709 Table 1. The genomes of 78 modern horses, publicly available, were also accessed as indicated in their
710 corresponding original publications, and in Supplementary Table 1.

711
712 The maps presented in Figure 1 were generated using QGIS 3.36 software (available at
713 <https://www.qgis.org/en/site/>) and utilized free raster images obtained from Natural Earth
714 (<https://www.naturalearthdata.com/>). The maps in Extended Data Figure 3cd were automatically
715 generated through the R scripts embedded within the Locator software package ([https://github.com/kr-](https://github.com/kr-colab/locator)
716 [colab/locator](https://github.com/kr-colab/locator)).

717 718 **Code availability**

719 The software to calculate generation time changes based on the recombination clock is available without
720 restriction on Bitbucket (<https://bitbucket.org/plibradosanz/generationtime/src/master/>) and Zenodo
721 (10.5281/zenodo.10842666; <https://zenodo.org/records/10842666>).

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767
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825

826 **Additional Information**

827 **Supplementary information** The online version contains supplementary material available at EDITOR-
828 HTTP-LINK-NEEDED. Correspondence and requests for materials should be addressed to Pablo Librado
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830

831 **Extended Data Figures**

832 **Extended Data Fig. 1. QC filtering.** **a)** Histogram showing the distance between adjacent nucleotide
833 transversions, if separated by less than 1Kbp. This revealed an excess of mutations at contiguous genomic
834 positions (*ie.* 1bp away). Although these could correspond to true single nucleotide polymorphism (SNPs)
835 or multiple nucleotide variants (MNVs), they could also be enriched for spurious variants resulting from
836 mis-mapping around small DNA insertions and deletions. **b)** Proportion of mutations within pre-defined
837 MAF bins (Minor Allele Frequency), as a function of missingness across the specimens. Pre-defined MAF
838 bins range from low- (pink) to high-frequency variants (green). The dashed line delimits the positions
839 included (left) or excluded (right) from the analyses. The identifiability of low-frequency variants decreases
840 with greater missingness, as expected. **c)** Same as panel a), for the ~7.1M nucleotide transversions of the
841 downsampled data set. **d)** Same as panel b), for the ~7.1M nucleotide transversions of the downsampled
842 data set.
843

844 **Extended Data Fig. 2. Relative error rates.** Missing mutations per site in a test genome (y-axis), relative
845 to a modern Icelandic horse (P5782_Ice_Modern) used as high-quality reference. **a)** for the full data set and
846 SNP_pval 0. **b)** for the downsampled data set and SNP_val 0.
847

848 **Extended Data Fig. 3. On the origins of CWC horses.** **a)** Consensus admixture graph generated from
849 the posterior distribution of AdmixtureBayes⁶⁴, when applied to the same horse populations considered in
850 Extended Data Fig. 4. The values between brackets summarize the proportion of graphs sampled from the
851 posterior distribution that support a split or admixture node. Admixture from unsampled (ghosts)
852 populations is not represented, in contrast to Extended Data Fig. 4. **b)** Best Admixtools²⁴ population model
853 assuming 8 migration edges. The drift and admixture estimates are based on our extended dataset. **c)**
854 Reference panel used for modeling pre-CWC clines of genetic diversity. **d)** Geospatial projection of the six
855 CWC horse genomes analyzed in this study, in 10Mb-long windows.
856

857 **Extended Data Fig. 4. Most supported population graph⁶⁴.** This graph summarizes the evolutionary
858 history of pre- and post-domestication horse lineages, with CWC horses not receiving any direct genetic
859 contribution from the steppe. The model is split into 2 panels for clarity. The numbers reported within
860 boxes reflect the admixture contributions from the nodes specified, while those adjacent to arrows indicate
861 the amount of genetic drift leading to individual nodes. Population groups are detailed in Table S1 and
862 colors are according to Fig. 1a.
863

864 **Extended Data Fig. 5. Visual embedding of Struct-f4 affinities²⁴.** **a)** The two first dimensions of a
865 Metric MultiDimensional Scaling (MDS) analysis, summarizing the genomic affinities between horses, based
866 on Struct-f4. To improve visualization, this excludes the five outgroup specimens. Samples are color-coded
867 following Fig. 1a, and population groups are labelled accordingly. Horses projecting intermediate to large
868 population groups reflect ancient clines of ancestry, stretching from the East (closer to Botai) to the West
869 (closer to Europe). CPONT individuals, from the Central Steppe, are the closest to DOM2 horses. **b)** Same
870 as a) for the downsampled dataset. **c)** First and third dimension of the same MDS analysis, which reveals
871 CWC horses as the most distant European horses to DOM2 horses. **d)** Same for the downsampled dataset.
872

873 **Extended Data Fig. 6. Struct-f4 ancestry profiles.** Ancestry proportions for the 558 individuals
874 considered in this study, assuming from K=8 (left) to K=10 (right) components. A total of 272 horses

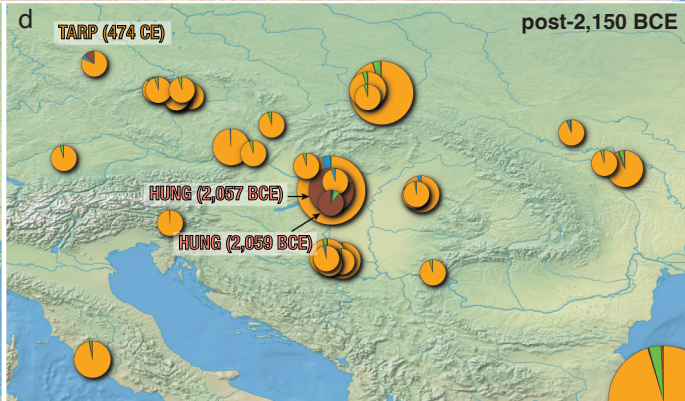
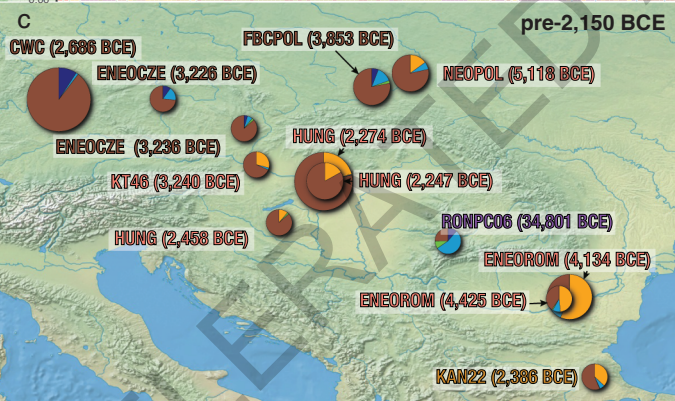
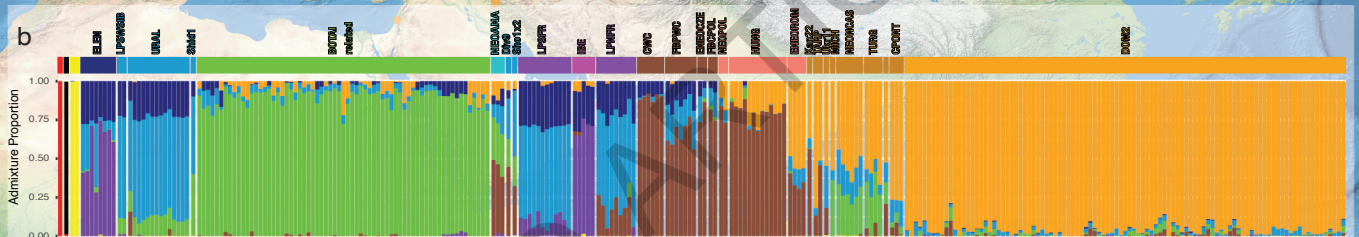
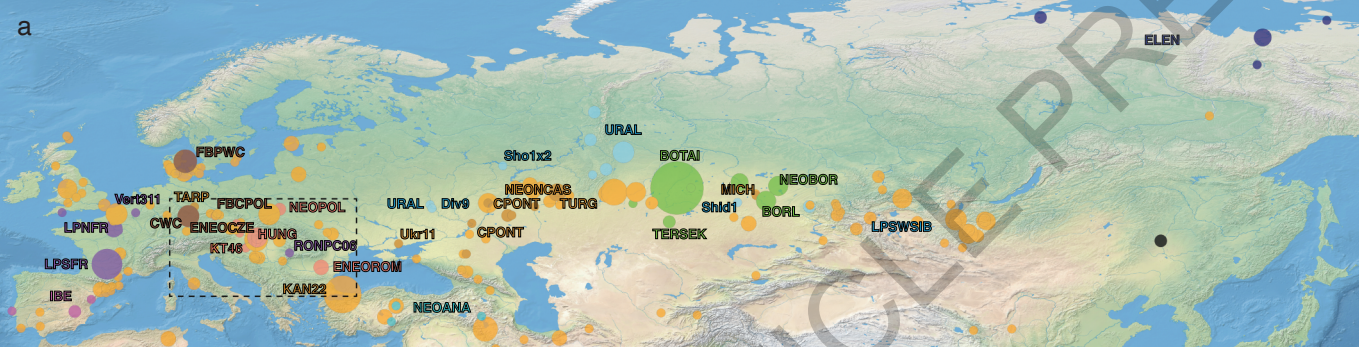
875 previously identified as DOM2 were merged into a single population (DOM2), including all modern breeds,
876 to reduce computational costs. CWC horses show the typical ancestry profile of pre-domestication Europe.
877

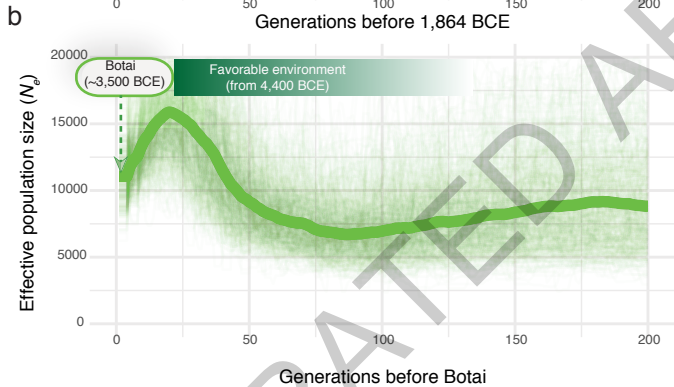
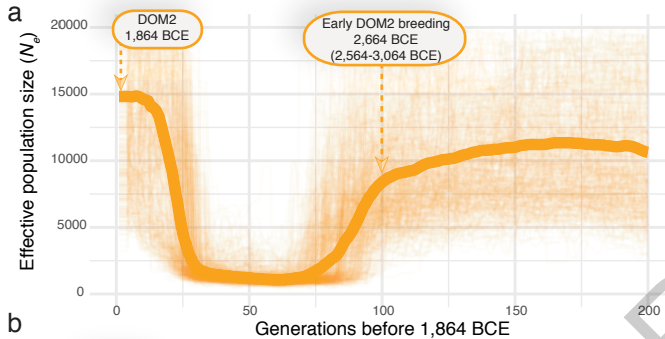
878 **Extended Data Fig. 7. GONE demographic reconstruction**²⁶. Effective population size (N_e) estimated
879 from the patterns of linkage disequilibrium (LD) present in a nearly contemporaneous population of 14
880 horses affiliated to the Sintashta culture, up to 200 generations before their existence. **b)** Example of local
881 ancestry for a TURG horse genome (LR18x15_Rus_m2763), modeled with Admixfrog as a mixture of Botai
882 and early DOM2 horses. **c)** Raw generation time estimates for ancient horses from the steppe, the
883 Carpathian and Transylvanian Basins, without correcting for population structure and uneven sequencing
884 depths (Supplementary Information). TURG* represents the group of TURG horses, after masking their
885 genomes for tracts introgressed from Botai horses. **d)** Same for Botai horses, which involved more
886 generations than past and contemporaneous horses from the region, with the exception of BORL and
887 Przewalski's horses (PRZW), previously inferred to descend from Botai and saved from extinction through
888 captive management. The dates reported correspond to rounded means of the different samples present in
889 each group.
890

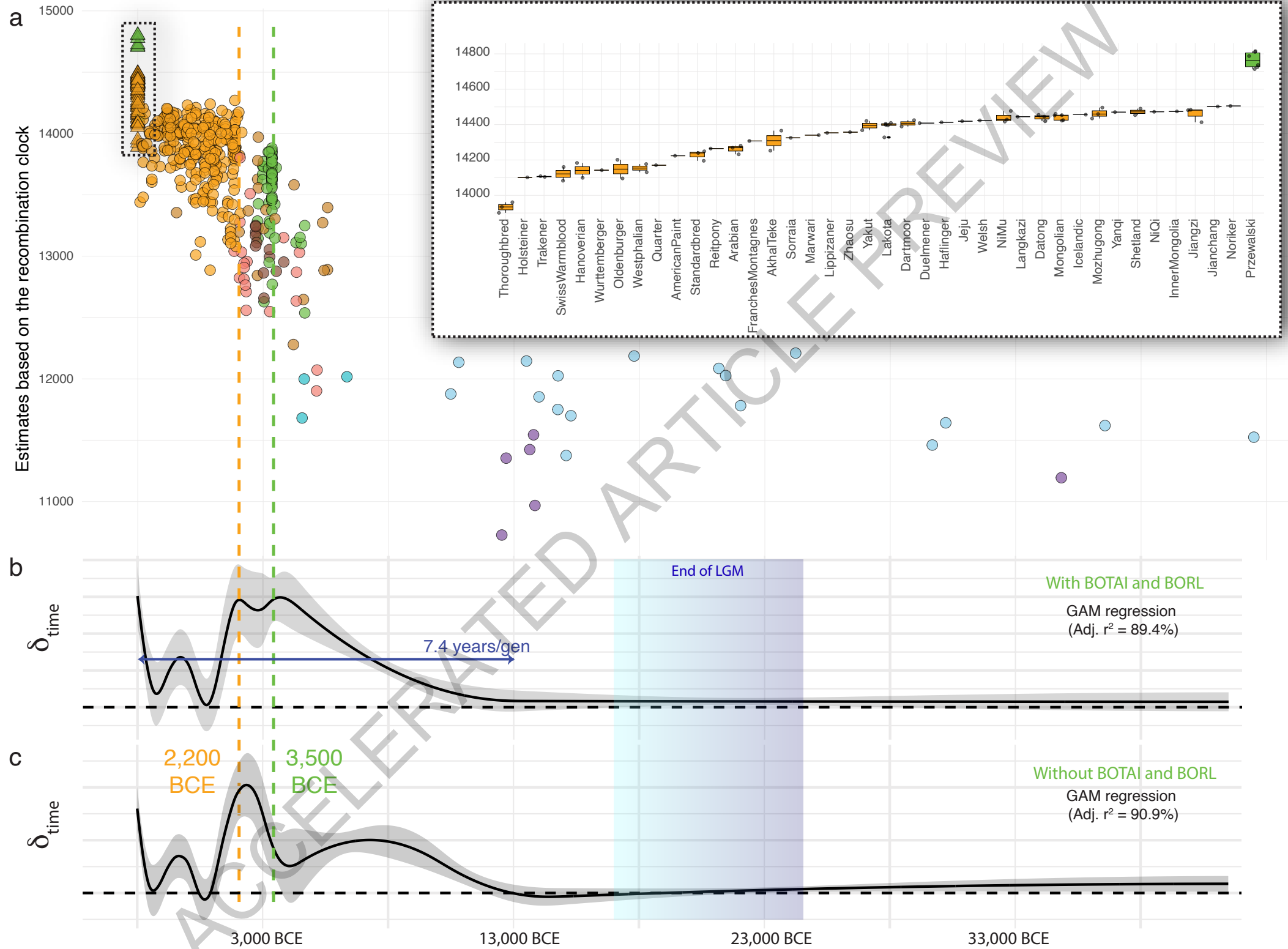
891 **Extended Data Fig. 8. Mutation clock estimates.** **a)** Relationship of the ingroup Eurasian horses to the
892 outgroups considered in this study, including non-caballine equids (*E. ovodovi* and the donkey) and ancient
893 horses from North America (LP_NAMR). Leveraging this topology, we counted the number of mutations
894 (represented as stars) that occurred in the branch leading to every single Eurasian horse. Following
895 pseudohaploidization, positions that are truly heterozygous in Eurasian horses become ancestral or derived,
896 and both outcomes are expected at equal probabilities. This approach is, thus, insensitive to the underlying
897 heterozygosity of the sample, and, hence, to their demographic history. **b)** Estimates of the number of
898 generations evolved from the outgroups, based on the full data set. **c)** Estimates based on the downsampled
899 dataset.
900

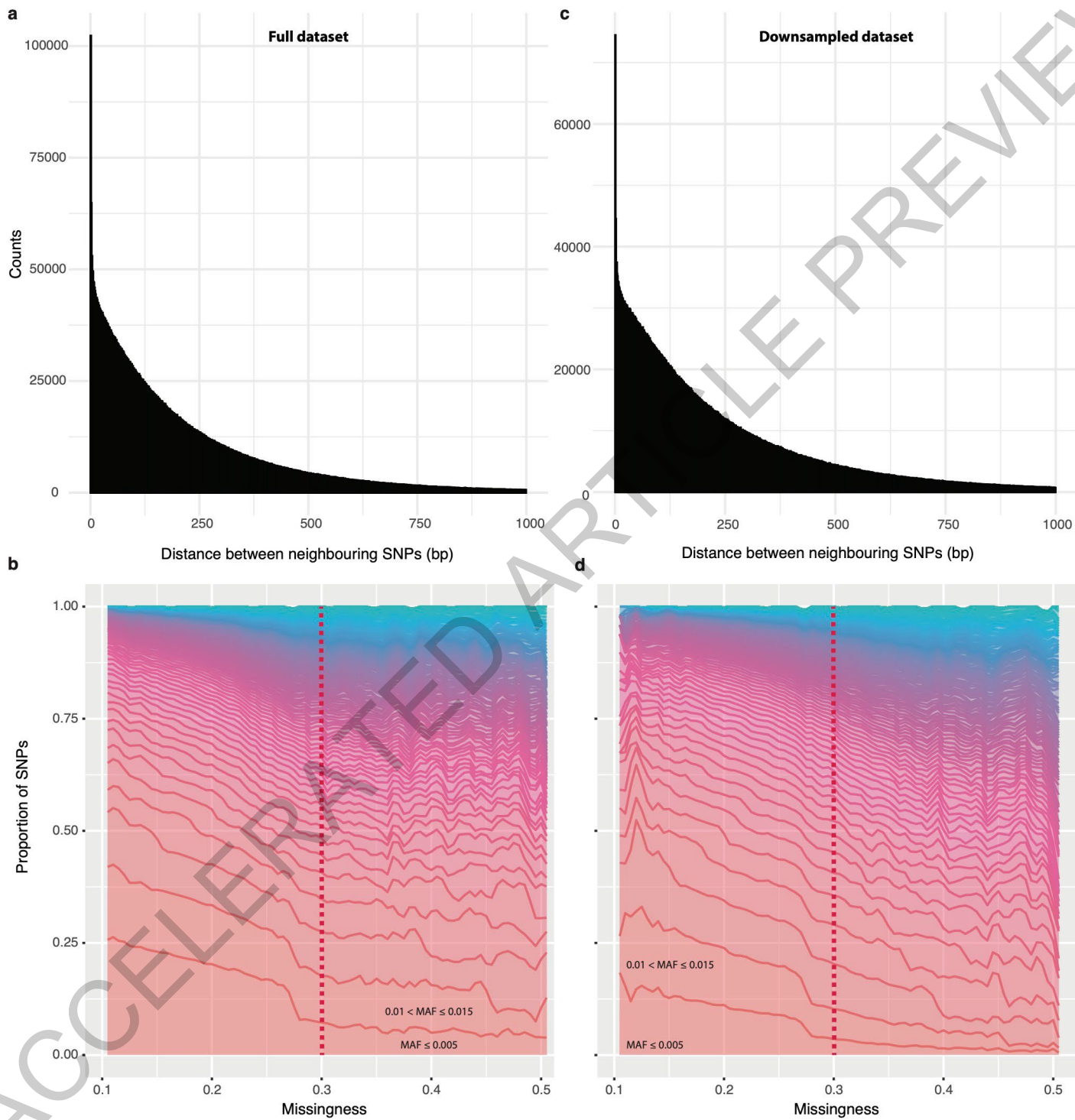
901 **Extended Data Fig. 9. Recombination clock estimates.** **a)** Schematic representation that illustrates the
902 expectation that the variance along the genome is greater in an older specimen (left) as the result of more
903 generations of evolution and, hence, more recombination events than in younger specimens with regards to
904 the time to the most common recent ancestor (MRCA) of the whole sample set. It is thus expected that the
905 distribution of mutations (stars) is less even in the younger specimen (right), which underwent fewer
906 recombination events, and thus carry longer haplotype blocks, in which mutations are equally likely to have
907 occurred or not. **b)** Schematic visualization of the t (time to the MRCA) and T (total length of the genealogy)
908 parameters constituting the recombination clock model, for an illustrative sample of four genomes. **c)**
909 Number of generations evolved from the MRCA, as estimated by applying the recombination clock model
910 to the full data set.
911

912 **Extended Data Fig. 10. Coalescent simulations to validate both methods.** **a)** Illustration of the 10
913 simulated scenarios (A-J), together with their underlying parameters. **b)** Each boxplot summarizes the
914 estimates obtained from $n=10$ diploid samples, when using the method relying on the recombination clock
915 (in generations of evolution from the MRCA). Boxplots are comprised of their corresponding centres
916 (median), box boundaries (interquartile ranges), and whiskers (1.5 times the interquartile ranges). The
917 estimated age of the samples perfectly correlates with the simulated age of sampling (Pearson correlation; r
918 = 0.999; two-tailed p -value = 0). **c)** Same as b) for the mutation clock (Pearson correlation; r = 0.999; two-
919 tailed p -value = 0).
920

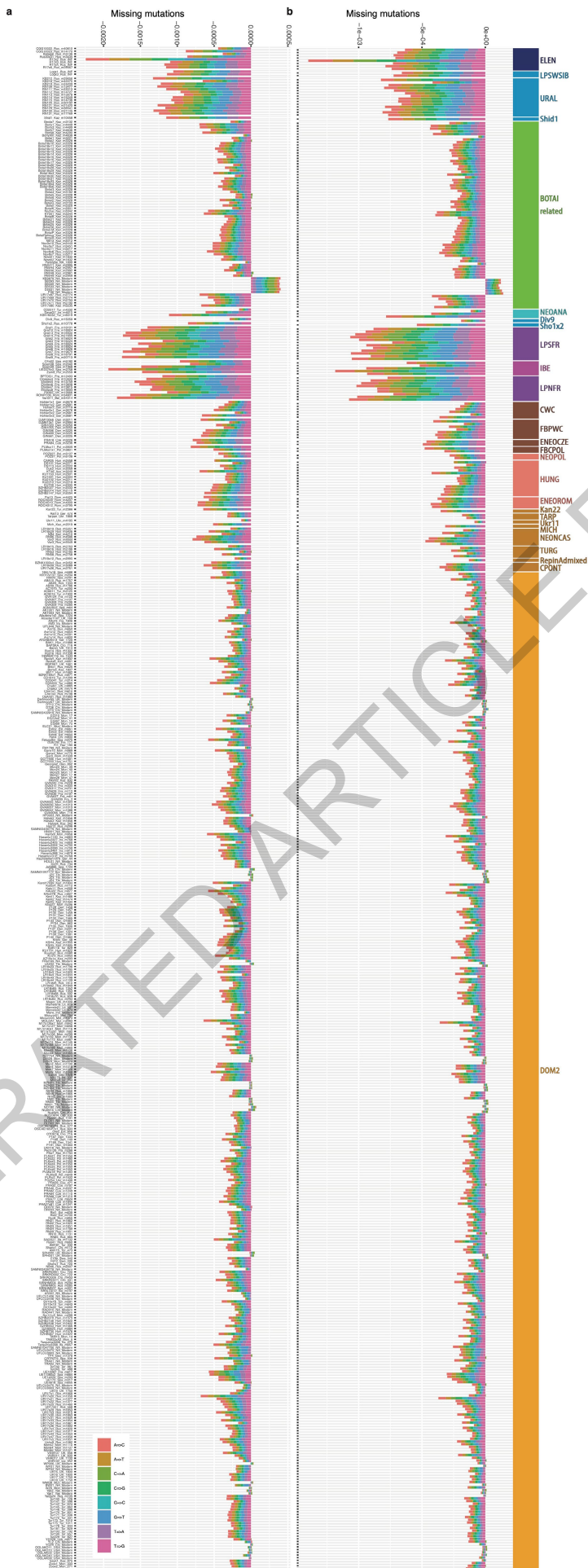




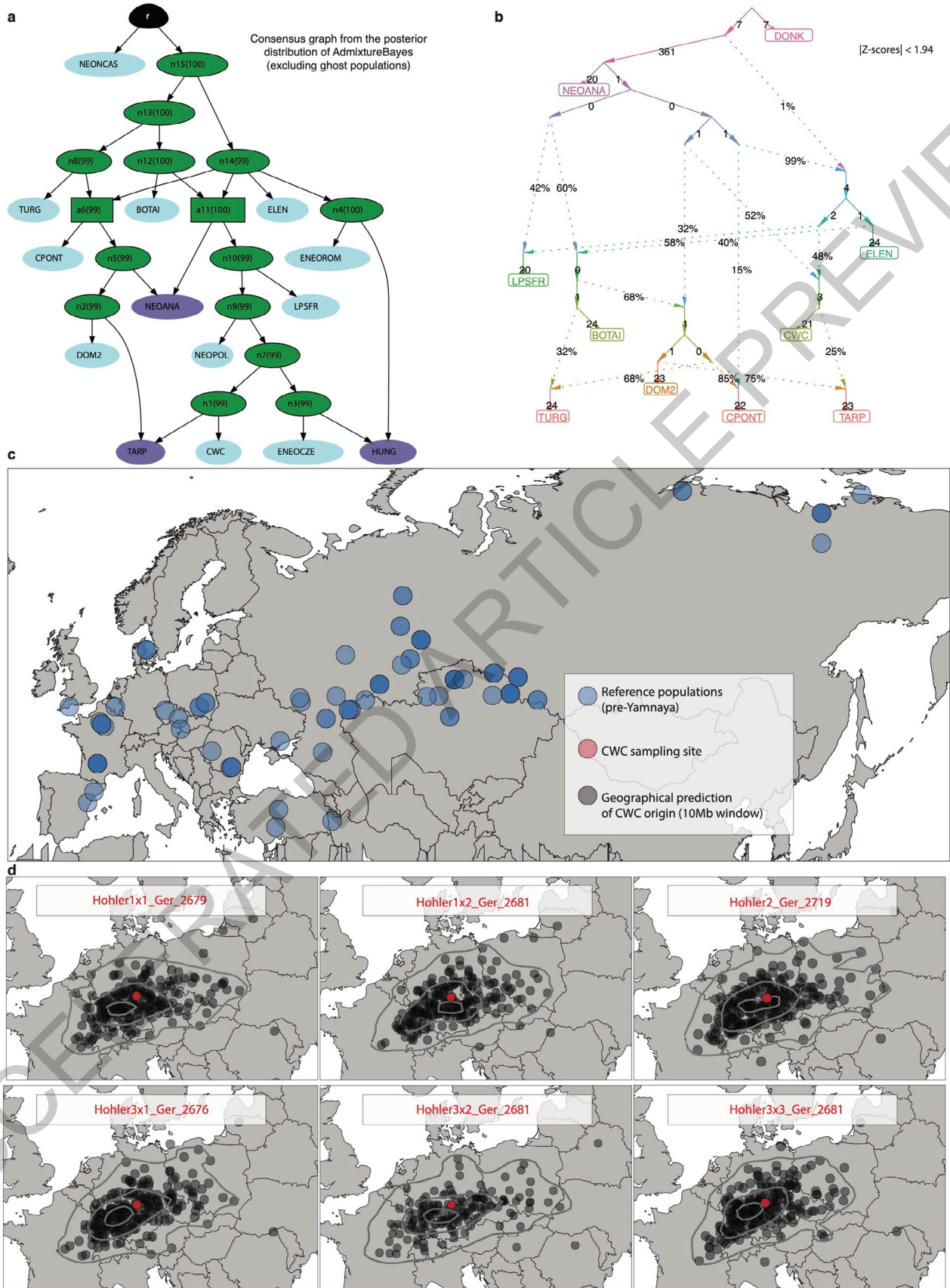




Extended Data Fig. 1



Extended Data Fig. 2



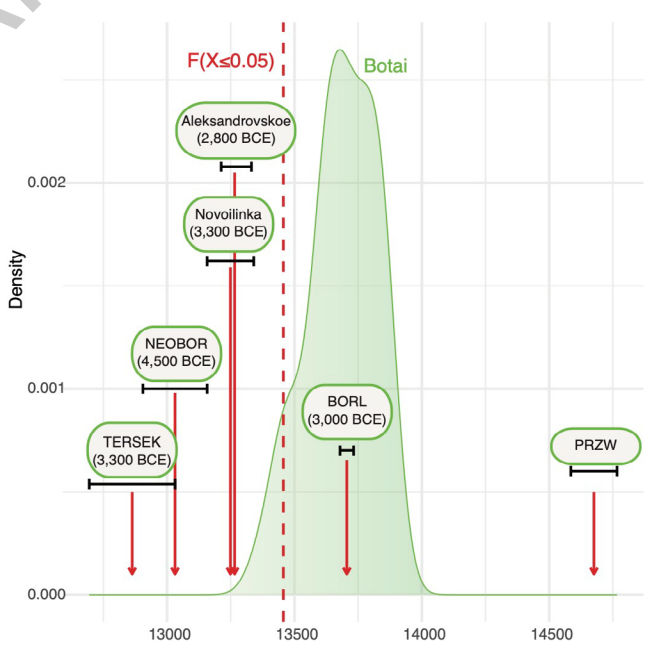
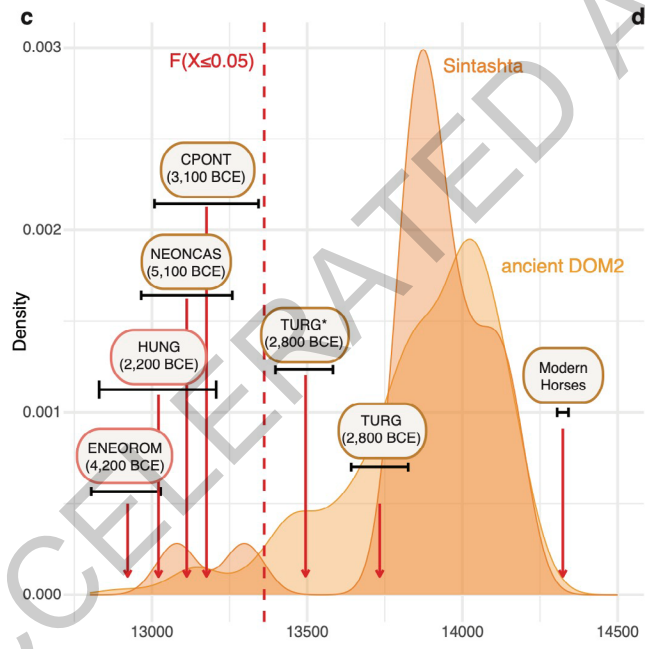
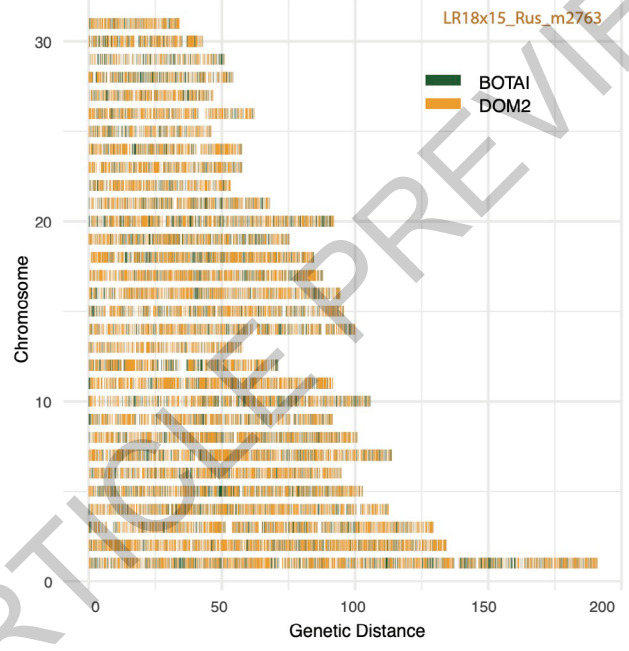
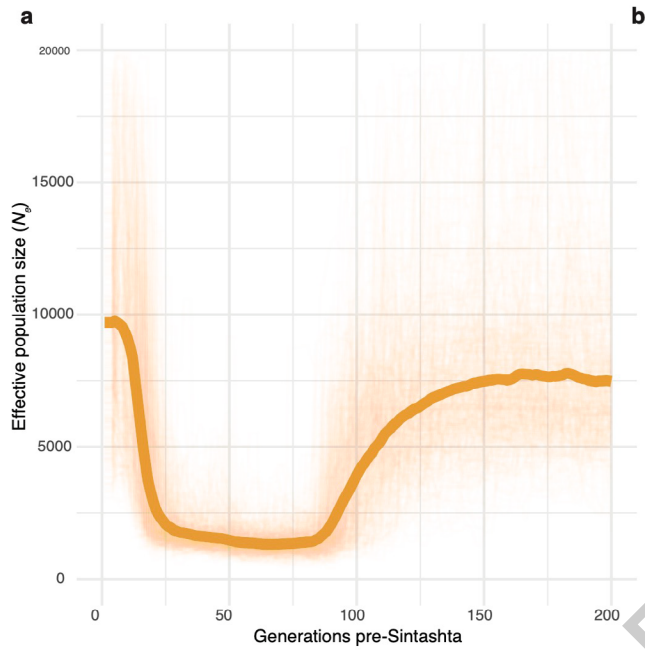
Extended Data Fig. 3



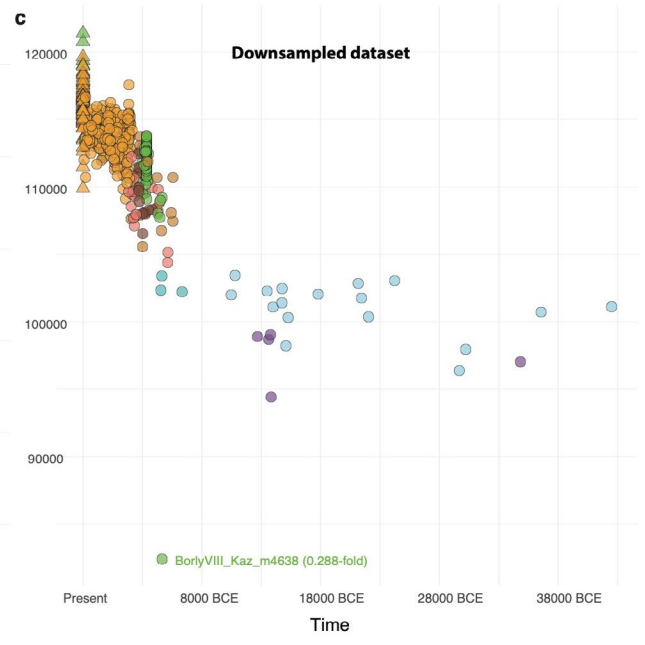
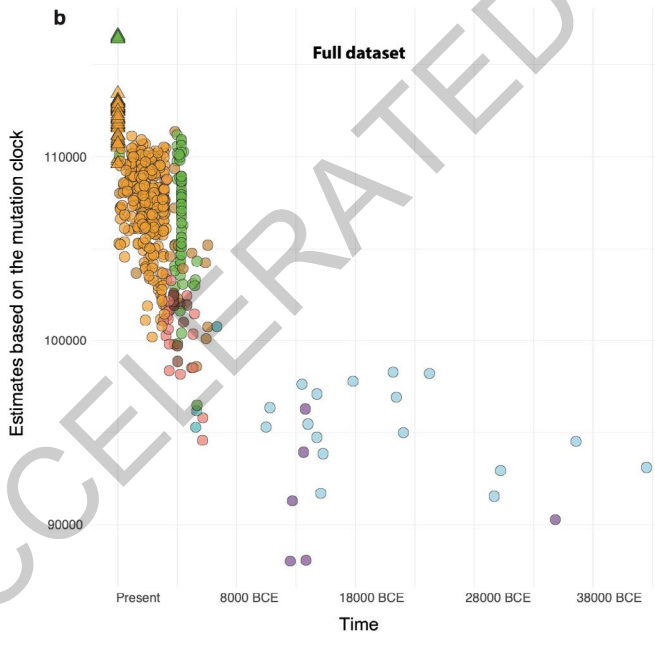
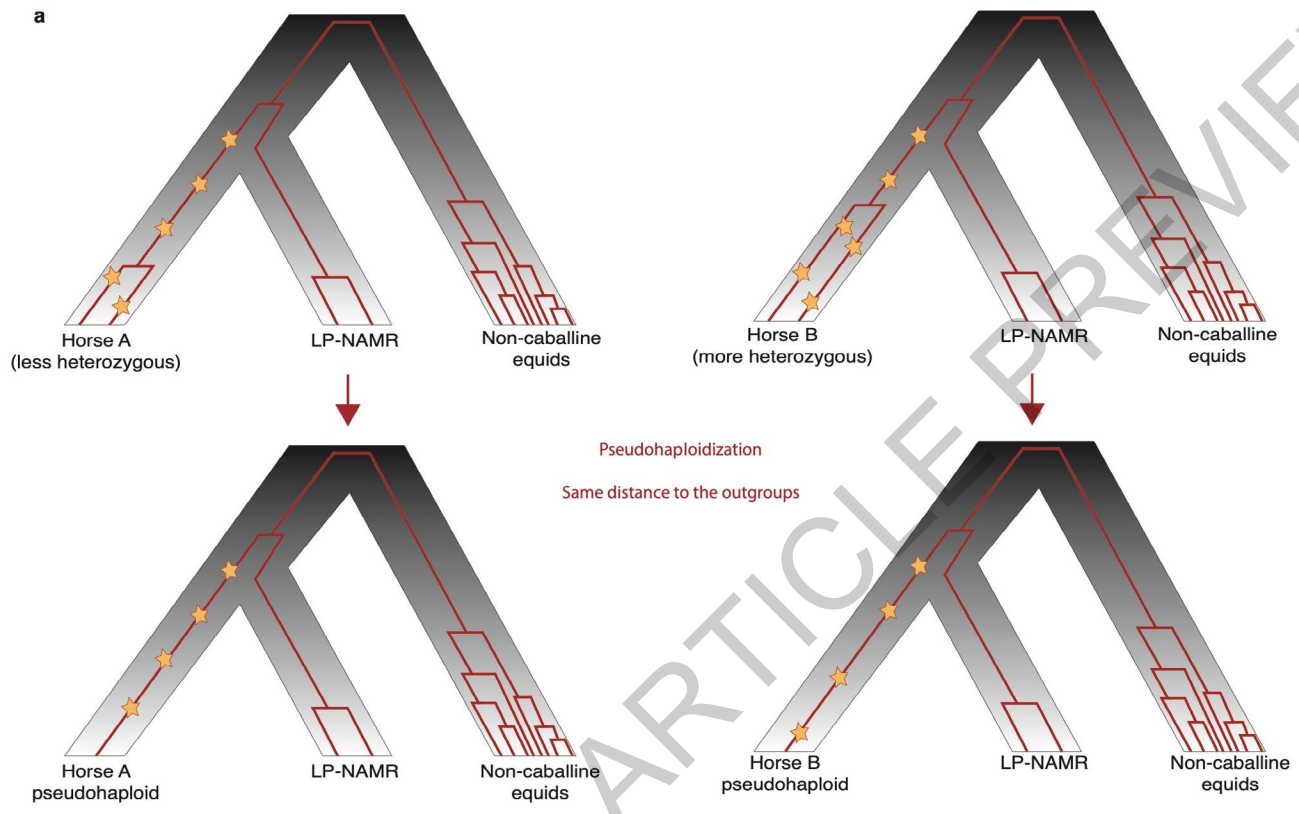
Extended Data Fig. 5



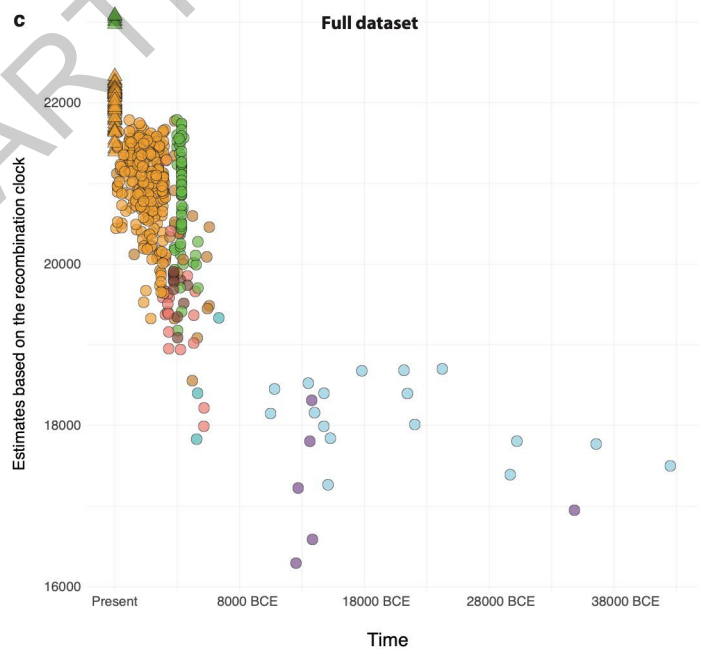
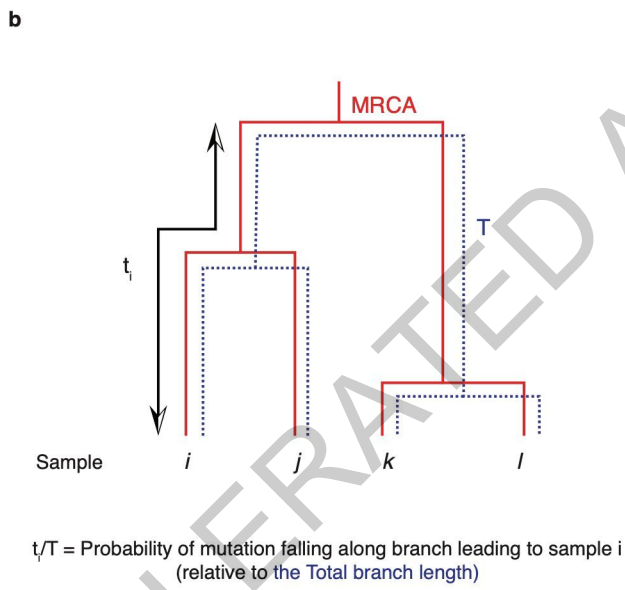
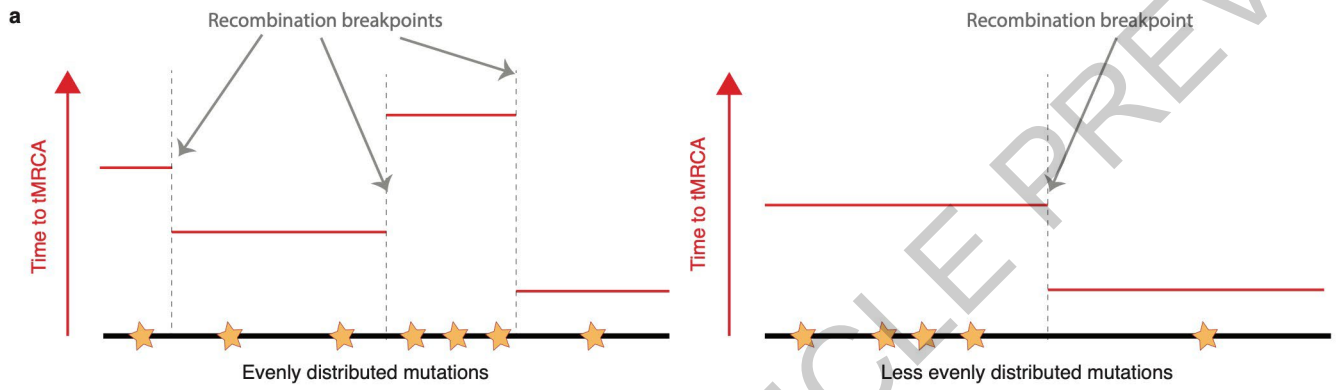
Extended Data Fig. 6



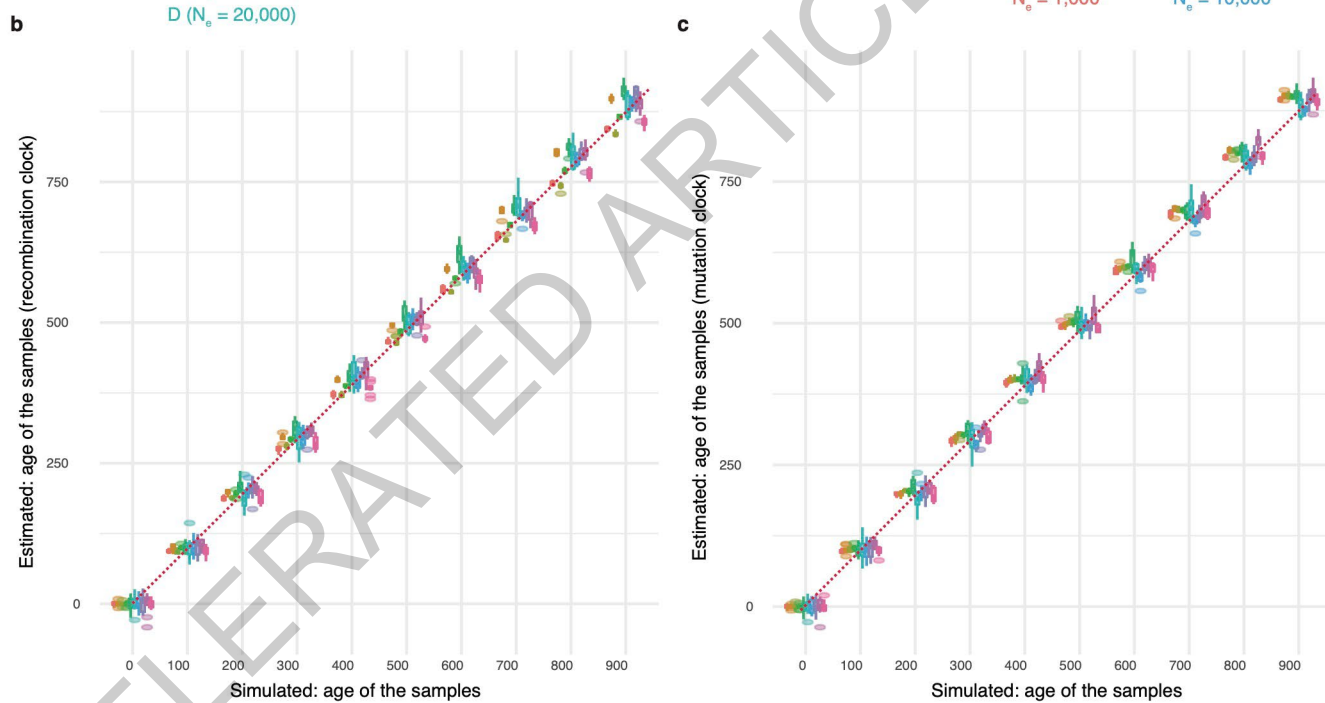
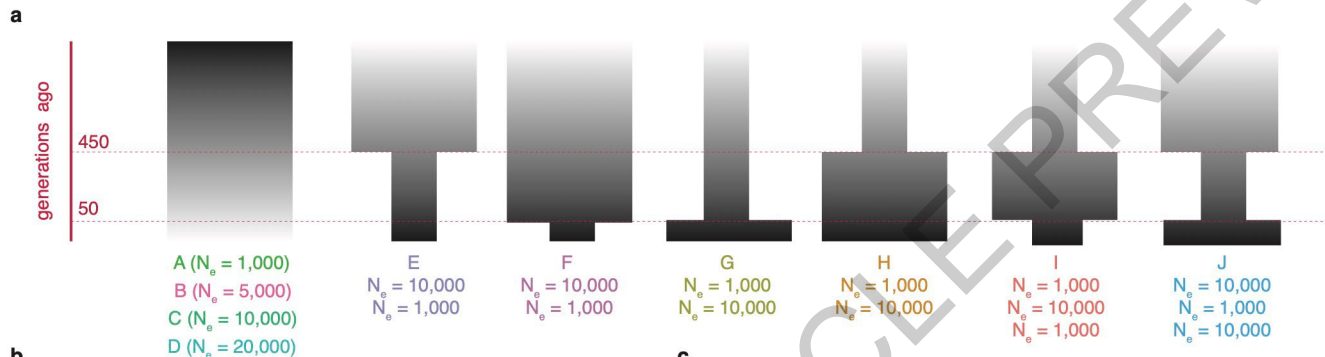
Extended Data Fig. 7



Extended Data Fig. 8



Extended Data Fig. 9



Extended Data Fig. 10

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

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Software and code

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Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

QGIS 3.36 (<https://www.qgis.org/en/site/about/index.html>)
 Angsd v0.927 (<https://www.popgen.dk/angsd/index.php/ANGSD>)
 Plink v1.9 (<https://www.cog-genomics.org/plink/1.9/>)
 Locator (<https://github.com/kr-colab/locator>)
 Admixtools2 (<https://uqrmaie1.github.io/admixtools/articles/admixtools.html>)
 Admixtools v7.0.2 (<https://github.com/DReichLab/AdmixTools>)
 AdmixtureBayes (<https://github.com/avaughn271/AdmixtureBayes>)
 Struct-f4 (<https://bitbucket.org/plibradosanz/structf4/src/master/>)
 generation_time (<https://bitbucket.org/plibradosanz/generationtime/src/master/>)
 GONE (<https://github.com/esrud/GONE/>)

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
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All collapsed and paired-end sequence data for samples sequenced in this study are available in compressed FASTQ format through the European Nucleotide Archive under accession number PRJEB71445, together with rescaled and trimmed bam sequence alignments against both the nuclear horse reference genomes. Previously published ancient data used in this study are available under accession numbers PRJEB7537, PRJEB10098, PRJEB10854, PRJEB22390, PRJEB31613, and PRJEB44430, and detailed in Supplementary Table 1. The genomes of 78 modern horses, publicly available, were also accessed as indicated in their corresponding original publications, and in Supplementary Table 1.

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

We generate 124 new ancient genomes, and combined them with 434 previously available to identify when domestic horses were extensively used for long-distance mobility

Research sample

The 550 horse genomes used in this study were used to provide a comprehensive representation of the horse genetic diversity prior and during domestication across Eurasia. The rationale was to identify shifts in the evolutionary trajectory of horses induced by humans, following their domestication. Eight outgroups were also included to polarise alleles as ancestral or derived. A full description of each new sample is provided in Table S1.

Sampling strategy

In the field of ancient DNA, sampling strategies are conditioned by the levels of endogenous DNA preservation in ancient remains. We attempted to (and succeeded) generate the largest genomic time-series for a non-human species. This was larger than our own

previous studies (eg. Librado et al. 2021), where the corresponding sampling size was already proven more than sufficient to perform evolutionary analyses

Data collection Fossil remains were collected from across Eurasia, from coauthors of this study. Sequencing of these remains was performed at the dedicated facilities of CAGT (Toulouse). The contribution of each coauthor is detailed in the corresponding section of the main manuscript, and the whole process was registered in our internal database.

Timing and spatial scale Ancient DNA samples were processed for whole genome sequenced as they arrived to our laboratory, shipped by our coauthors. No particular strategy was followed in this regard.

Data exclusions All data included in this study was analysed, with the only exception pertaining to a few samples showing signatures of introgression from basal lineages (true outliers) or not radiocarbon-dated in the regression analyses, as openly explained in the supplementary information.

Reproducibility All our experiments were found to be highly reproducible. Those experiments with lower reproducibility, pertaining to complex statistical inference, were repeated multiple times with different starting values and parameters to check for concordance between runs (eg. Locator runs). Only solid analyses are reported.

Randomization We followed the population group assignment from Librado et al. (2021; Nature)

Blinding Blinding was not relevant in our ancient DNA study.

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions Fossil remains were collected across Eurasia during years, if not decades, during a diversity of conditions. These do not impact our conclusions as fossils have been buried for hundreds to thousands of years before being sampled during field work.

Location All relevant parameters, including radiocarbon dates and GPS coordinates for each new ancient sample sequenced in this study, are provided in Table S1

Access & import/export All fossils were collected strictly following the highest standards in ancient DNA research, and in close coordination with the archaeologists responsible for the material and the corresponding excavations, with all local and international permits in place. All these archaeologists are coauthors in our study.

Disturbance This study caused no disturbance

Reporting for specific materials, systems and methods

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Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input type="checkbox"/>	<input checked="" type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance The samples that were analyzed in this study were collected from a range of archaeological contexts, as detailed in the Supplementary Information and summarized in Supplementary Table S1. As this involved sampling from across Eurasia and different procedures between countries and institutions, key contact persons were identified in each country so as to access relevant material and coordinate legal authorization to sample material for DNA analysis and radiocarbon dating. Samples were collected with permission from the organizations holding the collections and documented through official agreement letters provided by the named archaeologists and/or curators and/or directors of relevant institutions, named below. As DNA and radiocarbon dating techniques are partially destructive, we sought every opportunity to access samples as part of collaborations with other research projects so as to both save resources and avoid double sampling, and, thus, ultimately minimize destruction. The following list provides the sites and

names of those key contact persons, who granted access to the corresponding material, with reference to letters and permits where appropriate:

-Albufeira (Silo 1, Rua Henrique Calado), Portugal. Sample: Albufeira1x2_Spa_1224. Key contacts: Maria João Valente, and Luís Paulo (Museu Municipal de Arqueologia, Albufeira). Collection of the Museu Municipal de Arqueologia, Albufeira.

-Alorda Park, Calafell, Spain. Sample: H9020_Spa_m291 (SU 9020 - Bottom level of the filling of the ditch surrounding the fortified aristocratic residence). Key contact: Silvia Valenzuela-Lamas (Archaeology of Social Dynamics, Institutió Milà i Fontanals - Consejo Superior de Investigaciones Científicas (IMF-CSIC), C/ Egipcíacques 15, 08001 Barcelona, Spain).

-Arzhan-2, Russia. Sample: Rus8_Rus_m855. Key contacts: Aleksei K. Kasparov, Vladimir V. Pitulko (Institute of Material Culture, Russian Academy of Sciences). Sampled through the project 21-18-00457 from the Russian Science Foundation, with permission and all proper authority (confirmation letter nb 14102/33-772.4-263).

-At Daban, Yakutia. Sample: ATDABAN13_Yak_1725. Key contact: Éric Crubézy (Centre d'Anthropobiologie et de Génomique de Toulouse, CNRS UMR 5288, Université Paul Sabatier, Faculté de Médecine Purpan, 37 Allées Jules Guesde, 31000 Toulouse, France).

-Bakonszeg-Kádárdomb, Hungary. Sample: BAK1_Hun_m1686. Key contacts: Lajos Lakner, Dani János, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Déri Museum.

-Berettyóújfalu-Szilhalom, Hungary. Sample: BS11_Hun_m1682. Key contacts: Lajos Lakner, Dani János, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Déri Museum.

-Biluut 2, Zeerdegchingiin Khoshuu, Zunii Gol, Zuunkhangai, Ulaan Tolgoi, Mongolia: Samples M17x152x1_Mon_m80 and M17x156_Mon_m299. Key contact: Jamsranjav Bayarsaikhan (Institute of Archaeology, Mongolian Academy of Science, Ulaanbaatar 13330, Mongolia), and Will T. T. Taylor (Museum of Natural History, University of Colorado Boulder, Boulder, CO 80309, USA; Fulbright US Student research award nb 34154234, National Geographic Young Explorer's grant nb 9713-15), National Science Foundation Doctoral Dissertation Improvement Grant nb 1522024). Exported in 2015 and 2015 under research agreement nb 20150315. Collections from the National Museum of Mongolia, Ulaanbaatar

-Bitozeves, Czechia. Sample: PRA29_Cze_471. Key contact: René Kysely, Institute of Archaeology of the Czech Academy of Sciences, Prague. Excavated by Věra Sušická, ÚAPPSZČ, Most, Czechia.

-Bleachfield Street, Alcester, Warwickshire, United Kingdom. Key contact: Jacobo Weinstock (Faculty of Arts and Humanities, Department of Archaeology, University of Southampton, UK). Collection from the site curated by Warwickshire Museum.

-Bled, Pristava necropolis, Slovenia. Samples: SRSLO012_Slo_m197. Key contact: Peter Turk (Narodni muzej Slovenije, Prešernova 20, SI-1000 Ljubljana, Slovenia). Samples were made available through the Innovation Fund of the Austrian Academy of Sciences (ÖAW) (Grant agreement IF_2015_17).

-Borly IV, Kazakhstan. Sample: BorlyXIII_Kaz_m4638 and Borly9_Kaz_1807 (SQ 7G (33-44cm)). Key contact: Alan Outram (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Collections from the A. Kh. Margulan Joint Archaeological Research Centre Toraighyrov University (Director: Viktor K. Merts), sampled with permission and all proper authority from the Acting Deputy Chairman of the Board for Academic Work (confirmation letter 0605-2021 nb. 107-1232).

-Botai, Kazakhstan. Samples: Botai18x30_Kaz_m3328 and BotaiB_Kaz_m3228. Key contact: Prof Alan Outram (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Collections from the Al Farabi Kazakh National University, sampled with permission and all proper authority from the Dean of Faculty of History M.S. Nogaibayeva (confirmation letter 605-2021 nb. 1523-602).

-Bradgate Park, Leicestershire, United Kingdom. Sample: BGPSK1_UK_1661. Key contact: Richard Thomas (School of Archaeology and Ancient History, University of Leicester). Permission for analysis was provided by the Bradgate Park Trust. The specimen is curated at the School of Archaeology and Ancient History, University of Leicester, but will eventually be deposited with Leicestershire County Council Museums Service under the accession code XA19.2015.

-Bredholm, Denmark. Samples: P128_Den_1458, P129_Den_1457, P130_Den_1459, and P131_Den_1461. Key contact: Peter Pentz (museum inspector; National Museum of Denmark, Ny Vestergade 10, 1471 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Brusyany IV, Kurgan 2, mound fill, Russia. Sample: LR18x70_Rus_608. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Brusyany IV, Kurgan 1, Russia. Sample: LR18x68_Rus_592 (Grave 1, horse 1). Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Budapest-Királyok Útja 293, Hungary. Samples KU1153_Hun_m2301, KU1591_Hun_m2304, KU1701_Hun_m1822, KU2102_Hun_m2211, KU2210_Hun_m2218, and KU709_Hun_m2335. Key contacts: Paula Zsidi, Alice Choyke, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Aquincum Museum.

-Burgast, Mongolia: Samples GVA9046_Mon_716. Key contacts: Sébastien Lepetz (CNRS, Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France), Tsagaan Turbat (Archaeological Research Center and Department of Anthropology and Archaeology, National University of Mongolia, Ulaanbaatar, Mongolia), and Bayarkhuu Noost (Archaeological Research Center and Department of Anthropology and Archaeology, National University of Mongolia, Ulaanbaatar, Mongolia). Excavation campaign from 2016, Program MEAE – Institut of Archaeology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia.

-Çadır Höyük, Yozgat, Türkiye. Sample: CD5041_Tur_m314. Key contact: Benjamin Arbuckle (Department of Anthropology, Alumni Building, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA). Exported in 2013 to Benjamin Arbuckle via the Yozgat Müze Müdürlüğü under grant (NSF BCS-1311551).

-Can Roqueta-Torre Romeu, Spain. Sample: CRTR279_Spa_506 (Barcelona, Spain - Late Roman, structure CRTR-279). Key contact: Silvia Albizuri (Institut d'Arqueologia, Universitat de Barcelona).

-Chekon settlement, Russia. Sample: KUZ3_Rus_m954. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Dava Goz, Iran. Sampled: DavaG1_Ira_m4615. Key contact: Marjan Mashkour (CNRS, Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France).

-Derkul, Russia. Sample: NB4_Kaz_m4210. Key contacts: Pavel Kosintsev (Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia), and Mélanie Pruvost (UMR5199, PACEA, Université de Bordeaux, France).

-Dunakeszi-Székesdülő, Hungary. Samples: DS101_Hun_m2271 and DS113_Hun_m2222. Key contacts: Paula Zsidi, Alice Choyke, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Aquincum Museum.

-Eketorp, Sweden. Sample: F612_Swe_495. Key contacts: Key contacts: Johnny Karlsson (curator, The Swedish History Museum), Marie Sundquist (Östra Greda Research Group, 38791 Borgholm, Sweden), and Gabriella Lindgren (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden). Loan agreement: 331-2017-958.

-Egying Gol, Mongolia. Samples: EGI10_Mon_17 (Tomb 10), EGI12x2_Mon_41 (Tomb 12.2), EGI67_Mon_14 (Tomb 67), and EGI69_Mon_75 (Tomb 69). Key contacts: Éric Crubézy (Centre d'Anthropobiologie et de Génomique de Toulouse, CNRS UMR 5288, Université Paul Sabatier, Faculté de Médecine Purpan, 37 Allées Jules Guesde, 31000 Toulouse, France), and Tsagaan Turbat (Archaeological Research Center and Department of Anthropology and Archaeology, National University of Mongolia, Ulaanbaatar, Mongolia).

-El Graell (Vic), Spain. Sample: ADNUB33_Spa_m64 (Ibero Roman, Spain; structure E-42). Key contacts: F. Javier López-Cachero (Can Roqueta Project Manager, Ref: ARQ001SOL-178-2022), and Silvia Albizuri (Institut d'Arqueologia, Universitat de Barcelona).

-Filippovka II, Kurgan 1, Grave 2, Russia. Sample: LR18x84_Rus_m294. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Følenslev Mose, Denmark. Sample: P133_Den_O1663. Key contact: Peter Pentz (museum inspector; National Museum of Denmark, Ny Vestergade 10, 1471 Copenhagen K, Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Gáborján-Csapszékpart, Hungary. Samples: GC77486_Hun_m1681 and GC77550_Hun_m1751. Key contacts: Lajos Lakner, Dani János, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Déri Museum.

-Ginnerup, Denmark. Samples: GIN1020_Den_m3000 (structure A4), GIN1055_Den_m3000 (structure A4), GIN396_Den_m3000 (structure A1), GIN489_Den_m3000 (structure A1), GIN561_Den_m3000 (structure A1). Key contact: Lutz Klassen (Museum Østjylland, Randers, Denmark).

-Gørlev, Denmark. Samples P187_Den_1334. Key contact: Peter Pentz (museum inspector; National Museum of Denmark, Ny Vestergade 10, 1471 Copenhagen K, Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Halvai, Kazakhstan. Sample: Halvai4_Kaz_342. Key contact: Prof Alan Outram (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Sampled with permission and all proper authority from the Acting Vice-Rector on Science, Internationalization and Digitalization Gulshat Shaikamal (confirmation letter 11.05.2021 nb. 15-20-09/1052). Collections from the A. Baitursynov Kostanay State University, KSU (Kostanay).

-Hereuet, Seró, Spain. Sample: H2012x137_Spa_m204 (SU 2012 - Filling of silo SJ-8). Key contact: Silvia Valenzuela-Lamas (Archaeology of Social Dynamics, Institució Milà i Fontanals - Consejo Superior de Investigaciones Científicas (IMF-CSIC), C/ Egipcíacques 15, 08001 Barcelona, Spain).

-Hjortspringkobbøl, Denmark. Sample: P137_Den_m291. Key contact: Collections of the Natural History Museum of Denmark). Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

- Hovmarken, Denmark. Sample: P138_Den_1224. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.
- Hungate, United Kingdom. Sample: VEM107_UK_956. Key contact: Terry O'Connor (Department of Archaeology, University of York, c/o Kings Manor, York YO1 7EP, UK). Excavated by York Archaeological Trust between 2006 and 2011 from whom permission was granted to Terry O'Connor, University of York, for ancient DNA study in 2013 under exit documentation X0903 and X0937.
- Husiatyn, Ukraine. Sample: POZ54_Ukr_m1498 (Double burial). Key Contact: Daniel Makowiecki (Institute of Archaeology, Faculty of History, Nicolaus Copernicus University, Toruń, Poland). Two horse skeletons were discovered during rescue excavations (2015) of barrow by Vasyl Ilchyshyn (Zalitsi Museum, Ternopil Region, Ukraine, and the Security Archaeological Service of the Institute of Archeology, National Academy of Sciences of Ukraine, Kyiv, Ukraine). Samples were collected during zooarchaeological research (2021) by Daniel Makowiecki and Przemysław Makrowicz (Faculty of Archaeology Adam Mickiewicz University, Poznań), and stored since at the Institute of Archaeology, Nicolaus Copernicus, Torun. One of the two horses was sampled for the DNA and radiocarbon analysis presented in this study.
- Idzhil, Russia. Sample: IDZH_Rus_734. Marjan Mashkour (CNRS, Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France).
- Industriya, Russia. Sample: KAU27B_Rus_m627. Key contacts: Sabine Reinhold and Svend Hansen (Eurasia Department of the German Archaeological Institute, Berlin, Germany). Excavation carried out by Dr. D. S. Korobov (Institute of Archaeology, Russian Academy of Sciences, Moscow, Russia, licence nb 2001-868), with material curated by Ltd. 'Nasledie'. Exported in 2016 with proper authorization to the German Archaeological Institute, Berlin, Germany.
- Ipatovo 3, Russia. Sample: KAU22_Rus_m877 (Kurgan 2, Animal Complex 13). Key contacts: Sabine Reinhold and Svend Hansen (Eurasia Department of the German Archaeological Institute, Berlin, Germany). Excavation carried out by Dr. A.B. Belinskij (Stavropol, excavation Ltd. 'Nasledie' & DAI, Eurasia-Department, license nb 1998-177). Exported in 2013 with proper authorization to the German Archaeological Institute, Berlin, Germany.
- Katanda II, Russia. Samples: Kat2x4_Rus_m112 and Katx11_Rus_m269. Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia: interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).
- Karatomar Burial ground, Kazakhstan (Kurgan 1). Sample: Karat17039_Kaz_m1834. Key contact: Prof Alan Outram (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Sampled with permission and all proper authority from the Acting Vice-Rector on Science, Internationalization and Digitalization Gulshat Shaikamal (confirmation letter 11.05.2021 nb. 15-20-09/1052). Collections from the A. Baitursynov Kostanay State University, KSU (Kostanay).
- Khankarinsky dol, Russia. Sample: Han12_Rus_m296. Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia: interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).
- Kittsee settlement, Steinfeldäcker, Austria. Sample: KT46_Aus_m3240 (campaign 1997, pit 289). Key contact: Christian Mayer (Federal Monuments Authority Austria, Department for Digitalization and Knowledge Transfer, Vienna, Austria). Excavation documentation and excavated material accessible through the Federal Monuments Authority Austria, Department of Archaeology.
- Køge A ved Spanager, Denmark. Sample: P134_Den_862. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.
- Krasnosamarskoe settlement, Russia. Sample: RN85_Rus_865. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).
- Krasnosamarskoe IV, kurgan cemetery, Kurgan 7, Russia. Sample: RN10_Rus_1131 (horse bone in the mound fill layer). Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).
- Krasny Gorodok settlement, Russia. Sample: ABA3_Rus_1333. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).
- Krefeld-Gellep, Germany. Sample Kref4_Ger_48. Key contact: Sabine Deschler-Erb and Monika Schernig Mráz (Integrative Prehistory and Archaeological Science, University Basel). Sample made available through Project HumAnimAl: Swiss National Science Foundation 178834. Sample reference NI 2017/0030 2708-12, Museum Burg Linn, Germany.
- Le Cendre – Gondole, France: Samples GVA629_Fra_m112, and GVA636_From_m197. Key contact: Sébastien Lepetz (Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France). Excavation campaign from 2003.
- Langhøj, Denmark. Samples P189_Den_1541. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the

collection manager at the Collections of the Natural History Museum of Denmark.

-Maison Alfort Museum of the Veterinarian School, France (7 Av du Général De Gaulle, 94704 Maisons Alfort, France). Sample: Alfort3_Fra_1806: Key contacts: Christophe Degueurce, and Céline Robert (Ecole Nationale Vétérinaire d'Alfort, 7 Avenue du Général De Gaulle, 94704 Maisons-Alfort, France). Sampled with permission from the collections of the Maison Alfort Museum of the Veterinarian School.

-Miciurin (Odaia), Moldavia. Sample: Miciurin01_Mol_794. Key contact: Arne Ludwig (Leibniz-Zentrum für Archäologie (LEIZA), Ludwig-Lindenschmit-Forum 1, 55116 Mainz, Germany).

-Noviye Kluchi III cemetery, Bronze Age Pokrovka Culture, Kurgan 1, sacrificial complex, Russia. Sample: LR18x3_Rus_m1820. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Nytorv, Denmark. Sample: P139_Den_1381. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Orcet – La Roche Blanche – L'Enfer, France: Samples GVA637_Fra_m64 and GVA639_Fra_2. Key contact: Sébastien Lepetz (CNRS, Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France). Excavation campaign from 2003.

-Pech Maho, France. Sample: Pech126_Fra_m288. Key contact: Armelle Gardeisen (CNRS, Archéologie des Sociétés Méditerranéennes, Archimède IA-ANR-11-LABX-0032-01, Université Paul Valéry, Montpellier 34090, France).

-Puig de Sant Andreu, Spain (Ullastret). Sample: UE14029_Spa_m293 (SU14029 - abandoned layer of the main residence; zona 14 of this urban site of more than 10Ha). Key contact: Silvia Valenzuela-Lamas (Archaeology of Social Dynamics, Institució Milà i Fontanals - Consejo Superior de Investigaciones Científicas (IMF-CSIC), C/ Egipcíacs 15, 08001 Barcelona, Spain).

-Rathewitz 13, Burgenland district, Saxony-Anhalt, Central Germany. Sample: Rat13_Ger_474 (deposited in grave 13 of a burial ground from the Migration period, 5th/6th century). Key contact: Hans-Jürgen Döhle (former curator of State Museum of Prehistory, Halle (Saale), Germany). Stored in the State Office for Heritage Management and Archaeology Saxony-Anhalt - State Museum of Prehistory, Halle (Saale), Germany.

-Rislev, Denmark. Sample: P188_Den_149. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Roseldorf, Austria. Samples: SRNHM007_Aus_m301 and SRNHM004_Aus_m294. Key contact: Erich Pucher (Naturhistorisches Museum Wien, Austria). Samples were made available through the Innovation Fund of the Austrian Academy of Sciences (ÖAW) (Grant agreement IF_2015_17).

-Sadgorod IV, Kurgan 2, sacrificial complex 2, Russia. Sample: LR18x5_Rus_1810. Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Saregrad-Klopare, Croatia. Samples: SRKRO001_Cro_720 and SRKRO002_Cro_722. Key contact: Mario Novak (Centre for Applied Bioanthropology, Institute for Anthropological Research, Ljudevita Gaja 32, 10 000 Zagreb, Croatia), and Andrea Rimpf. (Ilok Town Museum, Šetalište o. Mladena Barbarića 5, 32236 Ilok, Croatia). Samples were made available through the Innovation Fund of the Austrian Academy of Sciences (ÖAW) (Grant agreement IF_2015_17).

-Százhalombatta-Földvár, Hungary. Samples: SZHB2027_Hun_m2033, SZHB2074_Hun_m2115, SZHB2079_Hun_m1575, SZHB2147_Hun_m2054, SZHB2158_Hun_m1820, SZHB2438_Hun_m1602, SZHB553_Hun_m1566, SZHB625_Hun_m984, SZHB734_Hun_m1576, and SZHB967_Hun_m1822. Key Contact: Magdolna Vicze, Katherine Kanne (Department of Archaeology and History, University of Exeter, Exeter EX4 4QE, UK). Exported in 2008-2009 under National Science Foundation Dissertation Improvement Grant nb 0833106, Wenner-Gren Foundation for Anthropological Research Dissertation Fieldwork Grant nb 7896). Collections Institution: Matrica Museum.

-Sepphoris, Israel. Sample: MV243_Isr_294. Key contact: Liora Kolska Horwitz (National Natural History Collections, Edmond J. Safra Campus, Givat Ram, The Hebrew University; Jerusalem 9190401, Israel) Sampled with permission and all proper authority from Zeev Weiss (Eleazar L. Sukenik Professor of Archaeology, Institute of Archaeology, The Hebrew University of Jerusalem), archaeologist in charge of site.

-Shanmava, China. Sample: Shanx1_Chi_m112. Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia: interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).

-Shahr-i-Qumis, Iran. Sample: AM3_Ira_Modern. Key contact: Marjan Mashkour (CNRS, Muséum national d'Histoire naturelle, Archéozoologie, Archéobotanique (AASPE), CP 56, Paris, France).

-Shohidon, Tajikistan. Sample: Shohx1_Rus_720 (Grave 20). Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia:

interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).

-Shumaevo I, Kurgan 5, ditch, Russia. Samples: LR18x65_Rus_1352 (SE sector, skull 5) and LR18x66_Rus_1353 (NE sector, Skull 1, depth -123). Key contact: Pavel Kuznetsov (Department of Russian History and Archaeology, Samara State University of Social Sciences and Education, Samara, Russia). Sampled with proper authorization (confirmation letter nb 03-01-Myzea).

-Skedemosse, Sweden. Sample: F139_Swe_342. Key contacts: Johnny Karlsson (curator, The Swedish History Museum), Marie Sundquist (Östra Greda Research Group, 38791 Borgholm, Sweden), and Gabriella Lindgren (Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden). Loan agreement: 331-2017-958.

-Sosnovka 1, Russia. Samples: UR17x27_Rus_568. Key contact: Pavel Kosintsev (Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia).

-Tamiryn Ulaan Khoshuu, Mongolia. Samples: TAM13_Mon_14 (Tomb 13) and TAM22xA2_Mon_5 (Tomb 22). Key contacts: Éric Crubézy (Centre d'Anthropobiologie et de Génomique de Toulouse, CNRS UMR 5288, Université Paul Sabatier, Faculté de Médecine Purpan, 37 Allées Jules Guesde, 31000 Toulouse, France), and Tsagaan Turbat (Archaeological Research Center and Department of Anthropology and Archaeology, National University of Mongolia, Ulaanbaatar, Mongolia).

-Tarquinia monumental complex, Italy. Sample: Tarquinia3206_Ita_275. Key contact: Giovanna Bagnasco Gianni (Dipartimento di Beni Culturali e Ambientali Etruscologia, Università Degli Studi di Milano, Italy).

-Tominy, Poland. Samples: POZ327_Pol_m5127 (EQ_288, EQ_To6_07; No inv P18/09 (ob. 108), and POZ37_Pol_m5108 (No inv. 55/09; feature 115, layer 163). Key contact: Daniel Makowiecki (Institute of Archaeology, Faculty of History, Nicolaus Copernicus University, Toruń, Poland). Samples are from animal remains excavated (2006 – 2016) by Marcin Szeliga (Institute of Archaeology, Maria Curie-Skłodowska University, Lublin, Poland), and were collected during zooarchaeological research (2018) by Daniel Makowiecki, and stored since at the Institute of Archaeology, Nicolaus Copernicus, Torun.

-Tuse Skole, Denmark. Sample: P135_Den_1806. Key contacts: Kirsten Christensen (museum inspector; Museum West Zealand, Forten 10, 4300 Holbaek, Denmark), and Lone Claudi-Hansen (museum inspector; Museum West Zealand, Forten 10, 4300 Holbaek, Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Tyubyak, Russia. Samples: ABA9_Rus_m1786 and ABA10_Rus_m1757. Key contact: Pavel Kosintsev (Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia).

-Ulvehøj, Denmark. Sample: P191_Den_O1663. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Vejen, Denmark. Sample: P140_Den_O1660. Key contact: Kristian M. Gregersen (former collection manager of Quaternary Zoology, Natural History Museum of Denmark, Gothersgade 130, 1123 Copenhagen K., Denmark). Sampled with permission from the collection manager at the Collections of the Natural History Museum of Denmark.

-Vinkovci, Na-ma, Croatia. Samples: SRKRO009_Cro_m453 and SRKRO011_Cro_331. Key contact: Hrvoje Vucic (Vinkovci Municipal Museum, Trg bana Josipa Šokčevića 16, 32 100 Vinkovci, Croatia). Samples were made available through the Innovation Fund of the Austrian Academy of Sciences (ÖAW) (Grant agreement IF_2015_17).

-Yaloman-II, Russia. Sample: Yal2x24_Rus_m105. Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia: interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).

-Yenikapi, Türkiye. Sample: KSK11b_Tur_829. Key contact: Vedat Onar (Osteoarchaeology Practice and Research Center and Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul 34320, Turkey).

-Zayukovo 3, Russia. Samples: OSCAE16SP7x1_Rus_604 and OSCAE16SP8_Rus_577. Key contact: Anna Kadieva (State Historical Museum, Department of Archaeological Monuments, Moscow, Red Square 1, Moscow 109012, Russian Federation).

-Zoolongiyin am, Mongolia. Samples: Zoox1_Mon_333 and Zoox2_Mon_271. Key contact: Alexey A. Tishkin (Department of Archaeology, Ethnography and Museology, Altai State University, Prospekt Lenina, 61, 656049 Barnaul, Russia). Sampled with proper permission and authority by Alexey A. Tishkin from auxiliary collection of the Department of Archaeology, Ethnography and Museology of the Altai State University, under the framework of the Russian Science Foundation project "The world of ancient nomads of Inner Asia: interdisciplinary studies of material culture, sculptures and economy" (No. 22-18-00470).

Specimen deposition

Ancient remains were 3D-scanned in our facilities to ensure future morphometric studies, if needed, and stored in our laboratory facilities unless the bone fragment was inevitably destroyed during ancient DNA extraction. Based on the detailed archaeological metadata provided in Table S1, scholars are encouraged to contact Prof. Ludovic Orlando if aim to obtain further sample information.

Dating methods

140 samples were radiocarbon-dated. Their calibrated and uncalibrated dates, 95% confidence intervals, and relevant laboratory codes are provided in Table S1.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.