Reconstructing the origin and spread of horse domestication in the Eurasian steppe

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Despite decades of research across multiple disciplines, the early history of horse domestication remains poorly understood. On the basis of current evidence from archaeology, mitochondrial DNA, and Y-chromosomal sequencing, a number of different domestication scenarios have been proposed, ranging from the spread of domestic horses out of a restricted primary area of domestication to the domestication of numerous distinct wild horse populations. In this paper, we reconstruct both the population genetic structure of the extinct wild progenitor of domestic horses, *Equus ferus*, and the origin and spread of horse domestication in the Eurasian steppes by fitting a spatially explicit stepping-stone model to genotype data from >300 horses sampled across northern Eurasia. We find strong evidence for an expansion of *E. ferus* out of eastern Eurasia about 160 kya, likely reflecting the colonization of Eurasia by this species. Our best-fitting scenario further suggests that horse domestication originated in the western part of the Eurasian steppe and that domestic herds were repeatedly restocked with local wild horses as they spread out of this area. By showing that horse domestication was initiated in the western Eurasian steppe and that the spread of domestic herds across Eurasia involved extensive introgression from the wild, the scenario of horse domestication proposed here unites evidence from archaeology, mitochondrial DNA, and Y-chromosomal DNA.

demic spread | microsatellites | demography

The origin of horse domestication has been studied intensively for decades, yet the key question of whether horse domestication originated in a small number of geographically defined areas or whether numerous wild populations were domesticated independently remains unanswered. An increasing body of archaeological evidence points to an origin of horse domestication in the steppes of modern-day Ukraine and Kazakhstan (1) (see ref. 2 for review). However, there is as yet no corroborating molecular evidence for a geographically restricted origin of horse domestication anywhere in the Eurasian steppes, the main distribution area of wild horses at the time of domestication.

A related question concerns the spread of horse domestication across the Eurasian steppes: Did the spread of horse domestication involve actual movement of herds ("demic spread"), as appears to have been the case in most other domestic animal species (3)? Or was it primarily the knowledge of horse domestication techniques that spread, thus enabling pastoralist societies throughout the steppes to domesticate locally available wild stock? Whereas a demic spread of small herds of domestic horses out of a single, geographically restricted area has been put forward as one possible explanation for the observed low Y chromosome diversity in modern horses (4), the multiple-origins scenario is commonly invoked to account for the large number of female lineages in the domestic horse gene pool (2, 5, 6).

In this paper, we use a spatially and demographically explicit model, parameterized with autosomal genotype data from >300 horses, sampled in 12 localities distributed throughout northern Eurasia (Fig. 1A), to investigate the origin and spread of horse domestication. The model presented here allows us to distinguish between horse domestication in a single geographic area vs. multiple geographic areas, to pinpoint the geographic origin of domesticated horses in the former case, and to determine the relative roles of demic spread and recruitment of local wild stock in the spread of horse domestication.

Results

The wild progenitor of domestic horses, *Equus ferus*, is extinct; we therefore used a stepping-stone dynamic that allowed us to simultaneously reconstruct both the population genetic structure of *E. ferus* and that of its domestic descendants (see Fig. 1B for a diagrammatic representation of the model). We considered three population origins of *E. ferus*—western, central, and eastern Eurasia (Fig. 1C)—and combined each of the three wild horse origins with four putative origins of horse domestication (Fig. 1D), yielding a total of 12 combined scenarios. Depending on the choice of parameters, the model can cover a wide range of possibilities, spanning from populations having undergone range expansions to populations at migration–drift equilibrium. In the latter case, all three putative origins of *E. ferus* in Eurasia would be equally well supported. In this paper, we define domestication as a process whereby founder populations of domestic stock are established de novo and distinguish it from introgression as a process of re-stocking already domesticated herds with wild individuals.

For each of the 12 combined scenarios, we investigated a variety of population dynamics in both wild and domestic horse populations, as well as the full spectrum of possible modes of spread of domestication, ranging from a purely demic spread without wild horse introgression to numerous local domestications without population movements. In the latter case, all four putative

domestication origins should fit the data equally well. We used an approximate Bayesian computation framework to determine the likelihood of each of the 12 scenarios by comparing the model predictions to the observed data (Fig. 1*A* and Table S1).

The scenarios positing an origin of *E. ferus* in eastern Eurasia received the strongest support, regardless of the assumed domestication origin (Fig. 2, red bars). This finding is in agreement with paleontological evidence suggesting that Pleistocene horse species, including *E. ferus*, originated in North America and migrated to Eurasia via the Bering land bridge (7). Of the four domestication scenarios assuming an origin of *E. ferus* in eastern Eurasia, the scenario proposing a domestication origin in western central Eurasia received the strongest support (Fig. 2). Western central Eurasia, as defined here, comprises the steppes of modern-day Ukraine and northwest Kazakhstan, where some of the earliest archaeological evidence for managed horse populations has been found (1, 2, 8); here we provide additional evidence for the importance of this region as a primary area of horse domestication.

It should be noted that our dataset does not include populations west of Ukraine. Horses in central and western Europe tend to either belong to a specific recognized breed or be considerably breed admixed. Unfortunately, the modeling framework used here is not well suited to deal with the complex demographic histories experienced by horse populations in central and western Europe; the role of these areas in horse domestication can therefore not be confidently established here. Whereas central Europe is unlikely to have played an important role in horse domestication due to a lack of suitable wild horse habitat (9), there is increasing evidence that horse domestication may have also occurred in the Iberian Peninsula (9, 10).

Next, we explored the demographic details of the most strongly supported scenario, which posits an expansion of *E. ferus* out of eastern Eurasia and a domestication origin in western central Eurasia. The parameter combinations that best fitted the data strongly suggest that the expansion of *E. ferus* occurred ~160 kya (Fig. 3*A*). Although the credibility interval (CI) for this date is relatively wide (95% CI: 51–180 kya; Table S2), our estimate is very similar to the estimated age of 130–160 kya for the common maternal ancestor of modern domestic horses (10). The scenario of an expansion of *E. ferus* out of eastern Eurasia ~160 kya is furthermore consistent with paleontological data indicating that *E. ferus* first appeared in East Asia ~200 kya (7). Thus, the expansion captured by our model likely reflects the colonization of Eurasia by this species. Our results further suggest that this expansion was characterized by relatively strong founder effects (small cK, Fig. 3*B*), large effective population sizes (K, Fig. 3*C*), and a rate of spread in the order of ~100 km in 240 y (growth rate $r = 0.05$, Fig. 3*D*). Due to the early date of this expansion, a wide range of migration rates in wild horses are consistent with the data (mK, Fig. 3*E*).

Both the scenario postulating a purely demic spread ($q = 1$) and the one postulating a spread based solely on local recruitment ($q = 0$) are outside the 95% credibility interval for q (the proportion of already domesticated horses in the founding population of subsequent domestic populations; Table S2), suggesting that the spread of horse domestication out of western central Eurasia involved both actual population movement and introgression from the wild. The best-supported values of q ranged between 0.2 and 0.7 (Fig. 3*F*), suggesting that both mechanisms were approximately equally important. The demography of horses changed markedly following domestication. Compared with the expansion of *E. ferus*, the spread of domestic horses was characterized by weaker founder effects (larger $c_d K_d$ in Fig. 3*B*) and smaller effective sizes of established populations (K_d, Fig. 3*C*). We obtained strong support for high migration rates between domestic populations
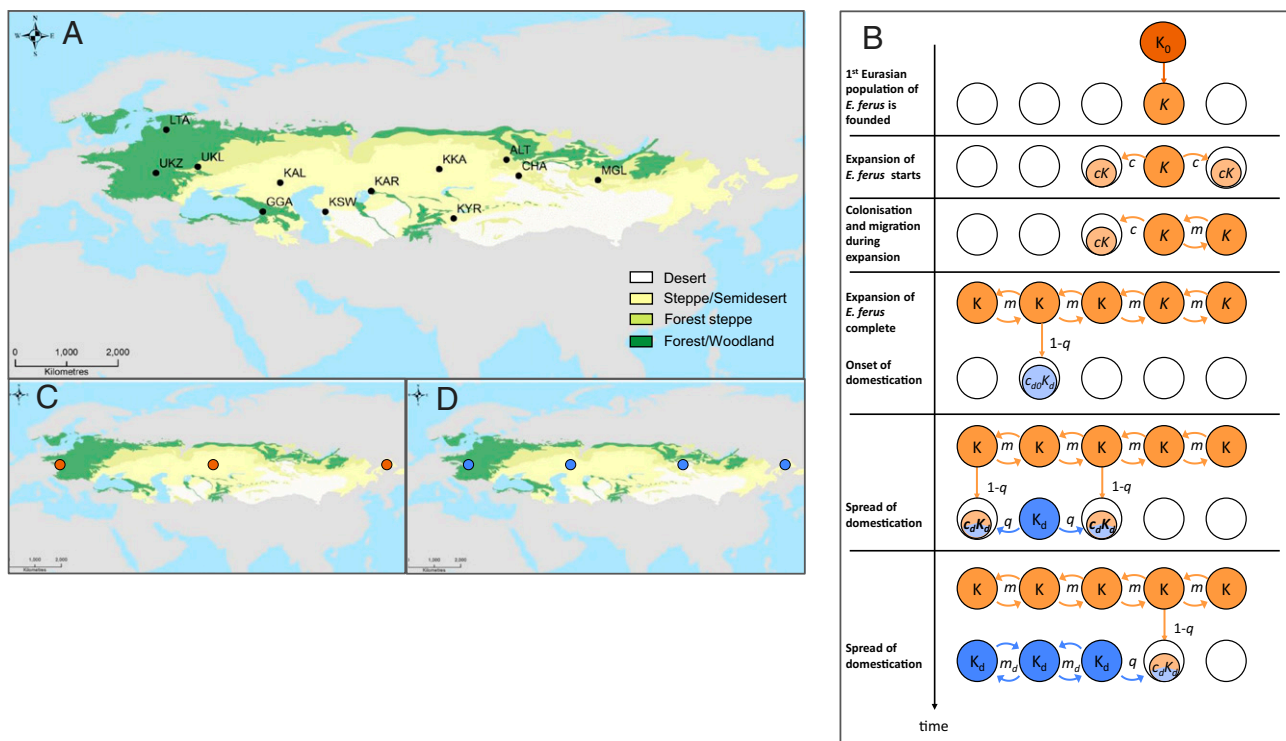

Fig. 1. Geographic distribution of sampling locations (country and administrative unit) and the model. (*A*) LTA, Lithuania, Vilnius; UKZ, Ukraine, Zakarpattia; UKL, Ukraine, Lviv; KAL, Russia, Kalmykia; GGA, Georgia, Samegrelo; KSW, Kazakhstan, Mangystau; KAR, Kazakhstan, Kyzylorda; KKA, Kazakhstan, Karagandy; KYR, Kyrgyzstan, Naryn; ALT, Russia, Altai Republic; CHA, China, Xinjiang; MGL, Mongolia, Övörkhangai. (*B*) Schematic representation of the linear stepping-stone model used in this study (see *Materials and Methods* for a detailed description of the parameters). Note that for illustrative purposes only five demes are shown. (*C*) Location of the three putative origins of *E. ferus*. (*D*) Locations of the four putative domestication origins.
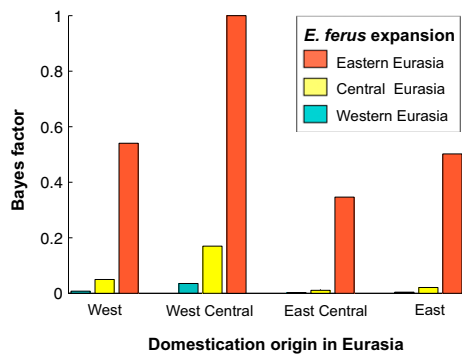
Fig. 2. Bayes factors for 12 scenarios describing the origin of the wild progenitor of domestic horses, *Equus ferus*, and the origin of horse domestication in Eurasia. Bayes factors are relative to the best-supported scenario, which posits an origin of *E. ferus* in eastern Eurasia and a domestication origin in western central Eurasia. The scenarios are grouped by domestication origin and colored by the origin of *E. ferus*.

($m_d K_d$, Fig. 3E), consistent with the increased mobility of human societies following horse domestication.

Discussion

Previous research into the origin of horse domestication has led to different, sometimes contradictory, conclusions regarding the number of domestication origins and the mode of spread of horse domestication. Here we provide additional support for an origin of horse domestication in a geographically defined area in the western Eurasian steppe as suggested by archaeology (1, 2). We furthermore show that the spread of horse domestication out of the western Eurasian steppe was characterized by high levels of introgression from local wild populations. In light of the exceptionally high levels of matrilineal diversity in horses (e.g., ref. 11), we suggest that introgression from the wild was mainly female mediated. The repeated capture of wild females for the purpose of maintaining or growing domestic herd sizes may seem counterintuitive, given that in other domestic animal species, introgression from the wild typically involved domestic females being impregnated by wild males (12). However, given the initial difficulties in breeding the most closely related wild relative of domestic horses, Przewalski's horse, in captivity (13), it can be speculated that, for an indeterminate amount of time, herd sizes could not be maintained solely through breeding existing stock (14). Because stallions are inherently more difficult to handle than mares, the easiest way to maintain or grow herd sizes would have been to restock existing herds with wild females. Whereas the high levels of diversity and limited geographic structure in the horse mitochondrial genome (11) may thus reflect the continued augmentation of domestic herds with wild mares from a wide geographic area, the observed low levels of Y chromosome variability (15) might reflect the strong domestication bottleneck in western central Eurasia (low $c_{d0}K_d$, Fig. 3B) (4). Additional losses of patrilineal diversity may have occurred as a consequence of successive bottlenecks as domestic herds spread out of the western steppes (4) and of breeding practices reducing the effective size of the male gene pool (16).

The geographic pattern of within- and between-population heterozygosity observed in contemporary horses from the Eurasian steppes reflects the combined effects of an east-to-west expansion of *E. ferus* and repeated introgression of local wild animals into spreading domestic herds. The expansion of *E. ferus* out of eastern Eurasia set up an isolation-by-distance pattern (off-diagonal elements in Fig. S1A) and a weak east-to-west decline in within-population genetic diversity (on-diagonal elements in Fig. S1A). The pattern of isolation-by-distance has been preserved in modern Eurasian steppe horses (off-diagonal elements in Fig. S1B)
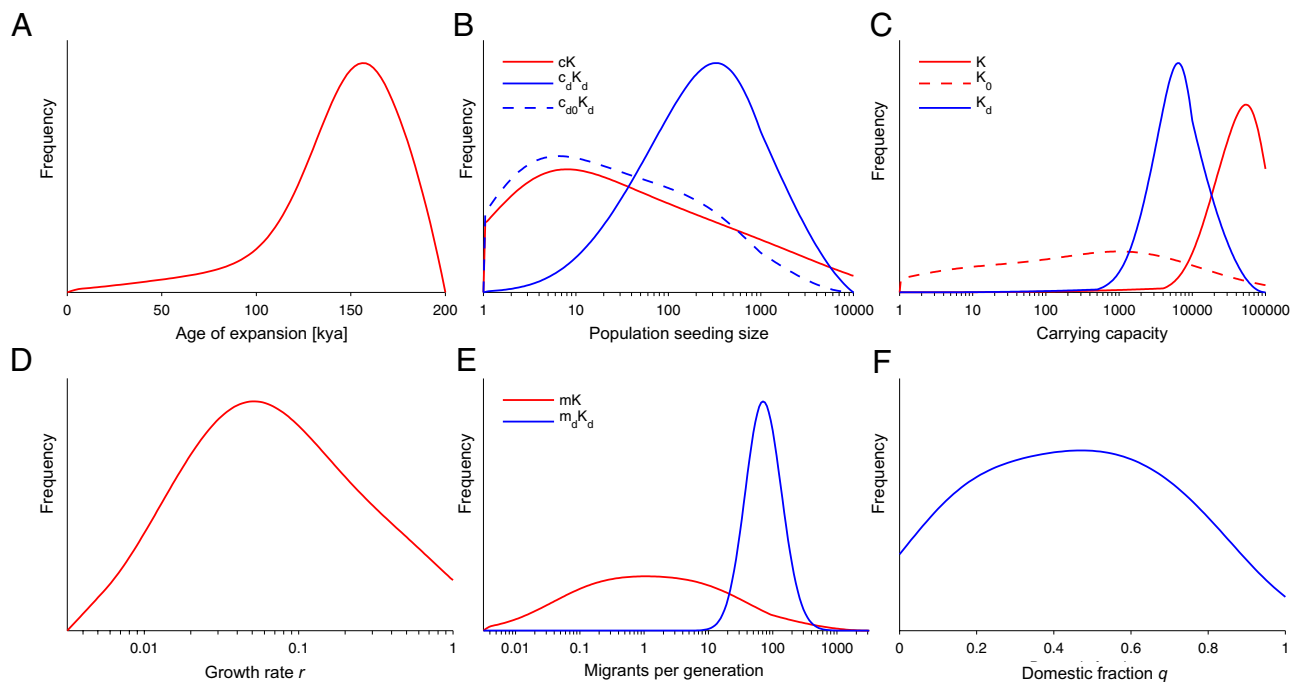

Fig. 3. Posterior distributions of demographic parameters for wild and domestic populations estimated through ABC analysis. (A) Timing (*t*) of the expansion of *E. ferus* out of eastern Eurasia. (B) Effective founder population sizes of the wild horse (*cK*, red line), the first domestic population ($c_{d0}K_d$, blue dashed line), and subsequent domestic populations ($c_d K_d$, blue solid line). (C) Carrying capacity (effective population sizes) of the ancestral (K_0, dashed red line), wild (*K*, solid red line), and domestic populations (K_d, blue line). (D) Growth rate, *r*, of wild horse populations. (E) Migration rates in wild (*mK*, red line), and domestic populations ($m_d K_d$, blue line). (F) The proportion of domestic (*q*) and wild (1 − *q*) horses in domestic horse founder populations as a function of their effective size, $c_d K_d$.

due to extensive backcrossing of domestic herds to wild populations from across the steppes. The demic spread of domestic horses out of western central Eurasia, combined with the continued genetic input from local wild populations, has resulted in an accentuation of the original east-to-west decline in within-population genetic diversity (on-diagonal elements in Fig. S1B) because hybridization involved increasingly genetically differentiated populations. Whereas migration rates in domestic horses were found to be high (Fig. 3E), the relatively recent occurrence of horse domestication (from ~5.5 kya) (1, 17) means that there has not been enough time for increased population movements to obscure the genetic signature of the initial colonization process on large geographic scales, an observation that also holds true for other species (18–20), including humans (21, 22).

In the past decade, spatially explicit models have successfully been used to unravel complex demographic scenarios in the context of the spread of agricultural practices. Using a modified wave-of-advance model, Ackland et al. (23) identified the factors influencing the hitchhiking of cultural traits alongside beneficial subsistence technologies in heterogeneous landscapes. Itan et al. (24) used a demic computer model to trace the spread of lactase persistence and therefore dairying in Europe. More recently, Van Etten and Hijmans (25) used a landscape model, parameterized with archaeobotanical and genetic data, to trace the origin and routes of spread of maize in the Americas. In this paper, the combined use of autosomal markers and spatially explicit modeling has allowed us to reconstruct the population genetic structure of the extinct wild ancestor of domestic horses and to unravel the complex processes involved in horse domestication. The scenario of horse domestication proposed here unites evidence from archaeology, mtDNA, and Y-chromosomal sequence data that has previously given rise to conflicting scenarios.

Materials and Methods

Sample Collection and DNA Analysis. Our dataset consists of 322 nonbreed horses sampled in 12 geographically localized (within a radius of 100 km) sampling areas spanning eight countries (Fig. 1A and Table S1). Populations of nonbreed horses (local village horses that are mainly used for work and do not belong to any particular breed) have not been subjected to the same degree of intense artificial selection and closed breeding as many modern breeds and are thus expected to be more informative regarding horse population history.

Genomic DNA was extracted from 15–20 hair roots per individual according to a protocol adapted from ref. 26. DNA extracts were purified (QIAquick purification kit; Qiagen) and standardized to a concentration of 10 ng of DNA/μL. A total of 26 microsatellite markers were amplified in two multiplex reactions (Table S3), using the Type-it Microsatellite PCR kit from Qiagen. PCR amplifications were carried out according to the manufacturer's instructions using 10 ng of template DNA and a total volume of 12.5 μL. Samples were run on an ABI 3730 Genetic Analyzer (Applied Biosystems). Alleles were assigned with GeneMapper Software v.3.7 (Applied Biosystems).

Modeling Framework. We used a general stepping-stone framework that can represent a wide variety of demographic scenarios, including populations at migration–drift equilibrium and populations having undergone range expansions, depending on the choice of parameters. Eurasian populations of *E. ferus* were represented by a linear chain of 80 demes (numbered 0–79, from east to west), each 100 km apart, stretching from east to west and starting from latitude 50° N and longitude 125° E (deme 0). Sampling locations were assigned to demes on the basis of their shortest distance on land from deme 0, such that, for example, a location 1,200 km from deme 0 would be assigned to deme 12. The shortest distances on land between populations were calculated as in ref. 22. To avoid boundary artifacts, we appended 20 demes on both sides of the chain (see Fig. 1D for a schematic representation of the model). To test the sensitivity of our results to deme spacing, we repeated the full analysis using 160 demes with a spacing of 50 km as well as 40 demes with a spacing of 200 km, respectively; sampling locations within the chain of demes were recalculated on the basis of the new deme spacings.

We considered three putative population origins of *E. ferus*, one in eastern Eurasia (deme 0), one in central Eurasia (deme 40), and one in western Eurasia (deme 80) (Fig. 1C). In each scenario, the deme corresponding to the population origin of *E. ferus* in Eurasia was populated by randomly sampling cK_0 diploid individuals from a hypothetical ancestral population of size K_0. The initial founder population grows linearly at rate rK horses per generation until it reaches carrying capacity K. Demes at carrying capacity send out a fraction c of colonizers to neighboring empty demes. In addition, occupied neighboring demes exchange mN_{min} migrants per generation, where N_{min} represents the smaller of the two population sizes.

The domestication process was initiated 450 generations before present (t – 450), the number of generations that have elapsed since domestication started, assuming an average generation time of 12 y for wild horses (27) and a start date for horse domestication around 6,000 y ago (1, 16). Populations of domestic horses were represented by a linear chain of demes parallel to the one representing the wild horse populations and with the same spatial structuring. The sampled horse populations (Fig. 1A) were placed on this chain according to their geographic distance from the easternmost deme as described above. We considered four putative origins of horse domestication, one in eastern Eurasia (deme 0), one in eastern central Eurasia (deme 25), one in western central Eurasia (deme 50), and one in western Eurasia (deme 75) (Fig. 1D). In each run, domestication was initiated by randomly sampling $c_{d0}K_d$ individuals from the deme representing the wild population closest to the putative domestication origin. The initial founder population grows within one generation to size K_d, the carrying capacity of the domestic demes. Subsequently, empty demes (i.e., domestic herds) are established from c_dK_d effective individuals representing a mixture of already domesticated horses from the previous deme and local wild horses in proportions q and $1 - q$, respectively. The parameter q thus describes the proportion of already domesticated horses in the founding population of subsequent domestic populations, with $q = 1$ corresponding to a pure demic spread of domestication and $q = 0$ corresponding to multiple independent domestications without a demic component. Once established, neighboring domestic populations exchange migrants at rate m_d.

Given the large number of parameters and scenarios, running a suitable number of stochastic simulations is challenging. We optimized computing time by finding the analytical solution predicting the expected values of within- and between-population heterozygosity for any given combination of parameter values for each scenario (*SI Materials and Methods*). Given the expected values, we generated approximate stochastic samples from the model by adding noise generated with the same correlation structure between the elements of the matrix of heterozygosity values as in the observed data (estimated using the bootstrap method and 10,000 replicates). A validation of this approach is given in *SI Materials and Methods*.

Model Fitting. We fitted our model by using an approximate Bayesian computation framework, using the ABC-GLM algorithm implemented in the ABCtoolbox software (28). We used six summary statistics to describe our dataset: After assigning the sampled populations to three groups (west, central, and east, Fig. S2), we computed average within-population heterozygosities within each of the groups (three estimates) and average between-group heterozygosities (west vs. central, west vs. east, and central vs. east, three estimates).

We started by randomly sampling 55 million combinations of parameter values within the following ranges: $t \in$ [500, 15,000] generations (corresponding to 6–180 kya), $m \in$ [10^{-6}, 10^{-3}], $cK \in$ [1, 10^4], $r \in$ [0.005, 1], $K \in$ [4,000, 10^5], $K_0 \in$ [1, 10^5], $m_d \in$ [10^{-4}, 0.5], $K_d \in$ [500, 10^4], $c_dK_d \in$ [1, 10^3], $q \in$ [0, 1], and $c_dK_{d0} \in$ [1, 10^3]. Whereas t and q were sampled according to the uniform distribution of their untransformed values, all other parameters were sampled from the uniform distribution of their log-transformed values. For each parameter value combination, we then generated our six summary statistics for each of the 12 scenarios, combining all possible origins for wild horses and domestication events in our model. In these calculations we took the mutation rate (μ) to be 1.5×10^{-4} per generation (the average of mutation rate estimates for two microsatellite markers, AHT4 and HTG10) (29). Because horse domestication occurred relatively recently, the only parameters that are considerably affected by the mutation rate are the time of the initial expansion of *E. ferus* in Eurasia (t) and the ancestral population size of *E. ferus* (K_0). For each scenario, we ran ABC-GLM on the accepted parameter combinations (on the basis of the 0.1 percentile of Euclidean distances between simulated and observed summary statistics) to estimate posterior distributions of the model parameters and the likelihood of the summary statistics as estimated from the genetics data (Table S2). We then used Bayes factors, given by the ratio of estimated likelihoods for each pair of scenarios, for model comparison. Because both Bayes factors and posterior distributions

for a deme spacing of 100 km (Figs. 2 and 3) were in close agreement with those obtained for deme spacings of 50 km and 200 km, respectively (Figs. S3–S5), we refer to the former only in the main text.

1. Outram AK, et al. (2009) The earliest horse harnessing and milking. *Science* 323: 1332–1335.
2. Olsen SL (2006) *Documenting Domestication: New Genetic and Archaeological Paradigms*, eds Zeder MA, Bradley DG, Emshwiller E, Smith BD (Univ of California Press, Los Angeles), pp 245–269.
3. Clutton-Brock J (1999) *A Natural History of Domesticated Mammals* (Cambridge Univ Press, Cambridge, UK).
4. Lindgren G, et al. (2004) Limited number of patrilines in horse domestication. *Nat Genet* 36:335–336.
5. Vilà C, et al. (2001) Widespread origins of domestic horse lineages. *Science* 291: 474–477.
6. Jansen T, et al. (2002) Mitochondrial DNA and the origins of the domestic horse. *Proc Natl Acad Sci USA* 99:10905–10910.
7. Prothero DR, Schoch RM (2002) *Horns, Tusks, & Flippers: The Evolution of Hoofed Mammals* (The John Hopkins Univ Press, Baltimore), pp 213–227.
8. Brown D, Anthony D (1998) Bit wear, horseback riding and the Botai site in Kazakhstan. *J Archaeol Sci* 25:331–347.
9. Achilli A, et al. (2012) Mitochondrial genomes from modern horses reveal the major haplogroups that underwent domestication. *Proc Natl Acad Sci USA* 109:2449–2454.
10. Warmuth V, et al. (2011) European domestic horses originated in two holocene refugia. *PLoS ONE* 6:e18194.
11. Cieslak M, et al. (2010) Origin and history of mitochondrial DNA lineages in domestic horses. *PLoS ONE* 5:e15311.
12. Luikart G, et al. (2001) Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc Natl Acad Sci USA* 98:5927–5932.
13. Boyd L, Houpt KA (1994) *Przewalski's Horse, the History and Biology of an Endangered Species*, eds Boyd L, Houpt KA (State Univ of New York Press, Albany, NY), pp 195–228.
14. Levine MA (1999) Botai and the origins of horse domestication. *J Anthropol Archaeol* 18:29–78.
15. Ling Y, et al. (2010) Identification of Y chromosome genetic variations in Chinese indigenous horse breeds. *J Hered* 101:639–643.
16. Lippold S, et al. (2011) Discovery of lost diversity of paternal horse lineages using ancient DNA. *Nat Commun* 2:450.
17. Ludwig A, et al. (2009) Coat color variation at the beginning of horse domestication. *Science* 324:485–485.
18. Linz B, et al. (2007) An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 445:915–918.
19. Tanabe K, et al. (2010) *Plasmodium falciparum* accompanied the human expansion out of Africa. *Curr Biol* 20:1283–1289.
20. François O, Blum MGB, Jakobsson M, Rosenberg NA (2008) Demographic history of European populations of *Arabidopsis thaliana*. *PLoS Genet* 4:e1000075.
21. Manica A, Amos W, Balloux F, Hanihara T (2007) The effect of ancient population bottlenecks on human phenotypic variation. *Nature* 448:346–348.
22. Prugnolle F, Manica A, Balloux F (2005) Geography is a better determinant of human genetic differentiation than ethnicity. *Curr Biol* 15:R159–R160.
23. Ackland GJ, Signitzer M, Stratford K, Cohen MH (2007) Cultural hitchhiking on the wave of advance of beneficial technologies. *Proc Natl Acad Sci USA* 104:8714–8719.
24. Itan Y, Powell A, Beaumont MA, Burger J, Thomas MG (2009) The origins of lactase persistence in Europe. *PLoS Comput Biol* 5:e1000491.
25. van Etten J, Hijmans RJ (2010) A geospatial modelling approach integrating archaeobotany and genetics to trace the origin and dispersal of domesticated plants. *PLoS ONE* 5:e12060.
26. Allen M, et al. (1998) Mitochondrial DNA sequencing of shed hairs and saliva on robbery caps: Sensitivity and matching probabilities. *J Forensic Sci* 43:453–464.
27. Sokolov VE, Orlov VN (1986) *The Przewalski Horse and Restoration to Its Natural Habitat in Mongolia* (Food and Agriculture Organization of the United Nations, Agriculture and Consumer Protection Department, Rome).
28. Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L (2010) ABCtoolbox: A versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics* 11:116.
29. Bowling AT, et al. (1997) Validation of microsatellite markers for routine horse parentage testing. *Anim Genet* 28:247–252.