

## RESEARCH ARTICLE

## DOMESTICATION

## The genomic history and global expansion of domestic donkeys

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Donkeys transformed human history as essential beasts of burden for long-distance movement, especially across semi-arid and upland environments. They remain insufficiently studied despite globally expanding and providing key support to low- to middle-income communities. To elucidate their domestication history, we constructed a comprehensive genome panel of 207 modern and 31 ancient donkeys, as well as 15 wild equids. We found a strong phylogeographic structure in modern donkeys that supports a single domestication in Africa ~5000 BCE, followed by further expansions in this continent and Eurasia and ultimately returning to Africa. We uncover a previously unknown genetic lineage in the Levant ~200 BCE, which contributed increasing ancestry toward Asia. Donkey management involved inbreeding and the production of giant bloodlines at a time when mules were essential to the Roman economy and military.

Domestic donkeys (*Equus asinus*) have facilitated the movement of goods and people for millennia, enabling trade and transport across a broad spectrum of landscapes (1). Despite their importance to ancient pastoral societies, little is known about the deep history of donkeys and the impact of human management on their genomes. This is most likely due to their undervalued status and loss of utility in modern industrialized societies. Donkeys are, however, extraordinary working animals and remain essential for

developing communities, especially in semi-arid environments (2). Understanding their genetic makeup is not only key to assessing their contribution to human history but also to improving their local management in the future.

The current archaeological record of early donkeys is limited (1, 3), which makes their domestic origins and spread through the world contentious. The reduced body size of zooarchaeological ass remains in Egypt at El Omari (4800 to 4500 BCE) and Maadi (4000 to

3500 BCE) has been interpreted as early evidence of domestication (4–7). Carvings on the Libyan palette that were found in Abydos, Egypt (3200 to 3000 BCE), depict lines of walking asses, cattle, and sheep, which also suggests a domestication context (8, 9). Together with contemporary remains from the same region that show morphological evidence for load carrying (10), these findings suggest that donkeys could have been first domesticated within a range extending from the Northeastern Sahara, the Nile Valley, the Atbara River, and the Red Sea Hills to Eritrea. In this model, donkeys were domesticated by pastoralists to assist with mobility around 5500 to 4500 BCE because of the large-scale aridification of the Sahara (1). Independent evidence based on patterns of mitochondrial (11, 12) and nuclear sequence variation (13) also points to the African origins of the donkey, owing to their closer proximity to African wild asses (*Equus africanus* spp.) than to Asian wild asses (*Equus hemionus* spp.).

However, candidate regions outside of Africa are also proposed as alternative domestication centers. In Ash Shuman (Yemen), for example, ass remains of disputed domestic status predate those from Egypt by 2000 years (~6500 BCE) (14). Likewise, textual, iconographic, and zooarchaeological material indicates a possible additional center in Mesopotamia during the fourth and third millennia BCE (15–19), a context in which first-generation hybrids of donkeys and Syrian onagers have been identified genetically (20). The segregation of mitochondrial variation in two main clades may also support a dual domestication process, for which the Nubian wild ass (*Equus africanus africanus*) is securely identified as the progenitor of Clade I (11, 12). As for the ancestor of Clade II, it could either be the extinct Atlas wild ass (*Equus africanus atlanticus*), which was endemic to Northern Africa, or another undescribed subspecies that potentially ranged outside of Africa. Whether a single, maternally

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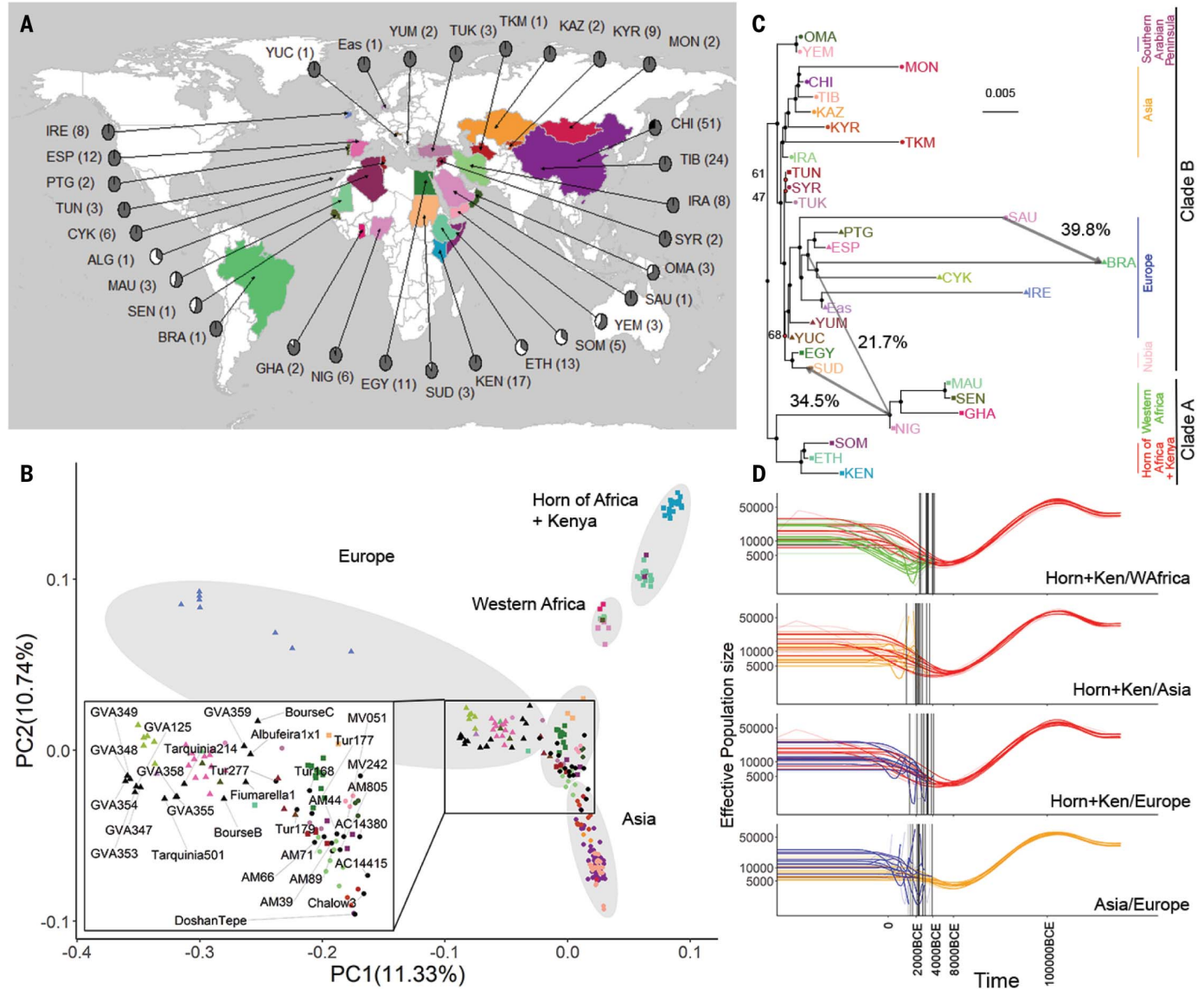
inherited marker captures the whole complexity of underlying ancestries can, however, be questioned after recent results from other animals [e.g., horses (21)]. Furthermore, previous analyses of nuclear genetic variation in African and non-African donkeys have failed to disentangle their origins (13, 22). Overall, this lack of consensus between genetic and archaeological data means that the geographic and temporal origin of donkeys and whether they

were domesticated more than once remain uncertain. The global spread of donkeys is also unclear, as their worldwide patterns of genomic diversity lack extensive characterization.

**Modern donkeys originated in Africa and spread into Eurasia**

To address these issues, we sequenced 49 modern donkey genomes from underrepresented regions and combined these with 158 public-

ly available genomes to capture worldwide diversity (Fig. 1A and table S1) (13, 23–25). We constructed a fine-scale recombination map from genomes that encompassed all phylogenetic groups, which we used to phase 13,013,551 variants (fig. S1 and tables S3 and S4). Principal component analysis (26) revealed strong geographical substructuring, with donkeys from Africa, Europe, and Asia forming distinct genetic clusters (Fig. 1B and figs. S2 and S3).



**Fig. 1. Modern donkey dataset and population evolutionary history.**

(A) Number and geographical distribution of modern donkey samples ( $n = 207$ ). Pie charts show the ADMIXTURE proportion of domestic ancestry (gray), African wild ass ancestry (white), and kiang ancestry (black) averaged across all individuals from each country (56). For visualization, the total surface of each pie chart is scaled to 2%. (B) SmartPCA (57) of modern donkeys, with the imputed ancient samples in black. PC, principal component. (C) Treemix phylogeny of modern domesticates (excluding individuals with high wild introgression,  $n = 201$ ) (27). Node supports are estimated from 100 bootstrap pseudo-replicates (confidence <90% in red). The percentage values indicate admixture proportions inferred from Treemix (27). (D) SMC++ demographic trajectories (colored)

and split time estimates (black) for pairs of main geographic regions (28), repeating the analysis on two datasets of three individuals per population (the second dataset is shown in semitransparency). Modern donkeys are colored and shaped according to geographical location and continents in all panels. Country Codes: Algeria (ALG), Brazil (BRA), China (CHI), Canary Islands (CYK), Denmark (Eas), Egypt (EGY), Spain (ESP), Ethiopia (ETH), Ghana (GHA), Iran (IRA), Ireland (IRE), Kazakhstan (KAZ), Kenya (KEN), Kyrgyzstan (KYR), Mauritania (MAU), Mongolia (MON), Nigeria (NIG), Oman (OMA), Portugal (PTG), Saudi Arabia (SAU), Senegal (SEN), Somalia (SOM), Sudan (SUD), Syria (SYR), Tibet (TIB), Turkmenistan (TKM), Turkey (TUK), Tunisia (TUN), Yemen (YEM), Croatia (YUC), Macedonia (YUM).

A Treemix phylogenetic reconstruction that grouped modern donkeys according to sampling locations (27) confirms the earliest split between African (Clade A) and mostly non-African donkeys (Clade B) (Fig. 1C). Further structure within Clade A separates donkeys from the Horn of Africa (Ethiopia and Somalia) plus Kenya from those from Western Africa (Ghana, Mauritania, Nigeria, and Senegal). Within Clade B, we find another major divergence between European and Asian donkeys, with east-to-west affinities in Europe from the Balkans (Croatia and Macedonia) to Iberia, Denmark, and Ireland. Conversely, Asian subpopulations show west-to-east substructuring from Iran and Central Asia to China and Mongolia. Combined, these findings suggest expansions from a central source into both continents.

In Clade B, some of the most basal donkeys are from the Southern Arabian Peninsula (Oman and Yemen), whereas the single donkey from Saudi Arabia analyzed here shows European affinities, which is indicative of a secondary translocation. Similarly, the Pega donkey from Brazil is nested within Iberia, which mirrors the colonization history of the Americas. Clade B also includes donkeys from Nubia (Egypt and Sudan) that show affinities to the Levant (Syria) and Anatolia (Turkey), as well as donkeys from Maghreb (Tunisia), with closer genetic proximity to European subpopulations. This suggests gene flow into Africa from donkeys that were native to Anatolia and the Levant, but not to the Arabian Peninsula. Overall, this phylogenetic reconstruction is compatible with both models of donkey domestication: a unique origin in Africa followed by dispersals out and back, or dual origins in Africa and the Southern Arabian Peninsula.

The unique origin model posits a demographic expansion in Africa first and subsequent waves into Europe and Asia. By contrast, dual origins would result in an earlier split of demographic trajectories between African and Eurasian subpopulations, given their deep phylogenetic divergence. To test these, we first performed demographic modeling using SMC++ (28), which revealed the first expansion around 5200 BCE ( $7186 \pm 742$  years ago), in line with archaeological evidence of domestication occurring at this time (Fig. 1D and fig. S6). Additionally, when modeled from a possible African source, SMC++ trajectories indicated more recent and nearly coincidental expansions into Asia around 2600 BCE ( $4573 \pm 577$  years ago) and Europe around 2800 BCE ( $4806 \pm 671$  years ago) (Fig. 1D). This is in line with the unique origin model and the earliest archaeological evidence of donkeys in Asia (Iranian Plateau and the Indus Valley) and Europe (Portugal, Greece, and Cyprus) in the mid-to-late third millennia BCE (29–34).

Furthermore, the Yemen and Oman subpopulations do not branch basal to Clade B according to fineSTRUCTURE (35), in contrast to the expectations of the dual origins model, but within Asian subpopulations (Fig. 2, A and B). Lastly, the pairwise genetic distances between Ethiopia and non-African subpopulations were greater than those from Yemen (fig. S5). They both increased linearly with geographic distances and supported identical dispersal rates (fig. S5;  $p = 0.775$ ), which is in line with a single wave of expansion at a constant pace. Therefore, our analyses support an early domestication in Africa that spread at an even rate into the Arabian Peninsula and Eurasia and flowed back into Nubia and Maghreb. Modern subpopulations from the Horn of Africa and Kenya so far best represent the descendants of earliest donkeys.

#### **Ancient donkey genomes reveal early and rapid dispersal into Asia and secondary contacts between Europe and Western Africa**

The patterns of genetic variation within modern subpopulations may reflect recent breeding history rather than early domestication (36). Additionally, they could underrepresent the contribution of lineages that were once important but have since declined (37). Dating population splits also assumes constant yet unknown generation intervals. To address these caveats and validate the domestication history reconstructed above, we generated a genomic time series spanning the past ~4000 years that included 31 ancient donkeys from 11 different sites, which ranged from the Atlantic shores (Portugal) to Central Asia (Eastern Iran/Turkmenistan) (Fig. 3A and table S2).

Ancient genomes sequenced to 0.77- to 5.05-fold coverage (table S6) were analyzed by using two complementary methods: pseudo-haploidization following (21) that resulted in 4,833,570 nucleotide transversion sites and genotype imputation following (38), at 7,161,029 polymorphic sites present at >5% frequency in modern donkeys (38, 39). Imputation accuracy was confirmed by the high consistency rates between imputed and observed genotypes after down-sampling of high-coverage modern genomes and downstream analyses that were largely consistent with those that were based on pseudo-haploidized data (figs. S7 to S15 and table S6).

The three oldest samples from our dataset consist of donkeys from Anatolia (Acemhöyük, Turkey), which were radiocarbon dated to 2564 to 2039 BCE. Their age and phylogenetic placement within Clade B confirm an early expansion out of Africa by ~2500 BCE, which is in agreement with SMC++ time estimates (Fig. 2A and fig. S10). These samples, as well as a donkey from Eastern Iran/Turkmenistan that is affiliated to the Bactria–Margiana Ar-

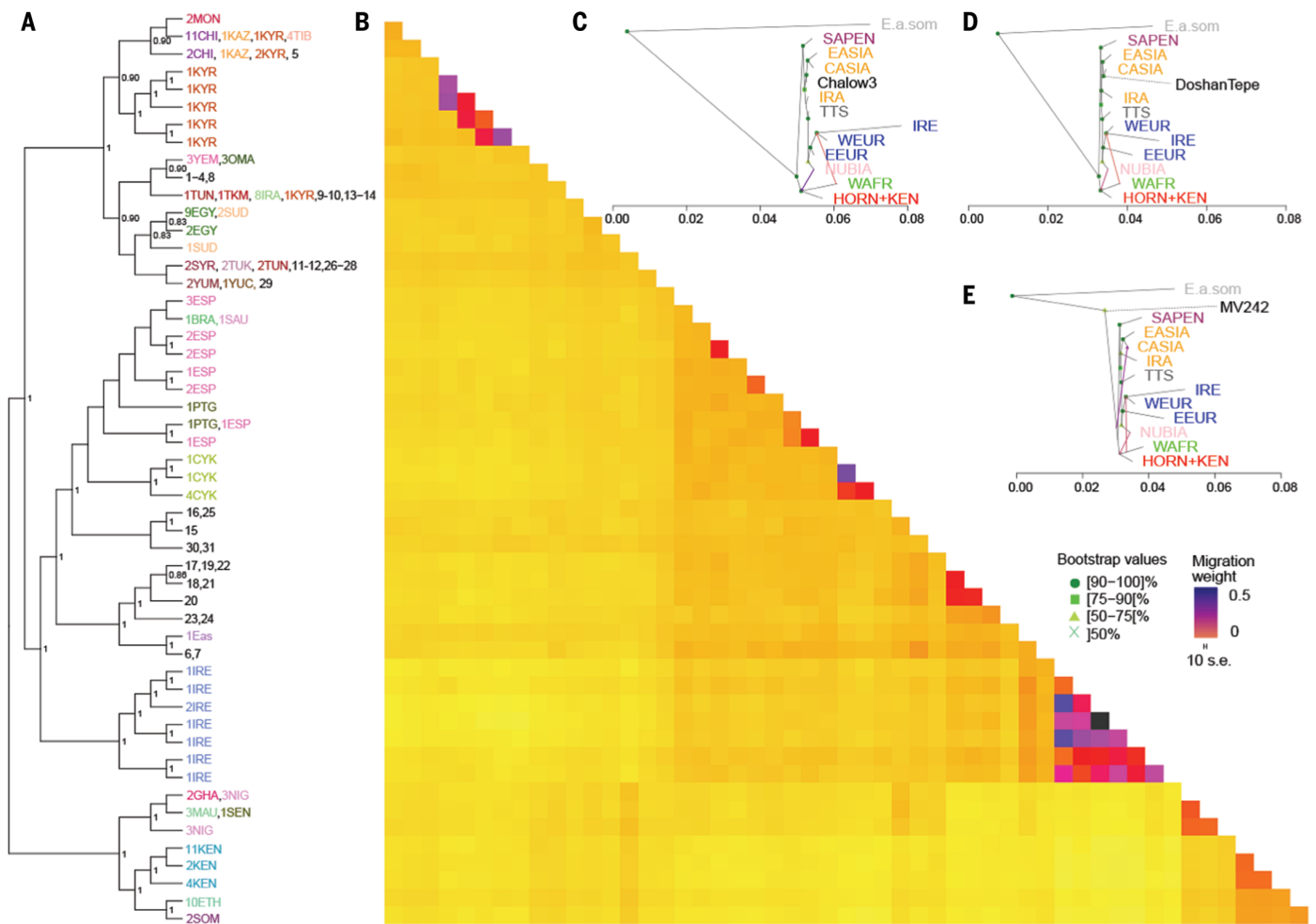
chaeological Complex (BMAC) (~2050 BCE; sample Chalow3), branch before the formation of modern subpopulations from Central Asia (Kazakhstan, Kyrgyzstan, Turkmenistan) and Eastern Asia (China, Mongolia, Tibet) (Fig. 2C). These subpopulations thus diverged after 2050 BCE, but potentially before the radiocarbon age of the donkey from Doshan Tepe (1049 to 928 BCE), which appears closer to modern subpopulations from Central Asia in one Treemix analysis (Fig. 2D and fig. S10).

Ancient samples from Iran (Shahr-i-Qumis, 800 BCE to 800 CE), including one Sassanid (sample AM805), are not more closely related to Central than to Eastern Asian modern subpopulations, although their exact phylogenetic placement remains unclear (Fig. 2, A and B, and fig. S10). Their fineSTRUCTURE affinities to modern Iran, Anatolia (Turkey), the Levant (Syria), and Maghreb (Tunisia) support different genetic ancestry profiles from those inferred at the nearby site of Doshan Tepe. This indicates a population turnover in Iran after ~1000 BCE but before ~500 CE, corresponding to the radiocarbon time interval of Doshan Tepe and a single specimen from Shahr-i-Qumis.

Notably, all our ancient specimens from Europe cluster within modern European domesticates, supporting differentiation within this continent before the oldest European samples analyzed (Tarquinia, 803 to 412 BCE, ~2500 years ago; Fig. 3C). However, a donkey from a Roman context in Marseille, a major seaport trading center in Southern France (Centre Bourse Marseille, 0 to 500 CE), displayed strong genetic affinities with modern individuals from Western Africa (Fig. 3, B and D). Additionally, SNP and haplotype sharedness with modern Western Africa were also found in European donkeys from the Islamic era in Portugal (Albufeira, 1228 to 1280 CE) and Roman times in Northern France (Boinville-en-Woëvre, 200 to 500 CE) (Fig. 3, B and E). This reveals multiple contacts between Europe and Western Africa from the Antiquity to Middle Ages. Despite ancient European donkeys showing Western African ancestry, these contacts have affected Western Africa more than Europe, in line with Treemix inferring gene flow predominantly in this direction rather than the reverse (Fig. 1C). All modern Irish donkeys and the two Etruscan samples from Tarquinia are devoid of Western African ancestry. This suggests the preservation of old European genetic lineages, at least in some modern subpopulations of this continent.

#### **Donkey management involved inbreeding and introgression from divergent lineages**

Inbreeding is a common reproductive strategy for breeding animals with desirable traits (40).



## Fig. 2. Haplotype sharedness and phylogenetic placement of ancient European donkeys.

(A) Haplotype sharedness clustering of modern ( $n = 168$ ) and ancient donkeys ( $n = 31$ ) reconstructed using fineSTRUCTURE (35). Modern domesticates are colored following Fig. 1, and ancient individuals are numbered according to Fig. 3A. Cluster supports are shown as probabilities on each node if  $>0.8$ . MV242 placement is incongruent with Treemix (Fig. 2E) because of the

limited representation of divergent ancestries in the modern reference panel used for imputation. (B) Co-ancestry matrix based on haplotype sharedness. Co-ancestry values are averaged for co-clustered individuals. (C to E) Treemix phylogenies of three ancient specimens shown in black (C: Chalow3, D: Doshan Tepe, and E: MV242) placed within the subpopulations defined in Fig. 1C (27). Branches that are not scaled are shown as dashed lines. s.e., standard error.

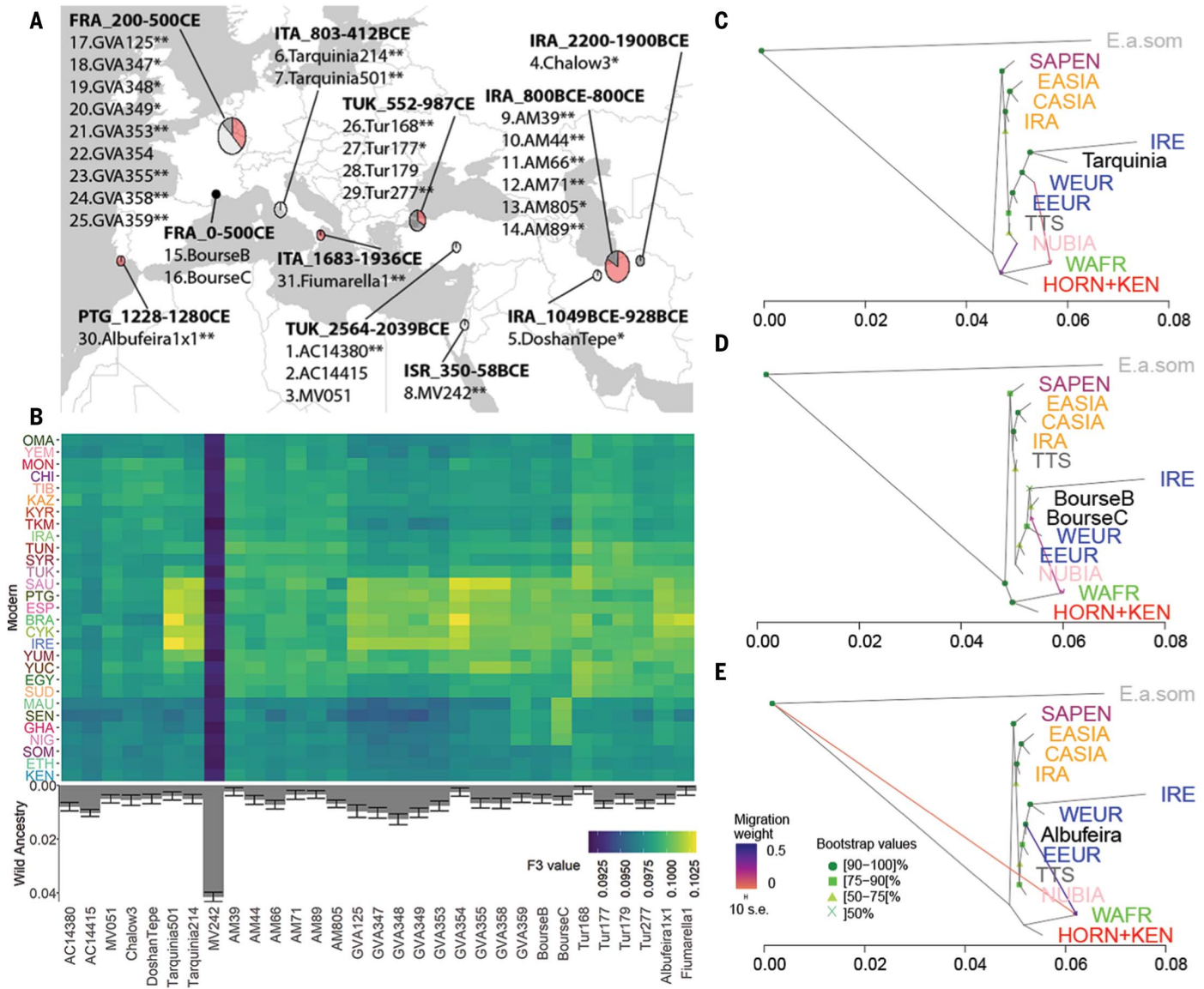
To assess whether ancient donkey breeders made use of inbreeding, we measured the proportion of autosomal runs of homozygosity (ROH) using three independent techniques, all of which provided consistent results (figs. S13 and S14). We detected inbreeding, but no significant changes in levels between modern and ancient donkeys (Wilcoxon rank sum test,  $p = 0.395$ ) (Fig. 4, A, B, and C). Conversely, modern horses show higher inbreeding levels than their ancient counterparts (Wilcoxon rank sum test,  $p < 0.001$ ), which mirrors previous reports of reduced heterozygosity and increased deleterious mutation load in recent times (Fig. 4, D, E, and F, table S11) (21, 41). Longer ROH tracts are more common in modern horses and donkeys than in the past, which is consistent with inbreeding from closer generations in their genealogies (Fig. 4, C and F). Overall, our analyses sup-

port recent major changes in reproductive management inflating inbreeding in horses, but not in donkeys.

Admixture modeling suggests ongoing introgression from African wild asses into modern donkeys from Africa and the Southern Arabian Peninsula (with between 0.24 to 6.99% of admixture) (Fig. 1A, fig. S4, and table S5). This is in line with free-ranging local management practices that allow for continued interbreeding with wild and feral subpopulations (4, 42). The limited but substantial amount of wild genetic material from kiangs in one modern donkey from China also supports admixture between taxa generally regarded as separate species. This confirms previous reports of mitochondrial introgression (43) and genomic admixture despite different karyotypes (24). Notably, all but one ancient donkey (sample Tur168) carried remnants of outgroup mate-

rial (0.21 to 4.15%; Fig. 3B), which potentially results from recent range contractions of wild subpopulations and ancient management practices providing more opportunities for wild introgression.

The genome of MV242, a donkey from Israel dating to the Hellenistic period (350 to 58 BCE), displayed the largest fraction of divergent genetic material (Fig. 3B, 4.15%  $\pm$  0.019). In Treemix, this sample showed a deeper placement than all donkeys present in our panel, except the Somali wild ass [*Equus africanus somaliensis* (*E.a.som*)] (Fig. 2E). Significantly positive  $f_4$  (*E.a.som*, MV242; Horn+Ken, x) statistics revealed MV242-related genetic ancestry in some modern subpopulations (x), especially toward Central and Eastern Asia (Fig. 5E). This ancestry was already present in the BMAC sample from Iran (sample Chalow3, Fig. 5F), which indicates contact  $\sim$ 2050 BCE at the



**Fig. 3. Ancient donkey dataset, genetic affinities to outgroups, and modern donkeys.** (A) Geographical distribution, estimated age, and sample names of ancient donkeys ( $n = 31$ ). Pie charts represent the proportion of individuals with dun coat color (white), heterozygotes (gray), and derived coat color (red) at each site. Genotype probabilities  $\geq 0.99$  are denoted with \*\* and  $\geq 0.9$  with \*. (B) Heatmap displaying outgroup  $f_3$ -statistics in the form of (modern, ancient; kiang) (58). Bar charts represent the proportion

of wild ancestry (kiang, onager, zebra, *E.a.som*) in each ancient individual with standard errors estimated from ADMIXTURE with 100 bootstrap pseudo-replicates (56). (C to E) Treemix phylogenies of ancient specimens from three archaeological sites shown in black [C: Tarquinia (Tarquinia214, Tarquinia501), D: Bourse (BourseB, BourseC), E: Albufeira 1x1] placed within the subpopulations defined in Fig. 1C (27). Branches that are not scaled are shown as dashed lines. s.e., standard error.

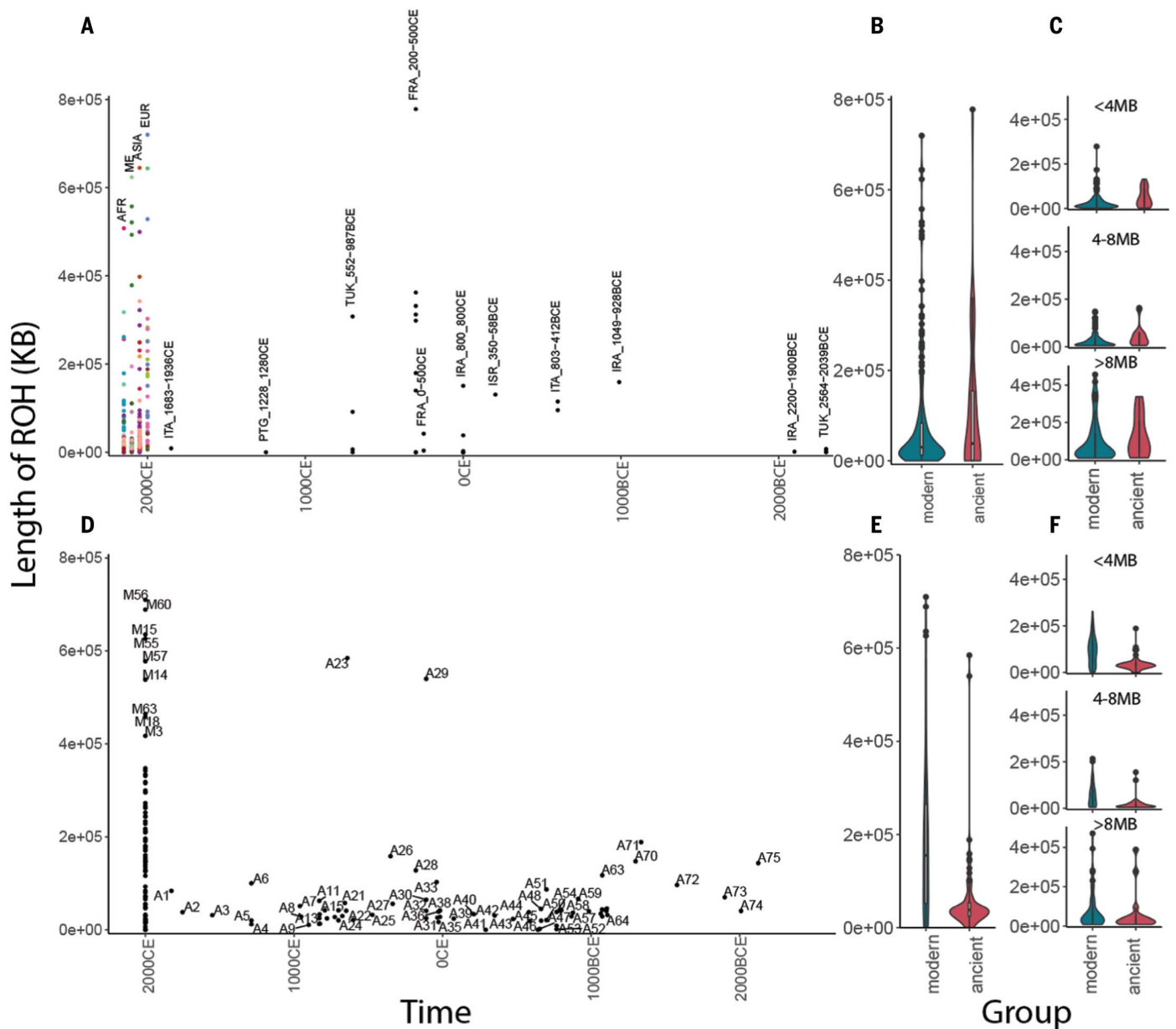
latest. It was, however, absent in Acemhöyük at that time, which suggests that the MV242 divergent lineage ranged into Eastern Iran/Turkmenistan, but not Turkey. This lineage also left genetic material in modern Anatolia, the Levant, Nubia, and Maghreb, but not in Western Africa, which is consistent with donkeys carrying MV242-related ancestry flowing back into some African regions. In addition, this ancestry was also present in Southwestern European subpopulations (Canary Islands, Portugal, and Spain), but neither in the modern Balkans and Ireland nor in any ancient

European sample analyzed here (Fig. 5, E and F). Combined, our results suggest a range for the MV242-related lineage from the Levant into Asia rather than Europe and Africa.

Despite its divergent genetic makeup, MV242 carries a mitochondrial haplotype characteristic of Clade II (Fig. 5A). Our tip-calibrated coalescent analyses revealed that the time to the most common recent ancestor of that Clade was 32,226 BCE and not 332,580 to 142,980 BCE (Fig. 5B), as previously reported (12, 44). Because the same holds true for Clade I, both clades could have coexisted in

sympatry 25,000 years later when donkeys were first domesticated (Fig. 5B). Additionally, no phylogeographic structure is apparent in patterns of mitochondrial variation, both in modern and ancient subpopulations, as ancient specimens from Asia and Europe, sometimes from the same archaeological sites, were placed across both clades (Fig. 5A). Y-chromosomal variation was also associated with little, if any, population structure (Fig. 5, C and D). Combined, our results dismiss mitochondrial DNA and the Y-chromosome as reliable markers of domestication history in donkeys.

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**Fig. 4. Inbreeding in domestic donkeys and horses.** (A) Distribution of total ROH length in modern versus ancient donkeys. (B) Total length of ROH in donkey genomes through time. (C and D) Same as (A) and (B), but for 79 modern and 75 ancient horses. ROH tracts were identified using the program ngsF-HMM (59).

#### Romans bred improved donkeys for producing mules essential for their military power and economy

Beyond documenting domestication history at the global scale, our genomic dataset also included three jennies (females) and six jacks (males) from the same archaeological site (Boinville-en-Woëvre) (Fig. 3A). These were found in a dedicated farming area of a Roman villa, providing insights into local management practices in Roman Northern France (200 to 500 CE). One jack (sample GVA349) appeared particularly inbred, with long ROH indicative of recent consanguinity (Fig. 4A), and was genetically related to four jacks and one jenny

(family group GVA1, including samples GVA125, GVA347, GVA348, GVA349, GVA353, and GVA354; table S10). Additionally, two jennies showed genetic relatedness coefficients equivalent to full siblings (family group GVA2, including samples GVA355 and GVA358; table S10). This indicates breeding management within close kin, potentially aimed at selecting for desirable traits. Genotype imputation at *TBX3* (13) revealed the presence of dun and derived colored coats, but no evidence for the dominant alleles associated with white spots or long hair was found in the sequence alignments at *KIT* (45) and *FGF5* (fig. S11 and tables S7 to S9) (46). The latter two phenotypes are,

however, relatively common in modern breeds from France, which suggests post-Roman selection for these traits.

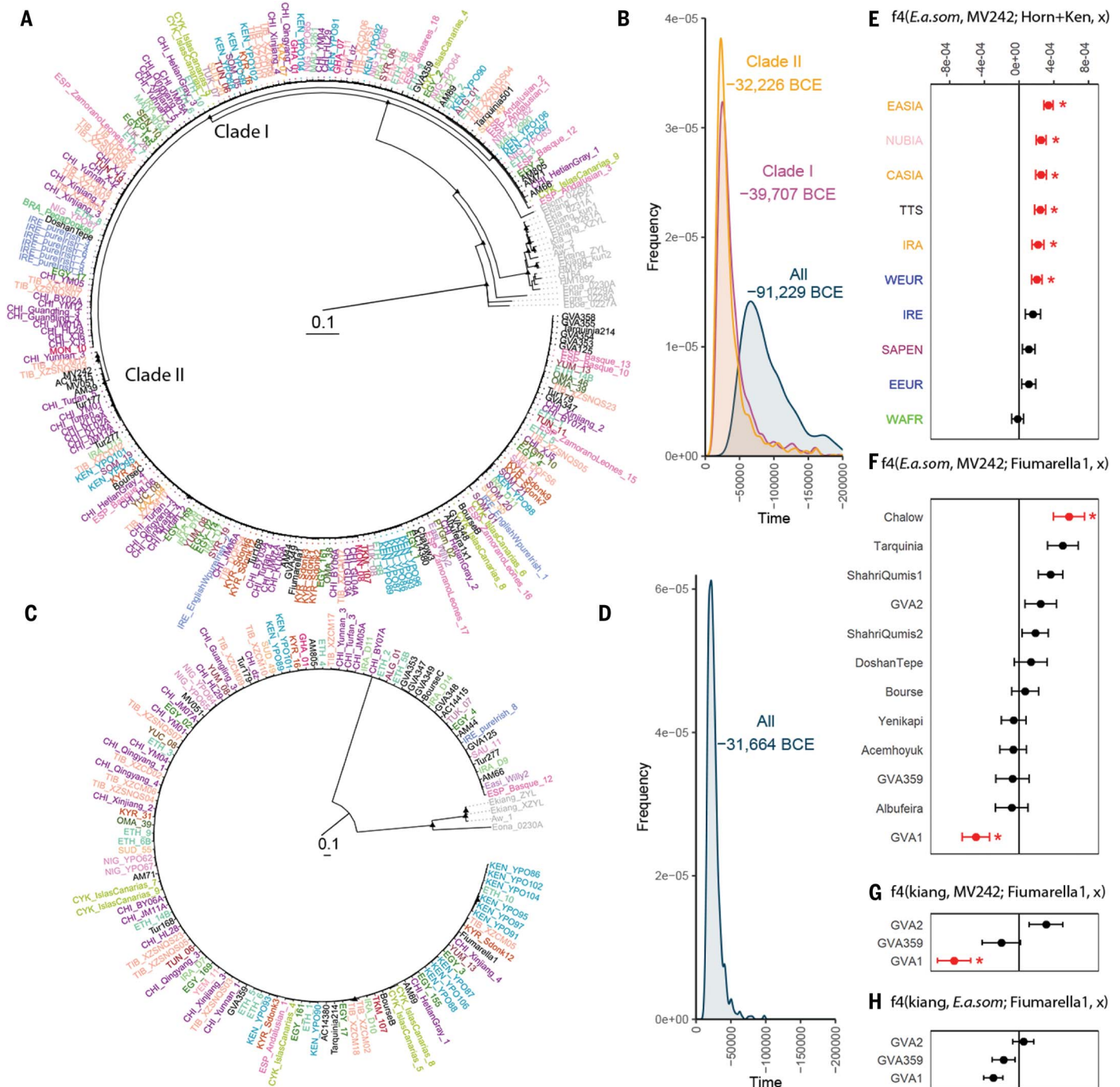
The abundance of donkeys at Boinville-en-Woëvre stands as an exception in Roman France, as mules dominated all other assemblages from this time (47). Contemporaneous Roman sites report mules of a large and uniform size, indicating selective breeding in the parental species for expensive animals of exceptional stature [Varro (2, 6)] (48). Morphometric measurements previously revealed five donkeys from family group GVA1 as giant (148 to 156 cm at the withers) (47). We found that sample GVA359 had a similarly large size

(144 cm) and genetic affinities to Western Africa. This may indicate restocking to enhance body size from distant bloodlines carrying divergent ancestry or from wild populations.

Outgroup admixture was significantly higher at Boinville-en-Woëvre than in other ancient donkeys except the divergent MV242 specimen ( $p = 0.045$ ). Significantly negative  $f_4(\text{kiang,}$

MV242; Fiumarella1, Boinville-en-Woëvre) statistics support restocking into family group GVA1 only, from a lineage more divergent than MV242 (Fig. 5G). Additionally,  $f_4(\text{kiang, E.a.som; Fiumarella1, GVA1})$  statistics reject unbalanced allele sharedness between *E.a.som* and GVA1, ruling out restocking from *E.a.som* or more divergent populations (Fig. 5H). Com-

bined, these findings uncover a lineage, phylogenetically intermediate between MV242 and *E.a.som*, that contributed to the genetic makeup of some Roman donkeys at Boinville-en-Woëvre. Together with the evidence of genetic relatedness and inbreeding, this suggests Boinville-en-Woëvre as a likely mule production center that maintained the bloodlines of giant donkeys



**Fig. 5. Uniparental marker phylogenies and introgression of divergent lineages.** (A) Mitochondrial phylogeny constructed using IQ-TREE (60) with 100 bootstrap pseudo-replicates marked with a black triangle if >90%. (B) Posterior distributions of the time to the most recent common ancestors of all mitochondrial

haplotypes, Clade I and Clade II labeled with their modes. (C and D) Same as (A) and (B) for the Y-chromosome. (E to H)  $f_4$ -statistics (58) exploring the genetic contribution of divergent lineages into modern and ancient donkeys. Z scores were corrected for multiple testing, and red bars with asterisks show  $p < 0.05$ .

that were selected through familial breeding and restocking. This center may illustrate how Romans sustained the enormous demand for mules, which is documented in the nearby Rhine frontier (49), and fueled transportation networks throughout the Empire (47).

## Discussion

Our study solves long-standing debates about donkey domestication. We support domestication starting from a unique African source ~5000 BCE. Donkeys subsequently spread into Eurasia from ~2500 BCE, and Central and Eastern Asian subpopulations differentiated ~2000 to 1000 BCE. Genetic affinities characteristic of modern Western Europe were already formed by 500 BCE. After early domestication, African donkeys further differentiated in the West and the Horn of Africa plus Kenya but also received streams of genetic ancestry from Western Europe as well as a region encompassing the Levant, Anatolia, and Mesopotamia. Donkey domestication involved limited but detectable wild introgression. It did not entail inflated inbreeding in recent times, in contrast to horses. The processes of donkey and horse domestication differed substantially, as horses were domesticated twice (50) and rapidly spread across Eurasia from the lower Don-Volga region ~2000 BCE (21). Their regional differentiation remained relatively limited owing to strong connectivity at continental distances early on and until oriental bloodlines were propagated throughout the world during the past 1000 years (41, 51). The extent to which the different domestication trajectories of donkeys and horses were only driven by their respective roles in human societies or also reflected management practices adapted to their respective mating and social behavior (52) remains to be explored.

This work clarifies global patterns of donkey domestication and movements but also highlights many directions for future research. For example, it remains unknown whether domestic donkeys only dispersed out of Africa by land through the Sinai Peninsula or across the Red Sea from Ethiopia to Yemen. Additionally, modern subpopulations from the Horn of Africa plus Kenya were found to be the first expanding. This may suggest early domestication there or that donkeys domesticated elsewhere in Africa entering the region more recently. Further research is needed to clarify the timing of pastoral spread into the Red Sea Sudanese region and the Horn of Africa. Current dates range from ~2500 BCE in Ethiopia and Eritrea (53) to ~3000 BCE in Northern Kenya (54). Donkeys are not present in the archaeological record of Western Africa before the beginning of the common era, either (55), which postdates by 3000 years the time when donkey populations from the Horn of Africa

plus Kenya and Western Africa are inferred to have split genetically. This may indicate an early yet undocumented arrival in the region or a slow migration westward, only reaching the modern range later. Improving the current African archaeological record thus appears paramount to refining the exact context underlying early donkey domestication and subsequent population movements.

Further genomic studies in other regions would also largely benefit the understanding of donkey diversity and history. Resolving the genetic structure of equine remains from the third millennium BCE of Southwest Asia will be challenging because of postmortem DNA decay but essential to mapping the geographic range of the divergent lineage identified here (sample MV242), as well as to understanding dispersal mechanisms in greater detail. The same holds true for Chalcolithic and Bronze Age Europe, which remain genetically undocumented in our dataset, and onwards. Developing genetic knowledge of ancient European donkeys will further clarify patterns of exchange across the Mediterranean region, including during and after Roman times, as revealed in this study. It will also provide insights into the dispersal mechanisms underpinning the genetically supported presence of donkey remains in Portugal ~2200 BCE (33). Genetic characterization of local archaeological sites at the population scale may uncover additional mule breeding centers other than the one reported here. This will shed light on the diversity of breeding management strategies that were developed by Romans to supply their continental-wide economy and military with adequate animal resources (49). For now, both the absence of mules and the rarity of horse mares at Boinville-en-Woëvre (47) suggest that mares were brought in for mating before returning pregnant to their owners. Alternatively, donkey breeders may have visited other farms with their jacks to cover mares.

Efforts should continue to characterize the modern donkey diversity around the world, especially in Saudi Arabia, which is currently characterized by a single individual, as well as in Africa, for which no populations located south of the Equator have been sampled. Such efforts may not only refine the historical legacy of past populations into the modern world but also uncover the genetic basis of desert adaptations, which could prove invaluable for future donkey breeding in the face of global warming.

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The authors declare that they have no competing interests. **Data and materials availability:** The sequence data generated in this study are available for download at the European Nucleotide Archive (accession no. PRJEB52849). The variant data for this study have been deposited in the European Variation Archive (EVA) at EMBL-EBI under accession number PRJEB55549. The recombination map can be downloaded from <https://osf.io/k7x8b/>. The accession numbers for each individual sample and all other data used in this study are included in tables S1, S2, and S11 of the supplementary materials. **License information:** Copyright © 2022 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. <https://www.science.org/about/science-licenses-journal-article-reuse>

#### SUPPLEMENTARY MATERIALS

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## The genomic history and global expansion of domestic donkeys

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### Donkeys' African origins

Donkeys have been important to humans for thousands of years, being the primary source of work and transport for many cultures. Unlike horses, little was known about the origin and domestication of donkeys. Todd *et al.* sequenced the genomes of modern and ancient donkeys and found evidence of an eastern African origin over 7000 years ago, with subsequent isolation and divergence of lineages in Africa and Eurasia. They also reveal the imprint of desertification on divergence among groups and specifics about donkey breeding and husbandry, including selection for large size and the practice of inbreeding. —SNV

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