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The palaeogenetics of cat dispersal in the ancient world

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The cat has long been important to human societies as a pest-control agent, object of symbolic value and companion animal, but little is known about its domestication process and early anthropogenic dispersal. Here we show, using ancient DNA analysis of geographically and temporally widespread archaeological cat remains, that both the Near Eastern and Egyptian populations of *Felis silvestris lybica* contributed to the gene pool of the domestic cat at different historical times. While the cat's worldwide conquest began during the Neolithic period in the Near East, its dispersal gained momentum during the Classical period, when the Egyptian cat successfully spread throughout the Old World. The expansion patterns and ranges suggest dispersal along human maritime and terrestrial routes of trade and connectivity. A coat-colour variant was found at high frequency only after the Middle Ages, suggesting that directed breeding of cats occurred later than with most other domesticated animals.

The domestic cat is present on all continents except Antarctica, and in the most remote regions of the world, and its evolutionary success is unquestioned. While it is nowadays one of the most cherished companion animals in the Western world, for ancient societies barn cats, village cats and ships' cats provided critical protection against vermin, especially rodent pests responsible for economic loss and disease[1]. Owing to a paucity of cat remains in the archaeological record, current hypotheses about early cat domestication rely on only a few zooarchaeological case studies. These studies suggest that ancient societies in both the Near East and Egypt could have played key roles in cat domestication[2,3].

Wildcats (*Felis silvestris*) are distributed all over the Old World. Current taxonomy distinguishes five wild, geographically partitioned subspecies: *Felis silvestris silvestris*, *Felis silvestris lybica*, *Felis silvestris ornata*, *Felis silvestris cafra* and *Felis silvestris bieti*[4]. Modern genetic data analyses of nuclear short tandem repeats (STR) and 16% of the mitochondrial DNA (mtDNA) genome in extant wild and domestic cats revealed that only one of them, the north African/southwest Asian *F. s. lybica*, was ultimately domesticated[5].

Wildcats are solitary, territorial hunters and lack a hierarchical social structure[6,7], features that make them poor candidates for domestication[8]. Indeed, zooarchaeological evidence points to a commensal relationship between cats and humans lasting thousands of years before humans exerted substantial influence on their breeding[2,3,9]. Throughout this period of commensal interaction, tamed and domestic cats became feral and/or intermixed with wild

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F. s. lybica or other wild subspecies as is common today[10]. These regular genetic exchanges may have contributed to the low level of differentiation observed between modern wild and domestic cat genome sequences[11]. Accordingly, the domestication process seemingly has not profoundly altered the morphological, physiological, behavioural and ecological features of cats[9], in contrast to what has been observed, for example, for dogs[12].

To address questions related to the contribution of the two purported centres of cat domestication, the Near East and Egypt, and the history of human-mediated cat dispersal, we analysed ancient and modern cats from Europe, north and east Africa, and southwest Asia (SWA), spanning around 9,000 years, from the Mesolithic period to the twentieth century AD. We analysed ancient DNA (aDNA) to explore whether a fine phylogeographic structure of maternal lineages existed prior to the domestication of *F. s. lybica* and whether, when and how it was reconfigured over time in response to human intervention, thereby documenting the domestication process of the cat. We also studied a genetically defined coat-colour marker, the blotched tabby marking[13], to monitor a phenotypic change reflecting human-driven selection along the domestication pathway.

Results

Strategy for data acquisition. The mtDNA phylogeny reconstructed from extant wild and domestic cats[5] identified five geographically distinct clades (I–V, Supplementary Fig. 1), representing the five *F. silvestris* subspecies. The modern domestic cat mtDNA pool was traced back to five deeply divergent subclades (IV-A to IV-E) of the *F. s. lybica* clade, representing multiple wildcat lineages incorporated over time and space[6]. These subclades lack a phylogeographic structure, which may reflect either poor sampling of the truly wild modern *F. s. lybica*, particularly in its African range, or multiple domestication events and/or extensive gene flow between wild and domestic populations following the dispersal of domestic cats. In order to screen and analyse a large number of ancient samples in parallel, many of which were expected to be poorly preserved owing to higher-temperature burial environments, we applied an ultrasensitive high-throughput approach[14] to target informative single-nucleotide polymorphisms (SNPs) on the mtDNA that recapitulate the most salient features of the previously obtained phylogeny (Supplementary Fig. 1). Although mtDNA alone cannot assess possible hybridization between different populations at the individual level, the absence of recombination and the high copy number make it a useful genetic marker for ancient population analyses involving a large number of poorly preserved samples. The mtDNA phylogeny (Fig. 1b) reconstructed from 286 bp sequenced in our ancient samples alongside modern data from the literature[5] clearly separates the five clades of *F. silvestris* (posterior probabilities >0.88, Supplementary Fig. 3, Supplementary Methods) and the five subclades of *F. s. lybica* (posterior probabilities >0.77). We examined the phylogeographic pattern and its changes across time by grouping the mtDNA haplotypes from our study into nine time bins (Fig. 1c).

Ancient European wildcats. We found the mtDNA clade I, representative of European wildcats (*F. s. silvestris*), exclusively in Europe. From the Mesolithic period to the 8th century BC in Western Europe (geographic locations 1-5 in Fig. 1a, c), all cats analysed (9 out of 9) carried clade I mtDNA, whereas in southeast Europe (6-8) we observed similar frequencies of clade I ($n = 13$, 42%) and clade IV ($n = 18$, 58%), representative of *F. s. lybica*. The latter was mostly represented by one of the lineages of subclade IV-A, hereafter IV-A1 (Supplementary Figs 1, 3), the earliest occurrence of which, in our dataset, dates back to 7700 BC in Romania (7) (Supplementary Data 1), and which is still present today in European wild (8) and domestic cats[5]. The occurrence of a *F. s. lybica* mitotype in pre-Neolithic southeast Europe indicates that the native range of this subspecies extended beyond the Bosporus.

Anatolian cats from the Neolithic period to the Bronze age. A mitotype belonging to subclade IV-A (hereafter IV-A*, see Methods section) was predominant (12 out of 14) from around 8000 to 800 BC in Anatolia (10-13) (Fig. 1a–c). Its range may have also extended to Lebanon (15). The frequencies of IV-A1 and IV-A* found in southeast Europe and Anatolia, respectively, are significantly different (Fisher's exact test; $P < 0.001$), suggesting a phylogeographic structure that mirrors the original distribution of genetically distinct wildcat populations carrying *F. s. lybica* mtDNA. The earliest occurrence of IV-A* outside the Anatolian range in our dataset was detected in two directly radiocarbon-dated specimens from southeast Europe, in Bulgaria (4400 BC) and Romania (3200 BC), clearly postdating the introduction of Neolithic farming practices, and in two Late Bronze/Iron age cats (around 1200 BC) from Greece. The range expansion of this mitotype suggests human-mediated translocation.

Ancient Levant and Africa. Owing to very poor DNA preservation, we could not explore the phylogeographic structure of *F. s. libyca* in this area prior to the Bronze Age. Therefore, we inferred the original distribution of the other subclades (IV-B/E) by taking into account their temporal appearance in our dataset. We found IV-B in three ancient cat remains dated to the 1st millennium BC from southeast Anatolia and Jordan (13, 16, Fig. 1a–c), the 6th century BC in Syria and later in Jordan (15, 16). This clade is still found in modern wildcats from Israel[5,15]. These data suggest that this subclade was mainly restricted to a Levantine range, throughout history. Outside of this range, IV-B was found only in Medieval Iran (17) at very low frequencies (7%).

In Africa, two lineages of IV-C (named IV-C1 and IV-C*) were detected in five out of seven cats (including three mummies) from Egypt with dates ranging from the 7th century BC to the 4th century AD (20, 21, Fig. 1a–c). The original range of IV-C may have extended from Egypt along the Nile River as far south as Congo and Burundi (27, 28), where we detected a novel sub-lineage of IV-C (IV-C2, Fig. 1) in modern wildcats that had not yet been described in the mtDNA pool of present-day domestic cats.

Subclades IV-D and IV-E were found at low frequencies solely in recent temporal bins of our ancient dataset (1, 9–11), most likely as a result of human-mediated dispersal. Their basal position in clade IV, shared with lineages found in ancient African cats (light pink symbols in Fig. 1b, c; 20, 25, 28, 29) and not detected so far in domestic cats, may suggest an African origin.

The dispersal of Egyptian cats. Outside Africa, from the 8th century BC to the 5th century AD, we found IV-C1 in five Classical Antiquity period cats from Bulgaria, Jordan and Turkey (8, 11 and 16, respectively, Fig. 1a–c). This range expansion is more evident between the 5th and 13th centuries AD, when the two IV-C lineages found in ancient Egyptian cats became substantially more frequent both in Europe (78%; 7 out of 9) and in SWA (46%; 32 out of 70). By contrast, none of the 41 European and 18 southwest Asian cats from archaeological contexts predating the 8th century BC possessed IV-C haplotypes (Fisher's exact test; $P < 0.001$ in both cases).

The territorial behaviour of cats and the rapid reconfiguration of the phylogeographic pattern observed in Europe and SWA suggest that cats carrying IV-C haplotypes were spread by humans throughout the eastern Mediterranean region in Classical antiquity. Further expansion occurred during the Medieval period, whereby the IV-C1 haplotype was found at the Viking trading port of Ralswiek on the Baltic Sea (1, Fig. 1a–c) by the 7th century AD, and at the Iranian port of Siraf by the 8th century AD (17). In the Balkans, IV-C1 persisted throughout Medieval times up to the present (8). Translocation of cats over even longer distances was observed by the presence of Asian wildcat (*F. s. ornata*) mtDNA at the Roman–Egyptian port of Berenike on the Red Sea (1st–2nd century AD; 23, Fig. 1a–c) and at Medieval coastal sites in Turkey (9, 10).

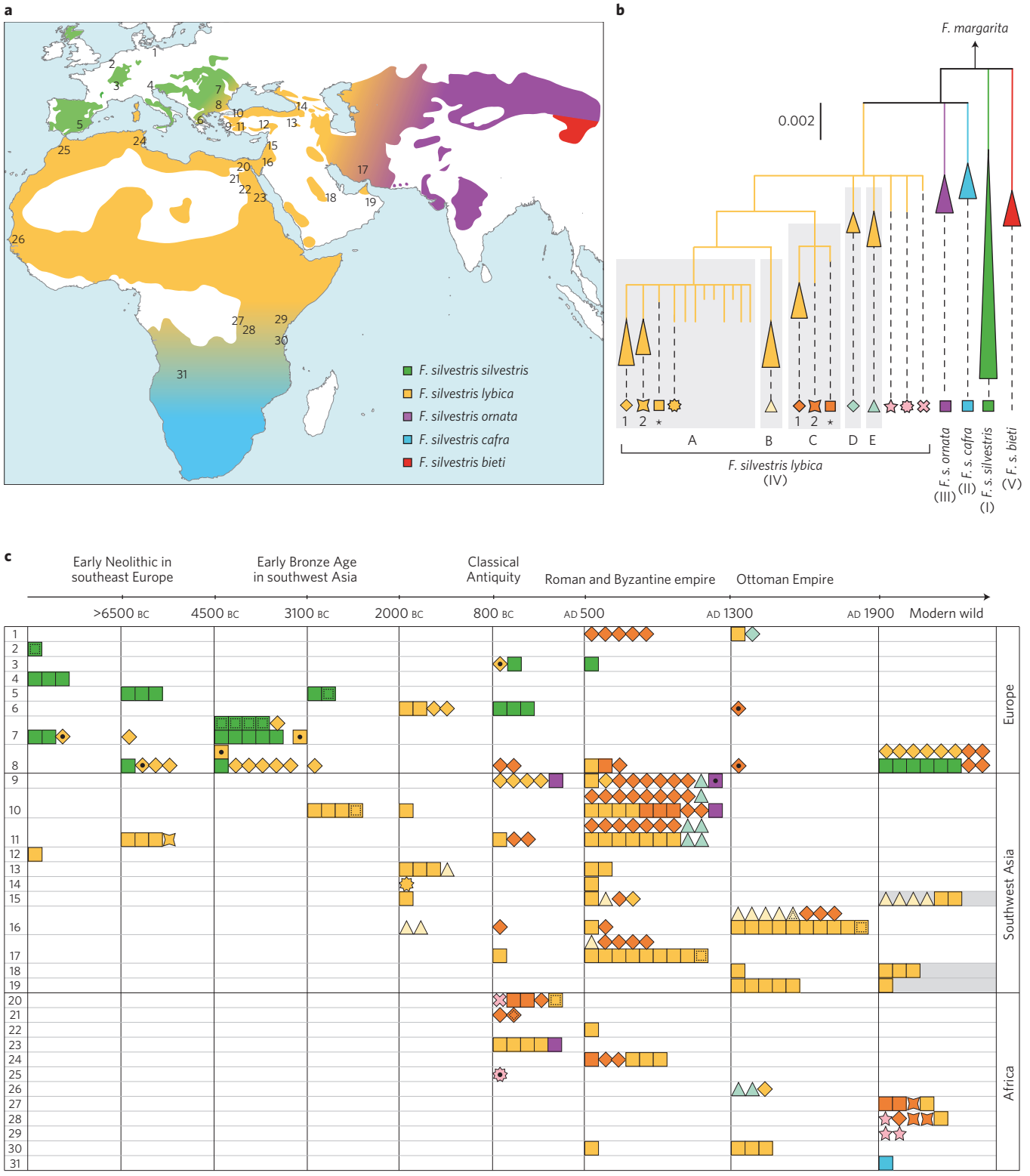

Figure 1 | Spatio-temporal representation of cat maternal genealogies. a, Map showing the present-day distribution of *Felis silvestris*[4] with the geographic range of each subspecies as reported in literature[5] and inferred from the data presented herein. **b**, Tree of mtDNA lineages observed in our ancient samples and in modern wild and domestic cats from literature[5]. **c**, Spatio-temporal depiction of ancient cat haplotypes as depicted with symbols from the tree in **b**. Rows represent the approximate geographic provenance of the samples as reported in the map in **a** whereas the columns pertain to chronological periods, the limits of which were selected to separate the prehistoric and historical periods evenly, to unambiguously assign each sample to a single bin and to take historic events into account that could have affected human–cat interactions, as indicated on the timeline above. A dot inside the symbols indicates AMS-radiocarbon-dated samples; dashed lines inside the symbols indicate incomplete mtDNA profiles; Near Eastern modern wildcats from literature[5] are indicated by grey-shaded bins. Numbers in **a** and in **c** represent the approximate geographic locations of the sites from which the samples are derived as reported in Supplementary Table 5.

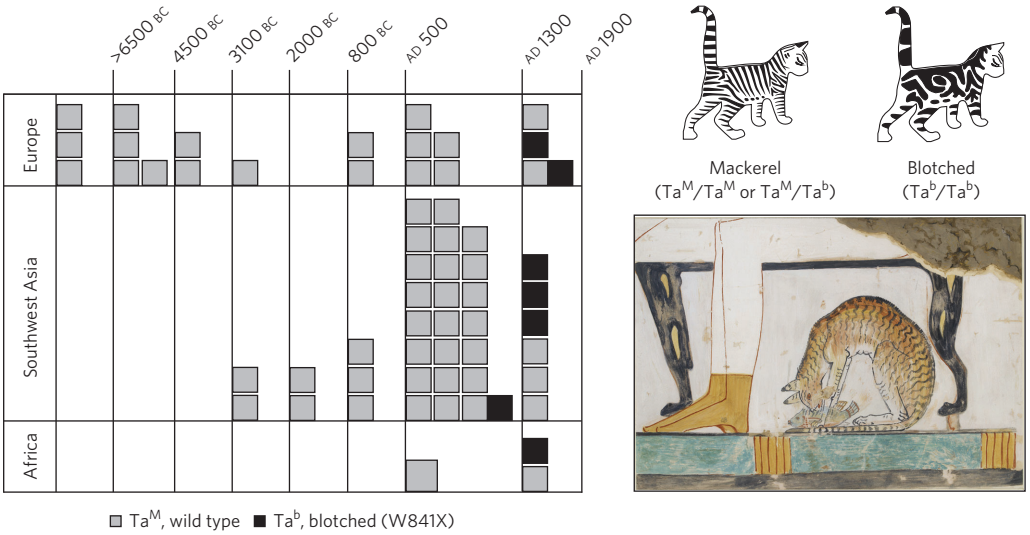

Figure 2 | Spatio-temporal representation of the alleles determining the phenotypic variation in the shape of tabby patterns, mackerel (Ta^M) and blotched (Ta^b). To overcome issues of potential allelic drop-out, each individual is defined by at least one observed allele, except for the few instances in which both alleles were detected. The image shows a 'cat under the chair' with a tabby mackerel marking, typical of *F. silvestris lybica* (Anna (Nina) Macpherson Davies, *Copy of Wall Painting from Private Tomb 52 of Nakht, Thebes (I, 1, 99–102) Cat Eating Fish.* Photo: © Ashmolean museum, Oxford, UK).

Coat pattern. The domestication process has not markedly changed the morphology of cats, and few traits can be used today to identify wild or hybrid populations. Of the few traits available, the most widely used is the tabby coat marking[16]. The transmembrane aminopeptidase Q (*Taqpep*) gene is responsible for the tabby phenotypic variation in cats, with a single SNP distinguishing most of the mackerel and blotched patterns that are characteristic of the wild and domestic patterns, respectively[13]. To develop a temporal framework for the emergence of a variation in coat pattern typical of domestic cats, we investigated the three SNPs in the *Taqpep* gene[13]. We found that the recessive allele responsible for the blotched-tabby pattern in 80% of present-day cats (W841X) occurred in our ancient dataset not earlier than the Medieval period in SWA (3%, minimum number of total alleles, see Methods) (Fig. 2). Thereafter, its frequency increased in Europe, SWA, and Africa (50% in total), showing late expansion of this typically domestic allele.

Discussion

Zooarchaeological and iconographic evidence for early cat domestication. Owing to the paucity of cat remains in the archeological record and the lack of established osteometric features distinguishing remains from wild and domestic *F. s. lybica*[2], current hypotheses about early cat domestication are grounded in scanty evidence when compared to other domesticated animals. A complete skeleton found in Cyprus in association with a human burial dated to around 7500 BC suggests that cats were probably tamed by early Neolithic sedentary communities that had been growing cereals in SWA, concomitant with the emergence of commensal rodents[3]. Similarly, the skeletons of six cats in an elite Predynastic cemetery in Egypt, around 3700 BC, may suggest a close cat–human relationship in early ancient Egypt[2].

The iconography of Pharaonic Egypt constitutes a key source of information about the species' relationship with humans, and has motivated the traditional belief that cat domestication took place in Egypt[1,17]. Numerous depictions in Egyptian art from the 2nd millennium BC document a progressive tightening of the relationship between human and cat, as illustrated in particular by the popularization of the motif of the 'cat under the chair' of women after around 1500 BC[1,17].

Here, we show that mitochondrial lineages corresponding to these two purported domestication centres contributed at different times to the gene pool of modern domestic cats. We deduced this by establishing the ancestral phylogeography of wild cats in the Old World and by observing its reconfiguration through time, which reveals the spread of cats through human agency following ancient land and maritime trade routes.

Distribution of wildcats. Our aDNA data (Fig. 1a and Supplementary Fig. 4) show a clear phylogeographic structure. *F. s. silvestris* was confined to Europe, whereas *F. s. lybica* was found in SWA and southeast Europe. A clear understanding of the present distribution of wild *F. s. lybica* in Anatolia has proven elusive until now owing to a lack of genetic data. It has commonly been assumed that the native range of the modern European wildcat includes Anatolia[5,18,19]. Our phylogeographic reconstruction demonstrates that mtDNA clade IV, corresponding to *F. s. lybica*, was predominant in Anatolia for many millennia beginning in the Neolithic period at the latest. Not a single instance of clade I, corresponding to *F. s. silvestris*, was detected in our samples from SWA (Fig. 1). Nevertheless, we cannot exclude its presence in the wilds of Anatolia, in particular in the forest and mountain refuges of northern Anatolia and the Caucasus.

We found two distinct IV-A mitotypes on either side of the Bosporus. In Anatolia, from around 8000 BC to 800 BC, almost all cats (12 out of 14) belonged to the IV-A* mitotype. By contrast, cats carrying a distinct mitotype, IV-A1, were present in southeast Europe by the beginning of the 8th millennium BC. This suggests that *F. s. lybica* was distributed across Anatolia from the early Holocene epoch at the latest, prior to the formation of the present-day extension of the Black Sea, and that it made its way to southeast Europe before the onset of farming in the Neolithic period. A split in an ancestral Anatolian cat population in the late Pleistocene epoch, presumably during the Last Glacial Maximum, followed by local differentiation and/or drift and founder effect, might have been responsible for the distribution of distinct clade IV mtDNA lineages in Anatolia and southeast Europe. *F. s. silvestris* and *F. s. lybica* occur across different biotopes that include, respectively, temperate woodland and open bushland[4]. The expansion of open bushlands during the Late Pleistocene epoch might have attracted *F. s. lybica* into the

Balkans when the Bosporus was a land bridge and the Balkans represented a refuge for warm-adapted species[20,21].

Currently, IV-A1 is found in the European wildcat population and also in modern domestic cats[5] (Fig. 1). Our data imply that admixture episodes potentially occurring through time between overlapping populations of wild *F. s. silvestris* and *F. s. lybica* could be in part responsible for *F. s. lybica* mtDNA introgression in present-day European wildcat populations. Conservation programs should also take into account past natural admixture when aiming at neutering and removing hybrids that are believed to have a role in cryptic extirpations of wild *F. s. silvestris* populations[4].

Origin and dispersal of domestic cats. Our data show that mitotype IV-A* had a wide distribution stretching across Anatolia from west to east throughout the Neolithic, Bronze age and Iron age. Its range may have extended as far south as the Levant, where we inferred the presence of subclade IV-B. These findings suggest that in the Fertile Crescent, cats that developed a commensal relationship with early farming communities during the Neolithic period carried at least mitotypes IV-A* and IV-B. Mitotype IV-A* later spread to most of the Old World, representing the Near Eastern contribution to the mtDNA pool of present-day domestic cats. This spread may have started as early as around 4400 BC into southeast Europe, the date of the first appearance of IV-A* in our European dataset, and therefore subsequent to the neolithisation of Europe. This suggests that the human-mediated translocation of cats began in prehistoric times, corroborating the interpretation of the finding of a cat buried around 7500 BC in Cyprus[3]. We also found IV-A* in cat remains from the Roman–Egyptian port of Berenike on the Red Sea and in one Egyptian mummy (Fig. 1a–c), which may hint at an introduction of cats from SWA to Egypt.

Our data provide the first evidence for an African origin for one of the mitochondrial lineages of present-day domestic cats, namely clade IV-C. Indeed, we found the lineages C1 and C* in the majority of Egyptian cat mummies. These cats were worshipped and, during the Greco–Roman period, kept in temple precincts to be mummified[17]. We show that, despite a local ban on cat trading being imposed in Egypt as early as 1700 BC[22], cats carrying IV-C mtDNA spread to most of the Old World. The increasing popularity of cats among Mediterranean cultures and particularly their usefulness on ships infested with rodents and other pests presumably triggered their dispersal across the Mediterranean[22]. Indeed, depictions of cats in domestic contexts, already frequent during the New Kingdom in Egypt around 1500 BC ('cat under the chair', Fig. 2), are found on Greek artifacts from as early as the end of the 6th century BC (Supplementary Methods). The Egyptian cat must have been very popular, as IV-C1 and C* represented more than half of the maternal lineages in Western Anatolia during the 1st millennium AD, and occurred twice as frequently as the local mitotype IV-A*. This suggests that the Egyptian cat had properties that made it attractive to humans, presumably acquired during the tightening of the human–cat relationship that developed during the Middle and New Kingdoms and became even stronger afterwards[1,17]. As the most pronounced genetic changes that distinguish wild and domestic cats are apparently linked to behaviour[11], it is tempting to speculate that the success of the Egyptian cat is underlain by changes in its sociability and tameness.

North of the Alps, domestic cats appeared soon after the Roman conquest, yet remained absent outside the Roman territory until Late Antiquity[23]. In medieval times it was compulsory for seafarers to have cats onboard their ships[24], leading to their dispersal across routes of trade and warfare. This evidence explains, for example, the presence of the Egyptian lineage IV-C1 at the Viking port of Ralswiek (7–11th century AD)[24]. The expansion of the domestic cat may have been fostered by a diversification in their cultural usage, which in Medieval Europe included the trade of domestic cat pelts as cloth items[25]. Spread of the black rat (*Rattus rattus*) and house mouse (*Mus musculus*) by sea routes as early as the Iron age, documented by zooarchaeological and genetic data[26], probably also encouraged cat dispersal for the control of these new pests.

Increased translocation as a result of long-distance trade is also witnessed by the finding of Asian *F. s. ornata* mtDNA in cats from the Roman Red Sea port of Berenike (1st–2nd century AD) and from Turkey in the 6–7th century AD. This was probably the result of increasingly intensive and direct trade connections between south Asia and the Mediterranean basin via the Indian Ocean and Red Sea[27], but possibly also via the Silk Road connecting central Asia with Anatolia[28]. Long-distance maritime routes[29], as described for instance in the 1st century AD *Periplus of the Erythraean Sea*, probably explain the occurrence of IV-A*, typical of SWA, as far south as East Africa (30, Fig. 1a–c).

Upon arrival in these various new locations, introduced cats reconfigured the phylogeographic landscape of the species through admixture with local tame or wild cats, leading to a transfer of deeply divergent mitochondrial lineages in the domestic pool (IV-D/E and possibly III-*F. s. ornata* although these lineages are found only at low frequency in modern domestic cats[5]). Modern genetic data have shown that admixture with domestic cats still occurs today in European wildcat populations[10,16], and intensive conservation programs have been implemented to preserve the integrity of *F. s. silvestris*[4,30].

Evolution of the tabby pattern. Our study also sheds light on the late emergence in domestic cats of a key phenotypic trait, the blotched coat marking caused by a SNP in the *Taqpep* gene[13]. Wildcats exhibit a mackerel-like coat pattern, whereas the blotched pattern is common in many modern domestic breeds[13]. In our dataset, the first occurrence of the recessive allele W841X, which is associated with the blotched markings, dates to the Ottoman Empire in SWA and later increases in frequency in Europe, SWA and Africa (Fig. 2). This result is in agreement with the iconography from the Egyptian New Kingdom through the European Middle Ages, where cats' coats were mainly depicted as striped, corresponding to the mackerel-tabby pattern of the wild *F. s. lybica*[1,17] (Fig. 2). It was only in the 18th century AD that the blotched markings were common enough to be associated with the domestic cat by Linnaeus[13], and physical traits started to be selected only in the 19th century AD for the production of fancy breeds[15]. Thus, both our data and recent genomic data[11] suggest that cat domestication in its early stages may have affected mainly some behavioural features, and distinctive physical and aesthetic traits may have been selected for only recently. A similar pattern of late emergence of other phenotypic traits has been observed in chicken[31], but contrasts with what has been observed in horses, where coat-colour differentiation appeared at an early stage of domestication[32].

Conclusive remarks. The comprehensive aDNA genetic study of cats across time and space that we present, provides answers to longstanding questions concerning the domestication process of the cat and contributes to a better understanding of how humans have reshaped global biodiversity through species translocations[23,26,33]. By revealing the original phylogeographic distribution of wildcats and its profound modification through human-mediated dispersal of tamed cats through time, we show that both Near Eastern and Egyptian cat lineages contributed at different times to the maternal genetic pool of domestic cats, with one or other present in the vast majority of present-day cat breeds. Cat domestication was a complex, long-term process featuring extensive translocations that allowed admixture events between geographically separated cat populations at different points in time.

Methods

Ancient DNA analyses. Ancient DNA analysis was performed in dedicated aDNA facilities in Paris and Leuven from bone, teeth, skin and hair samples (the last two when available in Egyptian mummies) of 352 ancient cats. The ages of the archaeological remains were determined using direct accelerator mass spectrometry (AMS) radiocarbon dating (KIK-IRPA, Belgium), stratigraphic associations with AMS dates, and contextual archaeological evidence (Supplementary Data 1). All dates in the text are reported in calibrated radiocarbon years BC. DNA was also extracted from claws and skin samples of 28 modern wildcats from Bulgaria and east Africa (Supplementary Methods).

Amplification of nine mtDNA and three nuclear DNA fragments in the *Taqpep* gene was preceded by the elimination of carry-over contamination based on the dUTP/UNG system[34] and carried out in three separate multiplex PCRs. Phylogenetically informative SNPs in the mtDNA were selected following the most up-to-date worldwide cat phylogeny[5] (Supplementary Fig. 1). We targeted 42 informative SNPs in nine short regions distributed across the mitochondrial *ND5*, *ND6* and *CytB* genes that recapitulate the most salient features of the phylogeny obtained with longer portions of these genes (Supplementary Fig. 1). The diagnostic SNPs were screened with Pyrosequencing assays (Biotage, Qiagen) and sequencing on a PGM Ion Torrent platform (Institut Jacques Monod, Paris) of amplicon libraries following the 'aMPlex Torrent' workflow and downstream sequence analysis with a bash script described elsewhere[14] (Supplementary Code). The aMPlex Torrent approach combines the sensitivity of multiplex PCR with the power and throughput of next-generation sequencing[14] and made it possible to screen and analyse a large number of poorly preserved ancient samples in parallel. We obtained mtDNA sequences from 209 out of 352 ancient cats (59%; Supplementary Data 1, 2), with expectedly lower success rates for old samples from hot environments (Supplementary Fig. 2). The mtDNA profiles ranged from 286 to 449 bp, 12 of which were incomplete profiles generated from two to seven mtDNA fragments. The authentication criteria adopted rely on: (i) strict contamination prevention controls including physical containment as well as material and reagent decontamination[34–36]; (ii) extensive replications performed through independent PCRs (at least two, but up to eight with one up to three independent DNA extracts). For samples where the low DNA content decreased data reliability, we increased the number of replicates and used different PCR assays, multiplex and simplex PCR, pyrosequencing and the aMPlex Torrent method performed in independent laboratories (Paris and Leuven) so that samples with different preservation levels could be genotyped with similar reliability. More details about DNA extraction, amplification, sequencing and the authentication criteria can be found in the Supplementary Methods.

Phylogeographic analyses. Each specimen was assigned to an mtDNA clade using the terminology previously proposed[5], including specimens with an incomplete profile (shapes with an inner dashed line in Fig. 1c). Owing to our streamlined sequencing assay, some of the subclades and lineages of IV-A and IV-C observed in the 2007 study were collapsed into a single haplotype, which we named IV-A* and IV-C*, respectively (Supplementary Fig. 1). The ancient and modern sequences generated here were aligned to 159 sequences from Driscoll's study[5]. A Bayesian tree of 66 unique 286 bp-long haplotypes (Supplementary Fig. 3) was constructed as described in detail in the Supplementary Methods. An ML tree, obtained as described in the Supplementary Methods, had the same topology. Frequencies of haplotypes A* and A1 in Anatolia and southeast Europe, and of clade C in before and after the 8th century AD in SWA, were tested using a Fisher's exact test.

Nuclear markers. We typed allelic variations within the *Taqpep* gene associated with coat-colour pattern differences—W841X, D228N and T139N[13]. The results presented here are intended to be indicative of allele frequencies. Given the low level of independent replications of our assay and the risk of allelic dropout, especially in ancient degraded samples, we could not ascertain genotypes, except for a few heterozygous samples showing a fairly high number of reads in at least two independent amplifications (Fig. 2, Supplementary Methods and Supplementary Data 2). Assuming that none of the alleles is amplified preferentially, and adopting a conservative strategy that accounted for the minimum number of alleles observed, our data across the spatial and temporal framework showed that 7 out of 67 successfully amplified cat samples possessed at least one mutant Tabby-W841X allele, of which two were heterozygotes (BMT2 and MET9). In 88 cats we could screen the allele D228N and in all instances we observed the wild-type. Among 63 cats successfully screened for T139N, we detected the mutant allele (C to A) in three specimens.

Code availability. A bash script and accessory fasta and gff files for data analysis of the aMPlex Torrent data are provided as Supplementary Code.

Data availability. Sequence data that support the findings of this study have been deposited in Dryad (http://dx.doi.org/10.5061/dryad.g4p30).

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Author contributions

The project was initiated by W.V.N., E.-M.G., C.O., T.G. and R.D. The ancient DNA study was conceived and designed by T.G., E.-M.G. and C.O. C.O. carried out the molecular laboratory work, with support of S.G. and analysed the data. J.D. generated the aMPlex Torrent data. The archaeological bone samples were provided by W.V.N., B.D.C., J.P., N.S., M.E.P., N.Bo., A.M.-M., A.Bă., C.B., N.Be., A.Bo., H.B., J.C., A.C., L.L., N.M., H.M., V.O., M.O., M.O., O.P., E.M.Q.M., J.S., U.W., and W.V.N. and B.D.C. were responsible for their curation and archaeozoological recording. The authors' list from A.Ba. to U.W. is in alphabetical order. C.O., E.M.G. and T.G. wrote the paper. W.V.N., B.D.C., J.P., N.S., M.E.P., N.Bo., A.M.-M. contributed to further discussion about the interpretation of the data and the outline of the paper. N.Bo. and M.E.P. revised the English. All the authors gave final approval for publication.

Additional information

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Competing interests

The authors declare no competing financial interests.