Current Biology

Limited historical admixture between European wildcats and domestic cats

Highlights

- Domestic cats and wildcats cohabitated in Europe for at least 2,000 years

- During this period, interbreeding between domestic cats and wildcats was extremely limited

- This reproductive isolation likely stems from behavioral and ecological differences

- Today this reproductive isolation is being eroded due to anthropogenic activities

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In brief

Jamieson, Carmagnini, et al. generate ancient genomes from European domestic cats and wildcats. Their results show that interbreeding between domestic and wildcats was limited (less than 10%) despite cohabitating for over 2,000 years. They argue that this reproductive isolation was likely the result of behavioral and ecological differences.

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Report

Limited historical admixture between European wildcats and domestic cats

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SUMMARY

Domestic cats were derived from the Near Eastern wildcat (*Felis lybica*), after which they dispersed with people into Europe. As they did so, it is possible that they interbred with the indigenous population of European wildcats (*Felis silvestris*). Gene flow between incoming domestic animals and closely related indigenous wild species has been previously demonstrated in other taxa, including pigs, sheep, goats, bees, chickens, and cattle. In the case of cats, a lack of nuclear, genome-wide data, particularly from Near Eastern wildcats, has made it difficult to either detect or quantify this possibility. To address these issues, we generated 75 ancient mitochondrial genomes, 14 ancient nuclear genomes, and 31 modern nuclear genomes from European and Near Eastern wildcats. Our results demonstrate that despite cohabitating for at least 2,000 years on the European mainland and in Britain, most modern domestic cats possessed less than 10% of their ancestry from European wildcats, and ancient European wildcats possessed little to no ancestry from domestic cats. The antiquity and strength of this reproductive isolation between introduced domestic cats and local wildcats was likely the result of behavioral and ecological differences. Intriguingly, this long-lasting reproductive isolation is currently being eroded in parts of the species' distribution as a result of anthropogenic activities.

RESULTS AND DISCUSSION

Previous analyses of microsatellite markers indicated that modern domestic cats (*Felis catus*) derive most of their ancestry from Near Eastern wildcat (*Felis lybica*).[1] Despite the fact that the remains of domestic cats, their wild progenitor (Near Eastern wildcats), and other wild relatives, such as European wildcats (*Felis silvestris*), are difficult to distinguish zooarchaeologically,[2] the

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archeological record suggests that domestic cats dispersed into Europe and reached France and Britain by ∼2800–2200 years before present (BP).[3]

Mitochondrial and nuclear genetic approaches have been used to distinguish domestic cats from European and Near Eastern wildcats.[1,4] These data have been used to address both domestic cat migration and the degree of introgression between European and Near Eastern wildcats at the boundary of their overlapping natural distributions in southeastern Europe.[4–7] In addition, numerous studies have demonstrated that European wildcat populations across Europe have experienced gene flow from domestic cats as a consequence of anthropogenic factors.[8–11]

This recent gene flow between introduced domestic cats and European wildcats is perhaps unsurprising for two reasons. Firstly, Near Eastern wildcats and domestic cats are morphologically even less divergent than many other pairs of wild and domestic populations (e.g., O'Connor[2,12]), suggesting a lack of physical barriers to hybridization. Secondly, previous studies have demonstrated that numerous domestic animal taxa, including pigs,[13,14] sheep,[15] goats,[16,17] bees,[18] chickens,[19]

and cattle,[20,21] have all acquired a proportion of their genomic ancestry via gene flow with sympatric, closely related wild populations. The notable exception to this rule is domestic dogs, which, despite their sympatry with wolves across the Northern Hemisphere, have received little to no gene flow for at least the last 11,000 years.[22–24]

Here, to ascertain the spatial and temporal pattern of hybridization between European wildcats and domestic cats following their arrival into Europe, we extracted DNA from 258 archaeological cat samples (8,500–100 years BP) excavated from 85 archaeological sites (Figure 1; Table S1; method details). We then generated and analyzed 75 mitochondrial genomes (>2× coverage) as well as 14 low- to medium-coverage nuclear genomes (0.1x–4x) (Figure 1; Table S1). To better understand patterns of hybridization between cat species, we also generated 31 modern (Figure 1; Table S2), full nuclear genomes (>15× coverage), including 16 European and four Near Eastern wildcats.

Ancient reticulation in the genus Felis

To establish the genetic ancestry of modern and ancient domestic cat genomes, we first reconstructed the evolutionary relationships
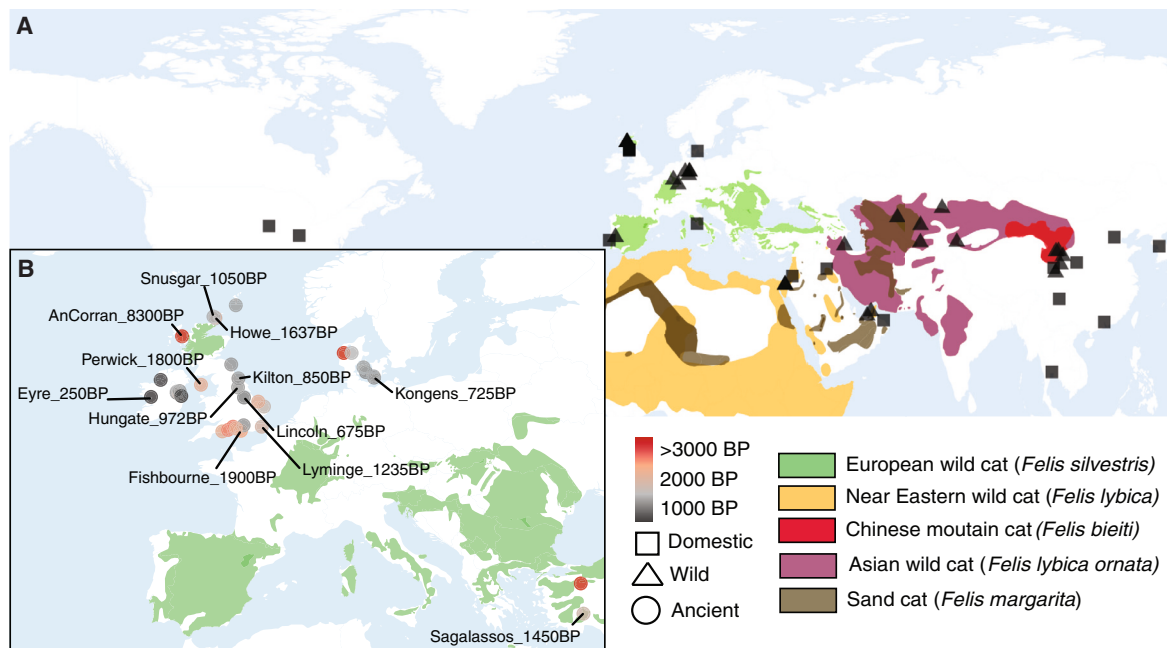
Figure 1. Location of cat samples sequenced in this study
Maps representing the geographical range of the *Felis* species investigated in this study (except for *Felis chaus*, which was used as an outgroup). The data (shape files) for this plot were obtained from the IUCN Red List database.[25]
(A) Location of modern genomes analyzed in this study.
(B) Location and age of ancient cat samples for which DNA information (either mtDNA, nuclear, or both) was obtained. The archeological sites from which samples possessing enough DNA to generate nuclear data are highlighted (time before present [BP]). See also Figures S1 and S4 and Tables S1 and S2.

of *Felis* lineages using complete nuclear genomes using the jungle cat (*Felis chaus*) as an outgroup (Figures 2A,S1B, and S1C; Table S2). Phylogenetic analyses showed that the Near Eastern wildcat represents an outgroup to all other wildcats, including the European, Asian (*Felis lybica ornata*), and Chinese mountain cats (*Felis bieti*; Figure 2A). This indicates that Asian and Near Eastern wildcats do not form a monophyletic group, suggesting that the current IUCN taxonomy,[26] which implies that *Felis lybica ornata* and *Felis lybica* are sister taxa, requires reconsideration. In addition, D-statistics analyses support at least two past admixture events: one involving Near Eastern and European wildcats and another involving Near Eastern and Asian wildcats (Figure 2B).

Though these admixture signatures could result from modern instances of gene flow from domestic cats into wildcat individuals, we found the same, significant D-statistics signal (Z > |3|) using the genome of a Mesolithic Scottish wildcat (8,459–8,272 cal. BP; AnCorran_1; Table S1; method details) that predates both the emergence of domestic cats and their arrival to Scotland. This result indicates that at least a portion of the admixture signal is due to ancient reticulation between wildcat lineages. Our phylogenomic analyses also further confirmed that domestic cats and Near Eastern wildcats are sister clades, corroborating previous studies using microsatellites and mtDNA data.[1]

Resolving the origins of the Near Eastern ancestry present in European wildcats

ADMIXTURE analyses (both K = 4 and K = 6; method details) suggested that none of the European wildcats in this study possess Near Eastern wildcat/domestic cat ancestry

(Figures S1D and S1E). Results of clustering-based analyses can be difficult to interpret,[27] although our analysis did not include samples previously identified as recent domestic cat-wildcat hybrids from Scotland, these results contradict previous analyses that show that modern Scottish wildcats possess some degree of domestic cat ancestry due to recent gene flow.[28]

Recent work on human data has shown that ADMIXTURE may not be able to detect admixture events if all analyzed admixed individuals possess similar ancestry proportions.[27] This would mean that ADMIXTURE may not be able to detect admixture if most European wildcats possessed similar levels of domestic cat ancestry. To address this issue, we analyzed the genome from the Scottish Mesolithic wildcat (8,459–8,272 cal. BP; AnCorran_1; Table S1; method details). We computed D-statistics of the form D (outgroup, Near Eastern, European wildcat, and AnCorran_1) in order to take advantage of the fact that the AnCorran_1 cat could not possess any domestic cat ancestry. We found that other than two individuals (Kilton_2, a 16th century cat from Kilton Castle in Northeast England, and FSX360, a modern Portuguese wildcat), all D-statistics involving the other nine European wildcats analyzed here yielded significantly negative Z values (|Z| > 3), indicating that all nine individuals possess a degree of Near Eastern/domestic ancestry (Figure S2A; method details).

The Near Eastern wildcat ancestry fraction in both ancient and modern European wildcats was computed using f4 ratios (Figure S3A; method details). We used AnCorran_1, Kilton_2, and FSX360 as representatives of non-admixed European wildcats. The Near Eastern ancestry fraction in European wildcats ranged
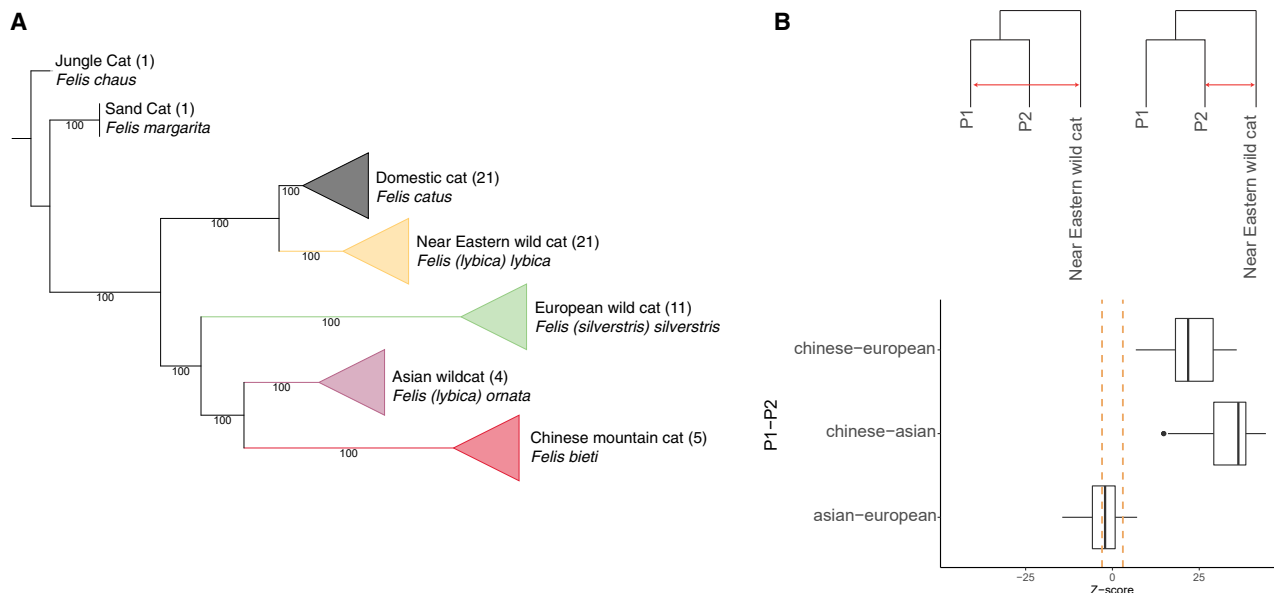
Figure 2. Nuclear phylogeny and admixture between wildcat species

(A) Maximum likelihood tree built using genome-wide SNP data (method details). Support values based on 100 bootstrap replicates are indicated near the nodes; values in brackets represent the number of samples in each collapsed clade. Species name reflects the two most used taxonomic nomenclatures by Driscoll et al.[1] and Kitchener et al.[26]

(B) Z score obtained from D-statistics of the form D(outgroup [Jungle cat]; Near Eastern wildcat; P1, P2). See also Figures S1, S2, and S4 and Tables S1 and S2.

from ∼3.5% in an Eastern German wildcat to ∼21% in Scottish wildcats (Figure S3C). Although the high level of ancestry in Scottish wildcats is likely due to recent hybridization with domestic cats,[28] it remains unclear whether the same is true for German wildcats, which possess low levels of Near Eastern wildcat ancestry. It remains possible that European wildcat populations that live closer to the edge of their distribution in southeast Europe naturally possess some degree of Near Eastern wildcat ancestry due to admixture and that the ancestors of these populations may not have experienced any hybridization with introduced domestic cats. In fact, mitochondrial analyses have shown that haplogroup IV, which is typically associated with Near Eastern wildcats, has been found in southeastern Europe in ancient European wildcats predating the introduction of domesticates (e.g., Romania, dated to 7,700 BC) and in modern wildcats (n = 10, 62%),[4] suggesting the existence of an admixture zone.

Low levels of European wildcat ancestry in modern and ancient domestic cats

Principal component analysis (PCA) of nuclear genomes, including 14 ancient and 52 modern genomes, further demonstrated the affinity of both AnCorran_1 and Kilton_2 with modern European wildcats, particularly FSX360 (Figure 3). This analysis also indicated that all 12 archaeological cats (excluding ancient wildcats) analyzed here, most of which (10/12; Figure 1) originated in Europe, likely derived the majority of their ancestry from Near Eastern wildcats/domestic cats rather than European wildcats. The earliest evidence of nuclear Near Eastern wildcat ancestry north of the Alps was found in a Roman domestic cat from the site of Fishbourne dated to 1,926–1,827 cal. BP

(Fishbourne_2), confirming that domestic cats with primarily Near Eastern wildcat nuclear ancestry were present in northwest Europe by at least the Roman period.

Our PCA further indicated that some ancient cats, including a Roman cat from the Isle of Man (Perwick_1) and a 17th century cat from Ireland (Eyre_3), were slightly shifted toward European wildcats on the PCA. This positioning suggests a possible history of gene flow with European wildcats. To test this, we first used ADMIXTURE. At K = 4, ADMIXTURE detected European wildcat ancestry in all but one of the ancient domestic cats (from the site of Dhzankent, Kazakhstan, 1,175–1,010 cal. BP)[29] and in most (8/9) modern European and Middle Eastern samples (Figures S1D and S1E). At K = 6, ADMIXTURE only found detectable levels of European wildcat ancestry in two ancient samples, a 17th century cat from Ireland (Eyre_3) and a Roman cat from the Isle of Man (Perwick_1; Figure S3).

We then computed D-statistics of the form D (outgroup, FSX360/AnCorran_1/Kilton_2, Near Eastern wildcat, and domestic cat). All tests involving modern and ancient domestic cats from Europe, Turkey, and North America, including Denmark, England, Ireland, Italy, Portugal, Scotland, and the USA, yielded significant Z values (|Z| > 3). This result shows that all European (ancient and modern), Turkish (ancient), and North American (modern) domestic cats analyzed in this study possessed a degree of European wildcat ancestry (Figure S2B).

The same D-statistics computed using modern cat data from the Middle East, including Iraq, Jordan, and Oman, and when using the genome of a Madagascan cat, were either non-significant or borderline significant (Figure S2B). This result suggests that these non-European cats possessed either fleetingly small or no European wildcat ancestry. In addition, a central Asian
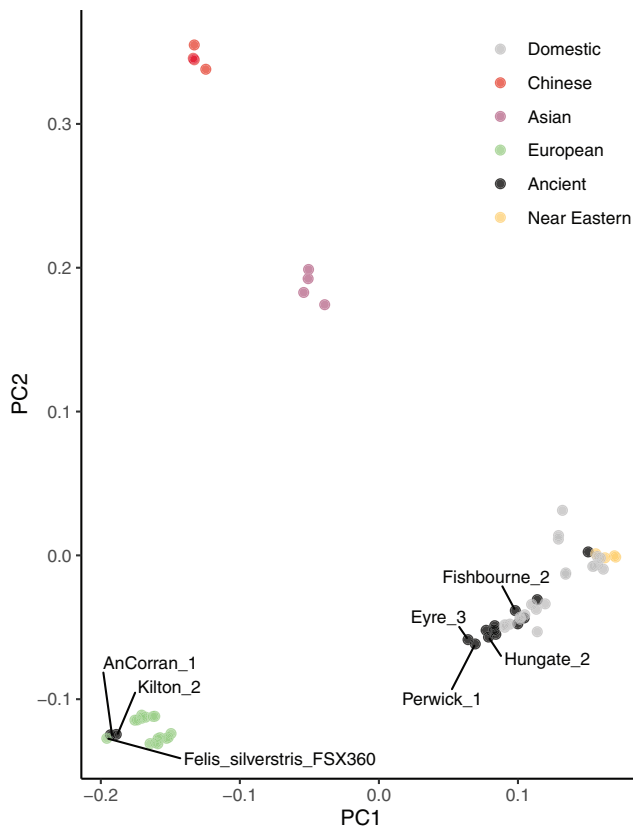
Figure 3. Principal component analysis (PCA)
Built using 67 genomes, including 14 ancient cat genomes (projected). See also Figures S2–S4 and Tables S1 and S2.

ancient domestic cat from Kazakhstan and modern cats from China, South Korea, and Thailand all yielded negative, significant, D-statistics values. This result suggests that these cats may possess either or both Chinese and Asian wildcat ancestry (neither of which were represented in this test), corroborating a previous genomic study of wild and domestic Chinese cats.[30] This was confirmed through D-statistics analyses of the form D (outgroup, Chinese mountain cat, Near Eastern wildcat, and domestic cat), which showed that these individuals share significantly more alleles with Chinese mountain cats than expected under a model with no gene flow (Figure S2C).

To improve our power to estimate the contribution of European wildcat ancestry in domestic cats, we computed ancestry proportions using f4 ratios (Figure S3A; method details). We used an explicit model (Figure S3A) in which each domestic cat received its ancestry from European wildcats represented by An-Corran_1, Kilton_2, and FSX360 (all un-admixed wild individuals; Figure S2A), and Near Eastern wildcats represented by three modern wild individuals: two Israeli cats (FSI48 and FSI51) and a wildcat from the United Arab Emirates (FSI7). This analysis detected significant levels of European wildcat ancestry in eight ancient cats (Kongens_3, Perwick_1, Lyminge_20, Lincoln_1, Hungate_2, Timberyard_1, Eyre_3, and Snusgar_7; Figure 4), including the two detected by ADMIXTURE at K = 6 (Figures S1D and S1E). The proportion of European wildcat

ancestry in cats from Ireland and Britain, identified by both f4 ratio and ADMIXTURE at K = 6 (Eyre_3 and Perwick_1) was 11%–14%, while those only identified by f4 ratio had values less than 10%. Similar results were obtained using qpAdm (Figure S3B)[31] and struct-f4 analyses (Figures S3E–S3G; method details).[32]

Overall, the proportion of European wildcat ancestry in ancient domestic cats ranged from 0% to 14% (+−2%) (Figure 4). Slightly lower levels of European wildcat ancestry were also identified across modern domestic cats (between 0% and 11%; +−1%) (Figure 4). This is consistent with the results of recent analyses that estimated the proportion of Chinese mountain cat ancestry in Chinese domestic cat genomes,[30] though the restricted distribution of the Chinese mountain cat significantly lowers opportunities for introgression into domestic cats. Overall, the relatively low proportion of wildcat ancestry found in domestic cats is much more similar to dogs and substantially less than the reported percentages in pigs,[13,14] sheep,[15] goats,[16,17] bees,[18] chickens,[19] and cattle.[20,21]

Near Eastern mitochondrial haplogroups present in European wildcats

These low levels of hybridization between wildcats and domestic cats raises the possibility that the Near Eastern wildcat ancestry identified in the European wildcats was not mediated by admixture with domestic cats. If true, this would undermine the use of mitochondrial data as a definitive proxy to determine the domestic status of ancient cats. Domestic cats, and Near Eastern and European wildcats, belong to two mitochondrial haplogroups: I and IV.[1] Haplogroup I is found exclusively in European wildcats, while haplogroup IV is found in all domestic and Near Eastern wildcats, as well as in multiple European wildcats. The presence of haplogroup IV in European wildcats has traditionally been interpreted as the result of hybridization with domestic cats,[1] though it is possible that these signatures were introduced into European wildcats through hybridization with Near Eastern wildcats living in the admixture zone at the edge of their distribution.[11]

We found that all four Near Eastern wildcats (labelled *Felis lybica* in Figures S4A and S4B) and all 22 modern domestic cats analyzed here belonged to haplogroup IV (Figures S4A and S4B). Haplogroup I was found in 10 out of the 16 modern European wildcats, while the other six possessed haplogroup IV (IV-A; see individual highlighted in Figures S4A and S4B). Intriguingly, haplogroup IV was also found in the three eastern German wildcats, two of which had low levels of Near Eastern wildcat ancestry (3%–4%; Felis_silverstris_ex9 and Felis_silverstris_ex19; Figure S3C), and one possessed >10% Near Eastern wildcat ancestry (Felis_silverstris_ex38). This suggests that they did not acquire their mitochondrial genome through recent admixture with domestic cats (i.e., ~3–5 generations ago). All modern European wildcats with haplogroup IV, except a single individual from Scotland, however, possessed sub-haplogroup IV-A, which was also most frequently identified in previous studies of domestic cats.[1]

This sub-haplogroup was also the most common in our total dataset (~74% of all domestic cats) and in specific geographical populations, including ancient cats from Britain and Ireland (28 of 43 cats dated between 339 BCE and 1,799 CE). This pattern suggests that sub-haplogroup IV-A in wildcats could have been
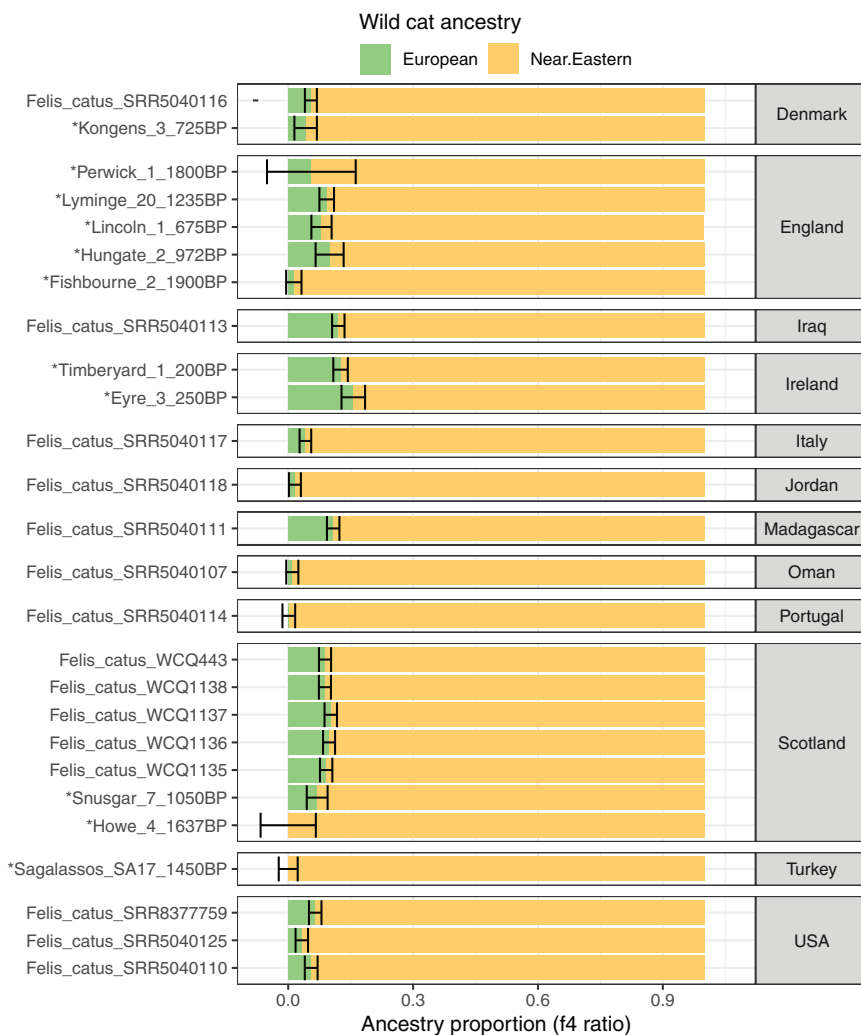
Figure 4. Admixture fractions computed using f4 ratio

Proportion of European wildcat ancestry in modern and ancient (denoted by an *) domestic cat genome computed using an f4 ratio as described in Figure S3A. Error bars represent standard errors computed across multiple f4 ratios using different European (AnnCorran_1, Kilton_2, and Felis_silvestris_FSX360) and Near Eastern (Felis_lybica_lybica_FSI47, Felis_lybica_lybica_FSI48, Felis_lybica_lybica_FSI51, and Felis_lybica_lybica_FSI7) wildcats as sources. See also Figures S2 and S3 and Tables S1 and S2.

analyses of one of the wildcats from Kilton Castle that possessed haplogroup I (Kilton_2) revealed a lack of any domestic cat ancestry (Figure S2A; method details), indicating that introgression from domestic cats into European wildcats was limited in the past.

These results indicate that although domestic cats were not subjected to strong, conscious selection by people until the last century, they acquired biological adaptations as the result of their association with people that led to at least partial reproductive isolation from European wildcats. While domestic cats possess the ability to tolerate living in anthropogenic environments at much higher population densities,[33] they are also likely to be competitively excluded by larger, more aggressive wildcats in other habitats with less abundant food. Combined with differences in male repro-

acquired through gene flow with domestic cats. While the presence of haplogroup IV-A in European wildcats is, at least to some degree, due to admixture with domestic cats, the low-level Near Eastern wildcat ancestry in the eastern German cats raises the possibility that ancient, natural hybridization in the admixture zone of southeastern Europe and Anatolia resulted in the spread of haplogroup IV into European wildcats. Taken together, this pattern suggests that mitochondrial data alone are inadequate for determining the domestic status of ancient cats.

Our analyses indicate that domestic cats with Near Eastern nuclear ancestry were present in Britain by at least ~2,000 years ago (1,926–1,827 cal. BP; Fishbourne_2) and that European wildcats and domestic cats have been coexisting throughout Northern Europe ever since. Despite this long-term co-existence, 30 of the 35 domestic cats analyzed in this study possessed less than 10% of their ancestry from European wildcats, and 17 out of 35 possessed less than 5%. In addition, mitochondrial analyses of four cats from the English medieval site of Kilton Castle (Table S1) revealed individuals with both sub-haplogroup IV-A (2/4), the most globally common haplogroup in modern domestic cats, and haplogroup I (2/4), which is only found in European wildcats, co-existed at the site. Nuclear

ductive behavior (i.e., male wildcats are only reproductively active in winter and spring, while domestic cats are active all year), this ecological separation likely reduced both the opportunity for hybridization and the ability of hybrids to survive.[34] These factors may explain why the proportion of wildcat ancestry in both ancient and modern domestic cats is substantially less than many other introduced domestic taxa and more similar to dogs.[35] One hypothesis is that given the behavioral modification required for carnivorous animals such as dogs and cats to survive in an anthropogenic environment, those that possess substantial wild ancestry have historically been much less able to survive and reproduce relative to their herbivorous domestic counterparts.

Despite this general and long-term resistance to introgression, several studies have presented evidence for recent gene flow from domestic cats into indigenous European wildcat populations in both France[11] and Scotland.[28] In Britain, this was likely triggered by a drastic reduction of the distribution of wildcats, which began prior to the 19th century[36,37] and intensified in the second half of the 20th century.[38] This indicates that habitat degradation and the encroaching human presence have led to the erosion of the reproductive isolation that had maintained

the genomic integrity of domestic and European wildcats for at least 2,000 years.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.08.031.

AUTHOR CONTRIBUTIONS

Conceptualization, A.J., G.L., and L.F.; methodology, A.C., A.H., A.J., D.L., E.D., J.H.-C, L.F., N.S., O.L., R.A., R.B., and P.L.; resources, A.S., A.F., A.C.K., A.B.G., A.H., B.D.C., C.D., C.N., C.M., C.O., C.B., D. Griffith, D. Groß, E.D., E.G., F.B., G.T., H.R., H.S., H.A., I.M., I.M.R., J.H., J.B., J.P.,
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DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
259 archeological felid samples	This paper	See Table S1
31 modern felid samples	This paper	See Table S2
Chemicals, peptides, and recombinant proteins		
Proteinase K	Roche	#03450376103
Proteinase K	Qiagen	#19131
Critical commercial assays		
MinElute PCR Purification Kit	Qiagen	#28004
Qiaquick gel extraction kit	Qiagen	#28706X4
T4 DNA Polymerase (5 U/μL)	Thermo Scientific	#EP0062
T4 Polynucleotide Kinase	NEB	#M0201 S/L
T4 DNA Ligase	New England Biolabs	#M0202S
T4 DNA Ligase (5 U/μL)	Thermo Scientific	#EL0011
Invitrogen™ AccuPrime™ SuperMix I	Thermo Scientific	#12342010
SPRIselect	Beckman Coulter Life Sciences	#B23317
AMPure XP	Beckman Coulter Life Sciences	A63881
SpeedBeads™ magnetic carboxylate modified particles	Sigma-Aldrich	#GE45152105050250
DNeasy Blood & Tissue Kits	Qiagen	#69504
NEBNext® Ultra™ II DNA Library Prep Kit	New England Biolabs	#E7103
Oligonucleotides		
Illumina-compatible adapters	N/A	N/A
myBaits Mito v4 (*Felis catus*)	Arbor Biosciences	#303008.v4
Deposited data		
Short read data for archeological and modern cats	This paper	PRJEB57412
Short read data from Felis_chaus_SRR2062187	European Nucleotide Archive (ENA)	SRR2062187
Short read data from Felis_margarita_SRR2062538	European Nucleotide Archive (ENA)	SRR2062538
Short read data from Felis_catus_SRR5040114	European Nucleotide Archive (ENA)	SRR5040114
Short read data from Felis_catus_SRR5040111	European Nucleotide Archive (ENA)	SRR5040111
Short read data from Felis_catus_SRR5040120	European Nucleotide Archive (ENA)	SRR5040120
Short read data from Felis_catus_SRR5040116	European Nucleotide Archive (ENA)	SRR5040116
Short read data from Felis_catus_SRR5040126	European Nucleotide Archive (ENA)	SRR5040126
Short read data from Felis_catus_SRR5040110	European Nucleotide Archive (ENA)	SRR5040110
Short read data from Felis_catus_SRR5040107	European Nucleotide Archive (ENA)	SRR5040107
Short read data from Felis_catus_SRR5040113	European Nucleotide Archive (ENA)	SRR5040113
Short read data from Felis_catus_SRR5040117	European Nucleotide Archive (ENA)	SRR5040117
Short read data from Felis_catus_SRR5040118	European Nucleotide Archive (ENA)	SRR5040118
Short read data from Felis_catus_SRR5040125	European Nucleotide Archive (ENA)	SRR5040125
Short read data from Felis_catus_SRR7621254	European Nucleotide Archive (ENA)	SRR7621254
Short read data from Felis_catus_SRR7621252	European Nucleotide Archive (ENA)	SRR7621252
Short read data from Felis_lybica_ornata_SRR7621238	European Nucleotide Archive (ENA)	SRR7621238
Short read data from Felis_bieti_SRR7621235	European Nucleotide Archive (ENA)	SRR7621235
Short read data from Felis_bieti_SRR7621229	European Nucleotide Archive (ENA)	SRR7621229
Short read data from Felis_bieti_SRR7621227	European Nucleotide Archive (ENA)	SRR7621227
Short read data from Felis_bieti_SRR7621226	European Nucleotide Archive (ENA)	SRR7621226
Short read data from Felis_catus_SRR7621225	European Nucleotide Archive (ENA)	SRR7621225

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Short read data from Felis_catus_SRR7621222	European Nucleotide Archive (ENA)	SRR7621222
Short read data from Felis_catus_SRR7621212	European Nucleotide Archive (ENA)	SRR7621212
Short read data from Felis_catus_SRR8377759	European Nucleotide Archive (ENA)	SRR8377759
Software and algorithm		
AdapterRemoval2	Schubert et al. (2012)[39]	https://github.com/MikkelSchubert/adapterremoval
Trimmomatic	Bolger et al. (2014)[40]	https://github.com/usadellab/Trimmomatic
BWA aln v0.7.17	Li and Durbin (2009)[41]	http://bio-bwa.sourceforge.net/
MapDamage2.0	Jónsson et al. (2013)[42]	https://ginolhac.github.io/mapDamage/
Bowtie2	Longmead and Salzberg (2012)[43]	https://github.com/BenLangmead/bowtie2
samtools v1.9	Li et al. (2009)[44]	http://samtools.sourceforge.net/
FilterUniqueSAMCons	Kircher (2012)[45]	NA
GATK v4.0.8.1	McKenna et al. (2010)[46]	https://gatk.broadinstitute.org
ANGSD	Korneliussen et al. (2014)[47]	https://github.com/ANGSD/angsd
Plink 2.0	Purcell et al. (2007)[48]	https://github.com/chrchang/plink-ng/tree/master/1.9
RAxML v8.2.4	Stamatakis (2014)[49]	https://github.com/stamatak/standard-RAxML
smartPCA	Price et al. (2006)[50]	https://github.com/chrchang/eigensoft/tree/master/POPGEN
ADMIXTURE	Alexander and Lange (2011)[51]	http://dalexander.github.io/admixture/download.html
qpDstat	Patterson et al. (2012)[31]	https://github.com/DReichLab/AdmixTools
qpF4ratio	Patterson et al. (2012)[31]	https://github.com/DReichLab/AdmixTools
qpAdm	Patterson et al. (2012)[31]	https://github.com/DReichLab/AdmixTools
Struct-f4	Librado and Orlando (2022)[32]	https://bitbucket.org/plibradosanz/structf4/src/master/
BEAST v2.6.3	Bouckaert et al. (2019)[52]	https://github.com/CompEvol/beast2
Tracer	Rambaut et al. (2018)[53]	https://github.com/beast-dev/tracer/releases

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Laurent Frantz (laurent.frantz@lmu.de).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The sequence reported in this paper is available at https://www.ebi.ac.uk/ena/browser/view/PRJEB57412 under the accession number ENA: PRJEB57412.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Archaeological site descriptions
258 cat bones were collected from 85 sites across Britain, Ireland and Denmark, mainland Europe, the Middle East and Tanzania (Table S1). All of the samples were collected either directly from the excavator or from a zooarchaeologist or museum where the bones were housed. The sites range from the Mesolithic through to the post-medieval period. Nineteen of the above samples were radiocarbon dated by either the Oxford Radiocarbon Accelerator Unit or Beta Analytic (Table S1). All radiocarbon dates

reported are calibrated using OxCal 4.4 and the INTCAL20 calibration curve.[54] Two samples (AJ517, AJ513) had a high delta 13C value so have been calculated using the mixed curve approach using the Marine as well as the INTCAL20 calibration curve.

Denmark

Almosen, Tyvelse (contact: Anne Birgitte Gotfredsen). A single cat tibia was obtained from this site and radiocarbon dated. The radio-carbon date (1904 AD) indicated that this sample was an intrusion into a Bronze Age site.

Kongens Nytorv, Z.M.K. 19/2011, KBM 3829 (contact: Anne Birgitte Gotfredsen). The Kongens Nytorv excavations was carried out in the years 2009 to 2016 as one of three major excavations, which took place within the medieval city walls of Copenhagen in connection with the Metro City Ring constructions.[55] The huge Kongens Nytorv excavation, divided into 12 main phases, covered the time from establishment (1050-1200 CE) of the early medieval town to present time. The cat bones, in general well-preserved, came from fill in boundary ditches; fill in of moats or levelling out of moats. Retrieval was carried out with trowels through single context excavations. Some selected deposits were either fine sieved or sieved on 3 mm or 5 mm mesh sizes. The actual cat bones, deposited together with primarily household waste, lay scattered in the deposits and had all skeletal elements represented, although one bone (P135) came from a single deposit (SD60568), holding 71 cat remains out of 320 mammal bones. A single cat bone (P135) derived from a ditch dated to phase 6, i.e., 1050-1550 CE, two cat bones (P136 and P138) dated to phase 5A-1 and 5B-1, i.e., 1550-1660 CE and iii) and finally two cat remains (P139 and P140) derived from deposits dated to phase 45B, i.e., 1550-1845 CE.[55,56] A total of six cat bones (3 tibias & 3 humeruses) were screened for aDNA (Table S1).

Læderstræde 4, Roskilde, Z.M.K. 61/2015, ROM 2982 (contact: Anne Birgitte Gotfredsen). Læderstræde 4 located in Roskilde was excavated by Roskilde Museum (ROMU) during August through October 2015. The town of Roskilde located at the head of Roskilde Fjord, Zealand, Denmark and founded around the year 1000 functioned as a royal and clerical center. By the 12th century, Roskilde was already densely built and Læderstræde was established as a craft district at the southern periphery of the medieval town. The site comprised several disposal deposits and pits among which Pit A82 provided a large number of cat remains. The pit had steep vertical sides and a depth of c. 1.3 m and seemingly was in use c. 1200 to 1400 CE as dated by Baltic-ware pottery. The well-pre-served bone material, recovered by trowels and to some extent by sieving, was analyzed as part of a MSc thesis.[57] This analysis concluded that the Pit A82 primarily contained waste from various craft production, among others butchery and skinning of cats – the 434 cat bones making cat the third most frequent taxon of the pit.[57] A total of four cat femurs were screened for aDNA (Table S1).

Svendborg, land. Reg. No. 609 Z.M.K. 111/1950 a Møllergade 6 Z.M.K. 154/1977 (contact: Anne Birgitte Gotfredsen). Svendborg Land Reg. No. 609 derives from a minor urban excavation in Svendborg, south Funen, Denmark carried out by Svendborg Museum (formerly Svendborg og Omegns Museum). The material, dated to 1200-1300 CE, was sent to the Zoological Museum, Copenhagen in 1950 where Ulrik Møhl identified part of the material in 1977 Knud Rosenlund completed the work. The well-preserved cat remains comprising 59 bones of c. 2000 mammal bones exhibited cut marks on calvaria and mandibles, indicating skinning. Møllergade 6, Land Reg. No. 607a located centrally in the town came to light in 1976-1977. It formed but one excavation of a huge interdisciplinary archaeological investigation of Medieval Svendborg and the Stronghold of Ørkild northeast to the town, during the years 1972 to 1980, led by Henrik M. Jansen, Svendborg Museum. Preservation conditions of house foundations and midden deposits of Møller-gade 6 were exceptionally good for leather, wood, plant macrofossils and bones.[58] The culture layers, dated to 1200-1500 CE, comprise a very large only provisionally published animal bone assemblage of a diversity of fish, birds and mammals.[58] Cats comprise 251 bones of more than 12.000 mammalian remains scattered by layers and representing different parts of the cat skeleton (Tove Hatting, unpublished). A total of four cat femurs were screened for aDNA (Table S1).

Overgade 1 and 3, Odense, Z.M.K. 142/1970, OBM 8201 (contact: Anne Birgitte Gotfredsen). Overgade 1 and 3, comprising two medieval basements and a pit, are located in Odense on the island Funen, Denmark. The stratigraphic position of the pit provided a preliminary dating to the Viking Age, subsequently supported by a radiocarbon date of 1070 ± 100 CE (K-1887). Excavations in 1970 commenced by Odense Bys Museer (OBM) and continued by the Zoological Museum in Copenhagen (now Natural History Museum of Denmark, University of Copenhagen) produced at least 1783 well-preserved cat bones and a few remains of other species. The pit, almost circular and with a diameter exceeding 1 m, was about 80 cm deep narrowing towards the base. Most of the cat remains derived from a 30 cm thick clay layer overlaying a c. 15 cm thick reddish greasy clay layer close to the bottom of the pit. Throughout the pit lumps of lime and concentrations of fly puparia of which some were from *Musa domestica* L. indicated that the pit was left open for some time prior to being sealed. The contents of the pit comprising remains of at least 68 young skinned cats was subsequently analyzed and published by Tove Hatting, Zoological Museum.[59] A total of five cat femurs were screened for aDNA (Table S1).

Vejleby, Z.M.K. 108/1942, NM1 326/24 (contact: Anne Birgitte Gotfredsen). Vejleby (NM1 326/24) comprises a Viking Age burial site with at least 10 graves and settlement deposits from early medieval time, located on the island of Møn, southeastern Denmark. The site was due to railway constructions excavated by H. Kjær, the National Museum (NM) in September 1923. In 1942, the bone material was handed over to the Zoological Museum, Copenhagen. The cat bones found amongst other domestic animal bones were well preserved and recovered from pits found in connection with the burial site. Detector searching near the site have subsequently pro-vided artefacts mainly dated to early medieval time but also from the Viking Age. According to Tage Christensen NM the cat bones derived from layers dating to early medieval time, although with a risk of being mixed with underlying layers from the Viking Age. A total of one cat ulna was screened for aDNA (Table S1).

Viborg Søndersø, Z.M.K. 14/1988, VSM 881D & VSM 51E (contact: Anne Birgitte Gotfredsen). The Viborg Søndersø site (VSM 881D & VSM 551E) is located close to lake "Viborg Søndersø" in the old Thing-stead and capital of the diocese Viborg in Mid-Jutland, Denmark. Excavations during 1981 and 1984 to 1985 conducted by Viborg Museum (VSM) included culture layers from a presumed workshop area dated to c. 1000-1300 CE. A later multidisciplinary excavation at the site in 2001, however, revealed

that the workshops were not ordinary workshops producing goods for the town - but for the King, his housecarls and their peers (Pers. Comm. J. Hjermind 2022). The oldest houses, with dendrochronological dates of 1015 and 1018 CE, mark the approximate time of establishment of the workshop area. Around 1300 CE and probably in connection with King Erik Menved's erection of a stronghold in 1313 a damming up and raising of the lakes water level took place, resulting in the flooding of large parts of the workshop area. For long periods, the culture layers were therefore waterlogged thus providing excellent preservation conditions, especially in the lower layers, of wood, skin, textiles and bone.[60] A large number of recovered animal bones primarily from layers dated to c. 1100-1300 CE included cat remains from all parts of the skeleton of which many exhibited skinning marks.[60] A total of five cat femurs were screened for aDNA (Table S1).

Syltholm II (contact: Theis Jensen, Daniel Groß, and Mikkel Sinding). For this study three wild cat canines were gently extracted from well-preserved mandibles from the site Syltholm II (MLF00906-I). The site is located in an ancient fjord on the southern part of Lolland, near the town of Rødby. Beginning in 1872 the area was reclaimed after a series of devastating floods, so that filling up and dike constructions reclaimed an area of 187 ha.

Due to the construction of the fixed-link connection between Denmark and Germany, the Femern Belt tunnel, nearly the entire area was scheduled to be turned into a construction site for the tunnel. Therefore, beginning in 2012, Museum Lolland-Falster carried out several surveys and large scale excavations in the context of development-led archaeology.[61,62] In total, around 57 ha have been professionally excavated and revealed more than 50sites from various periods.

The canines for this study come from a c. 4.200 m^2 large excavation of a late Mesolithic/early Neolithic site (c. 6500-5500 cal. BP) where a riparian lagoon was excavated in the south while the northern part is the former beach and tidal zone. The lagoon was protected from the sea by a shifting barrier island and had a low water depth (c. 1–1.5 m) with 50-100 cm of gyttja covering the Pleistocene sediments. Organic remains are well preserved in the gyttja, with antler, bone and wood being in pristine condition.

The site is attributed to the late Ertebølle (6.500 BP) period up until Funnel Beaker Culture (5.500 BP) and shows vast evidence for fishing activities in the form of nearly 500 bone points as well as hundreds of wooden leister prongs. However, an area of the site was also interpreted as having been used to perform ritual activities,[61,62] based on *in situ* finds of faunal skeletal remains, predominantly mandibles of several different animal species. These were purposely deposited in a small, confined area underwater ("structure A") and - as the chronology shows - over a longer period of time. Other numerous animal remains were also found in the feature, but also decorated wood and antler, as well as hafted axes. The analyzed cat mandibles come from "structure A." A total of three cat samples were screened for aDNA (Table S1).

England

Bamburgh Castle, Northumberland (contact: Naomi Sykes). Bamburgh Castle is situated on the north-east coast of Northumberland, south of Lindisfarne (NGR NU182351). The cat bones examined in this study were recovered from excavations in the West Ward by Brian Hope-Taylor during the 1960s and 1970s, and later excavations by the Bamburgh Research Project from 1998 onwards. These revealed a deep stratigraphy (2.74 m) with evidence of occupation from at least the Iron Age to the modern period, within which seven discrete phases were identified.[63] Specimens Bamburgh_1 and Bamburgh_2 come from Phase 7, a substantial 12th to 14th-century stratigraphic sequence largely composed of midden material. Zooarchaeological identification and analysis were conducted by Naomi Sykes. A total of two cat mandibles were screened for aDNA (Table S1)

Caistor Roman town, Norfolk (contact: Will Bowden). Caistor St Edmund is located in Norfolk, 5km south of Norwich on the banks of the River Tas (NGR TG230035). The site is one of only three "greenfield" Roman regional capitals in Britain that were not succeeded by mediaeval and modern towns (the others being Silchester and Wroxeter). Excavations in the 1920s and 1930s led by Donald Atkinson concluded that the town was founded and its streets laid out in the CE 70s as part of the Roman pacification of the Iceni following the Boudican Revolt (c.AD 60-61).[64] This Flavian date has been challenged by recent excavations led by Will Bowden, which suggest that the streets were likely laid out in the first half of the 2nd-century.[65] Numismatic evidence shows that the Roman town survived in some form into the very early 5th-century. Early-Middle Saxon cemeteries to the north and east of Caistor suggest that occupation of the area continued until as late as the 8th century, and coin finds from the adjacent field to the west of the River Tas also indicate significant Middle Saxon occupation. Whether occupation continued within the walled town itself is unclear; however, this appears plausible as a cat pelvis (Caistor_4) returned a radiocarbon date of 653-708 AD (Beta-589634) at 51.6% probability (all specimens analyzed came from this deposit). A total of four cat pelvises were screened for aDNA (Table S1).

Danebury, Hampshire (contact: Naomi Sykes). The Iron Age hillfort at Danebury (NGR SU323376) was built in the 6th century BCE and occupied for almost five hundred years.[66] Shortly after 300 BCE, Danebury underwent major structural changes involving the rebuilding of its eastern gateway with large external hornworks. A total of one cat humerus was screened for aDNA (Table S1), and one (Danebury_1) was radiocarbon dated (OxA-38897) to 392-208 cal. BCE.

Old Methodist Chapel and Greyhound Yard, Dorchester (contact: Naomi Sykes). Excavations in Dorchester (NGR SY806848) uncovered evidence of the Roman town of *Durnovaria,* founded around c. AD 70, likely on the site of Legio II Augusta's garrison. The town expanded over the subsequent centuries, developing from timber structures in the second-century to stone-founded buildings with painted wall-plaster and tessellated floors in the fourth century.[67] Ten cat skeletons were recovered from the site, all absent of evidence of skinning or butchery.[68] A total of eight bones of various elements were screened for aDNA (Table S1) and one (Dorchester_4) was radiocarbon dated (OxA-39001) to 132-317 cal. CE.

Fishbourne, West Sussex, UK (contact: Naomi Sykes). Fishbourne Roman Palace (NGR SU839047) is located to the west of the city of Chichester at the head of a navigable creek leading to the English Channel. The earliest archaeological evidence at the site comes from a pre-AD 43 ditch, containing Late Iron Age pottery and the top of a gladius scabbard of an early style atypical to those found in

Claudio-Neronian military sites in Britain.[69] Granaries and residential buildings were constructed soon after the conquest in AD 43. These were demolished around AD 60-65 and replaced with a large stone-walled proto-palace, likely occupied by the local chieftain and legatus Augusti, Tiberius Claudius Togidubnus. Construction of the Claudian palace—the largest Roman residence north of the Alps—was undertaken around AD 75-80,[70] incorporating the proto-palace in the south-east corner. The site was furnished with a large ornamental garden to the south, which from the 1st century was home to a variety of imported fauna, including fallow deer[71] and chickens.[72] However, by the 3rd-century, the palace was in an increasing state of disrepair and was abandoned in c.CE 270 following a fire. The early date and short duration of occupation is supported by direct radiocarbon date of cat remains to 96 BCE-CE 17 (Fishbourne_1; OxA-38875, 2038 ± 16) and 4-85 AD (Fishbourne_2; OxA-38877, 1950 ± 17) respectively, at 95.4% probability. A total of five cat bones from various elements were screened for aDNA (Table S1).

Flaxengate, Lincolnshire, UK (contact: Naomi Sykes). The site of Flaxengate lies within the Roman walls of Lincoln, due south of the Cathedral and Bishops Palace (NGR SK977714). The earliest structures date from the Roman period, with an apparent gap in occupation from the late-4th-century until the late 9th-century when a series of timber buildings and streets were established.[73] The site was reorganised in the 10th with the addition of glass and copper-alloy workshops, and again in the 11th century, which contributed to the decline of these industries. The successive phases of timber buildings were brought to an end in the late 12th or early 13th century with the construction of stone buildings. The majority of the animal bone assemblage was recovered from the Saxon (*c.*870-900 CE) and Angevin (*c.*1180 CE) period. A total of five cat bones Table S1.

<i>Guildford Castle, Surrey, UK (contact: Naomi Sykes).* Excavations at Guildford Castle (NGR SU997493) uncovered evidence for the construction and development of the Royal complex during the 11th–14th century.[74] The original Norman bailey was replaced and expanded in the later-12th century, principally for the construction of domestic apartments for the king and his court following the establishment of the royal hunting park at Guildford in 1154 AD. The castle remained an important strategic fortification until the mid-14th century, after which it fell into disrepair. A total of eleven cat bones recovered from contexts dating to the 12th-14th century were screened for aDNA (Table S1).

Gussage All Saints, Dorset, UK (contact: Naomi Sykes). The Iron Age Settlement of Gussage All Saints on Cranborne Chase (NGR ST998101) was excavated in 1972 by G.J. Wainwright.[75] The site is roughly circular in plan, approximately 3 acres in extent with a single entrance in the east and was occupied between 4th-1st century BCE. A large number of pits were excavated, producing evidence for grain processing and storage and a bronze foundry. The zooarchaeological assemblage is noted for the recovery of a large number of young felid bones. With the exception of one specimen, all were immature, with one pit containing at least five new-born kittens interpreted by Harcourt[75,76] as a single litter. The presence of juvenile bones within the settlement led Harcourt to conclude that: a) they were domestic cat (*Felis catus*) rather than the European wildcat (*Felis silvestris silvestris*); and b) the Roman were not responsible for the introduction of the domestic cat to Britain. The cat tibia analyzed in this study (Guassage_4) returned a radiocarbon date of 361-168 BC at 95.4% probability (OxA-38874, 2125 ± 17), consistent with its archaeological phasing. This specimen was from the same assemblage of kitten bones as Guassage_2, Guassage_3. Guassage_7 (BETA-568254) was dated to 361-168 cal. BC. A total of six cat bones were screened for aDNA (Table S1).

Lincoln Castle, Lincoln, UK (contact: Naomi Sykes). Construction of Lincoln Castle (NGR SK974717), located in the city of Lincoln, began shortly after the Norman Conquest. The Castle was the focus of attention during the First Battle of Lincoln (1141) as King Stephen warred with his cousin Empress Matilda over the English crown. During this period (c.1138-c.1154), enhanced fortification saw the construction of a timber stable block. Following the Second Battle of Lincoln (1217), during the course of the First Barons' War and the signing of *Magna Carta*, the stable block was redeveloped in stone—c.1223-c.1272[77]— with the addition of a hay loft, brewhouse and horse mill. The cat bones analyzed in this study were recovered from deposits associated with the later-13th century construction of the stable block. A total of three cat bones (tibia. femur radius) were screened for aDNA (Table S1).

Lyminge, UK (contact: Zoe Knapp). Excavations between 2008 and 2015 at Lyminge (NGR) were led by the University of Reading. A total of 31 cat bones were screened for aDNA (Table S1). One of the Lyminge cats was radiocarbon dated. Lyminge_28 (OxA-31749) was dated 656-775 cal. CE.

Owslebury, Hampshire, UK (contact: Naomi Sykes). Excavations between 1962 and 1972 at Bottom Pond Farm, Owslebury, 8km southeast of Winchester (NGR SU525246), uncovered an Iron Age and Roman farming settlement occupied from the 4th-century to the 4th-century AD. In the early and middle Iron Age (c.4th-2nd Century BC), the site was characterized by a banjo farm enclosure with storage pits, chalk quarries and inhumations.[78] The settlement underwent considerable change in the late Iron Age (c.1st century BC to mid-1st Century AD), trebling in size from 3-4 acres to 10-15 acres, with enclosures and linear gullies circling much of site and the addition of a ditch trackway leading to the main complex. While the Roman conquest (AD 43) did not bring about substantial changes in the site's morphology, the material culture indicates the increased presence of imported pottery, such as Samian Ware from Southern France. By the middle of the 2nd-century AD the range of imported material began to decrease, and burials were scattered across much of the site instead of being enclosed in a restricted area. While there is no evidence for occupation later than c.380, a cat tibia (Owslebury_1) recovered from a "Roman" deposit, returned a radiocarbon date of 678-770 AD at 94.5% probability (OxA-38872, 1274 ± 16). A cat metatarsal (Owslebury_2) from a "4th-century cesspit" was radiocarbon dated to 128-233 CE at 94.5% probability (OxA-38873, 1847 ± 16). A total of two cat bones (tibia and metatarsal) were screened for aDNA (Table S1).

Whitehall Farm, Northamptonshire, UK (contact: Naomi Sykes). Whitehall Farm in Nether Heyford, Northamptonshire (NGR SP659585) was excavated by the Community Landscape Archaeology Survey Project (CLASP) between 2000 and 2012. Occupation of the site began in the Late Iron Age (c.-late-1st century BCE to 1st century AD) with the construction of roundhouses within a deep ditched enclosure (~1.5 acres). In the late-1st century AD, a Roman-style timber dwelling with ancillary buildings was erected,

followed by two new stone roundhouses and the beginnings of an associated field system for a mixed farming economy in the c.2nd-3rd century AD. In the late-3rd to early-4th century AD, a 5-acre rectilinear ditched and banked enclosure containing stock management areas, a bath-house and associated halled-range between the stone roundhouses was constructed. The zooarchaeological evidence indicates a shift from mixed farming to intensive sheep-rearing. The main buildings were expanded and modified in the 4th-5th century, which is thought to mark a transition from the complex being a "tenanted" farm on a larger villa estate to an independent self-contained estate. In the 5th-6th Century AD, the main range was demolished and replaced with a timber hall, which was occupied into the Saxon period. A total of five cat bones were screened for aDNA (Table S1). Whitehall_4 phased to the 2nd-3rd century AD, a date corroborated by the radiocarbon dating of a hare from the same deposit to 176-42 AD (OxA-38817, 2096 ± 25).

Hungate, York (contact: Ellie Drew). Hungate is located in the centre of York near the river Foss (NGR SE606518). The multiperiod site was excavated by York Archaeological Trust between 2006 and 2011, revealing significant features including a Roman cemetery, half-cellared Anglo-Scandinavian buildings, substantial medieval occupation, and 19th-20th century terraced housing. Clare Rainsford's in-depth study of the animal bone from Hungate shows that in Anglo-Scandinavian contexts, cats and dogs occur consistently but infrequently, largely as scattered elements. In general, there is little evidence for the lives of cats and dogs at this period, nor anything to suggest whether these animals were feral or domestic. Most cat and dog elements appeared fully adult, which may suggest either a degree of maintenance by humans, or alternatively an environment that was not densely populated by other scavengers, and thus not highly competitive. Cats and dogs are also present over the 12th-14th centuries. These primarily represent scattered elements of adult animals, but semi-articulated remains of adult cats, kittens, and a young puppy were also present in various contexts. The occurrence of elements of younger cats and dogs may indicate a greater degree of population management by humans in the area than was the case in the Anglo-Scandinavian period. A total of 14 cat bones of various elements were screened for aDNA (Table S1). Hungate_2 (BETA - 589632) was radiocarbon dated to 978-1151 cal. CE.

Kilton Castle, UK (contact: Terry O'Connor). Kilton Castle is in the North-East corner of Yorkshire (NGR NZ701175). Built in the 12th century on a strongly-defended promontory, it changed hands between local noble families and was ruinous by the 14th century. The castle was seized by the Crown following the Pilgrimage of Grace (1536-37) and was described as totally abandoned by the late 16th century. Excavations in the 1960s included a well, from the lower levels of which a mixed assemblage of rodent and felid bones was recovered 50, apparently by sieving. Deposits were wet and apparently anoxic: bone preservation is excellent. Bones from the well were taken in hand by Jennie Coy, who passed them on to Terry O'Connor in the early 1980s, describing the context as "from the Civil War" (i.e., 1642-1651). Subsequent correspondence with the excavator, the late Arthur ApSimon, indicated that the bone material was available for further research but did not further clarify the date. Given the known history of the building a Civil War date is unlikely, and the filling of the well is more likely to derive from episodes of ruination in the 14th century. This is supported by a direct radiocarbon date of 1303-1402 CE at 95.4% (OxA-36673, 604 ± 23) on a left rat femur in the same deposit as the cat remains.[79] The cats from Kilton Castle have been previously studied, see O'Connor 2007.[2] A total of 8 cat mandibles were screened for aDNA (Table S1).

Ireland

Ballyhanna, Ireland (contact: Fiona Beglane). A single-celled church and graveyard were excavated in 2003-2004 (ITM 588022 861197, DG107-119, 03E1384) in advance of the construction of a bypass at Ballyhanna on the outskirts of the town of Ballyshannon, Co. Donegal, Ireland. These works, directed by Brian O'Donnchadha of IAC Ltd, demonstrated that the graveyard was in use from the second half of the 7th century into the first quarter of the 17th century; with approximately 86% of those individuals radiocarbon dated being laid to rest between the mid-13th and 17th centuries. All faunal remains recovered from the site were considered to be stratigraphically insecure and were analyzed as a group. There were a total of 849 faunal fragments, including two cat bones, and the sample analyzed was an adult femur which was directly dated to the post-medieval period (OxA-40490: 1656-1804 cal. CE). A total of one cat femur was screened for aDNA (Table S1).

Bective, Ireland (contact: Fiona Beglane). Bective Abbey, Co. Meath, Ireland (ITM 685903 759974, NM187, ME031-026), was excavated between 2009 and 2012 (E4028) by Geraldine Stout and Matthew Stout. This Cistercian abbey was founded in 1147 by Murchad Ua Máelsechlainn, King of Mide and was the first daughter house of Mellifont Abbey. It was suppressed in 1536 and the site then leased to Thomas Agard, before being bought by Andrew Wyse in 1552. During the sixteenth century the buildings were remodeled to provide residential accommodation. After passing through a number of hands, Bective Abbey was bought by Sir Richard Bolton in 1638 and remained in that family until it became state property in 1894. This was the largest faunal assemblage ever analyzed from an Irish monastic site, with 38,128 bone and tooth fragments recovered. Unusually for an Irish assemblage, sheep/goat (33.8% NISP) were the most frequently occurring species among the medieval samples, although the assemblage reverted to the more usual pattern of cattle being the most frequent (44.9% NISP) for the post-Dissolution period. There were a total of 218 cat elements, distributed through all phases of occupation. Many were in articulated or semi-articulated groups, reflecting the disposal of dead cat carcasses with refuse. Both adults and juveniles were present, and the metrical data suggests that the individuals were relatively small in size. The samples analyzed were both femurs from individuals aged less than 7-9 months, and both came from contexts dated to Phase 8, the sixteenth-century post-Dissolution phase. A total of fourteen cat bones of various elements were screened for aDNA, (Table S1).

Eyre Square, Galway, Ireland (contact: Fiona Beglane). Eyre Square (ITM 529972 725468) is the main square at the heart of the city of Galway, Ireland. In advance of enhancement works within the square, small-scale excavations (04E1561) in 2004 by Billy Quinn of Moore Group revealed a number of archaeological features dating from the 16th century onwards, situated at the northern end of the square. These included wall-footings of the 18th century market house, a stone-lined latrine, an ash pit and three successive metaled

surfaces. The first sample analyzed was the femur of a juvenile cat aged less than 7-9 months, which was found in a Phase 5 dump layer dating to the 18th century. The second sample was an ulna from a Phase 4 peaty deposit, dating to between the mid-17th and 18th centuries. A total of three cat bones (2 femurs, 1 ulna) were screened for aDNA (Table S1).

Navan, Ireland (contact: Fiona Beglane). In 2006, excavations (06E0274 and 06E0024) were carried out under the direction of Antoine Giacometti and Stuart Halliday of Arch-Tech Ltd at Moathill townland, Navan Co. Meath, Ireland (ITM 685817 767731), in advance of road construction. The excavations revealed an early medieval landscape in a strip of c.130m curving around the western side of what is believed to be a late-12th century Anglo-Norman motte. Activity was divided into three phases. Phase I, dating possibly from the Iron Age through to the mid-7th century yielded evidence for a burial area and a dwelling on the top of the slope, with animal bone in this area suggesting consumption waste (Site 2). During Phase II (mid-7th to early-8th centuries), a series of rectilinear ditches were constructed, which defined craft working areas at the northern extent, near the base of the hill (Site 1), with evidence for iron working and for animal butchery. Also, during Phase II, the burial area was defined within a rectangular enclosure (Site 2). During Phase III (8th to 9th centuries), at the southern extent, a new boundary ditch, new structures and two key-hole shaped cereal processing kilns were constructed (Site 3). Faunal remains were dominated by cattle (55-75% NISP). The first sample was a humerus from semi-articulated remains found in a Phase III (8th to 9th centuries) pit on Site 3. This was from a juvenile aged between 3–4 months and 1.5–2 years. The second sample was a tibia in a fill of a Phase I ditch (Iron Age to mid-7th century) on Site 2 and came from an individual aged over 1–1.5 years. A total of four cat bones from various elements were screened for aDNA (Table S1).

Ratoath, Ireland (contact: Fiona Beglane). Excavation in advance of housing development in Ratoath, Co. Meath, Ireland (ITM 701779 751796, 03E1781) was carried out by Angela Wallace of Arch-Tech Ltd. The main features of the site were an early medieval enclosure, incorporating both domestic and burial areas, and with adjacent associated field systems, however the earliest feature was a Bronze Age ring barrow. Activity continued in the Iron Age with a cremation inserted into the upper fill of a large ring ditch, and some pre-enclosure activity in the area of the enclosure. During the Anglo-Norman period activity appears to have consisted mainly of re-cutting of ditches, whilst a further ditch was added in the 15th to 17th centuries. In addition to these features, a number of corn-drying kilns and pits were recovered from various locations on the site. This relatively large assemblage was dominated by cattle bones (50-73% NISP) with sheep and pig being of secondary importance. During the early medieval period the settlement was well organized, with clearly defined areas for butchery, craftworking and waste disposal. Six cat bones were recovered from the early medieval and Anglo-Norman periods. The analyzed sample was a juvenile femur from an individual aged less than 7-9 months; this came from an isolated burnt spread and was directly dated to 602-653 cal. CE (OxA-40489). To our knowledge this (Ratoath_2) is the earliest directly dated cat bone from Ireland. A total of two cat femurs were screened for aDNA (Table S1).

Stalleen, Ireland (contact: Fiona Beglane). Stalleen townland, Donore, Co. Meath, Ireland (ITM 703318 773135, ME020-067) lies within the early Medieval kingdom of northern Brega, in a sub-kingdom controlled by the Síl nAedo Sláine and ruled from a chief residence at Knowth, Co. Meath. Placename evidence suggests that the townland was the site of an early ecclesiastical foundation and historical records show that in the later medieval period Stalleen was a monastic grange or "model farm" owned by the nearby Cistercian abbey of Mellifont. Typically, these estates changed over time from direct exploitation by lay brothers to letting of the lands to tenants. After the dissolution of the monasteries, extents from 1540 record three "messuages," or houses with land, held by Hugh Myller, Walter Bogard and Robert Conton, a fishing weir held by Hugh Dodall and a watermill in need of repair. Excavation (08E0456) in 2008 by Mandy Stephens of CRDS in advance of construction of a wastewater treatment plant revealed evidence for an early medieval ecclesiastical site and a later medieval monastic grange, including a substantial enclosure with a stone gateway. The excavation yielded a large faunal assemblage, with a total of 15,539 fragments of bone. It was dominated by cattle (48% NISP) with sheep of relatively high significance and pigs of much lower significance. Only seven cat bones were found on the site, distributed between early medieval and later medieval phases. Most notable was a mandible from an early medieval souterrain that displayed evidence of skinning. The analyzed element was the tibia of an adult cat from a later medieval phase dating to between the 11th and 13th centuries. A total of three cat bones (tibia,ulna, femur) were screened for aDNA (Table S1).

Timberyard (contact: Fiona Beglane). The site at Timberyard, Coombe Bypass, Dublin 8, Ireland (ITM 714638 733480) was excavated in 2006 by Antoine Giacometti (06E710) in advance of housing development in this inner-city area. The archaeological findings dated from pre-1700 to post-1880 and were divided into three areas: Mutton Lane, Atkinson's Alley, and the Foundry. Prior to the late 17th century, the area was used for agriculture, after which time both Mutton Lane and Atkinson's Alley were laid out for domestic development. Residential occupation continued through the 18th century although the area became poorer over time and was gradually given over to industrial uses in the form of a foundry and a blacking works. The most notable aspect of the faunal assemblage was the retained sub-sample of 42 cattle horn cores including short, medium, and long-horned cattle, but with short-horned cattle restricted to the post-1750 period. There was also evidence for hide-working waste associated with an adjacent tannery. Two cat bones were recovered from the site, both in Atkinson's Alley. The analyzed bone was a femur from an adult and dated to the post-1750 period. A total of one cat femur was screened for aDNA (Table S1).

Isle of Man

Perwick cave, Rushen, Isle of Man (contact: Allison Fox). The site is a small cave or rock shelter, which lies in a small south-facing bay near Port St Mary on the south coast of the Isle of Man. Archaeological excavations in 1969 uncovered the remains of a possible habitation site dating to 1880 +- 150 BP. Mammal bones of goat/sheep, pony, ox, pig, cat, rabbit were found, along with bones of fifteen species of bird (including the now extinct Great Auk), shellfish, and a human burial. There were traces of cooked food and cooking fires. The reasons for the remains being in the cave are unclear. The site may have been a domestic place, though there remains the possibility that the deposits are the result of ritual activity.[80]

Scotland

An Corran (contact: Alison Sheridan). An Corran, Staffin, is a rock shelter on the Isle of Skye in the Inner Hebrides, Highland Region, Scotland (NGR NG 4915 6848; Canmore ID 11349).[81] It is located on a narrow ledge on the north-east coast of the Trotternish peninsula, overlooking the sea. First recognized as an archaeological site by Martin Wildgoose in 1982, it was explored, in advance of its total destruction through blasting for roadworks, over a few days during the winter of 1993–4 by Roger Miket and Martin Wildgoose, together with volunteers. They found deposits including shell midden layers, with evidence for multi-phase human use from the Early Mesolithic period to the very recent past, with most activity occurring between the Mesolithic and the Iron Age. Eighteen radiocarbon dates, obtained between 1994 and 2009, confirmed that activities at the rock shelter occurred from as early as the 7th millennium BCE. Conditions for bone preservation in the rock shelter deposits were good, thanks to the alkaline micro-environment created by the abundant limpet and other shells. Over 7,000 vertebrate faunal bone fragments, of a wide range of species, were found in the Mesolithic levels (Bartosiewicz 2012), including three wildcat bones, the latter with an overall weight of 2.8g. The radiocarbon date of 7540 ± 27 BP (OxA-40487, 6459–6272 cal BCE at 95.4% probability) obtained for one of the wildcat bones in the current study is in line with the other earliest radiocarbon dates for the site. It is not known whether the wildcat/s had been killed and their remains used by the human inhabitants, but this is a distinct possibility. A total of one cat ulna was screened for aDNA (Table S1).

Jarlshof (contact: Alison Sheridan). Jarlshof, Shetland, is an important multi-phase (Neolithic to post-medieval) settlement site at the southern end of the largest island in the Shetland archipelago, close to Sumburgh Airport (NGR HU 39819 09551; Canmore ID 513).[82] Parts of the site have been excavated at various times between 1931 (when James Curle excavated on behalf of the Ministry of Works, 1931–35) and 2010, with the most extensive fieldwork being that undertaken by Dr James S Richardson (1936–39) and J R C Hamilton (1949–52). The cat bones sampled for the current research come from features excavated by Curle in 1935 and by Hamilton in 1949 and 1952. The sample that was radiocarbon-dated for the current project, Jarlshof_6, comes from the "Red Peat Midden" deposits associated with the Viking to late Norse phase of occupation, and the cat bone date of 1044 ± 18 BP (OxA-40488, cal CE 989–1029 at 95.4% probability) is in line with expectations for this period of occupation at the site. A total of six cat bones (4 femurs, 2 humeri) were screened for aDNA (Table S1).

Earls Bu (contacts: Ingrid Mainland and Colleen Batey). The Earl's Bu in Orphir (Canmore ID 1970 Site Number HY30SW 2; NGR HY 3346 0442 is known historically to have been the one of the main residences and estate farms of the Earls of Orkney (Crawford 2013). The Earl's Bu complex includes a Church and nearby dwellings which were the subject of antiquarian interest during the 19th and early 20th centuries. More recent excavations on an area adjacent to the Earl's Bu site have revealed further evidence for Late Norse and earlier settlement (Batey 1992, 1993). The stone-built underhouse, lade, or head-race, and tail-race of a horizontal mill, constructed in the Viking period, have been recovered along with associated bioarchaeological material. The mill was overlain by midden deposits dating to the eleventh and twelfth centuries, which are probably contemporary with the occupation of the "hall-like'" structure at the Earl's Bu, and by later middens from the thirteenth century.

The mammalian assemblage has been recorded and analyzed by Ingrid Mainland (Mainland in prep). A total of 89717 fragments were recovered of which 213 were identified as cat. The presence of butchery on limb extremities may be indicative of skinning and the use of this species for fur. A total of two cat bones (1 pelvis, 1 femur) were screened for aDNA (Table S1). Earls_2 (BETA - 589633) was radiocarbon dated to 772 - 973 cal. CE.

Howe (contact: Ingrid Mainland). Located on the West Mainland of Orkney (HY 2759 1092) (Canmore ID 1731; Site Number HY21SE 41), Howe is a large Atlantic round house, or broch, and subsequent "village" type settlement, which was excavated between 1978 and 1982 (Ballin Smith 1994). The main periods of occupation span the Scottish Iron Age (phases 3-8) and likely continue into the early phases of 'Viking' or Scandinavian occupation of the islands. The broch was built upon and incorporated within its structure, an earlier Neolithic chambered tomb (phases 1-2). A series of 25 radiocarbon dates suggested the following chronology for the Iron age occupation: Phase 3, of unknown duration, possibly 6 and 5th centuries cal. BC; Phase 4 – 5th and 4th centuries cal. BC; Phase 5 – probably 4th and 3rd centuries cal. BC; phase 6 – at least the 2nd and 1st centuries cal. BC; phase 7 – 1st to 4th centuries cal. AD; phase 8 – 4th to 7th centuries cal. AD, possibly as late as 9th century cal. AD. Catherine Smith (1994) recorded and analyzed the sizeable assemblage of animal bones. These are now archived at the Orkney Museums (Kirkwall, Orkney). A total of 144 cat remains were recovered from Howe. Two fragments are reported from Early Iron Phases (phase 4-6 and Phase 6) but the majority are recovered in phases 7 (n = 13) and 8 (129). A total of four cat bones of various elements were screened for aDNA (Table S1). Howe_1 (BETA-570176) was radiocarbon dated to 661-774 cal. CE, Howe_3 (OxA-40465) to 1053 - 1317 cal. CE and Howe_4 (OxA-40486) to 247-380 cal. CE.

Snusgar (contact Ingrid Mainland and David Griffiths). The site of Snusgar is situated in the Bay of Skaill, Sandwick, Orkney (Canmore ID 1674, Site Number HY21NW 21; NGR HY 2361 196). Excavations between 2004 and 2011 found evidence for Viking and Norse longhouses and associated middens on two large settlement mounds, the "Castle of Snusgar" and "East Mound" (Griffiths et al. 2019). Radiocarbon and OSL dates indicated a general chronology AD c950–1200 for the site as a whole, spanning eight phases 2-8), with the occupation of the better-preserved East mound longhouse focused on the early 11th century AD. The mammalian faunas were recorded by Ingrid Mainland and Vicki Ewens (Mainland et al. 2019) who reported a total of 15, 628 bone fragments in the hand collected assemblage. Wet sieving was undertaken, but only the mammal bone assemblages from the East mound longhouse floor were analyzed in detail (n = 1172). 110 cat bones were hand recovered from Snusgar with a further 2 present in the wet-seived samples from the East mound longhouse floor. Cat is represented in each of phases 2-9, with the highest concentration in phase 5 (1.98%) (11th-early12 centuries AD). Numbers in this phase are however elevated by the presence of a pair of partial cat

skeletons, which were interred under the threshold of the byre and passageway into the domestic longhouse. A total of nine cat bones of various elements were screened for aDNA (Table S1).

Turkey

Daemircihöyük (contact: Bea De Cupere). Demircihüyük is a prehistoric dwelling mound, about 25 km northwest of the town of Eskişehir (NW-Anatolia). The archaeological finds date from the Early and Middle Bronze Age. The Early Bronze Age settlement ranges from the late 4th to the 1st half of the 3rd millennium BC. Between the last phase of the Early Bronze and the Middle Bronze occupation (ca. 1900-1500 BC) the settlement remained inhabited. The mound represents an important place in the Neolithic-Early Bronze Age culture development. Excavations were carried out in 1975-1978 under the directorship of M. Korfmann. Faunal remains were studied by J. Boessneck, A. von den Driesch and H. Rauh.[83] A total of three cat bones (ulna, tibia, radius) were screened for aDNA (Table S1).

Sagalassos (contact: Bea De Cupere). The ruins of the ancient city of Sagalassos are located in southwestern Turkey near the village of Ağlasun (Burdur province), about 110 km north of Antalya. In Hellenistic times, Sagalssos was one of the major cities in Pisidia. In 25 BC, the region came under the authority of the Roman Empire and Sagalassos greatly expanded and flourished during the next three centuries. As a result of the strategic location of the town's territory (through which passed the Via Sebaste, connecting Pisidian Antiochia with harbors in Pamphylia), it developed into an economic center based on its local ceramic industry ("Sagalassos Red Slip Ware"). Large-scale excavations started in 1990 under the direction of Marc Waelkens (https://www.arts.kuleuven.be/sagalassos). Throughout the years, the animal remains were studied by Bea De Cupere and colleagues (e.g.,[84–86] A total of ten cat bones (7 humerus, 2 mandible, 1 fibula) were screened for aDNA (Table S1).

North America

Emanuel Point II shipwreck (contact: John Bratten). The Emanuel Point II shipwreck (8ES3354) is located in Pensacola Bay, Florida. Associated with the 1559 colonization attempt of Don Tristán de Luna y Arellano, Emanuel Point II was part of a 12-vessel fleet that sailed from Vera Cruz, Mexico, in 1559 to establish a colony in Florida and safeguard the northern frontier of New Spain for the Crown. In addition to 1,000 colonists, 500 cavalry and foot soldiers and 240 horses were also present. Royal financing provided livestock and agricultural and construction tools and food supplies meant to last a year. The settlers came ashore at Pensacola and soon suffered through a powerful hurricane that destroyed all but three of the ships anchored in the bay, many still loaded with supplies. The catastrophe doomed the colony, which Luna abandoned in 1561. Archaeologists discovered the well-preserved remains of the first Emanuel Point ship in 1992 and the second ship in 2006. Multiple campaigns of excavation and analysis of Emanuel Point II revealed a variety of faunal specimens including cow, pig, sheep/goat, chicken, rat, and the cat remains analyzed for this study. A total of two cat vertebrae were screened for aDNA (Table S1).

Ancient genomic data. All samples were prepared in a dedicated ancient DNA facility at the University of Oxford or at the University of Leuven. Standard ancient DNA laboratory practices were followed to minimise contamination, including the use of blanks at each stage from extraction to amplification.

Extraction (Oxford). All material analyzed at Oxford underwent the following treatment. Bone samples were cut using a Dremel 3000 electric hand-drill to between 50 and 200 mg. The surface of the bone was removed using a circular cutting disk to eliminate any surface contamination of modern DNA. The bones were powdered using a Retsch MM400 micro-dismembrator. The DNA was extracted using a protocol based on[87] with modifications from.[88]

Extraction (Leuven and Paris). DNA previously extracted[4] was used in this study. Briefly, DNA extractions and purifications were performed on aliquots of 100-300 mg of bone or tooth powder, hair tuft (BM03, 5 mg) and skin (BM02, 230 mg). Bone or tooth powder was incubated for 24-48 h at 37°C on a rotating wheel in 1.8 mL digestion buffer containing 0.5 M EDTA (Sigma Aldrich), 0.25 M Na$_2$HPO4^{3-} (Sigma Aldrich) and 1% 2-mercaptoethanol (Sigma Aldrich) at pH 8, or in 1.8 mL digestion solution of 0.5 M EDTA pH 8 (Invitrogen, Carlsbad, CA, USA) and 0.25 mg/mL proteinase K (Roche, Penzberg, Germany). Skin and hair tuft samples of Egyptian mummies from the British Museum (BM02 and BM03_II; Table S1) were incubated for 24 h at 56°C on a rotating wheel in 1.8–3.6 mL digestion solution of Tris-HCl 100 mM pH 8, NaCl 100 mM, CaCl$_2$ 3 mM, N-Lauroylsarcosine 2%, DTT 40 mM and 0.4 mg/mL proteinase K.[89]

After pelleting, DNA was purified following a protocol based on the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany), including additional washing steps with 2 mL QG binding buffer and 2 mL PE wash buffer (Qiagen). Total volumes of 8–36 mL of extract in binding buffer were passed through the silica columns on a vacuum manifold (Qiagen) using 15-25 mL tube extenders (Qiagen). Final DNA elution was done in two steps, each using 27 μL EB elution buffer (Qiagen) heated to 65°C. Each independent extraction batch contained one blank control for every five archaeological samples.

Library building and sequencing. Illumina libraries were built following either[90] or,[91] but with the addition of a six base-pair barcode added to the IS1_adapter.P5 and IS3_adapter.P5+P7 adapter pair. The libraries were then amplified on an Applied Biosystems StepOnePlus Real-Time PCR system to check that library building was successful, and to determine the minimum number of cycles to use during the indexing amplification PCR reaction. A six base-pair barcode was used during the indexing amplification reaction, resulting in each library being double-barcoded with an "internal adapter" directing adjacent to the ancient DNA strand, which would be the first bases sequenced, and a traditional external barcode that would be sequenced during Illumina barcode sequencing. The number of PCR cycles was determined by performing qPCR (Quantitative PCR) using the StepOne Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions. The amplified libraries were then pooled together in three batches and purified using the QIAGEN MinElute columns following the manufacturer's instructions. This was followed by size selection using SPRI beads (Beckman Coulter), 32 μL of DNA extract using Carøe et al.'s method.[91] SPRI beads (Beckman Coulter) were used

for purification of the libraries as instructed in the protocol. The prepared libraries were then assessed for the optimal number of cycles for PCR and quality control conducted on a TapeStation 2200 (Agilent Technologies) prior to being sent for sequencing. The first batch was sequenced on a single lane of a HiSeq 4000 instrument at the Crick Institute, London. The second and third batches were sequenced on a single lane each of a HiSeq4000 instrument at Novogene, Sacramento.

Ancient mitochondrial DNA capture. In-solution targeted capture of the *Felis* mitochondrial genome was performed on 78 samples following the myBaits v.4 (Arbor Biosciences) Hybridization Capture for Targeted Next-Generation Sequencing (NGS) protocol with the following conditions: a hybridization temperature of 60°C and a hybridization time of 48 h. The samples for capture were chosen based on the screening results. The *Felis catus* mitochondrial reference genome (NCBI: NC_001700.1) was used to synthesize the baits for capture. Between 8 and 19 ancient, indexed libraries were pooled equimolarity per reaction and the pools were decided based on the percentage of endogenous DNA with mapping quality above 30. Three reactions were pooled for the first sequencing lane, which consisted of a total of 40 libraries and then two reactions were pooled for the second lane, which consisted of a total of 38 libraries. The two lanes were sequenced on an Illumina HiSeq 4000 at Novogene, Sacramento.

Ancient, full-genome DNA deeper sequencing. Libraries with >15% endogenous DNA were selected for deeper sequencing. In total, this included eleven individuals. These libraries were sequenced in two batches, the first consisted of five indexed libraries sequenced alongside another five indexed libraries from another project, the second batch consisted of six indexed libraries sequenced alongside another ten indexed libraries from another project. Both were sequenced on one lane each of a NovaSeq 6000 at Novogene, Sacramento.

Modern genomic data
Publicly available data
We downloaded data from 24 previously published genomes (see Table S2 for accessions), including 17 domestic cats, 1 *Felis ornata*, 4 *Felis bieti*, 1 *Felis chaus* and 1 *Felis margarita* from the European Nucleotide Archive (ENA).
Unpublished modern genomes
Scottish wild and domestic (contact: Helen Senn). The Scottish cats were chosen based on their hybrid Q scores, which suggested they were "pure" domestic or pure European wildcat respectively.[10] A further 17 previously unpublished sequences were made available for this study to add to the modern comparative dataset. These samples were collected from roadkill or Scottish Wildcat Action live-trapped cats, as part of routine *in situ* conservation management, between 1997 and 2018. DNA was extracted by the WildGenes laboratory, Royal Zoological Society of Scotland, as described by.[10]

German wild cats (contact: Violeta Munoz Fuentes). DNA was extracted from tissue samples from wild caught cats from Eastern Germany (Solling, and Hainich) and Western Germany (Eifel and Pfälzerwald) using Qiagen Dneasy Tissue Kit and library building and and sequencing was conducted at Edinburgh Genomics on a HiSeq X platform.

Diverse wild cats (contact: Carlos Driscoll). Additional information about the sampling protocol for these individuals can be found in[92] (Chapter 2). A sample from FBI4 was obtained from a wildcat caught in Qinghai, Huzhu County, China. FMA8 was obtained from Woodland Park Zoo, Washington, USA. FSI204 was obtained from a wildcat caught near the Syr Daria river in Kazakhstan. FSI47, FSI48 FSI51 was obtained from Nahal Zihor in Israel. FSX360 was obtained from Portugal through Margarida Fernandez. FSX392 and FSX405 were captive Scottish wild cats. DNA extraction and library build was conducted by BioServe (9000 Virginia Manor Rd., Beltsville, MD 20705 www.bioserve.com) and sequencing was performed on Illumina HiSeq 2500.

Diverse wild cats (contact: William Murphy). Samples FLI3 and FLI4, were from wild caught in Tajikistan, and Kazakhstan respectively, both were obtained from Tallinn Zoo (Estonia). FS1 was wild caught from Azerbaijan and obtained from Rotterdam Zoo (Netherlands). DNA was extracted from skin fibroblasts using Qiagen Puregene Kit. Libraries were built using the NEB Ultra II library kit for Illumina and sequenced on an Illumina NovaSeq S4 platform.

METHOD DETAILS

Data processing
Modern data
Poor quality read data were removed from FASTQ files using Trimmomatic.[40] A sliding window approach removed bases from the 3′ end of any sequence of four positions with a mean quality score less than 20. Bases at the leading or trailing ends of forward or reverse reads with a quality score less than three were removed. Trimmed read data were aligned to the domestic cat reference genome v9.02 (accession: GCA_000181335.3) using Bowtie2[43] and the default parameters for paired-end data. Samtools[44] was used to sort and index BAM files per sample.

Read group information was added and duplicate reads were identified and flagged for each BAM file using GATK AddOrReplaceReadGroups and GATK MarkDuplicates[46] (GATK v4.0.8.1).

GATK was used to call variants per sample, using HaplotypeCaller in GVCF mode. GATK GenomicsDBImport was used to aggregate per-sample GVCFs for joint-genotyping (per chromosome) with GenotypeGVCFs.

An existing set of high-quality reference data are not available for *Felis sil vestris*, so a hard-filtering approach was taken instead of variant recalibration with GATK. Two rounds of filtering were carried out. Firstly, using GATK SelectVariants, filtering sites with low quality by read depth (QD < 2), poor mapping quality (MQ < 40,MQRankSum< -12.5, ReadPosRankSumTest< -8), or strand bias (FS > 60, SOR>3). Read depth, variant quality and SNP density were then assessed per chromosome to inform a second round

of filtering using VCFtools[93] and BCFtools, removing sites with low-quality calls (QUAL<50) or excessive read depths (DP > 2000). Multi-allelic sites and sites with a genotyping rate of less than 100% were discarded and a minor allele count of three imposed. After this, the modern panel in vcf format consisted of 20,267,231 biallelic SNPs.

Ancient data

Raw reads were filtered, allowing one mismatch to the indices used in library preparation. Adapter sequences were removed using AdapterRemoval.[94] Reads were aligned using Burrows-Wheeler Aligner (BWA aln) version 0.7.17[41] to felCat9 with the following parameters ("–l 1024,-n 0.01, -o 2").[95] FilterUniqueSAMCons[45] was then used to remove duplicates. BAM files from different sequencing lanes were merged using Samtools.[44] Molecular damage was assessed using MapDamage2.0 using default parameters[42] (Figure S1A).

After the alignment stage, we performed a pseudo-haploid calling in ancient samples at those biallelic sites previously ascertained in modern individuals (20,267,23 , see above), using the -doHaploCall utility in ANGSD (29). During this procedure, for each ancient genome and each site, we sampled a random base with a minimum base quality of 20, considering only reads with mapping quality greater than 20 and trimming the first and last five bases of each read to remove deaminated sites. For efficiency reasons we performed this procedure in parallel on multiple intervals by splitting the full list of genomic coordinates from the modern vcf into different region files, which were then passed as arguments at each ANGSD call in the job array. The full command used to perform pseudo haploidization is reported below:

```
angsd -doHaploCall 1 -doCounts 1 -minMapQ 20 -minQ 20 -minInd 1 -setMinDepth 1 -b bam_list.txt -minMinor -trim 5 -out Ancient_Cats_Haploid_''$Input_region'' -rf $Input_region
```

The ANGSD output for each region was converted to a plink file set with the haploToplink utility from ANGSD and PLINK (64) was then used to merge all intervals and create the pseudo-haploid ancient panel also consisting of 11,863,892 SNPs.

Pseudo-haploidization of modern data, SNPs panel merging, and filtering

In order to compare ancient and modern data, we firstly converted the modern vcf into a plink file set and then performed a pseudo-haploidization procedure on the modern SNP panel using a custom python script. The two panels (ancient and modern) were then merged and only biallelic sites on autosomes were retained.

To ummariz issues arising from ancient DNA damage, we excluded all transitions employing the following three step approach. We firstly listed the two segregating alleles at each site using –freq flag in PLINK, we then parse this output with a custom python script identifying sites involving transversions and finally we extracted from the panel the desired list of SNPs using the –extract flag in PLINK.

We further prune this file set for linkage disequilibrium and minor allele frequencies in PLINK using the following set of parameters: –maf 0.01 –indep-pairwise 10kb 2 0.5. The final panel (cats_all_hap_pruned) comprised 69 individuals and 2,023,923 variants with a genotyping rate of 0.87.

QUANTIFICATION AND STATISTICAL ANALYSIS

Nuclear DNA analyses

Supermatrix analyses

A supermatrix tree, containing only modern data (both domestic and wild) was constructed using SNP data based on a VCF file (excluding chromosome X and mtDNA). We selected SNPs, with MAF of at least 10% at least 20kb apart – this resulted in 116,959 SNPs. We used RaxML v8.2.4[49] to build the tree using the Lewis ascertainment correction and the rapid bootstrapping algorithm (-f a, -m ASC_GTRCAT –asc-corr = lewis) with 100 bootstrap replicates (-# 100) (Figure S1B).

Species tree

A species tree, containing only wild modern data, was constructed using SNP data based on the pre-phased VCF (excluding chromosome X and mtDNA). We sampled every 5th 10kb window across the genome (4,661 windows; excluding chromosome X) and constructed a phylogenetic tree using all SNPs in each window. Each window contained on average ∼800 SNPs. We used RaxML v8.2.4[49] to build a tree in each window using the Lewis ascertainment correction (-m ASC_GTRCAT –asc-corr = lewis). These "gene trees" were then used as input for ASTRAL-III[96] (Figure S1C).

Principal component analysis

The PCA (Principal Component Analysis) was performed using smartpca by projecting ancient data onto axes defined by modern high-coverage data.[50,97] To better ummarizing the variation in wild and domestic we excluded from the analysis the distant outgroups (*Felis Margarita* and *Felis Chaus*). We then converted this subset of the cats_all_hap_pruned panel (see above) into eigenstrat format using the utility ummari from Admixtools[31] and ran smartpca with the option LsqProject = YES.

Admixture

ADMIXTURE 1.3.0 was run using the same SNPs panel that was used for the PCA analysis.[51] Cross-validation results indicate K = 4 and K = 6 as the best models (Figures S1D and S1E).

D-statistics

D-statistics were run on the final pseudo-haploid panel of SNPs using qpDstat (1.3.0).[98] We first calculated D (outgroup, Near Eastern, Chinese, European/Asiatic) to assess admixture between wild cat species. In the case of both European and Asiatic cats we found significant results (|Z|>3) – suggesting secondary contact with both species since their TMRCA with Chinese cats. This

was also significant using an ancient Scottish Mesolithic cat (AnCorran_1) (|Z|>3), which pre-date cat domestication, indicating that at least part of this signal is not due to recent admixture with domestic cats.

We assessed whether some of this could still be due to gene flow from domestic cats using the AnCorran_1 genome as an unadmixed genome. D-statistics of the form D(outgroup, Near Eastern, AnCorran_1, Kilton_2) and D(outgroup, Near Eastern, AnCorran_1, FSX360) were not significant (Figure S2A) indicating that both Kilton_2, a 16th century cat from Kilton Castle (Northeast England), and FSX360, a modern Portuguese cat genomes also did not have recent Near Eastern/domestic cat ancestry. These were used in both f4 and qpAdm analyses as representative of the European wild cat ancestry (see below). D-statistics, however, were significant in the case of all other European wild cats in this study (Figure S2A), indicating that they likely possess some level of ancestry from domestic cats.

To test for European wild cat ancestry in modern and ancient domestic cats, we computed D-statistics of the form D (outgroup, FSX360/AnCorran_1/Kilton_2, Near Eastern, test). All domestic cats, aside from an ancient cat from Kazakhstan (Dhzankent) returned a significant positive value (Z > 3), indicating European wild cat admixture into domestic cats (Figure S2B). The Dhzankent sample, however, returned a negative value, suggesting it possessed ancestry from another source. To test if the source of this could be another species sequenced here, we computed D(outgroup, Chinese mountain cat, Near Eastern, Asian domestic cat (Figure S2C). Most, except one comparison, led to a negative significant Z value (Z < -3). The pattern was more pronounced when using Chinese mountain cats as P3 (source).

F4 ratio statistics

We estimated the proportion of silvestris ancestry in all ancient samples using qpF4ratio (1.3.0). Under the phylogenetic model displayed in Figure S3A the proportion of silvestris ancestry (alpha) is estimated by taking the ratio of two f4 statistics:

$$\alpha = f4(A, O; X, C)/f4(A, O; B, C)$$

where A = Chinese mountain cat, B = European wild cat, X = ancient sample, C= Near Eastern wild cat and O = *Felis chaus*. For each ancient sample, we estimated alpha using a single representative of each population (A,B,C,O) and repeated the procedure using different combinations of modern wild-cat individuals. Alpha values were then averaged and plotted along with the qpAdm results (Figures S3B and 4).

Using the same procedure and assuming the same phylogeny in Figure S3A, we computed the proportion of silvestris ancestry in modern domestic cats including only individual genomes that showed positive D values in Figure S2B. Results were plotted along with the ones obtained for ancient samples in Figure S3B. Finally, we estimated the proportion of domestic cat ancestry in modern European wild cats as 1-α (Figure S3A). The three individuals chosen as representative of the silvestris population, namely AnCorran_1, Kilton_2, and FSX360, were obviously excluded from the list of target individuals. Results were then plotted in Figure S3C.

qpAdm

Admixture proportions were also calculated using qpAdm (v 1.3.0). We used as input the same SNPs panel in eigenstrat format that was used for the D-statistics, PCA, and F4-ratio estimates. The program requires two additional input files, one containing a list of reference populations (referred to as "right" populations) and a second list containing the target of admixture modelling and the two admixture sources ("left" populations). Our right populations list consisted of *Felis margarita*, *Felis chaus*, Asian wild cats and Chinese mountain cats while the left population list consisted of target, European, and Near Eastern wild cats. Each ancient or modern domestic individual was specified as the target of admixture modelling in a separate qpAdm run. As for F4-ratio, we chose AnCorran_1, Kilton_2, and FSX360 as representative of the European wild cat population while we included FSI47, FSI48, and FSI51 as representative of the Near eastern wild cat population. The admixture proportions for each target were plotted along with the F4 ratio results (Fig. F4QP).

Struct-f4

We used the Rcpp package *struct-f4* to ummarizing population structure in our dataset.[32] We first converted the pseudo-haploid dataset using the perl script *Tped2Structf4.pl* provided in the repository, which generates a series of TreeMix-like files ummarizing allele counts within 10Mb-long blocks across the genome.

We then estimated f4-statistics for all possible quadruplets of individuals in our dataset by running:

calc-f4 -I TreeMix-like.filelist -n 69 -t 28 | gzip -f > cats_calcf4.gz.

Finally, we ran the MCMC optimization algorithm (Struct-f4.r) to model individual profiles as mixtures of K ancestral populations (for k = 4, … ,10).

The Rccp package along with a detailed explanation of the pipeline is available here:

https://bitbucket.org/plibradosanz/structf4/src/master/

The results of the multidimensional scaling analysis based on pairwise shared drift are broadly consistent with the PCA and the Maximum likelihood tree (see Figure S3D). The analysis at higher K further revealed some level of geographic population structure within the domestic and European wild cat clades (compare Figures S3E and S3G). In fact, European Scottish wild cats and modern domestic cats from Scotland appear different from their closest relatives, which seems to suggest that the separation between British Islands and mainland cats was present prior to domestication. This makes the individual ancestry proportions estimated at different K values harder to interpret, especially for modern domestic cats.

In the case of the ancient samples included in our dataset, the proportion of domestic cat ancestry estimated by Struct-f4 are very similar and strongly correlated with values obtained from qpAdm and F4ratio. Values obtained assuming 7 ancestral components are reported in the following table.

Ancient Sample ID	Domestic Ancestry (min)	Domestic Ancestry (max)
Dhzankent	0.97054	0.97177
Eyre_3	0.79136	0.88472
Fishbourne_2	0.90103	0.99402
Howe_4	0.98410	0.99834
Hungate_2	0.88293	0.93414
Kongens_3	0.95723	0.97454
Lincoln_1	0.91192	0.96461
Lyminge_20	0.88231	0.97090
Perwick_1	0.81393	0.81394
Sagalassos_SA17	0.95657	0.99955
Snusgar_7	0.90329	0.96608
Timberyard_1	0.83559	0.94008
AnCorran_1	0.00580	0.00581
Kilton_2	0.00006	0.07147

Mitochondrial DNA analyses
Nuclear mitochondrial DNA (numts) and mitogenome processing

Multiple studies have found numerous mtDNA insertions in the nuclear genome of felid species.[99] We tested for a possible impact of Numts on mitogenome analyses. Numts could result in wrong haplotyping of mtDNA if incorporated in majority consensus filtering, only if nuclear molecules sequenced are as or more numerous than those coming from the mitochondria. To assess this we computed, for each of our ancient samples, the number of reads of nuclear and mitochondria origin scaled by the size of their respective genome. In non-captured libraries, we found 16-5,000x more mtDNA molecules indicating that mtDNA molecules were far more numerous than those of nuclear origin in our library. This indicates that molecules of nuclear origin are unlikely to be incorporated in the mtDNA genome sequences generated through majority consensus using ancient DNA (see below).

To address whether NUMTS could also affect the analysis of modern genomes, we computed the edit distance between each read mapping to the reference and the mtDNA reference genome. Reads that are of mtDNA origin are likely to be less divergent than those of nuclear origin, potentially resulting in a bimodal distribution of edit distance. In most samples we found two modes, one between 1 and 3% divergence and one above 4-5%. Removing reads that possess >4% mismatch to the reference, as well as reads below 90bp filtered out most heterozygous calls were filtered.

Based on this results we decided to filter mitochondrial genomes using the following steps: mitochondrial genomes from both the ancient and modern datasets were extracted from BAM files complete sequence files using Samtool view (v.1.6).The consensus mitochondrial sequences were then generated using HTSbox pileup (https://github.com/lh3/htsbox) with the following strict filters to ensure the exclusion of erroneous base calls and damage from terminal ends: -l25 -T6 -q0 -Q30 -M.

Phylogenetic analyses

We used RAxML v 8.2.9 (GTR+GAMMA) to construct a ML phylogenetic tree using samples with over 2x (Figure S4A) and 5x (Figure S4B) using fast bootstrap to assess support. The trees were similar but bootstrap support was overall lower in the 2x dataset.
BEAST analysis

To avoid issues arising from NUMTS and low coverage, only 10x genomes from *F. lybica*, *F. catus* and *F. silvestris* were used in this analysis. The aligned full mtDNA sequences were partitioned using custom scripts (https://github.com/lin-at/mt_partition). The partitions were defined using the NCBI annotation (accession number: NC_001700.1) for D loop, RNAs, the 1st, 2nd, and 3rd codon sites. The alignments were then visually inspected and blocks of unresolved nucleotide sequences were removed.

Bayesian phylogenetic analyses based on the partitioned mtDNA alignments were computed using BEAST v2.6.3.[52] We used the strict molecular clock and a lognormal distribution with a mean in real space of $1.0 \cdot 10^{-8}$, upper bound of $1.0 \cdot 10^{-6}$ substitutions/site/year, and a lower bound of $1.0 \cdot 10^{-10}$ substitutions/site/year (these bounds are actually part of a separate uniform prior and are not part of the lognormal distribution itself). HKY+Γ substitution model was used with four rate categories for gamma-distributed rates across sites. An exponential prior for kappa and a lognormal prior was selected for the gamma shape prior, with default parameters. Mean date estimates for all the mtDNA sequences for the analysis were used because accounting for age uncertainty has negligible

or minimal impacts on the resulting estimates in BEAST.[100] Constant coalescent population model was selected as the tree prior. Default settings were used for all other parameters. Posterior distributions of parameters were estimated by Markov chain Monte Carlo (MCMC) sampling. Samples were drawn every 10,000 steps over a total of at least 1 billion steps. The first 15% of samples were discarded as burn-in. Sampling was considered sufficient when the effective sample size of each parameter exceeded 100. When required, additional MCMC analyses were run to achieve sufficient sampling. The trace files were assessed using Tracer[53] and samples from independent runs were merged using LogCombiner.[101] The results are presented in Figure S4C.