Ancient mitochondrial DNA connects house mice in the British Isles to trade across Europe over three millennia

**CURRENT STATUS:** POSTED

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**DOI:**

10.21203/rs.2.14061/v1

**SUBJECT AREAS**

- Evolutionary Biology
- Evolutionary Developmental Biology

**KEYWORDS**

- Ancient DNA
- phylogeography
- mitochondrial DNA
- house mouse
- *Mus musculus*
- Britain
Abstract
The earliest records in Britain for the western house mouse (Mus musculus domesticus) date from the Late Bronze Age. As a commensal species, the arrival to Britain is suggested to be related with human transport and trade. In order to study the arrival of the species in Britain, we collected a total of 16 ancient mouse mandibulae from some of the rare early sites with the species in the four British archaeological sites, ranging from the Late Bronze Age to the Roman period. From these, we obtained the first ancient mitochondrial DNA (mtDNA) house mouse sequences from the British Isles, including eight house mice from three different archaeological sites ranging from the Late Bronze Age to the Middle Iron Age. We also obtained five ancient mtDNA wood mouse (Apodemus spp.) sequences from across all four sites. Modern British house mouse mtDNAs are primarily characterised by haplogroups E, F and, much less commonly, haplogroup D. The presence of haplogroups D and E in our samples provides evidence of an early house mouse colonisation that may be related with Late Bronze Age/Iron Age trade and/or expansions of humans. Our results confirm the hypothesis, based on zooarchaeological evidence and modern mtDNA predictions, that house mice with haplogroups D and E were established in Britain by the Iron Age and, in the case of haplogroup E, possibly even by the Late Bronze Age.

Background
The western house mouse (Mus musculus domesticus) is today a widely distributed commensal species that is closely associated with human settlements. Although likely a commensal since Neolithic times in the Near East, with rare evidence for its presence in Europe during the Bronze Age [1,2], it has been argued, from zooarchaeological analyses, that it probably did not spread widely into western Mediterranean and northern Europe until the first millennium BC, during the Late Bronze Age or even the Iron Age [3]. As a non-native species, the earliest records in Britain have been thought to date from the Late Bronze Age [4–6]. As these are not from securely dated stratigraphic contexts [7], it is possible that the house mouse did not arrive in Britain until the emergence of the denser human settlements of the Iron Age [3], and then firstly into southern England [8,9], where the presence of structures to store grain, which represent an ideal niche for house mice, would have helped their
introduction [10]. In addition, identification by faunal analysis is not always straightforward. Genetic evidence can, therefore, give clearer results, but no genetic data from prehistoric house mice have been obtained before now.

Within the last 3000 years, the British Isles have experienced multiple waves of human immigration and a long history of contact, trade and exchange with continental Europe. Due to the close relationship between the two species, demographic changes in human populations are likely paralleled by similar changes in house mouse numbers; indeed, the house mouse niche has largely been shaped by humans [11]. Phylogeographic studies have shown that historical human movements impacted on current house mouse population patterns [11–15]. However, to date, the founding populations and colonisation routes have been identified based on modern phylogeography alone. Ancient DNA (aDNA) analyses of early house mice from Britain can test these conclusions and help us to understand the origins of the earliest house mouse populations in the British Isles.

Islands are interesting for the varied ways in which they can be colonised. Species can arrive by natural ‘sweepstake’ dispersal [16] or by human introduction [17]. In this study, we aim to characterise the first colonisation of the western house mouse in Britain, and the possible route that these first arrivals followed, based on ancient DNA. To this end, we analysed mitochondrial DNA (mtDNA) control-region sequences of some of the rare ancient house mouse remains from four different archaeological sites in southern England, dating from the Late Bronze Age to the Middle Iron Age and Roman period, and compared these with published modern European mtDNA control-region data. We show that two of the house mouse mtDNA haplogroups present in northern Europe today were already present in Britain by the first millennium BC.

Material And Methods

Archaeological samples

The archaeological record of small rodents is adversely affected by their small size, and sieving of sediment using a minimum mesh of 2mm is necessary to recover them [10,18]. The small size of the bones also makes them problematic for radiocarbon dating, as a single bone will rarely yield enough collagen for direct analysis. Therefore, the dating of mouse material often requires dating by context
and association with artefacts and, consequently, lacks precision.

We collected a total of 16 ancient mouse mandibulae from four British archaeological sites, ranging from the Late Bronze Age to the Roman period (see Additional File 1: Figure S1; Table S1)—Potterne, Wiltshire (n = 7); Battlesbury Bowl, Wiltshire (n = 5); North West Farm, Dorset (n = 2); and Druce Farm Roman Villa, Dorset (n = 2).

The site of Potterne, near Devizes, Wiltshire, was excavated by Wessex Archaeology between 1982 and 1984, and comprises an extensive accumulation of dark anthropogenic soil deposits, up to 2 metres deep in places, covering an area of 3.5 hectares. The ‘midden-like’ deposits were rich in artefacts and biological remains, resulting from the accumulation of manure and refuse from stock keeping, and the repeated dumping and trampling of waste from human occupation and activities on and around the site over a 500-year period. Pottery typology, and radiocarbon dating of charcoal from different levels within the deposit and other cut features, suggest a date of 1200–600 BC, encompassing the Late Bronze Age into the very Early Iron Age period [6]. In addition to a large animal bone assemblage dominated by domestic mammals, small mammal remains were recovered, mainly from sieved environmental samples, and house mouse remains were identified from every level [19]. Although it was not possible to obtain direct radiocarbon dates from the mice themselves to confirm the Late Bronze Age date, a radiocarbon date of 1,460–990 cal. BC (2σ; lab number HAR–8938) was obtained from charcoal that came from the same posthole as mouse mandible OG06 [6]. The assumption is that all seven mouse samples (OG01–OG07) are contemporaneous with the associated archaeological materials of the Late Bronze Age (c. 1200–600 cal. BC) and date from the same layers and contexts; although we note that Locker [19] does caution that some small mammal remains may have filtered down the deposit from higher levels.

The later prehistoric site of Battlesbury Bowl lies along a narrow chalk ridge immediately to the north of Battlesbury Camp, an Iron Age hillfort near Warminster, Wiltshire. Excavations, by the commercial archaeological unit Wessex Archaeology in 1999, revealed features of Late Bronze Age to Middle Iron Age dates (base on ceramic style), including ditches, post holes, and almost 200 pits [20]. The faunal assemblage is one of the largest collections of Early to Middle Iron Age faunal material from Britain.
Hambleton and Maltby [21] report the presence of both house mouse and wood mouse in both the hand-recovered assemblage and the environmental sieved samples. The mouse mandibulae included in this study came from pit fills (OG08, OG09, OG11, OG12) and a posthole (OG10), all of which were assigned Early to Middle Iron Age dates. Radiocarbon dating of a pig humerus, from the same context as mouse mandible OG11, provided a date of 420–100 cal. BC (2σ; lab number NZ-13634) [20].

The site at North West Farm is just outside the village of Winterborne Kingston to the north of Bere Regis. The site’s archaeology represents multiple phases spanning Bronze Age to Roman periods. It forms part of a programme of archaeological fieldwork, the Durotriges Project, designed to investigate native and Romano-British settlement across Dorset, focusing specifically on the archaeologically distinct Iron Age Durotriges tribe. Two mouse remains (OG13 and OG14) were recovered from a chalk deposit (340) within one of three large storage pits in Trench H of the 2017 fieldwork season, and, based on preliminary pottery attributions and the form of the pits, may date to the Bronze Age.

Druce Farm Villa, Puddletown, Dorset, comprises a series of stone and flint constructed and timber-post built buildings arranged on a courtyard plan, surrounded by a series of ditched enclosures with features associated with industrial use (e.g. kilns/ovens and pits) [22]. The site displays a number of phases of use in Romano-British times, between the 1st and 4th centuries AD. Two mouse samples (OG15 and OG16) were obtained from an extensive deposit of remains of microfauna, which lay on the intact mosaic floor of a room in the main range of buildings, sealed by a deposit of degraded plaster and roof tiles. Analysis of the site and the deposit are ongoing [22], but this appears to represent a deposit of owl pellets, most likely derived from barn owls, which accumulated when the building was going out of use, and was sealed by the collapsed roof. The mosaic floor has been typologically dated to the 4th century AD. Two water vole mandibles from the deposit were successfully subjected to radiocarbon dating (which was possible because this is a relatively large rodent that can yield enough collagen for AMS radiocarbon dating) to elucidate the date of the building collapse, and returned dates of cal. AD 249–391 and cal. AD 208–346 (both 2σ; [22]).

Morphological identification
The morphological identification of our 16 mouse mandibles was not easily resolved, as the
characters published to distinguish house mouse (Mus spp.) from Apodemus spp. [23,24] are not wholly reliable. Unlike, for example, red deer (Cervus elaphus) versus fallow deer (Dama dama)25, have not been tested against a number of individuals of each species. Furthermore, some of the specimens were either missing the M1 tooth (OG05 and OG16), where the distinguishing characters are present, or the M1 was in an advanced wear stage (OG04, OG05, OG07, OG08 and OG09).

The character we used to determine species identification was the presence of tubercles on the buccal side of the M1 in Apodemus spp., which are absent in Mus spp. [23]. Some of the specimens analysed were suspected to be Apodemus spp., but were sampled for DNA to see if the presence of tubercles could be a reliable diagnostic trait (Table 1).

Table 1. Details of the ancient murid samples analysed in this study. BA? = possible Bronze Age; LBA = Late Bronze Age; EIA = Early Iron Age; MIA = Middle Iron Age.

Extraction and amplification of ancient DNA

We undertook sample processing at the Ancient DNA Facility of the University of Huddersfield (UK) under dedicated clean-room conditions supplied by a positive air pressure system. Researchers wore full body suits, hairnets, gloves and face masks throughout the sampling, extraction and PCR set-up processes, and constantly cleaned all tools and surfaces with LookOut® DNA Erase (SIGMA Life Sciences), as well as with bleach, ethanol and long exposures to UV light.

We decontaminated the surface of the mandibulae by UV radiation for 10 minutes on each side. We shook whole or partial jaws with a zirconium oxide grinding ball inside a zirconium oxide jar in a Mixer Mill (Retsch MM400) for 15 seconds at 30Hz/s. We extracted DNA from the resulting 10–50 mg of powder produced following the protocol by Yang et al. [26], with modifications by MacHugh et al. [27]. We included blank controls throughout the sampling procedure, extraction, and PCR set-up to monitor for possible contamination.

We amplified and sequenced the mtDNA sequences in 12 overlapping 121–150 bp fragments (Table 2) covering a 915-bp fragment of the control region. We designed four primer pairs specifically for this study, took six from Jones et al. [28] with minor modifications, and designed new reverse primers for two primer sets (see Table 2 for more detail). Each primer pair amplified overlapping fragments,
including the most variable region between positions 15381 and 15663 (when compared to the reference *Mus musculus domesticus* mitochondrial genome, accession number NC_006914).

We designed three of the pairs of primers (2b, 2c and 3) to also amplify other similarly sized murids of the *Apodemus* spp. (*Table 2*). This aided identification at the genus level of those mandibulae that presented morphological identification issues, and could not be clearly attributed to the species level.

**Table 2.** Primer pairs used to amplify the ancient mtDNA control-region sequence.

**Analyses of ancient and modern house mouse data**

Phylogenetic reconstruction from *Mus* control-region data is challenging, because a large fraction of the variation is due to indels rather than base substitutions, generating even more homoplasy than is seen in modern humans. We estimated phylogenies in two different ways. We firstly aligned the eight ancient sequences (OG01, OG02, OG03, OG04, OG07, OG08, OG09 and OG10) obtained for *Mus musculus domesticus* in this study with 111 previously published house mouse sequences from Britain (*see Additional File 1: Figure S2* [15, 28-31]) to create a Bayesian inference phylogenetic tree with MrBayes [32], using the parameters previously calculated in JModelTest [33]. We ran the analysis for five million generations with four chains and with a 25% burn-in. We used FigTree v.1.3.1. in order to visualise the tree and haplogroups were assigned and named following previous nomenclature [29,34]. There is an almost exact correspondence between the main haplogroups described (1 = C1; 2 = C; 3, 5 and 9 = B; 4 = F; 7 = D; 8 = D1; 10 = A; and 11 = E) [35].

Secondly, in addition to the 111 published modern British house mouse sequences, we also selected a further 613 modern sequences from across Europe (*see Additional File 1: Table S2;* [15, 28-31, 34,36-39]). We used these data to estimate haplogroup phylogenies using the Network software, constructing separate networks for lineages from haplogroups D and E using Network v.5.0.1.1 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)). After an initial run to separate the major haplogroups (not shown), we first made the data binary where necessary (at positions with both a transition and a transversion, or a transition and a deletion), and then ran the reduced-median algorithm, followed by the median-joining algorithm on the pre-processed dataset. We included indels, as a proportion of the variation in the mouse control region comprises insertion events; however, where there were tracts of contiguous
indels (such as the 11 base pair insertion seen in some D1 individuals between positions 16089 and 16090 when compared to the reference mitogenome, NC_006914), we only counted these as a single event. We estimated the position of the root of each haplogroup network from the larger network.

Results
Sample identification
In total, we obtained mtDNA sequence data from 13 of the 16 samples, of which we identified eight individuals as *Mus musculus domesticus*, four as *Apodemus sylvaticus*, and one as *Apodemus flavicolis* (Table 2). The Bronze Age North West Farm site only yielded DNA from one individual, attributed to *A. sylvaticus*, and the Roman period Druce Farm site also yielded only *A. sylvaticus* (*n* = 2). Other *Apodemus* spp. samples were found at the Late Bronze Age/Early Iron Age site of Potterne (*A. sylvaticus; n = 1*) and the Iron Age Battlesbury site (*A. flavicolis; n = 1*). However, in both of these sites, eight *M. m. domesticus* samples were also found (five at Potterne and three at Battlesbury). These results highlight the uncertainty in the identification of murid species in the archaeological record based on mandible morphology, particularly if the M₁ is either worn or absent. We tested the reliability of the presence of tubercles on the buccal side of the M₁ being a diagnostic trait for *Apodemus* spp., as these are absent in *Mus* spp. [23]. In one case, OG03, the M₁ was present, and unworn tubercles appeared also to be present, suggesting that the specimen belonged to *Apodemus*. However, the specimen was partially obscured by sediment, and the mtDNA indicated the specimen to be *Mus* spp. (Table 2). In all other cases, the diagnostic nature of the tubercles proved reliable, and aDNA was shown to be a useful tool for species identification if wear or a lack of M₁ did not allow the trait to be used. Therefore, the tubercles on the buccal side of the M₁ may not be 100% reliable in distinguishing between *Apodemus* spp. and *Mus* spp. and certainly not if the M₁ is worn (or missing).

House mouse phylogeography in Britain
The longest fragment obtained from *M. m. domesticus* was 772 bp (OG04), from positions 15508 to 16279 of the reference house mouse mitogenome (NC_006914; Table 2). The shortest fragment (OG01) had a length of only 74 bp. All house mouse individuals analysed here date to the Late Bronze Age or the Iron Age, and they clustered within two main haplogroups (D and E; Table 3; Additional File
Figure S2), described previously in modern samples from Britain. Six individuals (from across both sites) belonged to haplogroup E and two more (from Battlesbury Bowl) clustered in haplogroup D. Along with two modern Spanish haplotypes, these lineages share some, but not all, variants with D1, and are distinct from the main D1 expansion lineages that also carry a large deletion around position 16089, which includes a single modern British individual within a cluster of mainly German and Scandinavian lineages (Figure 1; Additional File 1: Figure S2). Interestingly, haplogroup F, the most widespread cluster in Britain today, was not present in our sample set. Three samples (OG04, OG07 and OG08) belonged to the same haplotype within E (the most common haplogroup E type seen in Britain today), and the rest of the sequences were unique.

Table 3. Variable positions in control region sequences of archaeological Mus musculus domesticus samples from the British Isles between positions 15508 and 16279, compared with the reference house mouse mitogenome (NC_006914; haplogroup E), and with reference sequences from haplogroups D, D1 and F. Differences are indicated, whilst a full stop denotes identity, and an asterisk denotes a deletion. Haplogroup D1 exhibited an 11bp insertion (ins.) at position 16089i, which is a repeat of the sequence seen between 16079 and 16089 (TTTTAACTCTC). Missing sequence data from the ancient samples are denoted by question marks. Sequence codes are given in the first column. In the final column, each sample has been assigned to a mitochondrial clade by means of its relative position in the larger network, and the mutations sites compared with modern samples.

Figure 1. Phylogenetic networks for haplogroups D and E, constructed from 772 bp of house mouse control region sequences (from positions 15508 to 16279 of the reference house mouse mitogenome, NC_006914). Circles represent sequence haplotypes, the area being proportional to the frequency. Small grey points are median vectors, i.e. reconstructed intermediate nodes introduced by the network algorithm, and links between haplotypes represent mutations, with mutational positions labelled in red. Recurrent mutations are denoted by the suffixes ‘a’ and ‘b’. Circles are coloured by the location of the samples, as seen in the accompanying key. We reconstructed haplogroup D using 211 modern samples from Europe (8 British Isles; 3 Ireland; 2 France; 69 Germany; 54 Denmark and 30 Sweden; 9 Portugal and 6 Spain; 3 Bulgaria and 27 Italy), and two of the ancient house mouse
sequences (both from Battlesbury Bowl). We reconstructed haplogroup E using 116 modern samples from Europe (40 British Isles; 25 Ireland; 25 France; 21 Germany; 1 Norway; 1 Portugal; 3 Greece), and six of the ancient house mouse sequences (5 Potterne and 1 Battlesbury Bowl). R denotes the likely position of the root of each network, as determined from the full dataset.

The haplogroup networks (Figure 1) locate the ancient British samples within haplogroups D and E. All five Potterne samples are securely located within a French/British/Irish subclade within haplogroup E. Four of them directly match the root haplogroup of this subclade, found both in modern Britain and France, which is ancestral to lineages seen in Potterne, modern Britain and Ireland. The remaining Potterne, and single Battlesbury Bowl, samples fall within haplogroup D, sharing some, but not all, variants with haplogroup D1. Haplogroup D is itself rare in present-day Britain and confined to the north in current datasets [29,34].

Unfortunately, the fragments obtained for the Apodemus spp., whilst distinctive, were too short for meaningful integration into wood mouse (A. sylvaticus) or yellow-necked mouse (A. falvicolis) phylogeographies.

Discussion

If, as believed, M. m. domesticus started its spread from the Near East, associated with modern humans, during the Early Neolithic [3,40], Britain is at the periphery of the western European expansion of the subspecies. The phylogeography of the western part of the Atlantic geographical range of the house mouse has been particularly well studied [15,29,41] and, based on haplotype diversity and the presence of different clades, Britain has a relatively high genetic variation [15]. This study complements the understanding of the colonisation of the British Isles by the western house mouse by showing that two haplogroups, D and E, were present in southern Britain during the Iron Age, with E most likely arriving even earlier (Figure 2). These two clades may represent several different mouse migrations linked to different human movements.

Figure 2. Localities and mtDNA haplogroups for all samples of Mus musculus domesticus, the western house mouse, from the British Isles and northern France. Colours indicate the proportion of individuals of each control region haplogroups (red: haplogroup E; blue: haplogroup F; dark green: haplogroup D;
white: haplogroup C). Circles, squares, stars and triangles show previously published sequence data. Crosses show the localities where ancient DNA has been recovered as part of this study.

Haplogroup E is the most common *M. m. domesticus* lineage in modern southern and central British samples, and is also present in Scotland and southern Ireland (*Figure 1; Figure 2*) [15,29]. Searle *et al.* [15] suggested that the distribution of haplogroup E might reflect the colonisation of Britain from the European mainland during the Iron Age. As this clade is not well represented in central Europe, it has been suggested that it did not arrive with people overland but via a maritime route, possibly transported from the Mediterranean by the seafaring Phoenicians in the Late Bronze Age/Early Iron Age [35]. The network of E (*Figure 1*) points to a proximal source in Germany for French, British and Irish lineages, with several distinct founder subclades, most of which Britain shares with France, and a suggestion of a possible ancestry further back in Portugal and ultimately Greece. Although zooarchaeological evidence has been used to argue that there was little or no house mouse presence in western Europe until the urban developments of the Iron Age [3], our results suggest the possibility that the extant haplogroup E lineages may have reached southern Britain as early as the Late Bronze Age.

Haplogroup D is much less common in Britain today (<10%). It is distributed at low frequencies from the Levant to the central and western Mediterranean, and north into Germany, northern Britain and the Baltic. Its derived subclade, D1, co-occurs with the remainder of D in Germany, but has replaced it in Denmark and Sweden (but not Norway), where D1 reaches 100%. D1 is also seen in Madeira and the Canary Islands [34,36,41], and the colonisation of these islands has been attributed to Danish Viking movements, first to Madeira and then to the Canary Islands, following Portuguese settlement [34,41]. The latter would be similar to the colonisation route of the common vole to Orkney (known as the Orkney vole) [17]. The presence of D1 in the Faroe Islands [29] also demonstrates a more complex scenario than a simple Norwegian Viking colonisation (related with haplogroup F), and has, instead, pointed to a more southern Norwegian, or even a possible continental, origin [28]. Only a single instance of D1 has been recorded in the British Isles, from the far north, and haplogroup D overall is restricted to Scotland in current extant data.
The presence of haplogroup D in Britain in the Iron Age, and more particularly in southern England, may suggest an introduction from continental Europe—either Germany or Denmark, or possibly even Iberia, to which the lineages appear most closely linked in the network. These lineages are phylogenetically distinct from extant British D lineages, which appear more directly linked to lineages from Germany, and may be unconnected to them historically (Figure 1). They most closely match a small Spanish sub-cluster that, like the Iron Age samples, lacks the large deletion around np 16089 and might potentially be ancestral to D1. However, this phylogenetic reconstruction is necessarily very tentative given the very high level of homoplasy in the mouse control region. Haplogroup D may, therefore, have been introduced to Britain on at least three distinct occasions, all of them probably separate from the introduction of haplogroup E, and one of which may not have survived to the present day.

Three main haplogroups are represented in modern British samples (D, E and F, with F being the most frequent), while only two of these (D and E) have been found in the archaeological sites analysed here. The presence of haplogroups D and E in Britain since at least the Iron Age had previously been hypothesised from the analysis of modern samples [28,29,35], but this study provides the first direct evidence of the presence of D and E in Britain, and specifically southern England, from at least the Iron Age period. The lack of haplogroup F in our data is consistent with its later introduction, possibly associated with the Vikings [15].

Conclusion
The presence of haplogroups D and E during the Late Bronze Age and Iron Age in Britain has provided evidence of an early house mouse colonisation that may be related with first-millennium expansions of humans. This is broadly in agreement with what has been previously suggested on the basis of modern mtDNA data, although the presence of haplogroup E possibly in the Late Bronze Age would predate, by several centuries, the most widely accepted model for its expansion in Europe. The absence of haplogroup D from southern England today represents a partial turnover, similar to that recently identified in common vole during the Late Glacial/Holocene transition in eastern parts of Europe [42].
Declarations
Authors’ contribution
O.G-R, C. J.E, M. B.R, and J. R.S wrote the manuscript. C. J.E, O.G-R and E. A.H did the lab work and/or analyses of the data. E.H, J.M and C.R provided information about the zooarchaeological context and the material. J. R.S and O.G-R did the identification of the material. All authors read and approved the final manuscript.

Competing interest
The authors declare that they have no competing interests.

Availability of data and materials
All of the ancient British house mouse mtDNA sequences generated in the course of the study have been deposited in GenBank (accession numbers XXXXXX).

Acknowledgements
The authors would like to thank the Wiltshire Museum for the zooarchaeological material. The authors also would like to thank Maria Pala and Jarek Bryk for commenting on the manuscript.

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Tables

Table 1. Details of the ancient murid samples analysed in this study. BA? = possible Bronze Age; LBA = Late Bronze Age; EIA = Early Iron Age; MIA = Middle Iron Age.

| Specimen | Location         | Period      | mtDNA Species ID | Total length (bp) | Morphological ID          |
|----------|------------------|-------------|------------------|-------------------|---------------------------|
| OG01     | Potterne, Wiltshire | LBA/EIA    | Mus musculus domesticus | 74 | Mus spp. |
| OG02     | Potterne, Wiltshire | LBA/EIA    | Mus musculus domesticus | 772 | Mus spp. |
| OG03     | Potterne, Wiltshire | LBA/EIA    | Mus musculus domesticus | 576 | Apodemus spp.? |
| OG04     | Potterne, Wiltshire | LBA/EIA    | Mus musculus domesticus | 772 | Mus spp./Apodemus spp.? |
| OG05     | Potterne, Wiltshire | LBA/EIA    | no amplification products | --- | Mus spp./Apodemus spp.? |
| OG06     | Potterne, Wiltshire | LBA/EIA    | Apodemus sylvaticus | 304 | Apodemus spp. |
| OG07     | Potterne, Wiltshire | LBA/EIA    | Mus musculus domesticus | 744 | Mus spp./Apodemus spp.? |
| OG08     | Battlesbury Bowl, Wiltshire | EIA/MIA | Mus musculus domesticus | 772 | Mus spp. |
| OG09     | Battlesbury Bowl, Wiltshire | EIA/MIA | Mus musculus | 744 | Mus spp./Apodemus spp.? |
| Site Code | Site Name          | Region   | Genus    | Species | mtDNA Amplification Product Size (bp) | Taxon/Genus          |
|----------|--------------------|----------|----------|---------|---------------------------------------|----------------------|
| OG10     | Battlesbury Bowl, Wiltshire | EIA/MIA | Mus      | domesticus | 354                                   | Mus spp.?           |
|          |                    |          | musculus | domesticus |
| OG11     | Battlesbury Bowl, Wiltshire | EIA/MIA | no amplification products | --- | Mus spp.? |
| OG12     | Battlesbury Bowl, Wiltshire | EIA/MIA | Apodemus | flavicolis | 259 | Apodemus spp. |
| OG13     | North West Farm, Dorset | BA? | Apodemus | sylvaticus | 255 | Apodemus spp. |
|          |                    |          |          |          | 255 | Apodemus spp. |
| OG14     | North West Farm, Dorset | BA? | no amplification products | --- | Apodemus spp. |
| OG15     | Druce Farm, Roman Dorset, Roman | Roman | Apodemus | sylvaticus | 131 | Apodemus spp. |
|          |                    | Period  |          |          | 131 | Apodemus spp. |
| OG16     | Druce Farm, Roman Dorset, Roman | Roman | Apodemus | sylvaticus | 104 | Mus spp./Apodemus spp. |

Table 2. Primer pairs used to amplify the ancient mtDNA control-region sequence.
| Fragment name | Primer Name | Primer sequences (5'-3') | Size (bp) | Reference |
|---------------|-------------|--------------------------|-----------|-----------|
| Fragment 1    | Mm-1F       | GCACCCAAAGCTGGTATTCT     | 146       | [28]      |
|               | Mm-1R       | TTTTATGACCTGAACCATTGATT  |           | Modified from [28] |
| Fragment 2    | Mm-2F       | CCAAGCATATAAGCAAGTACAT   | 141       | [28]      |
|               | Mm-2R       | GTATGTCAGATAACACAGATAT   |           | Modified from [28] |
| Fragment 2a   | Mm-2aF      | CAATATATATACCATGAATATTTATCTTAA | 121       | This study |
|               | Mm-2aR      | AAGGGGATAGTCATATG       |           | This study |
| Fragment 2b   | Mm-2bF      | ATCTGTGTATCTGACATACACC  | 150       | This study |
|               | Mm-2bR      | TTTAAATGGGCCCAGGAGCAGAAGA |           | This study |
| Fragment 2c   | Mm-2cF      | ACTATCCCCCTCCCATTGG      | 143       | This study |
|               | Mm-2cR      | GTAAGAACCAGATGTCTGATA   |           | This study |
| Fragment 3    | Mm-3F       | TCTACCATCTCCGTGA         | 145       | Modified from [28] |
|               | Mm-3R       | TATGGGCAGATACCCCTTTGAT  |           | [28]      |
| Fragment 4    | Mm-4F       | CTTTATCAGACATCTGGTCTTT | 124       | [28]      |
|               | Mm-4R       | CACAGTTATGTGGATCTAGGG   |           | This study |
| Fragment 4b   | Mm-4bF      | CTTAAATAAGACATCTCGATGG  | 142       | This study |
|               | Mm-4bR      | TAGACTGTGTGCTGCTCCTT    |           | This study |
| Fragment 5    | Mm-5F       | CTTTCATCAACATAGCCGTCAA  | 129       | [28]      |
|               | Mm-5R       | CATTTATGCTAACAAGCATGAA |           | This study |
| Fragment 6    | Mm-6F       | CACCTACGGTGAAAGATCATT   | 146       | [28]      |
|               | Mm-6R       | TGGTTTTGGGTGGTGCATTAAA  |           | [28]      |
| Fragment 7    | Mm-7F       | CTCAAATACAAAATTAAACTCTC | 144       | [28]      |
|               | Mm-7R       | GTCATATTTGGAAACTCTAG   |           | [28]      |
| Fragment 8    | Mm-8F       | CTATCAAACCTATGCTCCTGA  | 140       | [28]      |
|               | Mm-8R       | CTTGTTAATGGTTTTAGCTGAA |           | Modified from [28] |

Due to technical limitations, Table 3 is only available as a download in the supplemental files section.

Figures
Figure 1

Phylogenetic networks for haplogroups D and E, constructed from 772 bp of house mouse control region sequences (from positions 15508 to 16279 of the reference house mouse mitogenome, NC_006914). Circles represent sequence haplotypes, the area being proportional to the frequency. Small grey points are median vectors, i.e. reconstructed intermediate nodes introduced by the network algorithm, and links between haplotypes represent mutations, with mutational positions labelled in red. Recurrent mutations are denoted by the suffixes ‘a’ and ‘b’. Circles are coloured by the location of the samples, as seen in the accompanying key. We reconstructed haplogroup D using 211 modern samples from Europe (8 British Isles; 3 Ireland; 2 France; 69 Germany; 54 Denmark and 30 Sweden; 9 Portugal and 6 Spain; 3 Bulgaria and 27 Italy), and two of the ancient house mouse sequences (both from Battlesbury Bowl). We reconstructed haplogroup E using 116 modern samples from Europe (40 British Isles; 25 Ireland; 25 France; 21 Germany; 1 Norway; 1 Portugal; 3 Greece), and six of the ancient house mouse sequences (5 Potterne and 1 Battlesbury Bowl). R denotes the likely position of the root of each network, as determined from the full dataset.
Localities and mtDNA haplogroups for all samples of Mus musculus domesticus, the western house mouse, from the British Isles and northern France. Colours indicate the proportion of individuals of each control region haplogroups (red: haplogroup E; blue: haplogroup F; dark green: haplogroup D; white: haplogroup C). Circles, squares, stars and triangles show previously published sequence data. Crosses show the localities where ancient DNA has
been recovered as part of this study.

Supplementary Files

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Table 3.jpg