The colonisation history of British water vole (*Arvicola amphibius*[L., 1758]): origins and development of the Celtic Fringe.

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Abstract

The terminal Pleistocene and early Holocene, a period from 15 to 8 KA, was critical in establishing the current Holarctic fauna, with temperate-climate species largely replacing cold-adapted ones at mid-latitudes. However, the timing and nature of this process remain unclear for many taxa, a point which impacts on current and future management strategies. Here, we use an ancient DNA dataset to test more directly postglacial histories of the water vole (*Arvicola amphibius*, formerly *A. terrestris*), a species that is both a conservation priority and a pest in different parts of its range. We specifically examine colonisation of Britain, where a complex genetic structure can be observed today. Although we focus on population history at the limits of the species' range, the inclusion of additional European samples allows insights into European postglacial colonisation events and provides a molecular perspective on water vole taxonomy.

Introduction

The period from the end of the last (Weicheselian/Devensian) glaciation c.14700BP until the mid-Holocene 8200 BP (1) was one of extensive climatic and environmental change, including the presence of two minor temperate-climate episodes (the Bølling and Allerød interstadials) and a full cycle of glacial re-advance and retreat, known as the Younger Dryas (YD), 12800-11500 BP (collectively referred to as the Lateglacial), followed by the rapid climatic amelioration and ensuing vegetation change that accompanied the start of Holocene interglacial warming. The Lateglacial was a period of rapid

vegetational change and widespread faunal extinction and translocation across Eurasia (2-3). It may be viewed as the most recent example of the dramatic climatic fluctuations associated with the Pleistocene, where over the last 2.6 million years ice sheets periodically spread down from the north, leaving Northern Europe almost fully glaciated and permafrost extending throughout Central Europe with only the southernmost peninsulae ice free (4). During the last full glacial cycle, the maximum extent of glaciation (the last glacial maximum or LGM; c.22500BP) extended across Scandinavia and the British Isles, with large parts of Europe becoming too cold for many mammal species to survive (5, 2, but see 6). Hewitt (7-9) made significant progress in the development of a model of organismal response to Holarctic climate change, proposing that in Europe temperate populations survived periods of climatic deterioration in three refugial peninsulae (Iberia, Italy and the Balkans), before recolonising northwards as glaciers retreated. While this model has been central to our understanding of the population histories of the European biota, subsequent work suggests a more complex pattern of recolonisation that can vary in relation to particular taxa, regions and time points (10). One example is the proposal by Searle and colleagues (10), who found that a general pattern for the recolonisation of Britain can be inferred from studies of multiple small mammal species. Based on a range of genetic markers, they identified pairs of population groups in five different species, and in each case the two populations form either "core" (roughly England, sometimes excluding the south coast) or "peripheral" (Scotland, Wales, and sometimes the south coast of England) populations. This pattern has been referred to as the "Celtic fringe", since it bears a strong resemblance to the cultural and linguistic distinctions that today separate

Scottish, Irish, Welsh, Manx and Cornish peoples from those in central and eastern England (10). While it has been proposed to stem from multiple colonisation events from different populations, the exact timing and nature of this process remains unclear.

The application of ancient DNA in reconstructing species history has played a significant role in identifying source populations and postglacial recolonisation events (e.g. 2, 11-12). Here, we focus on one of the 'Celtic fringe' species, the northern water vole, *Arvicola amphibius* (also referred to as *A. terrestris*), a widely distributed species found across Europe (excluding Ireland and Central and Southern Spain), east through Siberia to the Lena River Basin, and from the Arctic Sea south to Lake Baikal and North West China through North West Iran, Iraq, North Israel, the Caucasus and Turkey (13).

We identified northern water vole as the most suitable organism to explore the formation and origins of the "Celtic fringe", as it (i) exhibits a very clear spatial pattern of mitochondrial DNA differentiation across the present-day Scottish-English border (14); (ii) is the largest of the small mammal fauna with a proposed "Celtic Fringe" distribution (10), thereby increasing the volume of bone available for each analysis and (iii) both English, and especially Scottish, water vole populations are the focus of considerable conservation efforts, and an improved understanding of the origins of these populations could therefore assist in targeting resources.

Water voles are clearly sufficiently polymorphic in both ecology and morphology to present a long-standing taxonomic problem. Membership of the water vole genus has fluctuated, ranging from one all-encompassing species (*terrestris*; (15)), more commonly two (*sapidus* and *terrestris*; (16)), but also four

93 (*amphibius*, *sapidus*, *scherman* and *terrestris*; (17)) and at its peak seven
94 (*amphibius*, *illyricus*, *italicus*, *musignani*, *sapidus*, *scherman* and *terrestris*; (18)).
95 Current taxonomic determinations recognise three species: *A*. *amphibius*,
96 (Northern water vole, distributed across Eurasia), *A*. *sapidus* (Portugal, Spain and
97 France) and *A*. *scherman* (European mountains: Alps, Carpathians, Cantabrian
98 Mountains, Massif Central and Pyrenees) (13), with a caveat that authors
99 anticipate convergence towards the greater diversity recognised by Miller (18).
100 Several studies have sought to resolve the taxonomy and evolution of water vole
101 lineages through molecular analyses (14, 19). Piertney *et al.* (14) specifically
102 targeted water vole from across Britain. The resulting phylogeny identified the
103 presence of two distinct clades, one with haplotypes from England/Wales and
104 the second with haplotypes from Scotland. A geographic and genetic division of
105 this nature suggests that two colonisation events occurred in Britain. Inference
106 from the within-clade association of five representative European samples
107 highlighted that the Scottish population was derived from an Iberian population,
108 and the English/Welsh population from Eastern Europe. However, due to the
109 limitations of an exclusively modern DNA-based dataset, it was impossible to
110 discern whether the two colonisation events were separated geographically but
111 occurred at the same time, or, whether events were temporally distinct, the
112 second colonisers replacing the first, in one or other of the geographical regions.
113 The application of an ancient DNA approach therefore provides an ideal
114 mechanism by which to explore water vole colonisation of Britain. Through the
115 analysis of Pleistocene, early Holocene and additional modern water vole
116 samples, we have tested some of the proposals arising from the Celtic fringe
117 hypothesis of postglacial colonisation of Britain, namely that:

(1) There was an initial re-occupation of the mammal fauna after the LGM, in a temperate-climate period dating sometime within the interval 19000 – 12900. Within Britain, this pre-YD population is inferred to have been small and dispersed.

(2) The climatic deterioration of the Younger Dryas would have played an important role in the subsequent replacement of these lineages a process that would have taken place prior to the formation of the English Channel (and severance from continental Europe) at 8200-8000 BP (Before Present AD 1950; (20)).

(3) The replacement populations came westwards, via Doggerland, presumably from a source population located in either the Balkans or European Russia.

(4) The post-YD population would have been prone to replacement by incoming populations from Continental Europe during the Holocene, which would have expanded quickly in size due to more favourable climatic conditions. Thus the displacement of mitochondrial clades is due to drift, rather than any ecotypic advantage for life in lowland environments.

Furthermore, and although not the focus of this study, the use of cross-species samples from a range of locations across Europe provides an opportunity to include a molecular perspective on water vole taxonomy and systematics and in particular, to examine the extent to which mitochondrial DNA data are congruent with the currently proposed three species taxonomy.

Materials and methods

Sample Collection

A total of 82 water vole samples were collected from across Europe (*Figure 1* & *Table S1*). Sample choice was restricted by availability of material for destructive purposes but was designed to source material from the Late Pleistocene through to the present day. Britain and surrounding areas were of highest priority, but sampling, particularly for modern materials, extended throughout Europe to allow a wider comparison with extant European haplotypes. Modern samples were obtained from archived museum sources, collected within the last 100 years. Mandibles were used throughout, with species level identifications conducted by the source museums (*Table S1*).

DNA extraction and sequencing

All DNA extractions were conducted in a dedicated ancient DNA laboratory, physically separated from the post- PCR laboratory. Mandibles were ground into a fine powder and DNA was extracted using silica spin columns based on Yang *et al*. (21), with the inclusion of 1M urea in the extraction buffer. MtDNA was amplified using overlapping fragments spanning 643 base pairs of the control region, tRNA-Phe gene and 12S ribosomal RNA regions. Six primer pairs were designed specifically for this study (*Table S2*), each pair amplifying short (150 – 200 base pair) overlapping fragments. PCR reactions, amplicon purification and sequencing were performed as described in (2) with PCR primer specific annealing temperatures ranging from 50^{o}C to 52^{o}C. Standard ancient DNA protocols (22) were followed throughout these extraction procedures to prevent contamination, with repeated PCR amplification and sequencing of fragments to ensure DNA authenticity and the absence of miscoding lesions.

Phylogenetic analyses

DNA sequences obtained from this study were aligned with additional sequence data, 27 unique modern haplotypes from the Piertney *et al*. (14) dataset.

Phylogenetic relationships were estimated using Bayesian analysis. The DNA substitution model selected with ModelTest3.7 (23) under Akaike Information Criterion (AIC) was General Time Reversible (GTR) with proportion of invariable sites (I) set to 0.6802 and gamma distribution (G) shape parameter 0.8091. Bayesian trees were constructed and approximate posterior probabilities performed using MrBayes 3.1 (24) implementing nucleotide substitution model GTR, four chains (three heated one cold) were run for one million generations. Southern water vole (*A*. *sapidus*) (sample 208) from Portugal was employed as the outgroup in analyses.

Sequence data were partitioned into haplogroups and Southern water vole species to establish sequence divergence between haplogroups and the Southern water vole. These were calculated in Arlequin Ver. 3.11 (25), using pairwise estimates of corrected average population sequence divergence.

Radiocarbon dating

Where there was a sufficient mass of sample material, water vole mandibles extracted in this study were also sent for accelerator mass spectrometry (AMS) dating at the Oxford radiocarbon accelerator unit (16 samples). Dates were received as uncalibrated radiocarbon years BP, calibrated calendar ages were generated using Oxcal v4.1 (26) with IntCal09 calibration curve (27).

Results

DNA recovery

In total, 70 of 82 specimens (85%) successfully amplified water vole mtDNA. From the modern samples, 36 amplified DNA (97%), the 14 samples dating to the Holocene returned a 100% success rate and a total of 20 samples from the Pleistocene (65%) also amplified water vole mtDNA. Three of these samples generated insufficient coverage (< 200 base pairs) and were therefore excluded from analyses. Of the remaining 67 samples, 62 amplified the entire 643 base pair region of interest; a further five successfully amplified all but one PCR fragment (*Table S1*). To test whether the un-amplified regions contained informative data, phylogenies were generated using both the entire region of interest and with the un-amplified regions omitted. Trees produced were identical; the five partially amplified samples were therefore included in all further analyses.

Phylogenetic analyses

The Bayesian analyses (*Figure 1*) supports three clades of water voles, exhibiting division between samples from the Pleistocene and older Holocene samples. Further to clade identification, sequence data were partitioned to assess the percentage of sequence divergence between the haplogroups identified and the sister species, the Southern water vole (*Table 1*).

Discussion

Confirmation of a Celtic fringe distribution in Arvicola

A primary aim of this study was to test predictions made about the so-called "Celtic fringe" distribution of small mammal haplotypes in Britain, using ancient

DNA, with water vole as a model system. We also incorporated original, recent samples from across Britain, to enable exploration of a major (molecular) division between modern water vole in England and those in Scotland (14). The resulting phylogeny (*Figure 2*) places all modern Scottish samples as part of a single clade, identified here as clade 1, and nearly all recent English samples in an additional clade, denoted clade 2. There are two exceptions to this pattern: sample 195 (Read's Island) and 200 (Northumberland) are both from English locations but phylogenetically are placed within clade 1. The Northumberland sample is directly adjacent to the Scottish border and that close proximity likely accounts for its association with the 'Scottish' clade. Read's Island, however, is more of an anomaly. Today, an RSPB reserve situated on the Humber Estuary in East Yorkshire, it is considerably (>200 km) south of the Scottish border, this sample could therefore represent a recent translocation of individuals from Scotland to the Humber. Despite this, the overall trend of a major population division between water vole in the north and the south of Britain persists, with strong support for the nodes that define lineage separation into haplogroup clusters.

Post-LGM recolonisation of Britain

Confirmation of the genetic division observed by Piertney *et al.* (14) can be achieved through modern sampling efforts, but to better establish the timing and mode of colonisation, ancient Pleistocene and Holocene water vole samples were included in the analysis. Our AMS dating of these samples shows water vole presence in southern England immediately prior to the LGM (e.g. sample 157; median calibrated date 27955 BP see *Table S1*). While it was not possible to

242 establish a haplotype for sample 157, ancient samples that were successfully
243 haplotyped are indicated in the phylogeny (*Figure 1*) by geographic location and
244 coloured blue (Pleistocene) and pink (Holocene). The phylogenetic placement of
245 these samples is highly informative; Pleistocene samples from England share the
246 same haplogroup as those currently restricted to Scotland, clade 1. In England,
247 only Holocene samples post-dating the Younger Dryas cluster within the modern
248 English water vole clade (clade 2). This supports the two-phase colonisation
249 proposal (10), with members of clade 1 as the pre-LGM colonisers of Britain,
250 subsequently distributed throughout England. Following the end of the
251 Pleistocene, this group was displaced by a second wave of colonisers that
252 remained in England throughout the Holocene to the current day.
253 The combination of radiocarbon dating and phylogenetic inference provides a
254 clear indication that water vole colonised Britain on (at least) two separate
255 occasions, with a resulting population structure that can be attributed to
256 temporally, rather than spatially, distinct colonisation events. This
257 reconstruction also suggests a potential explanation for the anomalous sample at
258 Read's Island, as it could represent a relict population of the initial colonisers, a
259 now isolated remnant of the original colonisers prior to their displacement to
260 Scotland.
261
262 *Timing of the post-YD colonisation*
263 The timing of the second colonisation event can be inferred from the direct
264 dating of samples used in this study (*Table S1* and *Figure 1*). Only three samples
265 from Britain could be directly dated; two of these (187 and 158) were clade 1
266 individuals, with dates prior to the end of the Younger Dryas (median calibrated

dates); 14621 and 12081 BP respectively. A third sample, 159, dates to 2900 BP and is associated with the second wave of colonisers, clade 2. Three further clade 2 samples (104, 106, 107) are undated, but were excavated from a Bronze Age barrow, which would provide a maximum date boundary at 4500 BP. Thus the second colonisation event occurred after 12081 BP, possibly before 4500 BP, and definitely before 2900 years BP. These dates are thus compatible with natural colonisation (rather than human translocation) via the landbridge between England and continental Europe that is estimated to have been inundated c.8000 BP (28).

The process of replacement

The most plausible timing for the second colonisation would be before the loss of the landbridge with continental Europe, between 12 and 8 Kyr BP. This was a period of oscillating climate, spanning the end of the last glaciation, the intermediate climatic transitions of the Lateglacial and finally, the start of the Holocene interglacial. Population displacements are most commonly associated with one population outcompeting another, through some ecotypic advantage. Water voles from Scotland are generally considered smaller and typically darker in colour than those from England (29), but morphological studies have shown there to be a continuum across Britain and the variability insufficient to support species or subspecies level differences (16, 30). However, in the early 1900s, water vole in Scotland were considered a subspecies, *A*. *terrestris reta* (31) based on their darker melanic pelage. Additionally, a study by Turk (32) found Bronze Age water vole skulls from Derbyshire, England to be more akin to the Scottish *reta* subspecies than to the English subspecies, *A*. *t amphibius*. Turk postulated

292 that either the early Bronze Age population differentiated into two subspecies or
293 that the *reta* population was once common in England, but was replaced by a
294 second population (*amphibius*) after the Bronze Age. This led Van den Brink (33)
295 to allocate two species of British water vole, suggesting that the northerly
296 species had been driven back to the Scottish Highlands by the species from the
297 south. Both authors were derided for their claims; Montgomery (30) asserted
298 that the insufficient sample size of Turk (32) had led to the erroneous
299 identification of two species, and that only one species of water vole had been
300 present in central England during the past 12,000 years. However, in light of the
301 findings from this study, Turk and Van den Brink appear to have been correct
302 with regards to a displacement event due to a second colonisation, even if the
303 proposed timings were inaccurate and the two populations are not sufficiently
304 genetically diversified (Table 1) as to warrant recognition as separate species.
305
306 The evidence from this study indicates that a second wave of colonisers did
307 indeed displace the first populations of water vole in England. As there are no
308 reports of discernable ecological or physiological differences between the two,
309 an alternative supposition, also suggested for other 'Celtic fringe' species (10), is
310 that the colder climate of the Younger Dryas heralded a severe reduction in
311 water vole numbers. As climate warmed, the second colonisers arrived to a
312 region virtually devoid of water voles, resulting in complete genetic replacement
313 in the south. However, as climate warmed, remnant populations could recover
314 and repopulate, meaning that sufficient numbers were in place to avoid genetic
315 replacement.
316

317 *Sources of the two British populations and the possible existence of Cryptic*
318 *Northern Refugia for water voles*

319 Taking a broader geographical outlook, the Pleistocene/Holocene division can be
320 observed across continental Europe (*Figure 1*). Pleistocene samples from
321 Slovakia, Belgium and Germany are found in haplogroup 1, whereas Holocene
322 samples from Belgium and Germany fall within haplogroup 2. This suggests that
323 population replacement in these regions occurred after Younger Dryas. Belgium
324 is an interesting case, as the modern sample reverts to a clade one haplotype.
325 This could indicate that both haplotypes remain in the region, highlighting the
326 possibility of a Belgian suture zone. As only one modern Belgian sample
327 successfully yielded DNA, there is insufficient support to test this line of
328 investigation, but with greater sampling effort in the region, this question might
329 be resolved. Germany also warrants additional discussion, as dated Pleistocene
330 samples from the same site (Fuchsloch im Krockstein) can be found in both clade
331 1 and clade 2. Sample 99, a clade 1 haplotype, dates to immediately prior to the
332 Younger Dryas, while sample 111, a clade 2 haplotype, has a median calibrated
333 date of 11,538 BP; the very cusp of the Younger Dryas/Holocene boundary. The
334 temporal interval between these two samples could therefore represent a more
335 accurate estimate of the timing of the second colonisation event. An alternative
336 interpretation is that Germany also forms part of a suture zone with both
337 haplotypes present, a question as with Belgium that could be resolved with
338 further sampling efforts.

339

340 Although not exhaustive, the inclusion of additional modern samples illustrates a
341 clear lineage division between eastern and western Europe. This is in agreement

342 with the previous proposals (Piertney et al 2005) that major divergent lineages
343 in Europe are derived from Iberian, and eastern refugia.
344 Expansion from an eastern, rather than peninsular, refugia is in contrast to the
345 standard "Hewitt" model of post-glacial recolonisation, and provides further
346 support for the presence of refugia north of the European continental divide (34-
347 35). It also underlines the importance of examining a wide spectrum of species,
348 including small mammals, as water vole appear to exhibit an unusual pattern of
349 genetic diversity. In contrast to almost all other species so far studied (36), the
350 Pleistocene-Holocene transition did not result in a major decline in genetic
351 diversity in this species.
352
353 *Taxonomy and conservation status of European Arvicola*
354 Our results indicate clear confirmation of the species level status of the southern
355 water vole (*A. sapidus*), through the monophyly of samples and the high
356 percentage of sequence divergence between southern and northern water vole,
357 (3.66%; *Table 1*). However, on the basis of mtDNA, we find no basis for elevating
358 the montane water vole (*A. scherman*) to species level. Three modern samples
359 (213, 221, 222) identified as montane water vole failed to form a cohesive
360 monophyletic association - in fact, they were designated to different haplogroups
361 (213 and 221 (France) were clade 1, while 222 (Slovenia) was clade 2. A far
362 clearer lineage separation was apparent in samples from Italy (203 and 215) and
363 southern Switzerland (219). Together, these recent samples form a
364 monophyletic clade, clade 3, with relatively high sequence divergence with
365 respect to either clade 1 (2.42%) or clade 2 (2.49%), and with a divergence not
366 much lower than the species level difference between Northern and Southern

367 water vole (3.66%; *Table 1*). From a mitochondrial DNA perspective, the
368 geographical distribution of this haplogroup, coupled with sequence divergence
369 from other groups, provides a more convincing argument that it should be
370 considered as a separate taxonomic unit distinct from the montane water vole.
371 However, an analysis of the nuclear genome will be required to fully understand the
372 history and taxonomic status of these lineages, as well as the relationship between the
373 lineages currently found in Britain (see e.g 37).
374
375 In summary, this study highlights the benefits and increased depth of knowledge
376 that can be obtained by incorporating ancient DNA approach into studies of
377 population history. While modern data was sufficient to identify molecular
378 distinction between English and Scottish water voles, this study was able to
379 reveal a more comprehensive explanation of lineage separation observed in
380 British water vole today, through phylogenetic analysis of ancient and modern
381 DNA. We also suggest an order and timing of the colonisation of Britain, defining
382 two temporally distinct events, with a second lineage of water vole replacing the
383 first in England ca. 12 – 8 Kyr BP, leaving the initial British colonisers restricted
384 to Scotland.
385

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References

1. Walker MJC, Berkelhammer M, Bjorck S, Cwynar LC, Fisher DA, Long JA, Lowe JJ, Newnham RM, Rasmussen SO, Weiss H (2012) Formal subdivision of the Holocene Series/Epoch: a Discussion Paper by a Working Group of INTIMATE (Integration of ice-core, marine and terrestrial records) and the Subcommission on Quaternary Stratigraphy (International Commission on Stratigraphy). *Journal of Quaternary Science* **27**, 649-659.
2. Brace S, Palkopoulou E, Dalen L, Lister AM, Miller R, Otte M, Germonpre M, Blockley SPE, Stewart JR, Barnes I (2012) Serial local extinctions in a small mammal indicate Late Pleistocene ecosystem instability. *Proceedings of the National Academy of Sciences, USA* **109**, 20532-20536.
3. Stuart AJ (2015) Late Quaternary megafaunal extinctions on the continents: a short review. Geological Journal **50**, 338–363.
4. Svendsen JI, Alexanderson H, Astakhov VI*, et al.* (2004) Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23**, 1229-1271.
5. Sommer RS, Nadachowski A (2006) Glacial refugia of mammals in Europe: evidence from fossil records. Mammal Rev. 36, 251–265.
6. Stewart JR, Lister AM (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology and Evolution* **16**, 608-613.
7. Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.
8. Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**, 87-112.

9. Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.
10. Searle JB, Kotlík P, Rambau RV, Markova S, Herman JS, McDevitt AD (2009) The Celtic fringe of Britain: insights from small mammal phylogeography. *Proceedings of the Royal Society B: Biological Sciences* **276**, 4287-4294.
11. Valdiosera CE, García N, Anderung C, *et al.* (2007) Staying out in the cold: glacial refugia and mitochondrial DNA phylogeography in ancient European brown bears. *Molecular Ecology* **16**, 5140-5148.
12. Meiri M, Lister AM, Collins MJ, *et al.* (2014) Faunal record identifies Bering isthmus conditions as constraint to end-Pleistocene migration to the New World. *Proceedings of the Royal Society B: Biological Sciences* **281**.
13. Musser GG, Carleton MC (2005) Superfamily Muroidea. In: *Mammal Species of the World. A Taxonomic and Geographic Reference* (eds. Wilson DE, Reeder DM), pp. 963 - 966. Johns Hopkins University Press, Baltimore.
14. Piertney SB, Stewart WA, Lambin X, Telfer S, Aars J, Dallas JF (2005) Phylogeographic structure and postglacial evolutionary history of water voles (Arvicola terrestris) in the United Kingdom. *Molecular Ecology* **14**, 1435-1444.
15. Ellerman JR, Morrison-Scott TCS (1951) *Checklist of Palaearctic and Indian mammals 1758 to 1946* British Museum of Natural History (London).
16. Corbet GB, Cummins J, Hedges SR, Krzanowski W (1970) The taxonomic status of British Water voles, genus *Arvicola*. *Journal of Zoology* **161**, 301-316.
17. Hinton MAC (1926) *Monograph of the voles and lemmings (Microtinae) living and extinct.* British Museum of Natural History (London).
18. Miller GS (1912) *Catalogue of the mammals of western Europe (Europe exclusive of Russia) in the collection of the British Museum* British Museum of Natural History (London).
19. Taberlet P, Fumagalli L, Wust-Saucy AG, Jean-François. C (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7**, 453-464.

20. Shennan I, Horton B (2002) Holocene land- and sea-level changes in Great Britain. *Journal of Quaternary Science* **17**, 511-526.
21. Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* **105**, 539-543.
22. Gilbert MTP, Bandelt H-Jr, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541-544.
23. Posada D, Crandall K (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.
24. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.
25. Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Libertas Academica.
26. Ramsey BC (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337-360.
27. Reimer PJ, Baillie MGL, Bard E*, et al.* (2009) INTCAL09 AND MARINE09 radiocarbon age calibration curves, 0-50,000 years cal BP. *Radiocarbon* **51**, 1111-1150.
28. Weninger B, Schulting R, Bradtmöller M*, et al.* (2008) The catastrophic final flooding of Doggerland by the Storegga Slide tsunami. *Documenta Praehistorica* **35**, 1-24.
29. Woodroffe GL, Lambin X, Strachan R (2008) Genus Arvicola. In: *Rodents Order Rodentia* (compiled by Gurnell, J. Hare, EJ) In: *Mammals of the British Isles: Handbook, 4th Edition* (eds. Harris S, Yalden DW), pp. 110 - 117. The Mammal Society, Southampton, England.
30. Montgomery WI (1975) On the relationship between sub-fossil and recent British Water voles. *Mammal Review* **5**, 23-29.
31. Miller GS (1910) Brief synopsis of the Water rats of Europe. *Proceedings of the Biological Society of Washington* **23**, 19 - 22.
32. Turk FA (1964) On some Bronze Age remains of the water-rat (*Arvicola terrestris amphibius* L). *Proceedings of the Zoological Society of London* **143**, 345-350.

33. Van den Brink FH (1967) A Field Guide to the Mammals of Britain and Europe, pp. 98 - 100. Collins, London.
34. Bilton DT, Mirol PM, Mascheretti S, Fredga K, Zima J, Searle JB (1998) Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. Proceedings of the Royal Society of London B: Biological Sciences **265** (1402), 1219-1266.
35. Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277**, 661-671.
36. Hofreiter M, Barnes I (2010) Diversity lost: are all Holarctic large mammal species just relict populations? *BMC Biology* **8**, 46.
37. Kotlík P, Marková S, Vojtek L, Stratil A, Šlechta V, Hyršl P, Searle JB (2014) Adaptive phylogeography: functional divergence between haemoglobins derived from different glacial refugia in the bank vole. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20140021.
38.

Data Accessibility

DNA sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/), with accession numbers XXX. Additional supporting information may be found in the online version of this article.

Author Contributions

IB, SB and JS designed the project. DS, JS, RM and MR provided samples and contextual information. SB performed the research. SB and IB analysed the data and wrote the manuscript, with contributions from the other authors.

Tables and Figures

Table 1 Population average pairwise estimates of sequence divergence with Kimura-2 Parameter between clades 1-3 as defined by the phylogeny (Figure 2) and the Southern water vole (*A. sapidus*).

		Clade			All
		1	2	3	*A. amphibius*
Clade	1				
	2	2.12			
	3	2.42	2.49		
All	*A. sapidus*	3.9	4.56	3.3	3.66

Figure 1 Sampling locations for water vole used in this study. British numbered locations key: England: 1=Somerset (187); 2=Surrey; 3=Hertfordshire; 4=Cambridgeshire; 5=Norfolk; 6=Shropshire; 7=Staffordshire; 8=Derbyshire (104,106,107); 9=Lincolnshire Read's Island (195); 10=Yorkshire; 11=Northumberland (200). Wales: 12=Gwent (158,159). Scotland: 13=Fife; 14=Morayshire. European sampling locations are denoted by country name. Bracketed numbers refer to the individual sampling numbers of water vole that are directly referred to in the text.

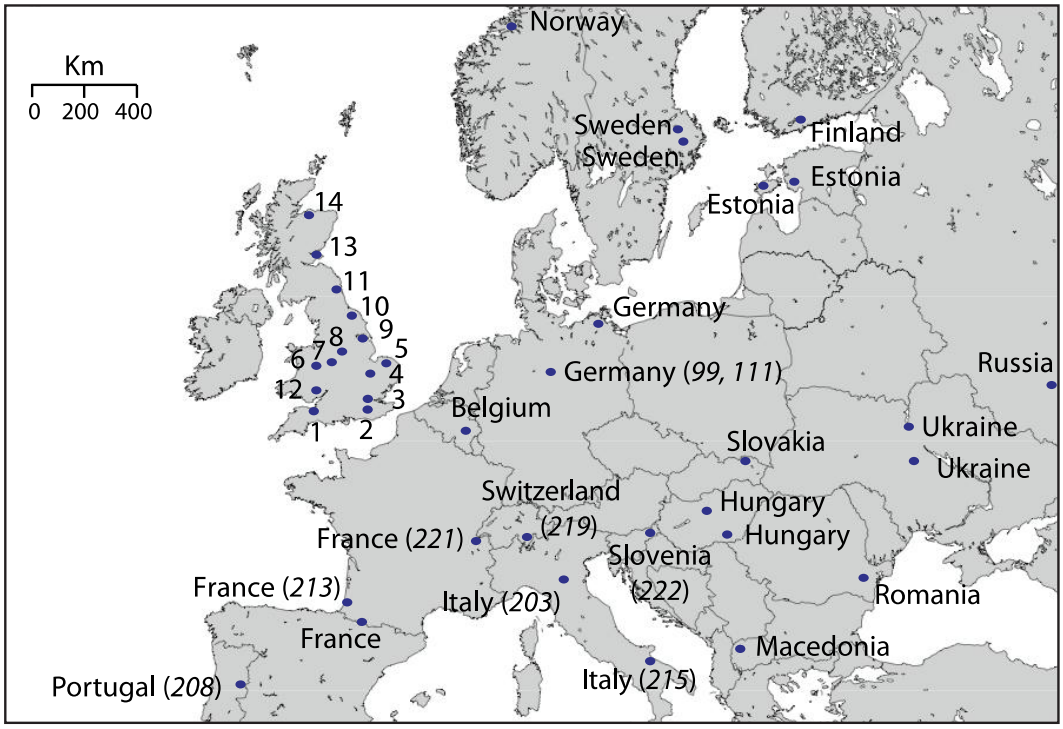

Figure 2 Phylogenetic relationships of water vole samples: Bayesian tree constructed in MrBayes. Nodal support is shown through approximate Bayesian probabilities (only values above 50% shown). AMS dates are given as the median calibrated year BP. Southern water vole used as the outgroup (not shown). Nomenclature: Sample location written entirely in uppercase = data taken from Piertney and colleagues (14); all other data are from this study and 1st number = unique sample identifier, followed by sample location and time period, where blue = Pleistocene (> 10 Kyr BP, oldest dated sample 27 Kyr BP), pink = archaeological Holocene (10 Kyr BP to 250 yr BP), black = recent/museum samples (250 yr to present). Green star denotes that the sample is directly referred to in the text.