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How did pygmy shrews colonize Ireland? Clues from a phylogenetic analysis of mitochondrial cytochrome *b* sequences

Silvia Mascheretti[1]†, Margarita B. Rogatcheva[1]¶, İslam Gündüz[2], Karl Fredga[3] and Jeremy B. Searle[1]*

[1]*Department of Biology (area 2), University of York, PO Box 373, York YO10 5YW, UK*
[2]*Department of Biology, Faculty of Arts and Sciences, University of Balikesir, Balikesir, Turkey*
[3]*Department of Conservation Biology and Genetics, University of Uppsala, Norbyvägen 18D, SE-752 36 Uppsala, Sweden*

There is a long-standing debate as to how Ireland attained its present fauna; we help to inform this debate with a molecular study of one species. A 1110 base pair fragment of the mitochondrial cytochrome *b* gene was sequenced in 74 specimens of the pygmy shrew, *Sorex minutus*, collected from throughout its western Palaearctic range. Phylogenetic analysis of these sequences revealed several well-supported lineages. Most of the 65 haplotypes belonged to a northern lineage, which ranged from Britain in the west to Lake Baikal in the east. The other lineages were largely limited to Iberia, Italy and the Balkans. One exception, however, was a lineage found in both Ireland and Andorra. This affinity, and the large difference between the mitochondrial sequences of Irish and British individuals, suggest that pygmy shrews did not colonize Ireland via a land connection from Britain, as has been previously supposed, but instead were introduced by boat from southwest continental Europe. All the Irish pygmy shrews analysed were identical or very similar in cytochrome *b* sequence, suggesting an extreme founding event.

Keywords: colonization; Ireland; mtDNA; cytochrome *b*; pygmy shrew; *Sorex minutus*

1. INTRODUCTION

There is considerable uncertainty about when and how Ireland attained its current terrestrial fauna. The first uncertainty is whether Ireland acquired these species before or after the last glacial maximum (20 000 years ago). At that time the north European ice sheet covered almost the whole of the present landmass of Ireland. A small unglaciated area in southwest Ireland may have been a glacial refugium for temperate species (e.g. Stewart & Lister 2001), although there is no direct evidence for this. In fact, most workers believe that conditions so close to an ice sheet would have been too harsh for temperate species to survive (e.g. Yalden 1999), and that these organisms must have colonized Ireland after the ice sheet retreated. It is also uncertain whether any land connections linked Ireland to a British source of temperate terrestrial species at the end of the last glaciation (Stuart 1995). Yalden (1982) and Devoy (1985) suggested the possibility of a single short-lived land-bridge between southwest Scotland and the north of Ireland, and Wingfield (1995) proposed a moving temporary land-bridge that first joined southeast Ireland and southwest England and ultimately joined eastern Ireland and northern England. As an alternative to overland colonization, Ireland could have acquired its current fauna by a combination of active dispersal using airborne or aquatic routes and passive dispersal mediated by human introductions. Certain species are shared by and limited to Ireland, western France and Iberia (e.g. the Kerry slug, *Geomalacus maculosus*), and Corbet (1961) argued that this 'Lusitanian element' is best explained by accidental human transport. The cultural links between these Lusitanian areas extend back to the Mesolithic and Neolithic, as indicated by the genetic resemblance of the people of these areas, the similarity of the megalithic monuments and the sharing of trade items (Corbet 1961; Kinnes 1984; Yalden 1999; Hill *et al.* 2000; Sheridan 2003).

Species-distribution data have low power to distinguish between these various hypotheses for the colonization of Ireland by temperate fauna. It is more powerful to use a species-by-species approach involving genetic comparisons of populations of the same species in Ireland, Britain and elsewhere in Europe to infer the source of colonization of Ireland. Molecular data of this type (Davison *et al.* 2001) indicate that the pine marten (*Martes martes*) has a Lusitanian distribution in terms of genetic composition. Davison *et al.* (2001) suggest that the pine marten was introduced to Ireland because of its fur, but the striking finding is that it appears to have been introduced from populations in southwest continental Europe and not from Britain. Although Davison *et al.* (2001) do not discuss this aspect of their results, their data provide support for Corbet's (1961) contention that trade links with southwest continental Europe had a role in the colonization of Ireland by temperate species.

Another of the mammals currently found in Ireland is the pygmy shrew (*Sorex minutus*). This species is known to have been accidentally introduced onto various islands around Britain (e.g. the Outer Hebrides and Orkney;

*Author for correspondence (jbs3@york.ac.uk).
† Present address: 1st Department of Medicine, Christian-Albrechts University, Schittenhelmstrasse 12, 24105 Kiel, Germany.
¶ Present address: Department of Animal Sciences, 329 Edward Madigan Research Laboratory, 1201 West Gregory Drive, University of Illinois, Urbana, IL 61801, USA.

Yalden 1982), so this is one way that it may have colonized Ireland, perhaps from a distant source (as suggested for the pine marten). It is also a species that might have been able to colonize overland from Britain; Yalden (1981, 1982, 1999) has studied the habitat requirements and behaviour of this species and concluded that it would have been able to travel over a low-lying marshy land-bridge of the sort that may temporarily have linked Britain and Ireland.

To examine whether the pygmy shrew colonized Ireland by human introduction (potentially from a distant locality) or over a land-bridge (from Britain), we conducted a genetic study of the species, comparing specimens from Ireland, Britain and elsewhere in its western Palaearctic range. Mitochondrial DNA (mtDNA) sequences are particularly valuable for such studies (Avise 2000), and in our analysis we examined variation in the mitochondrial cytochrome *b* gene. This study is based on 74 pygmy shrews from 46 localities and represents, to our knowledge, the first large-scale phylogenetic analysis of this species. The only previous study of this type (Bilton *et al.* 1998) was based on a shorter length of sequence from only 14 specimens collected in 11 localities that did not include Ireland.

2. MATERIAL AND METHODS

The collection localities of the pygmy shrews used in this study are given in table 1 and figure 1. Specimens were generally supplied as tissue samples (usually tail tips) in 100% ethanol. *Sorex volnuchini*, one of the closest relatives of the pygmy shrew (Zima *et al.* 1998), was used as the outgroup.

Total DNA was extracted using a standard phenol–chloroform method (Sambrook *et al.* 1989). Three partially overlapping PCRs were used to amplify the complete cytochrome *b* gene. The primer pairs used were: L14724/H15149, L15057 (5′-CGGACGAGGTCTCTACTA-3′)/H15548(5′-AGAAAG TACCATTCTGGTTAAA-3′), and L15408 (5′-GCAGA TAAAATTCCTTTCCA-3′)/H15915. All primers are named according to Anderson *et al.* (1981). L14724, H15149 and H15915 were derived from Irwin *et al.* (1991), L15057 and H15548 were new and L15408 was modified to be specific for pygmy shrews. PCR amplifications were performed in a Primus 96 plus thermal cycler (MWG Biotech), and the programme consisted of 30 cycles of 1 min denaturation at 93 °C, 1 min annealing at 50 °C and 2 min extension at 72 °C. PCR products were purified with the Nucleon for PCR/Oligo clean up kit (Scotlab Bioscience) and directly sequenced using an Applied Biosystems 377a automated sequencer. Each PCR product was sequenced using the light-strand primer, with approximately half of the individuals also sequenced with the heavy-strand primer to confirm the results.

The sequences were aligned and edited using AutoAssembler or the Seqman II module of the Lasergene 99 program (Applied Biosystems). Nucleotide and amino acid compositions were assessed using MacClade (Maddison & Maddison 2000). The overall consistency of the sequence data (comparison of heavy- and light-strand sequences and overlapping PCR products), the absence of multiple bands in the PCR reactions, the amino acid composition and the overall pattern of nucleotide variation indicated that our data reflected true mitochondrial sequences and not nuclear pseudogenes, which have been found in some other small mammals (Mirol *et al.* 2000). The sequences were deposited in the EMBL database (accession numbers AJ535393–AJ535458).

For the phylogenetic analysis both distance and maximum-parsimony trees were constructed using PAUP* (Swofford 1998). Maximum-parsimony analyses were conducted using a heuristic search algorithm, 100 random addition replicates, tree bisection–reconnection swapping and the steepest descent option. A range of transition–transversion ratios were used. The number of trees was constrained to 30 000.

Distance trees were constructed by the neighbour-joining method (Saitou & Nei 1987). The most appropriate DNA substitution models for our dataset were established using Modeltest (Posada & Crandall 1998). For the data including the outgroup, the hierarchical likelihood ratio tests and the Akaike information criterion estimates (Akaike 1974) both supported the Tamura–Nei model (Tamura & Nei 1993) with the frequency of invariable sites set at 0.6838 and a gamma correction of 1.0812. For the data without the outgroup, the HKY85 model (Hasegawa *et al.* 1985) with a gamma correction of 1.1404 and the General Time Reversible model (Rodríguez *et al.* 1990) with the frequency of invariable sites set at 0.5812 and a gamma correction of 1.1453 were selected by the hierarchical likelihood ratio tests and the Akaike information criterion estimates, respectively. We also applied the most widely used substitution model for generating neighbour-joining trees, the Kimura two-parameter model (Kimura 1980), with a variety of transition–transversion ratios, on the grounds that complex models do not necessarily produce better results for phylogenetic inference than simple models (Nei & Kumar 2000).

When generating distance and maximum-parsimony trees, bootstrapping (10 000 pseudoreplicates) was used to assess the support of tree nodes.

Both for the total dataset and for specific lineages identified in the phylogenetic analysis, nucleotide diversities were calculated according to Nei (1987) using the Proseq program (Filatov 2000).

3. RESULTS

(a) *General sequence characteristics*

A near-complete sequence (1110 base pairs (bp)) of the mitochondrial cytochrome *b* gene, from positions 14 777 to 15 886, was obtained for all 74 pygmy shrews analysed, and 65 haplotypes were identified. The overall level of nucleotide diversity (± s.d.) was 1.5% ± 0.7% and out of the 175 variable positions, 96 were informative for parsimony analysis. Twenty-nine variable positions occurred at the first codon position, eight occurred at the second and 138 occurred at the third; the transition–transversion ratio was 5.5. Third-codon position transitions represented 67% of substitutions. Altogether 18% of the changes were non-synonymous and 32 out of the 369 amino acids coded by the 1110 bp fragment of cytochrome *b* were variable. Most of these variable amino acids were located in the transmembrane domains as defined by Irwin *et al.* (1991). No indels were detected.

(b) *Phylogenetic analysis*

All the phylogenetic trees that we produced contained the same set of lineages with bootstrap support of 60% or more. For illustration, figure 2 shows a neighbour-joining tree (Kimura two-parameter model) based on the observed transition–transversion ratio. Using the same

Table 1. Collection sites of 74 pygmy shrews and one *Sorex volnuchini* used in this study and the cytochrome *b* haplotypes found at these sites. The haplotypes are labelled by country/geographical region/island of origin. The sites are mapped in figure 1.

locality	coordinates	map reference	*n*	cytochrome *b* haplotype
Rascafría, Spain	40°54′ N, 03°53′ W	1	3	Spain 1,2,3
Encamp, Andorra	42°35′ N, 01°35′ E	2	2	Andorra 1,2
Morlaix, France	48°35′ N, 03°50′ W	3	2	France
East Hendred, England	51°34′ N, 01°20′ W	4	1	England 1
Grittenham, England	51°33′ N, 01°58′ W	5	1	England 2
Macclesfield, England	53°15′ N, 02°02′ W	6	1	England 3
East Cottingwith, England	53°52′ N, 00°55′ W	7	1	England 4
Longnor, England	53°10′ N, 01°53′ W	8	1	England 5
Lyn Conwy, Wales	53°17′ N, 03°50′ W	9	2	Wales 1,2
Isle of Man, UK	54°09′ N, 04°29′ W	10	2	Man (UK) 1,2
Gask, Scotland	56°21′ N, 03°40′ W	11	2	Scotland 1,2
Slane, Eire	53°45′ N, 06°30′ W	12	2	Eire 1
Cloghan, Eire	53°15′ N, 07°45′ W	13	2	Eire 1,2
Camolin, Eire	52°35′ N, 06°25′ W	14	2	Eire 1,3
Castlebridge, Eire	52°25′ N, 06°30′ W	15	2	Eire 1,4
Whiting Bay, Eire	51°50′ N, 07°50′ W	16	4	Eire 5,6,7[a]
Islandmagee, N. Ireland	54°50′ N, 05°40′ W	17	2	Eire 1
Boxtel, Netherlands	51°36′ N, 05°20′ E	18	1	Netherlands 1
Wageningen, Netherlands	51°58′ N, 05°40′ E	19	1	Netherlands 2
Hartz Mountains, Germany	51°45′ N, 10°40′ E	20	2	Germany
Bassin, Switzerland	46°32′ N, 06°39′ E	21	1	Switzerland
Abruzzo, Italy	42°00′ N, 14°00′ E	22	2	Italy 1,2
Trento, Italy	46°15′ N, 11°50′ E	23	2	Italy 3,4
Cesky Jiretin, Czech Republic	50°41′ N, 13°34′ E	24	1	Czech Republic
Amager, Denmark	55°35′ N, 12°35′ E	25	1	Amager (Dk)
Langeland, Denmark	54°57′ N, 10°43′ E	26	1	Langeland (Dk)
Nyborg, Denmark	55°19′ N, 10°48′ E	27	1	Fyn (Dk)
Bornholm, Denmark	55°02′ N, 15°00′ E	28	2	Bornholm (Dk) 1,2
Revinge, Sweden	55°35′ N, 14°20′ E	29	2	Sweden 1,2
Jämjö, Sweden	56°10′ N, 15°52′ E	30	2	Sweden 3,4
Öland, Sweden	57°16′ N, 17°05′ E	31	2	Öland (Se) 1,2
Västergarn, Sweden	57°28′ N, 18°10′ E	32	2	Gotland (Se) 1,2
Tingstäde, Sweden	57°42′ N, 18°36′ E	33	1	Gotland (Se) 3
Uppsala, Sweden	59°45′ N, 17°40′ E	34	1	Sweden 5
Saortu, Finland	61°44′ N, 27°15′ E	35	1	Finland 1
Lammi, Finland	61°04′ N, 25°07′ E	36	1	Finland 2
Vilnius, Lithuania	54°40′ N, 25°19′ E	37	2	Lithuania 1,2
Blizocin, Poland	51°36′ N, 22°16′ E	38	1	Poland
Cherkassy, Ukraine	49°43′ N, 31°30′ E	39	1	Ukraine
Pelister Mountain, Macedonia	41°00′ N, 21°10′ E	40	1	Macedonia
Strandzha Mountains, Turkey	41°45′ N, 27°41′ E	41	2	Turkey 1,2
Pertozero, Russia	62°05′ N, 34°00′ E	42	1	Russia 1
Brjansk, Russia	52°20′ N, 34°00′ E	43	2	Russia 2,3
Tambov, Russia	51°55′ N, 42°15′ E	44	2	Russia 4,5
Novosibirsk, Russia	54°49′ N, 83°06′ E	[b]	2	Siberia 1,2
Lake Baikal, Russia	53°40′ N, 108°00′ E	[b]	1	Siberia 3
Artvin, Turkey	41°12′ N, 41°48′ E	[b]	1	*Sorex volnuchini*

[a] Two individuals had the Eire 7 haplotype.
[b] Not on the map.

ratio, parsimony analysis resulted in 30 000 minimal trees of 225 steps and a consistency index of 0.60.

In our phylogenetic trees most haplotypes from northern and central Europe and Siberia occurred in a 'northern' lineage with moderate-to-high bootstrap support (77% for the tree in figure 2). Three further well-supported lineages were identified: the 'Turkish–Macedonian' lineage groups the three haplotypes found in the Balkans; the 'Italian' lineage groups three of the four haplotypes found in Italy—the fourth haplotype from northern Italy is part of the northern lineage; and the 'Andorran–Irish' lineage groups nine haplotypes found in the Pyrenees and Ireland. Finally, three haplotypes from Spain group together in the tree, but without strong bootstrap support. The geographical distributions of these groupings are shown in figure 1.

(c) *Within-lineage variation*

The most widely distributed and best represented of the cytochrome *b* lineages is the northern, which has a range from Britain in the west to Lake Baikal in the east. Although there is some evidence for grouping of haplo-

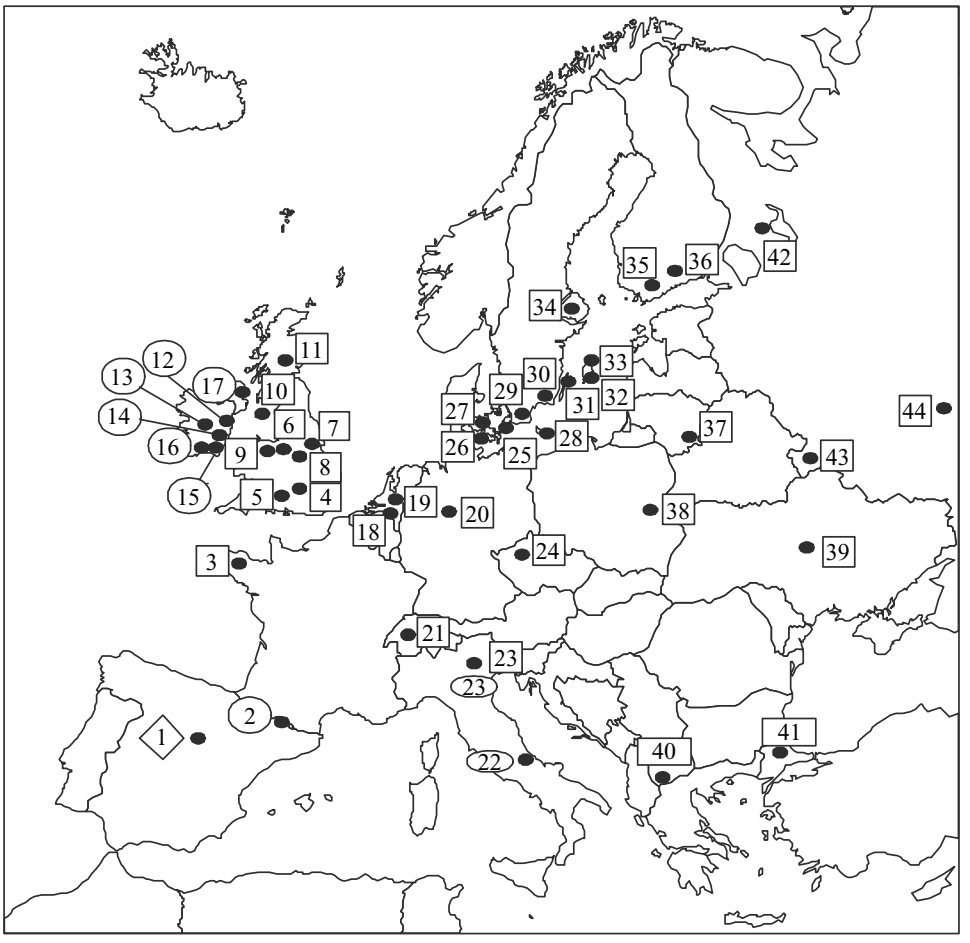

Figure 1. Map of European collection sites of the pygmy shrews used in this study (see table 1 for further details). The species is distributed throughout Europe (except southern Iberia, Iceland and the Mediterranean islands) and into Siberia (as far east as Lake Baikal). The symbols indicate different cytochrome *b* lineages: 'northern' (squares), 'Andorran–Irish' (circles), 'Italian' (ellipses) and 'Turkish–Macedonian' (rectangles). The grouping of Spanish haplotypes is also indicated (diamond).

types from particular geographical areas within the northern lineage, there are counter examples and generally a lack of bootstrap support for such within-lineage structure (figure 2). The nucleotide diversity (± s.d.) for the northern lineage was 0.70% ± 0.39%.

Most of the pygmy shrews from islands in northern Europe (Britain and the Isle of Man, the Danish islands and the Swedish islands of Gotland and Öland) had haplotypes belonging to the northern lineage (figures 1 and 2). All the haplotypes from Gotland cluster with one of the Öland haplotypes; this is the only interesting and reasonably well-supported clade within the northern lineage. It is notable because the populations of pygmy shrews on these islands are uniquely characterized by Robertsonian fusions and reduced chromosome numbers relative to the standard $2n = 42$, with one fusion shared between the Gotland and Öland populations (K. Fredga, unpublished data, presented as a poster at the Kew Chromosome Conference in 1987 (Zima *et al.* 1998)).

The lineage of particular interest is the Andorran–Irish. Figure 3 shows an unrooted minimum-parsimony network constructed by hand from the single most-parsimonious tree for the haplotypes within this lineage. From our data, the most widespread and common haplotype in Ireland is Eire 1, occurring in five out of the six localities and seven out of the 14 individuals typed (table 1; figure 1). This haplotype is also basal to the other Irish haplotypes defined, with Eire 2–7 each differing from Eire 1 by one or two nucleotides, generating a star phylogeny (figure 3). The nucleotide variation in Ireland is much lower than elsewhere in the range of the pygmy shrew. For example, the nucleotide diversity (± s.d.) in Ireland is 0.11% ± 0.08% (14 individuals, six localities) and on the British mainland it is 0.63% ± 0.37% (nine individuals, seven localities).

4. DISCUSSION

The primary focus of this paper is on the colonization of Ireland by pygmy shrews. However, the mtDNA haplotypes in Ireland need to be considered in the context of the variation throughout the species range. We found that the pygmy shrews in northern Europe have a different mtDNA lineage from those in southern Europe, and that the northern European lineage is very widespread. Despite the presence of pygmy shrews in Iberia, Italy and the Balkans (the classic refugial areas for temperate species during the last glacial maximum), our study implies that pygmy shrews colonized northern Europe and Siberia from a refugium in central or eastern Europe (Bilton *et al.* 1998). However, further sampling in southern Europe is needed to rule out completely Mediterranean glacial refugia as source areas for the northern lineage.

Most of the island populations of pygmy shrews

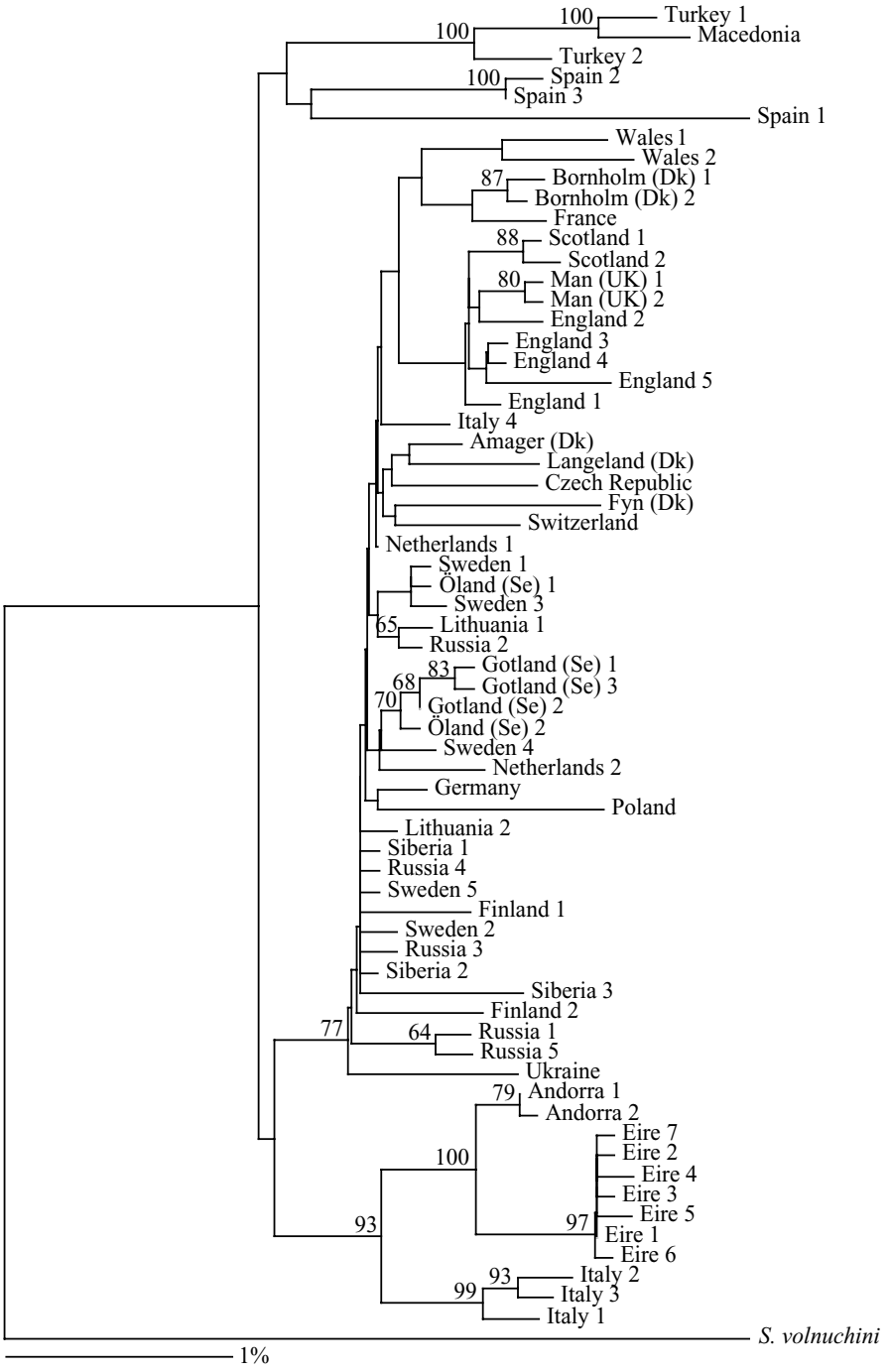

Figure 2. Phylogenetic tree based on all the cytochrome *b* haplotypes of the pygmy shrews used in this study (see table 1 and figure 1 for further details of geographical locations). The tree was generated by the neighbour-joining method applying the Kimura two-parameter substitution model (Kimura 1980) with a transition–transversion ratio of 5. All bootstrap values over 60% are shown. The *Sorex volnuchini* haplotype was used as the outgroup.

sampled in northern Europe had mtDNA haplotypes of the northern lineage, i.e. the lineage found on the nearest mainland. This consistency of mtDNA lineage between island and nearest mainland can be explained by colonization of the island over a former land-bridge or by human introduction by boat from a nearby landmass.

The populations of pygmy shrews in Ireland, however, show completely different mtDNA haplotypes from those found on the nearest landmass of Britain. Shrews from Britain have haplotypes that belong to the northern lineage, whereas Irish shrews have haplotypes similar to those found in southwest continental Europe. This result provides strong evidence that the pygmy shrew did not use any land-bridge that might have joined Britain and Ireland. If the shrews had crossed such a land-bridge, the mtDNA sequences in Ireland would be a subset of those found in Britain.

In Wingfield's (1995) moving land-bridge model, the connection between Britain and Ireland at one stage encompassed what is currently the Isle of Man. However, while the mtDNA data suggest that the Isle of Man was colonized from Britain, there is no similarity between the mtDNA of pygmy shrews on the Isle of Man and those in Ireland.

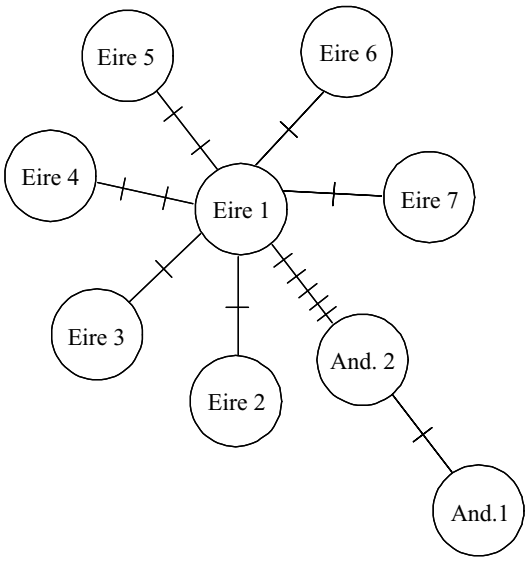

Figure 3. Unrooted minimum-parsimony network of all haplotypes within the Andorran–Irish lineage based on the single most parsimonious tree (see table 1 for geographical origins of haplotypes). Bars indicate mutation events. There are no homoplasies within this dataset.

The mtDNA dataset is not the only evidence that the pygmy shrew failed to cross a land-bridge from Britain to Ireland at the end of the last glaciation, if such a connection existed. Out of the four small rodents and insectivores currently found in Ireland, house mice and bank voles are known to be introductions, leaving only the pygmy shrew and the wood mouse as possible natural colonists. This compares with three species of vole, two or three species of mouse, one species of dormouse, three species of shrew and one species of mole that are native to Britain (Yalden 1982, 1999). As Stuart (1995) has argued, it seems implausible that pygmy shrews could have colonized Ireland over a temporary land-bridge from Britain, while other species, particularly the voles and the other shrews, failed to do so.

The difference in cytochrome *b* sequences between pygmy shrews in Ireland and in Britain and the similarity in sequences between pygmy shrews in Ireland and in southwest continental Europe adds the pygmy shrew to the 'Lusitanian element'. Like humans and pine martens it is not the whole species distribution that is Lusitanian in the pygmy shrew, but only particular lineages defined with molecular markers (mitochondrial: pygmy shrew, pine martens; Y-chromosomal: humans; Hill *et al.* 2000, Davison *et al.* 2001, this study). In all three species it is samples close to the Pyrenees that show affinity with those in Ireland.

Currently, there is no evidence of a temporary land-bridge between Ireland and continental Europe at the end of the last glaciation that could explain the Lusitanian element. Therefore, Corbet's (1961) suggestion that the Lusitanian element is attributable to human cultural exchanges between Ireland and southwest continental Europe is the most reasonable explanation of the data. During the process of human colonization of Ireland in the Mesolithic, or as a result of subsequent trading links, humans could have accidentally or deliberately transported a variety of species between these two areas.

Clearly, there is a need for further sampling of the pygmy shrews along the coast of southwest continental Europe (primarily Spain and France) to find populations with the greatest genetic affinity to the Irish populations. Scoring both mitochondrial and nuclear markers would aid this analysis. From such studies it should be possible to pinpoint the continental European source area of the Irish pygmy shrew in a way that is not possible from the data we present here. The findings would clearly be of great interest to archaeologists as the continental source area of Irish pygmy shrews may also represent the continental source area of Irish people or at least somewhere from where people embarked to trade with Ireland.

In the colonization of Ireland by pygmy shrews, the low level of mtDNA variation suggests that there were only a very few founders. Further sampling and molecular studies should help to pinpoint where these colonists arrived in Ireland and the manner of their spread over the island. From our results so far, the star phylogeny implies that the first shrews that derived from the colonists spread out over Ireland carrying the consensus Irish haplotype, with other haplotypes arising locally *in situ* by nucleotide substitution. However, this model needs to be tested with further data.

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REFERENCES

Akaike, H. 1974 A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* **19**, 716–723.

Anderson, S. (and 13 others) 1981 Sequence and organisation of the human mitochondrial genome. *Nature* **290**, 457–465.

Avise, J. C. 2000 *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Bilton, D. T., Mirol, P. M., Mascheretti, S., Fredga, K., Zima, J. & Searle, J. B. 1998 Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. *Proc. R. Soc. Lond.* B **265**, 1219–1226. (DOI 10.1098/rspb.1998.0423.)

Corbet, G. B. 1961 Origin of the British insular races of small mammals and of the 'Lusitanian' fauna. *Nature* **191**, 1037–1040.

Davison, A., Birks, J. D. S., Brookes, R. C., Messenger, J. E. & Griffiths, H. I. 2001 Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*. *Mol. Ecol.* **10**, 2479–2488.

Devoy, R. J. 1985 The problem of a late Quaternary landbridge between Britain and Ireland. *Quatern. Sci. Rev.* **4**, 43–58.

Filatov, D. 2000 *Processor of sequences manual*. University of Edinburgh.

Hasegawa, M., Kishino, H. & Yano, T. 1985 Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**, 160–174.

Hill, E. W., Jobling, M. A. & Bradley, D. G. 2000 Y-chromosome variation and Irish origins. *Nature* **404**, 351–352.

Irwin, D. M., Kocher, T. D. & Wilson, A. C. 1991 Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32**, 128–144.

Kimura, M. 1980 A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Kinnes, I. 1984 Microliths and megaliths: monumental origins on the Atlantic fringe. In *The archaeology of Carrowmore: environmental archaeology and the megalithic tradition at Carrowmore, Co. Sligo, Ireland* (ed. G. Burenhult), pp. 367–370. Stockholm: G. Burenhults Förlag.

Maddison, W. P. & Maddison, D. R. 2000 *MacClade 4: analysis of phylogeny and character evolution*. Sunderland, MA: Sinauer.

Mirol, P. M., Mascheretti, S. & Searle, J. B. 2000 Multiple nuclear pseudogenes of mitochondrial cytochrome *b* in *Ctenomys* (Caviomorpha, Rodentia) with either great similarity to or high divergence from the true mitochondrial sequence. *Heredity* **84**, 538–547.

Nei, M. 1987 *Molecular evolutionary genetics*. New York: Columbia University Press.

Nei, M. & Kumar, S. 2000 *Molecular evolution and phylogenetics*. Oxford University Press.

Posada, D. & Crandall, K. A. 1998 Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Rodríguez, F., Oliver, J. L., Marín, A. & Medina, J. R. 1990 The general stochastic model of nucleotide substitution. *J. Theor. Biol.* **142**, 485–501.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989 *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press.

Sheridan, A. 2003 French connections. I. Spreading the *marmites* thinly. In *Neolithic settlement in Ireland and western Britain* (ed. I. Armit, E. Murphy, E. Nelis & D. Simpson). Oxford: Oxbow Books. (In the press.)

Stewart, J. R. & Lister, A. M. 2001 Cryptic northern refugia and the origins of the modern biota. *Trends Ecol. Evol.* **16**, 608–613.

Stuart, A. J. 1995 Insularity and Quaternary vertebrate faunas in Britain and Ireland. In *Island Britain: a Quaternary perspective* (ed. R. C. Preece), pp. 111–125. London: Geological Society Special Publication 96.

Swofford, D. L. 1998 *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Sunderland, MA: Sinauer.

Tamura, K. & Nei, M. 1993 Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**, 512–526.

Wingfield, R. T. R. 1995 A model of sea-levels in the Irish and Celtic seas during the end-Pleistocene to Holocene transition. In *Island Britain: a Quaternary perspective* (ed. R. C. Preece), pp. 209–242. London: Geological Society Special Publication 96.

Yalden, D. W. 1981 The occurrence of the pygmy shrew *Sorex minutus* on moorland, and the implications of its presence in Ireland. *J. Zool.* **195**, 147–156.

Yalden, D. W. 1982 When did the mammal fauna of the British Isles arrive? *Mammal Rev.* **12**, 1–57.

Yalden, D. W. 1999 *The history of British mammals*. London: Poyser.

Zima, J., Lukáčová, L. & Macholán, M. 1998 Chromosomal evolution in shrews. In *Evolution of shrews* (ed. J. M. Wójcik & M. Wolsan), pp. 175–218. Białowieża, Poland: Mammal Research Institute, Polish Academy of Sciences.

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