



Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization

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There is a general perception that central and northern Europe were colonized by range expansion from Mediterranean refugia at the end of the last glaciation. Data from various species support this scenario, but we question its universality. Our mitochondrial DNA studies on three widespread species of small mammal suggest that colonization may have occurred from glacial refugia in central Europe–western Asia. The haplotypes on the Mediterranean peninsulae are distinctive from those found elsewhere. Rather than contributing to the postglacial colonization of Europe, Mediterranean populations of widespread small mammals may represent long-term isolates undergoing allopatric speciation. This could explain the high endemism of small mammals associated with the Mediterranean peninsulae.

Keywords: small mammals; colonization; glaciation; glacial refugia; endemism; Mediterranean

1. INTRODUCTION

At the height of the last (Weichselian) glaciation, 18 000 years BP (¹⁴C years before present), much of central and northern Europe was inhospitable to temperate species (Dawson 1992). However, there were regions in the three Mediterranean peninsulae of Iberia, Italy and the Balkans that did have a temperate climate and vegetation (Huntley 1988; Bennett *et al.* 1991). Therefore, it is a reasonable inference that the populations of temperate species that currently occupy central and northern Europe derive from Mediterranean ‘refugial’ populations that underwent range expansions in the late glacial and early postglacial (Hewitt 1996). (The Weichselian ended at approximately 10 000 years BP.)

Support for this sequence of events comes from the occurrence of temperate species currently consisting of subspecies or races with an eastern and western distribution in Europe. The eastern form can be viewed as deriving from the hypothesized Balkan refugium and the western form from Iberia, with genetic differentiation arising while the populations were in allopatry during the glaciation (figure 1a). The contribution of the Italian refugium to this colonization process may be considered to vary between species (figure 1a). Mayr (1963) famously used this model in relation to the crow *Corvus corone*, with

the black-and-grey ‘hooded crow’ (*C. c. cornix*), the east-European form, deriving from the Balkans and Italy, and the all-black ‘carrion crow’ (*C. c. corone*), the west-European form, from Iberia. In the case of the grass snake (*Natrix natrix*), Thorpe (1984) postulated that the eastern subspecies derived from the Balkans and the western subspecies from Iberia and Italy. A similar scenario can be suggested for the oak *Quercus robur–petraea* complex, which is subdivided into an eastern and western type on the basis of chloroplast (cp)DNA (Ferris *et al.* 1993; but see also Dumolin-Lapègue *et al.* 1997).

While the model of postglacial range expansion from Mediterranean refugia is well-supported for some temperate animals and plants (see also Huntley 1988), we suggest here an additional mode of colonization of central and northern Europe. Based on our studies of mitochondrial (mt)DNA sequences of three species of small mammal, Mediterranean refugial populations of these species have apparently remained within the Mediterranean peninsulae as geographical isolates. If such long-term isolation of Mediterranean populations has been common in small mammals, there is an expectation that new Mediterranean species should have evolved, and there are indeed a large number of endemic species of small mammals in Iberia, Italy and the Balkans. These concepts, and data in relation to small mammals, build on recent work by others for different taxa (e.g. Cooper *et al.* 1995), and contribute to an increasingly sophisticated understanding of the processes of colonization and speciation by animals and plants during the recent history of Europe (Hewitt 1996).

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2. MATERIALS AND METHODS

In this paper we describe similar data sets of mtDNA sequences for the common and pygmy shrews (*Sorex araneus* and *S. minutus*; Insectivora) and the bank vole (*Clethrionomys glareolus*; Rodentia). All these species have extensive ranges throughout Europe including two or three of the Mediterranean peninsulæ, with populations occurring over much of Siberia, as far east as Lake Baikal. MtDNA sequence variation over this distribution was analysed on the widely held assumption that such variation faithfully reflects population history undistorted by selection and gene flow (Avice 1994).

Specimens were sent to us from various localities by local mammalogists (full details can be obtained from J. B. Searle). In the laboratory, total genomic DNA was obtained from tail, heart or kidney stored in absolute ethanol or frozen at -80°C . The tissue was coarsely chopped and digested overnight at 55°C in 0.5 ml of 10 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.5% SDS to which 20 μl of proteinase K (10 mg ml $^{-1}$) were added. DNA was extracted using a mix of 1 part isoamyl alcohol:25 parts phenol:25 parts chloroform (Sigma), and precipitated either with absolute ethanol on dry ice or with a mix of 1 part 5 M ammonium acetate:10 parts isopropanol on ice. The DNA was washed in 70% ethanol, air-dried and resuspended in 10 mM Tris-EDTA pH 7.4.

We used 'universal' primers for polymerase chain reaction (PCR) amplification of particular mtDNA segments (Kocher *et al.* 1989; Irwin *et al.* 1991; Shields & Kocher 1991). For the shrews, primer pairs L14841-H15149 and L15162-H15915 were used to amplify two parts of the cytochrome *b* gene. For the bank vole, L14841-H15149 and L15774-H16498 (for part of the D-loop) were used. The PCR amplification cycle consisted of 1 min at 93°C , 1 min at 50°C and 2 min at 72°C , repeated 35 times. PCR products were purified with either the Wizard PCR Preps kit (Promega) or the QIAquick-spin PCR purification kit (Qiagen). For the shrews, the two parts of the cytochrome *b* gene were sequenced with the L14841 and H15915 primers, using a Taq DyeDeoxy Terminator cycle sequencing kit (ABI Applied Biosystems), before loading onto an ABI 373A automated sequencer. For the bank vole, the L14841 primer (cytochrome *b*) and L15774 and H16498 primers (D-loop) were used to generate manual sequences on a Biorad Sequigen GT Sequencing Cell using the Sequenase 2.0 T7 DNA Polymerase Sequencing kit (Amersham) and ^{35}S dATP (Du Pont NEN). Sequencing ambiguities of the type described by Zhang & Hewitt (1996) were not detected, leading us to conclude that contamination with nuclear copies of mitochondrial genes had not occurred.

For each species, the readable DNA from the two PCR-amplified segments was combined for the phylogenetic analysis. The lengths of sequence analysed were as follows: *S. araneus*, 581 bp; *S. minutus*, 447 bp; *C. glareolus*, 583 bp. In the case of *S. minutus* we present a phylogenetic analysis based on all 14 individuals for which this length of sequence was obtained. For *S. araneus* and *C. glareolus* we will present our complete data elsewhere (P. M. Mirol, S. Mascheretti and J. B. Searle, unpublished data). Here, for comparative purposes, we have extracted similar-sized data sets to that of *S. minutus*, based on 18 *S. araneus* and 20 *C. glareolus* individuals selected solely to provide a wide spread of geographical localities (not on the basis of knowledge of the sequences). For outgroups in the phylogenetic analyses we obtained and used sequences from closely related species (*Sorex volnuchini* for *S. minutus*, *Sorex coronatus* for *S. araneus* and *Clethrionomys rutilus* for *C. glareolus*).

In this study, we make the assumption that even single mtDNA sequences from particular geographical regions are likely to reflect, at least in a coarse manner, mtDNA characteristics of that region. For all non-commensal small mammals that we know of, this assumption would be valid because distributions of principal mtDNA lineages are strictly parapatric, i.e. clusters of closely related mtDNA haplotypes are found over continuous areas that border on other continuous areas where different clusters of related mtDNA haplotypes occur (see further discussion in Avice (1994)). Our own more extensive data on *S. araneus* and *C. glareolus* suggest that this is the pattern found in these species (P. M. Mirol, S. Mascheretti and J. B. Searle, unpublished data), as do the published studies of Taberlet *et al.* (1994) on *S. araneus* and Tegelström (1987) on *C. glareolus*. Other species that well illustrate such patterning are the field vole, *Microtus agrestis*, studied in northern Europe by Jaarola & Tegelström (1995) and the wood mouse, *Apodemus sylvaticus*, studied in western Europe by Michaux *et al.* (1996). These are species to which we will make further reference; they both broadly share the same distribution as *S. minutus*, *S. araneus* and *C. glareolus*.

For the sequences used for analysis, multiple alignments were done using the PILEUP function of the Genetics Computer Group package (Devereux *et al.* 1984). Phylogenetic analysis was done using PHYLIP 3.4 (Felsenstein 1991). Neighbour-joining (NJ) trees (Saitou & Nei 1987) were generated from Kimura two-parameter distances (Kimura 1980) with the standard transition:transversion ratio of 2:1. Identical trees were produced with a wide range of different ratios; also, very similar results were obtained with parsimony analysis using PAUP v. 3.1 (Swofford 1993). To assess the reliability of the NJ trees, bootstrap values were calculated for each clade after 100 replications.

3. RESULTS AND DISCUSSION

(a) DNA analysis of pygmy shrew and its interpretation

For the pygmy shrew, cytochrome *b* sequences were obtained from individuals from central Europe (Ukraine, Czech Republic, Poland (two from the same locality) and Germany (two from the same locality)), northern Europe (northern England, Finland) and eastern Siberia. The Mediterranean region is represented by individuals from European Turkey, Macedonia, central Spain (two from the same locality) and central Italy. Among the 13 haplotypes obtained from the 14 individuals analysed, there were 43 variable sites, representing 10% of nucleotides screened. As would be expected from other intraspecific studies of cytochrome *b* in mammals (see, for example, Taberlet *et al.* 1994), most of the base changes were transitions (84%) and silent (81%); also, most (seven out of nine) of the replacement substitutions were in the transmembrane regions, in keeping with the findings of Irwin *et al.* (1991) for a variety of other mammals.

The pairwise distances between the pygmy shrew haplotypes are given in table 1 and their phylogenetic relationship is shown in figure 2. Individuals from the same sites either had identical (central Spain) or very similar (Poland, Germany) haplotypes. All the non-Mediterranean haplotypes, except that from the Czech Republic, are grouped together in one of the best-supported branches (bootstrap: 89%) on the tree (hereafter known as the 'major branch'). What is notable about this major branch is that it links together individuals from a huge

Table 1. Pairwise sequence divergence (%) based on Kimura two-parameter distances between the 13 *Sorex minutus* and single *S. volnuchini* cytochrome *b* haplotypes obtained

haplotype	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Ukraine	0.2	1.1	1.1	1.8	1.3	1.6	1.4	3.7	2.5	4.2	3.5	3.5	8.9
2. E. Siberia	—	1.4	1.4	2.1	1.6	1.8	1.6	3.7	2.8	4.4	3.7	3.7	9.2
3. N. England	—	—	0.4	1.1	0.7	0.9	0.7	3.2	2.3	3.9	3.2	3.2	9.4
4. Finland	—	—	—	1.1	0.6	1.4	1.1	3.2	2.3	3.9	3.2	3.2	9.4
5. Poland1	—	—	—	—	0.9	2.1	1.8	3.9	3.0	4.6	3.9	3.9	9.7
6. Poland2	—	—	—	—	—	1.6	1.4	3.5	2.5	4.2	3.5	3.5	9.7
7. Germany1	—	—	—	—	—	—	0.2	3.7	2.8	3.9	3.7	3.7	9.4
8. Germany2	—	—	—	—	—	—	—	3.5	2.5	3.7	3.5	3.5	9.2
9. C. Italy	—	—	—	—	—	—	—	—	3.2	4.4	3.7	4.2	8.9
10. C. Spain	—	—	—	—	—	—	—	—	—	3.0	2.1	2.1	9.2
11. Czech Rep.	—	—	—	—	—	—	—	—	—	—	2.5	2.1	10.2
12. Macedonia	—	—	—	—	—	—	—	—	—	—	—	0.4	8.9
13. Turkey	—	—	—	—	—	—	—	—	—	—	—	—	8.9
14. <i>S. volnuchini</i>	—	—	—	—	—	—	—	—	—	—	—	—	—

geographical area extending from York (England) in the west to Baikal (Siberia) in the east, a distance of 7000 km!

Inspection of table 1 shows that the pairwise distances between haplotypes of the major branch are generally small, in the range 0.2–2.1%, whereas those between the major branch and all other pygmy shrew haplotypes are in the range 2.3–4.6%. Individuals within the major branch are characterized by three diagnostic nucleotides: a G in position 15601 (rather than a T), a G in position 15769 (rather than an A) and a T in position 15814 (rather than a C) (numbering of nucleotides follows Anderson *et al.* (1981)).

Among the pygmy shrews from the Mediterranean region, those from the same locality (central Spain) had the same haplotype, and those from nearby localities (Macedonia, European Turkey: both southern Balkans) had very similar haplotypes (differing by two base pairs). However, other comparisons among pygmy shrews from Iberia, Italy, the Balkans and the Czech Republic generate pairwise distances of 2.1–4.4% (table 1), of the same magnitude as those between haplotypes from the major branch and haplotypes from these four regions. The central Italian haplotype was particularly distinctive, with pairwise distances of at least 3.2% in comparisons with all other pygmy shrews. The central Italian haplotype differed from all others at seven nucleotide positions, i.e. 16% of those that are variable in the pygmy shrew. Incomplete sequence data from two additional pygmy shrews from the same locality in central Italy (D. T. Bilton, unpublished data) indicate that they also had all seven unique substitutions.

Thus, contrary to the traditional model of postglacial colonization of widespread species from Mediterranean regions northwards into central and northern Europe (figure 1a), our data for the pygmy shrew show little similarity between any Mediterranean populations and those from further north. Most of those haplotypes north of the Mediterranean differ little from each other and belong to a single well-defined monophyletic grouping: the major branch. Clearly, further analysis would be desirable to confirm the pattern revealed so far. In particular, mtDNA sequence from more pygmy shrews from more sites would be valuable to validate the assumption that the haplotypes

detected are truly representative of the region from which they were found. Also, studies with nuclear sequences would be of interest (Hewitt 1996). Nevertheless, as they stand, the data for the pygmy shrew do not fit well with the traditional biogeographical model for the postglacial recolonization of central and northern Europe.

Instead, an alternative biogeographical model (figure 1b) can be suggested in which, rather than expanding northwards, populations in the Mediterranean peninsulae remain as geographical isolates, continuing to diverge genetically from each other and from more northern populations. By this model, the colonization of Europe north of the Mediterranean at the end of the Weichselian is attributed to non-Mediterranean sources.

At first sight non-Mediterranean glacial refugia for temperate species may appear unlikely. At the height of the Weichselian (18 000 years BP) most of central and northern Europe and regions of Asia at similar latitudes would have been glaciated or else exposed to extremely cold and arid conditions (Velichko *et al.* 1984; Huntley 1988; Dawson 1992). Nevertheless, there is evidence that there were sheltered areas where moister conditions prevailed and tree cover could develop, on the slopes of mountain ranges and along river valleys (Soffer 1990; A. V. Kozharinov, personal communication); such areas may have been inhabited by temperate small mammals such as the pygmy shrew. For example, the southern slopes of the Carpathian Mountains, the hills of Crimea, the southwestern Ural Mountains and the northern slopes of the Altai Mountains may have been suitable areas (see Grichuk 1984; Markova 1984; Soffer 1990; Demesure *et al.* 1996). There is even an extraordinary record of one species normally considered to be 'temperate' (the field vole *Microtus agrestis*) from a site immediately south of the ice sheet in northern Russia in a deposit dated to 18 000 years BP (Markova 1984).

For the pygmy shrew, the fact that mtDNA haplotypes of the major branch are so prevalent over its range could be interpreted as indicating that there was a single main Weichselian refugium for this species for the colonization of Siberia and Europe north of the Mediterranean. However, the widespread occurrence of the major branch haplotypes may be the result of an earlier expansion from a single source

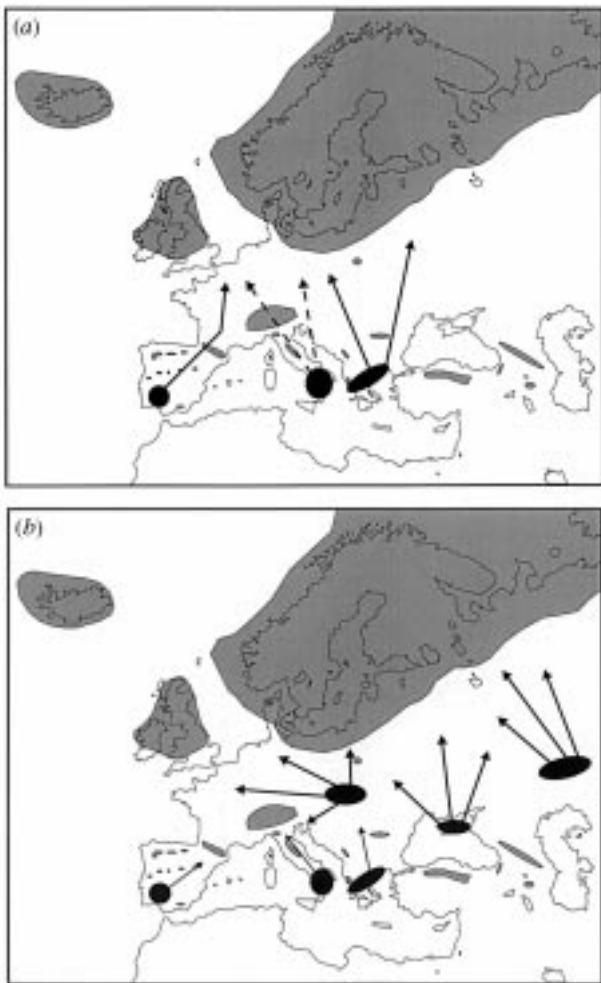


Figure 1. Recent biogeographical history of widespread temperate species in Europe. (a) *Traditional model*: occupation of Mediterranean refugia at the height of the last (Weichselian) glaciation (18 000 years BP) and colonization northwards from these refugia after the end of the glaciation (approximately 10 000 years BP) or during the late glacial. If the populations in the different Mediterranean isolates have become genetically differentiated, an eastern race (deriving from the Balkans) and a western race (deriving from Iberia) are expected after post-glacial colonization. The populations in the Italian refugium may contribute to either the eastern or the western race.

(b) *Model based on molecular studies of small mammals*: in this case, the populations that occupy Mediterranean refugia during the last glaciation essentially fail to expand their distribution; instead, more northerly areas of Europe become occupied by range expansion from one or more refugia in central Europe–western Asia. This model has also been suggested for the common beech (*Fagus sylvatica*) on the basis of cpDNA studies (Demesure *et al.* 1996). Glacial refugia and colonization routes shown schematically in black. Grey shading indicates ice sheets present at 18 000 years BP (after Huntley 1988).

(e.g. at the end of the previous glaciation 130 000 years ago). If so, at the height of the Weichselian there may have been several refugia occupied by pygmy shrews, with major branch haplotypes located in central Europe and Siberia.

(b) Support for the alternative biogeographical model from DNA analysis of bank vole and common shrew

Like the pygmy shrew, the bank vole (*Clethrionomys glareolus*) occupies all three Mediterranean peninsulae. The

NJ tree (figure 3a) is based on sequence data for bank voles from a wide range of localities, with each locality represented by a single individual. Although the detailed phylogeography suggested by the tree differs somewhat from that for the pygmy shrew (figure 2), there is once again strong evidence that the mtDNA haplotypes from the Mediterranean are distinctive: there is a branch (with 77% bootstrap support) that groups together the haplotypes from Turkey, Bulgaria, central Italy, north-eastern and central Spain. There are no haplotypes from north of the Mediterranean within this branch, although a haplotype from northeastern Italy lies outside it.

Thus, the data for the bank vole are broadly compatible with the model of postglacial colonization of Europe proposed for the pygmy shrew (figure 1b). The distinctiveness of the south European haplotypes, relative to those from further north, suggests that central and northern Europe was not colonized by postglacial expansion of populations from Iberia, central and southern Italy and the southern Balkans. Although north-eastern Italy, at the northern border of the Mediterranean zone, is represented by a haplotype with affinities to those from further north, this may actually be the result of colonization from more northerly regions upon deglaciation of the alpine ice sheet. The north-east Italian vole was collected from Trento in the Italian alps, an area that would probably have been covered by this ice sheet (see Dawson 1992).

In contrast to the pygmy shrew and bank vole, the common shrew (*Sorex araneus*) only occupies two out of the three Mediterranean peninsulae. In Iberia, *S. araneus* is replaced by the sibling species *S. granarius* in some areas and by *S. coronatus* in others (*S. coronatus* also occupies lowland France). We obtained a cytochrome *b* sequence of a common shrew from the southern Balkans and Taberlet *et al.* (1994) analysed common shrews from Italy (for a slightly different segment of cytochrome *b*). In addition, we studied common shrews from various parts of central and northern Europe and central and eastern Siberia. The samples from (Alpine) southern France and (Pyrenean) Andorra can also be classified as central European; there is no indication that they derive from populations that occurred in an Iberian refugium during the last glaciation.

The NJ tree for the mtDNA sequence that we collected is shown in figure 3b. The only substantial well-supported branch is that linking together all common shrew haplotypes, with the exception of that from European Turkey. Thus, as for the pygmy shrew there is a 'major branch' linking together haplotypes deriving from throughout central and northern Europe and Siberia. Although we do not include the data of Taberlet *et al.* (1994) in the tree, the Italian haplotypes that they examined diverge considerably more from the major branch than do the Turkish haplotypes, and would therefore appear outside all *S. araneus* branches on the present tree (P. M. Mirol and J. B. Searle, unpublished data).

Again these mtDNA data mirror precisely what we found with the pygmy shrew: the haplotypes from European Turkey and Italy are distinct from those found north of the Mediterranean, and the postglacial colonization model suggested for the pygmy shrew (figure 1b) appears to be more appropriate than the traditional model. Further support for the pygmy shrew model comes

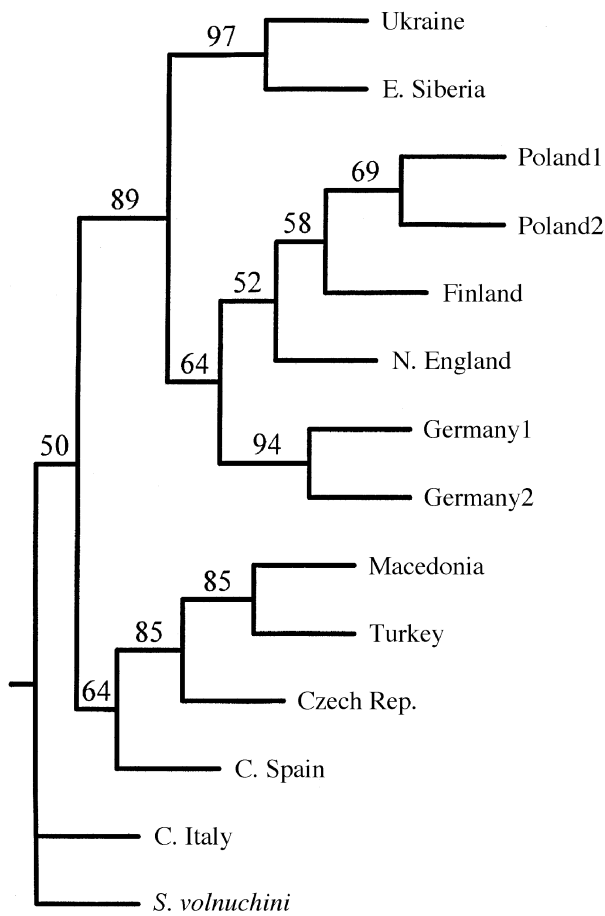


Figure 2. NJ tree (with standardized branch lengths) of pygmy shrew (*S. minutus*) haplotypes for a 447-bp segment of cytochrome *b*, based on specimens collected throughout the species range. There were two individuals from central Spain which had identical haplotypes. A haplotype of *S. volnuchini* represents the outgroup (collected from Artvin province in Asian Turkey by V. Vohralík). Numbers over branches indicate bootstrap support (100 replicates). Collection sites and collectors: C. Italy: La Maiella, Abruzzio (G. Amori); Macedonia: Mt Pellister (J. Z., B. Krystufek); Turkey: Istranca Daglari (J. Z.); C. Spain: Rascafria, Prov. Madrid (M. J. López Fuster); N. England: York (J. B. S.); Germany: Harz Mts. (J. Gahsche); Poland: Blizocin (A. Banaszek); Czech Rep.: N. Bohemia (J. Z.); Finland: Lammi (J. Saarikko); Ukraine: Kanev District, Cherkassk Region (A. Mishta); E. Siberia: Baikal Region, Russia (J. Z.).

from studies of chromosomal variation in the common shrew. As well as having distinctive mtDNA haplotypes, common shrews from Italy and European Turkey have very distinctive karyotypes (Tablerlet *et al.* 1994; Zima *et al.* 1997). Chromosomal data may also help infer more precisely the postglacial colonization of regions north of the Mediterranean. For example, the common shrews in the Ural Mountains, Finland, and western Siberia are chromosomally similar, and thus could have derived from a glacial refugium in or close to the Ural Mountains (Halkka *et al.* 1994; Polyakov *et al.* 1996, 1997).

(c) Data from DNA studies of other species and a general explanation for variation in colonization histories

As described in § 1, the cpDNA studies of Ferris *et al.* (1993) revealed a colonization history in the oak fitting

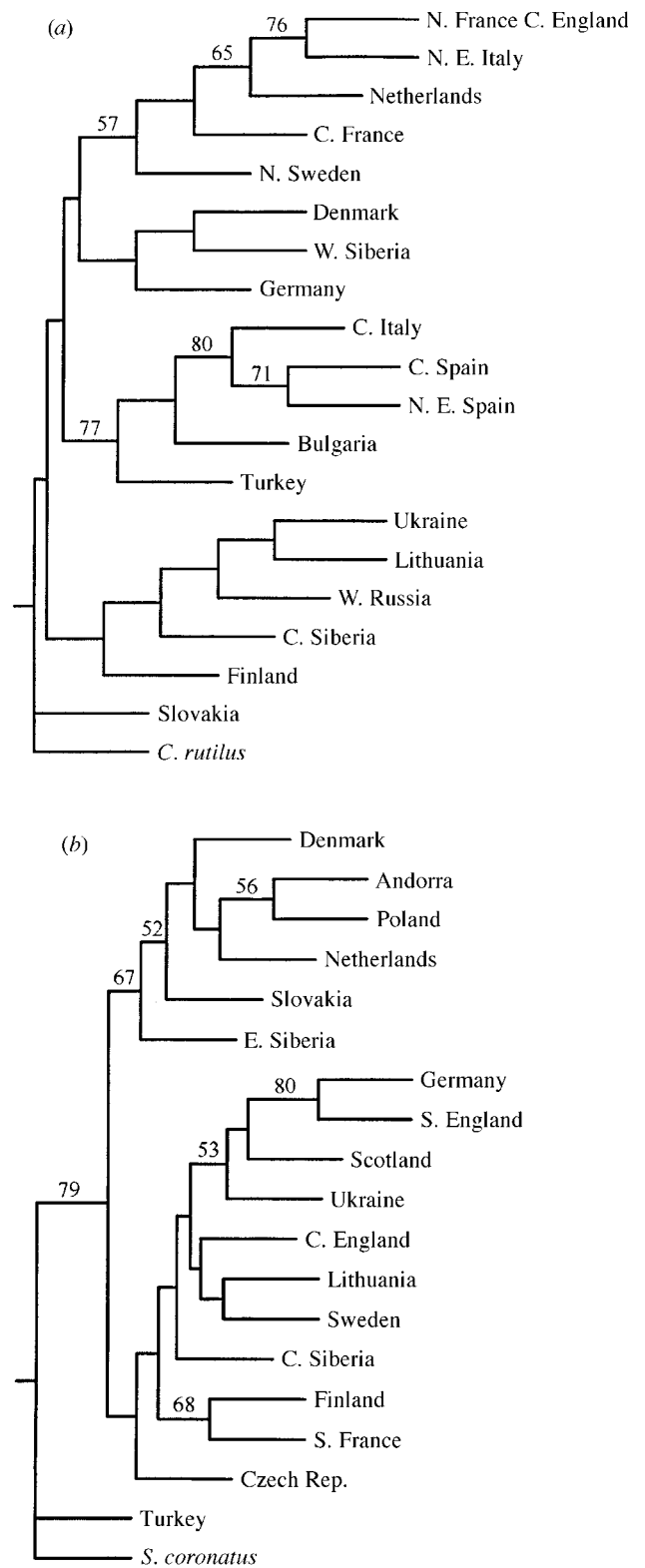


Figure 3. NJ trees of (a) bank vole and (b) common shrew cytochrome *b* haplotypes including *C. rutilus* and *S. coronatus* as outgroups.

the traditional model (figure 1a) of colonization northwards from all the Mediterranean peninsulae at the end of the last glaciation. In contrast, our mtDNA studies suggest that none of the Mediterranean peninsulae acted as source areas for the postglacial colonization of the

pygmy and common shrews and the bank vole. Instead, colonization of central and northern Europe occurred from non-Mediterranean glacial refugia. DNA studies of the meadow grasshopper *Chorthippus parallelus* (Cooper *et al.* 1995) suggest a similar situation, with the species colonizing central and northern Europe from refugial populations in the northern Balkans. Populations in the southern Balkans, Iberia, and Italy are distinct, and probably survived a number of ice-ages *in situ* (Cooper *et al.* 1995; Hewitt 1996). While the precise phylogeography of each species is individual to some extent, both *Chorthippus* and the small mammals studied here appear to have responded in broadly similar ways, with allopatric divergence in Mediterranean peninsular populations fail to play a significant role in northern colonization. In the case of the brown bear, *Ursus arctos* (Taberlet & Bouvet 1994), eastern Europe appears to have been colonized from non-Mediterranean refugia, whereas western Europe north of the Mediterranean was colonized from the Iberian peninsula, a situation somewhat intermediate between traditional and present models.

From another smaller-scale mtDNA study, Michaux *et al.* (1996) suggested that wood mice (*Apodemus sylvaticus*) from Italy did not contribute to postglacial colonization of central and northern Europe. However, they had no information on Iberia or the Balkans. The more extensive cpDNA study by Demesure *et al.* (1996) indicates that common beech (*Fagus sylvatica*) also failed to colonize central Europe from an Italian glacial refugium.

Thus, there are now several species for which a combination of the Mediterranean peninsulae apparently failed to contribute to the colonization of central and northern Europe at the end of the last glaciation. In each case it can be assumed that the northwards migration from the Mediterranean peninsula was impeded by a mountain range (Hewitt 1996; Michaux *et al.* 1996). Both the Alps (at the base of the Italian peninsula) and the Pyrenees (at the base of the Iberian peninsula) are high-altitude mountain chains (with most land above 2000 m), across which many species would not readily disperse. These mountain chains were also covered with ice sheets at the height of the glaciation (Dawson 1992) and any movements over the mountains (except aerial dispersal) would have depended on very substantial deglaciation. There is also high ground that could have slowed migration northwards from glacial refugia in the Balkans. The forested areas in the Balkans suitable for temperate species during the last glaciation existed along the coast of Greece, European Turkey, Albania and the former Yugoslavia (see, for example, Hewitt 1996). Migration northwards from these areas required passage over a substantial block of land mostly above 1000 m that covers much of the former Yugoslavia, Albania, northern Greece and Bulgaria.

The discrepancy between organisms that apparently followed the traditional model of postglacial range expansion of central and northern Europe for which the Mediterranean peninsulae are all-important (figure 1a), our model for which they are completely unimportant (figure 1b), and intermediate models suggested for the brown bear (Taberlet & Bouvet 1994) can be explained more generally. It depends on (i) whether the species occupied the Mediterranean peninsulae during the last

glaciation and the extent to which the Alpine, Pyrenean and Balkan mountains represented barriers to the species concerned; and (ii) whether there were any glacial refugia away from the Mediterranean peninsulae, the location of such refugia, and the ease with which the species could expand from such refugia. For example, while pygmy shrews and brown bears apparently occupied both Mediterranean and non-Mediterranean refugia during the last glaciation, in the case of the pygmy shrew we suggest that the Alpine, Pyrenean and Balkan mountains represented very significant barriers that could only be crossed very slowly (possibly over hundreds of years involving many generations of shrews) whereas in the case of the brown bear they could be crossed very quickly (possibly within an individual's lifetime). Therefore, we propose that at the end of the last glaciation, pygmy shrews from the non-Mediterranean refugia were able to colonize the whole of central Europe before individuals could migrate out of the Mediterranean peninsulae. In contrast, we suggest that Iberian brown bears were able to cross the Pyrenees and expand northwards through western Europe before colonization from any other refugium could take place.

(d) ***Rather than an important source area for postglacial colonization, the Mediterranean region as an important area for endemic small mammals***

Returning our emphasis to the pygmy and common shrews and the bank vole, the Mediterranean populations of these species appear to be diverging according to the allopatric speciation model (Mayr 1963). Thus, during the last glaciation, populations within each of the Mediterranean peninsulae would probably have been isolated from each other, as well as from other refugial populations. Certainly, for the pygmy shrew, the mtDNA haplotypes from the different peninsulae differ substantially. Subsequent to the Weichselian, these Mediterranean populations do not appear to have expanded out of the peninsulae. They may have rather little contact with central European populations deriving from different glacial refugia. This geographical isolation of the Mediterranean populations during both glacial and interglacial periods may have occurred for several glacial cycles, as discussed by Hewitt (1989, 1996). Certainly, the substantial nucleotide divergence between pygmy shrew haplotypes from the different Mediterranean peninsulae (2.1% or greater) would appear consistent with this, although given the uncertainties associated with the application of molecular clocks (Avice 1994), we will refrain from more precise estimates of population divergence times.

Thus, the populations of the small mammals that we examined on the different Mediterranean peninsulae may have been geographically isolated for a long period of time, permitting accumulation of new mutations accounting, for instance, for the (possibly selectively neutral) divergence in mtDNA sequence. More important adaptive changes may also have occurred. The Mediterranean is well-known to be a different climatic zone from the rest of Europe at the present time.

Taking all these factors into consideration, it would seem likely that the pygmy shrews, common shrews and

bank voles on the Mediterranean peninsulæ are evolving into separate species in allopatry. There is already clear evidence that the common shrews in Italy are strongly (though not completely) reproductively isolated from those in central Europe (Brünner & Hausser 1996). If there are signs that speciation is occurring for the small mammals that we have examined, it would be expected that some Mediterranean populations of small mammals have speciated already. There are indeed a large number of species of rodents of the subfamily Arvicolinae (voles, lemmings and the musk rat) and insectivores endemic to the Mediterranean peninsulæ (data from Mitchell-Jones *et al.* 1998). For Iberia, there are the following species: *Sorex granarius*, *Galemys pyrenaicus*, *Talpa occidentalis*, *Microtus cabreræ* and *Microtus lusitanicus*; for Italy, *Sorex samniticus*, *Talpa romana* and *Microtus savii*; for the Balkans, *Talpa stankovici*, *Dinaromys bogdanovi*, *Microtus felteni*, *Microtus guentheri* and *Microtus thomasi*. Out of a total of 26 species of insectivores and 25 species of arvicolines in Europe (excluding species on the Canary Islands), 23% of the former and 25% of the latter are endemic to one or other of the Mediterranean peninsulæ. Given that the Mediterranean peninsulæ only account for 15% of the land area in Europe, they are clearly extremely important in terms of the diversity of European small mammals.

Thus, for small mammals, the Mediterranean peninsulæ are a 'hot spot' of endemism (see Myers 1988), at least in a Palaearctic context. The same is true for other taxa. More than half the plant species of the Mediterranean region are endemic and four out of five European endemics are Mediterranean taxa (Gomez-Campo 1985). In insects a high percentage of Mediterranean taxa are endemic to the basin (Balletto & Casale 1991), with most of the European endemic species being found in one or more of the Mediterranean peninsulæ (e.g. hydraenid water beetles: Valladares & Montes 1991; Jäch 1992; Audisio *et al.* 1996). This aspect of the Mediterranean peninsulæ should be as important to biogeographers as their role in the postglacial colonization of some taxa.

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