

Comparative phylogeography and postglacial colonization routes in Europe

PIERRE TABERLET,* LUCA FUMAGALLI,† ANNE-GABRIELLE WUST-SAUCY,‡
JEAN-FRANÇOIS COSSON§

*Laboratoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France, †Department of Zoology, University of Queensland, Brisbane, Qld 4072, Australia, ‡Institut de Zoologie et d'Ecologie Animale, Université de Lausanne, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland, §Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Place Eugène Bataillon, C.C.63, F-34095 Montpellier Cedex 5, France

Abstract

The Quaternary cold periods in Europe are thought to have heavily influenced the amount and distribution of intraspecific genetic variation in both animals and plants. The phylogeographies of 10 taxa, including mammals (*Ursus arctos*, *Sorex* spp., *Crocidura suaveolens*, *Arvicola* spp.), amphibians (*Triturus* spp.), arthropods (*Chorthippus parallelus*), and plants (*Abies alba*, *Picea abies*, *Fagus sylvatica*, *Quercus* spp.), were analysed to elucidate general trends across Europe. Only a small degree of congruence was found amongst the phylogeographies of the 10 taxa, but the likely postglacial colonization routes exhibit some similarities. A Brooks parsimony analysis produced an unrooted area phylogram, showing that: (i) the northern regions were colonized generally from the Iberic and Balkanic refugia; and (ii) the Italian lineages were often isolated due to the presence of the Alpine barrier. The comparison of colonization routes highlighted four main suture-zones where lineages from the different refugia meet. Some of the intraspecific genetic distances among lineages indicated a prequaternary divergence that cannot be connected to any particular cold period, but are probably related mainly to the date of arrival of each taxon in the European continent. As a consequence, molecular genetics so far appears to be of limited use in dating Quaternary events.

Keywords: chloroplast DNA, glaciation, mitochondrial DNA, Quaternary, refugium, suture-zone

Introduction

De Candolle (1820) was the first author to propose that the current geographical distribution of living organisms depends upon both ecological and historical parameters. The influence of historical parameters can be assessed via historical biogeographic studies (Morrone & Crisci 1995; Wiley 1988b). Most studies of this type have compared the geographical distribution of taxa at or above the species level. With the development of molecular methods, it is now possible to investigate the geographical variation using molecular markers, and to deduce intraspecific phylogeographic structures (Avice *et al.* 1987). In this context, it is tempting to apply the methods of historical biogeography below the species level, and to compare the

intraspecific phylogeographic patterns of several taxa over the same area. Up to now, very few studies have been published in this field. The main goal of these studies has been to search for concordant geographical distribution among lineages within different species, which would indicate the influence of a common historical factor.

Thus, in south-eastern United States, a comparison of the intraspecific phylogeographies of four freshwater fishes (Bermingham & Avice 1986), and of 15 additional species including a bird, a reptile, a crustacean, a mollusc, and other marine and freshwater fishes (Avice 1992), has been carried out based on mitochondrial DNA (mtDNA) polymorphism. The most interesting result was that almost all the assayed species exhibited a deep genetic break between populations from the Atlantic Coast and populations from the Gulf of Mexico. The same kind of mtDNA concordance, with a deep genetic break at the intraspecific level, has also been recorded in

Correspondence: P. Taberlet. Tel. +33-476-51-46-00 ext. 3705; Fax. +33-476-51-42-79; E-mail: Pierre.Taberlet@ujf-grenoble.fr

the wet tropics of Australia for four species out of six tested (five birds, one reptile) (Joseph *et al.* 1995). Only one study so far has examined concordance amongst species at the continental level. The mtDNA intraspecific phylogeographic structure of five bird species has been compared throughout North America (Zink 1996). A lack of a congruent phylogeographic pattern was found, suggesting that the five codistributed species have not had a long history of coassociation.

Before discussing comparative phylogeography in Europe, it is important to: (i) briefly describe the Quaternary cold periods which appear to have had a dramatic influence on most organisms in temperate regions (Hewitt 1996); and (ii) mention the geographical particularities of this continent. Twenty years ago, it was recognized that changes in the earth's orbit are the fundamental causes of the Quaternary climatic oscillations (Hays *et al.* 1976). The ice sheets of the Northern Hemisphere began to grow about 2.5 Myr ago, and the major climatic oscillations occurred during the last 700 ky, with a dominant 100-ky cycle (Webb & Bartlein 1992). It has been demonstrated more recently that short-term and high-amplitude climatic variation also occurred during the late Pleistocene (130 ky to 10 ky), both within the glacial and the interglacial periods (Roy *et al.* 1996). A decrease of 10–14 °C in average temperature (above Greenland) may have occurred within only 10–20 y, and lasted for periods of 70–5000 y (Dansgaard *et al.* 1993; GRIP Members 1993). Such temperature shifts could be linked to enormous discharge of icebergs into the North Atlantic (Bond *et al.* 1993). In this context, the relative climatic stability recorded during the last 8000 y seems to be the exception rather than the rule. The consequence is that the European biota has experienced some dramatic climatic changes during the last few million years, with extensive oscillations during the last 700 ky. Figure 1 presents the maximum extension of the ice sheet during the last cooling, about 20–18 ky ago.

From a biogeographic point of view, Europe has some distinctive features. It is a large peninsula connected to Asia, with an east–west orientation. The Mediterranean sea in the south constitutes a strong barrier, and has limited the possibility of southern displacement of biota during cold periods. Furthermore, the east–west orientation of the main mountain ranges of the Alps and the Pyrenees acted as a barrier to northward expansion of species during warm periods. The effects of the ice ages on European species has been examined in detail by Hewitt (1996): during the Quaternary, each species went through many contractions/expansions of range, characterized by extinctions of northern populations when the temperature decreased, and a northward expansion from refugia involving spreading from the leading edge. Such a colonization process implies successive bottlenecks that

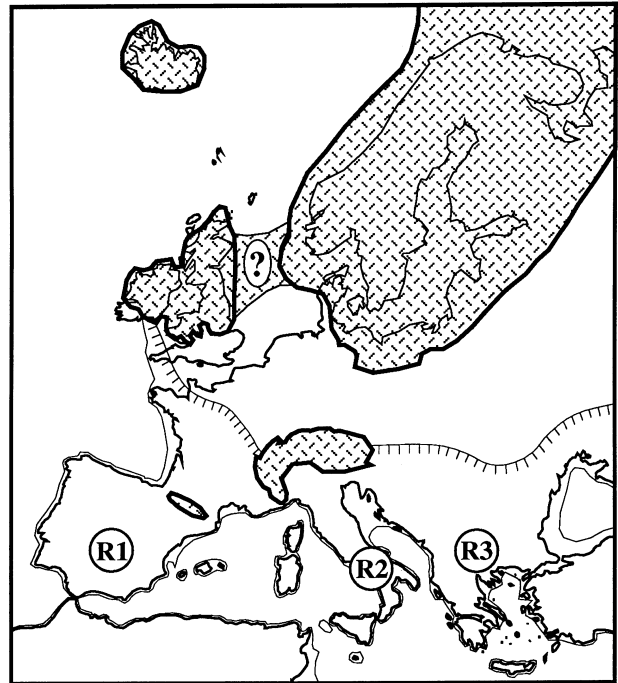


Fig. 1 Maximum extension of ice sheets in Europe during the last cold period, 20 000–18 000 y ago (redrawn from Frenzel *et al.* 1992; Lundqvist & Saarnisto 1995). R1, R2, and R3 indicate the three main potential refugia in Portugal–Spain, in Italy, and in the Balkans, respectively. The southern limit of the permafrost is indicated by the scaled line. Lowered sea shore is shown by a thinner line at the 100 m submarine contour.

may lead to a loss of genetic diversity in the northern populations, with the exception of cold-tolerant taxa. The refugia were localized in the mountains of southern Europe and, for the long-term presence of a species in Europe, survival in these refugia may have been as important during warm periods as during cold periods (Bennett *et al.* 1991). The disappearance of a species in southern refugia during warm periods could lead to the complete extinction of that taxon during the next cooling. The last northward expansion of European trees, which occurred from 13 ky BP, is well documented from the pollen record (Huntley & Birks 1983), and exhibited a rate of spread up to 2000 m/y (Bennett 1986).

However, the influence of the Quaternary cold periods on the geographical distribution of genetic diversity in Europe is still unclear. Do repeated cycles of isolation in southern refugia promote divergence and speciation? How is intraspecific biodiversity distributed? Are there common patterns of divergence and colonization? In this study, we present a comparative phylogeographic study of plants and animals at the European level. Our first goal is to elucidate general patterns in answer to the above questions. Our second goal is, assuming the existence of a molecular clock, to test if the depth of a concordant

genetic break observed within different species could be directly related to a particular historical factor such as a Quaternary cold period.

Materials and methods

The more taxonomically and ecologically diverse the species compared in this type of study, the more generally applicable the results will be (Zink 1996). Therefore, in order to extend the relevance of our results, the taxa compared were not limited by their taxonomic or ecological group, but were chosen only according to the availability of intraspecific phylogeographic studies over the geographical range considered. Studies where no clear phylogeographic pattern has been found (e.g. Estoup *et al.* 1996; Thomaz *et al.* 1996) or covering a restricted geographical range (e.g. Bernatchez *et al.* 1992; Garnery *et al.* 1995; Hardy *et al.* 1995; Jaarola & Tegelström 1995; Smith *et al.* 1991) were not taken into account for direct comparison, but are introduced where necessary. To have the opportunity to analyse as many data sets as possible, we considered phylogeographic patterns deduced from different molecular markers: mtDNA for animals, chloroplast DNA (cpDNA) or isozymes for plants. Due to clonal inheritance, cpDNA may be considered to be the plant counterpart for animal mtDNA. Isozymes are encoded by nuclear genes, which are characterized by biparental transmission and by pollen (haploid) or seed (diploid) dispersal, making phylogeographic inference more

complex. Nevertheless, in plants, isozyme data can be useful to identify populations which exhibit a common recent history.

A more restricted data set was used to test if the depth of genetic breaks observed within different animal species could be related to any historical factor. The taxa chosen for this purpose were those for which the mitochondrial DNA sequence divergence among lineages is known, and for which a time of divergence can be estimated assuming a molecular clock. The data used were composed essentially of intraspecific variation, but as the species boundaries are sometimes difficult to assess and can be questionable, data between closely related species were also considered when showing parapatric distributions. We simplified the original mtDNA phylogenetic trees by grouping the more closely related lineages to emphasize the main features of each taxon.

In order to highlight general patterns that are not obvious when the phylogeographic relationships are interpreted separately, a Brooks parsimony analysis (Brooks 1985, 1990; Wiley 1988a,b) was carried out. It corresponds to a Wagner parsimony analysis of an (area × taxon) matrix, where, in our case, each taxon is represented by a lineage. All the available data from the comparative study above were used, taking into account phylogenetic information when available, but excluding lineages present in all areas. The DELTRAN optimization routine of the PAUP program (Swofford 1993) was used to find the most parsimonious solution.

Table 1 The 10 taxa included in the comparative phylogeographic study

Scientific name	Common name	Taxonomic position	Techniques used	Reference
<i>Ursus arctos</i>	Brown bear	Mammalia, Carnivora	mtDNA sequencing	Taberlet & Bouvet (1994)
<i>Sorex araneus</i>	Common shrew	Mammalia, Insectivora	mtDNA sequencing	Taberlet <i>et al.</i> (1994)
<i>Sorex granarius</i>	Iberian shrew			
<i>Sorex coronatus</i>	Millet's shrew			
<i>Sorex samniticus</i>	Apennine shrew			
<i>Crocidura suaveolens</i>	Lesser white-toothed shrew	Mammalia, Insectivora	mtDNA sequencing	Cosson <i>et al.</i> (unpublished)
<i>Arvicola terrestris</i>	Northern water vole	Mammalia, Rodentia	mtDNA sequencing	Wust-Saucy <i>et al.</i> (unpublished)
<i>Arvicola sapidus</i>	Southwestern water vole			
<i>Triturus cristatus</i>	Crested newt	Amphibia, Urodeles	mtDNA RFLP	Wallis & Arntzen (1989)
<i>Triturus carnifex</i>				
<i>Triturus karekini</i>				
<i>Triturus marmoratus</i>	Marbled newt			
<i>Triturus pygmaeus</i>				
<i>Chorthippus parallelus</i>	Grasshopper	Insecta, Orthoptera	nuclear DNA sequencing	Cooper <i>et al.</i> (1995)
<i>Abies alba</i>	Silver fir	Gymnospermae, Pinaceae	protein electrophoresis	Konnert & Bergmann (1995)
<i>Picea abies</i>	Norway spruce	Gymnospermae, Pinaceae	protein electrophoresis	Lagercrantz & Ryman (1990)
<i>Fagus sylvatica</i>	Common beech	Angiospermae, Fagaceae	cpDNA PCR RFLP	Demesure <i>et al.</i> (1996)
<i>Quercus robur</i>	White oaks	Angiospermae, Fagaceae	cpDNA PCR RFLP	Dumolin-Lapègue <i>et al.</i> (1997)
<i>Quercus petraea</i>				
<i>Quercus pubescens</i>				
and related species				

The results of the Brooks parsimony analysis were visualized by drawing an unrooted area phylogram, with the different areas being placed to fit as much as possible to the geography.

Results

Table 1 gives details of the 10 studies considered in our comparative analysis. The original papers should be consulted to obtain details of the different phylogeographies. The simplified phylogeographies or postglacial colonization routes are presented in Figs 2, 3 and 4.

Ursus arctos (Taberlet & Bouvet 1994)

The brown bear exhibits two distinct mtDNA lineages in Europe. The eastern lineage is represented mainly by the large populations of Russia and Romania, whereas the western lineage, which includes the other European populations, appears to be organized into two clades corresponding to two different Quaternary refugia (Fig. 2). Contact zones between these two main lineages are localized in Scandinavia (Taberlet *et al.* 1995) and in Central Europe (Kohn *et al.* 1995). Sequence divergences in the cytochrome *b* gene amongst these lineages (Fig. 2) have been estimated by comparing results from different studies (Randi *et al.* 1994; Taberlet & Bouvet 1992), and are relatively low compared to intraspecific divergences in other taxa (Fig. 2).

Sorex spp. (Taberlet *et al.* 1994)

The shrews considered in our analysis were *Sorex samniticus* and the West European representatives of the *S. araneus* group. *S. granarius*, restricted to Spain, is closely related to a lineage of *S. araneus* found in western, northern and Central Europe. Another lineage of *S. araneus* found in Italy and the southern slopes of the Alps is slightly more divergent. *S. coronatus*, found in northern Spain and in France, and *S. samniticus*, restricted to Italy, differ from the other lineages by 3.7% and 9.1% in cytochrome *b* gene sequences, respectively (Fig. 2).

Crocidura suaveolens (Cosson *et al.*, unpublished)

The lesser white-toothed shrew is represented in Europe by two main lineages which differ from each other by a mean pairwise genetic distance of approx. 6.5% in the cytochrome *b* gene. The western lineage includes fragmented populations in western France and northern Spain. The eastern lineage is represented by the large populations in Central Europe, Italy, the Balkans, and Turkey (Fig. 2).

Arvicola spp. (Wust-Saucy *et al.*, unpublished)

The water voles exhibit four main lineages in Europe differing from each other by 3.8–7.6% in cytochrome *b* sequences: one lineage represented by *Arvicola sapidus* in Spain and western France, and three lineages of *A. terrestris* (Fig. 2). Each of the three lineages of *A. terrestris* were composed of populations belonging to the same clade and named according to a subspecies considered as representative of that clade: *A. terrestris italicus* in Italy, the aquatic form *A. terrestris terrestris* in northern and Central Europe, and the fossorial form *A. terrestris sherman* in northern Spain, Pyrenees, Massif Central, and the Alps.

Triturus spp. (Wallis & Arntzen 1989)

Five lineages of newts have been included: *Triturus pygmaeus* (southern Spain), *T. marmoratus* (Spain and France), *T. cristatus* (north-east France, Germany, Poland and Russia), *T. carnifex* (Italy), and *T. karelini* (the Balkans and Turkey). The entire mtDNA sequence divergence estimated from RFLPs amongst these lineages ranges from 4% to 9% (Fig. 2). The intralocus variation has not been considered, although it is substantial in *T. carnifex* and *T. karelini*. *T. dobrigicus*, only present in the Danube valley, has not been taken into account.

Chorthippus parallelus (Cooper *et al.* 1995)

The analysis of the variation at a noncoding nuclear marker in this species revealed geographical subdivision and an unambiguous pattern of postglacial expansion (Fig. 3). Most of the European populations of this grasshopper are homogeneous, originating after a range expansion from a Balkan refugium, and meet populations from Italian and Spanish refugia in the Alps and the Pyrenees, respectively.

Abies alba (Konnert & Bergmann 1995)

Silver fir occurs in mountainous regions of the southern part of Europe. The geographical distribution of allele frequencies at isozyme loci indicates five refugia during the last glaciation. Most of the current populations originate predominantly from two refugia in the Balkans and in Central Italy, with extensive mixing where these two lineages meet (Fig. 4). Another hypothetical refugium in south-eastern France could also have contributed to the isozyme polymorphism. The isolated populations in the Pyrenees and in Calabria correspond to two additional refugia, but did not expand.

Picea abies (Lagercrantz & Ryman 1990)

Norway spruce presents a phylogeographic pattern completely different from the other species examined here,

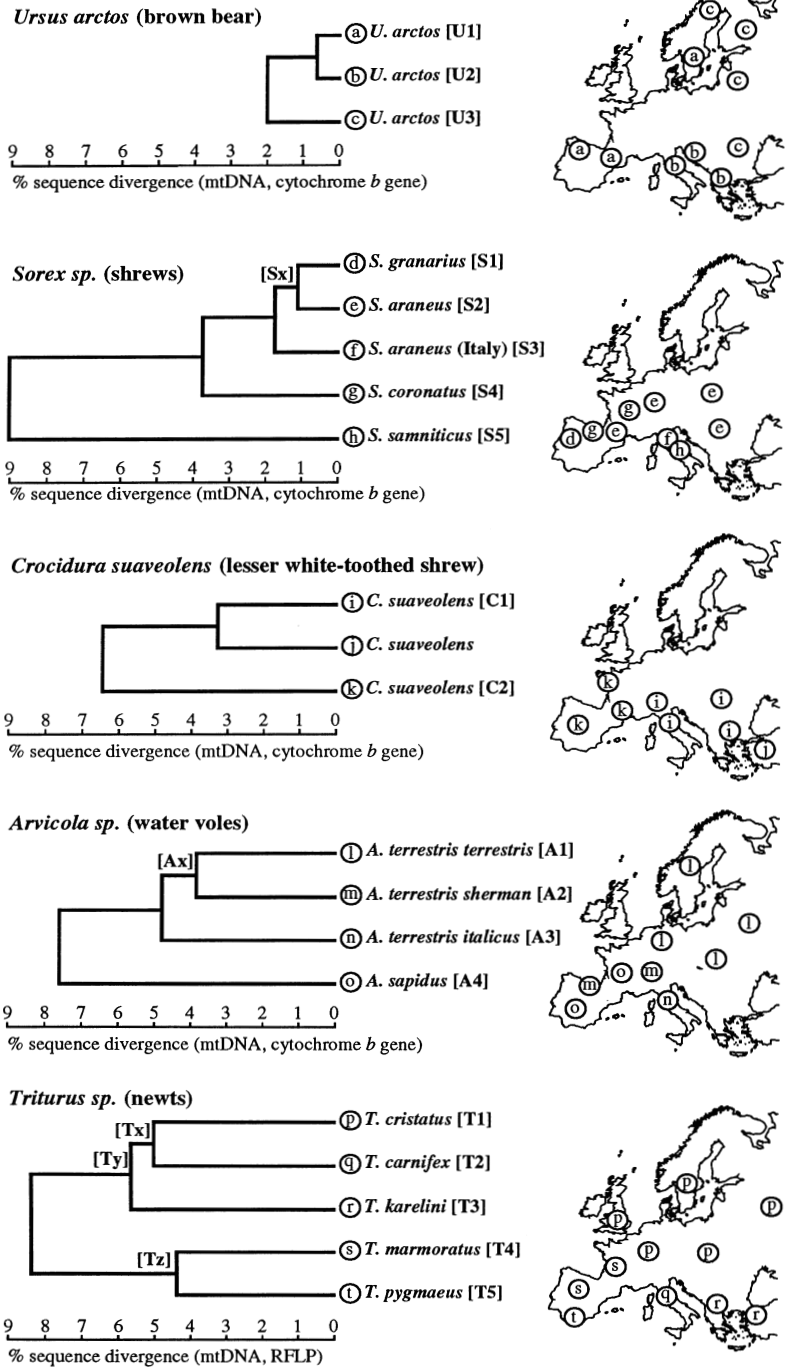


Fig. 2 Simplified phylogeographies of *Ursus arctos*, *Sorex* spp., *Crocidura suaveolens*, *Arvicola* spp., and *Triturus* spp. in Europe deduced from Taberlet & Bouvet (1994), Taberlet *et al.* (1994), Cosson *et al.* (unpublished), Wust-Saucy *et al.* (unpublished), and Wallis & Arntzen (1989), respectively. The lineages taken into account in the Brooks parsimony analysis are indicated in square brackets.

because it prefers cold climate and was not present in usual southern refugia (R1, R2 and R3 in Fig. 1). Isozyme polymorphism and fossil pollen analyses suggest postglacial westward colonization routes from three refugia in the present-day area of Moscow, in the Carpathians, and in the Dinaric Alps (Fig. 4) (Huntley & Birks 1983). Population bottlenecks in the two last refugia could account for the very low level of heterozygosity observed in Central Europe.

Fagus sylvatica (Demesure *et al.* 1996)

Common beech exhibits an outstanding homogeneity in chloroplast DNA amongst populations from northern Spain, France, Germany, Poland, and the Balkans. Only populations from Italy are distinct. The interpretation of these results together with fossil pollen data (Huntley & Birks 1983), which clearly localized the refugia in southern

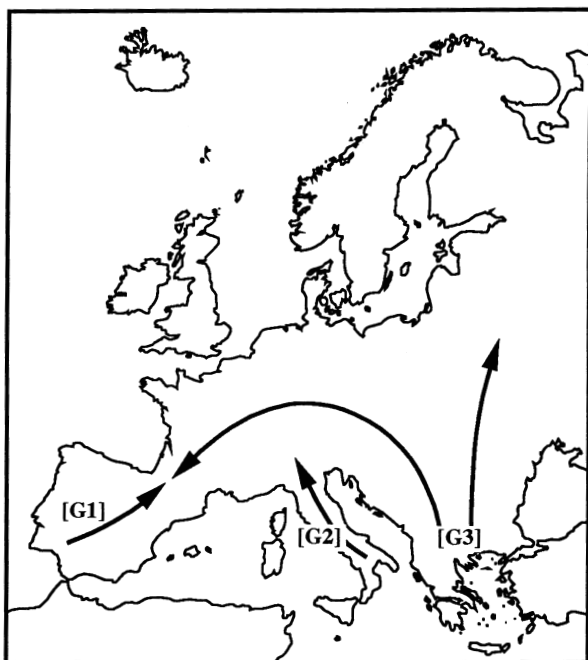
Chorthippus parallelus (grasshopper)

Fig. 3 Post-glacial colonization routes for *Chorthippus parallelus* (redrawn from Cooper *et al.* 1995). The lineages taken into account in the Brooks parsimony analysis are indicated in square brackets.

Italy and in the Balkans, allows us to infer the postglacial colonization routes (Fig. 4): all of the current geographical range except Italy was colonized from a refugium located in the Balkans (Carpathes), and the populations originating from a refugium in Calabria were confined to Italy.

Quercus spp. (Dumolin-Lapègue *et al.* 1997)

Study of chloroplast DNA variation in eight species of white oaks, which hybridize and share the same set of haplotypes, suggests some postglacial colonization routes originating from the three potential refugia in Spain, Italy, and the Balkans (Fig. 4). An extensive mixing of different lineages has been observed in the northern populations,

while some haplotypes did not expand outside of these refugia.

Results of the Brooks parsimony analysis

Five areas were taken into account for the Brooks parsimony analysis. Three of them correspond to the three potential refugia indicated in Fig. 1: Portugal–Spain, Italy, and the Balkans. Only two areas have been defined in the remaining regions and have been called France, and Germany–Poland, although they can include some other bordering areas. Due to the scarcity of available data, we were not able to consider a larger number of distinct areas, such as the British Isles, Fennoscandia, and the European portion of Russia. Furthermore, the lack of geographical accuracy of the available data set prevented us from defining more distinct areas in south-western and Central Europe. The lineages considered are all quoted in square brackets on Figs 2, 3, and 4. The construction of the data matrix (Table 2) involved some inevitable simplifications according to the chosen areas. For example, the lineage [T3] (*Triturus cristatus*) was not considered as present in France, despite its presence in the north-east portion of this area, and the lineage [F2] (*Fagus sylvatica*) was not considered as present in Italy, despite its limited occurrence in the northern part of the peninsula.

The parsimony analysis gave a single most parsimonious tree 39-steps long (consistency index: 0.821). The results are shown on an unrooted area phylogram where assigned branch lengths and bootstrap values (10 000 replicates) are indicated (Fig. 5). The grouping of Portugal–Spain/France vs. Germany–Poland/Italy/Balkans is supported by a bootstrap value of 97%. Another feature of this area phylogram is that Italy is connected via a very long branch length, whereas Portugal–Spain and the Balkans are connected via shorter branch lengths.

Discussion*Lack of congruence among phylogeographic patterns*

Ten taxa, including mammals, amphibians, insects, and plants, have been compared for phylogeographic patterns

Table 2 Data matrix of the Brooks parsimony analysis. The names of lineages or populations correspond to that indicated in square brackets in Figs 2, 3, and 4.

Areas	Lineages or populations																															
	U1	U2	U3	S1	S2	S3	S4	S5	Sx	C1	C2	A1	A2	A3	A4	Ax	T1	T2	T3	T4	T5	Tx	Ty	Tz	G1	G2	G3	F1	F2	Q1	Q2	Q3
Portugal–Spain	1	0	0	1	0	0	1	0	1	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	0
Italy	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0	1	0
Balkans	0	1	1	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	1
France	1	0	0	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0	1	1	1	1
Germany–Poland	1	0	1	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1

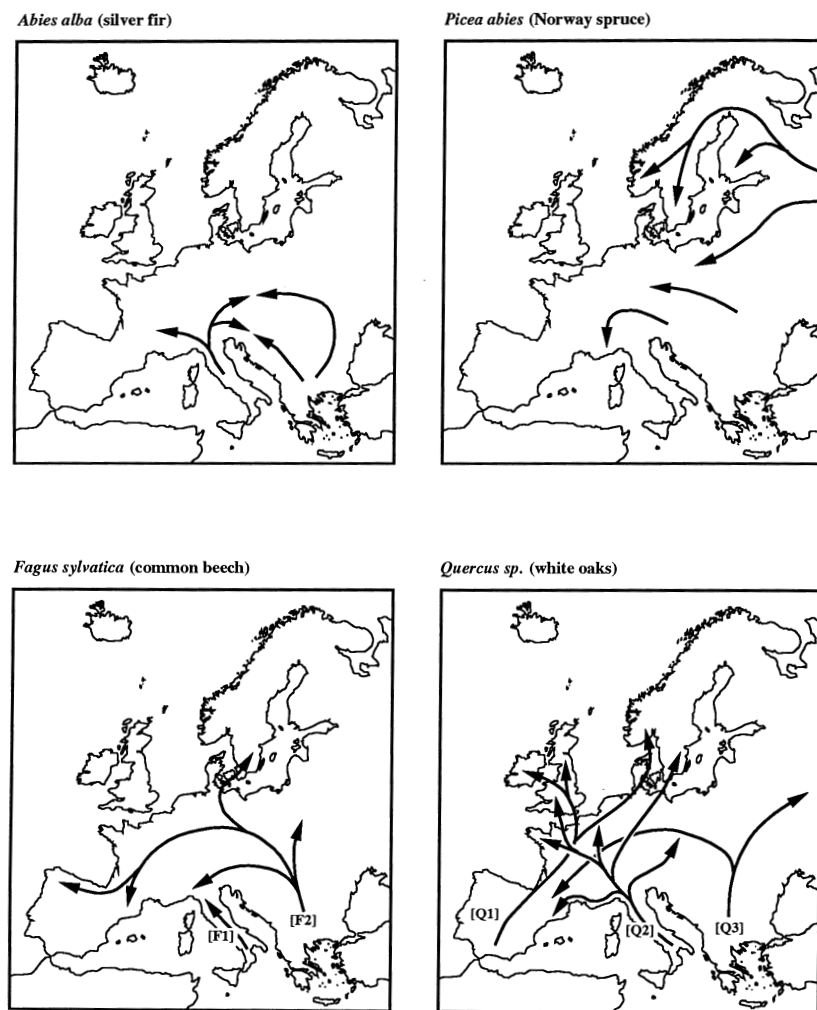


Fig. 4 Post-glacial colonization routes for four tree species: *Picea abies*, *Abies alba*, *Fagus sylvatica*, *Quercus* spp., deduced or redrawn from Lagercrantz & Ryman (1990), Konnert & Bergmann (1995), Demesure *et al.* (1996), and Dumoulin-Lapègue *et al.* (1997). The lineages or populations taken into account in the Brooks parsimony analysis are indicated in square brackets.

across Europe. The most striking result is the considerable dissimilarity among European-wide phylogeographic patterns, although the divergences amongst populations from the three refugia could be considered as a certain degree of concordance. It seems that each taxon has responded independently to Quaternary cold periods, and therefore is largely a unique case with its own history. For example, if we compare lineages present in Italy and in the Iberic peninsula, they are closely related in *Ursus* (less than 1% of sequence divergence in the cytochrome *b* gene), but much more distantly related in *Crocidura* (6.4%), in *Arvicola* (7.6%), and in *Triturus* (8.5%), while the *Sorex* species considered here exhibit two lineages in each of these two refugia. Populations occurring in France come either from a refugium in the Iberic peninsula (e.g. *Arvicola sapidus*, *Triturus marmoratus*), or from a refugium in the Balkans (e.g. *Chorthippus parallelus*, *Fagus sylvaticus*).

Although some spatial congruence has previously been found elsewhere in some restricted areas (Avisé 1992;

Bermingham & Avisé 1986; Joseph *et al.* 1995), the results obtained in Europe and in North America (Zink 1996) suggest that congruence is the exception at the continental scale. The consequence of an independent history for each taxon is that assemblages of plants and animals comprising particular communities are not stable over time, an observation consistent with previous findings based mainly on fossil pollen data (Bennett 1990).

In the context of repeated contraction/expansion of geographical ranges during Quaternary climatic fluctuations, the differing rates of colonization amongst taxa probably played an important role and could be at least partially responsible for the incongruences. It is likely that the first population colonizing a particular region can prevent the arrival of another one because the space is already occupied, and introgression or elimination by competition is always much slower than colonization of free areas. Extinction of a population in a potential refugium during a cold period, followed by a recolonization by another one, could also explain some incongruences.

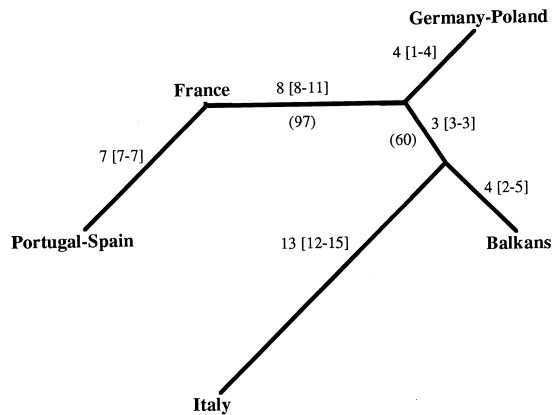


Fig. 5 Unrooted area phylogram illustrating the results of the Brooks parsimony analysis for 32 different European lineages or populations. Assigned branch lengths are given above each branch with the minimum and maximum possible lengths in square brackets. Bootstrap values are indicated in brackets.

The depth of intraspecific phylogenies

In our data set, we were able to estimate the depth of intraspecific phylogenies between populations from two refugia in Portugal–Spain and in the Balkans, for three taxa. These depths in mtDNA sequence divergence for *Ursus arctos*, *Crocidura suaveolens*, and *Arvicola* sp. are 2%, 6.4%, and 7.6%, respectively (Fig. 2). Assuming approximately equal rates of mtDNA sequence divergence as a working hypothesis (2% per Myr; Wilson *et al.* 1985), then if the depth in phylogenies was related to a common event, we may expect divergence times dating from the beginning of the strong climatic oscillations, i.e. 0.7 Mya. Our results do not reveal any evidence of a common time of divergence within these three taxa, except maybe for *C. suaveolens* and *Arvicola* spp. A divergence of 6.4% or 7.6% in the cytochrome *b* gene sequence for these two taxa even indicates a pre-Pleistocene population split, showing that Quaternary cold periods are unlikely to be a cause of the divergence. Fortunately, palaeontological data are available for these three species, indicating that *U. arctos* arrived in Europe 0.91–0.98 Mya (Mazza & Rustioni 1994), *Mymomys davakosi*, the ancestor of *Arvicola*, was present 4.0–3.4 Mya (Chaline & Graf 1988; Chaline *et al.* 1993), while *C. suaveolens* was known on the continent from the Plio-Pleistocene (Reumer 1983), i.e. approx. 2 Mya. One can imagine that populations of each species began to diverge among the three (subsequently refugium) areas, even as early as the date of first arrival of the species in Europe. The divergence may have occurred simply due to isolation by distance among Portugal–Spain, Italy, and the Balkans. The subsequent range contractions and expansions due to climatic oscillations may have eliminated only haplotypes present in northern areas, without affecting

those present in the refugia. Assuming no retention of ancestral polymorphism from the date of arrival, this scenario suggests that the depth of the deepest intraspecific divergences are related simply to the date of arrival of each taxon in the European continent, rather than to any subsequent historical event. This could also explain why no congruence was found in the dates of divergence even in cases of congruent phylogeographic patterns. Indeed, several authors (Avice 1992; Bermingham & Avice 1986; Joseph *et al.* 1995) pointed out the discrepancy in divergence date among lineages, and explained this either by a considerable heterogeneity in mtDNA evolutionary rates, or by the fact that the species reacted differently to the separate episodes of climatic variations (i.e. the more ancient the split, the more ancient the historical event involved in the divergence). We argue instead that, in the case of phylogeographies that are congruent due to the same historical event, the divergence times amongst lineages are expected to be different, and lie somewhere between the date of colonization and the date of the historical vicariant event. Consequently, the deepest divergence times between congruent intraspecific phylogeographies are unlikely to accurately date the historical event responsible for that congruence, although they may provide a minimum age.

Recently, several studies have emphasized that speciation events occurred mainly during the Pliocene (Bermingham *et al.* 1992; Zink & Slowinski 1995). The present study shows that this observation can be extended to the intraspecific level, and that many lineages within the same species can also stem from the Pliocene. In our data set, only the genus *Sorex* could have speciated during the Quaternary, but this could be related more to chromosomal incompatibilities rather than to genetic differentiation via gradual molecular divergence (e.g. Hausser 1994).

Refugia, colonization routes, and suture zones

Almost all the considered taxa used the three potential refuge areas in the south during Quaternary cold periods: the Iberic peninsula, Italy, and the Balkans. Only the Norway spruce and the common beech failed to follow this rule. The Norway spruce, a tree from a boreal climate, did not survive in the southernmost regions, but only in suitable habitats in the Dinaric Alps, in the Carpathes, and in the present-day area of Moscow (Huntley & Birks 1983; Lagercrantz & Ryman 1990). The common beech, a tree from a temperate climate, found refuge in the south as did other taxa, but it seems that it was not able to survive in the Iberic refugium; as a consequence, all the western part of the geographical distribution was colonized from the Balkans (Demesure *et al.* 1996). As predicted by the expansion/contraction model, a much higher intraspecific diversity in the southern areas than in recently colonized

northern regions has been documented for *Triturus carnifex* in Italy and *T. karelini* in the Balkans (Wallis & Arntzen 1989), for *Chorthippus parallelus* in the Balkans (Cooper *et al.* 1995), and for *Quercus* spp. and *Fagus sylvatica* in Italy (Demesure *et al.* 1996; Dumolin-Lapègue *et al.* 1997).

Although no evidence of common phylogeographic histories across Europe have been found, postglacial colonization routes exhibit some interesting concordances. The colonization route out of an Iberic refugium towards the south of Scandinavia seems to be identical for the brown bear and the white oaks, and the spread from a Balkanic refugium towards the southeast of France is similar for the grasshopper and the common beech (Figs 2, 3, and 4). Furthermore, the barrier of the Alps prevented the northward expansion of populations isolated in the Italian refugium for at least four taxa in our data set.

Even if some concordances in colonization routes are apparent, the dissimilarity of the global phylogeographic patterns makes the elaboration of a synthetic view difficult. In this regard, the Brooks parsimony analysis is valuable in that it integrates all the available data to produce the single picture presented in Fig. 5. The long branch length to Italy indicates how the scale of the barrier of the Alps, and the isolation of the Italian peninsula. Consequently, many lineages at the European level are present only in this area (Figs 2, 3, and 4). Italy and the Balkans form a clade, due probably to their geographical proximity, and to the connection across the Adriatic sea between these two refugia when the sea level was lower during cold periods (see Fig. 1). Shorter branch lengths to Portugal–Spain and to the Balkans convey the fact that northern areas were colonized generally from these two refugia. Although the Brooks parsimony analysis has been criticized in the field of historical biogeography (see Morrone & Crisci 1995), we feel that this approach is useful in our case by giving a consistent overview of a complex data set. Furthermore, using the parsimony approach, it is possible to produce not only a branching pattern, but also branch lengths which provide a valuable information concerning the degree of isolation of a particular region.

The term suture-zone has been introduced by Remington (1968) to describe 'a band, whether narrow or broad, of geographical overlap between major biotic assemblages, including some pairs of species or semispecies which hybridize in the zone'. The emergence of this term is due to the observation that hybrid zones are often concentrated within limited areas. This concept could easily be extended to the intraspecific level (see Jaarola & Tegelström 1995) to characterize areas where different populations meet after a postglacial expansion. In Europe four main suture-zones may be recognized (Fig. 6). The most obvious one corresponds to the Alpine barrier which

separates lineages occurring in Italy from lineages distributed in the west and in the north of the Alps. Many examples can be cited to illustrate this discontinuity: *Sorex araneus* (Taberlet *et al.* 1994), *Arvicola terrestris* (Wust-Saucy *et al.* 1997), *Triturus* sp. (Wallis & Arntzen 1989), *Salmo trutta* (Bernatchez *et al.* 1992), *C. parallelus* (Cooper *et al.* 1995), and *Apis mellifera* (Garnery *et al.* 1992). However, some Italian lineages of the white oaks were able to cross the Alps and to spread north-, east-, and westward (Dumolin-Lapègue *et al.* 1997). The second suture-zone corresponds to the junction between populations of the Iberic refugium with populations of eastern origin (the Balkanic refugium, or another easternmost refugium) somewhere around the border between France and Germany. As this region is relatively flat, without any substantial topographic barrier, this suture zone is not localized exactly in the same place for different organisms: it occurs in the northeast of France between *Arvicola sapidus*/*A. terrestris*, and *Triturus marmoratus*/*T. cristatus*, and in a more eastern position for *Sorex coronatus*/*S. araneus*. Previous morphological studies highlighted a suture zone in the same area, for instance for the ringed snake, *Natrix natrix* (Thorpe 1979). The third suture-zone is represented by the Pyrenees, and could be considered as a particular case of the second. In our dataset, only *C. parallelus* illustrates this Pyrenean suture-zone, but genetic data for the warbler *Phylloscopus collybita* (Helbig *et al.* 1993) also support it. The last suture-zone is located in



Fig. 6 Main postglacial colonization routes and subsequent suture zones in Europe.

Scandinavia, and indicates that this area may have been colonized from the north and from the south by different populations originating from different refugia. So far, four mammalian species have been shown to support this suture zone: *Sorex araneus* (Fredga & Nawrin 1977), *Clethrionomys glareolus* (Tegelström 1987), *Microtus agrestis* (Jaarola & Tegelström 1995), and *Ursus arctos* (Taberlet *et al.* 1995). As a consequence, the genetic diversity in Scandinavia may be higher than expected.

Conclusions and perspectives

Previous studies have shown that the Quaternary in Europe was characterized by many cycles of contraction/expansion of geographical ranges according to climatic fluctuations: contraction of ranges to southern regions during cold periods, and expansion from the leading edges during subsequent warmings (Hewitt 1996). Using this model, one can predict that: (i) the highest diversity should be found amongst the southern regions; and (ii) the distribution of intraspecific polymorphism in northern regions should be dictated by the colonization routes used from the refugia. Clearly, the available phylogeographic data confirm both predictions and strongly support the contraction/expansion model.

Although this study revealed no evidence of common phylogeographies amongst the 10 taxa compared, some general trends can be drawn. The current distribution of intraspecific polymorphism in Europe can be explained by the persistence of each taxon in three refugia (Fig. 1) and the subsequent colonization routes used (Fig. 6). The northern part of Europe has been colonized primarily from the Iberic and the Balkan refugia, whilst populations evolving in Italy were not usually able to spread northward due to the Alpine Barrier. The comparison of colonization routes suggested four main suture-zones where populations from different refugia meet (Fig. 6). However, it is noteworthy that the few data currently available for eastern Europe and Fennoscandia may underestimate the contribution of potential easternmost refugia localized in Europe and/or Asia.

These general trends have implications for conservation genetics, highlighting areas where conservation efforts should be concentrated. First, Italy has many endemic lineages. Second, the southern regions in general, where refugia are localized, are of particular interest: they support most of the current genetic variation, and for long-term conservation the preservation of genetic diversity in these areas seems desirable.

Traditionally, the history of species and communities over the last few million years was studied by using fossils and pollen data. As a result, the influence of Quaternary climatic oscillation has been estimated, and a general picture concerning the changes in faunas and

floras has been put forward. Molecular genetics can describe intraspecific geographical structure by identifying lineages, and consequently can reveal postglacial colonization routes provided that the location of the refugia are known. Palaeontology and palynology are not able to discern intraspecific phylogeographic structure and, in that, the molecular approach clearly provides an advance. However, molecular genetics based on polymorphism present in extant organisms cannot by itself localize refugia with precision. Furthermore, and contrary to widespread current belief, molecular genetic studies may be of limited use in dating a vicariant event that occurred during the Quaternary. Indeed, the intraspecific divergence amongst mtDNA lineages appears to often largely predate the Quaternary cold periods.

This study is a first attempt to synthesize our current knowledge, based on the limited number of available data sets. Although incomplete, it will help to define the appropriate scope for future phylogeographic studies in this region, and to design more pertinent sampling strategies. Advances in the field of comparative phylogeography will clearly come from a better integration of several complementary approaches including genetics, ecology, palaeontology, palynology, and climatology.

Acknowledgements

We wish to thank E. Bermingham, S. Lavery, C. Moritz, C. Schneider, L. Waits, and two anonymous reviewers for valuable comments on this work. We also thank R. Petit, B. Demesure, and S. Dumolin-Lapègue for kindly giving us submitted and in-press manuscripts. This study was partly funded by the Université Joseph Fourier (Grenoble, France), the Centre National de la Recherche Scientifique, the Institut National de la Recherche Agronomique, and was undertaken as part of an agreement in research collaboration between the Université Joseph Fourier and the Université de Lausanne. L.F. was supported by a post-doctoral fellowship of the Swiss National Science Foundation and the Agassiz Foundation. A.-G.W.-S. was supported by a Heim-Vöglin grant of the Swiss National Science Foundation.

References

- Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, **63**, 62–76.
- Avise JC, Arnold J, Ball RM, *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.
- Bennett KD (1986) The rate of spread and population increase of forest trees during the postglacial. *Philosophical Transactions of the Royal Society of London Series B*, **314**, 523–531.
- Bennett KD (1990) Milankovitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology*, **16**, 11–21.
- Bennett KD, Tzedakis PC, Willis KJ (1991) Quaternary refugia of

- north European trees. *Journal of Biogeography*, **18**, 103–115.
- Bermingham E, Avise JC (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113**, 939–965.
- Bermingham E, Rohwer S, Freeman S, Wood C (1992) Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 6624–6628.
- Bernatchez L, Guymard R, Bonhomme F (1992) DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Molecular Ecology*, **1**, 161–173.
- Bond G, Broecker W, Johnsen S, *et al.* (1993) Correlations between climate records from North Atlantic sediments and Greenland ice. *Nature*, **365**, 143–147.
- Brooks DR (1985) Historical ecology: a new approach to studying the evolution of ecological associations. *Annals of the Missouri Botanical Garden*, **72**, 660–680.
- Brooks DR (1990) Parsimony analysis in historical biogeography and coevolution: methodological and theoretical update. *Systematic Zoology*, **39**, 14–30.
- Chaline J, Graf J-D (1988) Phylogeny of the Arvicolidae (Rodentia): biochemical and paleontological evidence. *Journal of Mammalogy*, **69**, 22–33.
- Chaline J, Laurin B, Brunet-Lecomte P, Viriot L (1993) Morphological trends and rates of evolution in Arvicolids (Arvicolidae, Rodentia): towards a punctuated equilibria/disequilibria model. *Quaternary International*, **19**, 27–39.
- Cooper SJ, Ibrahim KM, Hewitt GM (1995) Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. *Molecular Ecology*, **4**, 49–60.
- Dansgaard W, Johnsen SJ, Clausen HB, *et al.* (1993) Evidence for general instability of past climate from a 250-kyr ice-core record. *Nature*, **364**, 218–220.
- De Candolle AP (1820) Géographie botanique. In: *Dictionnaire des Sciences Naturelles*, pp. 359–422.
- Demesure B, Comps B, Petit RJ (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution*, **50**, 2515–2520.
- Dumolin-Lapègue S, Demesure B, Fineschi S, Le Corre V, Petit RJ (1997) Phylogeographic structure of white oaks throughout the European continent. *Genetics*, **146**, 1475–1487.
- Estoup A, Solignac M, Cornuet J-M, Goudet J, Scholl A (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Molecular Ecology*, **5**, 19–31.
- Fredga K, Nawrin J (1977) Karyotype variability in *Sorex araneus* L. (Insectivora, Mammalia). In: *Chromosome Today* (eds DeLaChapelle A, Sorsa M), pp. 153–161. Elsevier/North Holland Biomedical Press, Amsterdam.
- Frenzel B, Pécsi M, Velichko AA (1992) *Atlas of Paleoclimates And Paleoenvironments Of The Northern Hemisphere. Late Pleistocene–Holocene*. Geographical Research Institute, Hungarian Academy of Sciences. Gustav Fischer Verlag, Budapest, Stuttgart.
- Garnery L, Cornuet J-M, Solignac M (1992) Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Molecular Ecology*, **1**, 145–154.
- Garnery L, Mosshine EH, Oldroyd BP, Cornuet J-M (1995) Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. *Molecular Ecology*, **4**, 465–471.
- GRIP Members (1993) Climate instability during the last interglacial period recorded in the GRIP ice core. *Nature*, **364**, 203–207.
- Hardy C, Callou C, Vigne J-D, *et al.* (1995) Rabbit mitochondrial DNA diversity from prehistoric to modern times. *Journal of Molecular Evolution*, **40**, 227–237.
- Hausser J (1994) The *Sorex* of the *araneus-arcticus* group (Mammalia: Soricidae): do they actually speciate? In: *Advances in the Biology Of Shrews* (eds Merritt JF, Kirkland GLJ, Rose RK), pp. 295–306. Carnegie Museum of Natural History, Special publication 18, Pittsburgh.
- Hays JD, Imbrie J, Shackleton NJ (1976) Variations in the Earth's orbit: pacemaker of the ice ages. *Science*, **194**, 1121–1132.
- Helbig J, Salomon M, Wink M, Bried J (1993) Absence de flux génétique mitochondrial entre les Pouillots 'véloces' médio-européen et ibérique (Aves: *Phylloscopus collybita collybita*, P. (c.) *brehmii*); implications taxinomiques. Résultats tirés de la PCR et du séquençage d'ADN. *Comptes Rendus de l'Académie des Sciences Série III*, **316**, 205–210.
- 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.
- Huntley B, Birks HJB (1983) *An Atlas Of Past And Present Pollen Maps For Europe: 0–13000 Years Ago*. Cambridge University Press, Cambridge.
- Jaarola M, Tegelström H (1995) Colonization history of north European field voles (*Microtus agrestis*) revealed by mitochondrial DNA. *Molecular Ecology*, **4**, 299–310.
- Joseph L, Moritz C, Huggall A (1995) Molecular support for vicariance as a source of diversity in rainforest. *Proceedings of the Royal Society of London Series B*, **260**, 177–182.
- Kohn M, Knauer F, Stoffella A, Schröder W, Pääbo S (1995) Conservation genetics of the European brown bear – a study using excremental PCR of nuclear and mitochondrial sequences. *Molecular Ecology*, **4**, 95–103.
- Konnert M, Bergmann F (1995) The geographical distribution of genetic variation of silver fir (*Abies alba*, Pinaceae) in relation to its migration history. *Plant Systematics and Evolution*, **196**, 19–30.
- Lagercrantz U, Ryman N (1990) Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. *Evolution*, **44**, 38–53.
- Lundqvist J, Saarnisto M (1995) Summary of project IGCP-253. *Quaternary International*, **28**, 9–18.
- Mazza P, Rustioni M (1994) On the phylogeny of Eurasian bears. *Palaeontographica Abstracts A*, **230**, 1–38.
- Morrone JJ, Crisci JV (1995) Historical biogeography: introduction to methods. *Annual Review of Ecology and Systematics*, **26**, 373–401.
- Randi E, Gentile L, Boscagli G, Huber D, Roth HU (1994) Mitochondrial DNA sequence divergence among some west European brown bear (*Ursus arctos* L.) populations. Lessons for conservation. *Heredity*, **73**, 480–489.
- Remington CL (1968) Suture-zones of hybrid interaction between recently joined biotas. *Evolutionary Biology*, **2**, 321–428.
- Reumer JWF (1983) Ruscian and early Pleistocene Soricidae (Insectivora, Mammalia) from Tegele (The Netherlands) and Hungary. PhD thesis, University of Leiden.
- Roy K, Valentine JW, Jablonski D, Kidwell SM (1996) Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. *Trends in Ecology and Evolution*, **11**, 458–453.

- Smith DR, Palopoli MF, Taylor BR, *et al.* (1991) Geographical overlap of two mitochondrial genomes in Spanish honeybees (*Apis mellifera iberica*). *Journal of Heredity*, **82**, 96–100.
- Swofford DL (1993) *Phylogenetic Analysis Using Parsimony Version 3.1.1*. Illinois Natural History Survey, Champaign, Illinois.
- Taberlet P, Bouvet J (1992) Génétique de l'Ours brun des Pyrénées (*Ursus arctos*): premiers résultats. *Comptes Rendus de l'Académie des Sciences Série III*, **314**, 15–21.
- Taberlet P, Bouvet J (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear (*Ursus arctos*) in Europe. *Proceedings of the Royal Society of London Series B*, **255**, 195–200.
- Taberlet P, Fumagalli L, Hausser J (1994) Chromosomal versus mitochondrial DNA evolution: tracking the evolutionary history of the southwestern European populations of the *Sorex araneus* group (Mammalia, Insectivora). *Evolution*, **48**, 623–636.
- Taberlet P, Swenson JE, Sandegren F, Bjärvall A (1995) Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear (*Ursus arctos*) in Scandinavia. *Conservation Biology*, **9**, 1255–1261.
- Tegelström H (1987) Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). *Journal of Molecular Evolution*, **24**, 218–227.
- Thomaz D, Guiller A, Clarke B (1996) Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society of London Series B*, **263**, 363–368.
- Thorpe RS (1979) Multivariate analysis of the population systematics of the ringed snake, *Natrix natrix* (L.). *Proceedings of the Royal Society of Edinburgh*, **78B**, 1–62.
- Wallis GP, Arntzen JW (1989) Mitochondrial-DNA variation in the crested newt superspecies: limited cytoplasmic gene flow among species. *Evolution*, **43**, 88–104.
- Webb T, Bartlein PJ (1992) Global changes during the last 3 million years: climatic controls and biotic response. *Annual Review of Ecology and Systematics*, **23**, 141–173.
- Wiley EO (1988a) Parsimony analysis and vicariance biogeography. *Systematic Zoology*, **37**, 271–290.
- Wiley EO (1988b) Vicariance biogeography. *Annual Review of Ecology and Systematics*, **19**, 513–542.
- Wilson AC, Cann RL, Carr SM, *et al.* (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26**, 375–400.
- Zink RM (1996) Comparative phylogeography in North American birds. *Evolution*, **50**, 308–317.
- Zink RM, Slowinski JB (1995) Evidence from molecular systematics for decreased avian diversification in the Pleistocene Epoch. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 5832–5835.

This synthesis forms part of a continuing programme on intraspecific phylogeographic studies of animals and plants carried out in the Laboratoire de Biologie des Populations d'Altitude (Grenoble, France). The four authors are currently working in four different laboratories, but share a common interest in studying intraspecific phylogeographies in Europe. Pierre Taberlet is also involved in conservation genetics and in developing new molecular techniques for population studies; Luca Fumagalli is working in the fields of conservation genetics and molecular evolution; Anne-Gabrielle Wust-Saucy is studying the phylogeography of the genus *Arvicola*; Jean-François Cosson recently joined the Laboratoire 'Génome and Populations' (Montpellier, France) and is interested in the phylogeography of the genus *Crocodyra*.
