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Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence

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Abstract

The geographic distribution throughout Europe of each of 32 chloroplast DNA variants belonging to eight white oak species sampled from 2613 populations is presented. Clear-cut geographic patterns were revealed by the survey. These distributions, together with the available palynological information, were used to infer colonisation routes out of the glacial period refugia. In western Europe in particular, movements out of the Iberian and the Italian Peninsulas can be clearly identified. Separate refugia are also present in eastern Balkans, whereas further west in this peninsula similarities with Italy were evident. Movements resulting in the exchange of haplotypes between refugia both during the present interglacial and probably also during earlier glacial cycles were therefore inferred. The consequences of these past exchanges is that phylogenetically divergent haplotypes have sometimes followed very similar colonisation routes, limiting somewhat the phylogeographic structure. Cases of geographic disjunction in the present-day distribution of haplotypes are also apparent and could have been induced by the existence of rapid climatic changes at the end of the glacial period (specifically the Younger Dryas cold period), which resulted in range restriction following an early warm period during which oak first expanded from its primary refugia. This cold phase was followed by a new period of expansion at the outset of the Holocene, involving in some cases 'secondary' refugia. It is expected that these short climate oscillations would have led to a partial reshuffling of haplotype distribution. Early association between haplotypes and oak species are also suggested by the data, although extensive introgression among species has ultimately largely blurred the pattern. This implies that colonisation routes may have been initially constrained by the ecological characteristics of the species hosting each chloroplast variant. We suggest for instance that two oak species distributed in the north of the Iberian Peninsula (*Quercus petraea* and *Q. pubescens*) are recent post-glacial immigrants there. When considered together, conclusions on the location of glacial period refugia and the colonisation routes derived from molecular information and fossil pollen data appear to be both largely compatible and complementary.

Keywords: Fossil pollen; Glacial period refugia; Phylogeography; *Quercus* sp.

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1. Introduction

Progress in our understanding of palaeoclimates, in particular that of the Pleistocene ice ages, together with the development of molecular markers for ecological studies, are leading to improved reconstruction of the past distribution of the European biota (Hewitt, 1999). For oaks, Brewer et al. (2002) have described the progress made in synthesising the location of refugia as well as post-glacial recolonisation dynamics using available pollen evidence. Simultaneously, molecular markers for the cytoplasmic genomes (chloroplast and mitochondria) have been applied to understand the recent history of these species. These genomes are known to be maternally inherited in oaks (i.e. transmitted by seeds only; Dumolin et al., 1995) and are therefore appropriate to track colonisation routes. As described in a previous paper in this issue (Petit et al., 2002a), an unprecedented effort to analyse a very large set of populations for chloroplast DNA (cpDNA) polymorphism, has been carried out by a consortium grouping 16 European laboratories. A total of 32 cpDNA haplotypes were described by PCR–RFLP from a sample of 11,937 trees belonging to 2613 populations of eight European white oak species. The phylogenetic relationships between these haplotypes have been described and patterns of diversity were studied as a function of the region and the species (Petit et al., 2002a).

In the present paper, attempts are made to identify refugia and colonisation routes by combining information from two sources: the distribution maps of individual chloroplast haplotypes and the fossil pollen sequences archived in the European Pollen Database (Brewer et al., 2002).

The considerable economic and ecological importance of oaks in Europe, and the practical consequences of large-scale surveys using molecular markers for future applications such as tracing the geographic origin of seedlots, identifying the source of plantations, checking the autochthony of oak woods or forests for conservation purposes or even tracing the origin of wood products (Dumolin-Lapègue et al., 1999b) would suffice to justify the efforts of the teams involved in the genetic analyses of these species. There are however several other reasons that explain why oaks have became popular for such studies.

Firstly, they are characterised by low levels of within population cpDNA diversity but relatively high levels of overall diversity and high population differentiation ($G_{ST} = 0.78-0.96$, depending on species, Petit et al., 2002a). Hence, a few individuals will generally be sufficient to permit characterisation of a population, which allows allocation of most of the sampling effort to recovery of the spatial structure rather than to characterisation of each population.

Secondly, they are widely distributed, and due to extensive introgressive hybridisation, the whole complex of species forms a suitable subject for investigations, potentially extending from northern Africa to the Caucasus and the Middle East, so that many different biogeographic questions can be tackled using the methods of intraspecific phylogeography.

Thirdly, they are abundant (and hence easy to sample) wind-pollinated species and prolific pollen producers. Their pollen may be retrieved from ancient sediments, which means that in addition to the molecular data, precise information can be independently obtained on their glacial period refugia, timing and speed of recolonisation and to some extent, recolonisation routes (Huntley and Birks, 1983; Brewer et al., 2002).

Fourthly, oaks represent key species, which shape the ecosystem and that host a particularly rich assemblage of other species (Speight and Wainhouse, 1989), because of their abundance and wide distribution (Kelly and Southwood, 1999). As a consequence, other organisms may have migrated with oaks during their post-glacial recolonisation, resulting in similar genetic partitioning across their ranges.

Despite these advantages, there are some limitations that should be taken into account in attempts to uncover the post-glacial history of oaks. The early recolonisation of oaks, compared to later migrants such as chestnut (*Castanea sativa*), walnut (*Juglans regia*), beech (*Fagus sylvatica*) or hornbeam (*Carpinus betulus*) (Huntley and Birks, 1983), means that comparatively less information will be available on the initial phase of their dispersal, because the pollen records are less numerous for the end of the glacial period and the early part of the Holocene (Brewer et al., 2002). In addition, their acorns have long constituted an important component of the diet of prehistoric populations (Lieutaghi, 1998), which could complicate the interpretations of post-glacial movements. Potential long-distance seed transfer and plantations during historical times should also be kept in mind when sampling present-day populations for molecular studies. Further information on the importance of artificial transfers and plantations with non-autochthonous material can be found in other papers in this issue, especially König et al. (2002), Jensen et al. (2002), and Cottrell et al. (2002). Finally, due to extensive cpDNA introgression between species (i.e., 'pollen swamping', Petit et al., 1997), early species-specific patterns of recolonisation may now be difficult to discern, although some footprints of these early associations may still be visible in favourable situations. It is also worth stressing at this point some of the discrepancies between the two data-sets, which must be taken into account in forming a comparative study. The data from the study of the cpDNA is based on modern material that may be precisely identified at the species level, and precisely located geographically. In contrast, whilst pollen studies provide thousands of years of vegetation history, the pollen of the oak is rarely identified to a taxonomic level higher than that of 'deciduous *Quercus*'. Further, the dispersal of the pollen grains limits the geographical precision of these studies (see Brewer et al., 2002, for further details). We have endeavoured to take into account these discrepancies whilst interpreting the data.

In this synthesis paper we will focus mainly on those results of significance at the continental level. More detailed analyses and interpretations are available for each of nine European regions (the Iberian Peninsula, Italy and Corsica, the northern Balkans, France, Great Britain, the Alpine region, Scandinavia, central Europe (Germany, Belgium and the Netherlands, and the Czech Republic), and Poland and the Baltic countries (see other papers in this issue)). Our goal is to integrate the molecular results with those of the joint palaeobotanical investigation (Brewer et al., 2002), in order to identify convergent interpretations or issues requiring further investigations. We therefore focus on the identification of glacial period refugia and natural routes of colonisation. We further consider some possible association between cpDNA lineages and oak species that may have existed at the outset of the present interglacial, in order to cast some light on the present distribution of oak species in Europe and to better interpret the migration patterns exhibited.

2. Material and methods

The distribution of samples and methods used to investigate cpDNA polymorphisms are described in Petit et al. (2002a). Maps of cpDNA variation were produced using the software MapInfo Professional Version 3.5. First, maps representing the distribution of individual haplotypes and oak species have been prepared (Fig. 1a–j). As shown in Dumolin-Lapègue et al. (1997) and in Petit et al. (2002a), few new haplotypes seem to have appeared by mutation during recolonisation, thus the haplotypes identified can be used to track movements of populations during post-glacial time (the so-called post-glacial recolonisation routes). Our interpretations of these recolonisation routes are provided in additional maps with arrows indicating the origin and direction of migration of individual lineages (Fig. 2A–C). High altitude areas may have acted as barriers to oak colonisation, and it is likely that oak populations could only survive in mountainous regions during the last glacial period (see Brewer et al., 2002 and the discussion below). Moreover, at the outset of recolonisation, landbridges existed between areas now separated by seas. Hence, the maps indicating the colonisation routes have been overlaid upon maps of relief (Row et al., 1995) and past sea levels. These latter maps were produced using the global gridded Pliocene and Quaternary sea-level data-set produced by Thompson and Schweitzer (1996). This data-set gives percentages of land above sea-level on a global 1° grid at 1000 year intervals. Three time periods were selected: 21 ka BP (18 ^{14}C ka BP), 15 ka BP (13 ^{14}C ka BP) and 12 ka BP (10 ^{14}C ka BP). These dates represent, respectively, the last glacial maximum, the start of the late-glacial interstadial, and the start of the Holocene period. The dates in brackets are the radiocarbon ages for these time periods. These are given to allow a comparison with the pollen maps that are all based on a radiocarbon timescale. Each data-set was interpolated onto a 15 min grid using bicubic interpolation, in order to obtain relatively smooth contours. A single contour was produced at 50% land availability because this contour provided the best fit between the modern data-set and the modern coastline. As the base data-set is at a low resolution, the resulting maps are relatively crude. For example, it should be noted that the Mediterranean was never completely closed during the glacial period. Corsica was above sea-level at all times, but was never attached to the Italian Peninsula.

The routes of recolonisation drawn here represent only one possible interpretation of the data gathered. Some of the alternative scenarios are described in the text. We generally favour interpretations of northward migration, although migration southward cannot be a priori excluded and appears to have occurred in several situations. Nevertheless, due to typical latitudinal effects and to the severe conditions characterising most of Europe during the last glacial period, it seems highly unlikely that more northern populations could have survived while appropriate sites located further south were devoid of the species (e.g., mountainous habitats).

3. Results and discussion

The distribution of the 32 haplotypes detected in this survey (described in Petit et al., 2002a) is shown in Fig. 1a–j. For most of them, specific hypotheses on post-glacial movements have been formulated as described in Section 2 (Fig. 2A–C). The main findings are described and possible interpretations are given below. Haplotypes are grouped according to their inferred refugial area and then by lineage. Frequent reference is made to their phylogenetic relationships presented in the previous paper (Petit et al., 2002a).

3.1. Haplotypes originating from the Iberian Peninsula

Four of the six chloroplast lineages recognised so far in Europe (A–D, Petit et al., 2002a) are represented in the Iberian Peninsula (Olalde et al., 2002). However, two of them are restricted to the north-east of the region and are only represented by a single haplotype (lineage C with haplotype 1, and lineage A with haplotype 7) and are described in the sections detailing the Italian and Balkan Peninsulas, respectively. In contrast, lineages B and D are well represented within the Iberian Peninsula (Olalde et al., 2002). These two lineages have very distinct, non-overlapping distributions. Haplotypes of lineage B occur to the west of a line running roughly from

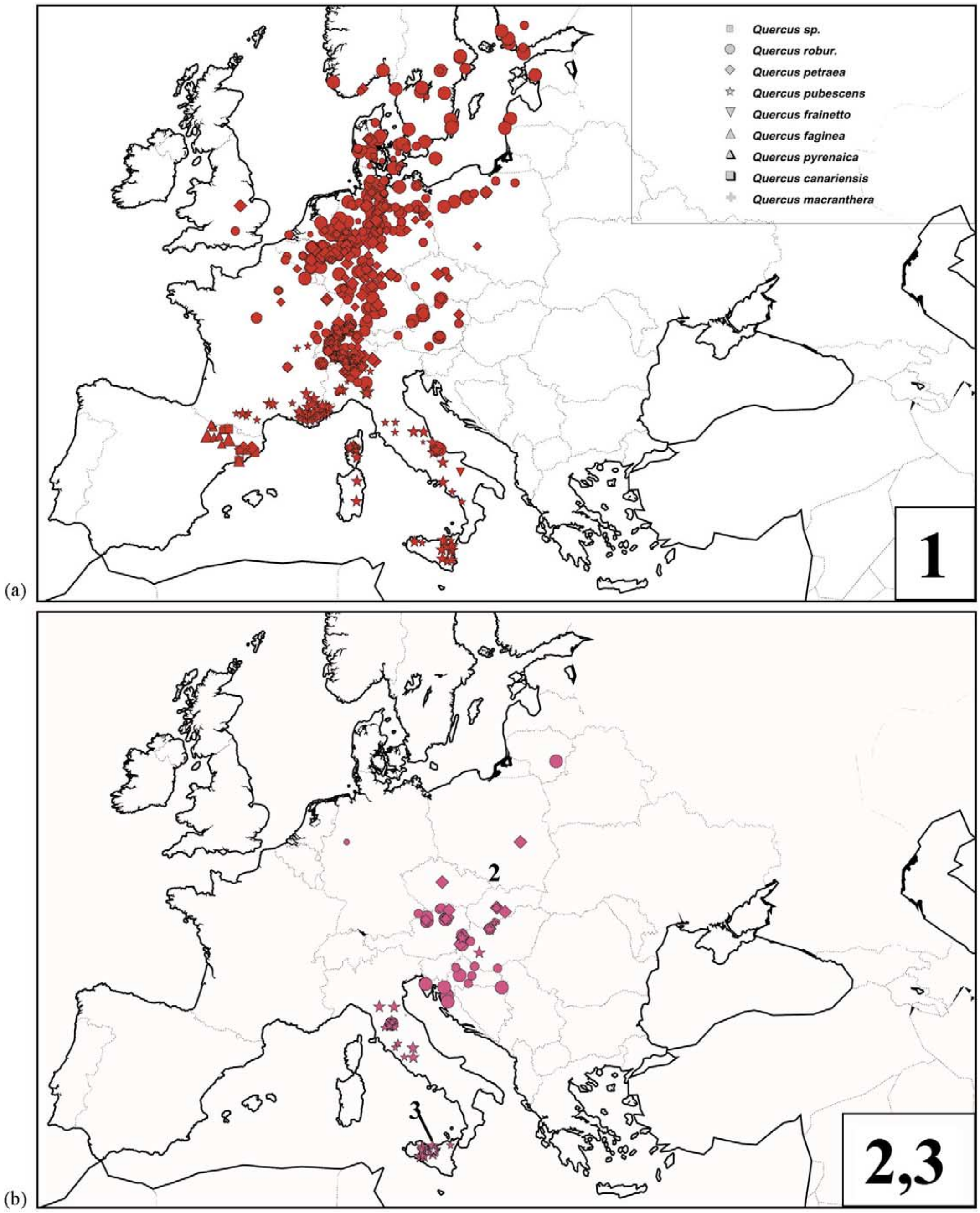
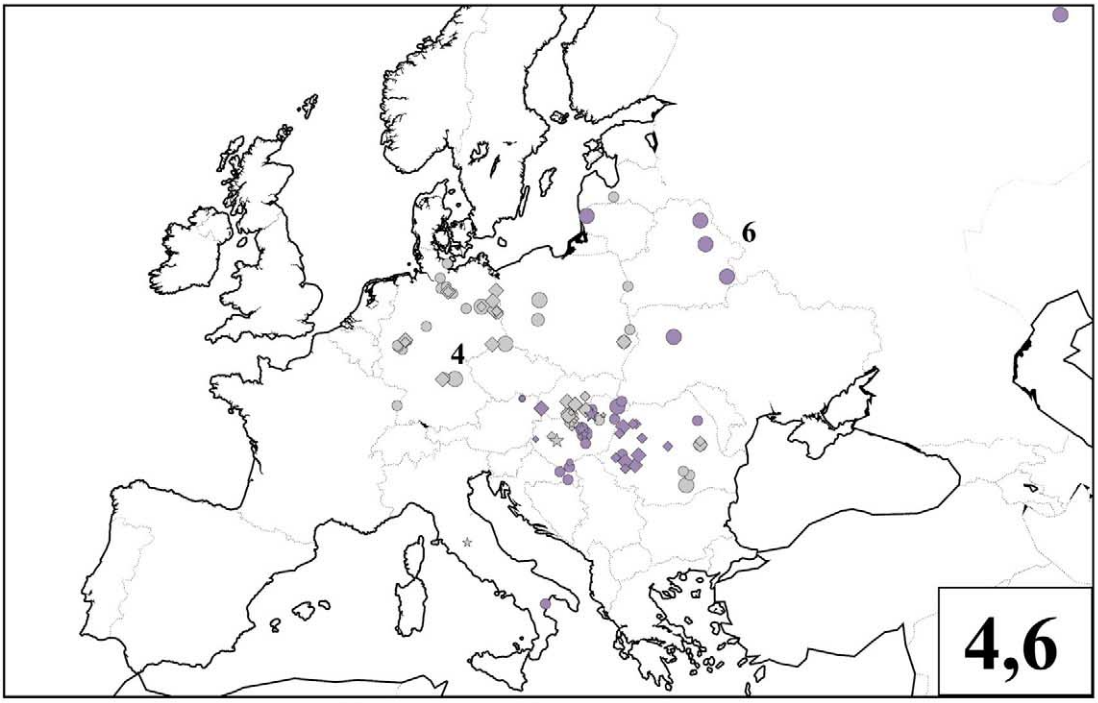
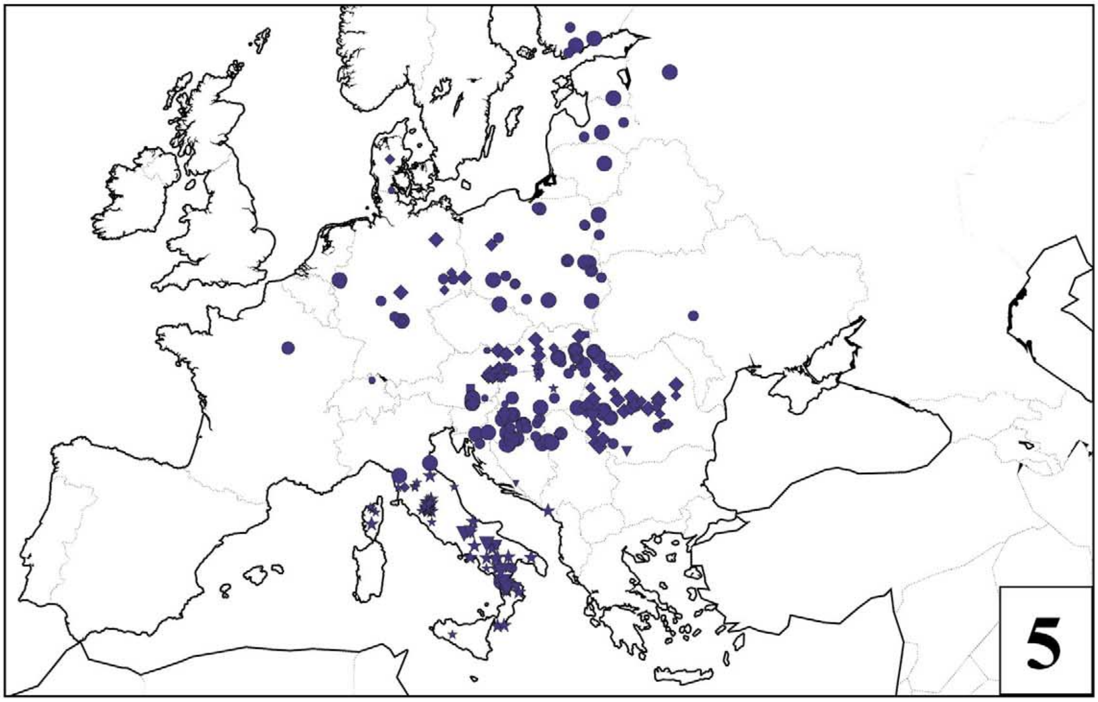


Fig. 1. Maps of haplotypes distribution in Europe, by species. Symbols sizes are roughly proportional to the likeliness that the population is autochthonous: populations fixed for a given haplotype are represented by the larger circles, those of dubious status by the smaller ones, and those populations where more than one haplotype have been identified are represented by symbols of intermediate size.



(c)



(d)

Fig. 1. (*Continued*).

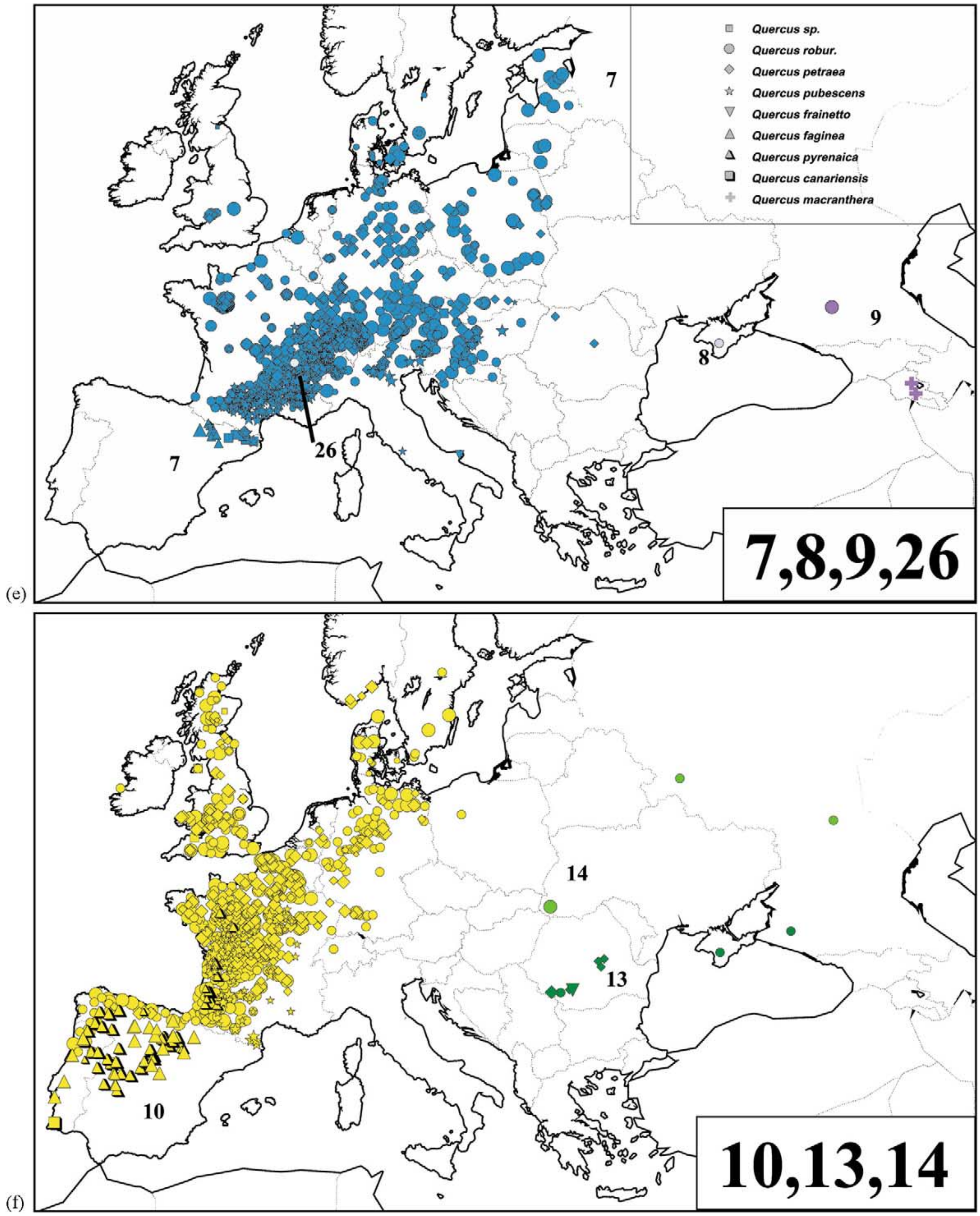
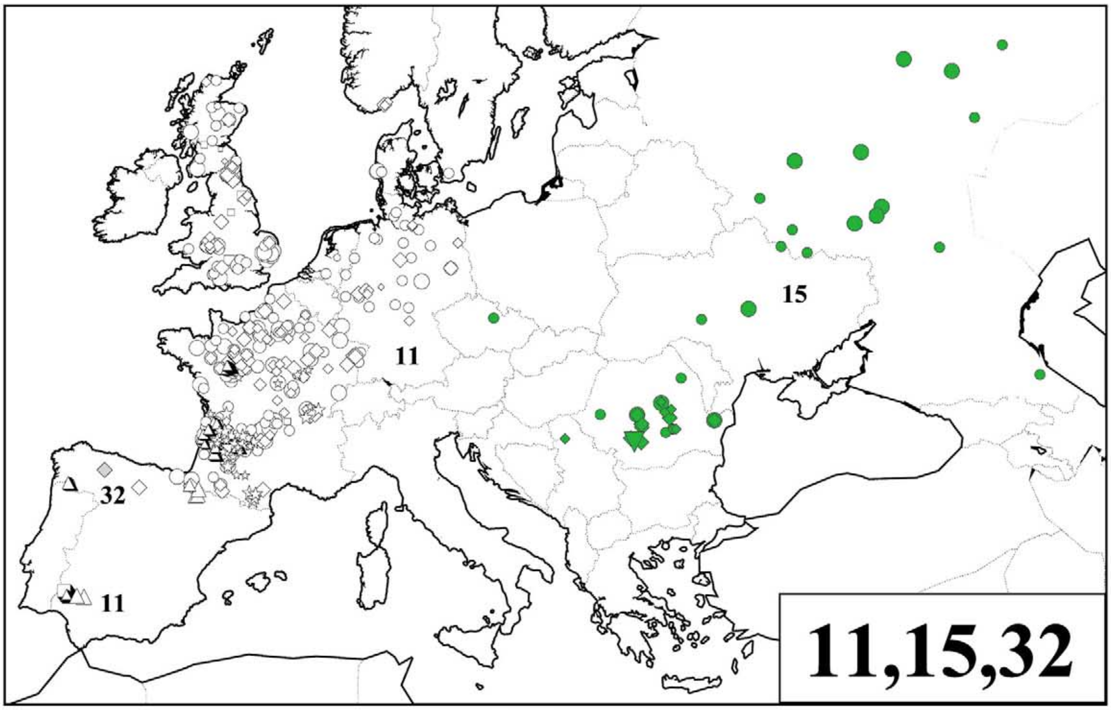
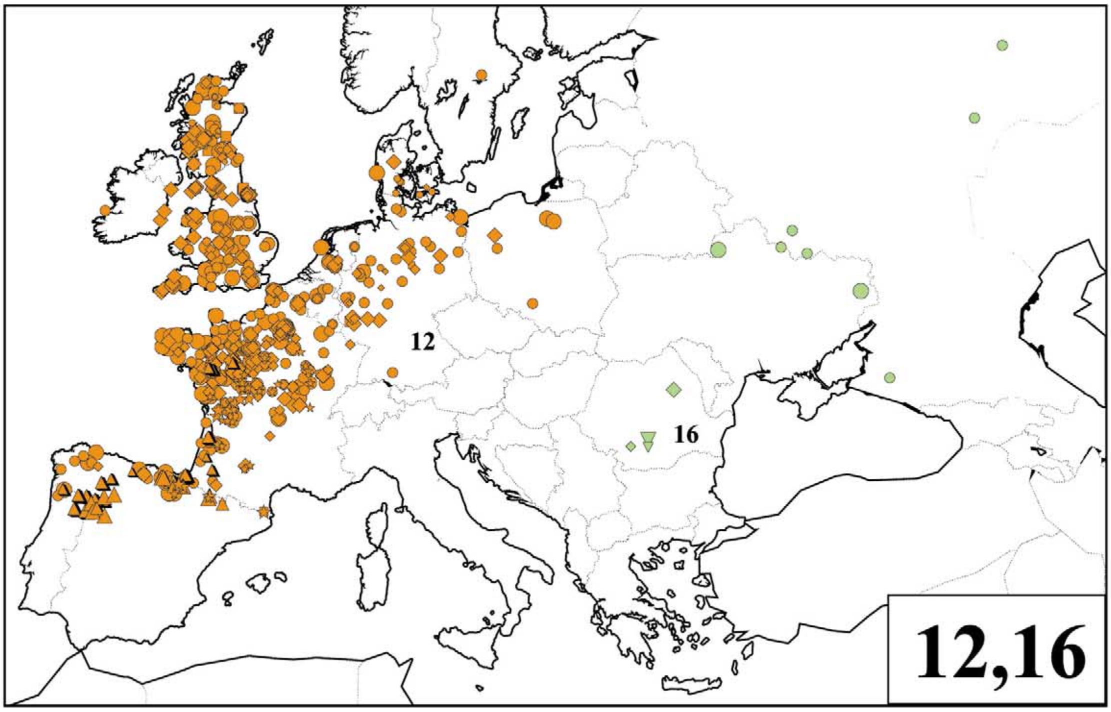


Fig. 1. (*Continued*).

(g)



(h)



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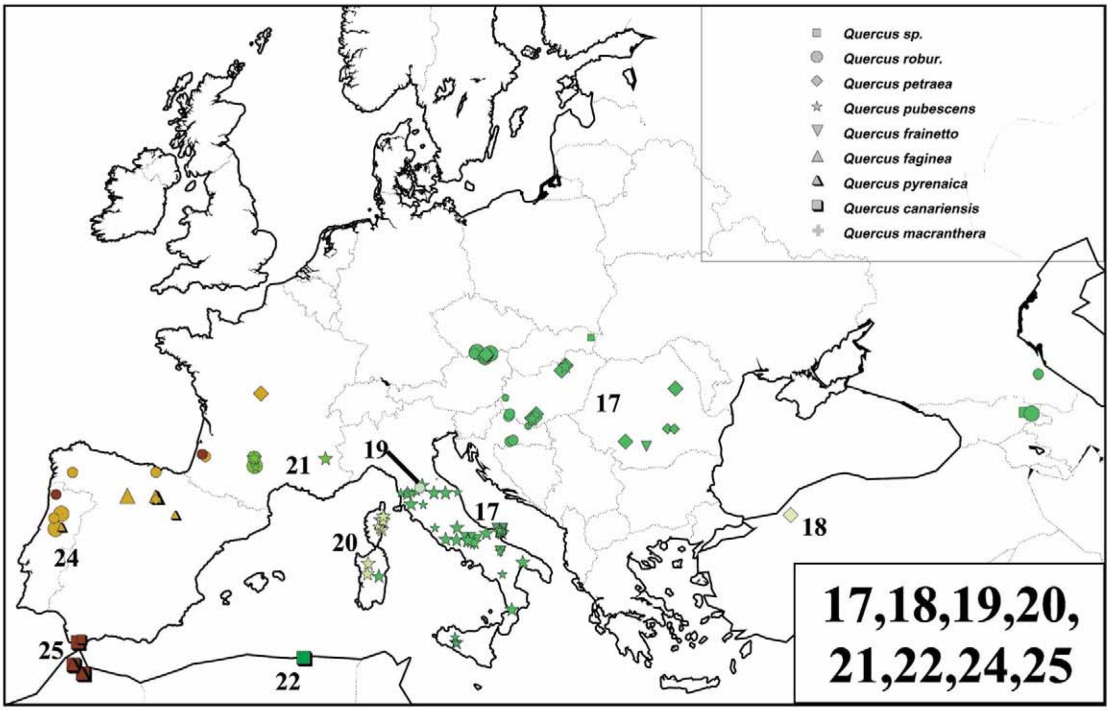
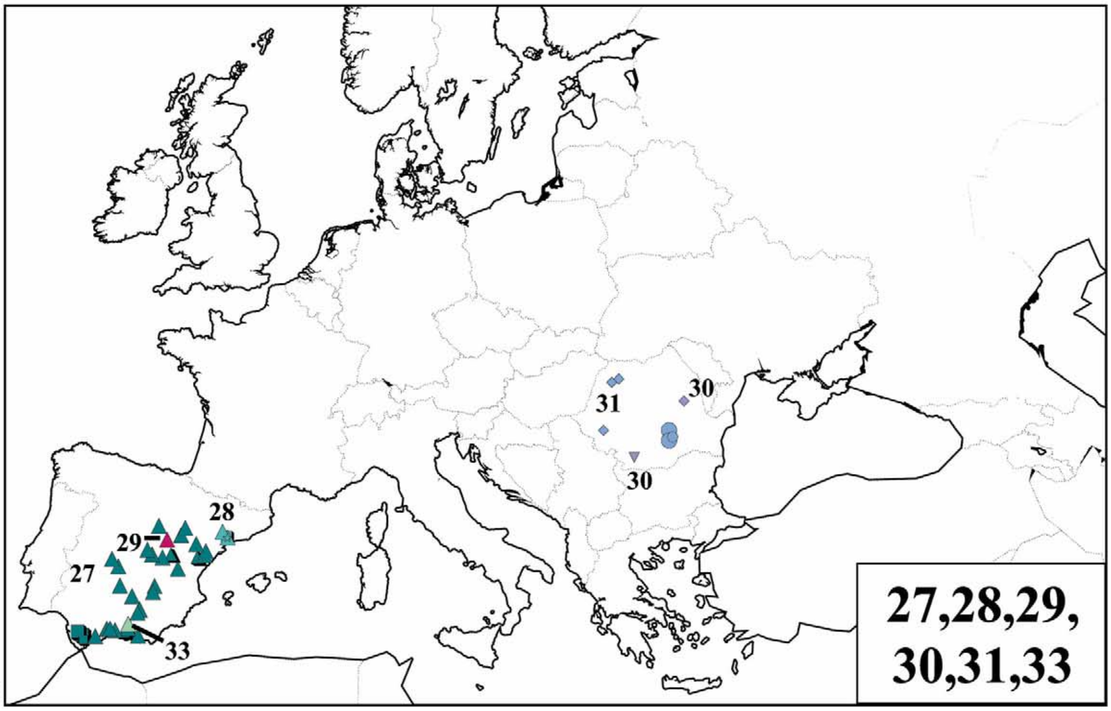


(i)



(j)

Fig. 1. (*Continued*).

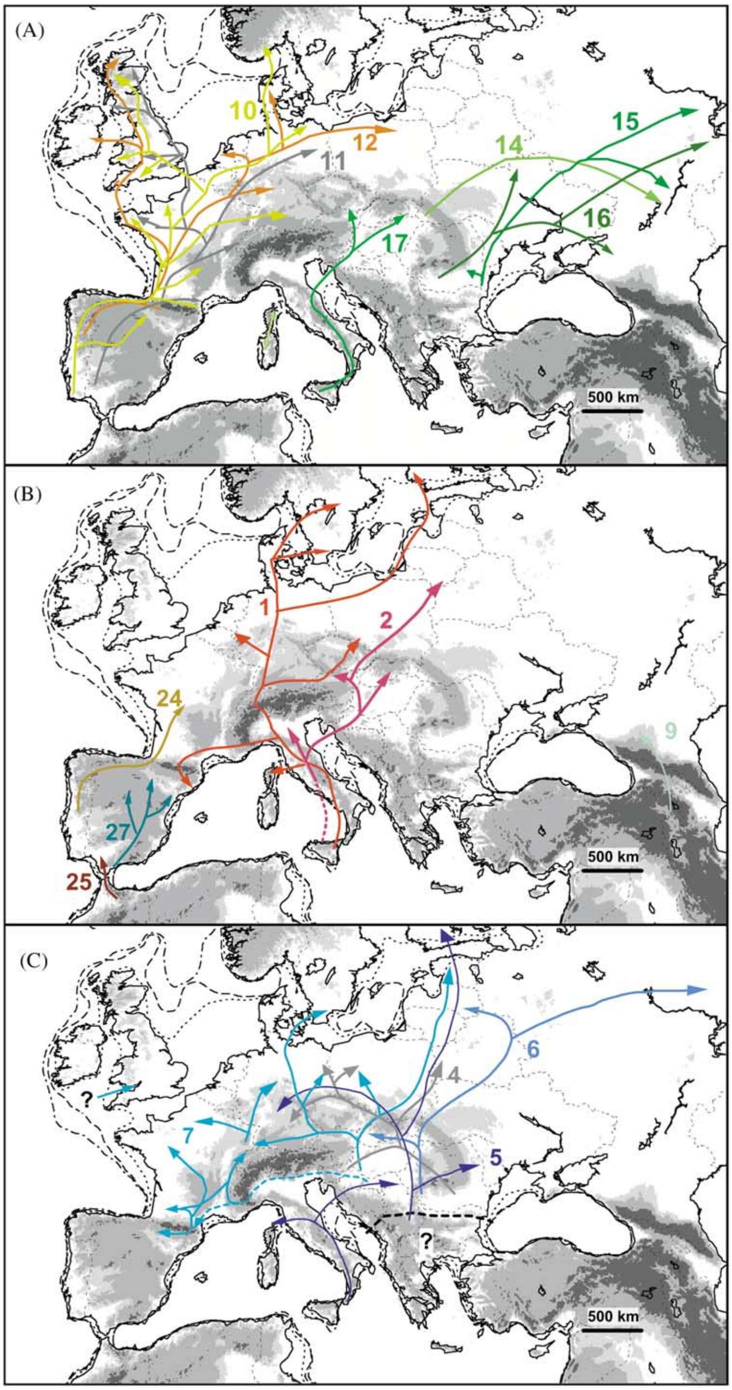


Fig. 2. Set of maps displaying the inferred colonisation routes for all frequent haplotypes. The altitude is indicated by grey shadings (250–500, 500–1000, >1000 m) and past sea levels at 21 ka BP (18 ^{14}C ka BP), 15 ka BP (13 ^{14}C ka BP) and 12 ka BP (10 ^{14}C ka BP) are indicated by dotted lines. (A) Lineages B (haplotypes 10–12) and E (haplotypes 14–17). (B) Lineages B (24–25), C (1–2), D (27) and F (9). (C) Lineage A (haplotypes 4–7).

Cádiz to Navarra, whereas haplotypes of lineage D are found exclusively east of this line.

3.1.1. Haplotypes belonging to lineage B

This lineage includes three abundant haplotypes (10–12) and six less frequent ones. The distribution of this lineage strongly supports the existence of a glacial refugium in the west coast of the Iberian Peninsula (Olalde et al., 2002; Petit et al., 2002a). From this refugium, a subset of haplotypes migrated through Europe, mostly following the Atlantic and North Sea coast of the continent (Cottrell et al., 2002; Jensen et al., 2002; Petit et al., 2002b). Movement past the Pyrenees seems to have occurred through the Basque country, and it is apparent that immediately after circumventing the Pyrenees, there was an easterly colonisation across to the Mediterranean Sea via the northern slopes of these mountains. In contrast, the southern slopes of the eastern Pyrenees were colonised by haplotypes 1 and 7 belonging to lineages C and A, respectively.

Haplotype 10 is the most common of the lineage B haplotypes and is distributed from southern Portugal and a large part of western Spain (Olalde et al., 2002), to France (Petit et al., 2002b), Great Britain (Cottrell et al., 2002), Belgium, the Netherlands, north-western Germany (König et al., 2002), and parts of Scandinavia (Jensen et al., 2002) (Fig. 1f). The spread north of the Pyrenees was extremely rapid and thus it is hard to discern from pollen data (Brewer et al., 2002). The timing, however, shows that the oaks migrated swiftly along the French Atlantic coast during the Holocene, and moved across the exposed bed of the English Channel from northern France into southern England (Fig. 2C). Further extension of oaks possessing these haplotypes to the east (south-east France in particular) would have been limited by the arrival of oak populations originating from Italian or Balkan refugia (Petit et al., 2002b). The spread of lineage B along the northern European coast into Belgium, the Netherlands, Germany and Denmark is most likely a result of its early rapid movement along the Atlantic border. Oaks migrating north from Italy appear to have been delayed by the difficult crossing of the Alps (Csaikl et al., 2002), and oaks from the Balkan refugium encountered delays in moving northward through central Europe (see Fig. 1a in Brewer et al., 2002).

The absence of trees possessing haplotype 12 south of the river Douro (northern Portugal; see Fig. 1h) could indicate that the Pleistocene refugium for this haplotype was located at a much higher latitude than the inferred distribution of oaks during this period (Brewer et al., 2002; Huntley and Birks, 1983). However, it is possible that southern populations characterised by this haplotype have either disappeared or have been overlooked. The distribution of haplotype 11 in Spain (Fig. 1g) illustrates this point; if the four populations located in western Andalusia (Sierra Morena) had been overlooked, the southern limit of this haplotype would lie instead in northern Spain, despite the fact that their is no pollen-based evidence to support the existence of a refugium in the northern half of Portugal (Brewer et al., 2002).

The distribution of haplotype 12 is slightly more westerly than that of haplotype 10, as haplotype 12 is particularly abundant in Galicia, the Cordillera Cantabrica (Olalde et al., 2002) and in western France (Brittany and Normandy) (Petit et al., 2002b). In Great Britain, it has also a more westerly distribution (in Cornwall, Wales and along the west coast of Scotland, Cottrell et al., 2002), although it is by no way restricted to these regions. A migration from north-western France to south-west England thus seems the most likely scenario for this haplotype. The map of sea-level at the start of the Holocene period (12 ka BP, Fig. 2) shows that the middle part of the English channel is below sea-level. The existence of such a barrier supports the contention that there were at least two different entry points into Great Britain characterised by haplotypes 10/11 and 12 (Cottrell et al., 2002).

There is an almost continuous distribution of populations containing haplotype 12 from northern France to the easternmost part of Germany, with some outlying populations as far east as Gdańsk in Poland (this population represents the easternmost limit of lineage B). This eastward spread is over a distance of approximately 2300 km, demonstrating that vegetation changes due to climate oscillations are by no means restricted to latitudinal movements. There are also some examples of bottlenecks occurring at the northern limit of this lineage. For example, haplotype 12 is not represented in the Scandinavian peninsula (except in one case where the native status of the population is dubious) and haplotype 11 is only present in a single Norwegian population along the south-eastern coast. However, haplotype 10 is not uncommon in Scandinavia, both along the south-eastern coast of

Norway and also in some scattered Swedish populations. The relative abundance of haplotype 10 in northern Denmark may explain this discrepancy (Jensen et al., 2002).

Haplotype 11 is less common overall than haplotypes 10 and 12 and has a very patchy distribution, particularly in France (Petit et al., 2002b) and in Great Britain (Cottrell et al., 2002) (Fig. 1g). One such patch occurs in the region between the Gironde estuary and the Massif Central, in south-west France (Petit et al., 2002b). Another example of such large grouping can be seen in East Anglia (Ferris et al., 1995; Cottrell et al., 2002), and further north along the east coast of Great Britain (Cottrell et al., 2002). Overall, the distribution of all three haplotypes is strikingly similar, even though they have usually mutually exclusive distribution ranges at a finer regional scale (Petit et al., 1997).

Among the less frequent cpDNA variants in lineage B is the newly recognised haplotype 25 that is common in southern Andalusia and the Rif region in Morocco (Fig. 1i). The distribution of this haplotype across the Straight of Gibraltar emphasises the importance of exchanges between the European and African continents. Actually, during the last glacial period, the sea-level was much reduced and the two coastlines were in closer proximity (Fig. 2B). The mountainous Rif region may have constituted a refugium from where recolonisation could have proceeded. Under such a scenario, it is likely that when the recolonising front reached southern Andalusia it would have met with other oak populations extending southward from Cádiz (Sierra del Ajibe and Sierra de Ronda, for instance). Due to the current scarcity of white oaks in the south-west of the Iberian Peninsula, this haplotype appears to be disconnected from the other haplotypes from lineage B, whereas it is surrounded by oaks possessing haplotype 27, the most abundant haplotype from lineage D (Fig. 1i).

Lineage B includes another haplotype of intermediate abundance that is restricted to the Iberian Peninsula and France: haplotype 24 (Fig. 1i) (Olalde et al., 2002; Petit et al., 2002b). It is also the most basal haplotype within that lineage and includes two haplotypes that have not been systematically distinguished during this survey (Petit et al., 2002a). The migration route that can be traced for this haplotype is similar to that of the most common haplotypes from lineage B (haplotypes 10–12).

Three further lineage B haplotypes have been detected in the Iberian Peninsula (10b, 12b, and 32), each is related to the three most abundant haplotypes (10a, 12a and 11, respectively) (Fig. 1g) (Olalde et al., 2002; Petit et al., 2002a). However, these rare haplotypes apparently did not migrate out of Iberia (Petit et al., 2002b).

3.1.2. Haplotypes belonging to lineage D

The investigations in Spain (Olalde et al., 2002) have revealed four new haplotypes belonging to lineage D (Fig. 1j) (Petit et al., 2002a). The most abundant, haplotype 27, is distributed all over eastern Spain, from southern Andalusia (where it is in contact with haplotype 25 of the B lineage) to Valencia. Haplotypes 29 and 33 are restricted to single populations in eastern Spain, where they are fixed, near other populations characterised by haplotype 27. The fourth, haplotype 28, is found at the mouth of the river Ebro in the two populations at the northernmost limit of that lineage. Given the relatively high density of sampling further south, this could imply that small oak refugia were present at relatively high latitudes along the Mediterranean coast of the Iberian Peninsula, which have not been detected so far by palynological investigations (Olalde et al., 2002). However, pollen data provide evidence for the presence of oaks only on the southern Mediterranean coast (Padul site; see Brewer et al., 2002), whereas oak pollen is only found from the middle Holocene on at a site further north on the Mediterranean coast (Carrion and Van Geel, 1999). Whilst this site may not be representative of the entire Mediterranean coast, this late arrival does raise questions about the establishment of the oak there (Brewer et al., 2002), and suggests that migration of this haplotype took place inland. This delay would explain not only the limited distribution of lineage D, but also why haplotypes originating from the Italian or Balkan refugia (haplotypes 1 and 7, see below) were able to get a foothold in the eastern Pyrenees. The two rarer haplotypes found only in the northern part of the country (28 and 29) may have been overlooked further south, and their finding seems therefore insufficient to demonstrate the existence of small refugia at high latitudes in central or north-eastern Spain, for which there appears to be little pollen evidence.

When considered together, the results of this survey indicate a clear phylogeographic split within the Iberian

Peninsula. The existence of two biogeographic regions in the Iberian Peninsula: an Atlantic and a Mediterranean one, is well established (Blanco Castro et al., 1997). Whether this biogeographic break is paralleled by a phylogeographic break in species having ranges encompassing both regions should be further investigated. However, the findings for the white oaks are shared by other species, such as maritime pine and its scale insect (Burban et al., 1999), and *Quercus suber* and *Q. ilex* (Jimenez, Gil and Petit, unpublished results). The recognition of lineage D represents a major biogeographic finding (Olalde et al., 2002; Petit et al., 2002a) compared to previous investigations (notably Dumolin-Lapègue et al., 1997). The relatively high frequency of oak pollen found at the Padul site (Andalusia) indicates the continuous presence of oak populations at this location throughout the last glacial period that could have harboured an eastern Iberian lineage (Brewer et al., 2002). In addition, there is limited evidence for a more westerly refugium in southern Spain (Stevenson, 1984), which points to the existence of at least two major refugia in Spain. The existence of oak species endemic to the Iberian Peninsula and to North Africa (*Q. faginea, Q. canariensis*) or shared only with western France (*Q. pyrenaica*) (see Fig. 3 in Petit et al., 2002a) points clearly to a diversified refugial zone in this part of Europe.

Finally two further haplotypes were characterised that, although they do not fall within lineage D, are most closely related to it phylogenetically (Petit et al., 2002a). Haplotype 21 was found in a restricted region in south-western France (north of Toulouse), more than 300 km from the northernmost limit of other haplotypes of lineage D. It was also detected in the French Alps. It may have originated from the Iberian Peninsula where it is either rare (and hence so far undetected) or has become extinct. Alternatively it may have originated from further east, for instance from the Italian Peninsula. Haplotype 22 is limited to a single population in Algeria. It had already been detected in a previous survey (Dumolin-Lapègue et al., 1997) but unfortunately no additional information has been gathered from this region. Its presence demonstrates however that several lineages are present in North Africa; further analyses in this region could reveal interesting phylogeographic relationships.

3.2. Haplotypes originating from the Italian Peninsula

Based on pollen data, the Italian Peninsula is thought to have harboured at least one oak refugium in southern Italy (at Laghi di Monticchio, Watts et al., 1996). However, the data do not preclude the existence

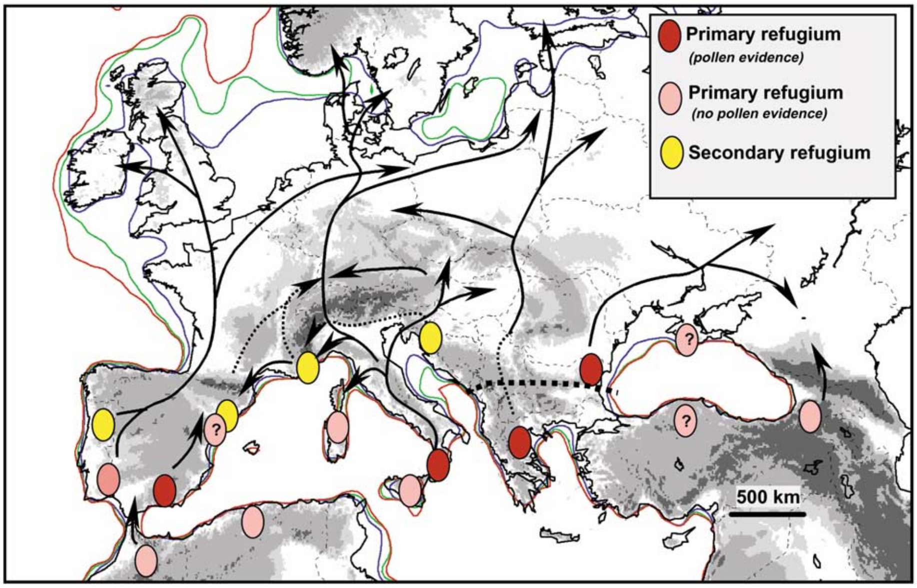


Fig. 3. Schematic distribution of primary and secondary oak refugia and post-glacial movements.

of other refugia, particularly in Sicily or Calabria, that have a rich endemic flora, or in Corsica/Sardinia. The cpDNA haplotypes detected in Italy (Fineschi et al., 2002) belong to three of the six recognised lineages (A, C and E, Petit et al., 2002a). From Fig. 1a and b, it appears that lineage C (in which three haplotypes are distinguished) was most probably restricted to an Italian refugium during the last glacial period. However, the situation for haplotypes belonging to lineages A and E is more complex, and some of them are shared by other refugial areas, particularly the Balkans.

3.2.1. Haplotypes belonging to lineage C

The three haplotypes of lineage C are all present in Italy. The southernmost limit of haplotype 1 (Fig. 1a) is in Sicily, where it is abundant, particularly in the east. This haplotype is also found along the Apennines (especially on the eastern side) and in Corsica and Sardinia. It is completely absent from the Balkan Peninsula but was detected on the south-eastern slopes of the Pyrenees and in the Catalonian region. The Pyrenees have an important component of endemic flora, however, they have also received many immigrating plant and animal taxa coming from the east, during post-glacial migrations (e.g., Hewitt, 1999; see also Olalde et al., 2002). Our preferred interpretation is therefore that haplotype 1 originated from Italy (possibly in Sicily, see Fineschi et al., 2002) and moved westward to reach south-western France (Petit et al., 2002b) and north-eastern Spain (Olalde et al., 2002). Oak migration is thought to have occurred in two well-differentiated stages (Brewer et al., 2002), and in line with this hypothesis, it is possible that the Catalonian area was colonised early (during the late-glacial interstadial) by oak populations originating from Italy via France. Catalonia would then have constituted a 'secondary refugium' for this haplotype during the colder period of the Younger Dryas (11–10 ka BP) (Brewer et al., 2002). In Italy, the oak migrated rapidly along the Apennine chain at the start of the late-glacial interstadial. A subsequent spread from there along the French Mediterranean coast appears to be supported by pollen analyses from the lower Rhone valley, which shows evidence of oak pollen from the middle of this period (Brewer et al., 2002). This movement may have been facilitated by the presence of oak populations possessing this haplotype on the eastern side of the Apennine range, from where migration could have proceeded easily to the Po plain without the need to cross these mountains. The location of this haplotype on the southern slopes of the Pyrenees suggests that it ultimately spread to this region by the end of the late-glacial interstadial, perhaps taking advantage of the exposed shelf of the gulf of Lions. The fragmentation of the migration route across the south of France may result from a decline in low-altitude populations during the climatically adverse Younger Dryas period (11–10 ka BP); alternatively, this pattern may have appeared as a consequence of the leptokurtic dispersal of seeds in these species (Hewitt, 1996; Le Corre et al., 1997; Petit et al., 1997). Despite the formidable barrier to migration that the Alps represent for species such as the oaks (which now rarely grow at altitudes higher than 1400 m at these latitudes), trees bearing haplotype 1 appear to have crossed this significant obstacle, necessarily via high elevation passes, such as for instance the Simplon pass (2006 m), and possibly even the Grand Saint Bernard pass (2469 m) (Mátyás, 1999; Mátyás and Sperisen, 2001). Indeed, through the intense sampling strategy implemented in the western Alps it has been possible to locate the colonisation routes into western Switzerland (Mátyás and Sperisen, 2001). Nevertheless, the pollen analysis suggests that the oak was unable to cross the Alps prior to the end of the late-glacial warm period. Temperatures at the start of the Holocene were warmer than at present due to an increase in insolation, the quantity of solar radiation received by the earth's atmosphere (Berger, 1978; COHMAP Members, 1988). This optimum climate for the migration of the oak probably enabled the trees to cross the mountains. More northerly colonisation, into south-western Germany, appears to have proceeded along the lowlands situated between the Jura mountains and the Swiss Alps. From there the migration continued all the way to southern Scandinavia, encompassing the centre of Germany with movements westward into Belgium and the Netherlands (König et al., 2002) and eastward to northern Poland and up to south-western Finland (Csaikl et al., 2001; Jensen et al., 2002). Movement into Austria probably involved a southward migration after oak populations had reached southern Germany from western Switzerland. This route is the most parsimonious as it does not involve another traversing of the Alps further east, for example at the border between Italy and Austria (Csaikl et al., 2002). In the north, colonisation of Scandinavia most probably took place via a landbridge connecting Denmark and Sweden (Jensen et al., 2002). The Norwegian colonisation of trees possessing haplotype 1 could have occurred through Sweden, but based on contemporary regional haplotype composition is more likely to have occurred directly across the North Sea (Jensen et al., 2002). The few trees found in Great Britain possessing haplotype 1 clearly represent artificially introduced material and are always found mixed with trees having locally abundant haplotypes (Cottrell et al., 2002).

Haplotype 2 was also found in many populations in Sicily (to which the related haplotype 3 is restricted). There is then an interesting disjunction, since haplotype 2 was not found in southern Italy but occurs just north of Rome (i.e., over 600 km further north; Fineschi et al., 2002). The haplotype may have been overlooked in intermediate populations. However, the sampling strategy for this area was fairly intensive and it would be interesting to investigate the possibility that we are dealing with different haplotypes (one in Sicily and the other in north Italy), by examining additional cpDNA fragments. Alternatively, the trees may have followed a coastal migration path within the Italian Peninsula, and the intervening populations may have been subsequently lost during the late-glacial cold period, the Younger Dryas. Outside Italy, haplotype 2 also occurs more or less continuously from Croatia to Austria, Hungary and Slovakia, with more isolated populations in Poland and Lithuania. This post-glacial migration path towards the north-east is in strong contrast to that of haplotype 1 that took a more westerly or central route. The extensive colonisation of eastern regions from Italy illustrates the ease with which oak populations could cross what is now the north of the Adriatic sea at a time when a landbridge existed between the Marche region in Italy and Croatia (Fig. 3). It remains to be explained why trees having haplotype 1 did not used this migration route, by examining more closely the timing of their migration.

3.2.2. Haplotypes belonging to lineage E

There are three other relatively common haplotypes found in the Italian Peninsula and adjacent islands; one belongs to lineage A (haplotype 5) and the other two belong to lineage E (haplotypes 17 and 20). Actually, some of these haplotypes also describe several sub-haplotypes that were not systematically resolved during this survey (haplotypes 5a–c and haplotypes 17a–f; Fineschi et al., 2002; Petit et al., 2002a). The wide distribution of these haplotypes from Italy throughout the eastern Balkans evidences important exchanges between these regions, although some may have been anterior to the last post-glacial. The case of haplotype 17 is complicated. It has been found in the centre of Sicily, where it is represented by a distinct type, 17e, also detected in north/central Italy (Fineschi et al., 2002). This haplotype also appears to be related to haplotype 19, which is rare but has been detected both in southern and north/central Italy (Fineschi et al., 2002; Petit et al., 2002a). Haplotype 17 (type 17a in particular) is then more or less continuously distributed up to Umbria. Thus a similar migration route to that of haplotype 2, i.e. across the northern Adriatic region and then further east and north into Croatia, Hungary and Austria, may be proposed (Fineschi et al., 2002).The presence of haplotype 17c in Romania south of the Carpathian mountains (Bordács et al., 2002), and in three populations from the Caucasus (two in Georgia and one in southern Russia), near the Caspian sea (Dumolin-Lapègue et al., 1997), indicates that this haplotype was present in at least two main refugia (probably Italy and the eastern Balkans). Further molecular analyses systematically distinguishing all the sub-variants of haplotype 17 would be useful.

Haplotype 20 was found only in eastern Corsica and in central Sardinia (Fineschi et al., 2002). The existence of an endemic type on these islands is of interest for two reasons. First, it points to the possible existence of a glacial refugium for white oaks on one or both of these islands, a finding that has so far not been borne out by pollen analysis. Indeed, the pollen data from Corsica show the presence of deciduous *Quercus* pollen during the late-glacial period (Reille, 1997), however, no conclusive evidence was found to propose the existence of a refugium. Taking into account the genetic evidence and the relatively early expansion of the oaks seen in the pollen analysis, it appears likely that a refugium did exist on the island, which has not yet been located by pollen analysis. The pollen evidence indicating a late-glacial colonisation comes from two high-altitude sites, and so the refugium may have been situated at a lower altitude (Reille, 1997). Alternatively, a refugium may have existed on Sardinia. The two islands were connected during the glacial period, and a single refugium could easily have acted as a source for contemporary populations characterised by haplotype 20. In Sardinia, one population possesses a distinct haplotype (17d), closely related to haplotype 20 (Fineschi et al., 2002). It was also detected on the Italian Peninsula, in Calabria. The phylogenetic relationships between these two haplotypes and the D lineage from Spain and Algeria need to be further elucidated (Petit et al., 2002a).

3.2.3. Haplotypes belonging to lineage A

The distribution of haplotype 5 (lineage A) in Italy is very similar to that of haplotype 17 (lineage E), and to some extent to that of haplotype 2 (lineage C), despite the fact that all three belong to different lineages. If we assume a southern Italian origin for all three haplotypes, then a similar route of migration into central Italy should have ensued, followed by the crossing of the Adriatic Sea via a northern landbridge (Fig. 3) to reach Slovenia and Croatia. However, none of these haplotypes were able to extend their range into northern Italy. Unfortunately, possible migrations further south across the Adriatic cannot be identified here due to a lack of samples of southern Balkan origin. For haplotype 5, in particular, which is nearly completely absent from Sicily and that belongs to an otherwise predominantly Balkan lineage (A), an early migration into Calabria from a southern Balkan refugium can also be conceived (Fineschi et al., 2002). It is also possible that there were two refugia, one in Italy and one in the Balkans. Further molecular characterisation may help to distinguish among these hypotheses. Haplotype 5 is not found in the southern most part of Romania, and it only extends into the region north and west of the Carpathian mountains. As for haplotype 2, further migration north-eastward has then occurred from either Croatia or Romania, but in the case of haplotype 5, migration has continued all the way up into the Baltic countries (Csaikl et al., 2001) and to eastern Finland (Jensen et al., 2002). Moreover, within Poland, the colonisation route appears to have split and a western colonisation route has branched off into central Germany. Points further west (where this haplotype has never been recorded in pure populations) are likely to be contamination due to human transport (König et al., 2002).

All three haplotypes that migrated east from Italy across the Adriatic may have made use of a secondary refugia during the Younger Dryas cold period, either within the mountains on the Balkan Adriatic coast, or further north on the southern slopes of the Alps. Separation into temporarily discrete refugia by these haplotypes may help to explain their varied pattern of spread during the post-glacial period. Furthermore, for haplotypes 5 and 17, exchanges across the Adriatic Sea during previous interglacials may explain the number of different but closely related sub-types on both sides of this sea.

3.3. Haplotypes originating from the Balkan Peninsula

3.3.1. Haplotypes belonging to lineage A

The southern European distribution of haplotype 7 raises some interesting questions. Indeed, it is present from Croatia to north-eastern Catalonia, and although present in northern Italy, it is absent from the south of the Italian Peninsula. In Croatia, it is present in the north of the country but is then absent further south. It does not extend very far to the east (the two points in Romania could represent contamination through plantation). On the basis of the previously published data (Dumolin-Lapègue et al., 1997), two conflicting hypotheses were proposed. Dumolin-Lapègue et al. (1997) by proposed a Balkan refugium for this haplotype, whereas Hewitt (1999), in a review of this work, suggested the existence of a refugium in north-eastern Spain. Olalde et al. (2002) also support this latter view. However, the distribution of haplotype 7 in the east indicates that it followed a migration route similar to that of haplotypes 2 and 5, towards the Baltic countries through Austria, Hungary, the Czech Republic and Slovakia. Another movement throughout central Germany up to the islands of Denmark also appears to have taken place. Based on palynological evidence, Huntley and Birks (1983) suggested an east-west migration of oaks across the northern flanks of the Alps. In particular, they describe a movement westward along the Danube lowlands, at 9 ka, which could have involved this haplotype. Finally, haplotype 7 belongs to lineage A, which is composed entirely of haplotypes of eastern origin (Balkans or Italy).

All these arguments support the existence of a refugium in the Balkans for this haplotype.

The arguments in favour of a second refugium located further west for haplotype 7 are the following. First, this haplotype is well-represented south of the Pyrenees, both in *Q. pubescens* and in *Q. faginea*. For other plant or animal species, these mountains have constituted a barrier that has blocked the progress of lineages originating from eastern refugia (Hewitt, 1999). Second, the period of time required for trees characterised by haplotype 7 to move along the Alps and to arrive in Spain before the region was fully colonised by other haplotypes seems too great, especially assuming a movement along the northern edge of the Alps, starting from the Balkans. Indeed, although palynological studies published to date provide no evidence for the presence of oaks during the glacial period in north-east Spain, oak pollen has been detected relatively early there, in the second half of the late-glacial interstadial (12–11 ka, Reille and Lowe, 1993). Third, these parts of the Pyrenees, as well as the Ebro valley, have high levels of endemism.

However, several arguments make this hypothesis unlikely. First, cases of migration of eastern lineages up to the Pyrenees and sometimes into the Iberian Peninsula have been reported in other organisms: *Chorthippus parallelus*, *Alnus glutinosa*, *F. sylvatica*, *Triturus cristatus* (see Fig. 4 in Hewitt, 1999). In oaks, the migration outlined above for haplotype 1 represents one such example. Second, haplotype 1 seems to have had sufficient time to establish itself south of the Pyrenees from an Italian refugium. For haplotype 7, the amount of time required for the oak to move west would be lower if the migration took place also along the southern flanks of the Alps. Indeed, movements along the Po plain may have brought this haplotype to the south-west corner of France much faster than a voyage across the northern flank of the Alps. There is indeed genetic evidence for the presence of haplotype 7 in this plain (Fig. 1e). Third, pollen studies have failed to reveal oak pollen during the glacial period in Catalonia and eastern Pyrenees. Although it could be argued that the refugium was further south, and that the oak used the Pyrenees as a secondary refugium, the lack of haplotype 7 to the south of the Iberian Peninsula suggests that it was not there, or alternatively that it has been lost in this area. Fourth, climatic reconstructions argue against the presence of refugia as far north as Catalonia (this argument is developed below). There is also genetic evidence for increased genetic variation within haplotype 7 in the Balkans and in Austria but not in Spain or in France (U. Csaikl and R.J. Petit, unpublished data). Although this observation should be confirmed, this is exactly the pattern that would be expected for a westward expansion of haplotype 7 from a Balkan refugium.

According to our preferred hypothesis, there was only one primary refugium for haplotype 7 and it was located in the Balkans. The subsequent spread of haplotype 7 westward but also eastward and northward would have resulted in a large distribution in western Europe. The migration may have followed two paths from the Balkans. One path led north to a secondary refugium in the south-east Alps, and a second westward across the exposed Adriatic sea basin and to the south-east of France or even the Pyrenees via the Po plain. Using the migration speed of 500 m/yr calculated by Huntley and Birks (1983) it is possible that this haplotype could have covered the distance to south-eastern France within the late-glacial interstadial. A higher speed would be required for the oaks to reach the southern Pyrenees.

At the start of the Holocene, haplotype 7 would have then migrated from the east of the Alps in a fan-like pattern, passing northwards through Germany and Poland to reach the west and east of the Baltic sea, respectively. Delays faced by the Italian haplotypes (notably haplotype 1) in crossing the Alps at the start of the Holocene would have offered the chance for haplotype 7 to spread west, this time following the northern slopes of the Alpine chain from a secondary refugium located in the eastern Alps. The direction of this movement appears to be represented by the westerly incursion into northern France. From the secondary refugium in the Pyrenees or in south-east France, the trees spread either towards the north-west (from the Pyrenees) or towards both the south-west and the north (from south-eastern France), thus entouring the Alps in both cases, as suggested by Mátyás and Sperisen (2001).

The northern distribution of this haplotype is also interesting. In western France, movements have also occurred towards the north-west, as shown by the existence of isolated populations or groups of populations (Petit et al., 1997, 2002b). On the other hand, the presence of trees characterised by this haplotype in

Great Britain may be due to recent human import of seeds or plants (Cottrell et al., 2002). In central Europe, the populations harbouring this haplotype split to colonise Denmark and southern Sweden on the one hand (Jensen et al., 2002), and Poland and the Baltic States on the other hand (Csaikl et al., 2001), although they fail to get into Finland (Jensen et al., 2002).

Finally, a haplotype closely related to haplotype 7 (haplotype 26) has been described earlier in the French Alps (Dumolin-Lapègue et al., 1998). It could represent a recent, post-colonisation mutation event of little biogeographic significance.

The south-eastern Carpathian mountains in Romania and northern Hungary is the southernmost extent of haplotype 4. The overall distribution of this haplotype suggests that its refugium was located in the south-eastern Balkans, possibly on the Bulgarian coast of the Black Sea (Bordács et al., 2002; Brewer et al., 2002). The most likely migration route from this refugium would then be along the northern side of the Carpathian mountains, from east to west, reaching the eastern side of Poland, from where further migration could have proceeded northwards, up into the Baltic countries, and westwards, to reach eastern Germany. Another colonising branch could have moved west along the border between Slovakia and Hungary. Expansion in the late-glacial, followed by fragmentation of the range in the Younger Dryas, may explain the observed distribution between Romania and Austria. Alternatively, a secondary refugium located in Slovenia may have existed, given the presence of oaks there during the late-glacial (Culiberg and Sercelj, 1996), from where a migration into Hungary would have taken place. From Hungary, further movements into Poland and Germany may also have occurred. Further sampling in the southern Balkans is required if this issue is to be resolved.

The southernmost occurrence of haplotype 6 is in eastern Croatia and south-western Romania. However, it was also found in one mixed population in Calabria. Such a distribution could point to a refugium in north-western Greece or southern Albania: indeed, palynological evidence exists for the continuous presence of oaks in the north-east of Greece (Brewer et al., 2002). From there, colonisation is most likely to have occurred towards the north-east, through the Dinaric Alps and then through the lowlands of Hungary, northern Serbia, eastern Croatia and eastern Romania. Further movements are apparent. There was probably migration eastwards towards Austria, and also across the Carpathian mountains, along the eastern border of Slovakia, into Ukraine, Belorussia, up into the Baltic countries and eastwards into Russia (3000 km eastward). However, given the lack of data from the southern Balkans, several other scenarios could also be imagined and a further study of material from this area is needed to elucidate the exact migration pathways.

3.3.2. Haplotypes belonging to lineage E

Haplotype 13 was only found in Romania, Crimea (Ukraine) and further east in Russia, along the coast of the Black Sea. The absence of this haplotype west of Romania (in Hungary or Croatia), in conjunction with the distribution of haplotype 4, support the existence of a deciduous oak refugium in the south-east Balkans, along the western coast of the Black Sea, or within the Bulgarian mountain ranges (Bordács et al., 2002). By analogy to the colonisation route inferred for haplotype 4, we suggest that the distribution of haplotypes 14, 15 and 16 in populations located from Romania to Russia also witnessed an eastern movement of populations initially located in an eastern Balkan refugium (rather than a movement from east to west). All of these haplotypes are present south of the Carpathian mountains in Romania, and could therefore have persisted along the western coast of the Black Sea. The sole population from Turkey (south of the Black Sea) that was included presents a distinct haplotype (haplotype 18); further analyses there may reveal interesting patterns and finer mapping of refugia.

3.3.3. Haplotypes belonging to lineage F

Two related haplotypes (8 and 9) were found east and north of the Black Sea (Crimea), suggesting other oak refugia in these areas (Dumolin-Lapègue et al., 1997; Petit et al., 2002a). As pointed out in Brewer et al. (2002), oak presence during the last glacial period was detected at increasingly high latitudes away from the Atlantic ocean (from southern Spain to Bulgaria); the detection of a separate lineage in this region (implying the presence of a refugium at relatively high latitude) fits well with this pattern.

3.4. Comparison between the distribution of the haplotypes and that of the oak species, and interspecific comparisons

In the above interpretations, we have not considered species status, because earlier studies had shown extensive cpDNA exchanges between species (e.g., Petit et al., 1997, 2002a; Dumolin-Lapègue et al., 1999a). Nevertheless, due to the small size of the inferred refugia and the distinct ecological conditions that prevailed in these widely separated regions during the last glacial period, an early relationship may have existed between species and haplotypes. The distribution of cpDNA lineages and that of the oak species may have then become further associated as a consequence of the different ecological requirements of the species undertaking recolonisation. Such relationships would have then become largely blurred as oak populations originating from these different refugia came into contact (see also Kremer et al., 2002). However, some of the hypotheses that can be proposed from the comparison of species and haplotype distribution are listed below.

3.4.1. Association between Q. pubescens and haplotype 1

In the Iberian Peninsula, *Q. pubescens* is found only in the river Ebro valley in northern Spain; it is replaced further south by *Q. faginea*, a species restricted to Morocco and to the Iberian Peninsula (see distribution maps in Petit et al., 2002a). Both species have somewhat similar ecological requirements. Many intermediate forms between the two species are found when they come into sympatry (Olalde et al., 2002). *Q. pubescens* clearly originates from the Balkan and the Italian Peninsula, where it is abundant and where related species, subspecies or forms have been described (Bussoti and Grossoni, 1997). Similarly, haplotype 1 is also widely distributed in Italy and in south-eastern France, where it is predominantly associated with *Q. pubescens*, the dominant species in these regions. As discussed above, haplotype 1 appears to have migrated into northern Spain. We therefore suggest that, after the last glacial period, *Q. pubescens* individuals characterised by haplotype 1 migrated into Spain, where introgression with other species such as *Q. faginea* took place. Migration of haplotype 1 into Switzerland also seems to have involved *Q. pubescens*, as suggested by the similarities between the distribution of this species and that of haplotype 1 in the western part of the country (Csaikl et al., 2002).

3.4.2. Association between Q. petraea and haplotype 7

In the Iberian Peninsula, *Q. petraea* is also restricted to the northern part of Spain, like *Q. pubescens*, although some isolated populations are found further south near Madrid. *Q. petraea* has clear eastern taxonomic affinities as it is widespread in the Balkans where many related species or subspecies are found (e.g., *Q. dalechampii*, *Q. polycarpa*, see Bussotti and Grossoni, 1997). However, unlike *Q. pubescens*, *Q. petraea* is very infrequent in Italy (Fineschi et al., 2002). Compared to the coastal migration route into Spain inferred above for *Q. pubescens* (along the French Mediterranean coast), a more inland colonisation route into Spain may be proposed for *Q. petraea* (Petit et al., 2002b). In fact, this species is found along the Alps and in the Massif Central, from where it could reach the Pyrenees. Actually, this species is found at relatively high altitudes in the Alps (up to 1400 m, i.e. much higher than *Q. pubescens* and *Q. robur* that are typically found in lowlands). A possible scenario, assuming that the westernmost (primary) refugium for this species/haplotype was located in Slovenia or in north-western Croatia, would be that *Q. petraea* and haplotype 7 migrated together, at least initially, as described earlier for haplotype 7, to reach the Pyrenees. Pollen swamping by *Q. pubescens* would then have occurred along the southern edge of distribution of the haplotype, whereas pollen swamping by *Q. robur* (initially associated with haplotypes of lineage B) would have taken place along the northern edge (see Fig. 1f). Finally, in northern Spain, pollen swamping by *Q. faginea* would also have occurred. Hence, given the contrasting ecological preferences of *Q. pubescens* and *Q. petraea*, initial associations with haplotypes 1 and 7 may account for the dominance of haplotype 7 over haplotype 1 in the south of France.

3.4.3. Association between Q. pyrenaica and lineage B

Q. pyrenaica is a typical Atlantic species. As shown in Petit et al. (2002a), it is most often associated with haplotypes of the B lineage (10–11–12, and 25 in Morocco). Only three populations along the Mediterranean coast (including two very close populations in the Sierra Nevada, a potential refugial area) are characterised by haplotype 27 (lineage D). Similarly, within the Iberian Peninsula, *Q. robur* is limited to the Atlantic coast, where it is associated with haplotypes of lineage B. Both species could therefore have persisted in a western refugium within the Iberian Peninsula.

3.4.4. Association between Q. faginea and lineage D

Although *Q. faginea* is characterised by a high cpDNA diversity, due to the presence of several divergent lineages (Petit et al., 2002a), they may have been gained through pollen swamping. Indeed, haplotypes of lineage D are the most frequent in *Q. faginea*, and are almost exclusive to this species.

3.4.5. Comparison with results obtained for beech (F. sylvatica)

Future comparative phylogeographic studies in Europe may prove fruitful, as shown by the first recent syntheses in the field of comparative phylogeography (Soltis et al., 1997; Taberlet et al., 1998; Hewitt, 1999). For instance, populations of the European beech (*F. sylvatica* L.) originating from the Italian refugium have been blocked south of the Alps (Demesure et al., 1996) similar to the situation of haplotypes 2, 5, and 17 for oak (but not haplotype 1). In both cases also, a haplotype of Balkan origin colonised northern Italy (haplotype 7 in the case of the oaks) faster than haplotypes of Italian origin did. The fact that oak populations originating from Italy did cross the Alps (cf. the discussion above concerning haplotype 1), whereas beech did not, may seem surprising, since beech grows presently at much higher altitudes than oaks (up to 1700 m, compared to a maximum of 1400 m for *Q. petraea*). Pollen analyses from the north of Italy suggest that beech was delayed in its northward migration by competitive interaction with *Abies* (Watson, 1996), whereas oak did not suffer such a delay and was present on the southern slopes of the Alps during the late-glacial interstadial. From this secondary refugium, oak was able to take advantage of the climatic optimum of the early Holocene, a period of increased temperature induced by a maximum in insolation, to cross the Alpine barrier between Italy and Switzerland.

4. General discussion

4.1. Location of refugia

As shown by Brewer et al. (2002) on the basis of fossil pollen evidence, the distribution of the deciduous oaks in Europe was limited during the glacial period to the extreme southern part of the continent. Reductions in temperature and in the available precipitation were the major reasons for the large-scale shift of their ranges. These conditions would have restricted deciduous oak to the three southern peninsulas i.e. Iberian, Italian and Balkan (Willis, 1996). However, even there, they were unable to form widespread forests due to the lack of moisture. Their presence was therefore restricted to smaller areas where suitable microclimates existed, termed refugia. These are generally believed to have been located at mid-altitude in mountainous regions, where the relief would induce greater precipitation (Beug, 1975). The fossil pollen sites containing the strongest evidence for the existence of oak refugia are all situated in or near mountainous regions (Brewer et al., 2002). This information has guided our attempts to locate potential refugia on the basis of the cpDNA investigation (Fig. 3).

4.1.1. Pollen and climate data

Unfortunately, the small size of these populations makes them difficult to detect; actually, refugia almost certainly existed that have not yet been discovered by pollen analysis. The strongest argument suggesting that the oaks were absent north of the 13 ka BP limit indicated in Fig. 1a of Brewer et al. (2002) is the advance of the North Atlantic Polar Front and the cold, arid conditions it brought to areas above approximately 40°N (this parallel runs from central Portugal to Sardinia, Calabria, and northern Greece) (Ruddiman and McIntyre, 1981). The North Atlantic Polar Front is the boundary between warm, high-saline water flowing north, and cold, low-saline water flowing south from the Arctic (Lowe and Walker, 1997). For comparison, this Polar Front is now located to the north of Norway, above the 70°N parallel, more than 1000 km from the northernmost European oak populations (located in Norway at approximately 63°N, Jalas and Suominen, 1976). However, as the polar front advanced southwards, the distance between it and the nearest oak populations would have diminished due to the increased insolation levels at lower latitudes.

Additional evidence from recent reconstructions of climatic variables for the period of the last glacial maximum (approximately 18 ka BP) indicate some significant regional differences in Europe, which may help to explain the proposed distribution of refugia. Winter temperatures were more reduced in the north of Europe than in the south. Reconstructions in the south of Spain, Italy and the Balkan Peninsula, as well as the near east show a reduction in winter temperatures by on average 15 ± 5°C in comparison to the modern day (Peyron et al., 1998). In the centre of Italy a reduction of 23 °C is noted. Above this, the fall in temperatures was greater: in the north of Spain, the Pyrenees and France were on average 30 ± 10°C lower than present. There is also a significant north/south difference in the reconstructed moisture availability. In the north of Spain, France and central Italy, the moisture availability was reduced by 60% in comparison to current levels. In contrast, in southern Spain and the near east, levels of moisture availability were similar to those of the present day. In the south of Italy and the southern Balkans, average reductions of 20% were observed (Peyron et al., 1998). The greater reduction in winter temperatures, and notably in the available moisture in the north of the continent make it unlikely that populations of the oak survived there.

The question of how far to the north were full-glacial period refugia located, remains open. On the basis of a re-evaluation of existing palaeobotanical evidence and on the climatic evidence discussed above, Brewer et al. (2002) consider it unlikely that oak populations would have persisted in northern Portugal or along the Atlantic coast, i.e., above 40°N of latitude. However, further palynological studies in critical sites during the last glacial period are badly needed, and climatic reconstructions of the potential northern limits of oak populations at the height of the last glacial period would help.

4.1.2. Molecular data

Molecular studies have the potential to reveal lineages whose modern distribution could help identify these so-called refugia. Indeed, the populations located at the northern edge of the distribution of a species during the glacial period are likely to contribute most to colonisation (the 'leading edge' hypothesis, Hewitt, 1996). If southern lineages exist that have been unable to contribute to the migration, then the existence of more northerly refugia may be inferred. Alternatively, if all southern lineages have been involved in post-glacial recolonisation, a rather synchronous spread from several refugia located at similar latitudes would constitute the most parsimonious explanation. Examples where southern lineages have been unable to proceed further north due to the existence of other more northern refugia have been provided in the recent literature, for instance in Greece and in Turkey (Hewitt, 1999). Here, in the two most well-studied peninsulas (Iberia and Italy), no evidence exists for such situations; with the exception of haplotype 20 in Corsica/Sardinia that has remained trapped on these islands, all the frequent southernmost haplotypes were able to migrate to the north. Lineage D remained trapped in Iberia, but it did colonise a large part of northern Spain from an inferred southern refugium, and moreover it seems to have been blocked in the north-east of Spain by incoming oak populations originating from other refugia, rather than by populations that had survived further north.

When taken together, these findings constitute a strong argument in favour of the model of oak refugia restricted to the extreme south of the European continent. Study of the southern Balkans may reveal a slightly different pattern because Turkish and probably also Greek haplotypes may have been prevented to migrate northwards by lineages situated in refugia located further north, e.g., southern Carpathians or Black Sea coast.

By contrast, using the southernmost distribution of a haplotype as evidence for the presence of a full-glacial refugium is more risky, because northern advance may have been accompanied by a southern retreat, i.e. the extinction of the southernmost populations of a given lineage, due to increased temperatures and drought in the south (Hewitt, 1999). After having served as a source for northern colonisation, lowland (e.g., coastal) refugial populations may have died out once the climate warmed, if they had no opportunity to move into nearby mountains. Southern limits of lineage distributions may therefore overestimate the latitude of the refugium for the corresponding lineage. Furthermore, cases of disjunction in the distribution of haplotypes can be observed, particularly in southern European regions, where more southerly populations may have persisted in favourable situations (e.g., at higher elevation) while intermediate populations have become extinct. In fact, a number of cases of disjunction (often involving several hundred kilometres) have been observed during this survey. In order to demonstrate that these large hiatuses in the distribution of haplotypes (such as for haplotype 11 in Iberia or for haplotype 2 in Italy) do result from the disappearance of intermediate populations, very detailed surveys are needed. For instance, we might have falsely concluded that a large hiatus existed for haplotype 1 between south-eastern France and Catalonia if we had sampled less intensively in south-western France. Additionally, given the low evolutionary rate of cpDNA, it is necessary to check that these disjunct haplotypes are indeed identical and do not exhibit cryptic molecular differences. Another possible explanation for these disjunctions is that intermediate populations have disappeared, not as a result of climate warming, but as a result of climate deterioration. Climate change is by no means a regular phenomenon, and transitions between glacial and interglacial periods often involve rapid shifts between warm periods with increased moisture availability (e.g., the late-glacial interstadial) and cold, arid periods (e.g., the Younger Dryas, Brewer et al., 2002). Migration from the primary glacial period refugia during warm periods may have left populations exposed between these primary refugia and favourable sites reached during the previous warm period (i.e., secondary refugia). These exposed populations would then have been unable to survive the climatic reversal, and may ultimately be replaced by trees migrating from a different area.

4.1.3. Communication between refugia

One finding from this study is that communications between refugia have occurred during the present interglacial (e.g., between the Iberian and the Italian and Balkan refugia for haplotype 1 and between the Italian and the Balkan refugia across the Adriatic for haplotype 7). Furthermore, the presence of haplotype 5 in the Italian refugium, despite the fact that the lineage to which it belongs (A) is centred in the Balkan refugium, seems to imply that exchanges across refugia during past interglacials have also occurred. Hence, even if the northernmost populations of the species die out at the onset of each new glacial period, as is classically emphasised (i.e. dead-end evolution, see e.g., Bennett, 1997), it seems dubious that populations located in the different refugia have evolved totally independently from each other throughout the Quaternary.

4.2. Post-glacial recolonisation

4.2.1. Shared routes

The pace of climate change during post-glacial recolonisation as well as the topography and ecology of Europe frequently induced roughly parallel migration routes for different haplotypes. This is the case for instance for haplotypes of lineage B migrating out of the Iberian Peninsula. However, a more unexpected result was the observation that widely divergent haplotypes (e.g., some of Italian and Balkan origin) exhibited common colonisation routes (for instance along the Italian Peninsula, but also across the Adriatic or from the Carpathians to the Baltic countries, see Fig. 2).

4.2.2. Maintenance of diversity

That few of the most frequent haplotypes were lost during such an extensive spread (up to a few thousand kilometres) along similar colonisation routes indicates that the mechanism of colonisation preserves cpDNA diversity relatively well. This seems to contrast with scenarios of colonisation through long-distance maternal founding events involving virtually a single seed and resulting locally in a complete loss of diversity (Petit et al., 1997). However, simulations of colonisation in two dimensions (i.e., excluding linear habitats) have shown that the *overall diversity* will be better preserved if colonisation takes place by a combination of local dispersal and long-distance jumps (stratified dispersal) rather than by means of diffusive dispersal: these long-distance jumps appear to maintain a mosaic of haplotypes (Le Corre et al., 1997). This would also explain the absence of well-demarcated areas of contact, the so-called suture zones, Hewitt (1996, 1999) between lineages expanding from divergent refugia at the continental scale (see Fig. 4 in Petit et al., 2002a), although at a more local scale, the transition between these areas can be quite sharp (Petit et al., 1997).

4.2.3. Human populations as vectors for oak dispersal

Besides climatic changes leading to the disappearance of intermediate populations, very long-distance seed transports could also account for the observed disjunction of some haplotypes. They could involve either natural vectors (birds or water drift, see Petit et al., 1997 or Le Corre et al., 1997 for putative mechanisms) or artificial ones. Indeed, in Europe, as in other parts of the world (e.g., North America), acorns have long constituted an important nutritional resource for man and his cattle. Most European white oak species have bitter tasting acorns due to the large amounts of tannins. However, some populations of *Q. pubescens* (var. *virgiliana*), *Q. pyrenaica* or especially *Q. frainetto* (called *aesculus* by Latin writers) can have sweet acorns. Even *Q. robur* trees may have sweet acorns, as deduced from wild-boar selection of the fruits and after tasting them (P.G. Goicoechea, pers. obs.). Moreover, techniques of detoxification (by roasting or by boiling) will render even the most bitter acorns suitable for human consumption. The great abundance of acorns and the facility to store them (despite irregular masting intervals in the northern part of the range of the oaks), explain the long-standing interest in this natural resource. In fact, discoveries of acorn caches in prehistoric habitats are frequent, dating back at least to the mesolithic (references in Lieutaghi, 1998). Hence man could have been involved in the recolonisation of oaks. However, it should be kept in mind that oaks were able to readily colonise Europe during the last interglacial (130–117 ka BP), at a time when human influence is unlikely to have played an important role (Van Andel and Tzedakis, 1996). More recent human influence on the geographic pattern of chloroplast variation has been acknowledged within Central Europe (König et al., 2002), Great Britain (Cottrell et al., 2002) and Scandinavia (Jensen et al., 2002).

4.2.4. Migration pathways may have been constrained by the ecology of the 'carrier' oak species

Initially, at the outset of recolonisation, many expanding lineages probably involved a single oak species (or differentiated oak population). This must have been particularly true for those lineages that migrated through mountains and underwent severe bottlenecks; however, considering the mechanism of stratified seed dispersal that has been suggested for oak recolonisation, and the resulting founding events (Petit et al., 1997; Le Corre et al., 1997), this may have been the rule. Because the oak species (or even oak populations of the same species) can have contrasting ecological behaviour, the direction and speed of colonisation may be affected. Indeed, the newly available land may better suit some oak species than others. Differential response to altitude may also play a role. During the present survey, initial associations between oak species and haplotypes have been suggested, such as between haplotype 1 and *Q. pubescens* or between haplotype 7 and *Q. petraea* in France and Spain. These interpretations, despite their speculative character, could be partly tested if significant amounts of usable DNA could be retrieved in key sites from ancient oak material (macrofossils) that could be identified to the species level, either by traditional taxonomic means or by the use of species-specific nuclear markers. Preliminary attempts to amplify DNA from recent and more ancient wood fragments uncovered during archaeological investigations have indeed given promising results (Dumolin-Lapègue et al., 1999b).

Despite some hints of such association, the overall picture is that of systematic cpDNA exchanges between species and hence nearly species-independence of cpDNA variation (when the species are sympatric). The hypothesis that oak species are able to colonise a site already occupied by other related species by pollen rather than by seeds (i.e., pollen swamping) (Petit, 1992; Bacilieri et al., 1996; Petit et al., 1997; Dumolin-Lapègue et al., 1999a) is therefore largely supported by the data presented here.

5. Conclusion

This synthesis of the work by researchers belonging to two communities (population geneticists and palaeoecologists) who had been previously rarely involved in direct co-operation (for an early exception see Cwynar and MacDonald, 1987) show the power of the approach and the necessity of extending this interdisciplinary study if new insights are to be gained on the past distribution and colonisation dynamics of forest trees. It turns out that the limitations of each approach are nicely supplemented by information from the other method; for instance, the taxonomic resolution is limited using palynological tools, whereas some insights on the dynamics of each species could be obtained on the basis of the genetic surveys. However, the genetic investigations have also their taxonomic limitations, because of the frequent pollen swamping of one oak species by another. Molecular analysis of macrofossils may ultimately provide a more direct multidisciplinary approach. The interpretation of colonisation routes provides another example: genetic surveys can have a greater spatial resolution than palynological surveys, but palaeobotanical investigations provide the indispensable temporal resolution. Once the migration routes meet, it becomes difficult to track subsequent movements with pollen data, whereas they can still be identified using the genetic approach. In the future, however, it will be necessary to better integrate climatic and ecological data, so that the processes that have led to the observed patterns of recolonisation can be better identified. This includes the competitive or facilitating interactions with other forest tree species (MacDonald, 1993) and with soil development. This effort should help in distinguishing the predictable from the unpredictable (the idiosyncrasies of the particular seed dispersal events). Computer simulations of postglacial recolonisation processes, following the work initiated by Le Corre et al. (1997), could provide a useful link between observations and interpretations.

Finally, it should be stressed that the rich information content of the cpDNA maps could not be described in full detail in the present European-wide review of the work. Further information on refugia, migration routes, and more extensive discussion on the effects of human management, forest fragmentation, mountain barriers, and on the situation at the periphery of the range can be found in the regional papers in this issue.

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