



# Glacial refugia influence plant diversity patterns in the Mediterranean Basin

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## ABSTRACT

**Aim** The aims of this study were to assess the distribution of putative Mediterranean refugia of plants, to compare the locations of refugia and those of regional hotspots of plant biodiversity, and to provide a critical analysis of the Mediterranean refugium paradigm. Furthermore, we consider how biogeographical and genetic results can be combined to guide global conservation strategies.

**Location** The Mediterranean region.

**Methods** We started from a detailed analysis of the scientific literature (1993–2007) in order to identify refugia in the Mediterranean region, based on intra-specific phylogeographical studies of plant species. We used population locations together with gene-pool identity to establish the database, comparing patterns of phylogeographical concordance with the locations of Mediterranean refugia. We then tested the biogeographical congruence between two biodiversity components, namely phylogeographical refugia and regional hotspots.

**Results** We identified 52 refugia in the Mediterranean bioclimatic region and confirmed the role played by the three major peninsulas, with a shared total of 25 refugia. We emphasize the importance of areas that have previously been attributed a lesser role (large Mediterranean islands, North Africa, Turkey, Catalonia). Of the 52 refugia identified, 33 are situated in the western Mediterranean Basin and 19 in the eastern part. The locations of the phylogeographically defined refugia are significantly associated with the 10 regional hotspots of plant biodiversity, with 26 of these refugia (i.e. 50%) occurring within the hotspots.

**Main conclusions** The locations of refugia are determined by complex historical and environmental factors, the cumulative effects of which need to be considered because they have occurred since the Tertiary, rather than solely during the last glacial period. Refugia represent climatically stable areas and constitute a high conservation priority as key areas for the long-term persistence of species and genetic diversity, especially given the threat posed by the extensive environmental change processes operating in the Mediterranean region. The refugia defined here represent 'phylogeographical hotspots'; that is, significant reservoirs of unique genetic diversity favourable to the evolutionary processes of Mediterranean plant species.

## Keywords

Biodiversity hotspots, conservation biogeography, endemism, Mediterranean islands, mountains, palaeoecology, phylogeography, plant persistence, Pleistocene ice ages.

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Une flore ne s'enfuit pas comme une armée, elle s'éteint sur place ou se déplace légèrement plutôt qu'elle ne fuit. Ce qu'elle laisse, ce ne sont point des trainards, mais des survivants, et parmi ces survivants, il y a beaucoup d'espèces endémiques.

(Battandier, 1894: p. 6).

Unlike an army, a flora doesn't flee; it expires where it is, or else moves slowly on, rather than fleeing. It leaves behind not stragglers but survivors, and among these survivors are many endemic species.

(Battandier, 1894: p. 6).

## INTRODUCTION

Since the importance of Mediterranean refugia in North Africa was first suggested by Battandier (1894), and the formalization of the general refuge theory (Haffer, 1982), the role that glacial refugia have played in structuring biodiversity at both global (e.g. Bennett, 1997; Hewitt, 2000) and regional (Svenning & Skov, 2007) scales has remained a key issue. Most of the existing palaeoecological and phylogeographical evidence underlines the impact of refugia on current patterns of biodiversity at mid and high latitudes (Taberlet *et al.*, 1998; Willis & Whittaker, 2000; Schönswetter *et al.*, 2005; Bhagwat & Willis, 2008).

First, several studies have shown the clear influence of Pleistocene climatic cycles on patterns of species richness and endemism (e.g. Jansson, 2003), and historical patterns of glaciations have left still-detectable traces, partly explaining the distribution and richness of tree species in both Europe and North America (e.g. Svenning & Skov, 2007). Second, full-glacial refugia have had a powerful influence on current patterns of genetic diversity in temperate Europe (Petit *et al.*, 2003; Hampe & Petit, 2005). Finally, in agreement with earlier evidence (e.g. Beug, 1975; Bennett, 1997), recent research has demonstrated that glacial refugia also played an important role in vegetation dynamics during interglacial periods of the Pleistocene in Europe (Cheddadi *et al.*, 2005; Tzedakis, 2007, 2009).

Hence, it is clear that ice age refugia are crucial for the long-term persistence and dynamics of temperate biodiversity (Hewitt, 1999; Tzedakis *et al.*, 2002). This is particularly true for the Mediterranean Basin, which constitutes one of the world's major biodiversity hotspots (Médail & Myers, 2004) and is an outstanding biogeographical crossroads for the European, Saharian and Irano-Turanian regions (Quézel, 1985). The interplay between complex historical processes and heterogeneous environmental conditions has given rise to considerable plant biodiversity and endemism in this region (Médail & Quézel, 1997; Thompson, 2005). The onset of the Mediterranean climate, major palaeogeographical events such as the Messinian Salinity Crisis (5.77–5.33 Ma), and the Milankovitch climate oscillations since the late Tertiary explain the heterogeneous evolutionary history of Mediterranean plant lineages (Thompson, 2005). The deterioration of Tertiary warm, moist climates during the Plio-Pleistocene resulted in the extinction of numerous cool- and warm-temperate plants (e.g. Suc *et al.*, 1999; Svenning, 2003), but Pleistocene climatic cycles also profoundly affected the phylogeographical footprint

of Mediterranean species (Taberlet *et al.*, 1998; Weiss & Ferrand, 2007). By providing suitable habitats during adverse climate periods, these refugia appear to have limited species extinction as well as favouring the emergence of new taxa (e.g. Hungerer & Kadereit, 1998). As a result, the whole Mediterranean region constitutes both a global refuge for relict plants and an area that encourages floristic exchange and active speciation by means of secondary contacts and hybridization.

During the late 1990s, several pioneering phylogeographical studies permitted an approximate location of these major southern European refugia, and emphasized the major role played by Mediterranean peninsulas (Taberlet *et al.*, 1998; Hewitt, 1999). However, the phylogeographical patterns of southern refugia are more complex than those found in northern regions (Petit *et al.*, 2003, 2005), and the exact geographical limits of Mediterranean refugia have never been defined. The phylogeographical picture appears incomplete because it is based mainly on the results of molecular analyses restricted to a few woody plant species. In order to improve this definition, we need to take into account recent molecular studies, including those focused on herbaceous species, whose patterns of local survival or dispersal may differ from those of tree species (e.g. Cozzolino *et al.*, 2003).

An accurate identification of the locations of glacial refugia is a high priority for conservation policies because these key areas for the long-term persistence of biodiversity are threatened by the rapid and wide-ranging environmental changes occurring around the Mediterranean (Blondel & Médail, 2009). Based on an important and unique set of 82 plant species, we here use a comparative phylogeographical approach to provide novel historical insights into phylogeographical congruence among species (Arbogast & Kenagy, 2001) that are extremely relevant to evolutionary biogeography. By providing, for the first time, an extensive and finer-scale delimitation of Mediterranean refugia, the present review also offers a sound framework for combining biogeographical and genetic results to guide conservation strategies in the Mediterranean Basin.

## WHAT IS MEANT BY REFUGIA IN THE MEDITERRANEAN REGION?

Confirming the early ideas about glacial refugia proposed by Mediterranean biogeographers (Battandier, 1894; Roi, 1937), the first full-glacial pollen diagrams from southern Europe confirmed the existence of tree populations in refugia located in the main Mediterranean peninsulas (e.g. Beug, 1975). Further palaeoecological studies pointed out the importance of glacial locations of trees for post-glacial vegetation dynamics (Reille *et al.*, 1996; Bennett, 1997). However, despite extensive palaeoecological (Elena *et al.*, 2000; Tzedakis, 2009) and phylogeographical syntheses (Petit *et al.*, 2003, 2005; Weiss & Ferrand, 2007), a detailed and consistently applied definition of Mediterranean refugia does

not exist. A related problem is that the locations of Mediterranean refugia may have been variable from one glacial to another (Reille *et al.*, 1996) and linked to differences in the long-term vegetation dynamics (but see Cheddadi *et al.*, 2005).

Insights from palynological studies have, nevertheless, made it possible to define three main types of refugia, according to their geographical and environmental characteristics (Reille *et al.*, 1996; Quézel & Médail, 2003).

(1) The most spatially extended areas are the moist mid-altitude refugia (c. 400–800 m a.s.l.), which would have allowed altitudinal shifts in response to climate changes or the *in situ* persistence of species (Beug, 1975). These areas were less arid than the surrounding dry plains owing to the orographic precipitation, and less cold than sites located at higher altitude or latitude. A good example of this kind of refugium is the Ioannina catchment in the Pindus Mountains (Greece), where several temperate and thermophilous trees have persisted through multiple glacial–interglacial cycles (Tzedakis *et al.*, 2002).

(2) The second type, which are more spatially limited, but relatively common, are refugia present in deep gorges or closed valleys with continued moisture availability owing to the protected microenvironment. In these areas, the climate stayed locally wet during periods of drought, enabling some Tertiary relict plants requiring a humid-warm climate to persist (e.g. the fern *Woodwardia radicans*), mostly confined to shaded cliffs and rocks. More generally, riverbanks clearly acted as ecological buffer zones against glacial drought, not only for warm-temperate and mesophilous plant taxa, but also for some typical Mediterranean sclerophyllous trees.

(3) Finally, there are some refugia of mesophilous trees located in low-altitude areas, notably in locally moist and warm sites such as valley bottoms, coastal plains and wetlands. However, these coastal sites have largely been flooded by eustatic sea-level rise, and little palaeobotanical evidence is available, except for a site in southern Spain (Pantaléon-Cano *et al.*, 2003). The relict alluvial forests of *Liquidambar orientalis* in south-west Anatolia and Rhodes Island represent a current example of this refugium type (Quézel & Médail, 2003). Such wetlands also served as refugia for Eurosiberian and boreal herbs, and were initially termed ‘colonies planitiales’ by Roi (1937); for example, some of the wetlands of the Camargue-La Crau (southern France), the Pontin marshes near Rome and the El Kala marshes (north-east Algeria).

The identification of the extent or grain of the refugia remains difficult, however, as the existing body of work on the refugium concept does not shed much light on this aspect. Here, we consider a refugium as an area where distinct genetic lineages have persisted through a series of Tertiary or Quaternary climate fluctuations owing to special, buffering environmental characteristics; in other words, their existence implies the local long-term (one or more glacial–interglacial cycles) persistence of a species or of one or more of its component populations within a well-defined geographical area (e.g. mountain range, gorge).

## MATERIALS AND METHODS

### The use of statistical phylogeographical models in the Mediterranean

The spatial delimitation of refugia remains a challenge that has not been widely addressed in the literature. Methods used to identify refugia have not been clearly defined, and at times appear to be in conflict (Waltari *et al.*, 2007). In contrast to the case for areas such as North America and temperate Europe, for which refugia predictions based on phylogeographical or ecological niche approaches have recently been made (e.g. Swenson & Howard, 2005; Carstens & Richards, 2007; Richards *et al.*, 2007; Waltari *et al.*, 2007), such studies are still scarce for the Mediterranean Basin (but see Benito Garzón *et al.*, 2007; Leroy & Arpe, 2007). This region is made up of three continents and encompasses a highly dissected sea with numerous islands, each with a distinct history. Hence this region is very heterogeneous in terms of climate and geography (Quézel & Médail, 2003), and will, as a consequence, be particularly challenging for refugium modelling.

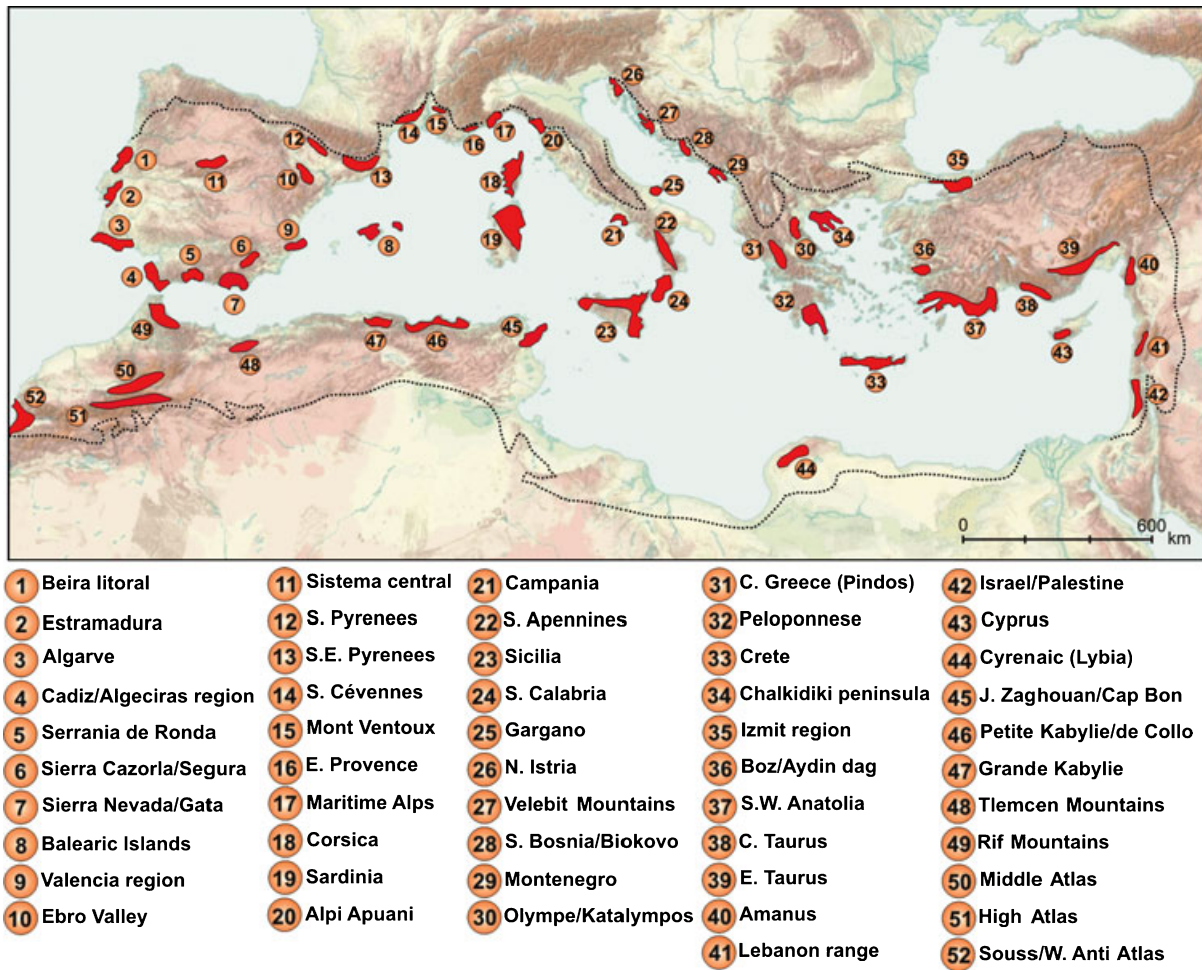
For example, Swenson & Howard (2005) defined refugium locations by drawing migration routes and using histograms from contact-zone distributional analyses in North America. Such an analysis is difficult to apply to the Mediterranean Basin owing to the lack of well-defined post-glacial expansion routes. Furthermore, this method does not predict the distribution of passive refugia, including islands, which contain relict populations not involved in post-glacial migrations. Another study combined species distribution and palaeodistribution models in North America, using coalescent simulation and empirical genetic data to generate alternative biogeographical hypotheses (Richards *et al.*, 2007). However, this kind of method cannot yet be applied to our area because palaeoenvironments are highly heterogeneous, and palaeoclimatic estimates are currently lacking for the southern and eastern coasts (J. Guiot & R. Cheddadi, personal communication) or are too coarse (Peyron *et al.*, 1998; Wu *et al.*, 2007). For the same reason, and because of the complex role of biogeographical barriers, the use of ecological niche models (ENMs) in conjunction with palaeoclimatic reconstructions (Waltari *et al.*, 2007) does not appear feasible for the Mediterranean area today.

To overcome these difficulties, we here consider all the available data from plant intraspecific phylogeographical studies (i.e. on populations rather than on species). Such intraspecific phylogeographical patterns are often influenced by the survival of populations during and after unfavourable climatic events, notably glacial periods (e.g. Taberlet *et al.*, 1998).

### Methodological outline

#### Literature base

We carried out a comprehensive selection and analysis of the scientific literature from 1993 to 2007 related to plant



**Figure 1** Fine-scale geographical distribution of the 52 putative refugia within the Mediterranean region (limits *sensu* Quézel & Médail, 2003, indicated by a broken line) obtained from the analysis of the phylogeographical patterns of 82 plant species (41 trees and 41 herbs).

intraspecific phylogeographical studies, selecting journals frequently publishing phylogeographical studies, as well as some papers published in other peer-reviewed journals (see references in Appendix S1). We limited our search to plant studies based on chloroplast DNA (cpDNA), ribosomal DNA (rDNA) and/or nuclear DNA that included at least one population located within the Mediterranean bioclimatic region *sensu* Quézel & Médail (2003) (Fig. 1) (i.e. a total land area of 2,300,000 km<sup>2</sup>). Therefore, some of the plant species selected here have distribution ranges that include non-Mediterranean biogeographical regions, such as central and northern Europe. These were included here in order to take account of the potential role of Mediterranean populations in the phylogeographical structure of extra-Mediterranean populations.

For each selected paper, we recorded the species distribution range, the number of populations used in the analysis, including those from the Mediterranean region, the type of marker obtained, and the location of putative refugia within

the nine broad geographical areas considered (Table 1, Appendix S1).

#### *Putative mapping of refugia*

Patterns of phylogeographical concordance were investigated for comparison against the locations of Mediterranean refugia. The data consist of a set of populations providing genetic information about putative refugia (e.g. unique haplotype, rare allele, private fragments). All the identified refugial populations of a given species (except for the genus *Quercus*, the *Androcymbium* sp. complex, *Hedera helix* complex and *Viola alba*: see Appendix S1) were georeferenced and entered onto a map of the Mediterranean region (i.e. non-Mediterranean populations were not included in the data set). Refugial polygons were then created using a two-step approach. First, a linear map was created by connecting the point populations with a straight line. Second, a geographical boundary for each reported refugium was placed around this straight line to

**Table 1** Number of papers, taxa and data sets (number of populations reporting intraspecific phylogeography and used for the identification of putative refugia, for each species and study) used to identify refugia for the main geographical areas.

Main geographical area	No. papers	No. taxa	No. data sets	No. refugia
Iberian Peninsula	36	39	79	11
Catalonia–Provence	20	18	24	4
Tyrrhenian islands	14	15	21	4
Italian Peninsula	30	27	37	6
Balkans	17	16	20	4
Greece–Crete	16	15	23	5
Turkey	8	8	17	7
Middle-Orient	6	4	8	2
North Africa	19	19	33	9

create a polygon representing the putative refugium. This was done taking the main topographical structures (e.g. mountain ranges) into account.

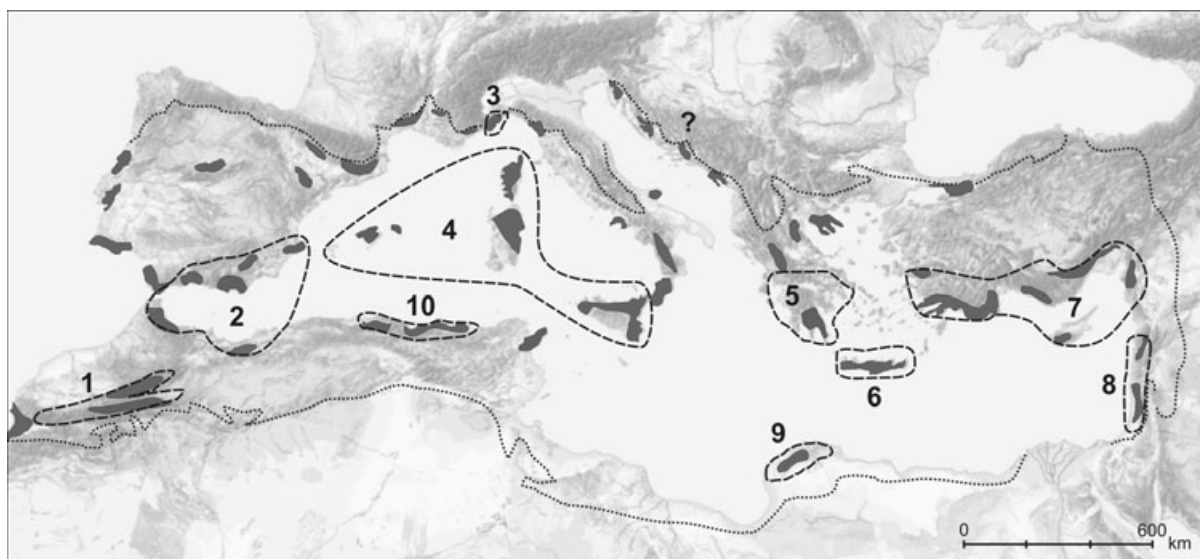
#### *Comparison between biodiversity hotspots and putative refugia*

We compared the distribution of the 10 Mediterranean regional hotspots of plant biodiversity, which represent key biodiversity areas (selected on the basis of high species diversity and endemism together with severe human-induced habitat modifications: see Médail & Quézel, 1997), with the locations of putative refugia within the Mediterranean region (Fig. 1). Delimitations of hotspots and refugial locations were

vectorized under GIS using ArcGIS 8.3 (Environmental Systems Research Institute, Redlands, CA, USA). The resulting map allowed us to estimate the spatial concordance between hotspots and putative refugia (Fig. 2). The percentage of the map covered by hotspot areas within the Mediterranean region was used to calculate the theoretical numbers of refugia expected to overlap with the hotspots by chance, according to a normal distribution. A chi-squared analysis was performed to test whether the amount of spatial congruence between hotspots and refugia identified from the phylogeographical survey was greater than that expected by chance.

## RESULTS

Our survey of intraspecific plant phylogeography studies selected 80 papers published between 1993 and 2007 (Table 1, Appendix S1). Eleven studies (13.8%) were published prior to 2000, and 69 (86.2%) after 2000, a pattern similar to that obtained for terrestrial plants by Beheregaray (2008) in his world-wide review of phylogeographic articles published since 1987. Our review takes into account 82 plant species, including 41 trees and 41 herbs, distributed across nine main geographical areas of the Mediterranean (Table 1, Appendix S1). Based on this unique data set including 262 refugial populations, we identified 52 refugia within the Mediterranean region (Fig. 1), 33 situated in the western Mediterranean Basin and 19 in the eastern part. Although the sample of plant species analysed to date clearly represents a very small proportion of the considerable Mediterranean plant biodiversity, we are confident that the phylogeographical picture depicted here has strong



**Figure 2** Locations of the 52 putative refugia identified in the present study (dark grey) and of the 10 regional hotspots of plant biodiversity (large broken line; data from Médail & Quézel, 1997, completed by Vela & Benhouhou, 2007). 1, High and Middle Atlas; 2, Baetic–Rifan complex; 3, Maritime and Ligurian Alps; 4, Tyrrhenian islands; 5, south and central Greece; 6, Crete; 7, south Anatolia and Cyprus; 8, Syria–Lebanon–Israel; 9, Mediterranean Cyrenaica; 10, Kabylies–Numidie–Kroumirie; ?, Dalmatian coast (T. Nikolic *et al.*, unpublished data).

biogeographical support. A first analysis performed with data available up to the end of the year 2006 (70 species examined) enabled us to define 50 Mediterranean refugia. The current analysis, based on a further data set of 12 plant species and 10 studies, has only led to the inclusion of two 'additional refugia': the High Atlas and Valencia regions. We therefore argue that most of the large or medium putative refugia of the Mediterranean region can now be considered to have been identified.

Our study indicates that several of the areas identified served as refugia for more than one plant species. With a joint total of 25 refugia, the role played during glacial periods by the three major peninsulas (Iberian, Italian and Balkans-Greece) is confirmed. However, the importance of other areas located on the main Mediterranean islands (6 refugia) and in North Africa (9), Turkey (6) and Catalonia-Provence (4) must be noted (Table 1). Furthermore, of the 52 refugia identified here, 33 are situated in Mediterranean submontane and mountain margins.

We found a strong biogeographical congruence between the 52 refugia and the major biodiversity areas of the Mediterranean region. First, the major plant endemism areas (i.e. with > 10% range-restricted endemics) of the Mediterranean Basin, which are mainly situated on islands and in mountainous regions (Médail & Quézel, 1997), are entirely included in the identified refugia. Second, half of these refugia (26) are included in the 10 Mediterranean regional biodiversity hotspots (Fig. 2). The GIS analysis indicates that these hotspots represent c. 20% (0.1999) of the total surface of the Mediterranean bioclimatic region. This suggests that, by chance, 10.4 refugia should be found within the hotspots and 41.6 refugia outside them. As we found an equal distribution of the number of refugia inside and outside of hotspots, we may conclude that refugia are significantly over-represented within the hotspots ( $\chi^2 = 29.26$ ; d.f. = 1;  $P < 0.001$ ).

## DISCUSSION

### The complex historical and environmental determinants of refugium locations

The common belief that refugia were confined to the major Mediterranean peninsula is not supported by these results. Instead, a more complex picture emerges, with numerous viable areas for thermophilous and mesophilous plant species, as partly suggested by several palaeobotanical studies (e.g. Carrión *et al.*, 2003; Tzedakis, 2009). The climatic changes during the Late Pliocene and the Pleistocene in the Mediterranean region are highly complex, and palaeoenvironmental data have demonstrated century-to-millennial climate variability, with rapid changes (e.g. de Beaulieu *et al.*, 2005; Martrat *et al.*, 2007). Variation in the Earth's obliquity and precession notably influenced the Mediterranean climate through dry-wet oscillations that were independent of glacial-interglacial variability (Tzedakis, 2007, 2009). Following the onset of the major Northern Hemisphere glaciations c. 2.8 Ma, the climate after 0.9 Ma was characterized by the intensification of glaciations

and lengthened glacial-interglacial cycles (Tzedakis, 2009). Several of the largest glaciations were recorded at c. 650 ka (Marine Isotope Stage (MIS) 16), 430 ka (MIS 12), 140 ka (MIS 6) and  $21 \pm 2$  ka (MIS 2: the Last Glacial Maximum, LGM) (Shackleton, 1987). From 450 ka, the Mediterranean climate underwent large changes between interglacial periods, when annual precipitation (Pann) was > 800 mm year<sup>-1</sup> and January temperature (Tjan) was > 2°C, and glacial periods, when Pann was < 400 mm year<sup>-1</sup> and Tjan oscillated between -10°C and -15°C (Cheddadi *et al.*, 2005). Recent improvements in the estimation of past climate suggest a complex spatial variation in the magnitude of cooling during the LGM (Wu *et al.*, 2007). Wu *et al.*'s study, based on an inverse modelling approach, indicated that, in the Mediterranean region, the mean temperatures of the coldest month were c. 12°C colder than today, with cold summers characterized by anomalies of c. -5°C. This represents a less extreme climate than that suggested by previous studies (e.g. Peyron *et al.*, 1998; who indicated a cooling of  $30 \pm 10^\circ\text{C}$  in western Europe; see also Jost *et al.*, 2005), owing to the fact that Wu *et al.* accounted for the effect of lowered glacial atmospheric CO<sub>2</sub> concentration on plant distributions (Wu *et al.*, 2007).

Hence, most of the phylogeographical studies examined here suffer from the adoption of an oversimplified model of Quaternary palaeoenvironmental history in the Mediterranean region. Indeed, the paradigm whereby Quaternary climates alternated between 'favourable' and 'adverse' periods is not verified by the varied ecological dynamics of plant taxa demonstrated by the Quaternary fossil records (de Beaulieu *et al.*, 2005; Tzedakis, 2009). Although it is certainly true that the higher latitudes of Europe generally suffered the most extreme impacts of glaciations, it is a fallacy to believe that southern Europe did not experience climatic fluctuations of large magnitude, and glacial erosive or depositional features have been found in several Mediterranean mountains, including the Sierra Nevada, Serra d'Estrala and Gran Sasso d'Italia. Rapid and major climate and vegetation variability existed, for example during the last 1.35 Myr in north-eastern Greece (Tzedakis *et al.*, 2006), and during the last glacial period in Italy, where vegetation changes often occurred in < 200 years (Allen *et al.*, 1999).

Therefore, it appears simplistic to divide refugia into two broad types as Nekola (1999) does, namely into palaeorefugia confined to small relict areas harbouring several Tertiary relict species, and neorefugia, defined as Quaternary refugia located in unglaciated areas. In both cases, refugia represent areas where the climate was locally warmer and more humid than that in nearby regions, but their relative age is still very difficult to ascertain. As genetic lineages possess a history that extends over past glacial conditions and often back into the Pliocene or earlier, we cannot label our defined refugia solely as 'glacial refugia'. These special areas should instead be considered as 'cumulative refugia' that have often persisted through several palaeogeographical and climatic events. For example, populations of cork oak (*Quercus suber*) may retain a very old genetic imprint dating back to Tertiary plate tectonics, between 25 and

15 Ma (Magri *et al.*, 2007). Furthermore, the Tertiary refugia of Mediterranean trees, defined by Quézel & Médail (2003) on the basis of current relict populations, are fully included in the 52 obtained refugia. Of these, 33 were situated in the western Mediterranean Basin and 19 in the eastern part. This longitudinal pattern may be related to the older palaeogeographical history of the western basin, which has also been subject to greater climatic changes during glacial periods, linked to changes in oceanic circulation. However, a possible bias resulting from the lower number of phylogeographical studies in the eastern basin prevents any further discussion.

On a smaller scale, climatic and topographical heterogeneities induced by mountainous or insular conditions have played a crucial role (Hewitt, 1999; Tzedakis *et al.*, 2002). Of the refugia identified, 33 are situated in Mountain ranges, notably the High and Middle Atlas, the Rif Mountains in north Morocco, the Sierras of Andalusia, the Maritime and Ligurian Alps, the Balkan Mountains, and the Taurus in Turkey. Mountain ranges and nunataks have played a determining role in the survival of small and isolated populations of herbs and shrubs, even mesothermic ones, during glacial periods. For example, the Sierra Nevada mountains (3482 m) – which represent the southernmost limit of the influence of the Quaternary glaciations in Europe – were glaciated only above c. 2500 m (Gómez *et al.*, 2001) and large areas remained free of permanent ice, favouring local alpine plant species persistence and diversification (Kropf *et al.*, 2006).

The local 'biogeographical stasis' of a plant species or of some populations is therefore linked to the capacity of the mountain range to provide a wide diversity of microhabitats, notably: (1) between sheltered and relatively humid gullies, and exposed and relatively dry ridges, (2) from south- to north-facing slopes or vice versa, or (3) between different altitudinal locations. These refugia appear to have been different microhabitats occupied by species at different periods, a refugium within a mountain range being in fact dynamic.

### Varied patterns of persistence, differentiation or migration of genetic lineages

Mediterranean plant species and genetic lineages experienced complex histories over the succession of glacial and interglacial periods: extinction, persistence, local differentiation or migration. It is an oversimplification to consider that all plant species and genetic lineages that were limited in their distribution to southern Europe during 'adverse' climate periods required similar ecological conditions for their survival. Furthermore, it can be argued that both fully glacial and fully interglacial conditions, being short-lived extremes of Quaternary climate fluctuations, are likely to represent 'adverse' periods for a majority of Mediterranean taxa. It seems better to consider the whole thing as a spatio-temporal dynamical system, with each plant species having an individual reaction.

The fact that part of the modern phylogeographical structure of some Mediterranean plants can be traced back to the Tertiary period (e.g. Magri *et al.*, 2007) implies

population persistence and genetic stability of a longer duration than the Quaternary. This pattern raises the question of how much of the genetic diversification of Mediterranean taxa is the result of isolation in Quaternary glacial refugia or of Tertiary palaeoenvironmental changes. Svenning (2003) demonstrated that the climate requirements of the European Tertiary and Quaternary cool-temperate trees were the major factors determining the persistence vs. extinction of these taxa. His analysis showed that genera now restricted to the Mediterranean region are cold-sensitive but relatively drought-tolerant, whereas more thermophilous or mesophilous trees have disappeared (Taxodiaceae, *Carya*, *Parrotia*, *Eucommia*, etc.) or are highly relictual in this region (*Pterocarya*, *Zelkova*) (Suc *et al.*, 1999; Quézel & Médail, 2003). However, plant responses to LGM climate conditions are complicated by the fact that maximum glacier size is not related to maximum drought. A significant degree of aridity probably occurred during the alpine deglaciation, somewhere between the end of the LGM and the beginning of the late glacial interstadial (Ravazzi, 2002).

It is difficult to compare the patterns of genetic lineage persistence and those of plant species on the basis of palaeoecological studies, as tree species are over-represented in such studies. The longest continuous Mediterranean pollen record of Tenaghi Philippon (northern Greece) shows robust evidence of long-term vegetation persistence after 650 ka (MIS 16) (Tzedakis *et al.*, 2006). Forests of reduced diversity and roughly similar to the modern forests with *Quercus*, *Carpinus* and *Ostrya* were established precociously, and the subsequent glacial–interglacial periods do not seem to have modified the composition of the arboreal vegetation of this refugium. Another high-resolution pollen record from western Greece demonstrates the persistence of temperate tree species in an ecologically stable area during the Quaternary (Tzedakis *et al.*, 2002). However, as there are only a few sites in Europe that cover the 900–400 ka period, the existence of long-term, stable tree populations during glacial–interglacial cycles cannot be generalized to other areas, given that the onset of greater glacial–interglacial amplitude occurred during MIS 22 (c. 900 ka) (P.C. Tzedakis, personal communication).

Glacial–interglacial episodes also induced several diversification events for a number of Mediterranean lineages, and glacial refugia were favourable areas for plant speciation during the Quaternary (e.g. Hungerer & Kadereit, 1998; Gutiérrez Larena *et al.*, 2002). At a large scale, vicariance results from the isolation of populations and subsequent allopatric differentiation, often with a considerable genetic distinctiveness between rear-edge populations of different low-latitude refugium centres (Petit *et al.*, 2003; Hampe & Petit, 2005). These populations experienced little gene flow and have probably been very stable from the time of their establishment, often prior to the Pliocene, allowing them to retain unique and high genetic variation (Petit *et al.*, 2003; Hampe & Petit, 2005). At a smaller scale, the 30 or so refugia identified in the Mediterranean mountains probably played a key role in speciation processes because they often acted as both a source and a sink

of genetic diversity, owing to the *in situ* survival of plants (Hewitt, 1999).

Such long-term survival was made possible by the locally high diversity of ecological niches and the opportunity for plant species to migrate in altitude, reducing the extent of range displacement necessary during glacial–interglacial cycles. Varied geological and topographical conditions also favour the isolation of populations, thus making them generally highly divergent even over short geographical distances (Petit *et al.*, 2003). Most of these well-isolated lineages have been identified in the mountains of southern Spain (Andalusia) (e.g. Hampe *et al.*, 2003) and Italy (e.g. Cozzolino *et al.*, 2003). Further evidence for the importance of vicariance processes between populations widely distributed during cold periods of the Quaternary comes from the distinctive intraspecific phylogroups observed among alpine plants in the mountains of south-western Europe (Kropf *et al.*, 2006). As these southern regions overall may have experienced less extreme climate change than higher latitudes, diverging gene pools may have finally resulted in the formation of neoendemic taxa.

With a global warming of temperatures from the LGM to the present that was effectively synchronous from south to north (P.C. Tzedakis, personal communication), southern populations often expanded their geographical distributions northwards, as new habitats became favourable. According to the leading-edge hypothesis (Hampe & Petit, 2005), populations involved in the post-glacial range expansions were mainly those at northern edges of refugia that were less isolated from deglaciated European territories. Petit *et al.* (2005) suggested that for most of the tree species of the cool-temperate Eurasian flora, the source of post-glacial colonization is located north of the Mediterranean region itself, at > 45° latitude. Nevertheless, northern edge populations of the Mediterranean could have contributed to these northward colonizations. Therefore, the initial paradigm of three post-glacial colonization roads from southern Europe (Taberlet *et al.*, 1998; Hewitt, 1999, 2000) constitutes an oversimplified biogeographical pattern. However, it remains difficult to generalize about the mode and tempo of recolonization of genetic lineages, owing to (1) differences in species dispersal capacities, (2) variation in the geographic locations of refugia or the existence of barriers, and (3) the complex processes of genetic structuring occurring during population expansions.

In the case of beech (*Fagus sylvatica*), the detailed analysis of a large genetic and palaeoecological data set showed that migration was not simultaneous, suggesting an important role for local environmental factors (Magri *et al.*, 2006). The migration rates of plant species are also under debate, as recent evidence suggests that the post-glacial colonization of some tree species was much slower than previously thought (McLachlan *et al.*, 2005). In contrast to the rapid migration of magnitude 100–1000 m year<sup>-1</sup> obtained with fossil pollen data sets, the genetic structures of modern tree populations provide evidence that colonization was driven mainly by slower dispersal from disjunct and cryptic glacial refugia, which are quite poorly identified by palynological data

(Stewart & Lister, 2001). However, the existence of cryptic refugia is still debated by several European palaeoecologists (J.-L. de Beaulieu & P.C. Tzedakis, personal communication), despite increasing fossil and genetic evidence for the full-glacial survival of some temperate trees in northern Europe (see the recent reviews of Bhagwat & Willis, 2008, and Provan & Bennett, 2008).

In trying to determine the migration routes of plants, the role of biogeographical barriers such as straits or mountain ranges must also be considered. Although the existence of barriers can be seen to have affected certain species, they do not explain the phylogeographical patterns of many others (for example, the Gibraltar strait for some legume species; see Arroyo *et al.*, 2008). An unexpected result of the phylogeographical study of beech (Magri *et al.*, 2006) was that mountain chains were found generally not to be geographical barriers but rather to have facilitated the diffusion of beech. In contrast, large continental plains (for example the Po), valleys (for example Ebro Valley) and rivers (for example Loukos River in northern Morocco, Guadalquivir River in southern Spain) acted as barriers. Differences in seed dispersal patterns and human transport of seeds may have provided ways to overcome these barriers. As a result of its long history of human occupation (Blondel, 2006), the Mediterranean region has had its biogeography significantly modified by man, who has shaped the modern genetic diversity of numerous taxa (e.g. *Quercus*; see Petit *et al.*, 2002). The most emblematic case is that of the stone pine (*Pinus pinea*), whose highly depauperate genetic diversity – in contrast to its widespread occurrence – is linked mainly to human-mediated dispersal (Vendramin *et al.*, 2008).

Finally, recent experimental evidence has suggested that surfing during recent range expansion (i.e. a strong genetic drift occurring in populations located on the edge of the expansion) might lead to multiple genetic revolutions of populations (Excoffier & Ray, 2008). It therefore appears that an expansion from a single refugium followed by surfing and spatial genetic structuration of populations can lead to the same phylogeographic pattern as several expansions from geographically distinct refugia. This mechanism should be considered in future studies in order to interpret complex phylogeographic patterns correctly.

### **Spatial congruence between Mediterranean refugia and biodiversity hotspots: implications for conservation biogeography**

We found a clear spatial congruence between the phylogeographically defined refugia and the 10 Mediterranean regional hotspots of biodiversity *sensu* Médail & Quézel (1997). Refugia are significantly over-represented within these hotspots, with, notably, the inclusion of nearly all southern and insular refugia. This latitudinal pattern requires further investigation but it is reasonable to hypothesize that the less drastic climate changes in the south and on large Mediterranean islands favoured the co-existence of distinct genetic lineages and of high plant richness and/or the local persistence of endemic plants.



A similar biogeographical congruence between refugia and areas of endemism has also been described at the periphery of the Alps (Schönswetter *et al.*, 2005). This suggests that similar evolutionary and biogeographical patterns have influenced different biodiversity components, but that this influence is perceptible only in areas where environmental changes have been of a lower amplitude (Vellend, 2003). There is growing evidence both at a global scale (Jansson, 2003) and for mediterranean-type ecosystems (Cowling *et al.*, 2005) that the areas with high contemporary diversity and endemism are those that have experienced long-term climate stability. Biodiversity hotspots coincide generally with areas that were buffered against climate extremes (Fjeldså & Lovett, 1997). Our results are consistent with these biogeographical patterns and suggest that Mediterranean refugia, which have been less affected by past environmental change than European refugia, constitute key areas for the long-term conservation of genetic and species diversity.

Therefore, the two major biodiversity levels, species and populations, must be accounted for in conservation plans. Identifying new areas for plant conservation policy at the population level is urgent, especially in the northern part of the area (Spain, France, Italy, Dalmatian coast). As the effects of future global change are likely to be particularly severe in the Mediterranean region (IPCC, 2007), identifying the precise locations of refugia – which we can define as ‘phylogeographical hotspots’ – may be critical for the optimal conservation of evolutionary potential in Mediterranean plants, because these refugia encompass the bulk of plant genetic diversity and retain their ancestral character (Petit *et al.*, 2003, 2005). If we consider only current climate change, most of the genetic lineages should be able to persist in Mediterranean refugia, because: (1) they are regions that are less affected by climate changes and have already withstood several past episodes of climate change; (2) even though future climate change is expected to take place on short time-scales, there is much evidence for rapid and major environmental changes in the past (Allen *et al.*, 1999); and (3) the present-day Mediterranean flora is composed of resilient and stress-tolerant taxa, notably the narrowly distributed endemic plants that have experienced several abrupt changes and population bottlenecks. Leading-edge populations will probably be better able to withstand future changes in climate, whereas rear-edge populations in the southern part of the area will continue to act as reservoirs of evolutionary history (Sechrest *et al.*, 2002). In order to reduce the impact of these changes, it seems *a priori* tempting to shift conservation areas northwards and to consider only leading-edge populations. However, such a conservation policy would be disastrous as it would obliterate the most ancient and unique relict plant populations located in the south (Petit *et al.*, 2005). Refugia that persisted throughout the Pleistocene will most probably continue to act as refugia in the future (Leroy & Arpe, 2007). It is therefore urgent to launch conservation programmes focused on these refugia, notably those located in North Africa, Turkey and in the Middle East because they are under a disproportionate threat from human activity.

In general, the level of human impact on biodiversity remains the most worrying problem in the Mediterranean (Blondel & Médail, 2009). A number of refugia are currently under severe threat from human activities: c. 25% of the total surface of the identified refugia coincides with the areas of highest human density (> 250 inhabitants km<sup>-2</sup>), mainly located along the coasts (Médail & Diadema, 2006).

### A need for detailed studies on Mediterranean refugia

The Mediterranean region is one of the most complex and heterogeneous areas of the world from an environmental and biogeographical point of view (Quézel, 1985), and this extreme diversity in space and time has been intensified by the multiple ecological consequences of occupation by human societies over several millennia (Blondel, 2006). It therefore remains difficult to establish precise regional phylogeographical patterns. We have preferred here to provide an empirical analysis based on objective refugium locations, as it would be premature to draw further inferences at the scale of the Mediterranean Basin from a relatively limited set of plant species, although the current phylogeographical data set, with 82 taxa, is now probably one of the largest world-wide for plants.

Several methodological problems exist in most of the phylogeographical analyses. Sampling artefacts include an over-sampling of some putative key populations within a predefined area and, even more problematic, the lack of consistent sampling across a whole biogeographical region. Several areas remain poorly explored (North Africa, the Middle East), and improved sampling of these regions must be a future goal. Another concern is pseudocongruence (i.e. the existence of *a priori* similar patterns) resulting from different causal factors operating at different times (Soltis *et al.*, 2006). Convergent phylogeographical patterns could, in fact, have emerged at different times during the diverse episodes of the Cenozoic, and therefore have different causes. Pseudocongruence is also apparent in the visual categorization of patterns by researchers, when roughly similar but distinct phenomena are artificially lumped together, leading to erroneous conclusions (Soltis *et al.*, 2006). It also remains difficult to make broad generalizations about the structure and functioning of refugia owing to the idiosyncratic nature of past vegetation dynamics over the Pleistocene (e.g. Cheddadi *et al.*, 2005) and the ecological discrepancies linked to the diverse impacts of climate cycles, disturbance regimes and the diversity of species-specific attributes.

Progress in Mediterranean phylogeography requires a more integrated approach, combining historical biogeography with palaeoecology and palaeoclimatology. It is necessary to go beyond simple juxtaposed patterns explaining coarse phylogeographical results related only to the role of some major historical event, notably the LGM and the Messinian Salinity Crisis. Although pioneering world-wide comparisons of the locations of phylogeographical refugia and palaeoecological refugia have been performed in southern Europe for a few common trees, these comparisons remain difficult for the main plant group, the herbs.

More generally, a comparative approach using different biodiversity levels also appears useful in order to better understand the imprint of historical biogeography on current biodiversity patterns of the Mediterranean region. For example, several modelling studies have recently demonstrated for Europe that historical events explain a significant part of the contemporary tree species richness and distribution patterns (Benito Garzón *et al.*, 2007; Montoya *et al.*, 2007; Svenning & Skov, 2007). The analysis of the spatial congruence of phylogeographical breaks with classical biogeographical barriers also needs further development (Arroyo *et al.*, 2008). These improvements should be made by combining phylogeography and landscape genetics, both at the local and at the regional scale, in order to examine the location and the functional roles of refugia.

## CONCLUSIONS

Given the need to refine the described methodological approaches, and the new challenges of modelling, to what extent do the identified putative refugia contribute to our understanding of Mediterranean plant biodiversity and biogeography? First, our results show the complexity of the definition 'refugium' in the Mediterranean region. This is because of the succession of environmental changes that have occurred since the Tertiary, rather than the sole influence of the last glacial period. Second, although several Mediterranean refugia have previously been identified for a single or a small group of species, this review clarifies the locations of multiple refugia both in the southern and in the northern parts, and not only in the three main peninsulas. The study has provided new biogeographical insights, especially in the little-studied regions of the southern and eastern Mediterranean. Third, we found a clear congruence between regional hotspots of plant biodiversity and refugia. This pattern emphasizes the critical importance of such areas for regional planning of *conservation biogeography* (Whittaker *et al.*, 2005). The identification of multiple refugia outside regional hotspots also highlights the need for ambitious conservation and sustainable management strategies in the northern Mediterranean countries. Therefore, refugia constitute crucial areas in today's context of global change, and future research using a modelling approach should serve to better disentangle their biogeographical roles within this highly complex, but fascinating, Mediterranean region.

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## REFERENCES

- Allen, J.R.M., Brandt, U., Brauer, A., Hubberten, H.W., Huntley, B., Kellerk, J., Kramlk, M., Mackensen, A., Minogram, J., Negendank, J.F.W., Nowaczyk, N.R., Oberhänsli, H., Watts, W.A., Wulf, W. & Zolitschka, B. (1999) Rapid environmental changes in southern Europe during the last glacial period. *Nature*, **400**, 740–743.
- Arbogast, B.S. & Kenagy, G.J. (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, **28**, 819–825.
- Arroyo, J., Aparicio, A., Albaladejo, R.G., Muñoz, J. & Braza, R. (2008) Genetic structure and population differentiation of the Mediterranean pioneer spiny broom *Calicotome villosa* across the Strait of Gibraltar. *Biological Journal of the Linnean Society*, **93**, 39–51.
- Battandier, M. (1894) *Considérations sur les plantes réfugiées, rares ou en voie d'extinction de la flore algérienne*. Association Française pour l'Avancement des Sciences, Congrès de Caen, Paris.
- de Beaulieu, J.-L., Miras, Y., Andrieu-Ponel, V. & Guiter, F. (2005) Vegetation dynamics in north-western Mediterranean regions: instability of the Mediterranean bioclimate. *Plant Biosystems*, **139**, 114–126.
- Beheregaray, L.B. (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology*, **17**, 3754–3774.
- Benito Garzón, M., Sánchez de Dios, R. & Sáinz Ollero, H. (2007) Predictive modelling of tree species distributions on the Iberian Peninsula during the Last Glacial Maximum and Mid-Holocene. *Ecography*, **30**, 120–134.
- Bennett, K.D. (1997) *Evolution and ecology*. Cambridge University Press, Cambridge.
- Beug, H.J. (1975) Changes of climate and vegetation belts in the mountains of Mediterranean Europe during the Holocene. *Biuletyn Geologiczny*, **19**, 101–110.
- Bhagwat, S.A. & Willis, K.J. (2008) Species persistence in northerly glacial refugia of Europe: a matter of chance or biogeographical traits? *Journal of Biogeography*, **35**, 464–482.
- Blondel, J. (2006) The 'design' of Mediterranean landscapes: a millennial story of humans and ecological systems during the historic period. *Human Ecology*, **34**, 713–729.
- Blondel, J. & Médail, F. (2009) Mediterranean biodiversity and conservation. *The physical geography of the Mediterranean* (ed. by J.C. Woodward). Oxford University Press, Oxford (in press).
- Carrión, J.S., Yll, E.I., Walker, M.J., Legaz, A.J., Chain, C. & López, A. (2003) Glacial refugia of temperate, Mediterranean and Ibero-North African flora in south-eastern Spain: new evidence from cave pollen at two Neanderthal man sites. *Global Ecology and Biogeography*, **12**, 119–129.

- Carstens, B.C. & Richards, C.L. (2007) Integrating coalescent and ecological niche modelling in comparative phylogeography. *Evolution*, **61**, 1439–1454.
- Cheddadi, R., de Beaulieu, J.-L., Jouzel, J., Andrieu-Ponel, V., Laurent, J.-M., Reille, M., Raynaud, D. & Bar-Hen, A. (2005) Similarity of vegetation dynamics during interglacial periods. *Proceedings of the National Academy of Sciences USA*, **102**, 13939–13943.
- Cowling, R.M., Ojeda, F., Lamont, B.B., Rundel, P.W. & Lechmere-Oertel, R. (2005) Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeography*, **14**, 509–519.
- Cozzolino, S., Cafasso, D., Pelegrino, G., Musacchio, A. & Widmer, A. (2003) Fine-scale phylogeographical analysis of Mediterranean *Anacamptis palustris* (Orchidaceae) populations based on chloroplast minisatellite and microsatellite variation. *Molecular Ecology*, **12**, 2783–2792.
- Elenga, H., Peyron, O., Bonnefille, R., Jolly, D., Cheddadi, R., Guiot, J., Andrieu, V., Bottema, S., Buchet, G., de Beaulieu, J.-L., Hamilton, A.C., Maley, J., Marchant, R., Perez-Obiol, R., Reille, M., Riollet, G., Scott, L., Straka, H., Taylor, D. & Van Campo, E. (2000) Pollen-based biome reconstruction for southern Europe and Africa 18,000 yr BP. *Journal of Biogeography*, **27**, 621–634.
- Excoffier, L. & Ray, N. (2008) Surfing during population expansions promotes genetic revolutions and structuration. *Trends in Ecology and Evolution*, **23**, 347–351.
- Fjeldså, J. & Lovett, J.C. (1997) Biodiversity and environmental stability. *Biodiversity and Conservation*, **6**, 315–323.
- Gómez, A., Palacios, A., Ramos, M., Tanarro, L.M., Schulte, L. & Salvador, F. (2001) Location of permafrost in marginal regions: Corral del Veleta, Sierra Nevada, Spain. *Permafrost & Periglacial Process*, **12**, 93–110.
- Gutiérrez Larena, B., Fuertes Aguilar, J. & Nieto Feliner, G. (2002) Glacial-induced altitudinal migrations in *Armeria* (Plumbaginaceae) inferred from patterns of chloroplast DNA haplotype sharing. *Molecular Ecology*, **11**, 1965–1974.
- Haffer, J. (1982) General aspects of the refuge theory. *Biological diversification in the tropics* (ed. by G. Prance), pp. 6–24. Columbia University Press, New York.
- Hampe, A. & Petit, R.J. (2005) Conserving biodiversity under climate change: the rear edge matter. *Ecology Letters*, **8**, 461–467.
- Hampe, A., Arroyo, J., Jordano, P. & Petit, R.J. (2003) Rangelike phylogeography of a bird-dispersed Eurasian shrub: contrasting Mediterranean and temperate glacial refugia. *Molecular Ecology*, **12**, 3415–3426.
- Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.
- Hewitt, G.M. (2000) The genetic legacy of Quaternary ice ages. *Nature*, **405**, 907–913.
- Hungerer, K.B. & Kadereit, J.W. (1998) The phylogeny and biogeography of *Gentiana* L. sect. *Ciminalis* (Adans.) Dumort.: a historical interpretation of distribution ranges in the European high mountains. *Perspectives in Plant Ecology, Evolution and Systematics*, **1**, 121–135.
- IPCC (2007) *Climate change 2007: the physical science basis*. Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Jansson, R. (2003) Global patterns in endemism explained by past climatic changes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 583–590.
- Jost, A., Lunt, D., Kageyama, M., Abe-Ouchi, A., Peyron, O., Valdes, P.J. & Ramstein, G. (2005) High-resolution simulations of the last glacial maximum climate over Europe: a solution to discrepancies with continental palaeoclimatic reconstructions? *Climate Dynamics*, **24**, 577–590.
- Kropf, M., Comes, H.P. & Kadereit, J.W. (2006) Long-distance dispersal vs vicariance: the origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. *New Phytologist*, **172**, 169–184.
- Leroy, S.A.G. & Arpe, K. (2007) Glacial refugia for summer-green trees in Europe and south-west Asia as proposed by ECHAM3 time-slice atmospheric model simulations. *Journal of Biogeography*, **34**, 2115–2128.
- Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gömöry, D., Latalowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., van der Knaap, W.O., Petit, R.J. & de Beaulieu, J.-L. (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist*, **171**, 199–221.
- Magri, D., Fineschi, S., Bellarosa, R., Buonamici, A., Sebastiani, F., Schirone, B., Simeone, M.C. & Vendramin, G.G. (2007) The distribution of *Quercus suber* chloroplast haplotypes matches the palaeogeographical history of the western Mediterranean. *Molecular Ecology*, **16**, 5259–5266.
- Martrat, B., Grimalt, J.O., Shackleton, N.J., Abreu de, L., Hutterli, M.A. & Stocker, T.F. (2007) Four climate cycles of recurring deep and surface water destabilizations on the Iberian margin. *Science*, **317**, 502–507.
- McLachlan, J.S., Clark, J.S. & Manos, P.S. (2005) Molecular indicators of tree migration capacity under rapid climate change. *Ecology*, **86**, 2088–2098.
- Médail, F. & Diadema, K. (2006) Biodiversité végétale méditerranéenne et anthropisation: approches macro et micro-régionales. *Annales de Géographie*, **651**, 618–640.
- Médail, F. & Myers, N. (2004) Mediterranean Basin. *Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions* (ed. by R.A. Mittermeier, P. Robles Gil, M. Hoffmann, J. Pilgrim, T. Brooks, C.G. Mittermeier, J. Lamoreaux and G.A.B. da Fonseca), pp. 144–147. CEMEX, Monterrey, Conservation International, Washington and Agrupación Sierra Madre, Mexico.
- Médail, F. & Quézel, P. (1997) Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. *Annals of the Missouri Botanical Garden*, **84**, 112–127.

- Montoya, D., Rodríguez, M.A., Zavala, M.A. & Hawkins, B.A. (2007) Contemporary richness of holarctic trees and the historical pattern of glacial retreat. *Ecography*, **30**, 173–182.
- Nekola, J.C. (1999) Paleorefugia and neorefugia: the influence of colonization history on community pattern and process. *Ecology*, **80**, 2459–2473.
- Pantaléon-Cano, J., Yll, E.-I., Perez-Obiol, R. & Roure, J.M. (2003) Palynological evidence for vegetational history in semi-arid areas of the western Mediterranean (Almería, Spain). *The Holocene*, **13**, 109–119.
- Petit, R.J., Brewer, S., Bordács, S., Burg, K., Cheddadi, R., Coart, E., Cottrell, J., Csaiikl, U.M., van Dam, B., Deans, J.D., Espinel, S., Fineschi, S., Finkeldey, R., Glaz, I., Goicoechea, P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G., Munro, R.C., Popescu, F., Slade, D., Tabbener, H., de Vries, S.G.M., Ziegenhagen, B., de Beaulieu, J.-L. & Kremer, A. (2002) Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecology and Management*, **156**, 49–74.
- Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martín, J.P., Rendell, S. & Vendramin, G.G. (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.
- Petit, R.J., Hampe, A. & Cheddadi, R. (2005) Climate changes and tree phylogeography in the Mediterranean. *Taxon*, **54**, 877–885.
- Peyron, O., Guiot, J., Cheddadi, R., Tarasov, P., Reille, M., de Beaulieu, J.-L., Bottema, S. & Andrieu, V. (1998) Climatic reconstruction in Europe for 18,000 YR B.P. from pollen data. *Quaternary Research*, **49**, 183–196.
- Provan, J. & Bennett, K.D. (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution*, **23**, 564–571.
- Quézel, P. (1985) Definition of the Mediterranean region and the origin of its flora. *Plant conservation in the Mediterranean area* (ed. by C. Gomez-Campo), pp. 9–24. Geobotany 7, W. Junk, Dordrecht.
- Quézel, P. & Médail, F. (2003) *Ecologie et biogéographie des forêts du bassin méditerranéen*. Elsevier (Collection Environnement), Paris.
- Ravazzi, C. (2002) Late Quaternary history of spruce in southern Europe. *Review of Palaeobotany and Palynology*, **120**, 131–177.
- Reille, M., Andrieu, V. & de Beaulieu, J.-L. (1996) Les grands traits de l'histoire de la végétation des montagnes méditerranéennes occidentales. *Ecologie*, **27**, 153–169.
- Richards, C.L., Carstens, B.C. & Knowles, L.L. (2007) Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. *Journal of Biogeography*, **34**, 1833–1845.
- Roi, J. (1937) *Les espèces eurasiatiques continentales et les espèces boréo-alpines dans la région méditerranéenne occidentale*. PhD Thesis, Université de Montpellier, Imprimerie Fournié, Toulouse.
- Schönswetter, P., Stehlik, I., Holderegger, R. & Tribsch, A. (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology*, **14**, 3547–3555.
- Sechrest, W., Brooks, T.M., da Fonseca, G.A.B., Konstant, W.R., Mittermeier, R.A., Purvis, A., Rylands, A.B. & Gitelman, J.L. (2002) Hotspots and the conservation of evolutionary history. *Proceedings of the National Academy of Sciences USA*, **99**, 2067–2071.
- Shackleton, N.J. (1987) Oxygen isotopes, ice volume and sea level. *Quaternary Science Review*, **6**, 183–190.
- Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S. & Soltis, P.S. (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–4293.
- Stewart, J.R. & Lister, A.M. (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology and Evolution*, **16**, 608–613.
- Suc, J.-P., Fauquette, S., Bessedik, M., Bertini, A., Zheng, Z., Clauzon, G., Suballyova, D., Diniz, F., Quézel, P., Feddi, N., Clet, M., Bessais, E., Bachiri-Taoufiq, N., Méon, H. & Combourieu-Nebout, N. (1999) Neogene vegetation changes in west European and west circum-Mediterranean areas. *Hominid evolution and climate in Europe* (ed. by J. Agustí, L. Rook and P. Andrews), pp. 370–385. Cambridge University Press, Cambridge.
- Svenning, J.-C. (2003) Deterministic Plio-Pleistocene extinctions in the European cool-temperate tree flora. *Ecology Letters*, **6**, 646–653.
- Svenning, J.-C. & Skov, F. (2007) Ice age legacies in the geographical distribution of tree species richness in Europe. *Global Ecology and Biogeography*, **16**, 234–245.
- Swenson, N.G. & Howard, D.J. (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist*, **166**, 581–591.
- Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.
- Thompson, J.D. (2005) *Plant evolution in the Mediterranean*. Oxford University Press, Oxford.
- Tzedakis, P.C. (2007) Seven ambiguities in the Mediterranean palaeoenvironmental narrative. *Quaternary Science Reviews*, **26**, 2042–2066.
- Tzedakis, P.C. (2009) Cenozoic climate and vegetation change in the Mediterranean Basin. *The physical geography of the Mediterranean* (ed. by J.C. Woodward). Oxford University Press, Oxford (in press).
- Tzedakis, P.C., Lawson, I.T., Frogley, M.R., Hewitt, G.M. & Preece, R.C. (2002) Buffered tree population changes in a Quaternary refugium: evolutionary implications. *Science*, **297**, 2044–2047.
- Tzedakis, P.C., Hooghiemstra, H. & Pälike, H. (2006) The last 1.35 million years at Tenaghi Philippon: revised chronostratigraphy and long-term vegetation trends. *Quaternary Science Reviews*, **25**, 3416–3430.

- Véla, E. & Benhouhou, S. (2007) Évaluation d'un nouveau point chaud de biodiversité végétale dans le bassin méditerranéen (Afrique du Nord). *Comptes-Rendus Biologies*, **330**, 589–605.
- Vellend, M. (2003) Island biogeography of genes and species. *The American Naturalist*, **162**, 358–365.
- Vendramin, G.G., Fady, B., González-Martínez, S.C., Hu, F.S., Scotti, I., Sebastiani, F., Soto, A. & Petit, R.J. (2008) Genetically depauperate but widespread: the case of an emblematic Mediterranean pine. *Evolution*, **62**, 680–688.
- Waltari, E., Hijmans, R.J., Peterson, A.T., Nyári, A.S., Perkins, S.L. & Guralnick, R.P. (2007) Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS ONE*, **2**, e563. Doi: 10.1371/journal.pone.0000563.
- Weiss, S. & Ferrand, N. (2007) *Phylogeography of southern European refugia. Evolutionary perspectives on the origins and conservation of European biodiversity*. Springer, Berlin.
- Whittaker, R.J., Araújo, M.B., Jepson, P., Ladle, R.J., Watson, J.E.M. & Willis, K.J. (2005) Conservation biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3–23.
- Willis, K.J. & Whittaker, R.J. (2000) The refugial debate. *Science*, **287**, 1406–1407.
- Wu, H., Guiot, J., Brewer, S. & Guo, Z. (2007) Climatic changes in Eurasia at the last glacial maximum and mid-Holocene: reconstruction from pollen data using inverse vegetation modelling. *Climate Dynamics*, **29**, 211–229.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** List of studies used in the comparative phylogeography analysis. Total species distribution, number of

investigated populations in total (and from the Mediterranean region), and molecular methods are given.

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