Journal of Biogeography (*J. Biogeogr.*) (2011) **38**, 619–630

SYNTHESIS

Biogeography and evolution of *Abies* (Pinaceae) in the Mediterranean Basin: the roles of long-term climatic change and glacial refugia

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ABSTRACT

Aim The genus *Abies* exemplifies plant diversification related to long-term climatic, geological and evolutionary changes. Today, the Mediterranean firs comprise nine species, one natural hybrid and several varieties. Here I summarize current knowledge concerning the origin and evolution of the genus *Abies* in the Mediterranean Basin and propose a comprehensive hypothesis to explain the isolation and speciation pattern of Mediterranean firs.

Location The Mediterranean Basin.

Methods The literature on *Abies* was reviewed, focusing on the morphology, fossil records, molecular ecology, phytosociology and biogeography of the genus in the Mediterranean Basin.

Results *Abies* fossils from the western Mediterranean indicate a wide Tertiary circum-Mediterranean distribution of the *Abies* ancestor. Palaeogeographical data also suggest a single eastern Mediterranean Tertiary ancestor. Following the Miocene to Pliocene climate crisis and marine transgressions, the ancestor of the northern Mediterranean firs is hypothesized to have separated into two eastern groups, one on the Balkan Peninsula and the other in Asia Minor. However, land bridges may have permitted gene flow at times. A southward migration of *A. alba* to refugia, where older fir species may have remained isolated since the Miocene, could explain recent findings indicating that morphologically distant species are more closely related than expected based on such morphological classification.

Main conclusions The *Abies* genus appears to have undergone significant morphological differentiation that does not necessarily imply reproductive isolation. That is, long-term Mediterranean Basin dryness along a south-eastern to north-western gradient may have caused an initial Miocene–Pliocene speciation sequence. Pleistocene glacial cycles probably forced migrations to occur, leading to repeated contact between fir species in glacial refugia.

Keywords

***Abies*, firs, fossil pollen, Mediterranean, migration, Miocene, phylogeny, plant macrofossils, Quaternary refugia, speciation.**

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INTRODUCTION

The current distribution area of a given species results from the adaptation of the species as it shifts in space (Willis & McElwain, 2002). The presence of a species in an ecosystem requires not only an explanation based on the species' current ecology, related to morphological and physiological adaptations to local conditions, but also an historical explanation, related to the sequence of events that allowed the presence of this species in this area and that drove evolution from an ancestral form (Hewitt, 1996; Svenning & Skov, 2005).

During the late Miocene and the Pliocene, the European Mediterranean flora underwent increasing differentiation (Palamarev, 1989; Kovar-Eder *et al.*, 2008). The main selective pressure for Mediterranean plant communities in the late Tertiary seems to have been a long-term decline in summer

http://wileyonlinelibrary.com/journal/jbi
doi:10.1111/j.1365-2699.2010.02458.x

precipitation (Suc, 1984). The drought associated with high temperatures during the growing season would have required specific adaptations and can be assumed to have led to the segregation of plant communities along elevational, latitudinal and topographic gradients, reflecting the ecophysiology of the constituent species. The orographic complexity derived from Alpine orogeny favoured this species segregation and the existence of local refugia around the Mediterranean Basin (Médail & Diadema, 2009). This sub-regional complexity appears to have been crucial in the evolution and persistence of the current Mediterranean flora (Taberlet & Cheddadi, 2002; Tzedakis *et al.*, 2002; Carrión *et al.*, 2003; Cheddadi *et al.*, 2009).

The firs (genus *Abies*, Pinaceae) exemplify plant diversification related to long-term climatic, geological and evolutionary changes (Pignatti, 1978). Today, the Mediterranean firs comprise nine species, one natural hybrid and several varieties (Vidakovic, 1991). However, the current relict status of these species constitutes an example of an endangered ecosystem, highly vulnerable to global change. Various hypotheses have been proposed to explain how the genus *Abies* evolved (Table 1). Some proposals are contradictory, perhaps because the explanations are based generally on a single trait and we do not have enough macrofossils to validate the arguments (Gaussen, 1964; Liu, 1971; Xiang *et al.*, 2009; Alba-Sánchez *et al.*, 2010).

Palaeoecological studies based on fossil pollen and plant macrofossils show that during the Pliocene (*c.* 5 Ma) the Mediterranean Basin was covered by vast forest ecosystems, presumably including a common ancestor of the current Mediterranean firs (Pignatti, 1978; Meyen, 1987; Palamarev, 1989). From this common ancestor, migrations and subsequent population fragmentation led to smaller, isolated fir forests around the current Mediterranean Basin (Farjon & Rushforth, 1989).

Mediterranean firs have been classically divided into two groups, based on the bracts of the cones, which are either longer or shorter than the scales (Dallimore & Jackson, 1923; Liu, 1971; Farjon & Rushforth, 1989; Vidakovic, 1991): a pinsapo fir-type archaic group and a silver fir-type modern group. The archaic group, which presents a southern Mediterranean distribution, is morphologically characterized by enclosed bracts. This apparently ancient group comprises the species *A. pinsapo* Boissier, *A. pinsapo* var. *marocana* (Trabut) Ceballos & Bolaños, *A. pinsapo* var. *tazaotana* (Cózar ex Huguet del Villar) Pourtet, *A. numidica* de Lannoy ex Carrière and *A. cilicica* (Antoine & Kotschy) Carrière. The modern group has a northern Mediterranean distribution and is morphologically characterized by protruding bracts. This group includes the species *A. alba* Miller, *A. borisii-regis* Mattfeld, *A. cephalonica* Loudon, *A. nebrodensis* (Lojac.) Mattei, *A. nordmanniana* (Steven) Spach, *A. equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen and *A. bornmuelleriana* (Steven) Spach.

However, recent evidence based on allozyme differentiation, chloroplast and mitochondrial DNA variation, as well as on other DNA markers, has shown that the speciation sequence and the Pleistocene overlap among different Mediterranean firs is not well understood (Vicario *et al.*, 1995; Scaltsoyiannes *et al.*, 1999; Vendramin *et al.*, 1999; Parducci *et al.*, 2001a; Ziegenhagen *et al.*, 2005; Xiang *et al.*, 2009). For instance, an unexpectedly close relationship between *A. pinsapo* and *A. alba* (two of the morphologically most distant Mediterranean fir species) was reported by Scaltsoyiannes *et al.* (1999). In addition to this molecular evidence, recent papers on wood anatomy (see Esteban *et al.*, 2009) and palaeopalynology and species distribution models (see Alba-Sánchez *et al.*, 2010) support these surprising results and underscore biogeographic issues that remain unsolved.

The present paper reviews the morphology, molecular ecology and biogeography of *Abies*, with a focus on the Mediterranean Basin, in order to summarize current knowledge concerning the origin and evolution of the genus *Abies* in this area. A comprehensive hypothesis to explain the isolation and speciation pattern of the Mediterranean fir is developed and discussed.

MATERIALS AND METHODS

This paper is based on a review of the literature on *Abies* fossil records, morphology, molecular ecology and biogeography. Science Citation Index (SCI) papers were used as far as possible, avoiding excessive use of references in German, Spanish and French in order to invite a wide readership to examine the presented hypothesis by means of the references used. The keywords used in the review were: *Abies*, biogeography, evolution, firs, fossil pollen, Mediterranean, Miocene, palaeogeography, phylogeny, plant macrofossils, Quaternary refugia.

RESULTS AND DISCUSSION

Palaeogeography, palaeoclimate and plant evolution in the Mediterranean Basin

The Mediterranean Basin represents the remnant of the ancient Tethys Sea, a Mesozoic ocean that underwent profound changes during the Alpine orogeny, beginning in the Middle Cretaceous, about 100 Ma (Gradstein *et al.*, 2004), and extending to the late Miocene, about 7 Ma. Regional trends towards decreasing rainfall and, to a lesser extent, towards a temperature decrease have been inferred at the end of the Oligocene (35–23 Ma), and mainly during several stages of the Miocene (23–5.2 Ma; Bruch *et al.*, 2006; Jost *et al.*, 2009). As a result, the Mediterranean Basin would have acquired the pattern of high atmospheric pressure that caused a water deficit in the Mediterranean summer (Pignatti, 1978; Suc, 1984; Mai, 1989; Fauquette *et al.*, 1999).

The Miocene (23–5.3 Ma) would have included periods during which there were large emerged areas, alternating with others during which, for instance, the current south-western edge of Europe (in the Iberian Peninsula) was a sea belt dotted by small islands (Popov *et al.*, 2006). This configuration led to

Table 1 Representative examples of features studied and hypotheses proposed to explain the evolution of the Mediterranean species of the genus *Abies*.

Species studied	Studied variables	Main hypothesis or conclusions	References
Mediterranean firs	Morphological and anatomical traits	Two groups based on the bracts of the cones, which are either longer or shorter than the scales	Farjon & Rushforth (1989); Dallimore & Jackson (1923); Liu (1971); Vidakovic (1991)
A. equi-trojani	Pollen analysis	Hybrid characteristics for *A. equi-trojani*	Aytug (1959)
Abies genus	Inter-specific hybridization	Artificial crosses suggest weak reproductive barriers among the Mediterranean *Abies* species as opposed to strict barriers between Mediterranean and North American fir species. Mediterranean firs are especially prone to hybridize, being closely related, suggesting recent speciation	Klaehn & Winieski (1962)
A. pinsapo	Phenolic composition	Spanish and Moroccan populations are very close	Granados & Rossell (1966)
Mediterranean firs	Phytosociology	Monophyletic origin followed by geographical fragmentation	Arbez (1969); Pignatti (1978); Barbero & Quézel (1975)
Greek firs	Morphology and monoterpene composition	Individual trait variation shows a south-east to north-west clinal pattern	Mitsopoulos & Panetsos (1987)
Eastern Mediterranean firs	Terpene composition	Single eastern Mediterranean Tertiary ancestor for present-day Aegean species	Fady *et al.* (1992)
A. borisii-regis	Segregation and linkage of allozymes	*Abies borisii-regis* populations are genetically very close	Fady & Conkle (1992)
Mediterranean firs	Allozyme differentiation	Great variation in heterozygosity (lowest in *A. pinsapo*, highest in *A. cephalonica*). Inter-population genetic diversity about 26% of the total genetic diversity. High affinity between the Calabrian fir population and the one from north-west Greece as well as between *A. equi-trojani* and southern Greek populations of *A. cephalonica*. Species-specific alleles in *A. cilicica*	Scaltsoyiannes *et al.* (1999); Pascual *et al.* (1993)
Mediterranean firs	Allozyme, chloroplast DNA and random amplified polymorphic DNA	Genetic variation easily detectable using isozyme loci, but less evidence of genetic divergence by single-primer DNA amplification at the chloroplast DNA level	Vicario *et al.* (1995); Ziegenhagen *et al.* (2005); Kaya *et al.* (2008)
A. alba	Polymorphic chloroplast microsatellites, allozyme markers	Genetic distances between most populations were high. Genetic distances increased with spatial distances. Present organizations of levels of allelic richness across the range of the species is likely have been shaped by the distribution of refugia during the last glaciation and the subsequent re-colonization processes	Vendramin *et al.* (1999); Parducci *et al.* (1996)
Abies genus	Restriction fragment-length polymorphism analysis of chloroplast DNA	The chloroplast genome was highly variable in most of the taxa investigated. Species-diagnostic markers were found for four of ten species analysed, but intra-specific variation was also detected in four species	Parducci & Szmidt (1999)
A. nebrodensis, *A. alba*, *A. numidica* and *A. cephalonica*	Chloroplast simple-sequence repeats	*Abies nebrodensis* differed from the other three *Abies* species investigated, supporting its classification as an independent taxon	Parducci *et al.* (2001a)
Mediterranean firs	Mitochondrial DNA markers	Five haplotypes were defined. Two different haplotypes were found in each of *A. alba* and *A. cephalonica*, one haplotype being shared among the two species. A single species-specific haplotype was found in *A. cilicica*. *Abies pinsapo* and *A. numidica* shared one haplotype, while eastern Mediterranean firs (*A. cephalonica*, *A. bornmuelleriana*, *A. equi-trojani* and *A. nordmanniana*) also shared one haplotype	Ziegenhagen *et al.* (2005)

Table 1 Continued

Species studied	Studied variables	Main hypothesis or conclusions	References
Western Mediterranean firs	Chloroplast microsatellites	High levels of genetic diversity. There was a strong effect of the Strait of Gibraltar on western Mediterranean *Abies* differentiation, leading to two groups corresponding to Spanish and Moroccan populations. High diversity within populations and low differentiation among populations in Spain and Morocco suggest a certain level of pollen-mediated gene flow. *Abies tazaotana* is genetically very close to *A. marocana*	Terrab *et al.* (2007)
Abies genus	Sequence of the complete nuclear ribosomal internal transcribed spacer region	Phylogenetic analyses showed a close relationship between European and Asian species, but with weak support	Xiang *et al.* (2009)
Mediterranean firs	Wood anatomy	*Abies alba*, *A. pinsapo*, *A. pinsapo* var. *marocana* and *A. pinsapo* var. *tazaotana* possess biometric features that distinguish them from the other Mediterranean firs. *Abies numidica* shows biometric features close to *A. pinsapo* and differs from the eastern firs (*A. borisii-regis*, *A. cephalonica*, *A. cilicica*, *A. nordmanniana* and *A. equi-trojani*)	Esteban *et al.* (2009)
A. alba and *A. pinsapo*	Palaeopalynology and species distribution models	*Abies alba* and *A. pinsapo* cannot be distinguished in palynological analyses; previous studies related to their glacial refugia or past distribution usually refer to the entire genus. Species-distribution models yielded a significant geographical and ecological segregation, suggesting that the two taxa remained isolated throughout the Quaternary	Alba-Sánchez *et al.* (2010)

the geographical isolation of several plant populations and eventually allowed the speciation of some taxa. Palaeoclimatic researchers studying the late Miocene (Messinian, 7.2–5.3 Ma) have identified an event of regional aridity, during which much of the Mediterranean Sea became a marsh (Kovar-Eder *et al.*, 2008). Thereafter, during the Pliocene (5.3–1.8 Ma), the Mediterranean seasonality and the regional cooling accentuated around 4.5, 3.6, 3.2, 2.8 and 2.4 Ma (Suc, 1984; Kovar-Eder *et al.*, 2008; Jost *et al.*, 2009). These climatic oscillations, ending with the Pleistocene glaciations, resulted in the cumulative loss of several thermophilic species from the European continent, as well as in episodic expansions of xerophytic communities (Pignatti, 1978; Suc, 1984; Palamarev, 1989). Palaeoclimatic analysis suggests the establishment of the current Mediterranean climate seasonality, characterized by two intra-annual stress periods for plant growth, in summer and in winter, during at least three climatic crises dated to 3.2, 2.8 and 2.4 Ma (Suc, 1984; Fauquette *et al.*, 1999, 2007; Bruch *et al.*, 2006).

The Pleistocene (1.8 Ma to the present) was characterized by cycles of fragmentation and re-expansion in the distribution range of several species (Thompson, 2005). This period has been analysed theoretically under three main evolutionary scenarios (Schluter, 2001). The first could be called *species persistence*. Under this approach, the species would have undergone contractions and re-expansions in their distribution area as a consequence of the glacial–interglacial cycles (Willis & McElwain, 2002). These biome shifts would have taken place without genetic differentiation or leading to a loss in local differentiation following the re-expansion and overlap of populations previously isolated. This hypothesis results in *evolutionary stasis* (Hewitt, 1999; Willis & Niklas, 2004). The second scenario involves *species extinction*. The species distribution area would have become reduced and progressively fragmented, whereupon subsequent population isolation would have led to extinctions (Svenning & Skov, 2007). The third possibility is that of *allopatric speciation*, whereby geographic isolation would have promoted local adaptations and genetic differentiation among populations (Turelli *et al.*, 2001). As a consequence, the new species would have shown reproductive isolation during re-expansion and possible geographic overlaps (Hewitt, 1996; Willis & McElwain, 2002; Willis & Niklas, 2004; Thompson, 2005). These three evolutionary processes appear to have operated during the Mediterranean fir history (Pignatti, 1978).

It has been shown that each species has responded independently to climate changes. The species patterns of differentiation, persistence in a refugium, migration rates and the expansion–retraction routes cannot easily be generalized (Willis & Whittaker, 2000; Willis & Niklas, 2004). Moreover, the genus *Abies* appears to have acquired significant morphological differentiation, which does not suggest reproductive isolation (Klaehn & Winieski, 1962). Introgression between the Mediterranean *Abies* species seems to have been common (Tzedakis, 1993; Scaltsoyiannes *et al.*, 1999; Xiang *et al.*, 2009; Alba-Sánchez *et al.*, 2010).

Evolution of the archaic Mediterranean firs

The discovery of *Abies* fossils in different parts of the Iberian Peninsula gives rise to the hypothesis that the arrival of *Abies* in the southern Iberian Peninsula as well as in northern Morocco and Algeria occurred across Tethys bridges from the northern Iberian Peninsula (Fig. 1). This evidence also supports the idea of a wide Tertiary circum-Mediterranean distribution of the *Abies* ancestor, prior to its range fragmentation and subsequent allopatric speciation (Liu, 1971). On the other hand, the above-mentioned fossil records suggest an apparent evolutionary stasis in the morphology of the western Mediterranean firs since the Miocene (Areitio, 1874; Depape, 1928; Dubois-Ladurantie, 1941; Gaussen, 1964; Menéndez & Florschütz, 1964; Pons & Reille, 1988; Carrión *et al.*, 2001).

Southward migration of *A. alba* to southern Iberian Peninsula refugia (Fig. 2a), where *A. pinsapo* would have remained isolated since the Miocene (Pignatti, 1978; Palamarev, 1989), could explain recent findings that establish closer than expected relationships between *A. alba* and *A. pinsapo*, paradoxically the most distant species among the Mediterranean firs, based on morphological classification (Dallimore & Jackson, 1923; Liu, 1971; Farjon & Rushforth, 1989; Vidakovic, 1991). According to allozyme differentiation, *A. pinsapo* shows greater genetic distance from *A. cilicica* and *A. numidica* than from *A. alba* populations from the Pyrenees, Alps or Apennines (Scaltsoyiannes *et al.*, 1999). Recent wood biometry studies (Esteban *et al.*, 2009) have shown high anatomical proximity between *A. alba* from the Pyrenees and *A. pinsapo*, despite the fact that they belong to two different morphological groups, suggesting that contact occurred between the two species in the past (Fig. 2a). Moreover, similar results were found in the genetic distance of different populations of the fir-root pathogen *Heterobasidion abietinum* (Basidomycota) isolated from *A. pinsapo*, which showed close relationships with *H. abietinum* populations isolated from *A. alba* in France and Italy (Luchi *et al.*, 2008).

Several pieces of evidence indicate that this hypothetical connection between *A. alba* and *A. pinsapo* would have been disrupted during the Pleistocene, with the insertion of a semi-arid wedge in the south-eastern Iberian Peninsula (Fig. 2b; Konnert & Bergmann, 1995; Combourieu-Nebout *et al.*, 1999; Burjachs & Allue, 2003; Carrión *et al.*, 2003). However, the fossil records are scant and do not validate the hypothesis of Pleistocene contacts between silver and pinsapo firs. In addition, a strong niche differentiation between the two Iberian firs has recently been found by Alba-Sánchez *et al.* (2010), suggesting an early ecological divergence of *A. alba* and *A. pinsapo*, and possibly indicating that the two species have followed independent biogeographic dynamics (Fig. 2b; see also Muller *et al.*, 2007).

In summary, for *A. pinsapo*, the findings suggest range overlaps (Fig. 2a), at the end of Pliocene or during early Pleistocene glacial periods, with the ancestors of *A. alba* (see Alba-Sánchez *et al.*, 2010, and references therein). However, for *A. cilicica* (in Turkey) and *A. numidica* (in Algeria), the

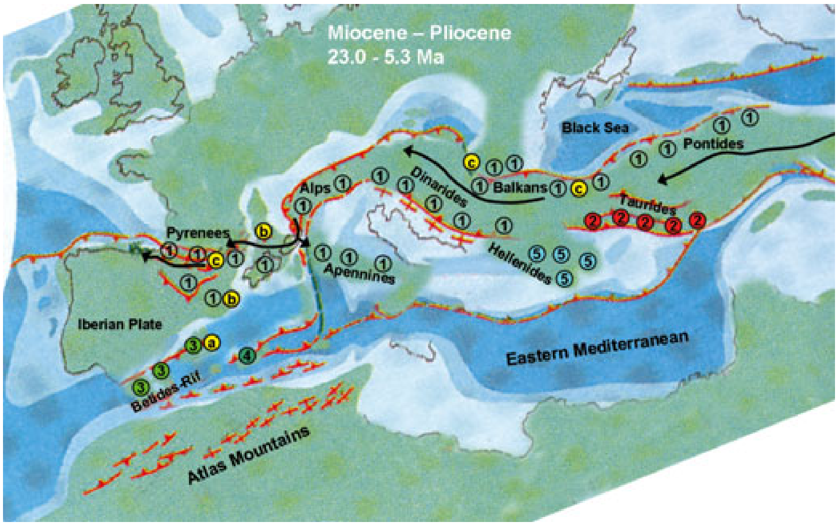

Figure 1 Hypothesized speciation sequence of Mediterranean fir (*Abies*) species during the Miocene–Pliocene. 1, *A. alba* ancestor; 2, *A. cilicica* ancestor; 3, *A. pinsapo* ancestor; 4, *A. numidica* ancestor; 5, *A. cephalonica* ancestor. Hypothesized expansion routes based on molecular data and fossil records appear as arrows, and uplift areas are indicated by the other lines. Letters denote Tertiary fossil findings. a, *Abietites latisquamosus* Ludw. Tertiary. Shrine of Saint Eulalia, Totana (Spain; Areitio, 1874). b, *Abies ramesii* Saporta. Early Miocene 20–16 Ma. Majorca Island (Spain; Depape, 1928); France (Saporta, 1865). c, *Abies saportana* Rerolle. Late Miocene. Santa Eugenia, Coll del Saig, Cerdaña (Spain; Dubois-Ladurantie, 1941; Kovar-Eder *et al.*, 2006); Poland; and Rhodope region, Bulgaria (Palamarev, 1989).

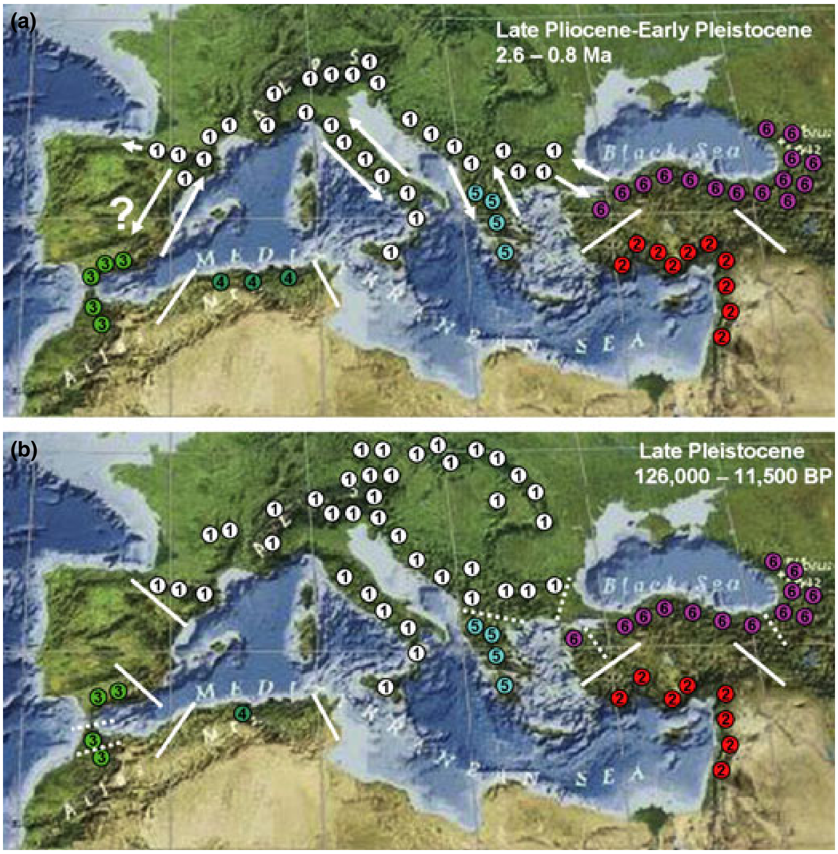

Figure 2 Hypothesized speciation sequence and migration routes of the Mediterranean fir (*Abies*) species (a) during the late Pliocene to the early Pleistocene and (b) during the late Pleistocene. 1, *A. alba* ancestor; 2, *A. cilicica* ancestor; 3, *A. pinsapo* ancestor; 4, *A. numidica* ancestor; 5, *A. cephalonica* ancestor; 6, *A. bornmuelleriana* ancestor. Migration routes appear as arrows; introgression areas appear as dotted lines; isolation areas appear as solid lines.

Tertiary isolation process would not have been broken during the Pleistocene (Fig. 2).

Evolution of the modern Mediterranean firs

Palaeogeographical data support the possible existence of a single eastern Mediterranean Tertiary ancestor for present-day Aegean species (Mitsopoulos & Panetsos, 1987). Following the Messinian climatic crisis (Suc, 1984; Palamarev, 1989) and the Pliocene marine transgression, the ancestor of the northern Mediterranean firs would have split into two groups, one on the Balkan Peninsula and the other in Asia Minor.

As the current eastern populations of *A. alba* are more similar to the Pontic fir (*A. bornmuelleriana*) than they are to *A. cephalonica*, it has been suggested that *A. alba* may have originated in the Pontic region during the Pliocene (see Fig. 2a; Fady *et al.*, 1992). This dynamic new taxon would have then spread throughout central and western Europe during the Pliocene (Fig. 2a). Indeed, Pliocene macrofossils attributed to *A. alba* have been discovered in western Europe (Gaussen, 1964; Liu, 1971). However, the occasional land bridges probably maintained gene flow between the Pontic populations of the *A. bornmuelleriana* ancestor and the eastern Balkan populations of the *A. alba* ancestor (see Fady *et al.*, 1992).

After the Pliocene marine transgression of the Aegean basin, *A. alba* would have appeared in the northern part of the Aegean and then colonized all of Europe, while *A. bornmuelleriana* would have appeared in the eastern part (Fig. 2a; Pignatti, 1978; Mitsopoulos & Panetsos, 1987). Frequent Pleistocene contacts between the two species (Fig. 2b) could have contributed to the individualization (the occurrence of geographic isolation, local adaptations and genetic differentiation of a new taxon) of *A. nordmanniana*, and thereafter to the individualization of *A. equi-trojani*, and furthermore could have given rise to the particular morphology of the south-eastern ecotypes of *A. alba* (Kaya *et al.*, 2008).

In the western Aegean basin, *A. cephalonica* populations would also have appeared after the Pliocene (Fig. 2a). Southern migrations of *A. alba* during the ice ages and contact with *A. cephalonica* in glacial refugia probably led to the development of *A. borisii-regis* hybrid populations (see Fig. 3). Indeed, evidence for gene flow with *A. alba* has been suggested for the southernmost *A. cephalonica* populations by Fady *et al.* (1992). Moreover, in northern Greece and the Balkans, hybrids between *A. cephalonica* and *A. alba* appeared (*A. borisii-regis*) during the glaciation period, when the two taxa came into contact (Fady & Conkle, 1992; Scaltsoyiannes *et al.*, 1999).

Based on allozyme differentiation, Scaltsoyiannes *et al.* (1999) also proposed a monophyletic evolutionary hypothesis for the genus *Abies* in the Mediterranean, according to which an ancient progenitor fir existed at the beginning of the Miocene in the Balkan Peninsula, in the Aegean, between the Ionian Sea and Turkey. During the evolution of *Abies* species, an ancient progenitor gave rise to *A. alba* and *A. nordmanniana* to the north and to *A. cephalonica* to the south in response to the wide range of ecological features, while the

Figure 3 Current distribution and diversity of Mediterranean fir (*Abies*) species. 1, *A. alba*; 2, *A. cilicica*; 3, *A. pinsapo*; 4, *A. numidica*; 5, *A. cephalonica*; 6, *A. bornmuelleriana*; 7, *A. nordmanniana*; 8, *A. equi-trojani*; 9, *A. borisii-regis* (*A.* × *borisii-regis* = *A. alba* × *A. cephalonica*); 10, *A. nebrodensis*; 11, *A. pinsapo* var. *marocana*; and 12, *A. pinsapo* var. *tazaotana*. Post-glacial expansion routes hypothesized for *A. alba* based on molecular data and fossil records appear as arrows.

central area remained occupied by the ancient progenitor (Fig. 2). Under this hypothesis, at the end of the Pliocene and during the Pleistocene, species such as *A. cephalonica*, *A. alba*, *A. nordmanniana*, *A. cilicica* and *A. pinsapo* would have become differentiated (Scaltsoyiannes *et al.*, 1999; see also Fig. 2a).

In summary, within the silver fir group, the eastern *A. alba* populations appear to be closely related to *A. nordmanniana* and *A. bornmuelleriana* (Scaltsoyiannes *et al.*, 1999; Ziegenhagen *et al.*, 2005). A relationship between *A. alba* and *A. bornmuelleriana* was also found by Arbez (1969), while the Calabrian silver firs show great differentiation from the rest of the *A. alba* populations (Konnert & Bergmann, 1995; Parducci *et al.*, 1996; Scaltsoyiannes *et al.*, 1999). On the other hand, central Apennines silver fir populations appear to be intermediate between the typical *A. alba* population and the Calabrian one (Scaltsoyiannes *et al.*, 1999). The Pleistocene glacial cycles probably forced the eastern populations of *A. alba* to migrate towards Asia Minor and, in glacial refugia, to come into repeated contact with the populations derived from the Pontic fir (*A. bornmuelleriana*). This was made possible by a general lowering of the sea level (100–120 m below the current sea level). Gene exchange probably occurred and could be responsible for the present biochemical and morphological differences between the eastern and western populations of *A. alba* (Konnert & Bergmann, 1995; Hewitt, 2004). For instance, eastern European *A. alba* specimens have brush-shaped needles on current-year branches comparable to those of *A. equi-trojani* and *A. bornmuelleriana* (Fady *et al.*, 1992).

On the other hand, the Greek *A. cephalonica* appears to be closely related to *A. equi-trojani* and *A. borisii-regis* (Kaya *et al.*, 2008). The great distinction within *A. cephalonica* populations (Scaltsoyiannes *et al.*, 1999) led to the proposal of two varieties (*A. cephalonica* var. *cephalonica* and *A. cephalonica* var. *graeca*, with *A. equi-trojani* belonging to *A. cephalonica* var. *graeca*; see Liu, 1971).

An alternative explanation to the above-mentioned relationships between *A. cephalonica* var. *graeca* and *A. equi-trojani* is provided by Fady *et al.* (1992), who hypothesized that gene exchanges between *A. nordmanniana* and *A. alba* could have been responsible for the emergence of *A. equi-trojani* (see Fig. 3), which has the characteristics of a relatively new species (Arbez, 1969) and strong similarities in its terpene composition to the *A. alba* populations of Romanian provenance (Fady *et al.*, 1992). On the other hand, southern Pleistocene migrations of *A. alba* must have led to gene exchange with *A. cephalonica* (which had hypothetically evolved from the Balkan firs) in glacial refugia (Figs 2 & 3). These exchanges probably contributed to the origin of *A. borisii-regis*, in which the present natural distribution is the result of multiple introgressions with its parent species (Fady & Conkle, 1992).

Although an eastern Mediterranean Tertiary ancestor for present-day Mediterranean fir species seems likely, the archaic characteristic of the western Mediterranean fir species (*A. pinsapo* and *A. numidica*; see Xiang *et al.*, 2009) is widely recognized, supporting the hypothesis that this common ancestor had a broad circum-Mediterranean distribution prior to the successive isolation and speciation of distinct populations (Figs 2 & 3).

The south-east to north-west speciation-sequence hypothesis

Probably since the middle Miocene, and mainly during the Pliocene (Fig. 1), increasing aridity led to a contraction in the range of the ancestral fir species (Kovar-Eder *et al.*, 2008). However, the establishment of this regional dryness trend would not have been spatially simultaneous throughout the Mediterranean Basin. Recent findings suggest that the long-term Mediterranean Basin dryness could have spread from the south-east to the north-west (Fauquette *et al.*, 1999, 2007;

Bruch *et al.*, 2006; Jost *et al.*, 2009). Studies such as those associated with the 'Environments and Ecosystem Dynamics of the Eurasian Neogene' (EEDEN) project of the European Science Foundation have focused on the palaeoclimatic reconstruction of the period from the mid-Miocene (*c.* 14 Ma) to the mid-Pliocene (*c.* 3.6 Ma). These studies have shown that this period was characterized by deep palaeogeographical changes and climatic shifts (Bruch *et al.*, 2006; Popov *et al.*, 2006; Kovar-Eder *et al.*, 2008; Jost *et al.*, 2009) related to plate movements (e.g. Alpine uplifts, closing and opening of marine gateways). The results show that the climate was warmer than it is today throughout the Neogene, that the transition from a weak latitudinal thermal gradient (from around 0.48 °C per degree in latitude to 0.6 °C per degree in latitude) took place at the end of the Miocene, and that the latitudinal precipitation gradient was, from the mid-Miocene to the mid-Pliocene, more accentuated than it is today, with higher precipitation than today in the north-western Mediterranean but conditions drier than or equivalent to today in the south to south-eastern Mediterranean region (Fauquette *et al.*, 1999; Jost *et al.*, 2009). In this sense, some of the above-discussed phylogenetic studies, based on allozyme differentiation, support a south-east to north-west *Abies* speciation sequence in the Mediterranean Basin (Scaltsoyiannes *et al.*, 1999; Fig. 1). At a smaller spatial scale, based on tree morphology and monoterpene composition, Mitsopoulos & Panetsos (1987) also found a south-east to north-west speciation pattern for Greek firs in the Peloponnesian peninsula.

Under this south-east to north-west speciation-sequence hypothesis (Fig. 1), the first isolated group would have been the ancestor of the current Turkish *A. cilicica*. Indeed, this species was identified as the oldest Mediterranean fir based on allozyme differentiation (Scaltsoyiannes *et al.*, 1999) and has enclosed bracts, characteristic of the morphological archaic group. However, it has been reported that *A. cilicica*, *A. cephalonica* and *A. nordmanniana* appear very close anatomically (see Esteban *et al.*, 2009), and have significant genetic proximity according to chloroplast DNA markers (see Parducci & Szmidt, 1999) and sequences of the complete nuclear ribosomal internal transcribed spacer (see Xiang *et al.*, 2009).

The second isolated group would have been the ancestor of the Algerian *A. numidica*, and finally, the Spanish and Moroccan *A. pinsapo* (Fig. 1; Xiang *et al.*, 2009). Analysis of chloroplast DNA markers (Parducci & Szmidt, 1999) and mitochondrial markers (Ziegenhagen *et al.*, 2005) have placed *A. numidica* genetically close to *A. pinsapo*, as these two species share haplotypes. The early isolation of *A. cilicica*, *A. numidica* and *A. pinsapo* would account for features such as their low heterozygosity and high gene differentiation.

Although *A. cilicica*, *A. pinsapo* and *A. numidica* belong to the same taxonomic subsection (*Pinsapones* Franco) and are characterized by enclosed cone-bract scales, great genetic distances between them were found by Scaltsoyiannes *et al.* (1999). These contrasting results could be attributed partially to the prolonged isolation of the species and also to their restricted distribution and the subsequent genetic drift (Parducci & Szmidt, 1999).

Abies cilicica, *A. numidica* and *A. pinsapo* grow in the same regions as *Cedrus* species (*A. cilicica* with *Cedrus libani*; *A. numidica* and *A. pinsapo* with *C. atlantica*), suggesting that firs and cedars may have a common biogeographic history in Africa, southern Turkey and the Middle East (Scaltsoyiannes, 1999; Scaltsoyiannes *et al.*, 1999; Qiao *et al.*, 2007). For instance, fossil records from the Miocene and Pliocene (see also Fig. 1) have identified *Cedrus* sp. (*Cedrus vivariensis* N. Boul. = *C. miocenica* Lauby) and *Abies saportana* Rér. in the eastern Iberian Peninsula (Alvarez Ramis & Golpe-Posse, 1981), France, Poland and Bulgaria (see Palamarev, 1989). Moreover, *Abies* species from the Himalayas (e.g. *A. pindrow*, which grows in mixed stands with *Cedrus deodara*) could possibly be related to the ancestral Mediterranean fir species. Indeed, *A. pindrow* has enclosed bract scales (Vidakovic, 1991), as in the pinsapo fir-type archaic group, and, with *A. pinsapo*, it forms an interspecific hybrid (*A. vasconcellosiana* Franco; see Klaehn & Winieski, 1962), despite the fact that both species (*A. pinsapo* and *A. pindrow*) have probably remained geographically isolated since their origin (Xiang *et al.*, 2009).

Phylogenetic studies show that *Abies* and *Cedrus* originated simultaneously (Magallón & Sanderson, 2005), although it is not possible to infer a common biogeographic history. The present revision suggests a northern Holocene expansion of the *Abies* genus over the entire Mediterranean Basin (Fig. 3), while *Cedrus* appears to have expanded throughout the southern Mediterranean Basin from glacial refugia, such as those located in the Rif Mountains of northern Morocco (Cheddadi *et al.*, 2009), where remnant *A. pinsapo* populations are located (see Fig. 4). However, *Cedrus* is not present today in Spain, Italy, or the Peloponnesian peninsula.

Based on the spatial climate patterns and gradients reported for the Neogene (Fauquette *et al.*, 1999; Bruch *et al.*, 2006), the early south-eastern to north-western isolation of some Mediterranean firs could also have affected the ancestor of the Greek *A. cephalonica* (Mitsopoulos & Panetsos, 1987) and even of the Sicilian *A. nebrodensis* (Parducci *et al.*, 2001a,b). However, repeated overlaps with northern species or subspecies that migrated following long-term climatic oscillations, from the Balkan range (to the southern Peloponnesian peninsula) and from the Alps (to the southern Italian peninsula), could explain modern features in these species (Mitsopoulos & Panetsos, 1987; Bennett *et al.*, 1991; Tzedakis, 1993).

The origin and the phylogenetic relationships between *A. nebrodensis* and *A. alba* have not been definitively identified. Some authors have hypothesized that *A. nebrodensis* originated from the southern silver fir (*A. alba*) populations as a consequence of isolation during post-glacial periods (Fig. 3; see Parducci *et al.*, 2001b, and references therein). Results based on allozyme, chloroplast DNA and random amplified polymorphic DNA show a clear differentiation among *A. nebrodensis* and *A. alba* populations and provide support for their classification into two distinct taxonomic groups (Vicario *et al.*, 1995). Nevertheless, *A. nebrodensis* showed a

Figure 4 The Rif Mountains (northern Morocco) experience high precipitation, essentially as a result of the orographic uplift of air charged with moisture from the nearby coast. These conditions probably also occurred during the glacial period, buffering the impact of regional aridity on tree populations. At present, these refugia may aid the persistence of relict species (*Abies pinsapo*, *Cedrus atlantica*, *Acer granatense*, *Sorbus aria*, *Taxus baccata*, *Ilex aquifolium*, *Pinus nigra*, etc.). Photograph: J. C. Linares.

closer affinity to *A. alba* populations from southern Italy, suggesting that this species may have originated through a past hybridization event (Parducci *et al.*, 2001b) or through genetic drift resulting from the prolonged isolation of southern populations.

Past and present roles of glacial refugia

The current natural distribution of *Abies* species around the Aegean and central Europe was inherited from Holocene colonization patterns from the last glacial-age refugia (see Fig. 3). The Mediterranean area may have been the starting point for a wave of *Abies* colonization towards central Europe (Konnert & Bergmann, 1995; Hewitt, 1999; Tzedakis *et al.*, 2002; Muller *et al.*, 2007; see also Fig. 3). The strong human pressure (agriculture and grazing), *c.* 7500–8000 yr BP (see Pons & Quézel, 1985), caused the decline and geographical disjunction of temperate fir forests.

As suggested by palaeopalynological and palaeoclimatic studies (see Tzedakis *et al.*, 2002, and references therein), the factors that lead to the currently high precipitation in some mountainous areas of the Mediterranean Basin (essentially, orographic uplift of air charged with moisture from the nearby coasts, see Fig. 4) also operated during the glacial period, moderating the impact of regional aridity on tree populations (Jost *et al.*, 2005). At present, the role that these refugia may play in allowing the persistence of relict species and the possibility of further migration in response to future climate change, as has happened repeatedly in the past, is subject to a number of uncertainties (Taberlet & Cheddadi, 2002; Terhürne-Berson *et al.*, 2004; Cheddadi *et al.*, 2006, 2009). The intense habitat fragmentation and degradation as a result of human action, in addition to such ecological factors as low genetic variability and low population sizes, increase the extinction risk of some of these Mediterranean firs, for example *A. numidica* and *A. pinsapo* (Linares & Carreira, 2009; Linares *et al.*, 2009; Rodríguez-Sánchez *et al.*, 2010). Further interdisciplinary research integrating climate models, biogeographic studies and palaeoecological as well as genetic data are needed for a better understanding of the role of these geographic areas, which have acted as refugia in both the past and present (Médail & Diadema, 2009; Alba-Sánchez *et al.*, 2010; Rodríguez-Sánchez *et al.*, 2010).

Concluding remarks

Since the Miocene, and mainly during the Pliocene, increasing aridity has led to increasing fragmentation and contraction of the range of ancestral Mediterranean fir species, probably following a south-eastern to north-western sequence. The speciation of the ancestor of the current Turkish *A. cilicica*, the ancestor of the Algerian *A. numidica*, and the ancestor of the Spanish and Moroccan *A. pinsapo* has been related to this regional dryness. The selective pressures over these early isolated species might be responsible for the current low heterozygosity and high gene differentiation among them, and also between them and the other Mediterranean fir species.

Based on palaeoclimate studies, the early isolation could also have affected the ancestor of the Greek *A. cephalonica* and even the Sicilian *A. nebrodensis*. However, repeated geographic overlaps with northern species or subspecies that migrated following long-term climatic oscillations, from the Balkans range to the southern Peloponnesian peninsula and from the Alps to the southern Italian peninsula, could explain the modern morphological features and complex genetic relationships for these species.

In the north-eastern Mediterranean Basin, following the Miocene to Pliocene climate crisis and marine transgressions, the ancestor of the northern Mediterranean firs would have been separated into two eastern groups, one on the Balkan

Peninsula and the other in Asia Minor, despite the occasional land bridges that probably maintained gene flow.

During the Pleistocene glacial cycles, the southward migration of *A. alba* to glacial refugia, where older fir species may have remained isolated, could explain recent findings that establish closer than expected relationships between distant species based on morphological classification.

The orographic complexity of the Mediterranean Basin played a key role in the evolution and the persistence of the Mediterranean firs. Factors that currently lead to high precipitation in some coastal mountain areas also operated during the glacial period, moderating the impact of regional aridity on tree populations. Today, however, the role of these refugia in the persistence of relict species could be limited by rapid climatic change and human-induced land degradation.

ACKNOWLEDGEMENTS

I would like to thank David Nesbitt for English revision, and F. Steininger and J. Eder for palaeoclimatic information. I also thank M. Ebach, R.J. Whittaker and A. Whittaker, and two anonymous referees for their very constructive and thorough comments on the manuscript.

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BIOSKETCH

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Editor: Malte Ebach