8. For a review of the development of Ru-based olefin metathesis catalysts, see [T. Trnka, R. H. Grubbs, *Acc. Chem. Res.* **34**, 18 (2001)].
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10. Over 80% of complex **A** can be recovered from the polymerization by column chromatography (neutral silica gel, pentane/diethyl ether as eluent).
11. The presence of even small amounts of acyclic olefins leads to linear polymer contamination. For example, linear polymer was observed when attempts were made at synthesizing cyclic polybutadiene from 1,5-cyclooctadiene, which contains approximately 0.1% of 5-vinylcyclohexene as an impurity.
12. When initial monomer concentrations of less than 0.2 M were used, only low molecular cyclic oligomers (MW < 2 kD) were obtained. The result is probably related to the critical monomer concentration of *cis*-cyclooctene (~0.25 M in toluene).
13. Olefin metathesis polymerization with extensive chain transfer approximates a step-growth polymerization where polymers with PDIs = 2.0 are expected. Chain transfer is known to occur during metathesis polymerizations when catalyzed by Ru complexes ligated with *N*-heterocyclic carbenes [C. W. Bielawski, R. H. Grubbs, *Angew. Chem. Int. Ed.* **39**, 2903 (2000)].
14. Viscosity measurements were performed in tetrahydrofuran (THF) at 30°C using a SEC-viscometer apparatus. The observed ratio of 0.4 is in accord with theory for cyclic and linear polymers in good solvents [W. Burchard, in *Cyclic Polymers* (Elsevier Applied Science, London, 1986), pp. 43–84].
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18. [1,2-diol-5-cyclooctene]$_0$/[*cis*-cyclooctene]$_0$ = 25; [total monomer]$_0$ = 0.5 M in CH_2Cl_2.
19. By assuming a continuous and random distribution of cleavable groups into infinitely long chains, the PDI was calculated to increase by a factor of 4/3 after cleavage.
20. The cyclic polyoctenamers were hydrogenated using either standard H_2/Pd/C procedures or tosylhydrazine decomposition [S. F. Hahn, *J. Polym. Sci. Polym. Chem.* **30**, 397 (1992)]. Either procedure resulted in hydrogenation of more than 99% of the olefins in the polyoctenamer's backbone, as determined by ^{1}H and ^{13}C NMR spectroscopy.
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23. No differences in θ were observed between high molecular weight (~200 kD) cyclic and linear polyoctenamers. This supports our belief that surface topology is affected by the presence (or absence) of end groups.

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Buffered Tree Population Changes in a Quaternary Refugium: Evolutionary Implications

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A high-resolution pollen record from western Greece shows that the amplitude of millennial-scale oscillations in tree abundance during the last glacial period was subdued, with temperate tree populations surviving throughout the interval. This provides evidence for the existence of an area of relative ecological stability, reflecting the influence of continued moisture availability and varied topography. Long-term buffering of populations from climatic extremes, together with genetic isolation at such refugial sites, may have allowed lineage divergence to proceed through the Quaternary. Such ecologically stable areas may be critical not only for the long-term survival of species, but also for the emergence of new ones.

The Pleistocene refugium hypothesis (*1*) proposed that population fragmentation during individual glacial stages of the Quaternary promoted speciation. A more recent view is that the accentuated environmental instability did not lead to increased speciation rates, with most species predating the Pleistocene (*2*, *3*). This evolutionary stability has been attributed to orbital (*2*) and millennial (*4*) climate fluctuations, which undo microevolutionary changes by forcing repeated population crashes, range shifts, and gene flow. Central to this view is that the alternation of climate phases does not generally allow sufficient time for genetic differentiation to species rank. However, molecular genetic data reveal considerable divergence between populations of many species in southern refugial centers in Iberia, Italy, the Balkans, and Greece, which took several glacial-interglacial cycles to accumulate (*5*–*7*). Analysis of DNA divergence in animals (*6*, *8*) shows that species have continued forming through the Pleistocene and that such divergence has proceeded apparently unhindered in some places (*7*, *9*). DNA divergence indicates that, whereas in lowland tropical forests most species formed before the Quaternary, clusters of recently diverged lineages along with older species are found in tropical mountain regions (*10*, *11*). It has thus been postulated (*10*) that these mountains are centers for speciation because they provide a relatively stable habitat through climate oscillations in which older species survive and new lineages are generated. Although little paleoecological evidence has been available, this long-term stability is thought to be a function of continued moisture availability and varied topography (*7*, *10*). A revised view of Quaternary evolutionary trends is that, while climate variability mostly inhibited speciation, species continued to form in places where presumed ecological stability allowed accumulation of genetic divergence over several glacial-interglacial cycles.

Here we present a new pollen record from a refugial site in Greece and test the extent to which certain habitats in mid-latitude areas remained immune from the extreme effects of Quaternary climate variability. The Ioannina basin (Fig. 1) is an intramontane plateau, 470 m above sea level (a.s.l.), situated in a topographically diverse landscape on the western flank of the Pindus mountain range, where the presence of refugial tree populations has been previously documented (*12*). The basin has a sub-Mediterranean climate with high annual precipitation [mean January temperature (T_{jan}) 4.9°C; mean July temperature (T_{jul}) 24.9°C; annual precipitation (p_{ann}) 1200 mm]. Core I-284 (39°45′N, 20°51′E; 319 m length) was drilled in 1989 near the previously studied I-249 site (*12*). An initial age model for I-284 (*13*) has here been modified by using calibrated radiocarbon ages for the last 21,000 ^{14}C years and an astronomical calibration of certain vegetation patterns for the rest of the sequence (*14*). Results of pollen analysis (*14*) from the upper 102 m of the I-284 core, spanning the past 130,000 years, are presented in Figs. 2 and 3B; mean sampling interval is ~225 years, 10 times as fine as the interval in I-249, and higher than other comparable European pollen sequences.

Three broad vegetation types are represented by the palynological assemblages of I-284: (i) forest communities [arboreal pollen (AP) > 70%] during the Last Interglacial [111.8 to 127.3 thousand calendar years ago (ka)], Interstadial 1 (88 to 104.5 ka), Interstadial 2 (68 to 83 ka) and the early Holocene (5 to 11.5 ka); (ii) communities of intermediate forest cover (70% > AP > 40%) during the Middle Pleni-

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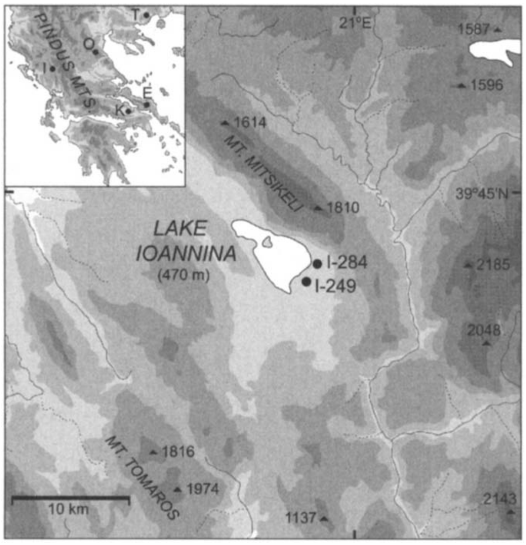
glacial (26 to 59 ka) and Stadials 1 (104.5 to 111.8 ka) and 2 (83 to 88 ka) of the last interglacial complex; and (iii) open vegetation communities with woodland of scattered trees (40% > AP > 21%) during the Early (59 to 68 ka) and Late (11.5 to 26 ka) Pleniglacial (and also over short intervals of the Middle Pleniglacial and during the late Holocene). During the glacial intervals, in addition to the well-represented deciduous *Quercus* and *Pinus*, the record shows continuous or near-continuous presence of pollen of *Abies*, *Ulmus*, *Corylus*, *Carpinus*, and *Ostrya*; the more thermophilous taxa show intermittent presence. These data clearly show that temperate tree populations persisted within the region throughout the last glacial period.

The summary pollen curves (Fig. 3B) show that the I-284 record captures the large-scale orbital variability over the last 130,000 years, but is characterized by less extensive AP decreases relative to other long pollen sequences from southern Europe (*15–18*) during stadial and glacial intervals. Recent paleoclimate results using a nested model (*19*, *20*) furnish some idea of the conditions that contributed to the survival of tree populations at Ioannina. During the last glacial maximum (LGM) at 21 ka, model simulations for the Ioannina region show decreases from modern values of 10°C, 6.8°C and 545 mm for T_{jan}, T_{jul}, and p_{ann}, respectively. In addition, annual growing degree days above 5°C were 920 at the LGM, compared with 2330 at present. Despite the magnitude of the decrease in precipitation, p_{ann} remained above 600 mm during the LGM, with an equable distribution throughout the year. Therefore, the decrease in LGM arboreal values represents the combined effects of reduced annual precipitation and winter temperatures, a shorter growing season and also lower atmospheric CO_2 concentrations, which led to tree population contraction, but were not severe enough to cause their elimination.

The detailed nature of the I-284 record also allows the identification of millennial-scale expansions and contractions of tree populations, similar in frequency to the climate variability seen in North Atlantic (*21*) and Greenland

Fig. 1. Topographic map showing the Ioannina basin and surrounding area. All heights are in meters above sea level. The sites of cores I-249 and I-284 are marked with circles. Inset: location of sites discussed in text. I, Ioannina; K, Kopais; T, Tenaghi Philippon; O, Mount Olympus; E, Island of Evvoia.



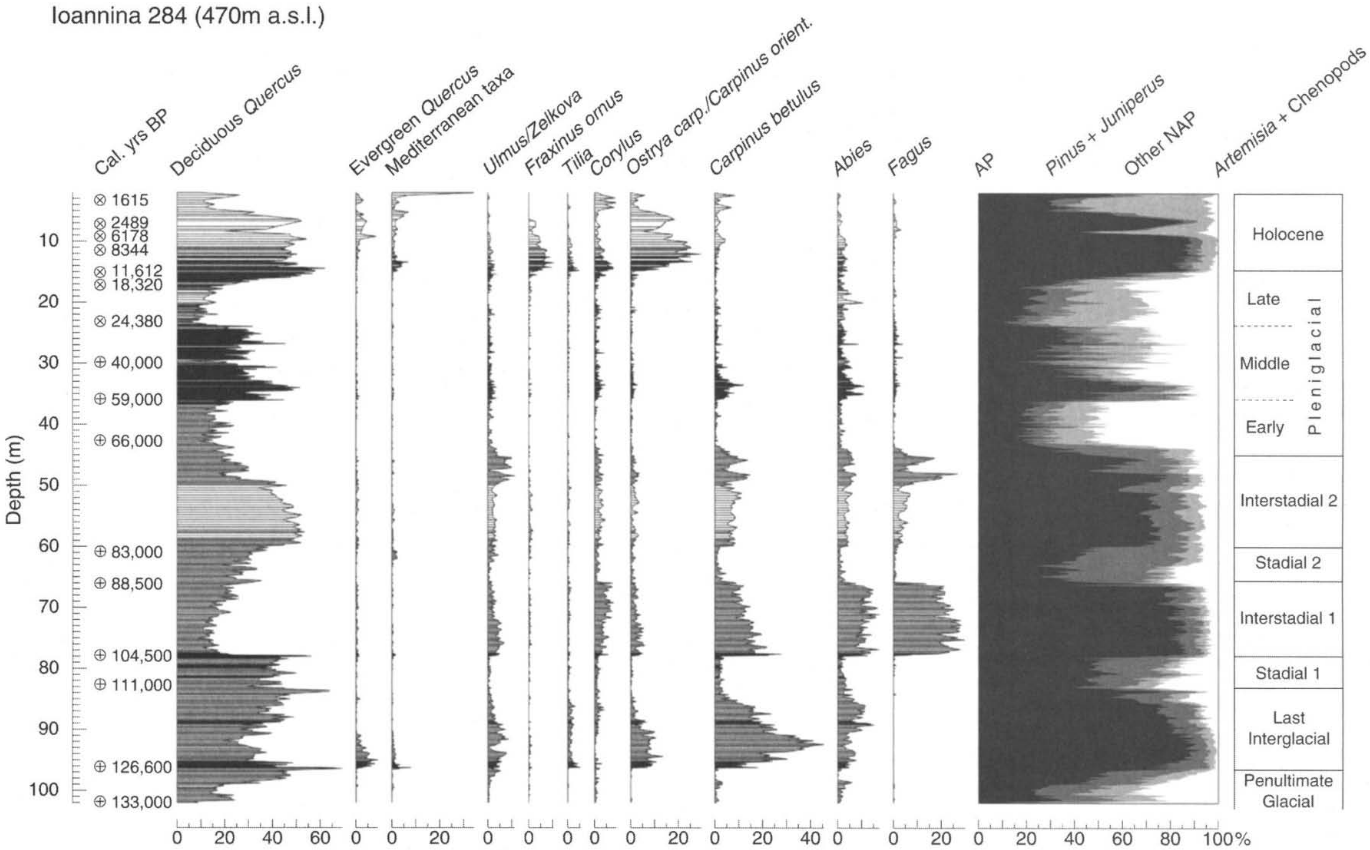


Fig. 2. Pollen diagram of the top 102 m of the I-284 sequence showing percentages of selected taxa. AP, arboreal pollen; NAP, nonarboreal pollen; *Ostrya carp.*, *Ostrya carpinifolia*; *Carpinus orient.*, *Carpinus orientalis*; Mediterranean elements, *Olea* + *Pistacia* + *Phillyrea*. Ages are presented in calendar years: ⊗ symbols refer to the midpoint of the calibrated AMS radiocarbon determinations (errors of 1σ omitted for clarity) (see supporting online text); ⊕ are derived by pollen-orbital tuning.

records (*22*) (Fig. 3, C and D). The precise phase relationship between North Atlantic climate oscillations and vegetation response in southern Europe has recently been clarified by pollen sequences in marine cores from the Portuguese margin (*23*) and the Alboran Sea, western Mediterranean (*24*), which show that fluctuations in Iberian tree populations closely tracked North Atlantic millennial-scale variability. Rapid transmission of this variability [reduced moisture content of eastward-moving low-pressure systems and increased advection of polar air (*24*)] would have led to abrupt changes in terrestrial ecosystems across S. Europe, with the largest tree population crashes associated with Heinrich events, and intermediate contractions corresponding to Dansgaard-Oeschger stadials, as documented for southeast Spain (*24*) and Italy (*17*). Hence, the largest reductions in AP values at Ioannina should correlate with the AP absolute minima in the Alboran marine pollen record (*24*), which are, in turn, synchronous with Heinrich events.

Although Fig. 3 shows evidence of millennial-scale variability, a consistent feature of I-284 is that the minimum AP values are always above 21%, with continuous curves for several temperate tree pollen types and, moreover, the amplitude of oscillations is relatively subdued. One explanation may be that this is a result of a gradual eastward attenuation of the North Atlantic climate signal. However, pollen records from Kopais in central Greece (*18*) and the island of Lesvos in the eastern Aegean (*25*) show much larger amplitude changes in AP values during the last glacial period, indicating no such attenuation. A more likely explanation for the subdued tree population contractions at Ioannina revolves around the importance of local intrinsic properties. As suggested by the paleoclimate simulations (*19*), factors leading to high precipitation in western Greece today (essentially, orographic uplift of air charged with moisture from the nearby Ionian Sea) also operated during the last glacial, moderating the impact of regional aridity on tree populations at Ioannina. In addition, high topographic variability provided a range of sheltered habitats, such that populations could migrate and survive within the Ioannina region. In contrast, at Kopais (38°26′N, 23°03′E; 95 m a.s.l.), located on the Boeotian plain in the rain-shadow of the Pindus with annual precipitation levels today at 470 mm, arid events had a more extensive

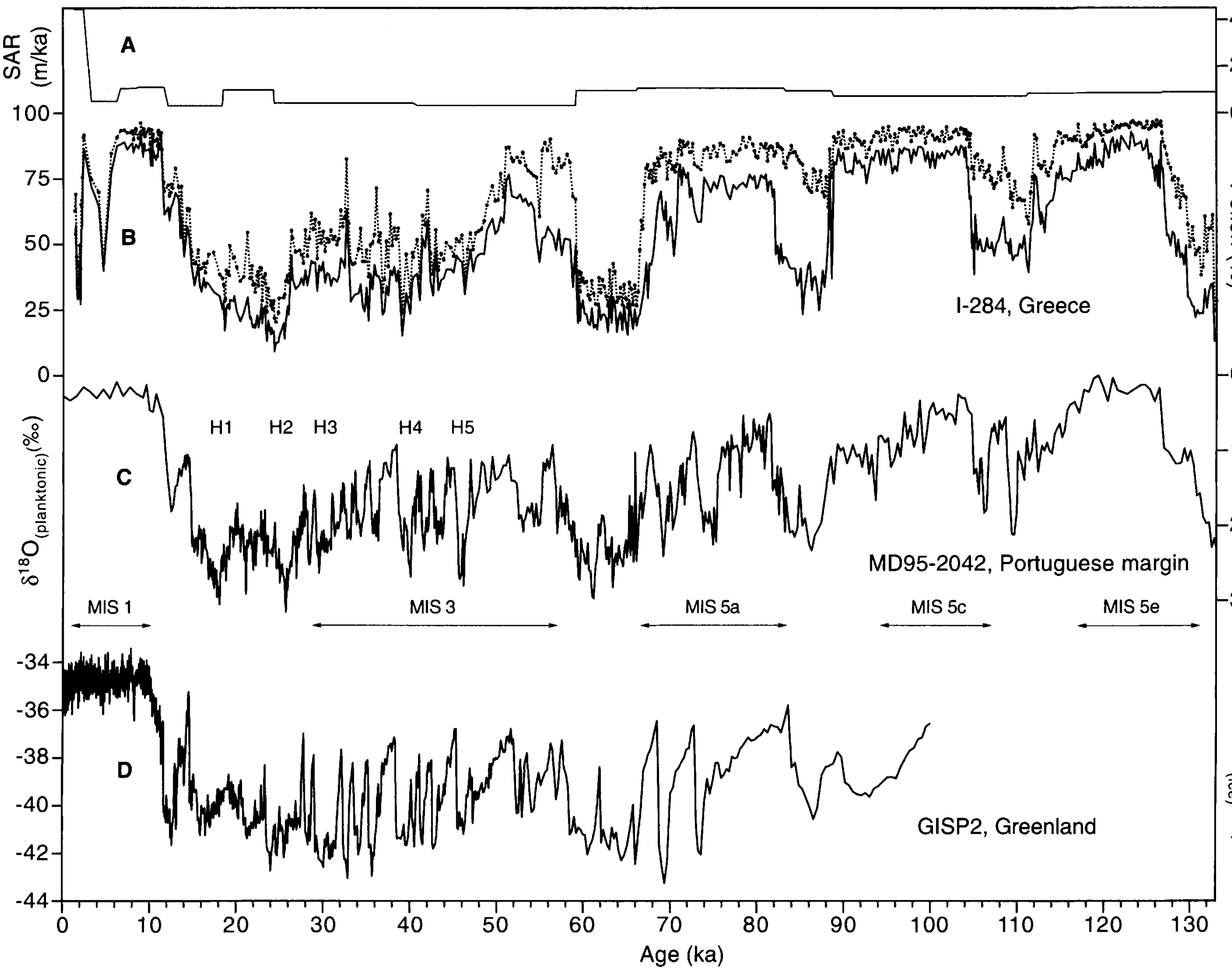


Fig. 3. (**A**) I-284 sediment accumulation rates. (**B**) Summary pollen percentage curves from the I-284 sequence; they represent changes in vegetation structure (relative degree of forest versus open vegetation communities). Dashed line, total AP; solid line, AP – (*Juniperus* + *Pinus*), representing the relative abundance of temperate tree populations (*Juniperus* and *Pinus* are not included as their ecological requirements are not always indicative of temperate conditions). The age model is based on control points shown in Fig. 2. (**C**) Variations in $\delta^{18}O$ composition of planktonic foraminifera in marine core MD95-2042 in the Portuguese margin (*21*). (**D**) Variations in $\delta^{18}O$ composition of ice in the GISP2 record, Greenland (*22*). H1 to H5 represent positions of Heinrich events. Marine isotope stages (MIS) are indicated. The GISP2 ice core chronology is based on counting annual layers, the MD95-2042 chronology on alignment to the GISP2 record and radiometric ages of sea-level stillstands, and the I-284 age model on radiocarbon ages and astronomical calibration (*14*).

impact on vegetation communities, crossing ecological thresholds. Although the magnitude of the paleoclimatically simulated LGM differences from modern-day values is smaller at Kopais (8°C and 3°C for T_{jan} and T_{jul}, respectively, and 295 mm for p_{ann}) than at Ioannina, absolute paleoprecipitation values are in this case critical. At Ioannina LGM p_{ann} is estimated at 655 mm; at Kopais the equivalent figure is 180 mm, below the ~300-mm threshold, which could have supported significant tree populations [e.g. (*26*)]. Similarly, at Tenaghi Phillipon (41°10′N, 24°20′E; 40 m a.s.l.) on the plain of Drama, northeastern Greece, where conditions are more continental, even the wetter phases could barely support temperate trees (*15*).

Hence, although all three sites experienced climatic oscillations throughout the last glacial, their ecological impact was largely determined by the extent to which (i) local moisture availability minima remained above the threshold for survival of temperate trees and (ii) topographic variability provided shelter from incursions of polar air. This resulted in strikingly different pollen records (fig. S1), which show a distinct biogeographic pattern east and west of the Pindus, with eastern arid and exposed lowlands having minimal presence of temperate tree populations during glacials and stadials, in contrast to the main refugial mid-altitude sites of western Greece, which provided the source for recolonization during each interstadial. Although there is no direct paleobotanical evidence, thermophilous Mediterranean taxa may have persisted along coastal plains, whereas small populations of pines and oaks may also have survived in favorable locations east of the Pindus (e.g., areas of high precipitation and variable topography near Mount Olympus and the Island of Evvoia).

The Ioannina data provide evidence for the existence of an ecologically near-stable area where local conditions appear to have buffered the extreme effects of Quaternary climate variability, contributing to the survival of residual tree populations. This conclusion is independent of any imprecisions in the I-284 age model: whether a particular AP minimum is in or out of phase with a Heinrich event does not alter the main feature of the pollen record, which is that curves of many tree taxa remain continuous throughout. When combined with the longer I-249 record (*12*), what emerges is that populations of many temperate tree species have persisted in this general area, over several hundred thousand years, albeit at varying abundances.

Given the present distinctive genetic character of Iberian, Italian, and Balkan populations, it has been argued (*6*) that each peninsula remained genetically isolated not only during glacials, but also during interglacials, thus preserving the products of evolutionary processes. Whether some communication occurred in northern parts of refugial peninsulas (*27*) is debatable, but in any event, populations in the south would have remained isolated. At a finer scale, the Ionian Sea and Pindus mountain range would act as barriers, limiting gene flow that might have originated from range expansion from areas further afield, thus preserving any accumulated differences. Recent DNA data from grasshoppers on separate mountain blocks, "sky islands" in the Rocky Mountains, USA, provides evidence of Pleistocene divergence and speciation in similar conditions of isolation and allopatry (*28*).

During glacials, reduced populations surviving in isolated habitats could have differentiated through selection and genetic drift. Such microallopatry would be repeated each cold period, interspersed with range expansion beyond the Pindus, varying selection, and parapatry with some hybridization. Under such changing conditions, each taxon would follow its own pathway of divergence and speciation (*6*). Whether speciation events did take place in the immediate Ioannina area has yet to be established, but the record presented here affords us a glimpse of the conditions that potentially could lead to divergence and speciation, when populations remained effectively isolated over several glacial-interglacial cycles.

The richness of the Mediterranean flora with its unusually high endemism is, in part, a reflection of its geographical position and geological history, as well as the extent to which Tertiary species managed to survive the effects of Quaternary climate variability (*29*). However, local buffering from extreme environmental effects, as illustrated by the Ioannina record, not only led to reduced extinction rates but may have also provided an opportunity for new species to emerge. The western Balkans and specifically the Pindus mountains consistently emerge as a hot spot of endemism not only in plants, but also in various animal groups (*30*). For example, the distribution of spiders shows that the highest number of endemic species in the Balkans is recorded in the Pindus region (150) and, moreover, half of these are woodland species (*31*). Thus, the persistence of tree populations in the Pindus may also have promoted genetic divergence in other organisms by providing relatively stable habitats through Quaternary climate variations.

Examination of the genetic structure of populations in the Pindus should provide a test of these ideas, by showing the degree of differentiation relative to nonrefugial areas in Greece and the Balkans. A combined paleoecological-genetic approach should be able to pinpoint those populations that are most important for conservation, not only for the long-term survival of species, but also for the emergence of new ones. Because most northern European populations are eliminated during glacials, the identification of the locations of southern long-term refugia should be a conservation priority.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5589/2044/DC1
Materials and Methods
Fig. S1
Tables S1 and S2

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