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Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity

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Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity

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Glacial refuge areas are expected to harbor a large fraction of the intraspecific biodiversity of the temperate biota. To test this hypothesis, we studied chloroplast DNA variation in 22 widespread European trees and shrubs sampled in the same forests. Most species had genetically divergent populations in Mediterranean regions, especially those with low seed dispersal abilities. However, the genetically most diverse populations were not located in the south but at intermediate latitudes, a likely consequence of the admixture of divergent lineages colonizing the continent from separate refugia.

During the long glacial episodes of the Quaternary, European forests were considerably more restricted than in the present interglacial, because the Mediterranean Sea in the south and unsuitable environment in the north restricted temperate tree and shrub taxa to the Iberian, Italian, and Balkan peninsulas. For instance, at the time of the last glacial maximum, 25,000 to 17,000 years ago, networks of fossil pollen data and macrofossil remains such as charcoals indicate that several tree species were localized in small favorable spots within the Mediterranean region but also at the southern edge of the cold and dry steppe-tundra area in eastern, central, and southwestern Europe (*1*–*5*). After climate warming, some of these surviving populations expanded, whereas others remained trapped and either became extinct or persisted by shifting altitude (*2*, *6*). As a consequence of prolonged isolation, extant tree populations situated close to refugia should be highly divergent, especially if they were not the source of the expansion. Another related prediction is that intraspecific diversity should decline away from refugia, as a consequence of successive founder events during postglacial colonization (*7*, *8*). However, species attributes such as colonizing ability may alter these predictions (*9*). Furthermore, the individualistic migration behavior of tree species during interglacial periods (*6*, *9*) and the presence of more northern refugia (*4*, *5*) may have blurred this pattern. In Europe, range-wide genetic surveys of a few well-investigated tree species have been performed (*10*–*12*), but it is difficult to generalize from these studies. To get a broader picture and to test the previous predictions, we gathered data from several woody angiosperm taxa across Europe using standardized sampling and molecular screening techniques. Such knowledge on the genetic consequences of the recent history of woody plant species may be critical for the conservation and sustainable management of their genetic resources.

Plastids are generally maternally inherited in angiosperms and, therefore, moved by seeds only. Because colonization of new habitats occurs through seeds, chloroplast DNA (cpDNA) markers provide information on past changes in species distribution that is unaffected by subsequent pollen movements (*13*). We have investigated patterns of cpDNA diversity in 22 woody species. These were sampled in the same 25 European forests selected on the basis of their high species richness and limited human influence (table S1). About 10 individuals per species were sampled from each forest, following a standard procedure (*14*). Polymorphisms were detected by polymerase chain reaction (PCR) techniques (*14*) in all 22 species [4 to 50 haplotypes per species, mean 16.9 (Table 1)]. The degree of subdivision of cpDNA diversity (G_{ST}) was estimated for each species (*15*, *16*). This measure partly reflects the dispersal ability of the species considered, although long-term range fragmentation should also play a role. Low G_{ST} values (indicative of high levels of gene flow through seeds) were found in *Salix* and in *Populus* (0.09 to 0.11), characterized both by light, wind-dispersed cottony seeds. The species characterized by animal-ingested seeds also tended to have below-average values. In contrast, species with animal-cached seeds (i.e., nuts) exhibited higher than average values (Table 1).

To compare forests with each other, we calculated the mean number of haplotypes and within-population gene diversity by averaging across species in each forest (table S2). We also calculated a measure that expresses the average genetic divergence of the forest from all remaining populations (*17*) (table S2). The highest values were observed in Corsica, Italy, and the Balkans, including Croatia and Romania, whereas average or below-average values were

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found in the rest of Europe (Fig. 1). Patterns of diversity across forests were very different; both mean number of haplotypes (Fig. 2) and gene diversity (table S2) were higher in Central France, southern Germany, and Slovakia, whereas the southern- and northernmost populations generally had low or average diversity, with the exception of southwestern Sweden.

To assess the consistency of these patterns across species, we tested whether the geographic pattern found in each species was congruent with the pattern formed by the remaining species. For divergence, the correlation (r_D) was positive in 18 of the 22 species and significant in 11 cases (Table 1). Those species with the highest G_{ST} estimates (such as *Carpinus* or *Corylus*) conformed best to the overall pattern of divergence (as shown by the significant and positive relation across species between G_{ST} and r_D: $r = +0.52$, $P < 0.01$). Species with low or medium G_{ST} and with more boreal distributions, such as *Betula*, *Calluna*, *Rubus*, or *Salix*, did not conform well to this pattern. For haplotype diversity, the correlation (r_H) was positive in all but one case (excluding *Carpinus betulus* for which within population diversity was not detected) and significant in 13 cases (Table 1), indicating a largely consistent pattern across species.

Despite their individualistic migration behavior, their varied ecologies (from southern temperate to southern boreal), and heterogeneous modes of seed dispersal, the species investigated here (i.e., a significant component of the woody flora from northern Europe) exhibited a largely congruent pattern of divergence, with the genetically most unique populations located in southern and central Italy, Corsica, and the Balkan peninsula, but extending into northern Italy, Croatia, and Romania, i.e., at relatively high latitudes. This is in agreement with recent findings of tree remains slightly north of the European peninsulas dated from the full glacial period (*4*, *5*). The species suspected to have the lowest dispersal abilities (e.g., *Carpinus* or *Corylus*) conformed best to the overall pattern of divergence. Founding events would have been strongest in these species, which would have left a major share of their genetic diversity in the refugia. On the contrary, taxa with a more boreal distribution did not conform well to the overall pattern, which may be attributed to the survival of more northern and diffuse populations of these species during ice ages (*18*–*20*).

The fact that the three Iberian forests are not as divergent as those from the other peninsulas may be due to any of the following causes. First, the Pyrenees may not have formed such a strong barrier to colonization after the last ice-age, compared with the Alps (*8*), because northern Spain, western France, Britain, and Ireland were united by a land bridge during the postglacial period, due to lower sea levels (Fig. 1). As a consequence, in several species such as *Quercus* (*12*), *Hedera* (*21*), *Fraxinus*, and *Ilex*, Iberian lineages could spread over large parts of western Europe, resulting in lower divergence of the Iberian populations. Second, the Iberian peninsula seems to have been exposed to particularly severe climatic episodes (i.e. arid and cold) during the Quaternary (*22*); therefore, the temperate tree populations that survived throughout successive ice ages would be smaller than in other parts of Europe and would be located further south. Third, for several species, especially those that conform best to the overall pattern of divergence (i.e., *Acer pseudoplatanus*, *Carpinus*, *Fagus*), present distribution, fossil pollen, cpDNA (*23*), or other genetic data (*24*) suggest that populations originating from Italy or the Balkans entered Iberia from the north. This

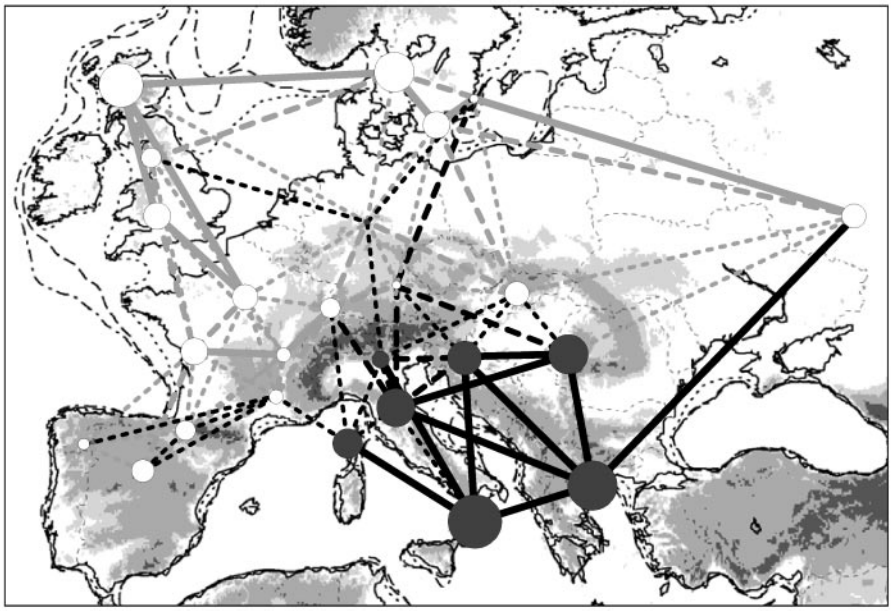


Fig. 1. Multispecies genetic divergence of each of the 25 European forests studied. Higher than average values are in black circles, lower than average are in white circles, and circle diameter is proportional to the difference from the mean value (exact values in table S2). For all forests, the level of divergence with each of the five nearest forests was represented by connecting lines, with continuous black lines indicating comparatively high divergence, dotted lines, intermediate divergence (black, higher than the mean; gray, lower than the mean) and continuous gray lines, low divergence (table S3). The altitude is indicated by gray shadings (lightest gray indicates 250 to 500 m, and the gray intensifies as the altitude increases from 500 to 1000, and >1000 m). Past sea levels at 21 ka BP (18 ^{14}C ka BP), 15 ka BP (13 ^{14}C ka BP), and 12 ka BP (10 ^{14}C ka BP) are indicated by black dotted lines (*12*).

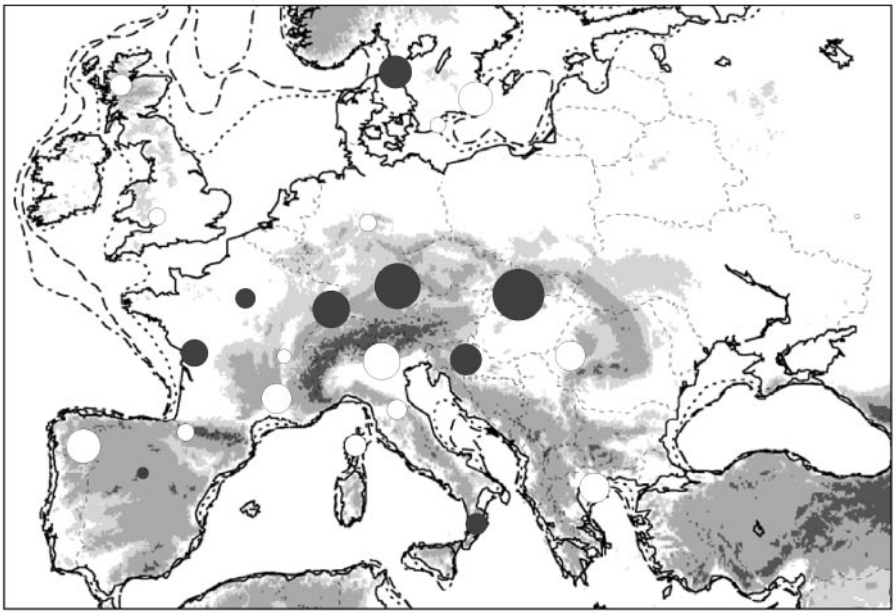


Fig. 2. Mean number of haplotypes per forest, averaged across species (exact values in table S2). Legend as for Fig. 1. Diversity is highest at relatively high latitudes, north of the three European peninsulas.

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Table 1. List and parameter values for the 22 species investigated. The seed dispersal mode was defined as follows: Ac, animal-cached; Ai, animal-ingested; Wc, cottony, wind-dispersed; Ww, winged, wind-dispersed; Wa, wind-dispersed and animal-ingested; Ed, explosive dehiscence. r_D measures the correlation between the pattern of divergence of a given species and the pattern of divergence for all species combined, excluding that particular species, whereas r_H corresponds to the same correlation for allelic richness (asterisk indicates $P < 0.05$). Nc, not computed.

Species	Family	Seed dispersal mode	No. of populations	Total no. of haplotypes	G_{ST}	r_D	r_H
Acer campestre	Aceraceae	Ww	16	14	0.71	0.65*	0.07
Acer pseudoplatanus	Aceraceae	Ww	19	22	0.66	0.62*	0.60*
Alnus glutinosa	Betulaceae	Ww	25	12	0.81	0.62*	0.26
Betula pendula	Betulaceae	Ww	23	9	0.42	−0.02	0.48*
Calluna vulgaris	Ericaceae	Wa	17	12	0.59	0.22	0.39
Carpinus betulus	Betulaceae	Ww	18	4	1.00	0.81*	nc
Corylus avellana	Betulaceae	Ac	24	5	0.89	0.73*	0.66*
Crataegus monogyna	Rosaceae	Ai	21	4	0.24	0.14	0.76*
Cytisus scoparius	Fabaceae	Ed	18	24	0.57	0.54*	0.61*
Fagus sylvatica	Fagaceae	Ac	23	6	0.74	0.70*	−0.07
Fraxinus sp.	Oleaceae	Ww	24	7	0.86	0.08	0.41*
Hedera sp.	Araliaceae	Ai	22	11	0.57	0.21	0.58*
Ilex aquifolium	Aquifoliaceae	Ai	16	8	0.60	0.18	0.47*
Populus tremula	Salicaceae	Wc	23	30	0.11	0.47*	0.54*
Prunus avium	Rosaceae	Ai	23	16	0.29	0.78*	0.42*
Prunus spinosa	Rosaceae	Ai	25	50	0.32	0.44*	0.57*
Quercus sp.	Fagaceae	Ac	25	10	0.84	0.40*	0.31
Rubus sp.	Rosaceae	Ai	23	15	0.31	−0.04	0.34
Salix caprea	Salicaceae	Wc	25	29	0.09	−0.16	0.01
Sorbus torminalis	Rosaceae	Ai	17	26	0.33	−0.11	0.69*
Tilia cordata	Tiliaceae	Ww	16	16	0.57	0.45	0.66*
Ulmus sp.	Ulmaceae	Ww	25	41	0.47	0.34	0.10
Mean			21.3	16.9	0.54	0.36	0.42

would also reduce the genetic divergence of northern Iberian populations.

The strikingly different pattern of intrapopulation diversity, which peaks north of the main mountain ranges, rather than south of them, may be due to one of the following causes. First, the mixing of colonization routes and the subsequent admixture of divergent cpDNA haplotypes could create such a pattern, especially if the glacial refugia that were the source of colonization were present not far to the south of these regions (*4*, *5*). This may be strengthened by the evolution of higher dispersal ability in newly colonized (as opposed to refugial) populations (*25*), resulting in increased levels of seed flow away from refugia. Finally, retreating southern edges of the ranges may have become dissected to the point that local populations lost diversity (*8*). Regardless of the underlying mechanisms, this pattern of diversity does contrast with that predicted under simple models of colonization, i.e., a gradual decrease in diversity away from the source populations (*7*, *8*). Recent theoretical models have shown how genetic diversity may be better preserved during colonization than previously assumed, but they do not predict increased diversity (*11*, *26*). In fact, increased diversity would be achieved mostly through the redistribution ("melting pot") of the genetic information already present among populations in refugia (the actual "hot spots," i.e., areas where diversity has been created). Because the contribution of a population to total species diversity depends more on its divergence from other populations than on its intrinsic within-population diversity (*27*), the genetic uniqueness of southeastern European populations should largely outweigh their low diversity for long-term conservation purposes.

Our study confirms the importance of glacial relict forest tree populations but warns against simple genetic criteria to identify them. Such results apply to intraspecific diversity only. It would now be of interest to contrast them with patterns of species richness, as these should also have been affected (but perhaps not to the same extent) by the legacy of past climate changes (*28*–*29*).

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16. For each species, we also computed N_{ST}, a parameter similar to G_{ST} but which takes into account similarities between haplotypes (*15*). Values are reported in table S4. Mean value across species was 0.60 for N_{ST}, compared with 0.54 for G_{ST}. N_{ST} was higher than G_{ST} in 17 out of 22 cases and significantly so ($P < 0.05$) in 9 cases, demonstrating the existence of a phylogeographic structure for these species (*15*).
17. A strong overall pattern of isolation-by-distance was detected through regression analysis of pair-wise measures of N_{ST} against distances in kilometers. To map areas showing intrinsically high or low divergence independently of the particular distribution of the populations sampled, we used a distance-free estimate of divergence. The residuals of the regression of pair-wise divergence against distance were used for this purpose (*14*).
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