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Rangewide phylogeography of a bird-dispersed Eurasian shrub: contrasting Mediterranean and temperate glacial refugia

A. HAMPE,*† J. ARROYO,* P. JORDANO† and R. J. PETIT‡
*Depto de Biología Vegetal y Ecología, Universidad de Sevilla, Apdo. 1095, E-41080 Sevilla, Spain, †Estación Biológica de Doñana, CSIC, Apdo 1056, E-41080 Sevilla, Spain, ‡UMR Biodiversity, Genes and Ecosystems, Institut National de la Recherche Agronomique, 69 route d'Arcachon, F-33612 Cestas cedex, France

Abstract

We studied the phylogeography of alder buckthorn (*Frangula alnus*), a bird-dispersed shrub or small tree distributed over most of Europe and West Asia and present in three of the four main refugia of West Palaearctic temperate woody plants: the Iberian Peninsula, the Balkans and Anatolia. A total of 78 populations from 21 countries were analysed for chloroplast DNA variation using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), and 21 different haplotypes were distinguished. We found a very strong overall population differentiation (G_{ST} = 0.81) and phylogeographical structure, and a sharp contrast between the haplotype-rich refugia and the almost completely uniform area of postglacial colonization. The haplotype network comprises three lineages made up of haplotypes from the Iberian Peninsula, Anatolia with the Caucasus, and temperate Europe. The Iberian and the Anatolian branches represent parts of a major lineage that spans over the whole northern Mediterranean Basin and some neighbouring areas and probably dates back to the Tertiary. Many haplotypes of this lineage are distributed locally and most populations are fixed for a single haplotype; these populations have apparently been very stable since their establishment, experiencing negligible gene flow and few mutations. The temperate European lineage consists of one very widespread and abundant plus six locally distributed haplotypes. Four of them are located in Southeast Europe, the putative refugium of all extant temperate European populations. Contrary to populations from Iberia and Anatolia, *F. alnus* populations from the southeastern European refugium have most genetic variation within populations. Bird-mediated seed dispersal has apparently allowed not only a very rapid postglacial expansion of *F. alnus* but also subsequent regular seed exchanges between populations of the largely continuous species range in temperate Europe. In contrast, the disjunct *F. alnus* populations persisting in Mediterranean mountain ranges seem to have experienced little gene flow and have therefore accumulated a high degree of differentiation, even at short distances. Populations from the southern parts of the glacial refugia have contributed little to the postglacial recolonization of Europe, but their long-term historical continuity has allowed them to maintain a unique store of genetic variation.

Keywords: chloroplast DNA, diversity, differentiation, fleshy fruits, postglacial recolonization, seed dispersal

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Introduction

The present-day distributions of temperate plant species and communities are a result of multiple range shifts driven by Quaternary climate changes. During glacial maxima, taxa suffered severe range reductions and fragmentations into refugia, from which they expanded more or less successfully during interglacial periods. The principal refugia of West Palaearctic plants have been localized on the Peninsulas that surround the northern

Correspondence: A. Hampe. Fax: + 34 954 557059; E-mail: arndt@us.es

Mediterranean Sea: Iberia, Italy, the Balkans and Anatolia (Hewitt 1996, 2001; but see also Steward & Lister 2001). Their central significance for the long-term persistence, genetic reshuffling and diversity of European plant and animal taxa has been documented broadly by the fossil pollen record (Huntley & Birks 1983; Tzedakis *et al*. 2002) and phylogeographical studies (Taberlet *et al*. 1998; Hewitt 2001; Petit *et al*. 2003).

However, comparatively little is known about the inner structure of the major refugial regions and the degree to which different populations persisting in these areas have been involved in the postglacial range expansions of widespread temperate species. Many phylogeographical studies conducted so far have concentrated on continental-scale phylogeographical patterns (reviews in Taberlet *et al*. 1998; Hewitt 2001). Some regional studies (Gömöry *et al*. 1999; Villani *et al*. 1999) have sampled glacial refuge areas intensively, but their small geographical scope has not allowed a global view of the species' history. As a consequence, although it is widely recognized that glacial relict populations have been experiencing unique ecological conditions and specific demographic and evolutionary processes (Hewitt 1996), the heterogeneity of conditions prevailing in the main refugia during past glacial maxima has been largely neglected.

For instance, the southernmost parts of the main refugia have been under the full influence of the Mediterranean climate; glacial relict populations from these areas are currently restricted to particular wet and cool habitats within a matrix of unsuitable landscapes and are highly isolated. For mountain plants, their response to past climate changes has mostly consisted in altitudinal shifts without migrating long distances (Tzedakis *et al*. 2002). Gene flow or metapopulation dynamics between disjunct populations would have been negligible under interglacial as well as under glacial maximum conditions, and mutations would have expanded little from their place of origin (Hewitt 1996, 2001). Moreover, during interglacial periods relict populations would have been exposed to bottleneck situations due to their small size and to climatic constraints on regeneration (see Hampe & Arroyo 2002 and references therein). In contrast, the northern parts of the main refugia have had a climate more similar to that of central and northern Europe. During glacial maxima, refuge populations would have inhabited lower altitudes and be less isolated than those from to the southernmost refugia. This probably allowed more horizontal range shifts and among-population gene flow. During interglacial periods, regeneration would be less limited by climatic conditions and population bottlenecks would be less common.

According to the 'leading edge' hypothesis, the postglacial range expansion of species involved mainly populations from the northern edges of the refugia, which filled the new territories rapidly with their progeny and largely precluded the northward expansion of later arriving lineages (Hewitt 2001). It is now widely acknowledged that long-distance dispersal events and the subsequent establishment and rapid growth of pioneer populations ahead of the continuous range have played a central role in the expansion of species and in the buildup of phylogeographical patterns (Hewitt 2000, 2001; Petit *et al*. 2001). Moreover, the demographic processes linked with range shifts have sometimes promoted microevolutionary improvements of dispersal mechanisms (Dynesius & Jansson 2000; for case studies see Cwynar & MacDonald 1987; Hampe & Bairlein 2000a).

The leading edge model predicts also a clear geographical separation of lineages expanding from different refugia and a successive northward decrease in genetic diversity due to multiple successive founder events (Hewitt 2001). Although these patterns have been found in several chloroplast DNA (cpDNA)-based phylogeographical studies, plant species characterized by efficient dispersal mechanisms (e.g. small, wind-dispersed or animal-ingested seeds) do not fit with these predictions. Instead, they usually exhibit weak phylogeographical structures and much variation within northern as well as southern populations (Raspé *et al*. 2000; Mohanty *et al*. 2001a,b; Oddou-Muratorio *et al*. 2001; Rendell & Ennos 2002; but see Grivet & Petit 2002). Because in many angiosperms the cpDNA is inherited maternally (Birky 2001) and therefore only propagated by seeds, these patterns have been explained commonly by an intense gene flow among populations during and after their establishment due to efficient seed dispersal. However, so far no studies have examined the phylogeographical structure of fleshy-fruited plant species across their entire range. In particular, the southern populations from putative glacial refugia have been represented very little. It is therefore unknown if gene flow has occurred between populations of the southernmost refugia located in the Mediterranean Basin or if it has been restricted to the areas of postglacial recolonization. Finally, many temperate bird-dispersed fruits ripen during the autumn migration period of their seed dispersers, but to our knowledge it has never been asked if the preferentially southward seed dispersal by migrating birds has affected current phylogeographical patterns of bird-dispersed plant species.

Frangula alnus Miller (Rhamnaceae) is a shrub or small tree that grows over most of temperate Europe and Western Asia (Fig. 1). The nominate subspecies occurs through most of the range, while two other subspecies have been described from Central and East Anatolia (*F. a. pontica* (Boiss.) Davis & Yalt.) and from South Iberia and North Morocco (*F. a. baetica* (Rev. & Willk.) Rivas Goday). The latter is treelike and individuals become about twice as tall and old as those of the other two shrublike subspecies (Hampe & Bairlein 2000a). *F. a. alnus* has recently been

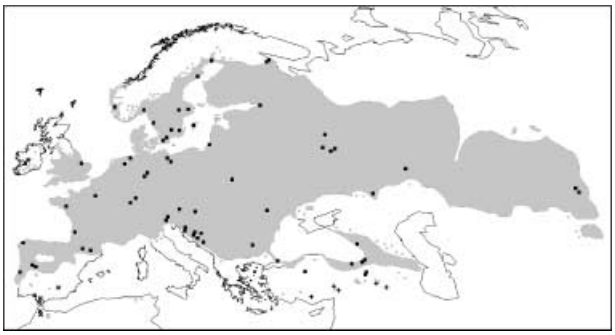

Fig. 1 Distribution of *Frangula alnus* (after Meusel *et al*. 1978). Black signs indicate sampling sites and differ according to the subspecies sampled (■ = *F. a. alnus*, †: *F. a. pontica*, × = *F. a. baetica*). Information on subspecies distributions are taken from regional standard floras (Yaltirik 1967; Muñoz 1987).

introduced into North America, is expanding quickly and becoming a pest in some regions (Catling & Porebski 1994).

Reproductive biology and organs of *F. alnus* have been described in detail by Medán (1994) and Hampe & Bairlein (2000a). This entomophilous species produces blackish two- or three-seeded fleshy fruits, which are eaten and dispersed by birds. In temperate Europe, *F. alnus* is a widespread woody pioneer species on acid, moist soils and can build up large populations on fens, clearcuttings or at forest edges, which are later overgrown and substituted by forest vegetation (Godwin 1943). In contrast, *F. alnus* populations in the Mediterranean Basin are restricted to special habitats with year-round water supply, mainly riparian forests in mountain ranges (Yaltirik 1967; Muñoz 1987).

Fossil pollen records show that *F. alnus* grew in northern Europe during the last interglacial (Moe 1984). Macrofossil records of *Rhamnus* sp., found in Slovenia and northern Italy and dated back to the last glacial maximum (Willis *et al*. 2000), belong most probably to *F. alnus* (sometimes still called *Rhamnus frangula*), as no other species of this genus grows in boreal plant communities such as those reported by the cited records. After the last glaciation, *F. alnus* was present in southern Scandinavia about 11 500 years BP, comprising one of the woody species that first recovered its current distribution range. The species reached its northern distribution maximum around 5000 BP, before it retreated to its current range (Moe 1984).

The present study investigates the phylogeography of *F. alnus* across its Eurasian and North African range. Specifically, our aims are (1) to locate and delimit its Quaternary refugia, (2) to assess phylogenetic relations and patterns of genetic variation within and among different refugia and recolonized areas and (3) to evaluate the role of the bird-mediated seed dispersal on colonization, phylogeographical structure and among-population gene flow of the species.

Materials and methods

Plant material and DNA isolation

A total of 322 individuals from 78 localities were sampled (see Table 1). Intact leaves and, when available, mature seeds were collected from up to five individuals growing at least 20 m apart from each other. In most populations samples were dried quickly and stored in silicagel or as herbarium specimens until DNA isolation. Samples from six populations could be used fresh or after deep-freezing and storage at −80 °C. Fifteen specimens provided by herbaria (B, GAZI, HUB and ISTE) were similarly included. DNA was isolated from leaf tissue in all but two populations, where seeds were used to improve the DNA yield. The DNeasy 96 Plant Kit (Qiagen) and the GenElute Plant Genomic DNA Kit (Sigma) were used for DNA isolation. Herbarium vouchers have been stored by the first author or the collaborating herbaria.

Identification of cpDNA variation

In a preliminary screening, 18 cpDNA primer pairs (*trn*H/*psb*A, *trn*S/*trn*G and *psb*B/*psb*F from Hamilton 1999); AS, B_1B_2, B_2B_3, BD, DT, HI, HK, K_1K_2, K_2Q, OA, QS, S_{fM}, SR, ST and TF from Grivet *et al*. 2001) were tested in combination with two restriction enzymes (*Hin*fI and *Taq*I) over a subset of 46 individuals from 23 populations distributed over most of the sampled range. Eleven combinations revealed polymorphisms and the three that performed best (in terms of amplification of the partly degraded DNA and readability of electrophoresis bands) were chosen to screen all *F. alnus* populations: DT with *Hin*fI, *trn*S/*trn*G with *Taq*I and SR with *Hin*fI. The remaining eight markers showed variation only in those populations that were also polymorphic according to the three finally chosen systems. Polymerase chain reaction (PCR) was carried out in a PHC-3 Techne thermal cycler using the following procedure: 1 cycle (4 min/94 °C), 30 cycles (45 s/94 °C, 45 s/annealing temperature, elongation time/72 °C), 1 cycle (10 min/72 °C). Annealing temperatures and elongation times were 54.5 °C/2 min for DT, 50 °C/1 min for *trn*S/*trn*G and 52 °C/3 min for SR, respectively. The digestion was run for 3 h at 65 °C for *Taq*I, and overnight at 37.5 °C for *Hin*fI in a total volume of 20 μL containing 5 μL of PCR product, 3 μL of buffer and 2 U of restriction enzyme. Enzymes and buffers were obtained from Life Technologies, Gibco. PCR–restriction length polymorphism (RFLP) fragments were separated on 8% acrylamide gel and stained with ethidium bromide.

Phylogenetic analyses

Statistical parsimony (Posada & Crandall 2001) was used to describe relationships among haplotypes. The haplotype

Table 1 Features of the *Frangula alnus* populations sampled

No.	Name	Country	Ssp.	Geogr. region	Latitude	Longitude	No. of samples
1	Jbel Bouhachem I	Morocco	B	1	35°12′ N	5°25′ W	5
2	Jbel Bouhachem II	Morocco	B	1	35°15′ N	5°27′ W	5
3	Puerto Oscuro	Spain	B	1	36°30′ N	5°35′ W	5
4	Medio	Spain	B	1	36°31′ N	5°36′ W	5
5	Doñana	Spain	B[1]	1	37°12′ N	6°36′ W	5
6	Huerta Vieja	Spain	B	1	38°08′ N	2°52′ W	5
7	Mondego	Portugal	A	1	40°08′ N	8°38′ W	5
8	Robledilla	Spain	A	1	40°20′ N	6°28′ W	3
9	Nava del Barco	Spain	A	1	40°35′ N	5°33′ W	5
10	A Coruña	Spain	A	1	43°22′ N	8°25′ W	5
11	Ermenek	Turkey	P	2	36°37′ N	32°53′ E	1
12	Engizek Dağı	Turkey	P	2	37°49′ N	37°10′ E	2
13	Muş	Turkey	P	2	38°43′ N	41°30′ E	2
14	Nemrut Dağı	Turkey	P	2	38°30′ N	42°18′ E	3
15	Pinarbaşi	Turkey	A	2	38°50′ N	36°20′ E	1
16	Ovaçik	Turkey	P	2	39°43′ N	40°37′ E	1
17	Aşkale	Turkey	A	2	39°57′ N	40°39′ E	1
18	Arifiye	Turkey	A	2	40°43′ N	32°49′ E	1
19	Tirebolu	Turkey	A	2	40°47′ N	38°55′ E	3
20	Durusu Gölü	Turkey	A	2	41°17′ N	28°40′ E	1
21	Hopa	Turkey	A	2	41°25′ N	41°23′ E	1
22	Djarnali	Georgia	A	2	41°33′ N	41°36′ E	5
23	Ritsa	Georgia	A	2	43°35′ N	40°40′ E	1
24	Banoviči	Bosnia	A	3a	42°24′ N	18°16′ E	5
25	Pasarel Gorge	Bulgaria	A	3a	42°41′ N	23°22′ E	5
26	Konjic	Bosnia-H.	A	3a	43°48′ N	17°54′ E	5
27	Sarajevo	Bosnia-H.	A	3a	43°49′ N	18°17′ E	5
28	Donji Vakuf	Bosnia-H.	A	3a	44°08′ N	17°23′ E	5
29	Ključ	Bosnia-H.	A	3a	44°33′ N	16°47′ E	5
30	Jastrebarsko	Croatia	A	3a	44°58′ N	18°44′ E	5
31	Vrbanja	Croatia	A	3a	44°59′ N	18°45′ E	5
32	Tagliamento	Italy	A	3a	46°20′ N	12°40′ E	5
33	Codru	Moldavia	A	3a	47°07′ N	28°22′ E	5
34	Gödöllö	Hungary	A	3a	47°36′ N	19°35′ E	5
35	Villobas	Spain	A	3b	42°31′ N	0°16′ W	5
36	Villanúa	Spain	A	3b	42°40′ N	2°52′ E	5
37	Pierroton	France	A	3b	44°45′ N	0°38′ W	5
38	Mooshamer Moos	Austria	A	3b	47°06′ N	13°42′ E	5
39	Mettnau	Germany	A	3b	47°44′ N	9°00′ E	5
40	Paimpont	France	A	3b	48°00′ N	2°14′ W	5
41	Dürrwien	Austria	A	3b	48°10′ N	16°04′ E	2
42	Fontainebleau	France	A	3b	48°25′ N	2°40′ E	5
43	Donaumoos	Germany	A	3b	48°28′ N	10°15′ E	5
44	Padok	Russia	A	3b	49°46′ N	43°22′ E	1
45	Edertal	Germany	A	3b	51°10′ N	9°04′ E	5
46	Poleski	Poland	A	3b	51°20′ N	23°20′ E	5
47	Hühnerfeld	Germany	A	3b	51°22′ N	9°46′ E	5
48	Ivanteevka	Russia	A	3b	52°18′ N	49°23′ E	1
49	Bruinehaar	Netherlands	A	3b	52°28′ N	6°42′ E	5
50	Woodbastwick Fen	Britain	A	3b	52°42′ N	1°27′ E	5
51	Lengenermoor	Germany	A	3b	53°13′ N	7°57′ E	5
52	Schorfheide	Germany	A	3b	53°20′ N	14°00′ E	5
53	Knockaunroe	Ireland	A	3b	53°30′ N	9°16′ W	5
54	Lecarrow	Ireland	A	3b	53°32′ N	8°03′ W	5
55	Anklam	Germany	A	3b	53°50′ N	13°40′ E	4
56	Novosibirsk I	Russia	A	3b	55°02′ N	82°55′ E	5

Table 1 *Continued*

No.	Name	Country	Ssp.	Geogr. region	Latitude	Longitude	No. of samples
57	Novosibirsk II	Russia	A	3b	55°12′ N	82°50′ E	5
58	Rybachy	Russia	A	3b	55°13′ N	20°46′ E	5
59	Yesino	Russia	A	3b	55°42′ N	38°22′ E	5
60	Höör	Sweden	A	3b	55°56′ N	13°32′ E	5
61	Filimonovo	Russia	A	3b	55°58′ N	39°16′ E	5
62	Volokolansk	Russia	A	3b	56°01′ N	35°57′ E	2
63	Johanneshus	Sweden	A	3b	56°05′ N	13°38′ E	5
64	Oskarshamn	Sweden	A	3b	57°25′ N	16°40′ E	5
65	Hejdeby	Sweden	A	3b	57°40′ N	18°21′ E	5
66	Eksjö	Sweden	A	3b	57°43′ N	14°58′ E	5
67	Marstrand	Sweden	A	3b	57°54′ N	11°28′ E	4
68	Borok	Russia	A	3b	58°03′ N	38°12′ E	5
69	St Petersburg	Russia	A	3b	59°36′ N	31°13′ E	5
70	Bysetermosen	Norway	A	3b	59°48′ N	10°59′ E	5
71	Tullgarn	Sweden	A	3b	59°58′ N	18°33′ E	5
72	Fagersta	Sweden	A	3b	59°59′ N	15°54′ E	5
73	Bergen	Norway	A	3b	60°19′ N	5°20′ E	3
74	Umeå	Sweden	A	3b	63°47′ N	20°17′ E	4
75	Snåsa	Norway	A	3b	64°15′ N	12°23′ E	5
76	Kalix	Sweden	A	3b	65°55′ N	23°14′ E	5
77	Khetolambina	Russia	A	3b	66°24′ N	33°25′ E	4
78	Kandalaksha	Russia	A	3b	66°47′ N	33°11′ E	1

Codes for *F. alnus* subspecies: A = *alnus*, B = *baetica*, P = *pontica*.
Geographic region: 1 = Iberia south of the Pyrenees; 2 = Anatolia and Caucasus; 3a = temperate Europe, area of documented forest refugia; 3b = temperate Europe, putative area of postglacial recolonization.
[1]Probably also some plants of ssp. *alnus* present (A. Hampe, pers. obs.)

network was drawn using the software ARLEQUIN version 2.0 (Schneider *et al*. 2000). The program PARSPROB version 1.1 (by D. Posada, available at: http://bioag.byu.edu/zoology/crandall_lab/programs.htm) was used to calculate the exact probability of a parsimonious linkage between two haplotypes that differ at a certain number of sites. A preliminary calculation evaluates the probability that homoplasious sites or multiple hits will reject strict parsimony. This estimate is based on the number of individuals studied and the number of polymorphic nucleotides relative to the total number of nucleotides examined (Templeton *et al*. 1992). If overall parsimony is rejected (as will often be the case in intraspecific data sets) the probability (Pj) of a parsimonious linkage between two haplotypes that differ at j polymorphic sites and share m sites can be calculated according to Templeton *et al*. (1992). Contrary to the commonly used program TCS, written by the same authors, PARSPROB has been designed and is recommended for handling RFLP data (Clement *et al*. 2000).

Gene diversity analyses

Parameters of population diversity and differentiation were estimated according to Pons & Petit (1995, 1996). The program PERMUT (by R.J. Petit, available at http://www.pierroton.inra.fr/genetics/labo/Software/) was used to calculate the average within-population gene diversity (h_S), the total gene diversity (h_T) and the gene differentiation over all populations (G_{ST}), as well as the equivalent parameters (v_S, v_T and N_{ST}) obtained by taking into account the similarities between haplotypes. To test for the existence of a phylogeographical structure, we checked if $N_{ST} > G_{ST}$ by comparing the directly measured N_{ST} values with those obtained after 1000 random permutations of haplotype identities (Burban *et al*. 1999). Only populations that contained at least three individuals (see Table 1) were included in this analysis. Tests were conducted on the entire data set as well as on regional population ensembles. Furthermore, a hierarchical analysis of molecular variance (AMOVA) was carried out to assess genetic differentiation within and between geographical regions. Finally, the relationships between geographical and genetic distances of populations were examined for each region. Pairwise N_{ST} were calculated first according to Grivet & Petit (2002), and Mantel tests were then conducted on the matrices containing the pairwise geographical distances between populations and their corresponding N_{ST} values. (The N_{ST} was used instead of the G_{ST} to account for potential

Table 2 Description and frequencies of the 21 haplotypes identified in *Frangula alnus* by PCR–RFLP. Columns 2–8 refer to the polymorphic bands produced by the three markers, and numbers indicate the different states detected. The last two columns give the number of individuals and populations in which the respective haplotype was observed

Haplotype	*trn*S/*trn*G 1	*trn*S/*trn*G 2	*trn*S/*trn*G 3	DT 2	DT 3	DT 5	SR 1	SR 3	No. individuals	No. populations
1	1	2	2	2	3	1	1	3	5	1
2	4	2	1	2	2	1	3	3	5	1
3	4	2	1	1	2	1	3	3	4	1
4	4	2	1	1	2	2	3	3	1	1
5	4	2	1	1	2	1	5	3	10	2
6	4	1	1	1	2	1	5	3	2	1
7	5	1	1	1	2	1	5	3	8	2
8	5	1	3	1	2	1	5	3	3	1
9	5	1	3	1	1	1	5	3	10	3
10	3	1	3	1	4	1	5	3	1	1
11	6	1	3	1	4	1	5	3	1	1
12	6	1	3	1	4	1	6	3	2	1
13	6	2	3	1	2	1	6	3	16	9
14	2	2	3	1	2	1	4	3	3	1
15	5	1	3	1	2	1	5	1	1	1
16	5	1	3	1	2	1	5	2	236	55
17	5	2	3	1	2	1	5	2	4	1
18	5	1	4	1	2	1	7	2	1	1
19	5	1	3	1	2	1	2	2	1	1
20	5	1	3	1	2	1	4	2	5	2
21	3	1	3	1	2	1	4	2	3	1

phylogeographical structures between the examined populations.) AMOVAS and Mantel tests were carried out with the software ARLEQUIN (Schneider *et al*. 2000), while the pairwise N_{ST} values were calculated using the program DISTON (by R.J. Petit, available at http://www.pierroton.inra.fr/genetics/labo/Software/).

The nested clade analysis of geographical distances (NCA) has received much interest during recent years (Cruzan & Templeton 2000), as it appears to make a better use of the information available in phylogeographical surveys. However, its statistical and conceptual properties have recently been questioned (Petit & Grivet 2001; Knowles & Maddison 2002), and we have therefore preferred not to use it on the present data set.

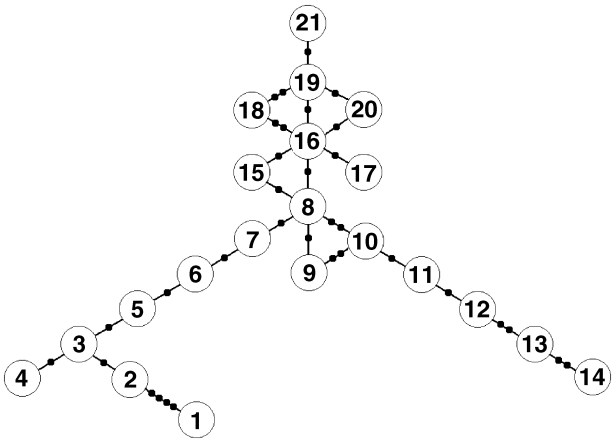

Fig. 2 Minimum-length spanning network of the 21 cpDNA haplotypes detected in *Frangula alnus*. Black circles indicate the number of mutations between haplotypes.

Results

Phylogenetic relationships and geographical distribution of chloroplast haplotypes

The three primer pairs distinguished a total of 21 haplotypes among the 322 individuals analysed (Table 2). Single DNA fragments exhibited up to seven different states, indicating a considerable accumulation of mutations within some DNA regions. The network of cpDNA haplotypes, as inferred by statistical parsimony, is shown in Fig. 2. Exact parsimonious probabilities for the linkages spanning one (17 cases), two (6) and four mutations (1) were 0.96, 0.87 and 0.68, respectively; in other words, 17 of 24 linkages showed a statistically significant (i.e. $P < 0.05$) level of confidence.

Three lineages could be distinguished that occupied different regions (Figs 2 and 3): the Iberian Peninsula south of the Pyrenees and northernmost Morocco (hereafter termed Iberia) (haplotypes 1–9), Turkey and the western Caucasus

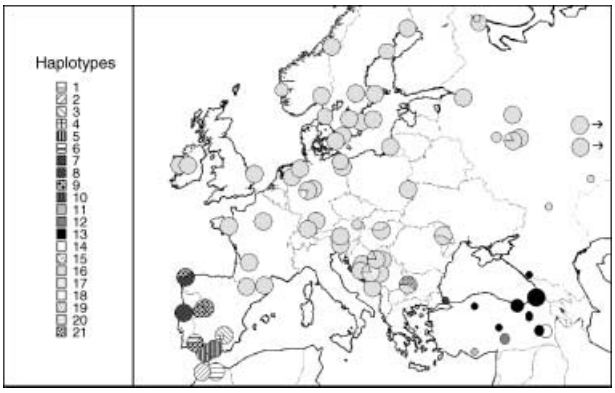

Fig. 3 Geographical distribution of *Frangula alnus* chloroplast haplotypes. Circle sizes are proportional to the number of analysed samples per population ($n = 1–5$).

(hereafter Anatolia) (haplotypes 10–14), and temperate Europe from the Pyrenees to West Siberia (haplotypes 15–21). Among the nine haplotypes found in Iberia, only haplotype 9 occurred over more than one mountain range. The Moroccan haplotype 1 differed strongly from the rest of the Iberian lineage. This haplotype was fixed in the population where it occurred, although another, larger population fixed for haplotype 2 was growing only some 6 km away. Generally, Iberian haplotypes were clearly structured and their divergence was correlated positively with the geographical distances between populations (Mantel test: $r = 0.34$, $P = 0.02$), after excluding the strongly divergent haplotype 1. This was also the case when Iberian and Anatolian populations were pooled ($r = 0.32$, $P = 0.003$). In Anatolia, one of the five haplotypes detected (haplotype 13) spanned over the Black Sea region, while the others were restricted to single populations in areas where the species has a disjunct distribution.

The third region, temperate Europe between the Pyrenees and West Siberia, showed a very different pattern. It was dominated by a single haplotype (16), which was present in 73% of the tested individuals and 70% of the populations, being almost the sole haplotype found north of the Alps. It occurred in admixture with other haplotypes from the Alps and the Pannonian Basin southeastwards; that is, from the range of documented forest refugia during the last glacial maximum. However, haplotype 16 remained the most widespread and abundant in this region as well (Fig. 3). The seven haplotypes detected across temperate Europe differed little and were assembled around the widespread haplotype 16 (Fig. 2). Three haplotypes (15, 18 and 20) were detected in a single individual. Contrary to the other two regions, no relation was evident between genetic and geographical distances ($P > 0.05$, both for whole temperate Europe and for its two subregions).

Regional differences in cpDNA diversity and differentiation

Diversity parameters (Table 3) were calculated on the basis of 63 populations (mean = 4.8 individuals per population) and 18 of the 21 detected haplotypes. The haplotype differentiation measured throughout the range of *F. alnus* was very high ($G_{ST} = 0.81$) and even higher when taking the relationships between haplotypes into account ($N_{ST} = 0.91$). The permutation test indicated that the observed N_{ST} was significantly higher than the G_{ST} ($P < 0.01$), indicating the existence of a phylogeographical structure at this scale (Pons & Petit 1996). In contrast, no phylogeographical structure was detected within any of the regional population subsets (i.e. $N_{ST} \cong G_{ST}$ for Iberia, Anatolia, whole temperate Europe, southeastern or remaining temperate Europe).

Results of the analysis of molecular variance (AMOVA) are presented in Table 4. When comparing Anatolia, Iberia and temperate Europe, most of the total variance (71%) was explained by differences among regions, while within-population variation accounted for only 5% of the total variance. In contrast, the comparison of the two subregions within temperate Europe almost reversed this pattern: 62% were explained by differences within populations and only

Table 3 Gene diversity parameters estimated according to Pons & Petit (1995) with standard error in brackets

	h-type parameters			*v*-type parameters			
Region	h_S	h_T	G_{ST}	v_S	v_T	N_{ST}	n
Anatolia	0.0 (0.0)	0.67 (0.22)	1.0 (nc)	0.0 (0.0)	0.67 (0.22)	1.0 (nc)	3
Iberia	0.16 (0.08)	0.93 (0.04)	0.83 (0.09)	0.20 (0.13)	0.98 (0.12)	0.79 (0.11)	10
Temperate Europe	0.07 (0.02)	0.12 (0.05)^{a}	0.42 (0.09)	0.04 (0.02)	0.07 (0.09)^{b}	0.39 (0.09)	50
Southeast	0.24 (0.08)	0.42 (0.13)	0.44 (0.07)	0.18 (0.07)	0.34 (0.12)	0.47 (0.03)	11
Recolonized part	0.02 (0.01)	0.02 (0.01)^{A}	0.0 (nc)	0.02 (0.01)	0.01 (0.01)^{B}	0.0 (nc)	39
Total	0.08 (0.02)	0.43 (0.08)	0.81 (0.05)^{A}	0.03 (0.01)	0.35 (0.08)	0.91 (0.31)^{B}	63

Different letters show significant differences between *h*-type and *v*-type parameters, where only *v*-type parameters take distance between haplotypes into account (lower case: $P < 0.05$, upper case: $P < 0.01$, nc: not calculated).

Table 4 Analysis of molecular variance (AMOVA) of *Frangula alnus* genetic variation for the whole range and, separately, each on the three regions occupied by different *F. alnus* haplotype lineages: Anatolia, Iberia and temperate Europe

Region analysed and source of variation	d.f.	SS	Variance components	% of total variance	*P*-value
Whole range (Anatolia, Iberia and temperate Europe) (Φ_{ST} = 0.95)					
Among regions	3	270.5	1.48	71.3	< 0.001
Among populations within regions	70	154.5	0.49	23.8	< 0.001
Within populations	242	24.8	0.10	4.9	< 0.001
Total	315	449.8	2.08		
Anatolia (Φ_{ST} = 1.00)					
Among populations	12	25.2	1.36	100	< 0.001
Within populations	8	0	0	0	< 0.001
Total					
Iberia (Φ_{ST} = 0.93)					
Among populations	9	133.53	3.05	92.9	< 0.001
Within populations	38	8.80	0.23	7.1	< 0.001
Total	47	142.33	3.28		
Temperate Europe (Φ_{ST} = 0.31)					
Among populations	54	13.25	0.04	30.6	< 0.001
Within populations	196	16.00	0.08	69.4	< 0.001
Total	250	29.25	1.20		

Table 5 Analysis of molecular variance (AMOVA) of *Frangula alnus* genetic variation for two subregions of temperate Europe, the Southeast (area of documented forest refugia) and the putative area of postglacial recolonization

Region analysed and source of variation	d.f.	SS	Variance components	% of total variance	*P*-value
Southeast and recolonized part (Φ_{ST} = 0.38)					
Among regions	1	2.16	0.022	16.7	< 0.001
Among populations within regions	53	11.09	0.028	21.3	< 0.001
Within populations	196	16.00	0.082	62.0	< 0.001
Total	250	29.25	0.131		
Southeast (Φ_{ST} = 0.36)					
Among populations	11	9.92	0.14	35.6	< 0.001
Within populations	45	11.20	0.25	64.4	< 0.001
Total	56	21.12	0.39		
Recolonized part (Φ_{ST} = −0.03)					
Among populations	42	1.17	−0.00	−2.8	NS
Within populations	151	4.80	0.03	102.8	NS
Total	193	5.97	0.03		

17% by differences between regions (Table 5). All observed results were highly significant ($P << 0.001$).

Discussion

Geographic distribution and phylogenetic relationships of chloroplast haplotypes

The current distribution range of *F. alnus* encompasses three of the four main glacial refuge regions of West Palaearctic plants: Iberia, Anatolia and the Balkans. The analysis of *F. alnus* cpDNA haplotypes and their phylogenetic relationships revealed that each region is occupied by a different lineage. These differ considerably. The Iberian and the Anatolian lineages consist of locally distributed haplotypes with clear geographical affinities. In contrast, the temperate European lineage is organized around one very abundant and widespread haplotype, and no relation exists between the geographical situation and the similarity of haplotypes. Only the Balkans harbour haplotypes of the temperate

European lineage, suggesting that the Holocene expansion of *F. alnus* across Europe originated exclusively from this refugium. The structural differences between lineages suggest that *F. alnus* populations in different regions have experienced markedly distinct evolutionary processes.

The populations distributed over the Iberian Peninsula and Anatolia were highly differentiated and fixed mainly for a single haplotype. Surprisingly, the haplotypes found in northern Spain and northwest Anatolia (respectively, haplotypes 8 and 9 and haplotype 10) are more similar to each other than to some of the other haplotypes within their respective regions; in other words, they bridge Iberia and Anatolia within a single large lineage distributed over the entire Mediterranean Basin and some neighbouring areas (for instance, West and North Iberia and the Black Sea region). The ample overall genetic divergence — 17 mutations between the Moroccan haplotype 1 and the East Anatolian haplotype 14 — the wide geographical distance and the geological history of the areas involved suggest that the formation of this 'Ibero–Anatolian' lineage could date back to the Tertiary, possibly even prior to the onset of the current Mediterranean climate in the Pliocene (see Palamarev 1989; Denk *et al*. 2001; for numerous similar cases). Three further inferences were supported by the phylogenetic structure of this lineage: first, the steady sequence of haplotypes separated by only one or two mutation steps distributed over southern Europe indicates that the current pattern may have been produced during a slow but continuous expansion of the species range. Second, few mutations have apparently become fixed after the initial population establishment (contrary to some expectations; see, e.g. Knowles 2001; Tzedakis *et al*. 2002), as all but one haplotype (4) coincided with the continuous linear haplotype sequence. Finally, populations seem to have experienced little dispersal or gene flow since their formation, as deduced by the correlation between genetic and geographical distances in Iberia and Anatolia. In other words, populations of this ancient Ibero–Anatolian lineage would have participated little in the continental-scale migration dynamics of the species throughout Quaternary climate cycles, as might be expected for refugia located relatively far south and away from the 'leading edge'.

Temperate Europe is dominated largely by haplotype 16. The two derived haplotypes detected in this area (15 and 18) were found in single plants and could be of local postglacial origin. The widespread haplotype 16 prevailed also in Southeast Europe, where it occurred mixed with four other haplotypes (17, 19, 20 and 21). Because most of the cpDNA diversity in this lineage was restricted to this region, it may be assumed that the glacial refugium for this lineage was located somewhere in this area. Interestingly, the northern range limit of these mixed populations in Hungary and Moldavia coincides with the location of the northernmost glacial refugia of forest vegetation documented so far (Willis *et al*. 2000). The observed distribution range of haplotype 16 indicates that *F. alnus* from Southeast Europe must have spread quite rapidly and in several directions to populate the new territories. This process would explain the strong differences in dispersal ability found between present-day *F. alnus* populations from South Iberia and from Central Europe (Hampe & Bairlein 2000a; see also Dynesius & Jansson 2000).

The strong dominance of haplotype 16 and its central phylogenetic position within the network of temperate European haplotypes suggests that it is the ancestor of this lineage. Moreover, the phylogenetic network situated haplotype 16 close to the node between the Iberian and the Anatolian lineage, which coincides with its geographical situation between these two regions. It is noteworthy that this haplotype is the only one that has clearly been involved in Quaternary migration dynamics and, at the same time, the only that has generated several other permanent haplotypes.

Genetic diversity and differentiation of populations

High genetic differentiation was observed within Iberia and Anatolia, but not in temperate Europe. Moreover, populations from Anatolia and particularly the Iberian Peninsula contained high haplotype richness: nine haplotypes were restricted to the Iberian Peninsula and North Morocco and seven of them were restricted to the endemic subspecies *F. a. baetica*. The smaller sample of Anatolian and Georgian populations revealed five haplotypes, two of which are restricted to the endemic subspecies *F. a. pontica*, while the widespread haplotype 13 occurred in both subspecies. The coefficients of differentiation between populations in Iberia (G_{ST} = 0.83) and Anatolia (G_{ST} = 1.0) are among the highest found so far for a fleshy-fruited temperate plant species (see below). With only two exceptions, the haplotypes of these regions were restricted to single mountain ranges. The first exception was haplotype 9, which was found in Northwest Spain as well as in the southern Spanish Doñana population. This population contains treelike *F. alnus* plants characteristic of the native subspecies *baetica* as well as some plants of small, multistemmed shrub habit typical of the northern subspecies *alnus* (A. Hampe, pers. obs.). These shrublike plants grow in a restricted area around a man-made lake, suggesting that they could have been introduced during historical times from northern Spain. The second exception involves haplotype 13, found along the Black Sea Coast of Turkey and Georgia. This region lies at the margin of the summer-dry Mediterranean climate and is known for its richness in Quaternary and Tertiary relict plant species (Denk *et al*. 2001). Here, *F. alnus* populations are not restricted to mountain habitats and therefore not as isolated as those in the dry mountain ranges of southern Anatolia.

Temperate European *F. alnus* populations had most of their cpDNA diversity restricted to the putative southeastern refugium. The comparatively high level of haplotype mixing in this region indicates that — in contrast to Iberia and most of Anatolia — gene flow between populations has been significant, although it has probably involved mostly the abundant haplotype 16. None of the 55 temperate European populations was fixed for any haplotype other than this haplotype, and only one of the six other haplotypes of this lineage (19) was found at more than one site.

At first sight, *F. alnus* appears to follow the 'southern richness vs. northern purity' paradigm (Hewitt 2001), because haplotype richness is highest in the stable populations of Iberia and Anatolia, decreases towards Southeast Europe and is extremely low in the northern areas of postglacial recolonization. However, the within-population diversity of *F. alnus* does not follow this south-north gradient: populations from Iberia and Anatolia, in fact, show values almost as low as those from the recolonized area of temperate Europe, and only the Southeast European refugium harbours a relatively high fraction of mixed populations. The validity of the 'southern richness vs. northern purity' paradigm for within-population haplotype diversity has been questioned recently by Petit *et al*. (2003), who found that many European woody species exhibit peak values of intrapopulation diversity not in southern but in central Europe, where populations from different refugia have merged. The present study illustrates that temperate refugia may similarly harbour markedly higher within-population diversities than refugia within areas of Mediterranean climate; this is due probably to higher rates of gene flow between populations during interglacial periods.

The role of bird-mediated seed dispersal during and after the postglacial expansion

Most phylogeographical studies conducted so far on fleshy-fruited tree species have reported low degrees of population differentiation at chloroplast markers (*Sorbus aucuparia*: G_{ST} = 0.26–0.29, Raspé *et al*. 2000; *Prunus avium*: 0.29, *P. spinosa*: 0.33, Mohanty *et al*. 2001a,b; *S. torminalis*: 0.34, Oddou-Muratorio *et al*. 2001) and weak phylogeographical structures. Instead, in *F. alnus*, the species' overall degree of differentiation (G_{ST} = 0.81) was much higher and a clear phylogeographical structure was detected. These patterns were due mainly to the strong divergence of Iberian and Anatolian populations, while temperate European populations (G_{ST} = 0.42) fitted better with the patterns known in other fleshy-fruited species. The previous studies had, however, scarcely sampled populations from the southernmost parts of the range. An exception is a recent study on bird-dispersed *Hedera* spp. (Grivet & Petit 2002), which included a number of southern populations and reported a relatively high G_{ST} (0.64) and a clear phylogeographical structure. This and the present case indicate that the relation between bird-mediated seed dispersal and low levels of cpDNA differentiation may not be as straightforward as has sometimes been assumed. Moreover, a representative sampling across the entire species range should be an important goal of future large-scale phylogeographical studies in order to detect existing regional variation in population differentiation.

Generally, results suggest that in several temperate plants with bird-ingested fruits, seed-mediated gene flow between populations may have been significant across the more or less continuous species range but negligible among the disjunct populations growing in Mediterranean climate areas (as well as across the unforested plains north of the Caucasus). In northern Europe many bird-dispersed fruits ripen in late summer and early autumn and much seed dispersal is carried out by migrating birds, whereas in southern Europe many seed dispersers are resident and territorial (Hampe & Bairlein 2000b; Hampe 2001).

Once *F. alnus* populations and bird migration routes had become established in the Holocene, more or less regular long-distance dispersal events by migrating birds may have promoted a step-by-step secondary expansion of northern haplotypes towards the south. This process would be reinforced by the fact that temperate European *F. alnus* populations usually grow in mid-successional plant communities (Godwin 1943) and used to experience frequent extinctions and colonizations (i.e. metapopulation dynamics). A secondary southward migration of the already common haplotype 16 across the area of recolonization might have reduced further the abundance of other haplotypes throughout the newly colonized range. This southward 'secondary haplotype migration' after the range establishment has to our knowledge never been hypothesized; its existence should be tested by comparing the phylogeographical patterns of fleshy-fruited species with different dispersers and/or fruit ripening seasons.

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References

Birky CW (2001) The inheritance of genes in mitochondria and chloroplast: laws, mechanisms and models. *Annual Review of Genetics*, **35**, 125–148.

Burban C, Petit RJ, Carcreff E, Jactel H (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. *Molecular Ecology*, **8**, 1593–1602.

Catling PM, Porebski ZS (1994) The history of invasion and current status of glossy buckthorn (*Rhamnus frangula*) in Southern Ontario. *Canadian Field Naturalist*, **108**, 305–310.

Clement MD, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Cruzan MB, Templeton AR (2000) Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. *Trends in Ecology and Evolution*, **15**, 491–496.

Cwynar LC, MacDonald GM (1987) Geographic variation of lodgepole pine in relation to its population history. *American Naturalist*, **129**, 463–469.

Denk T, Frotzler N, Davitashvili N (2001) Vegetational patterns and distribution of relict taxa in humid temperate forests of Georgia (Transcaucasia). *Biological Journal of the Linnean Society*, **72**, 287–322.

Dynesius M, Jansson R (2000) Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Acadamy of Sciences USA*, **97**, 9115–9120.

Godwin H (1943) Biological flora of the British Isles: Rhamnaceae. *Journal of Ecology*, **31**, 66–92.

Gömöry D, Paule L, Brus R *et al.* (1999) Genetic differentiation and phylogeny of beech on the Balkan peninsula. *Journal of Evolutionary Biology*, **12**, 746–754.

Grivet D, Heinze B, Vendramin GG, Petit RJ (2001) Genome walking with consensus primers: application to the large single copy region of chloroplast DNA. *Molecular Ecology Notes*, **1**, 345–349.

Grivet D, Petit RJ (2002) Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology*, **11**, 1351–1362.

Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*, **8**, 521–523.

Hampe A (2001) The role of fruit diet within a temperate breeding bird community in Southern Spain. *Bird Study*, **48**, 116–123.

Hampe A, Arroyo J (2002) Recruitment and regeneration in populations of an endangered South Iberian Tertiary relict tree. *Biological Conservation*, **107**, 263–271.

Hampe A, Bairlein F (2000a) Modified dispersal-related traits in disjunct populations of *Frangula alnus* (Rhamnaceae): a result of its Quaternary distribution shifts? *Ecography*, **23**, 603–613.

Hampe A, Bairlein F. (2000b) Nahrungssuche und Vergesellschaftung frugivorer Zug- und Brutvögel. *Journal für Ornithologie*, **141**, 300–308.

Hewitt GM (1996) Some genetic consequences of the ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt GM (2000) The genetic legacy of the ice ages. *Nature*, **405**, 907–913.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, **10**, 537–549.

Huntley B, Birks HJB (1983) *An Atlas of Past and Present Pollen Maps for Europe: 0–13 000 Years Ago*. Cambridge University Press, Cambridge, UK.

Knowles LL (2001) Did the Pleistocene glaciations promote species divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology*, **10**, 691–701.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology*, **11**, 2623–2635.

Medán D (1994) Reproductive biology of *Frangula alnus* (Rhamnaceae) in southern Spain. *Plant Systematics and Evolution*, **193**, 173–186.

Meusel H, Jäger E, Rauschert E, Weinert E (1978) *Vergleichende Chorologie der Zentraleuropäischen Flora*, vol. II. Fischer, Jena.

Moe D (1984) The late Quaternary history of *Rhamnus frangula*. Norway. *Nordic Journal of Botany*, **4**, 655–660.

Mohanty A, Martín JP, Aguinagalde I (2001a) A population genetic analysis of chloroplast DNA in wild populations of *Prunus avium* L. in Europe. *Heredity*, **87**, 421–427.

Mohanty A, Martín JP, Aguinagalde I (2001b) Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany*, **89**, 1223–1228.

Muñoz JM (1987) *Frangula*. In: *Flora Vascular de Andalucía Occidental* (eds Valdés B, Talavera S, Fernández Galiano E), vol. II, p. 245. Ketres, Barcelona.

Oddou-Muratorio S, Petit RJ, Demesure B (2001) Pollen- versus seed-mediated gene flow in a scattered forest tree species. *Evolution*, **55**, 1123–1135.

Palamarev E (1989) Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. *Plant Systematics and Evolution*, **162**, 93–107.

Petit RJ, Aguinagalde I, de Beaulieu J-L *et al.* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **3000**, 1563–1565.

Petit RJ, Bialozyt R, Brewer S, Cheddadi R, Comps B (2001) From spatial patterns of genetic diversity to postglacial migration processes in forest trees. In: *Integrating Ecology and Evolution in a Spatial Context* (eds Silvertown J, Antonovics J), pp. 295–318. Blackwell Science, Oxford.

Petit RJ, Grivet D (2001) Optimal randomization strategies when testing the existence of a phylogeographic structure. *Genetics*, **161**, 469–471.

Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. *Theoretical and Applied Genetics*, **90**, 462–470.

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered *versus* unordered allels. *Genetics*, **144**, 1237–1245.

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37–45.

Raspé O, Saumitou-Laprade P, Cuguen J, Jacquemart AL (2000) Chloroplast DNA haplotype variation and population differentiation in *Sorbus aucuparia* L. (Rosaceae: Maloideae). *Molecular Ecology*, **9**, 1113–1122.

Rendell S, Ennos RA (2002) Chloroplast diversity in *Calluna vulgaris* (heather) populations in Europe. *Molecular Ecology*, **11**, 69–78.

Schneider SD, Roesli D, Excoffier L (2000) *ARLEQUIN, version 2.0: a Software for Population Genetic Data Analysis*. *Genetics and Biometry Laboratory*. University of Genava, Switzerland.

Steward JR, Lister AM (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology and Evolution*, **16**, 608–613.

Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Templeton AR, Crandall AK, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence mapping. III. cladogram estimation. *Genetics*, **132**, 619–633.

Tzedakis PC, Lawson IT, Frogley MR, Hewitt GM, Preece RC (2002) Buffered tree population changes in a Quaternary refugium: evolutionary implications. *Science*, **297**, 2044–2047.

Villani F, Sansotta A, Cherubini M, Cesaroni D, Sbordoni V (1999) Genetic structure of natural populations of *Castanea sativa*. Turkey: evidence of a hybrid zone. *Journal of Evolutionary Biology*, **12**, 233–244.

Willis KJ, Rudner E, Sümegi P (2000) The full-glacial forests of central and southeastern Europe. *Quaternary Research*, **53**, 203–213.

Yaltirik F (1967) *Frangula* Miller. In: *Flora of Turkey and the Aegean Islands* (ed. Davis PH), Vol. II, pp. 525–526. University Press, Edinburgh, UK.

This study forms part of Arndt Hampe's PhD thesis on the reproductive ecology of *Frangula alnus* in glacial relict populations and postglacial 'recolonizer' populations, and on the large-scale geographical variation of the species' reproductive traits. Juan Arroyo is interested in the ecology of relictual plant populations and in phylogeography as a tool for the study of evolutionary processes and their geographical patterns. Pedro Jordano is studying the evolutionary ecology of plant–animal interactions and their demographic and genetic consequences at multiple spatial scales. Rémy Petit is studying phylogeography, postglacial migration processes and palaeogenetics of forest trees and shrubs. He is also working on the development of methods to be used for their conservation and the management of their genetic resources.
