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Range-wide phylogeography of *Juniperus thurifera* L., a presumptive keystone species of western Mediterranean vegetation during cold stages of the Pleistocene

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ABSTRACT

We investigate the range-wide population structure and phylogeography of thuriferous juniper (*Juniperus thurifera* L.), a species with a highly disjunct distribution in the western Mediterranean. We genotyped a total of 327 individuals from 20 populations using amplified fragment length polymorphisms (AFLP). Different analyses such as principal co-ordinate analysis (PCoA), nonmetric multidimensional scaling of F_{ST} distances among populations, unweighted pair group method with arithmetic mean (UPGMA), and Bayesian clustering revealed that the Strait of Gibraltar acted as an efficient barrier against gene flow between the Moroccan and European populations for a very long time, and consequently support that the Moroccan populations should be recognised as a distinct subspecies (*J. thurifera* L. subsp. *africana* (Maire) Romo and Boratyński). The Algerian population was genetically more closely related to the European than to the Moroccan ones, probably due to dispersal events from Europe to Algeria. With respect to the mainland European populations, our data are not conclusive to reject any of the two following hypotheses: (1) the Iberian Peninsula was subdivided into different gene pools, and was the source for the colonisation of the Pyrenees and the Alps; and (2) the pattern we see today is partly the result of immigration into the Iberian Peninsula, e.g. from the Alps. Finally, the Corsican population was closely related genetically to two northern Iberian populations most probably due to relatively recent long-distance dispersal.

1. Introduction

The Mediterranean basin, one of the global hotspots of biodiversity (Myers et al., 2000) constitutes a major Pleistocene refugium, a crossroads of plant migrations and a centre of active speciation (Quézel, 1978, 1985). Three events have had an especially large effect on the current distribution of western Mediterranean biota. (a) The cycles of desiccation and transgression of the Mediterranean Sea in the Messinian period (*c.* 5.59–5.33 million years ago) opened numerous transcontinental pathways between the marine basins, thereby enabling biotic interchange (Bocquet et al., 1978). Various studies applying a wide array of molecular markers (including nrITS, mtDNA, cpDNA-RFLPs and allozymes) unravelled genealogical relationships in amphibians, reptiles and beetles occurring on both sides of the Strait of Gibraltar (Sanmartín, 2003; Martínez-Solano et al., 2004; Carranza et al., 2006). All these groups predate the Messinian salinity crisis and underline the importance of a land bridge between Iberia and North Africa for faunal exchange (Palmer and Cambefort, 2000; Sanmartín, 2003; Veith et al., 2004). (b) The final separation of the Iberian Peninsula and Africa by the opening of the Strait of Gibraltar (around 5.33 mya; Krijgsman et al., 1999) has likely created an impermeable barrier for many species, resulting in vicariance speciation as previously reported (Castella et al., 2000; Lumaret et al., 2002; Broderick et al., 2003; Gantenbein and Largiadèr, 2003; Fromhage et al., 2004; Gantenbein, 2004; Hampe et al., 2003; Terrab et al., 2007). (c) Finally, the Quaternary climatic oscillations have likely modelled the genetic structure and spatial distribution of biota, eventually leading to speciation (Hewitt, 1999). In the slowly evolving tree species, the current genetic structure was suggested to reflect population divergence that predates the onset of the Mediterranean climate in the Pliocene (Petit et al., 2005).

Extensive knowledge of the range dynamics of typical Mediterranean tree species such as *Quercus ilex* (Lumaret et al., 2002) and *Olea europaea* (Rubio de Casas et al., 2006) contrasts with the virtual lack of range-wide phylogeographical data for species that dominated the area during cold and dry periods of the Pleistocene,

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when the Mediterranean vegetation receded into refugia. *Juniperus thurifera* L., the thuriferous juniper, is considered a keystone species of low-temperature adapted, open woodlands with steppe-like undercover, which showed a maximum expansion during the Late Glacial between 14,000 and 12,000 BP (Quézel, 1999; Jalut et al., 1992). Subsequently, Holocene climatic amelioration, along with anthropogenic activities have likely caused range fragmentation, thereby shaping the present distribution of *J. thurifera* (Quézel and Médail, 2003).

Juniperus thurifera is a dioecious bush or tree, and presently exhibits a disjunct distributional pattern, occurring from Algeria and Morocco over the Iberian Peninsula and the Pyrenees to the French and Italian Alps and to Corsica. In France, thuriferous juniper extends over approximately 1000 ha and is found in three areas: the Corsican highlands (Gamisans et al., 1994), the Alps where the stands are more extensive (Lathuillière, 1994), and the Pyrenees where only two locations have so far been identified (Dupias, 1960; Guerby, 1993). In the Italian Maritime Alps, thuriferous juniper has only recently been discovered (Barbero et al., 1988). The Iberian Peninsula harbors numerous stands, forming an arc from the Cantabrian Cordillera in NW Spain to the Betic Range in the southeast, with most populations being found in the Iberian Cordillera. In total, *J. thurifera* stands cover more than 125,000 ha, representing 1% of the whole Spanish forest area. In Morocco, thuriferous juniper stands are still extensive in the Middle and High Atlas mountains (around 30,000 ha). In contrast to the situation in Europe and Algeria, where *J. thurifera* occurs from 200 to 1800 m above sea level, the Moroccan stands are associated with subhumid cold winter bioclimates (Achhal et al., 1980) at the tree line, mainly between elevations of 1800 and 3150 m (Romo and Boratyński, 2005). In Algeria, thuriferous juniper is limited to the Aurès mountains with a number of scattered and often very large trees that are probably the remains of formerly more extensive stands (Lapie and Maige, 1914).

Stands of *J. thurifera* are seriously endangered (Conifer Specialist Group, 1998), especially in Morocco and Algeria as a result of intensive wood removal and grazing in these densely populated areas (Fromard and Gauquelin, 1993), and in Europe as a result of grazing and colonization of stands by pines or oaks (Gauquelin et al., 1999) and by forest fires (P. Schönswetter, pers. obs.).

Palaeoecological information for the genus *Juniperus* is limited because of the poor macrofossil record and the difficulty of distinguishing pollen of Cupressaceae species. However, several studies conducted in the Iberian Peninsula, the Pyrenees and the Alps have suggested that the distribution of *Juniperus* expanded and retreated repeatedly during the last glaciations. Periods of expansion are associated with continental, dry climate, whereas declines were presumably caused by competition with *Pinus* and angiosperm tree species during interglacials (Heinz and Barbaza, 1998; Carrión et al., 2001a,b, 2004; Pini, 2002).

Juniperus thurifera is a morphologically variable species, perhaps as a result of long-term isolation of disjunct populations. Maire (1926) was the first to distinguish the North African and European populations of *J. thurifera*. Later, based on morphometric characters such as the size of the cones and the number of seeds per cone, as well as differences in the prodelphinidin content in the foliage, Gauquelin et al. (1988; followed by Romo and Boratyńsky, 2007) recognized both entities as subspecies; *J. thurifera* subsp. *africana* (Maire) Romo and Boratyńsky in North Africa, and *J. thurifera* subsp. *thurifera* in the European range of the species. Studies based on essential oils and morphometric data (Barbero et al., 1994; Adams et al., 2003), supported the clear differentiation of *J. thurifera* subsp. *africana* from Morocco against the European populations, but without studying the Algerian ones. However, it has to be stressed that not all authors agree upon the distinctiveness of subsp. *africana* (Farjon, 2001, 2005).

Being a threatened species with a remarkably disjunct distribution pattern, *J. thurifera* has been the object of a few previous phylogeographic studies. Jiménez et al. (2003), were the first to report on genetic diversity and differentiation in Moroccan and Spanish *J. thurifera* based on RAPD markers and the (paternally transmitted) chloroplast *trn*L–*trn*F intergenic spacer. The former suggested the existence of three population groups, the first comprising two populations from northeastern Spain and two from southern Spain, the second group comprising the rest of the Spanish populations, and the third group consisting of the Moroccan populations. The chloroplast marker, however, was uninformative of any geographic pattern. Adams et al. (2003), also applying RAPD markers to Moroccan and some European populations, revealed that the Moroccan *J. thurifera* populations were most similar to those from southern Spain, while those from France (Pyrenees, Alps, and Corsica) were more distantly related. However, to date no study has attempted to infer the phylogeography of *J. thurifera* taking into account its entire distributional area.

Here, we used amplified fragment length polymorphism (AFLP) to assess the genetic structure of *J. thurifera* populations throughout the distributional range of the species. Specifically, our aims were to (1) test the hypothesis implied by previous taxonomic treatments that the main genetic split in *J. thurifera* follows the Strait of Gibraltar; and (2) to describe the phylogeographical relationships and patterns of historical range dynamics among the disjunct occurrences in the Alps, Pyrenees, Corsica, and Algeria, and the main distributional area on the Iberian Peninsula as well as in Morocco. As outlined above, *J. thurifera* was likely widespread during cold stages of the Pleistocene implying that exchange of gene flow between the currently isolated portions of the species' distribution area on the European mainland was possible until relatively recently. Consequently, we expect only weak phylogeographical structure in continental Europe in comparison with more warmth-demanding species which were restricted to isolated refugia during cold stages.

2. Materials and methods

2.1. Plant material

We obtained plant material from 20 *Juniperus thurifera* populations covering the entire distribution (Fig. 1 and Table 1). One population was sampled in Algeria (population 6, Aurès mountains), five in Morocco (1–5, Middle and High Atlas), nine in Spain (7–15, Betic, Central, Iberian and Cantabrian Cordilleras), one in the Pyrenees (16), three in the Alps (17–19), and one in Corsica (20). Based on preliminary analyses that included *J. foetidissima*, *J. phoenicea*, and *J. sabina* (Terrab et al. unpubl.), one population of *J. foetidissima* was used as outgroup. Table 1 provides details of the location of populations and number of sampled individuals. Young branches were collected from a total of 327 individuals, (8–20 in each population; mean = 16.4) and dried in silica gel. Vouchers are deposited in SEV and WU.

2.2. DNA extraction and molecular analyses

Genomic DNA was extracted following the CTAB protocol (Doyle and Doyle, 1987), with the following modifications: After precipitation with isopropanol and subsequent centrifugation, the DNA pellet was washed with 70% ethanol, dried in a vacuum centrifuge, and resuspended in TE buffer. DNA extracts were treated with RNAse (Fermentas, St. Leon-Rot, Germany) at 37 °C for 30 min. The quality of the extracted DNA was checked on 1% TAE agarose gels. The AFLP procedure followed established protocols (Vos et al., 1995) with modifications (Tremetsberger et al., 2004).

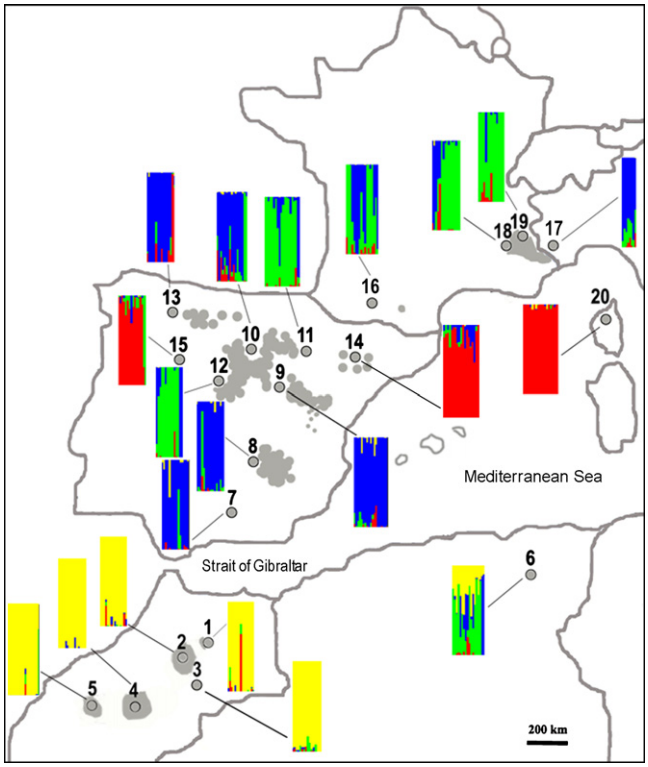

Fig. 1. Geographic distribution, sampled populations and genetic population structure of the 20 *Juniperus thurifera* populations analyzed. Grey areas indicate the present range of the species. Circles and numbers indicate the locations of the investigated populations (for details, see Table 1). The graphs next to each population indicate the proportional assignment of individuals to the genetic Clusters I (yellow), II (blue), III (green), and IV (red) as detected in an admixture analysis of AFLP data conducted with the program Bayesian Analysis of Population Structure (BAPS) .

No explicit data on the genome size of *J. thurifera* are available, but IC values of other *Juniperus* species were in the range of 10.82 to 12.15 pg (Hizume et al., 2001). Following the recommendations of Fay et al. (2005) who suggested to use the standard AFLP protocol with three selective bases for species with genome size $\leqslant$ 12.0 pg, but to increase the number of selective bases above this range (but not exceeding approximately 15 pg), we performed an initial screening of selective primers using 102 primer combinations with three and four selective nucleotides on eight individuals from eight different populations. The final three primer combinations for the selective PCR were (fluorescent dye in brackets): EcoRI (6-FAM)-AGG/MseI-CTGA, EcoRI (VIC)-ACG/MseI-CTCG and EcoRI (NED)-ACG/MseI-CTAG. As MseI primers with four selective nucleotides were chosen for the selective amplification, we used primers with two selective nucleotides in the preselective amplification following the recommendations of Vos et al. (1995). Thirty individuals were replicated in order to exclude non-reproducible bands and to calculate the error rate according to Bonin et al. (2004). The fluorescence-labeled selective amplification products were purified with Sephadex G-50 Superfine (GE Healthcare Bio-Sciences, Uppsala) as described in Dixon et al. (in press), combined with formamide and an internal size standard (GeneScan-500 ROX, Applied Biosystems, Foster City, CA, USA) and separated on a capillary sequencer (Applied Biosystems 3130*xl* Genetic Analyzer). Raw data were collected and aligned with the internal size standard using GENESCAN 2.1 (Applied Biosystems). Subsequently, the GENESCAN files were imported into GENOGRAPHER (Version 1.6.0, Montana State University 2001; available at http://hordeum.oscs.montana.edu/genographer) for scoring of the fragments. Each AFLP fragment was scored using the 'thumbnail' option, which allows comparison of the signal of each fragment (present or absent) over all samples. Criteria for choosing AFLP bands were (1) visual clarity, (2) straightforward interpretability, (3) similar fluorescence intensity, and, most importantly, (4) reproducibility between independent replicates. The results of the scoring were exported as a presence/absence matrix.

2.3. Data analysis

For each population, the total number of AFLP fragments present ($Frag_{tot}$), the percentage of polymorphic fragments ($Frag_{poly}$), and the average gene diversity (H_D) calculated with ARLEQUIN 3.01 (Excoffier et al., 2005) were estimated. Additionally, to eliminate the influence of uneven sample sizes per population, H_D was calculated for a standardised population size of 15 randomly selected individuals (population 16 was excluded). Analyses of molecular variance (AMOVAs) were calculated with ARLEQUIN 3.01 (Excoffier et al., 2005).

We used two complementary ordinations to investigate genetic distances and relationships between individuals and populations, (i) principal co-ordinate analysis (PCoA) of a matrix of Jaccard similarities among individuals, and (ii) nonmetric multidimensional scaling (NMDS) of pairwise F_{ST}-distances among populations. The PCoA was calculated and plotted with the program package NTSYS-pc 2.0 (programs DCENTER, EIGEN; Rohlf, 1997). We estimated the goodness of fit of the analysis by generating a model distance matrix from the eigenvector matrix (program SIMINT) and comparing it to the original F_{ST} matrix (using MXCOMP and 1000 permutations). Nonmetric multidimensional scaling (NMDS) provides a better fit to the data than many other commonly used clustering techniques because it can uncover, but does not assume, hierarchical relationships among populations (Lessa, 1990; Edwards and Sharitz, 2000). The NMDS runs were performed 100 times from an initial random configuration of populations in two-dimensional space to minimize the possibility of finding local rather than global minima. Analyses were performed with STATISTICA 6.0 (StatSoft, 2001).

A chord distance matrix (single-locus chord distance; Cavalli-Sforza and Edwards, 1967) among populations was constructed from allele frequency data (estimated in a Bayesian framework with a non-uniform prior derived from among-locus information; Zhivotovsky, 1999) using FAMD 1.08 (Schlüter and Harris, 2006). We then constructed a majority rule (50%) consensus UPGMA tree of 1000 bootstrap replicates using the same program. The tree was rooted with *J. foetidissima*.

The overall population structure was further explored using model-based Bayesian assignment, as implemented in BAPS v. 4.13 (Corander et al., 2003, 2004; available at http://www.rni.helsinki.fi/~jic/bapspage.html). BAPS performs equally well or even better than the widely used program STRUCTURE (Pritchard et al., 2000) and at a 400-fold speed (Corander and Marttinen, 2006). The program treats both the frequencies of the markers and the number of genetically diverged groups as random variables. Stochastic optimisation is used to infer the mode of the posterior distribution. As a Neighbour Joining analysis of individuals based on Nei and Li (1979) distances conducted with TREECON (Van de Peer and De Wachter, 1997; results not shown) indicated that most populations did not cluster together, we conducted clustering of individuals (instead of populations) without using the geographic origin of the samples as informative prior. BAPS was run with the maximal number of groups (K) set to 2–25 (i.e. a number larger than the sampled populations). Each run was replicated six times and the results were averaged according to the resultant likelihood scores. Subsequently, admixture analysis (Corander and Marttinen, 2006) was conducted with the following settings: minimal size of clusters five individuals; 100 iterations to estimate

Table 1
The 20 *Juniperus thurifera* populations sampled

Pop	Location	Coordinates	Collector, collection no.	*N*	Assignment of individuals to clusters	$Frag_{tot}$	$Frag_{poly}$	$H_D \pm SD$	$H_D \pm SD^*$
Morocco									
1	Jbel Bou-Iblan (Middle Atlas)	33°39′ N/4°09′ W	AT, 50/06	15	I: 14; IV: 1	193	88.60	0.153 ± 0.18	0.153 ± 0.18
2	Col de Zad (Middle Atlas)	33°05′ N/5°01′ W	AT, 67/06	15	I: 15	185	80.54	0.141 ± 0.18	0.141 ± 0.18
3	between Midelt-Er Rachidia (Eastern High Atlas)	32°34′ N/4°34′ W	AT, 88/06	16	I: 16	196	81.63	0.149 ± 0.18	0.147 ± 0.18
4	Jbel Azourki (Middle High Atlas)	31°47′ N/6°21′ W	AT, 156/06	16	I: 16	193	78.75	0.153 ± 0.19	0.153 ± 0.19
5	Oukaimeden (Western High Atlas)	31°46′ N/7°51′ W	AT, 149/06	17	I: 16; III: 1	179	83.80	0.136 ± 0.18	0.132 ± 0.18
Algeria									
6	Jbel Ahmar Khaddou (Aurés mountains)	35°22′ N/6°25′ E	EV, 60/06	18	I: 4; II: 6; III: 8	169	82.84	0.129 ± 0.17	0.118 ± 0.18
Iberian Peninsula									
7	Guadix, Granada (Betic Cordillera)	37°36′ N/3°21′ W	AT, 100/06	15	II: 14; III: 1	177	82.48	0.137 ± 0.18	0.137 ± 0.18
8	Osa de Montiel, Ciudad Real (Betic Cordillera)	38°48′ N/2°50′ W	ST, 195/06	16	II: 15; III: 1	187	84.49	0.149 ± 0.18	0.149 ± 0.18
9	Alcolea del Pinar, Guadalajara (Iberian Cordillera)	41°02′ N/2°19′ W	ST, 196/06	19	II: 19	194	87.62	0.153 ± 0.18	0.156 ± 0.19
10	Cañon del Rio Lobos, Soria (Duero Basin)	41°43′ N/3°02′ W	MJD, 71 /06	17	II: 15; III: 2	179	86.59	0.145 ± 0.18	0.141 ± 0.18
11	Calcena, Zaragoza (Iberian Cordillera)	41°39′ N/1°42′ W	MBG, 75/06	20	II: 3; III: 17	178	82.58	0.137 ± 0.18	0.129 ± 0.18
12	Hoces del Duratón, Segovia (Central Cordillera)	41°15′ N/3°57′ W	MJD, 198/06	15	II: 3; III: 12	166	84.94	0.136 ± 0.18	0.136 ± 0.18
13	Montes de León, León (Cantabrian Cordillera)	42°50′ N/5°51′ W	ST, 313/6	16	II: 14; IV: 2	177	85.87	0.150 ± 0.19	0.149 ± 0.19
14	Osera del Ebro, Zaragoza (Ebro Basin)	41°32′ N/0°34′ W	MBG, 70/06	19	IV: 19	171	88.30	0.130 ± 0.17	0.131 ± 0.18
15	Toro, Zamora (Duero Basin)	41°31′ N/5°23′ W	ER, 80/06	16	III: 2; IV: 14	150	89.33	0.116 ± 0.17	0.111 ± 0.17
Pyrenees									
16	Montagne de Rié, Haute-Garonne (Pyrennes)	43°47′ N/0°06′ E	PS, 11442	18	II: 9; III: 9	166	81.93	0.138 ± 0.19	0.125 ± 0.19
Alps									
17	Valdieri, Cuneo (Maritime Alps)	44°16′ N/7°23′ E	PS, 11401	8	II: 8	145	73.10	0.126 ± 0.19	-
18	W La Roche-des-Arnauds, Hautes-Alpes (Dauphiné Alps)	44°33′ N/5°54′ E	PS, 11444	16	II: 4; III: 11; IV: 1	176	80.68	0.144 ± 0.19	0.144 ± 0.19
19	St. Crépin, Hautes-Alpes (Cottic Alps)	44°42′ N/6°36′ E	PS, 11446	15	II: 1; III: 14	153	76.46	0.125 ± 0.19	0.125 ± 0.19
Corsica									
20	Calaccucia, Niolu	42°32′ N/9°03′ E	LH, 65/06	20	IV: 20	165	84.24	0.135 ± 0.18	0.136 ± 0.19
Outgroup									
J. foeti–dissima	Greece, Mt. Astraka, Ioanninon (Timfi)	39°59′ N/20°46′E	PS, 11650	4	n.a.	n.a.	n.a.	n.a.	

N represents the number of individuals analyzed. $Frag_{tot}$: number of AFLP fragments per population, $Frag_{poly}$: percentage of polymorphic AFLP fragments, $H_D \pm SD$: average gene diversity ± standard deviation; $H_D \pm SD^*$, average gene diversity calculated for a standardised population size of 15 ± standard deviation. *Abbreviations for collectors:* AT, A. Terrab; ER, E. Rico; EV; E. Vela; LH, L. Hugot; MBG, MB. García; MJD, M.J. Diéz; PS, P. Schönswetter; ST, S. Talavera.

the admixture coefficients for the individuals; 200 simulated reference individuals from each population; and 20 iterations to estimate the admixture coefficients for the reference individuals.

3. Results

The three AFLP primer combinations generated 326 unambiguously scorable fragments, EcoRI-ACG/MseI-CTAG: 91; EcoRI-ACG/MseI-CTCG: 142; EcoRIAGG/MseI-CTGA: 93, of which all but one were polymorphic. All 327 investigated individuals had unique AFLP profiles. The error rate, based on phenotypic comparisons among the 30 replicated individuals (Bonin et al., 2004), amounted to 0.8%.

3.1. Genetic diversity

Genetic diversity estimators are given in Table 1. There was no obvious geographic pattern in the distribution of genetic diversity estimated with H_D, and with the standarised H_D, calculated for a standarised population size of 15, e.g. Iberian populations included the genetically most diverse (population 9) as well as the least diverse (population 15) population.

A non-hierarchical AMOVA revealed that most of the overall genetic variation was explained by the within-population component (81.38%; consequently $\Phi_{ST} = 0.186$). A nested AMOVA (i.e. grouping populations 1–5 from Morocco vs. populations 7–20 from Europe) attributed 21.44% of the global variation to differences between the two continents, and 7.85% to among-population differentiation (both at $P < 0.001$).

3.2. Phenetic phylogeographical analyses

In the PCoA conducted at the level of individuals (Fig. 2A), the first two factors explained only 7.88% and 3.13% of the total variation, respectively, and mainly separated the Moroccan populations (1–5) from the rest of the data set. Iberian (7–15), Pyrenean (16), Alpine (17–19) and Corsican (20) populations formed a relatively unstructured cloud while population 6 from Algeria was situated in an intermediate position between Moroccan and European populations. The goodness-of-fit of the matrix of eigenvectors to the matrix of Jaccard distances was $r = 0.673$ ($P < 0.001$, based on 1000 permutations). Nonmetric multidimensional scaling of F_{ST}-distances among populations (Fig. 2B) produced a plot with a stress value of 11.4% deviation between the final NMDS result and the original distance matrix. The five Moroccan populations (1–5) as well as that from Corsica (20) were clearly separated. The Algerian population (6) was situated between the Moroccan and mainland European populations (7–19). Populations 11 and 12 from northern Spain clustered with the Pyrenean (16) and Alpine (17–19) populations.

The consensus UPGMA tree of populations rooted with *J. foetidissima* (Fig. 3) was roughly congruent with the results of the ordinations. *J. thurifera* clustered with moderate support (bootstrap support, BS 60%). The Moroccan populations (1–5) clustered with 100% BS and formed the sister group to the remaining populations (BS 94%). This remainder was a polytomy consisting of (a) population 17 from the Italian Alps; (b) population 6 from Algeria; (c) populations 14 and 15 from the Iberian Peninsula and 20 from Corsica (BS 75%); (d) populations 7–10 and 13 from the Iberian Peninsula (BS 70%); and (e) populations 11 and 12 from the Iberian Peninsula, population 16 from the Pyrenees, as well as populations 18 and 19 from the Alps (BS 77%).

3.3. Bayesian analyses

Bayesian clustering conducted with BAPS resulted in a best partition of four clusters with log marginal likelihood of −27802 at $P = 1$. The respective placement of individuals of the 20 investigated populations is given in Table 1. The following clusters were detected: Cluster I, all except for two individuals from the five populations from Morocco (1–5); Cluster II, prevalent in five populations from the Iberian Peninsula (7–10, 13) and the population from the Italian Alps (17); Cluster III, mainly in two populations from northern Spain (11, 12) and the populations from the Pyrenees and the French Alps (16, 18–19); and Cluster IV, dominant in two populations from the westernmost and the easternmost Iberian Peninsula (14, 15) and population 20 from Corsica. The admixture analysis (Fig. 1) detected extensive admixture, which was most pronounced in the Algerian, Pyrenean, Alpine, and some of the Iberian populations. The Moroccan populations as well as that from Corsica were more homogeneous.

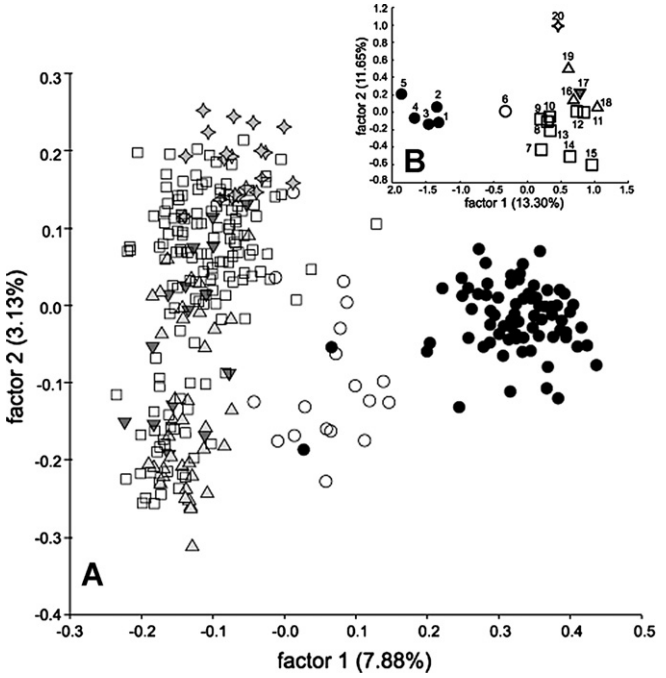

Fig. 2. Ordinations of AFLP data of individuals and populations of *Juniperus thurifera*. (A) Principal Co-ordinate Analysis (PCoA) based on a matrix of Jaccard similarities among the 327 investigated individuals. (B) Non-metric multidimensional scaling (NMDS) based on a matrix of *F*ST distances among 20 populations. Individuals and populations are labelled according to their geographical provenance. Black dots, Morocco (populations 1–5); circles, Algeria (6); squares, Iberian Peninsula (7–15); light grey triangles, Alps (17–19); dark grey upturned triangles, Pyrenees (16); diamonds, Corsica (20). Numbers in (B) refer to the populations described in Table 1 and illustrated in Fig. 1.

4. Discussion

The investigated 20 populations of *J. thurifera* maintained high levels of within population diversity (Table 1). This result is in line with previous studies of other conifers (Jiménez et al., 2003; Renau-Morata et al. 2005; Terrab et al. 2006, 2007) employing different marker systems (AFLPs, cpDNA SSRs, RAPDs). In contrast, the range-wide population divergence of *J. thurifera* (AMOVA-derived $\Phi_{ST} = 0.186$) is higher than in many other conifer species, which typically show Φ_{ST} values around 0.10 (Petit et al., 2005). This suggests that gene flow among the surveyed populations is infrequent, presumably due to the currently fragmented distribution of *J. thurifera*.

4.1. The main split follows the Strait of Gibraltar

In accordance with the most recent taxonomic treatments (Gauquelin et al. 1988; Barbero et al. 1994; Adams et al. 2003;

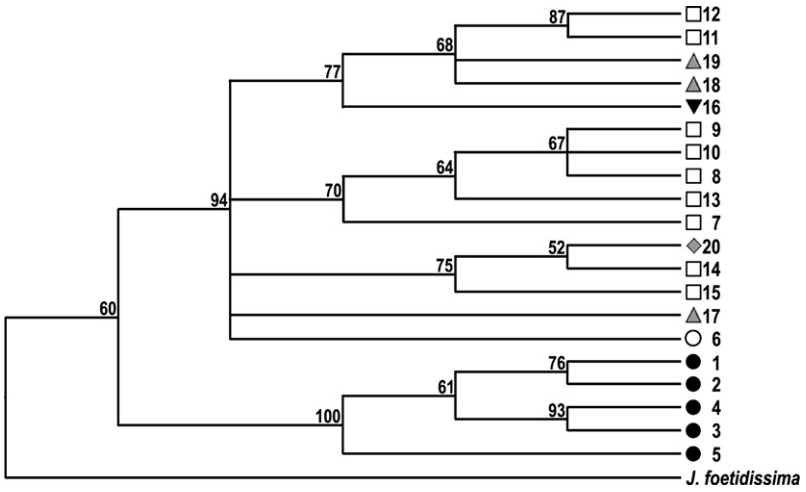

Fig. 3. Bootstrap majority rule (50%) consensus UPGMA tree (rooted with *J. foetidissima*) of AFLP data from 20 populations of *Juniperus thurifera*. Symbols refer to the same geographical grouping as in Fig. 2. Black dots, Morocco; circles, Algeria; squares, Iberian Peninsula; light grey triangles, Alps; dark grey upturned triangles, Pyrenees; diamonds, Corsica.

Jiménez et al. 2003; Romo and Boratyński, 2007) based on results from morphometric, chemical and DNA markers, our AFLP analysis revealed two major groups of populations in *J. thurifera*. One was distributed over the Middle and High Atlas ranges in Morocco (subsp. *africana*), the other comprised the European populations, including Iberian Peninsula, Pyrenees, Alps and Corsica (subsp. *thurifera*). In the UPGMA analysis (Fig. 3) both groups are separated with high bootstrap support (BS 100 for the Moroccan clade and BS 94 for the European/Algerian clade) and in the ordinations (Fig. 2) the clusters show no overlap (for the intermediacy of the Algerian sample, see below). The (non-hierarchical) Bayesian clustering analysis (Table 1 and Fig. 1) attributed all except for two Moroccan *J. thurifera* individuals to a separate cluster. One of these two individuals has been assigned to Cluster IV that is virtually absent from the otherwise strongly admixed Algerian population (Fig. 1), testifying that rare gene flow from the Iberian Peninsula to Morocco has occurred in the past. Our results thus globally suggest that the Strait of Gibraltar functioned as an efficient though not impermeable barrier against gene flow between the two groups of populations for a long time. In particular, there is no indication for geneflow from the Moroccan *J. thurifera* populations to the Iberian Peninsula after the glaciations. This is in agreement with other studies conducted across the Strait of Gibraltar in conifers (Terrab et al., 2007), Fagaceace (Lumaret et al., 2002), *Olea* (Rubio de Casas et al., 2006), and also in herbaceous plants (Vargas et al., 1999; Ortiz et al., 2007). The importance of the Strait of Gibraltar as a barrier to gene flow has also been documented in animals such as scorpions (Gantenbein and Largiadèr, 2003; Gantenbein, 2004), bats (Castella et al., 2000), and amphibians (García-París and Jockusch, 1999; Fromhage et al., 2004).

4.2. The Algerian population is genetically intermediate

Our analyses clearly reveal the intermediate position of the Algerian population between the Moroccan and the European population groups. While the ordination of individuals (Fig. 2A) suggested an equidistant placement, the ordination of populations (Fig. 2B) related the Algerian population rather to the European samples. The same was true for the UPGMA tree (Fig. 3), where the Algerian population clustered with European accessions with high support; and for the Bayesian admixture analysis (Fig. 1) that revealed that a large portion of the Algerian gene pool overlapped with the European Clusters II and III, the rest being constituted by the otherwise strictly Moroccan Cluster I. While the presence of the latter cluster may be explained by a formerly wider distribution of *J. thurifera* in Northern Africa, the similarity with the European populations requires invoking geneflow from the European mainland to Algeria, most probably via long distance dispersal. Migration over land bridges between the Iberian Peninsula, the African coast and the Balearic Islands during the Messinian event (Schüle, 1993; Jaeger, 1994) is less likely, because the pronounced differences in genetic divergence between Europe, and Morocco and Algeria, respectively, strongly argue against a simultaneous origin. Additionally, the presence of Clusters II and III in the Algerian population support several independent dispersal events. Only a few phylogeographic studies on Mediterranean woody species have included samples from Algeria, and mainly detected relationships to central or eastern Mediterranean populations. Dumolin-Lapègue et al. (1997) and Petit et al. (2002), using chloroplast DNA within different *Quercus* species, detected a single haplotype in the Algerian population, which was closely related to those from the Caucasus, northern Italy and Corsica. Based on variation in the mitochondrial *nad* 1 intron 2 in *Pinus pinaster*, Burban and Petit (2003) detected three different mitotypes, one in Morocco, the second in the western part, and the third one in the eastern part of the species' range including the northeastern-most Iberian population, the southeast of France, Italy, Corsica, Sardinia, Tunisia and the single Algerian population. It has to be stressed, however, that the mentioned species are typical elements of Mediterranean vegetation and will likely exhibit phylogeographical patterns that deviate from that of *J. thurifera*, a species adapted to drought and low temperatures. Interestingly, our results are supported by a morphometric study (Véla et al., unpubl.) on the Algerian *J. thurifera* population, which revealed that the length of the leaf scales as well as the cone diameter were more similar to subsp. *thurifera* from the Iberian Peninsula than to subsp. *africana* from Morocco.

4.3. The Iberian Peninsula: source area or melting pot of intra-European migrations?

The Bayesian analyses (Table 1 and Fig. 1) revealed three different Clusters (II–IV) in Europe, all of them present in the Iberian Peninsula and specifically in its northern part. Cluster II predominated in the Italian Alps, Cluster III in the French Alps, and the single investigated Pyrenean population (16) showed a high degree of admixture between Clusters II and III (Fig. 1). These results are congruent with the ordination of populations (Fig. 2B) and the UPGMA dendrogram (Fig. 3), the only exception being population 17

from the Italian Alps that did not fall into any other cluster in the UPGMA analysis (Fig. 3). Consequently, our data does not support the three chemivars proposed by Gauquelin et al. (1988), namely "thurifera" in Spain and the French Pyrenees; "gallica" in the Alps, and "corsicana" in Corsica. The presence of the three Clusters in the Iberian Peninsula allows for two alternative scenarios, none of which can be rejected by the present data. (1) Clusters II to IV originated on the Iberian Peninsula, most probably during a warm and humid interglacial period when the distribution area of *J. thurifera* which likely had its widest distribution during glacial periods (Peñalba et al., 1997; Stevensen, 2000) became fragmented. Later on, the Iberian populations served as source for the colonisation of the Pyrenees and Alps. Alternatively, (2) diversification may have partly taken place outside of Iberia and the pattern observed today is (partly) the result of immigration into Iberia, e.g. from the Alps. Such interchange likely happened during cold stages of the Pleistocene, and the intervening populations subsequently became extinct when the climate ameliorated. In any event, the strong current admixture between the Iberian Peninsula, Pyrenees and Alps (Fig. 1) suggests pronounced historic or – less likely – contemporary gene flow among all three areas.

4.4. Long-distance dispersal to Corsica?

The Corsican population exhibits strong genetic ties with the northern Iberian Peninsula, and not with the geographically much closer Alps. This is corroborated by the UPGMA dendrogram (Fig. 3) as well as by the Bayesian clustering (Table 1) and admixture (Fig. 1: Cluster IV) analyses that congruently identified a close relationship among the Corsican population and populations 14 and 15 from the northwestern and northeastern Iberian Peninsula. The low divergence among the three populations rejects the hypothesis that the Corsican population originated at the time when the Corsican-Sardinian microplate was still attached to the Iberian plate (until 29 mya; Bellon et al., 1977). A relatively recent long-distance dispersal, maybe from the Ebro valley where population 15 thrives, appears to be a more likely explanation for the colonization of Corsica by *J. thurifera*, a predominantly bird-dispersed tree (Debussche and Isenmann, 1989; Herrera, 1989; Jordano, 1993), thrushes being the most relevant dispersers (Santos et al., 1999). Gene flow in the opposite direction, i.e. from Corsica to Spain, cannot be excluded based on our data but appears less likely. Given that the disjunction between Corsica and Spain is certainly younger than that between the Moroccan and the European mainland populations which possibly relates to the end of the Messinian event, such a dispersal would imply two long-distance dispersal events, one from an unknown source population to Corsica and a second dispersal to northern Spain. Long-distance dispersal has also been documented using AFLP in *Armeria pungens*, which grows on the Atlantic coast of the Iberian Peninsula and in Corsica/Sardinia (Piñeiro et al., 2007). In this species, the populations from SW Portugal were more closely related to Corsica/Sardinia than to those from the SW of Spain. Congruent with our results, Fineschi et al. (2002) in the genus *Quercus* found two chloroplast haplotypes in Corsica and Sardinia, which were phylogenetically related to that detected in the Ebro valley (Olalde et al., 2002; Petit et al., 2002). Consequently, a long-distance dispersal during the postglacial colonization was suggested (Petit et al., 1997). In contrast, the herbaceous perennial mountain plant *Bupleurum stellatum* has likely colonized Corsica from the southeastern Alps (Schönswetter and Tribsch, 2005).

5. Conclusions

Altogether, our study suggests that the phylogeographic pattern of *J. thurifera* differs remarkably from that of typical Mediterranean tree species such as *Olea europaea* (Rubio de Casas et al., 2006) or *Quercus ilex* (Lumaret et al., 2002). The fairly weak genetic structure on the European mainland may be explained by the hypothesis that *J. thurifera* was more widespread during cold periods of the Pleistocene (Carrión et al., 2001a, b, 2003, 2004) enabling gene exchange between the currently isolated portions of the species' range. The continuous distribution on the European mainland was maybe disrupted only in the early Holocene, leaving little time for genetic differentiation. For the Moroccan, Algerian and Corsican populations, our data allow rather unambiguous inferences. Whereas the strong differentiation between the Moroccan and the European populations suggests old vicariance across the Strait of Gibraltar, the data indicate an opposing history for the Algerian stands which appear derived from several independent immigration events from Europe, most probably via long-distance dispersals. In contrast, the population on Corsica has its presumed geographical source in the lower Ebro valley of north-eastern Spain.

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