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Phylogeography of North African Atlas cedar (*Cedrus atlantica*, Pinaceae): Combined molecular and fossil data reveal a complex Quaternary history[1]

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Northwest Africa is a major hotspot of plant biodiversity, but very little is known about the Quaternary range dynamics of plant species in this region. Here we investigate the range-wide population structure and phylogeography of Atlas cedar (*Cedrus atlantica*), an emblematic forest tree endemic to Morocco and Algeria. We genotyped 261 individuals from 11 populations using AFLP markers. Data were analyzed using both conventional F_{ST}-based techniques and Bayesian clustering. Overall population differentiation was high ($F_{ST} = 0.25$). Two major groups of populations were identified, one distributed through the Rif and Middle Atlas mountains in Morocco and the other through the Algerian Tell Atlas and Aurès mountains as well as the Middle Atlas. Combined molecular and fossil data indicate that *C. atlantica* survived the Last Glacial Maximum in at least three disjunct refugia along the coast of the Mediterranean Sea, whereas the Middle Atlas, today the core of the species range, has been colonized relatively recently (<10 000 yr BP). The colonization history of individual populations has left clear imprints in their present-day diversity, which may vary greatly even between nearby stands. Our study illustrates how integrating different data sources and analytical approaches can help elucidate complex range dynamics that would otherwise remain undeciphered.

Key words: Algeria; AFLP; diversity; gene flow; glacial refugia; Morocco; nonmetric multidimensional scaling analysis; population divergence; range contraction; Structure program.

Phylogeographical studies have been conducted on thousands of terrestrial, freshwater, and marine organisms worldwide. The geographical coverage of the earth's surface remains highly unbalanced, however, and while some regions have been covered by very comprehensive and detailed accounts, others remain largely unexplored. A particularly sharp regional contrast exists between large parts of Europe and North Africa, which remains poorly explored in contrast to Europe. This imbalance is unfortunate, because North Africa harbors several major hotspots of plant biodiversity and endemism (Médail and Quézel, 1997) and is one of the regions of the world that will suffer the strongest negative effects from modern climate change (in particular a significant increase in aridity; IPCC, 2001). Phylogeographical patterns around the Mediterranean Basin are typically more complex and often span much longer time-scales than those at higher latitudes (Fady-Welterlen, 2005; Petit et al., 2005a, b; Gómez and Lunt, 2007). Due to the climatic history and heterogeneous topography of the region, phylogeographical surveys typically detect a much greater allelic richness and evolutionary divergence of populations than further north (e.g., Bilton et al., 1998; Fineschi et al., 2002; Hampe et al., 2003; Petit et al., 1993), and they may trace range dynamics dating back well into the Tertiary (e.g., Lumaret et al., 2002; Caujapé-Castells and Jansen, 2003; Magri et al., 2007). In surveys of Mediterranean taxa, North Africa is often underrepresented, and detailed rangewide population surveys of exclusively North African plant species are very scarce (but see El Mousadik and Petit, 1996a; Terrab et al., 2007). Because the fossil record of this region is also fragmentary (Elenga et al., 2000; Magri and Parra, 2002), the past vegetation dynamics in the area and its consequences on gene pools remain poorly known.

Here we investigate the genetic population structure and historical dynamics of Atlas cedar, *Cedrus atlantica* (Endl.) Carrière. This ecologically and economically important forest tree is endemic to the mountain ranges of Morocco and Algeria (being the only African member of the genus). Atlas cedar forests constitute some 2.8% of the total forested area of Morocco, and the major stands occur in two widely separated geographical areas: the North Moroccan Rif mountains (total forest area: 160 km^2) and the Middle and Eastern High Atlas (1160 km^2). In Algeria, Atlas cedar forests occur in distant areas and cover around 300 km^2 in the Tell Atlas and Aurès mountains, representing some 1.3% of the total forest surface of the country (Boudy, 1950). In comparison to the estimated original forest cover, the two countries have lost around 75% of their original cedar forests between 1940 and 1982 (Benabid and Fennane,

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1994), and the present species distribution shows a gap of ca. 500 km between the Moroccan and the Algerian parts of the range (Fig. 1). It seems noteworthy that the remaining Atlas cedar forests still contain a series of associated endemic plant species (Barbero et al., 2001).

Despite the ecological and economic importance of *C. atlantica* in the region, little information exists on the genetic structure and variability of its natural populations. Renau-Morata et al. (2005) were the first to report on genetic diversity and differentiation in eight Moroccan *C. atlantica* forests based on randomly amplified polymorphic DNA (RAPD) markers, while Terrab et al. (2006) used chloroplast DNA microsatellite (cpSSR) markers to analyze six stands of Moroccan *C. atlantica*. Both studies concluded that extensive gene flow seems to occur even among distant populations. However, to date, no study has considered the Algerian part of the species range nor attempted to infer its phylogeography.

In the current study, we use amplified fragment length polymorphism (AFLP) to assess the genetic structure of natural *C. atlantica* populations throughout the entire distribution range of the species and to reconstruct its historical population dynamics. Our aims are to explore the distribution of genetic variation within and among the disjunct areas that constitute the present range and to identify glacial refugia and reconstruct patterns of postglacial range expansions. We address these issues using conventional F_{ST}-based analyses focusing primarily at the population level, as well as a Bayesian approach that puts greater emphasis on the individual tree level. Finally, we complement our inferences about historical range dynamics of *Cedrus* with a review of Quaternary fossil records of the species in the region.

MATERIALS AND METHODS

Plant material—We obtained plant material from 11 natural *C. atlantica* stands (Fig. 1) representing the three major cores of the species distribution: the Middle Atlas (6 stands), the Rif (2), and the Algerian Tell Atlas and Aurès mountains (3). Table 1 provides details on the populations sampled. Needles were collected from 261 individuals (mean = 23.7 individuals per population). Only trees >100 years old were chosen (based on data from a dendrochronological survey in the same stands) to minimize any confounding effects that might arise from modern forest management. Samples were stored in silica gel until DNA isolation. Vouchers were taken for all populations and deposited in the herbarium of the University of Seville, Spain (SEV).

DNA isolation 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 Tris-EDTA (TE) buffer. DNA extracts were treated with RNAse at 37°C for 30 min. The quality of the extracted DNA was checked on 1% Tris-acetate-EDTA (TAE) agarose gels. The AFLP procedure followed established protocols (Vos et al., 1995; PE Applied Biosystems, Foster City, California, USA). Genomic DNA (≈0.5 μg) was digested with restriction endonucleases *Eco*RI and *Mse*I, then ligated to double-stranded *Eco*RI and *Mse*I adaptors in one step at 37°C for 2 h. Ligated DNA fragments were diluted 20-fold with TE 0.1% buffer. Preselective and selective amplifications were performed in a thermal cycler (Gene Amp PCR system 9700, PE Applied Biosystems). Polymerase chain reaction (PCR) protocols followed Vos et al. (1995). Preselective primers based on the sequences of *Eco*RI and *Mse*I adaptors with the addition of nucleotides (*Eco*RI-A and *Mse*I-C) were used to amplify a subset of fragments having the matching nucleotide downstream from the restriction sites, reducing the number of amplified fragments by approximately sixfold (4 × 4). The preselective PCR products were diluted 20-fold with TE 0.1% buffer. The preselective products were amplified with primers having two additional selective bases (three in total). The first base was the same as that used in the preselective amplification. The *Eco*RI-based primers were also labeled with fluorescent dyes. Selective primers using 72 primer combinations with three nucleotides were initially screened on 16 individuals of eight populations (two individuals per population). The final three primer combinations for the selective PCR were *Eco*RI (Fam)-ACC/*Mse*I-CAT, *Eco*RI (Hex)-ACG/*Mse*I CAG, and *Eco*RI (Ned)-AGC/*Mse*I-CAG. The fluorescence-labeled selective amplification products were separated on a sequencer (Applied Biosystems 3130*xl* Genetic Analyzer) with an internal size standard (GeneScan-500 ROX, PE Applied Biosystems). To test AFLP repeatability, we randomly selected three DNA samples per each population that were each run twice (starting from the same extraction). Raw data were collected and aligned with the internal size standard using ABI Prism GeneScan analysis software 2.1 (PE Applied Biosystems). Subsequently, the GeneScan files were imported into Genographer (version 1.6.0; 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. The results of the scoring were exported as a presence/absence matrix.

Data analysis—Genetic diversity was assessed for each population using the total number of AFLP fragments present ($Frag_{tot}$), the percentage of polymorphic fragments ($Frag_{poly}$), and the number of private fragments ($Frag_{priv}$). We also calculated the average gene diversity (H_D; Excoffier et al., 2005): $H_D = 1 - \Sigma x_i^2$; where x_i is the population frequency of each phenotype "allele" (1 or 0) at locus i. The average gene diversity is the resulting mean across all loci.

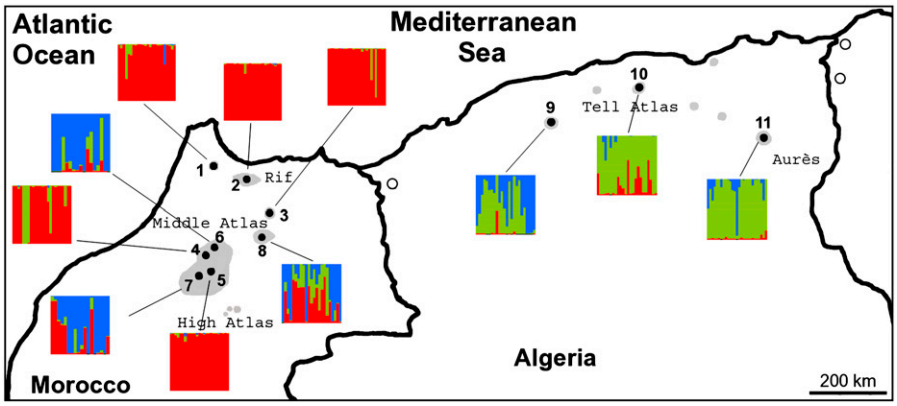

Fig. 1. Geographic distribution and genetic population structure of *Cedrus atlantica*. Gray areas indicate the present range of the species according to Quézel and Médail (2003), empty circles denote postglacial records of fossil *Cedrus* pollen or charcoal in areas outside the range. Black circles and numbers indicate locations of investigated populations. The graphs next to each population indicate the assignment of individual trees to the genetic clusters I (red), II (green), and III (blue) detected by the program Structure.

TABLE 1. Features of the *Cedrus atlantica* populations (Pop.) sampled. *N* represents the number of individuals sampled. Groups A and B refer to the population assemblages shown in Fig. 2. $Frag_{tot}$: number of fragments per population, $Frag_{poly}$: percentage of polymorphic fragments, $Frag_{priv}$: number of private fragments, H_{Sh}: Shannon diversity, H_D: average gene diversity.

Pop.	Location	Region	Elevation (m a.s.l.)	Coordinates	*N*	Group	$Frag_{tot}$	$Frag_{poly}$	$Frag_{priv}$	H_{Sh}	H_D
1	Jbel Bou-Hachem	Rif	1400	35°14′N 5°25′W	24	A	112	67.85	3	25.56	0.104
2	Ketama	Rif	1680	34°58′N 4°40′W	23	A	113	76.10	3	26.56	0.109
3	Tazzeka	Middle Atlas	1940	34°05′N 4°10′W	26	A	108	75.92	0	24.56	0.104
4	Azrou	Middle Atlas	1820	33°25′N 5°13′W	24	A	106	83.01	0	26.38	0.113
5	Col de Zad	Middle Atlas	2200	33°00′N 5°04′W	25	A	107	67.28	0	22.86	0.094
						Mean A	109	74.03	1	25.18	0.105
6	Ifran	Middle Atlas	1986	33°37′N 5°03′W	17	B	145	90.34	1	43.02	0.203
7	Aguelmam Aziza	Middle Atlas	1486	32°58′N 5°26′W	24	B	134	73.88	2	34.86	0.152
8	Jbel Bou-Iblan	Middle Atlas	1692	33°38′N 4°13′W	23	B	149	75.83	0	39.79	0.157
9	Theniet el Had	Tell Atlas	1457	35°52′N 1°56′E	24	B	140	82.85	5	41.67	0.179
10	Djurjura: Tikjda	Tell Atlas	1470	36°26′N 4°07′E	24	B	122	73.77	8	28.11	0.105
11	Jbel Cheliah	Aurès Mountains	1831	35°18′N 6°37′E	27	B	125	79.20	2	31.51	0.132
						Mean B	136	79.31	5	36.49	0.154

Finally, the Shannon diversity index was calculated as $H_{Sh} = -\Sigma(p_i \cdot \ln p_i)$, where p_i is the relative frequency of the i^{th} fragment in a population using the program FAMD version 1.08 (Schlüter and Harris, 2006).

The organization of the molecular variation within and among stands was assessed by analyses of molecular variance (AMOVA; Excoffier et al., 2005). After a global analysis considering the variation within vs. among all populations, we conducted a second analysis involving also the three major range cores (Rif, Middle Atlas, and Tell Atlas/Aurés mountains).

We used three complementary techniques to investigate genetic distances and relationships between populations based on population-pairwise F_{ST} (which were calculated with Arlequin version 3.0; Excoffier et al., 2005). First, a nonmetric multidimensional scaling analysis (NMDS) was undertaken. NMDS is an ordination procedure that identifies those positions of *n* individuals (populations in this case) in reduced space that best reflect their original genetic distances. It is an iterative process using a steepest descent algorithm to minimize the deviation between the final and the original distance matrix (termed stress). 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; O'Hanlon et al., 1999; Edwards and Sharitz, 2000). 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 the program Statistica version 6.0 (StatSoft, OK, USA). 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 nonuniform prior derived from among-locus information; Zhivotovsky, 1999) using FAMD 1.08 (Schlüter and Harris, 2006). We then constructed a majority rule (50%) consensus neighbor-joining tree of 1000 bootstrap replicates using the same program. Finally, we compared pairwise F_{ST} values with the geographical distances between populations using Mantel tests based on Spearman correlations (100 000 permutations; XLSTAT-PRO version 7.5.3, Addinsoft, Barcelona, Spain); this analysis was performed both on the entire sample and on the two subgroups identified by the phenetic analysis (as described later).

The overall population structure of our sample was further explored using a model-based Bayesian assignment technique without a priori information, as implemented in the program Structure version 2.1 (Pritchard et al., 2000; Falush et al., 2003). This approach uses individual multilocus genotypes to construct clusters of genetically similar individuals in a way that maximizes Hardy–Weinberg equilibrium and minimizes linkage disequilibrium within clusters. Thus, clusters represent the most "ideal" (yet virtual) populations that can be derived from the complete data set, and their distribution across the real populations allows us to make inferences about the genetic relatedness of the individuals they contain. The Structure program was initially developed for codominant data, and we therefore recoded the presence/absence matrix of our AFLP fragments (using 0/0 for absent bands and 1/–9 for present bands, following the procedure recommended by Evanno et al., 2005) using the AFLPDAT package of the program R (Ehrich, 2006). In line with the argumentation of Falush et al. (2003) and Evanno et al. (2005), we used the admixture model and the correlated allele frequency model; this combination is deemed to be the most appropriate when the aim is to infer subtle population structures, as in the present case. Test runs with the alternative models, assuming the no-admixture model (as tentatively suggested by Falush et al. [2007] for dominant markers), produced an unrealistically strong assignment of individuals to the extent that some populations contained only individuals completely assigned to either one or the other cluster (which would indicate that no gene flow at all exists between clusters within populations). In contrast, we found that using either the independent allele frequency model or the correlated allele frequency model made absolutely no difference to the analysis. For each run, we used 10 000 burn-ins followed by 50 000 iterations to ensure convergence of the Markov chain Monte Carlo algorithm. The optimal number of clusters was determined according to Evanno et al. (2005), performing analyses with $K = 1$–12 clusters and five independent runs for each value of *K*. Finally, all individuals were (probabilistically) assigned to clusters according to their genotype.

For purposes of comparison, we also analyzed our data with the program BAPS version 5.1 (Corander and Marttinen, 2006). The simulation was run from $K = 2$ to $K = 12$ as the maximum number of diverged groups (larger than the sampled populations), with five replicates for each *K*, and the option "clustering of individuals." The following settings were used: minimal size of clusters at five individuals, 100 iterations to estimate the admixture coefficients for the individuals, 200 simulated reference individuals from each population, and 20 iterations. Overall results of this analysis were very similar to those generated by Structure (discussed later).

RESULTS

The three AFLP primer combinations generated 203 unambiguously scorable DNA fragments [*Eco*RI(Ned)-AGC/*Mse*I-CAG: 90 fragments; *Eco*RI(Hex)-ACG/*Mse*I-CAG: 90; *Eco*RI(Fam)- ACC/*Mse*I-CAT: 23]. All but one were polymorphic within each of the 11 populations investigated and allowed us to distinguish unique multilocus phenotypes for each of the 261 trees analyzed. Our error rate assessment involving 33 of the 261 analyzed trees showed that average repeatability of AFLP fragment scores was 98%.

Population-level analyses of genetic diversity and geographical relationships—Detailed results of the genetic diversity analyses are shown in Table 1 (see also Fig. 1). Populations contained between 106 and 149 fragments, respectively. Seven of the 11 stands contained at least one private AFLP fragment; populations 9 and 10 of the Tell Atlas presented the highest number of private fragments (eight and five, respectively) followed by populations 1 and 2 of the Rif with three each. Within-population diversity was generally high but differed considerably among populations, including some nearby populations (4 and 6) in the Middle Atlas (Fig. 1).

Populations shared between 122 and 47 AFLP fragments, which resulted in pairwise F_{ST} distances ranging from 0.035 to 0.406 (Table 2) and an overall F_{ST} of 0.247. The AMOVAs assigned most of the overall molecular variation (>70%) to the within-population level, whereas only 8% were explained by differences among the three distant range cores (Table 3).

The NMDS analysis of the F_{ST} matrix produced a plot with a stress value of 24% (corresponding to the deviation between the final NMDS result and the original distance matrix). Two major groups of populations (hereafter termed groups) could be clearly distinguished (Fig. 2). Group A included the two Rif populations (1 and 2) and three populations of the Middle Atlas (3–5), whereas group B included the three other Middle Atlas populations (6–8) together with the three Algerian populations (9–11). While the populations of group A were very similar in either of the two principal dimensions, those of group B showed a much greater divergence along both axes (mean pairwise F_{ST}: group A = 0.053, group B = 0.185; *t* test for unequal variances: $t = -5.99$, df = 14.99, $P = 0.0001$).

The bootstrap consensus neighbor-joining tree of populations (Fig. 3) was congruent with the results of the NMDS analysis. The populations of group A (1–5) were completely separated from those of group B (6–11) with 100% bootstrap support (BS). Within group B, two subgroups were distinguishable, one formed by the Algerian populations (9–11) and a second one by the Moroccan ones (6–8). Some geographical trends were perceivable within group B, most notably a westward increase of scores in the second dimension of the NMDS plot (Fig. 2). This notion was corroborated by a strong correlation between pairwise F_{ST} values and geographic distance among populations in group B (Mantel test: $r = 0.76$; $P = 0.002$), whereas no correlations were detected either in group A ($r = 0.64$; $P = 0.16$) or across all populations ($r = 0.19$; $P = 0.92$). Finally, populations from group A were on average markedly less diverse than those from group B, as measured either by average gene diversity H_D (mean = 0.105 vs. 0.154; *t* test: $t = -3.46$, df = 5.51, $P = 0.015$) or by the Shannon index (mean = 25.18 vs. 36.49; *t* test: $t = -4.47$, df = 5.77, $P = 0.005$). However, no differences were observed when considering the index of frequency-down-weighted marker values (DW; mean = 21.61 vs. 23.02; *t* test: $t = -0.55$, df = 5.64, $P = 0.59$).

Bayesian analysis of the overall population structure—Using the method of Evanno et al. (2005), we found that the most informative representation of the overall genetic structure was achieved when considering $K = 3$ clusters. Figure 1 illustrates their distribution across the investigated stands. One cluster (hereafter termed cluster I) was strongly dominant within all five populations of group A but was much rarer in the populations belonging to group B. These were largely composed of individuals belonging to two different clusters (II and III, hereafter). A marked east–west trend existed in the relative dominance of clusters II and III within populations. The frequency of cluster III within populations was related with the second dimension of the NMDS plot (Fig. 2), whereas the first dimension was related with the dominance of cluster I within populations of group A, as well with as the relative frequencies of cluster I and cluster III within the significantly admixed populations. Finally, there was a clearly discernible relationship between the extent of cluster admixture within populations and their gene diversity as measured by the Shannon index (Fig. 1).

DISCUSSION

Overall population structure and patterns of gene flow—We found that the investigated *C. atlantica* stands retain high levels of within-population diversity. This result confirms previous surveys conducted with RAPD (Renau-Morata et al., 2005) and cpSSR (Terrab et al., 2006) markers. It is also in line with studies of other conifers (Hamrick et al., 1992). In contrast, range-wide population divergence of *C. atlantica* is markedly higher than in many other conifer species, which often have nuclear F_{ST} values around 0.10 (Petit et al., 2005a) to 0.15 (Hamrick, 2004) instead of the 0.25 observed here. Such a high value suggests that gene flow among populations would be relatively infrequent, although pollen dispersal by wind can span much greater distances than those involved in this study (Liepelt et al., 2002). Our results are in line with those reported for western Mediterranean populations of *Olea europaea* L. ($F_{ST} = 0.20$; Rubio de Casas et al., 2006), as well as for another emblematic northwest African tree with highly isolated populations, *Argania spinosa* Skeels ($F_{ST} = 0.25$; El Mousadik and Petit, 1996b; it should be noted, however, that this species is pollinated and dispersed by animals, which renders direct comparisons problematic). Remarkably similar patterns to those observed here have recently also been reported for *Cedrus libani* A.Rich. (Fady et al., 2007; 77% of the total molecular variation distributed within populations, 18% between the two range cores in Anatolia and Lebanon, and 5% among populations within each core). Although this species grows a few thousand kilometers east of *C. atlantica*, it seems noteworthy that the spatial configuration of its populations and the climatic history of the two regions are relatively similar (cf. Fady et al., 2007). These factors, together with the similar biology of the two species, might explain this coincidence in the organization of their rangewide

TABLE 2. Number of shared fragments (bold) and pairwise F_{ST} distances (italic) for the 11 *Cedrus atlantica* populations (Pop.).

Pop.	1	2	3	4	5	6	7	8	9	10	11
1	—	*0.054*	*0.043*	*0.080*	*0.062*	*0.391*	*0.341*	*0.234*	*0.368*	*0.242*	*0.301*
2	**59**	—	*0.037*	*0.073*	*0.035*	*0.406*	*0.361*	*0.254*	*0.373*	*0.254*	*0.331*
3	**53**	**51**	—	*0.048*	*0.048*	*0.396*	*0.356*	*0.225*	*0.370*	*0.235*	*0.316*
4	**54**	**65**	**55**	—	*0.054*	*0.356*	*0.319*	*0.210*	*0.327*	*0.181*	*0.277*
5	**59**	**54**	**57**	**47**	—	*0.406*	*0.361*	*0.245*	*0.388*	*0.282*	*0.342*
6	**81**	**75**	**80**	**66**	**75**	—	*0.049*	*0.110*	*0.126*	*0.367*	*0.293*
7	**91**	**78**	**80**	**85**	**87**	**125**	—	*0.066*	*0.132*	*0.334*	*0.246*
8	**83**	**81**	**74**	**83**	**79**	**106**	**122**	—	*0.114*	*0.201*	*0.160*
9	**79**	**80**	**76**	**70**	**77**	**103**	**116**	**101**	—	*0.225*	*0.133*
10	**66**	**67**	**64**	**67**	**64**	**91**	**103**	**90**	**80**	—	*0.154*
11	**67**	**72**	**66**	**58**	**67**	**93**	**109**	**93**	**121**	**74**	—

TABLE 3. Results of analyses of molecular variance (AMOVA) of AFLP data from the 11 *Cedrus atlantica* populations (pop.). Both models were highly significant ($P < 0.001$).

Structure analyzed and source of variation	df	Sum of squares	Variance component	Variation (%)	Φ_{ST}
Global analysis					0.247
Among pop.	10	1130	4.31	24.7	
Within pop.	244	3203	13.13	75.3	
Considering geographical range cores (Rif Mountains vs. Middle Atlas vs. Tell Atlas/Aurès Mountains)					0.268
Among regions	2	414	1.48	8.25	
Among pop.	8	715	3.32	18.54	
Within pop.	244	3203	13.13	73.21	

population genetic structure. Finally, a recent AFLP analysis of three *C. atlantica* populations (Dagher-Kharrat et al., 2007) found likewise roughly similar levels of gene diversity (polymorphic loci = 65%; $H_D = 0.196$), although its low sample size precludes detailed comparisons.

Inference of historical population dynamics—Our F_{ST}-based analyses revealed two clearly distinct groups of populations in *C. atlantica*, one (group A) distributed through the Rif and Middle Atlas in Morocco and the other (group B) through the Algerian Tell Atlas and Aurès mountains as well as the Middle Atlas. Within each group, the highest numbers of private alleles occurred in the two Tell Atlas populations (group B: 5 and 8 vs. 0–2 in the other populations), as well as the two Rif populations (group A: 3 vs. 0). The number of private fragments is commonly considered an indicator of population age and persistence in isolation (e.g., Stehlik et al., 2002; Schönswetter and Tribsch, 2005; Ortiz et al., 2007); hence, our data suggest that the Tell Atlas and the Rif Mountains would represent the most ancient range cores of the two population groups. Such interpretation agrees with the fossil record that situates glacial refugia of *C. atlantica* in these regions (discussed later).

Our analyses showed also that group B was considerably more differentiated than group A, and its populations were on average more diverse (though levels varied also greatly within group B). The assignment analysis with Structure corroborated this difference from a complementary viewpoint: It largely confirmed the existence and homogeneity of the group A populations assigning most of their individuals to a single cluster (cluster I, in red in Fig. 1). In contrast, the populations of group B contained a mixture of three different clusters. As might be expected, the mutual divergence of populations (as measured by the population-level analyses) coincided with the predominance of different clusters within each, while the extent of cluster admixture was closely related with their diversity, peaking in those stands that contained similar proportions of each cluster (Fig. 1).

The combined information on the spatial distribution of the three clusters and that of the private alleles detected allows a number of inferences about the history of the investigated populations. Cluster I was strongly dominant in the group A populations of the Rif and the Middle Atlas, less frequent in group B populations of the Middle Atlas, and even less so in the Algerian stands (except population 10). Such a distribution, together with the fact that all six private alleles of group A occurred in the Rif populations, indicates that cluster I is likely to reflect the history of *C. atlantica* populations that persisted through the Last Glacial Maximum in the Rif area and expanded subsequently from this region. The dominance of cluster II in the Algerian populations and its markedly lower frequency further west suggests that it would have had its origins in the eastern part of the species range. This hypothesis is notably supported by the fact that all eight private alleles detected in population 10 were found in individuals belonging to cluster II, thereby

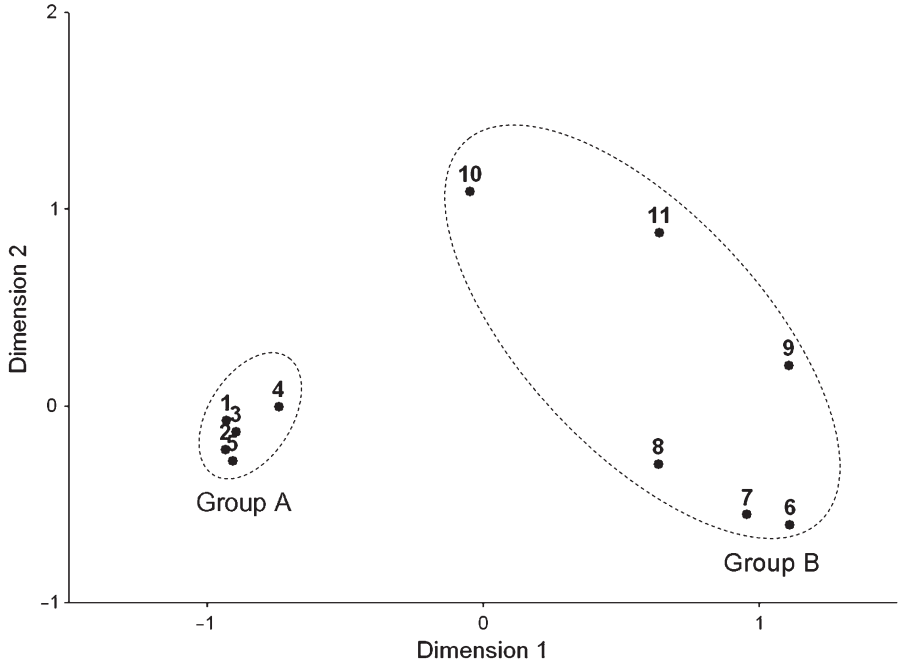

Fig. 2. Two-dimensional nonmetric multidimensional scaling (NMDS) plot of pairwise F_{ST} genetic distances among 11 populations of *Cedrus atlantica*. Numbers refer to the populations described in Table 1 and Fig. 1.

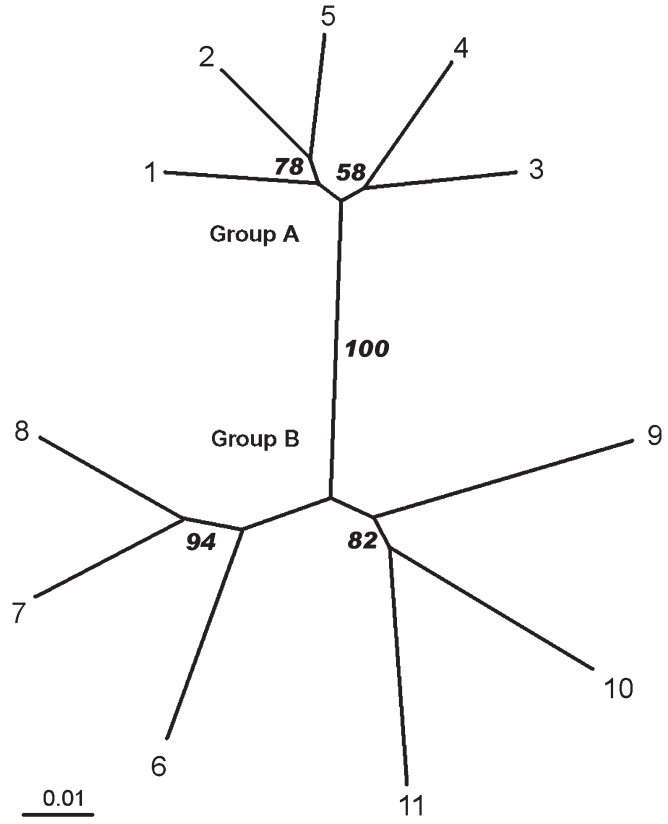

Fig. 3. Bootstrap majority rule (50%) consensus neighbor-joining tree of 1000 bootstrap replicates of AFLP data from 11 populations of *Cedrus atlantica*. Numbers refer to the populations described in Table 1 and Fig. 1.

underpinning its long-term persistence in the eastern Tell Atlas. Cluster III was most abundant in the western part of the species range; this might suggest that its postglacial expansion would have started from some (now orphaned) refugium close to the Atlantic coast and that populations would have undergone a successive admixture with populations containing other clusters. Some evidence speaks against such a scenario, however. First, the highest number of private AFLP fragments is not found in the westernmost populations but in the Algerian Tell Atlas: Four of the five private fragments found in population 9 occurred in individuals belonging to cluster III, which points to the long-term persistence of the cluster in this area. Second, the fossil record shows that *C. atlantica* arrived remarkably late in the Middle Atlas, certainly later than one could expect if a glacial refugium had existed near the Atlantic coast (Lamb et al., 1989). Hence, it seems most likely that cluster III would likewise have its origin in a glacial refugium near the coast of the Mediterranean Sea.

Although further analyses involving additional populations and complementary markers would be desirable to corroborate the inferences described, the observed patterns imply that *C. atlantica* would have persisted through the Last Glacial Maximum in at least three different glacial refugia located along the coast of the western Mediterranean Sea. Subsequently, populations would have expanded south to west and colonized the Middle Atlas, today the major core of the species range. The relatively marked directionality of this expansion might have arisen from a propensity of populations to spread along the major mountain chains of northern Algeria and Morocco (perhaps avoiding in this way competition with evergreen oak species at lower altitudes); such a migration along mountain ranges has recently also been documented for beech (*Fagus sylvatica* L.) in Europe (Magri et al., 2006).

A striking aspect of the patterns described in this study is seen in the disparate character of some stands in the Middle Atlas. It seems intriguing that three of the investigated populations have experienced an extensive admixture between the three clusters, whereas two other populations have remained remarkably homogeneous (Fig. 1): Population 5 lacks almost any sign of gene immigration from clusters II or III, whereas population 4 has only small traces of cluster II. These include moreover several pure individuals—a remarkable observation that indicates that the immigration of cluster II genotypes into population 4 is likely to be recent. The same might also be true for populations 4 and 5 themselves, because otherwise one would expect to find a markedly greater extent of gene immigration from nearby populations (cf. Liepelt et al., 2002; note that the smallest distance between "mixed" and "pure" populations is as short as 27 km). As a consequence, the Middle Atlas harbors today two types of *C. atlantica* stands with contrasting levels of genetic diversity: relatively low in the group A populations (4 and 5) and markedly higher in those belonging to group B (6–8). The origin of this striking pattern remains obscure because natural causes are difficult to imagine, whereas the age of the sampled trees (which established well before the onset of a systematic forest management) renders human interventions equally unlikely.

Overall, our study provides a compelling example of how combining different analytical approaches—in our case, F_{ST}-based and assignment techniques—can help elucidate complex range dynamics that would not have been decipherable with either approach alone. This study shows also that, although it is often most efficient to invest the major sampling effort in increasing the number of populations rather than the number of individuals within populations (Petit et al., 2005a), phylogeographical surveys should not neglect the potential information found within population, in particular when they are based on highly polymorphic markers such as AFLP.

Integrating molecular data and the fossil record—Macrofossils of the genus *Cedrus* have been found in several Eocene and Pliocene sediments across Europe (Gaussen, 1964), but the species disappeared from this region during the early Quaternary, most likely as a consequence of its temperature requirements (cf. Svenning, 2003). In contrast, the species has been continually present in the study region throughout the last 140 000 years (see Magri and Parra, 2002 for a review of *Cedrus* pollen records). Our genetic data suggest that *C. atlantica* has persisted through the Last Glacial Maximum in at least three distinct refugia located in the regions of the Rif and the central and eastern Tell Atlas. These results are corroborated by pollen records that demonstrate that several coastal mountain ranges of NW Africa harbored Atlas cedar forests shortly after the Last Glacial Maximum (Quézel, 1999; Magri and Parra, 2002). Such forests occurred also in areas where *C. atlantica* is absent today, most notably in Tunisia and eastern Algeria (Ben Tiba and Reille, 1982; Salamani, 1993). This fact suggests that additional glacial refugia may have existed further east of the three areas identified by us, whose populations would probably have disappeared during the species' range contraction in the Holocene. On the other hand, the current

midaltitude growing sites of *C. atlantica* were most likely covered by steppe vegetation with negligible tree cover during and shortly after the Last Glacial Maximum (Lamb et al., 1989; Elenga et al., 2000). Average temperatures in the region seem to have been at least 3–4°C lower than today during this period, which would have resulted in vegetation zones being around 1200 m lower than at present (Close and Wendorf, 1990). But whereas *C. atlantica* was relatively quick in recolonizing mountain ranges near the Mediterranean after the Last Glacial Maximum, its expansion further south clearly lagged behind the advance of suitable climate conditions, and the species arrived remarkably late at its present-day major range core in the Middle Atlas: Fossil *Cedrus* pollen does not appear in lake sediments of the central Middle Atlas as late as 8000 yr BP (Lamb et al., 1989, 1991), although even small *C. atlantica* populations growing in the region should have left some traces due to the abundant pollen production of this wind-pollinated species (Magri and Parra, 2002). In other words, the large Atlas cedar forests found today in the central Middle Atlas were established comparatively recently. This recent colonization could explain why some populations have experienced so little mixing, as described earlier. The phenomenon might have been further exacerbated by anthropogenic pressure because human activities started as early as 5000 yr ago to transform the forests of the Middle Atlas and to favor the increasing dominance of holm oak (*Quercus ilex* L.) forests at the expense of *C. atlantica* (Lamb et al., 1991), thereby favoring an early fragmentation of this species' populations.

Conclusions—The rangewide population genetic structure of *C. atlantica* is characterized by a series of particularities that can only be understood in the context of its Quaternary range dynamics, conditioned in part by the complex topography and climate of the study area. These dynamics include the persistence of the species during the Last Glacial Maximum in at last three refugia situated near the Mediterranean Sea, followed by an expansion toward the Middle Atlas region, which has since then become the major core of the species range.

Obviously, the postglacial range dynamics of *C. atlantica* differ greatly from the rapid, broad-scale, and poleward expansions that have been reported for many European temperate plant and animal taxa (Hewitt, 2004). The stark contrast underpins that North Africa has been subject to vegetation dynamics that cannot be easily extrapolated from processes that occurred in temperate Europe or North America. Further studies are clearly needed to achieve a better understanding of how the biota in this region have responded to past climate changes and will respond to those predicted for the near future (Hampe and Petit, 2005). Such knowledge is of vital importance given that North Africa is not only a major hotspot of plant biodiversity and endemism, both within and beyond the Mediterranean Basin (Médail and Quézel, 1997), but it is also one of the world's regions whose biodiversity seems most threatened by future climate change (IPCC, 2001).

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