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G. Besnard, P A Christin, D. Baali-Cherif, N. Bouguedoura, Fabien Anthelme. Spatial genetic structure in the Laperrine's olive (*Olea europaea* subsp. *laperrinei*), a long-living tree from the central Saharan mountains. *Proceedings of the National Academy of Sciences of the United States of America*, 2007, 104 (14), pp.5738-5742. 10.1073/pnas.0606292104 . hal-01625077

HAL Id: hal-01625077

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Submitted on 27 Oct 2017

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ORIGINAL ARTICLE

Spatial genetic structure in the Laperrine's olive (*Olea europaea* subsp. *laperrinei*), a long-living tree from the central Saharan mountains

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The Laperrine's olive (*Olea europaea* subsp. *laperrinei*) is an emblematic species of the Sahelo-Saharan Mountains. Populations of this tree are locally threatened by extinction due to climatic vicissitudes and human activities, particularly in Niger and Algeria. In order to study the spatial genetic structure and the dynamics of *O. e. laperrinei* populations, we sampled trees in four isolated mountain ranges (Tassili n'Ajjer and Hoggar (Algeria), Tamgak and Bagzane (Niger)). A total of 237 genets were identified using nuclear microsatellites. Phylogenetic reconstruction based on plastid DNA data supported a maternal origin of *O. e. laperrinei* populations in South Algeria, where a higher allelic richness was observed. Based on nuclear microsatellite data, two levels of structure were revealed: first, individuals from Niger and Algeria were separated in two distinct groups; second,

four less differentiated clusters corresponded to the four studied mountain ranges. These results give support to the fact that desert barriers have greatly limited long distance gene flow. Within populations, pairwise kinship coefficients were significantly correlated to geographical distance for Niger populations but not for Algerian mountains. Historical factors and habitat heterogeneity may explain the differences observed. We conclude that the Hoggar acts as an important genetic reservoir that has to be taken into account in future conservation programmes. Moreover, very isolated endangered populations (for example, Bagzane) displaying evident genetic particularities have to be urgently considered for their endemism.

Heredity (2007) **99**, 649–657; doi:10.1038/sj.hdy.6801051; published online 12 September 2007

Keywords: conservation genetics; microsatellites; olive tree; plastid DNA; spatial autocorrelation; threatened species

Introduction

The Saharan desert covers a large part of central Africa. Since the upper Miocene, it represents a natural barrier between Mediterranean and Tropical Africa which is difficult to cross for living organisms (Douady *et al.*, 2003). The number of species present in this area is particularly limited (Quézel, 1965), but the ratio of endemic species is relatively high in mountains that act as refugia for plants and animals due to higher rainfall in altitude. The Saharan flora has affinities with those from adjacent regions, mainly with the Mediterranean Basin, Arabia, Sahelian and Tropical Africa (Quézel, 1978, 1997). Genetic studies have confirmed that some Saharan species are closely related to Mediterranean taxa (for example, *Senecio*, Coleman *et al.*, 2003; *Atriplex*, Ortíz-Dorba *et al.*, 2005; *Erica*, McGuire and Kron, 2005). But they have also revealed complex biogeographic history for the Laperrine's olive populations (*Olea europaea* L. subsp. *laperrinei* (Cif.) Batt. and Trab.), which may be due to recurrent migration events from both Mediterranean and Tropical Africa (Besnard *et al.*, 2007). In the Saharan

mountains, long-term persistence of some species, particularly long-living trees, is uncertain, mostly due to their geographic isolation, their low regeneration associated with the impact of human activities and climatic vicissitudes. For instance, *Cupressus dupreziana* A. Camus (from the Tassili n'Ajjer) is classified as endangered taxon in the IUCN Red list (Abdoun and Beddiaf, 2002), while some others, such as the Laperrine's olive, are currently distributed in a highly fragmented habitat and locally threatened by extinction (Quézel, 1997). Moreover, an expected increase of local human activities (Anthelme *et al.*, 2006) as well as future global changes (Williams *et al.*, 2007) may add to the high isolation of the Saharan flora. It could then become even more vulnerable to a reduction of the favourable areas, particularly in the Niger mountains where the altitudinal range is smaller than in South Algeria.

The populations of *O. e. laperrinei* have regressed with the desert expansion during the Pleistocene (Wickens, 1976; Quézel, 1978; Maley, 1980). Small populations are present in mountains from central Sahara (Algeria: Hoggar, Tassili n'Ajjer, Mouydir; and Niger: Greboun, Tamgak, Bagzane) to northeast Sudan (Jebel Marra, Gourgueil). This tree grows at relatively high altitudes (generally from 1400 to 2700 m) on volcanic or eruptive rocks and its populations are often difficult to reach. It persists in very dry areas particularly in southern Algeria where mean rainfall can be less than 50 mm per year

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Received 17 November 2006; revised 26 July 2007; accepted 6 August 2007; published online 12 September 2007

(Quézel, 1965; Médail *et al.*, 2001). The persistence of this taxon could then be seriously affected by future environmental changes (Baali-Cherif and Besnard, 2005). Today, the wood of this tree is rarely exploited by humans but camels and goats intensively browse young shoots. This limits the possibility of regeneration of the *O. e. laperrinei* populations, which is considered as null by several authors (Maire, 1933; Quézel, 1978). However, even if survival of young trees has never been observed, a slow regeneration of populations cannot be excluded as reported for *C. dupreziana* in the Tassili n'Ajjer (Abdoun and Beddiaf, 2002; Abdoun *et al.*, 2005). The species is also considered as an emblematic tree for its very long persistence and rarity (Wickens, 1976). As a consequence, the preservation of *O. e. laperrinei* populations may have an increasing importance with the development of the eco-tourism in northern Niger and southern Algeria, but to date, the means given for their protection are still very limited.

For conservation purposes, the inventory of genetic resources of this Saharan relict tree is needed to understand the past and future evolution of its populations. Studying partition of the genetic diversity between and within populations can help unravel the dynamic of gene flow (for example, Baldoni *et al.*, 2006; Rubio de Casas *et al.*, 2006; Belaj *et al.*, 2007) and assess the importance of some geographic and biological features, such as habitat fragmentation, altitudinal distribution, clonal growth and lifespan of individuals. For instance, one can expect that desert barriers may have greatly limited gene flows between distant mountains and that small populations are more affected by stochastic effects (for example, genetic drift). Knowledge of population genetics may then provide baseline information for optimized management strategies (Escudero *et al.*, 2003) through the identification of areas that should preferentially benefit of a conservation programme, for example, because of a high genetic diversity or endemism.

In this context, a study on the genetic diversity of *O. e. laperrinei* populations from South Algeria and Niger is conducted with two main objectives: (1) to analyse the genetic structure of populations distributed in four isolated mountain ranges from Algeria and Niger using ptDNA and nuclear microsatellites. In particular, the allelic richness and the genetic differentiation between populations are assessed; and (2) to assess the importance of gene flow within each mountain range. A spatial autocorrelation analysis is performed to measure the degree of spatial genetic structure (SGS) at the population scale. Based on our observations, we discuss the dynamic of *O. e. laperrinei* populations in the central Saharan mountains and propose some possible conservation strategies.

Materials and methods

Plant material

To complete the data sample of subsp. *laperrinei* from South Algeria and North Niger previously analysed by Baali-Cherif and Besnard (2005) and Anthelme *et al.* (accepted), we prospected 64 additional tree samples in the Tassili n'Ajjer (southeast Algeria; Table 1). Clonal growth was previously shown (Baali-Cherif and Besnard, 2005; Anthelme *et al.*, accepted) and only appar-

ently unconnected ramets (individualized trunks) were sampled as already reported. Geographical coordinates of all trees were reported in each of the four mountain ranges sampled. Additionally, for four other localities (Assekrem, Jabbârene, In Acoulmou and Jebel Marra' crater), one to three ramets were also characterized (Table 1). A total of 311 ramets (including four herbarium samples from the Jebel Marra and the Tefedest) were analysed.

Genetic markers

To investigate the phylogenetic relationships between populations from Niger, Sudan and Algeria, polymorphism at 20 sites (10 microsatellite or indel loci and 10 PCR-RFLPs) of the plastid DNA (ptDNA) was investigated: *cemp5* and *cemp7* from Weising and Gardner (1999), 14 loci from Besnard *et al.* (2003) and four loci from Baali-Cherif and Besnard (2005). A total of 50 individuals (sampled in each mountain range) were characterized for each locus. The polymorphic loci were then used to characterize the whole sample. These ptDNA data allowed the investigation of (i) the phylogenetic relationships between populations and (ii) the genetic differentiation between populations based on a maternal marker.

Lastly, the SGS was investigated with nuclear microsatellite (simple sequence repeat (SSR)) markers. To characterize all sampled ramets from the Tassili n'Ajjer and the Assekrem, nine SSR loci were used as reported in Baali-Cherif and Besnard (2005) and Anthelme *et al.* (accepted): *ssrOeUA-DCA1*, *ssrOeUA-DCA3*, *ssrOeUA-DCA8*, *ssrOeUA-DCA9*, *ssrOeUA-DCA14*, *ssrOeUA-DCA15* (Sefc *et al.*, 2000), *GAPU45* (Carriero *et al.*, 2002), *PA(ATT)2* (Saumitou-Laprade *et al.*, 2000) and *EMO03* (de la Rosa *et al.*, 2002). For these nine loci, an absence or a very low frequency of null alleles was reported in subsp. *laperrinei* by Baali-Cherif and Besnard (2005). A total of 307 ramets (Table 1) were characterized with the nine SSR loci in the present work and in previous studies (Baali-Cherif and Besnard, 2005; Anthelme *et al.*, accepted).

Data analyses

The phylogenetic relationships between ptDNA haplotypes detected in subsp. *laperrinei* were analysed using a reduced Median network reconstructed with the NETWORK software (<http://www.fluxus-engineering.com/sharenet.htm>; Bandelt *et al.*, 1999). All haplotypes belonging to sub-lineage E1 not detected in subsp. *laperrinei* (haplotypes 1, 2 (subsp. *europaea*) and 8 (subsp. *cuspidata*); Besnard *et al.*, 2007) were also included in this analysis. In addition, haplotype no. 10 (sub-lineage E3 (subsp. *europaea*); Besnard *et al.*, 2007) was considered as out-group. This haplotype was closely related to sub-lineage E1 (Besnard *et al.*, 2007).

Based on nuclear SSR data, identical genotypes were identified on several independent ramets and we had to evaluate whether they represented the same individual (or genet). The probability that a zygote acquires a given diploid genotype, P_{gen} , was calculated, following Parks and Werth (1993). Population genetic analyses were then performed based on all distinct individuals. In order to account for different sample sizes of populations, allelic richness (R_S) was estimated for all SSR and ptDNA loci in each mountain range using the FSTAT software

Table 1 Geographic origin of the *O. e. laperrinei* populations and number of genets characterized for ptDNA and SSR genotyping

Mountain range, country	Locality	Altitude (m)	Latitude	Longitude	PtDNA ^a	SSR ^a
Hoggar, Algeria	Adjelella	1530	22°38'N	5°30'E	9	9 (10) ^b
	Adrar Heggueghene	1390–1600	22°47'N	5°37'E	57	57 (65)
	Issekrâm	1525–1605	22°50'N	5°33'E	16	16 (17)
	Tanarouatine	1580–1600	23°06'N	6°03'E	8	8 (13)
	Assekrem, Atakor	~2500	23°15'N	5°35'E	3	3 (3)
	In Acoulmou, Tefedest ^c	~2200	24°22'N	5°36'E	2	—
Tassili n'Ajjer, Algeria	Tamrit	1600–1710	24°37'N	9°35'E	45	45 (63)
	Jabbârene, Akba-Aroûm	~1750	24°28'N	9°43'E	1	1 (1)
Tamgak, Niger	Faille du Tamgak	1635–1810	19°03'N	8°39'E	45	45 (55)
Bagzane, Niger	Gomous—Bagzan'n'amas	1550–1850	17°49'N	8°46'E	52	52 (78)
Egalah, Niger	Egalah	~1650	18°10'N	8°41'E	1	1 (2)
Jebel Marra, Sudan	Jebel Marra' crater ^c	~2500	13°10'N	24°22'E	2	—

Abbreviation: SSR, simple sequence repeat.

^aThe number of genets per population characterized is given for each marker type.

^bThe number of ramets analysed using SSR data is given within parentheses.

^cFrom herbarium samples see Besnard *et al.* (2007).

(Goudet, 1995). The total genetic diversity (H_T ; Nei, 1987) was also estimated for each locus and each population. In order to get samples of comparable sizes and densities, individuals or patches distant from the core population of each mountain range were not considered. Thus, concerning the Hoggar, only the 57 individuals from the Adrar Heggueghene were used. A paired *t*-test (one-sided) was used to evaluate the significance of the differences of allelic richness (at nuclear loci) between each pair of populations. Similarly, a Wilcoxon test (non-parametric test) was used to evaluate the significance of the difference of gene diversity at nuclear SSR loci. Furthermore, the number of genetically homogeneous clusters (K) in the dataset was determined using a model-based clustering method implemented in the STRUCTURE software (Pritchard *et al.*, 2000). Bayesian analysis was run under the admixture model for 1 000 000 generations after a burn-in period of 100 000. Analyses were run for K between 1 and 10 with 10 iterations for each K -value. The most likely number of clusters was determined using the log probability of data as well as the absolute values of the second order rate of change of the likelihood distribution divided by the s.d. of the likelihoods (ΔK) following the recommendations given by Evanno *et al.* (2005). We also computed F_{ST} based on nuclear SSR data for each pair of populations according to Weir and Cockerham (1984) using FSTAT (Goudet, 1995). Given the lack of ptDNA polymorphism, we did not compute F_{ST} based on plastid markers. Significance of F_{ST} was tested using a permutation test. F_{IS} (fixation index) was also computed for each population using FSTAT. F_{IS} were tested for deviation from Hardy–Weinberg equilibrium by comparing the F_{IS} values computed from the observed data with values derived by randomly permuting (5000 times) the alleles among individuals.

The software BOTTLENECK version 1.2.02 (Piry *et al.*, 1999) was used to evaluate recent effective population size reductions in each mountain range. The two-phased model of mutation was applied with 5% of multi-step changes as recommended by Luikart and Cornuet (1998)

for microsatellite markers. The same model with 30% of multi-step changes and the stepwise mutation model were also tested.

To assess isolation by distance, the fine-scale SGS was investigated using a spatial autocorrelation method for each mountain range separately. In order to get samples of comparable sizes and densities, individuals or patches distant from the core population of each mountain range were not used for analyses. Thus, the individual from Jabbârene (Tassili n'Ajjer) was removed, and only the 57 individuals from the Adrar Heggueghene were considered in the Hoggar. Genetic relationships between all pairs of individuals within the same mountain range were regressed on the logarithmic geographical distance separating them by using SPAGEDi (Hardy and Vekemans, 2002). The kinship coefficient from Loiselle *et al.* (1995), robust against the presence of low frequency alleles, was used. Reference allele frequencies used to compute pairwise kinships were those of the whole sample. Significance of the regression coefficients was assessed through 10 000 permutations. In order to illustrate the trend of the relation between genetic and geographic distances, mean kinship coefficients were calculated for up to 10 distance intervals within each mountain range.

Results

Plastid DNA polymorphism and phylogenetic relationships between *O. e. laperrinei* populations

Six ptDNA haplotypes have been found in the *O. e. laperrinei* populations based on polymorphism of three ptDNA microsatellite loci (ccmp5, *trnT*-L-polyT and *psbK-trnS*-polyT/A; see Supplementary Material). One haplotype (no. 41) was observed for the first time in the present study. Five haplotypes were restricted to a specific mountain range: haplotype no. 7 to the Jebel Marra, haplotype no. 41 to the Bagzane and the Egalah, and haplotypes nos. 4, 5 and 6 to the Hoggar (Figure 1a).

Haplotype no. 3 was the most frequent one (73%) and was distributed in three mountain ranges (Hoggar, Tassili n'Ajjer and Tamgak). Phylogenetic relationships between haplotypes were inferred by Parsimony analysis (Figure 1b). Mediterranean haplotype no. 10 (out-group) allowed a polarization of the network. Interestingly, haplotype no. 3 displayed a basal position in sub-lineage E1, suggesting that it displays an ancestral state for the three microsatellite polymorphic loci.

Evidence of clonal growth in populations

A total of 109 alleles were revealed with the 9 nuclear SSR loci (Table 2). Among the 307 ramets analysed using SSRs, we recognized 237 distinct genets. A total of 54 (22.8%) genets were assigned to at least 2 ramets (13 (14%) in the Hoggar, 12 (26.1%) in the Tassili n'Ajjer, 21 (40.4%) in the Bagzane, 1 (100%) in the Egalah and 6 (13.3%) in the Tamgak). For these genets, the probability of obtaining the same genetic profile (P_{gen}) was extremely low, ranging from 7.2×10^{-5} (Bagzane no. 15 and 16) to 3.1×10^{-13} (Tamrit nos. 51 and 52). Moreover, in every case these ramets were sampled on the same site

and at a relatively small distance (less than 50 m). Consequently, we considered that two undistinguished ramets corresponded to different samples of the same plant and only one ramet per individual was considered for population genetic analyses.

Population genetic analyses

Gene diversity (H_T) and allelic richness (R_S) were compared between the four main mountain ranges investigated (Table 2). Similar gene diversity values (ranging from 0.60 to 0.65) were observed in each mountain range. In contrast, the Hoggar displayed the highest allelic richness for both nuclear DNA and ptDNA loci. Thus, for nuclear SSRs, R_S was significantly higher in the Hoggar than in the Bagzane after Bonferroni correction ($t=3.01$, 8 d.f., $P<0.05$). The fixation index (F_{IS}) was not significant for the four mountain ranges supporting a low level of consanguinity in the *O. e. laperrinei* populations. In addition, we did not find any recent reduction of effective population size in the four investigated populations whatever the model used (data not shown).

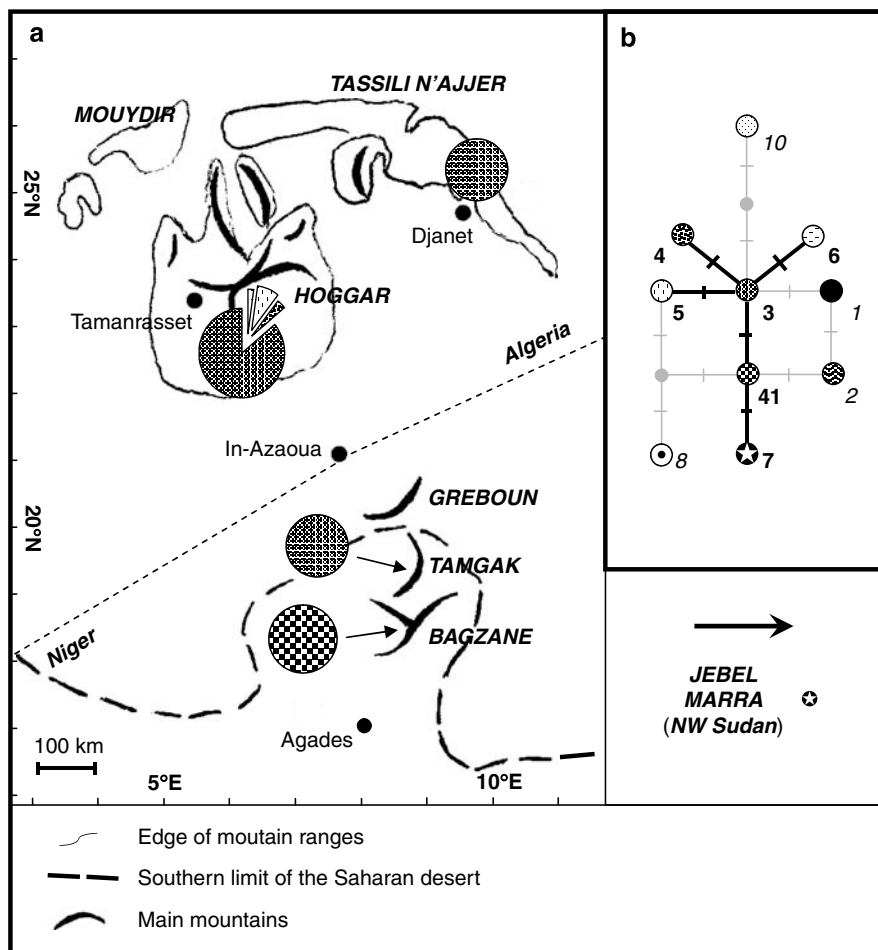


Figure 1 Plastid DNA polymorphism in the *O. e. laperrinei* populations. (a) Distribution of plastid DNA haplotypes (represented by pie charts) in each mountain range from southern Algeria and northern Niger. Size of pie charts is proportional to the number of individuals analysed. (b) Reduced Median Network (Bandelt *et al.*, 1999) representing phylogenetic relationships of plastid DNA haplotypes in the sub-lineage E1 (Besnard *et al.*, 2007). Each haplotype is represented by a particular symbol and its corresponding number. The six haplotypes found in *O. e. laperrinei* populations (nos. 3, 4, 5, 6, 7 and 41) are indicated by a number in bold and their relationships are represented by boldest links. Haplotype 10 (sub-lineage E3; Besnard *et al.*, 2007) is used as out-group.

Table 2 Total number of alleles (N_a) observed in *O. e. laperrinei* populations from Algeria and Niger, allelic richness (R_s), genetic diversity (H_T) and global fixation index (F_{IS}) computed on nuclear SSR loci and ptDNA haplotypes for each population

Locus	N_a	Bagzane			Tamgak			Tassili n'Ajjer			Hoggar ^a		
		R_s	H_T	F_{IS}	R_s	H_T	F_{IS}	R_s	H_T	F_{IS}	R_s	H_T	F_{IS}
<i>Nuclear SSR</i>													
ssrOeUA-DCA1	31	16.5	0.88		19.0	0.85		18.9	0.91		20.4	0.92	
ssrOeUA-DCA3	9	7.0	0.76		8.0	0.78		6.0	0.64		6.8	0.69	
ssrOeUA-DCA8	12	7.0	0.71		4.0	0.72		9.0	0.80		10.5	0.82	
ssrOeUA-DCA9	14	8.7	0.74		10.0	0.72		7.0	0.76		9.5	0.54	
ssrOeUA-DCA14	15	5.8	0.68		7.0	0.71		9.9	0.78		11.4	0.81	
ssrOeUA-DCA15	3	2.0	0.49		2.0	0.50		3.0	0.25		2.0	0.22	
PA(ATT)2	3	2.0	0.06		2.0	0.13		3.0	0.29		2.0	0.09	
GAPU45	6	3.0	0.39		3.0	0.52		5.0	0.64		4.8	0.71	
EMO03	16	8.0	0.68		8.0	0.78		7.0	0.78		12.4	0.83	
Total	109	60.0	0.60	0.015 ^{NS}	63.0	0.63	0.061 ^{NS}	68.8	0.65	0.010 ^{NS}	79.8	0.63	0.021 ^{NS}
ptDNA	5	1.0	0.00	—	1.0	0.00	—	1.0	0.00	—	4.0	0.29	—

Abbreviations: NS, non-significant; SSR, simple sequence repeat.

^aComputations were done using the 57 genets from the Adrar Heggueghene. Similar values were obtained when all genets sampled in the Hoggar were considered (data not shown).

We then investigated the genetic differentiation between each pair of mountain ranges using F_{ST} (Table 3). Based on nuclear SSR data, all F_{ST} values were significantly different from zero and ranged from 2.1 (Tassili n'Ajjer/Hoggar) to 11.6% (Tassili n'Ajjer/Bagzane). Based on ptDNA polymorphism, we only found a strong genetic differentiation between the Bagzane and all other mountains due to the fixation of a specific haplotype (41; Figure 1). Thus, the most distant mountains (Tassili n'Ajjer and Bagzane) were also the most differentiated. To investigate in more details the genetic structure in our nuclear SSR dataset, a model-based Bayesian procedure was used to identify clusters of individuals. According to the ΔK criteria of Evanno *et al.* (2005), the most likely number of clusters in our dataset is two (Figure 2b). However, ΔK also showed a peak at $K=4$. Together with the maximum likelihood and the low variability observed for this value (Figure 2a), this number of clusters also fits the required criteria. This suggests that $K=2$ detects the highest level of genetic structure whereas $K=4$ concerns a lower level. Thus, individual assignments to the different clusters averaged over the 10 iterations were considered for $K=2$ (Figure 2c) and $K=4$ (Figure 2d). For $K=2$ (Figure 2c), the clustering allowed the separation of most individuals from the Niger (cluster I) and Algerian (cluster II) mountains. This result indicates that the gene flow between Niger and Algerian populations is particularly limited. Only 17 (7.2%) individuals (8 from the Hoggar, 2 from the Tassili n'Ajjer and 7 from the Tamgak) were assigned to a cluster with a posterior probability inferior to 80%. Seven individuals (4 from the Tamgak and 3 from the Hoggar) were not assigned to the correct cluster according to their geographic origin with a posterior probability higher than 50%. For $K=4$ (Figure 2d), a more complex pattern was obtained. Individuals from the Bagzane were assigned to cluster IA with a mean probability of 86.7% and those from the Tamgak were assigned to cluster IB with a mean posterior probability of 79.5%. The assignment of individuals from the Hoggar and the Tassili n'Ajjer to a particular cluster was less efficient than in the Niger populations. Thus, individuals from the Hoggar were assigned to cluster IIA with a

Table 3 Pairwise genetic differentiation between populations (F_{ST} in percent) based on nuclear microsatellite data

	Bagzane	Tamgak	Hoggar
Tamgak	7.4*		
Hoggar	11.1*	9.7*	
Tassili n'Ajjer	11.6*	9.4*	2.1*

Significance values for pairwise test of population differentiation are based on 6000 permutations.

* $P < 0.001$.

mean posterior probability of 55.3% and to cluster IIB with a mean posterior probability of 36.1%. Similarly, individuals from the Tassili n'Ajjer were assigned to cluster IIB with a mean posterior probability of 73.9% and to cluster IIA with a mean posterior probability of 20.4%. These results demonstrate that populations from the Hoggar and Tassili n'Ajjer are poorly differentiated (as previously shown with F_{ST}).

Gene flow within population

Kinship coefficients calculated using SPAGEDi showed a significant decrease with logarithmic geographical distance in the Bagzane (intercept=0.1725, slope = -0.0089, P -value<0.0001, $R^2=0.02$) and the Tamgak (intercept = 0.2070, slope = -0.0166, P -value = 0.0018, $R^2=0.04$), indicating that individuals from these mountain ranges are subject to isolation by distance. In contrast, genetic coefficients were not related to logarithmic geographical distance in the Hoggar (P -value = 0.15) and in the Tassili n'Ajjer (P -value = 0.29). Mean kinship coefficients on distance intervals are presented together with the regression curves (Figure 3).

Discussion

Population dynamics of the Algerian and Niger populations

The plastid and nuclear DNA data attest that the Laperrine's olive has maintained a high genetic diversity in the South Algerian Mountains compared to Niger

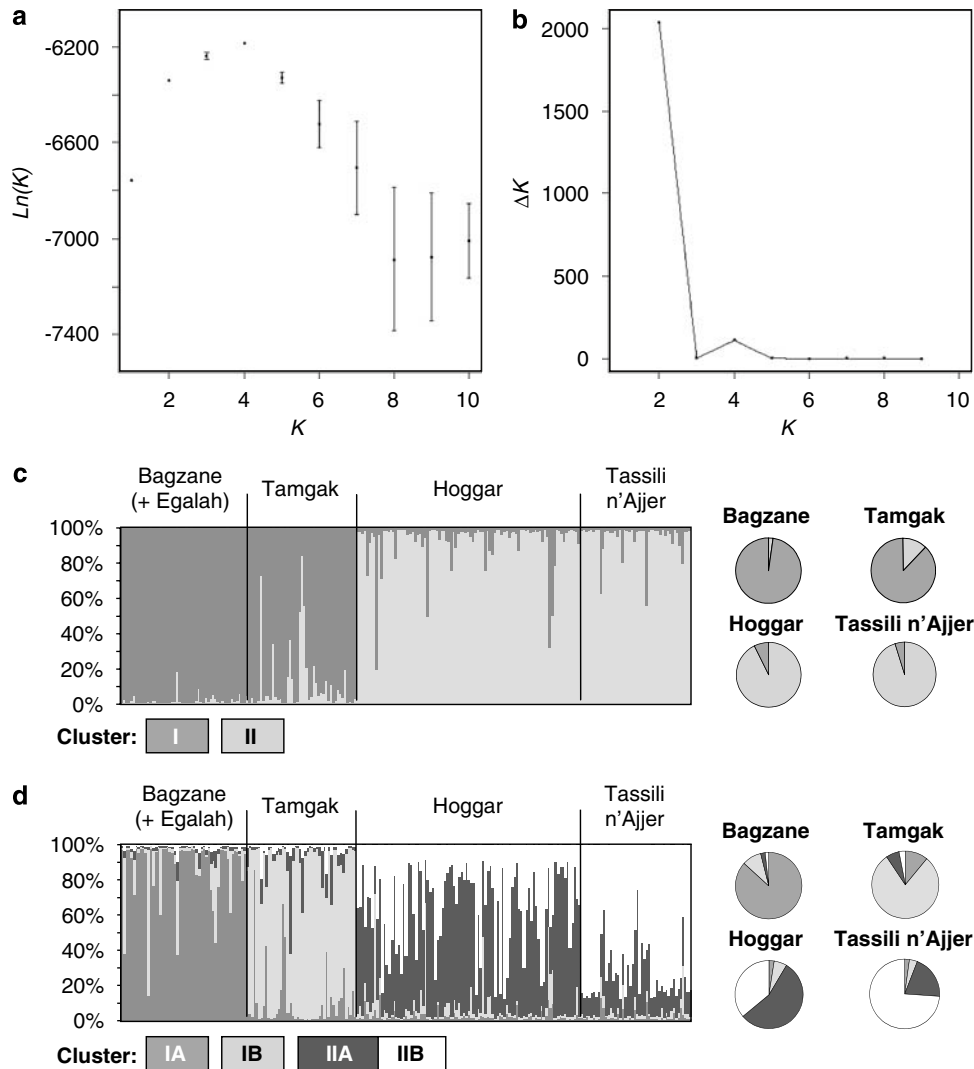


Figure 2 Inference of population structure based on nuclear SSR data and using Bayesian simulations (Pritchard *et al.*, 2000). (a) Mean log likelihood ($\ln(K) \pm \text{s.d.}$) averaged over the 10 iterations; (b) Absolute values of the second-order rate of change of the likelihood distribution divided by the s.d. of the likelihoods (ΔK in Evanno *et al.*, 2005). The percentage of assignment of each individual to the different clusters averaged over 10 iterations is shown for (c) two clusters and (d) four clusters. Each vertical bar represents an individual.

populations (Table 2). This high diversity (particularly in the Hoggar) could be due to an origin of the *O. e. laperrinei* populations in South Algeria as hypothesized by Wickens (1976). Haplotype no. 3 displayed a basal position in the ptDNA haplotype network (Figure 1), and its high frequency in South Algeria together with the presence of infrequent derived haplotypes (nos. 4, 5 and 6) suggests that the maternal lineage of subsp. *laperrinei* has probably diversified in the Hoggar. The highest genetic diversity in the Hoggar could also be due to the long persistence of a larger population in South Algeria, which would be less affected by stochastic effects than in other mountains. However, the population size in the Hoggar and the Tamgak are very similar (about several thousands of trees; Anthelme *et al.*, accepted), which would suggest that the low genetic diversity in the Tamgak population was not a consequence of recent loss associated with a reduced effective population size. Other factors, such as levels of populations regeneration (longevity of trees), the population density, the intensity

of gene flow between distant patches and the occurrence of barriers to dispersion may also be involved in the different levels of diversity found between the Niger and Algerian mountains (Escudero *et al.*, 2003). The long lifespan of individuals (associated with clonal growth) was proposed to maintain a high genetic diversity in Algerian populations (Baali-Cherif and Besnard, 2005). In the present study, we showed that the frequency of individuals with several ramets is not higher in Algeria (18%) than in Niger (28.9%) suggesting that this survival mode does not explain the highest diversity in Algerian mountains.

Our analyses of the genetic differentiation (Table 3, Figure 2) suggest that a combined effect of the geographic distance between the mountains with the presence of desert barriers has limited the occurrence of gene flow between populations. Thus, two levels of structure were found in our nuclear data. The first one allowed the identification of most individuals from Niger and Algerian mountains. These areas are strongly

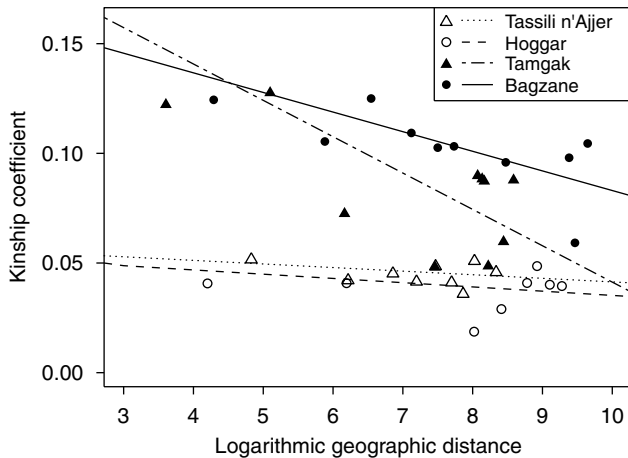


Figure 3 Comparative analysis of the intra-mountain range genetic differentiation (kinship coefficient) according to the logarithmic geographical distance. Mean kinship coefficients (Loiselle *et al.*, 1995) are shown for distance intervals in each mountain range. Regressions curves of the kinship coefficients between all pairs of individuals within a mountain range on the logarithmic geographical distances are added for each mountain range. Regression slopes for the Bagzane and Tamgak were significant (P -value = 0.0018 and 0.0001, respectively) whereas those of the Hoggar and Tassili n'Ajjer were not.

isolated by a desert barrier which may greatly limit long distance gene flow (> 500 km). The second level of structure showed a less pronounced differentiation among the two populations from Niger and Algeria, and in particular between the Hoggar and the Tassili n'Ajjer (Figure 2). From these observations, we cannot exclude possible exchanges between adjacent mountains in relatively recent periods when environmental conditions were more favourable (for instance during the last humid transition of the Sahara about 3500 years ago; Quézel, 1965; Crernaschi *et al.*, 2006). Finally, plastid genome data alone revealed a very strong differentiation of the Bagzane population compared to others (Table 3). This clearly indicates that long distance dispersion by seeds may be particularly infrequent, but unfortunately the low level of variation in the plastid genome did not allow any hypothesis testing for each pair of mountain ranges.

We showed that kinship coefficients were significantly correlated to geographical distance within Niger populations (Figure 3). The detection of a significant SGS at a local scale in this very long-living species is surprising since SGS in out-breeding trees is classically low (Veke-mans and Hardy, 2004). However, no SGS was observed within Algerian mountain ranges suggesting that the dynamic of Niger and Algerian populations is affected by different factors. Two non-exclusive explanations can be proposed:

(1) Occurrence of SGS in populations from Niger indicates that individuals from the same patch or from adjacent patches are constituted of relatives. A limited seed and/or pollen dispersal, due to the heterogeneity of the habitat, may explain this observation (Escudero *et al.*, 2003; Troupin *et al.*, 2006; Van Rossum and Triest, 2007). The Laperrine's olive trees are dispersed in patches often isolated by

several kilometres in both Niger and Algerian mountains. However, South Algerian mountains (particularly the Hoggar) display a higher altitudinal range allowing the species to persist in a larger area than in Niger (Anthelme *et al.*, accepted). Furthermore, the important network of wadis (interconnected temporary rivers) found in the Hoggar could have facilitated gene flow between distant patches, and thus reducing SGS in this mountain range. In contrast, some physical constraints to dispersion in Niger (due to the absence of a dense network of wadis between the different mounts) may have favoured the appearance and the maintenance of SGS.

(2) A supposed relatively recent colonization of subsp. *laperrinei* in Niger associated with limited seed dispersal and low density of maternal seed sources may also have created genetic structure within population (Dutech *et al.*, 2002; Jones and Hubbell, 2006). In contrast, the absence of SGS within Algerian populations could be due to long persistence of subsp. *laperrinei* in this area. Recurrent gene flow between distant patches (migration) may have then obscured SGS in this long-living species.

Based on our current knowledge, it is difficult to determine the exact importance of habitat heterogeneity (affecting dispersion) and historical factors in the maintenance of genetic diversity. A fine ecological description of the habitat is needed to model gene dispersal in each mountain range and to test if the genetic variation found is driven by ecological factors.

On the conservation strategy of populations of subsp. *laperrinei*

The long-term persistence of the Laperrine's olive is uncertain due to climatic vicissitudes and the strong human pressures in the Saharan mountains. However, it was previously demonstrated that this tree is able to survive in very dry areas during very long periods using an efficient vegetative growth (Baali-Cherif and Besnard, 2005). The main dilemma of long-living Saharan trees (as Laperrine's olive or *C. dupreziana*) is the low turnover of their populations. This biological characteristic makes these species vulnerable to environmental changes, and present and past human activities (for example, wood exploitation, browsing) have largely contributed to their regression (Quézel, 1965). Moreover, an absence of sexual reproduction and limited seed dispersal may affect the evolutionary potential of these taxa. Indeed, the present impossibility to assort genes and create new uniting genomes may reduce the opportunities of adaptation to future climatic changes (Honnay and Bossuyt, 2005). However, a great phenotypic plasticity has probably allowed them to persist for very long times (probably several thousand years; Abdoun *et al.*, 2005; Baali-Cherif and Besnard 2005). Seeds of the Laperrine's olive have recently been collected in the field (D Baali-Cherif, unpublished data). Successful germination trials indicated that it may be possible to produce seedlings which could be used to recreate populations. However, this strategy is heavily dependent on the possibility to collect seeds in the field. Most of trees generally do not flower every year, and only a few individuals were shown to be able to produce seeds (D Baali-Cherif, unpublished data).

Thus, only a few individuals may be involved in sexual reproduction and the use of seedlings issued from these trees could have some negative consequences on the genetic diversity of planted populations. Future seed collections should take into account the SGS described in the present study (for example, Chung *et al.*, 1998), and in order to maximize the genetic diversity, individuals must be collected from an area as wide as possible. Therefore, the management strategy of the Saharan relict trees is still an open question, and before to start any programme, the education of human populations will be an essential step to conserve the ecosystems of the Saharan mountains (Quézel, 1997). Our data also suggest that the Hoggar presently acts as an important gene reservoir for the Saharan olive populations, and due to its high altitudinal range, it probably played this role during previous driest episodes of the Pliocene and Pleistocene (Quézel, 1978). Consequently this area may be considered as a priority in future conservation programmes, but very isolated endangered populations displaying evident genetic particularities (for example, Bagzane; Figures 1 and 2) have also to be urgently considered for their endemism.

Acknowledgements

We thank G Evanno, N Galland, N Salamin and two anonymous reviewers for helpful comments on this manuscript, and A Aboukader for his help in collecting plant material in the Air.

References

- Abdoun F, Beddief M (2002). *Cupressus dupreziana* A. Camus: répartition, dépérissement et régénération au Tassili n'Ajjer, Sahara central. *C R Biol* **325**: 617–627.
- Abdoun F, Jull AJT, Guibal F, Thimon M (2005). Radial growth of the Sahara's oldest trees: *Cupressus dupreziana* A. Camus. *Trees Struct Funct* **19**: 661–670.
- Anthelme F, Abdoukader A, Besnard G: Distribution, shape and clonal growth of the rare endemic tree *Olea europaea* subsp. *laperrinei* (Oleaceae) in the Saharan mountains of Niger. *Plant Ecol* (accepted).
- Anthelme F, Waziri Mato M, de Boissieu D, Giazzi F (2006). Dégradation des ressources végétales au contact des activités humaines et perspectives de conservation dans le massif de l'Air (Sahara, Niger). *VertigO* **7**: 1–12. (<http://www.vertigo.uqam.ca/vol7no2/framerevue.html>).
- Baali-Cherif D, Besnard G (2005). High genetic diversity and clonal growth in relict populations of *Olea europaea* subsp. *laperrinei* (Oleaceae) from Hoggar, Algeria. *Ann Bot* **96**: 823–830.
- Baldoni L, Tosti N, Ricciolini C, Belaj A, Arcioni S, Pannelli G *et al.* (2006). Genetic structure of wild and cultivated olives in the Central Mediterranean Basin. *Ann Bot* **98**: 935–942.
- Bandelt HJ, Forster P, Röhl A (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* **16**: 37–48.
- Belaj A, Muñoz-Diez C, Baldoni L, Porceddu A, Barranco D, Satovic Z (2007). Genetic diversity and population structure of wild olives from the north-western Mediterranean assessed by SSR markers. *Ann Bot* **100**: 449–458.
- Besnard G, Rubio de Casas R, Vargas P (2003). A set of primers for length and nucleotide-substitution polymorphism in chloroplast DNA of *Olea europaea* L. (Oleaceae). *Mol Ecol Notes* **3**: 651–653.
- Besnard G, Rubio de Casas R, Vargas P (2007). Plastid and nuclear DNA polymorphism reveals historical processes of isolation and reticulation in the olive tree complex (*Olea europaea* L.). *J Biogeogr* **34**: 736–752.
- Carriero F, Fontanazza G, Cellini F, Giorio G (2002). Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). *Theor Appl Genet* **104**: 301–307.
- Chung MY, Chung GM, Chung MG, Epperson B (1998). Spatial genetic structure in populations of *Cymbidium goeringii* (Orchidaceae). *Genes Genet Syst* **73**: 281–285.
- Coleman M, Liston A, Kadereit JW, Abbott RJ (2003). Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). *Am J Bot* **90**: 1446–1454.
- Cernaschi M, Pelfini M, Santilli M (2006). *Cupressus dupreziana*: a dendroclimatic record for the middle-late Holocene in the central Sahara. *Holocene* **16**: 293–303.
- de la Rosa R, James CM, Tobutt KR (2002). Isolation and characterization of polymorphic microsatellites in olive (*Olea europaea* L. and their transferability to other genera in the Oleaceae. *Mol Ecol Notes* **2**: 265–267.
- Douady CJ, Catzeflis F, Raman J, Springer MS, Stanhope MJ (2003). The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). *Proc Natl Acad Sci USA* **100**: 8325–8330.
- Dutech C, Seiter J, Petronelli P, Joly HI, Jarne P (2002). Evidence of low gene flow in a neotropical clustered tree species in two rainforest stands of French Guiana. *Mol Ecol* **11**: 725–738.
- Escudero A, Iriondo JM, Torres ME (2003). Spatial analysis of genetic diversity as a tool for plant conservation. *Biol Conserv* **113**: 351–365.
- Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* **14**: 2611–2620.
- Goudet J (1995). FSTAT (version 1.2): a computer programme to calculate *F*-statistics. *J Hered* **86**: 485–486.
- Hardy OJ, Vekemans X (2002). SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population. *Mol Ecol Notes* **2**: 618–620.
- Honnay O, Bossuyt B (2005). Prolonged clonal growth: escape route or route to extinction? *Oikos* **108**: 427–432.
- Jones FA, Hubbell SP (2006). Demographic spatial genetic structure of the Neotropical tree, *Jacaranda copaia*. *Mol Ecol* **15**: 3205–3217.
- Loiselle BA, Sork VL, Nason J, Graham C (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *Am J Bot* **82**: 1420–1425.
- Luikart G, Cornuet JM (1998). Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv Biol* **12**: 228–237.
- Maire R (1933). Etudes sur la flore et la végétation du Sahara central. *Mémoires de la Société d'Histoire Naturelle de l'Afrique du Nord*, no 3, Mission du Hoggar II. pp 166–168, Alger.
- Maley J (1980). Les changements climatiques de la fin du Tertiaire en Afrique: leur conséquence sur l'apparition du Sahara et de sa végétation. In: Williams MAJ, Faure H (eds). *The Sahara and the Nile*. AA Balkema: Rotterdam. pp 63–86.
- McGuire AF, Kron KA (2005). Phylogenetic relationships of European and African *Ericas*. *Int J Plant Sci* **166**: 311–318.
- Médail F, Quézel P, Besnard G, Khadari B (2001). Systematics, ecology and phylogeographic significance of *Olea europaea* L. ssp. *maroccana* (Greuter & Burdet) P. Vargas *et al.*, a relictual olive tree in south-west Morocco. *Bot J Linn Soc* **137**: 249–266.
- Nei M (1987). *Molecular Evolutionary Genetics*. Columbia University Press: New York.
- Ortiz-Dorba J, Martínez-Mora C, Correal E, Simón B, Cenís JL (2005). Genetic structure of *Atriplex halimus* populations in the Mediterranean Basin. *Ann Bot* **95**: 827–834.
- Parks JC, Werth CR (1993). A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *Am J Bot* **80**: 120–126.

- Piry S, Luikart G, Cornuet JM (1999). Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J Hered* **90**: 502–503.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure from multilocus genotype data. *Genetics* **155**: 945–959.
- Quézel P (1965). *La végétation du Sahara. Du Tchad à la Mauritanie*. Gustav Fisher Verlag: Stuttgart.
- Quézel P (1978). Analysis of the flora of Mediterranean and Sahara Africa. *Ann MO Bot Gard* **65**: 479–534.
- Quézel P (1997). High mountains of the Central Sahara: dispersal, speciation, origin and conservation of the flora. In: Barakat HN, Hegazy AK (eds). *Reviews in Ecology: Desert Conservation and Development*. Metropole: Cairo, Egypt, pp 159–175.
- Rubio de Casas R, Besnard G, Schoenswetter P, Balaguer L, Vargas P (2006). Extensive gene flow blurs phylogeographic but not phylogenetic signal in *Olea europaea* L. *Theor Appl Genet* **113**: 575–583.
- Saumitou-Laprade P, Vassiliadis C, Epplen JT, Hardt C (2000). Isolation of microsatellite loci for paternity in *Phillyrea angustifolia* L. (Oleaceae). *Mol Ecol* **9**: 112–114.
- Sefc KM, Lopes MS, Mendonça D, Rodrigues dos Santos M, Laimer da Câmara Machado M, da Câmara Machado A (2000). Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. *Mol Ecol* **9**: 1171–1173.
- Troupin D, Nathan R, Vendramin GG (2006). Analysis of spatial genetic structure in an expanding *Pinus halepensis* population reveals development of fine-scale genetic clustering over time. *Mol Ecol* **15**: 3617–3630.
- Van Rossum F, Triest L (2007). Fine-scale spatial genetic structure of the distylous *Primula veris* in fragmented habitat. *Plant Biol* **9**: 374–382.
- Vekemans X, Hardy OJ (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol* **13**: 921–935.
- Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
- Weising K, Gardner RC (1999). A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome* **42**: 9–19.
- Wickens GE (1976). Speculations on long distance dispersal and the flora of Jebel Marra (Sudan). *Kew Bull* **31**: 105–150.
- Williams JW, Jackson ST, Kutzbach JE (2007). Projected distributions of novel and disappearing climates by 2100AD. *Proc Natl Acad Sci USA* **104**: 5738–5742.

Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)