Journal of Biogeography (*J. Biogeogr.*) (2006) **33**, 1916–1928

ORIGINAL ARTICLE

Genetic diversity and gene flow between the wild olive (oleaster, *Olea europaea* L.) and the olive: several Plio-Pleistocene refuge zones in the Mediterranean basin suggested by simple sequence repeats analysis

Catherine Breton†, Michel Tersac and André Bervillé*

UMR-DGPC, INRA Bât 33, Montpellier cedex 1, France

ABSTRACT

Aim The oleaster is believed to have originated in the eastern Mediterranean, implying that those in the western Mediterranean basin could be feral. Several studies with different molecular markers (isozymes, random amplified polymorphic DNA, amplified fragment length polymorphism) have shown a cline between the eastern and the western populations, which supports this hypothesis. To reconstruct the post-glacial colonization history and establish a relationship between olive and oleaster populations in the Mediterranean basin, analyses were carried out on the genetic variation of chloroplast DNA (chlorotype) and at 12 unlinked simple sequence repeat (SSR) loci, sampling a total of 20 oleaster groves.

Location This is the first known large-scale molecular study of SSR loci based on samples of both oleasters and cultivars from the entire Mediterranean basin.

Methods Samples were taken from 166 oleasters in 20 groves of modern populations, and 40 cultivars to represent molecular diversity in the cultivated olive. The Bayesian method and admixture analysis were used to construct the ancestral populations (RPOP; reconstructed panmictic oleaster populations) and to estimate the proportion of each RPOP in each tree. If one tree can be assigned to two or more RPOPs, it can be regarded as a product of hybridization between trees from different populations (i.e. admix origin).

Results On this first examination of the SSR genetic diversity in the olive and oleaster, it was found to be structured in seven RPOPs in both eastern and western populations. Based on different population genetic methods, it was shown that: (1) oleasters are equally present in the eastern and the western Mediterranean, (2) are native, and (3) are not derived from cultivars. Chlorotypes (one and three in the eastern and western Mediterranean, respectively) revealed fruit displacement for the oleasters.

Main conclusions Oleaster genetic diversity is divided into seven regions that could overlay glacial refuges. The gradient, or cline, of genetic diversity revealed by chloroplast and SSR molecular markers was explained by oleaster recolonization of the Mediterranean basin from refuges after the last glacial event, located in both eastern and western regions. It is likely that gene flow has occurred in oleasters mediated by cultivars spread by human migration or through trade. Animals may have helped spread oleasters locally, but humans have probably transported olives but not oleaster fruits over long distances. We found that cultivars may have originated in several RPOPs, and thus, some may have a more complex origin than expected initially.

*Correspondence: André Bervillé, UMR-DGPC, INRA Bât 33, 2, place Viala, F-34060 Montpellier cedex 1, France. E-mail: berville@ensam.inra.fr

†Present address: Université de Montpellier 2, Laboratoire Génome, Populations, Interactions, Adaptation, UMR 5171, F-34200 Sète, France.

www.blackwellpublishing.com/jbi
doi:10.1111/j.1365-2699.2006.01544.x

Keywords
Chlorotypes, crop evolution, gene flow, genetic diversity, glacial refugia, Mediterranean basin, *Olea europaea*, plant colonization, SSR analysis.

INTRODUCTION

According to botanists, the olive and oleaster correspond to *Olea europaea* subsp. *europaea* L. var. *europaea* and var. *sylvestris*, respectively. Oleaster is the wild form, while the olive is the cultivated form. The genetic relationships between the two remain unclear, as in many cases the trees cannot be assigned by morphology alone to either form (Besnard & Bervillé, 2000; Lumaret & Ouazzani, 2001). Confusion between the two variants may happen in abandoned olive groves, particularly due to the tree's morphology and leaf characteristics, and especially for juvenile plants. Feral trees can also originate from cultivars escaping from natural or agro ecosystems, or from female oleasters hybridizing with cultivars. As olive trees may live for at least several hundred years, isolated trees can occur when all traces of cultivation have disappeared. It is, therefore, assumed that the olive and oleaster cannot be distinguished in some locations.

The olive is economically important, while having historic and cultural significance in the Mediterranean basin. Recently, olive cultivar diversity has been documented with molecular markers, first isozymes (Lumaret *et al.*, 1997) and then amplified fragment length polymorphism (AFLPs) (Angiolillo *et al.*, 1999) and random amplified polymorphic DNA (RAPD) (Fabbri *et al.*, 1995; Besnard *et al.*, 2001a) and recently with simple sequence repeat (SSR) analysis for cultivar identification (Cipriani *et al.*, 2002) and breeding (Hatzopoulos *et al.*, 2002). Although Green (2002) has listed criteria to distinguish olive and oleaster trees, many cannot be classified by fruit and stone dimensions since continuous taxonomic variations occur naturally. Few studies have investigated the diversity of oleaster genetic structures and the origins of domesticated olive trees (Besnard & Bervillé, 2000). As little is known about the history of olive cultivars and their relationships with oleaster, we have developed an approach using molecular markers to address this gap.

Oleaster and most olive cultivars are self-incompatible (Villemur *et al.*, 1984). Out-crossing is wind mediated by pollen transported over long distances, with male-sterile cultivars pollinated efficiently by surrounding cultivars or even oleasters (Besnard *et al.*, 2000). Gene flow between cultivated olives and oleasters has not hitherto been subjected to detailed study (Bronzini *et al.*, 2002; Mekuria *et al.*, 2002; Breton *et al.*, 2006).

One common assumption in the literature is that the oleaster originated in the eastern Mediterranean basin where it was domesticated about 5850 years ago (Zohary & Spiegel Roy, 1975), which infers an absence in the western end. Domestication has been documented and previously reported by several authors (e.g. Zohary, 1994), with the assertion that cultivars moved westward with human migration supported by several authors (Besnard *et al.*, 2001a; Belaj *et al.*, 2002). Once cultivars were established in the western Mediterranean, birds may have played a role in disseminating seeds, resulting in feral olive trees (Spennemann & Allen, 2000). These trees may be considered as oleasters by botanists and therefore confused with genuine oleasters from the eastern Mediterranean. In the present paper we examine the genetic diversity patterns of oleasters and cultivars from the eastern and western Mediterranean, in order to evaluate the following model.

Cultivars that originated from olive domestication in the east probably inherited a restriction, or bottleneck, in genetic diversity in comparison to the oleaster diversity in the eastern Mediterranean. Transplantation to the western Mediterranean is likely to have further narrowed this bottleneck in western cultivars if just a single domestication event occurred in the east. It is expected that molecular markers would show the secondary differentiation in feral olives resulting from out-crossing among cultivars or between cultivars and oleasters. Putative feral olive trees may not differ from the eastern oleasters when using phenotypic descriptions, but they may be differentiated by molecular markers. This study compares the genetic diversity between eastern and western oleasters and between cultivars to confirm the accuracy of the model.

Microsatellite (SSR) markers have recently become available for the olive (Sefc *et al.*, 2000). When compared with isozymes these markers have the advantage of being more numerous and by comparison with RAPD or AFLP are co-dominant, as two alleles may be identified at each locus. Transferability of microsatellites among *O. europaea* has been demonstrated (Rallo *et al.*, 2003), but no study has been carried out on the relationships between olive and oleaster, except for a preliminary investigation by Breton *et al.* (2006).

For this study, 20 groves of oleaster (166 trees) were chosen in the Mediterranean basin. Genetic variation of the chlorotype was analysed to trace the gene flow via seed movement. Twelve microsatellite loci were also used to detect pollen-based gene flow, with Bayesian clustering employed to create reconstructed panmictic (random mating of individuals) oleaster populations (RPOPs). In addition, admixture analysis was used to describe the genetic oleaster structure and to determine the RPOPs. This model was designed to identify the K (unknown) populations (genetic clusters) of origin of individuals, while simultaneously assigning the individuals to the populations with explicit estimates of their 90% confidence intervals. Each lineage originated in an RPOP. We used these analyses to suggest that several modern populations of oleasters are

distinguished, which may match with glacial refuge populations (GRPs).

MATERIALS AND METHODS

Sample collection

A total of 166 oleasters from 20 groves were sampled in 10 areas in Algeria, France, Israel, Libya, Morocco, Spain, Tunisia and Turkey as well as Corsica and Sicily where oleaster populations are still important (Table 1, Fig. 1). Groves instead of populations were preferred as no information was available on gene exchange between individuals. Some oleasters bear fruits while others do not, which is common in extreme conditions such as growing on cliffs or rocks, suggesting that they had probably not been planted. Previous studies (Besnard & Bervillé, 2002a; Bronzini de Caraffa *et al.*, 2002) have revealed unusual patterns of diversity for Corsican oleasters and olives. It is questionable whether the genuine oleaster occurred in Corsica, and could therefore be feral, as historical and botanical data are lacking, although its presence has been observed in pollen records (Reille *et al.*, 1999). The sampling in Corsica was extended to address this question. Forty additional cultivars (not detailed here) were sampled from areas assumed to be lacking in oleasters. These were selected according to their common name in different countries (Greece, Italy, Crete, Cyprus), and in each of the 23 groups of the cultivars (Besnard *et al.*, 2001b), which represent most of the cultivated olive diversity, and consequently the oleaster diversity available and from which they were derived.

Molecular methods

DNA was prepared from leaves according to Besnard *et al.* (2000) with a CTAB buffer, and its concentration measured on agarose gel with a lambda ladder. The final DNA concentration was adjusted to 10 ng μL^{-1} and SSR amplification was performed in a PTC 100 (MJ Research, BIORAD, Hercules, CA, USA). We chose the SSRs from Sefc *et al.* (2000) labelled DCA (Departamento de Ciências Agrárias, University of Azores, Angra do Heroismo, Portugal). SSRs at 12 loci were revealed on 6% acrylamide gels, run in CBS Scientific Co. (Del Mar, CA, USA) sequencing plates and revealed by silver staining. Double visual reading was performed using the AFLP 30-330 (step 10 bp) ladder markers (Promega, Charbonnières, France). SSR polymorphism statistics (Table 2) were summarized according to Breton *et al.* (2006). Microsatellite polymorphism combinations using the primer pairs (ccmp5, ccmp7 and QR-indel-3) are specific to the chloroplast DNA according to Besnard & Bervillé (2002a) (see Appendix S1 in Supplementary Material). Polymerase chain reaction (PCR) fragments were separated as nuclear SSRs. These markers identified in two steps the three chlorotypes already characterized in olives: CE1, COM and CCK (Besnard & Bervillé, 2000). CE1 and COM are found, respectively, in the eastern and western Mediterranean basin, and CCK occurs in Kabylia (Algeria).

Table 1 List of oleaster groves analysed grouped by country and numbered by areas

Areas	*n* W/I/E	Country	Grove, location	Lat, Long	Chlorotypes*
1	7 W	Algeria	Belloua, Kabylia	2°30′ E, 36° N	6CCK, 1COM
2	9 W	France	Ostricone, Corsica	8° E, 38° N	2CCK, 7COM
2	10 W	France	Ogliastro, Corsica	8° E, 38° N	10CE1
2	10 W	France	Filitosa, Corsica	8° E, 36° N	1CCK, 9COM
2	8 W	France	Corte, Corsica	8° E, 38° N	8CE1
2	10 W	France	Sarrola Carcopino, Corsica	8° E, 38° N	5CE1, 5COM
2	10 W	France	Lama, Corsica	8° E, 36° N	3CE1, 7COM
2	5 W	France	Bonifacio	8° E, 38° N	5COM
2	4 W	France	Oletta, Casta, Pigna, Ogliastrello2	3° W, 37° N	4COM
3	10 W	Spain	Torviczon, Andalucia	14° E, 37°30′ N	3CE1, 7COM
4	10 W	France	Mont Boron, Nice	15° E, 38° N	3CCK, 1CE1, 6COM
5	8 W	Italy	Ali, Sicily	22° E, 32° N	1CE1, 7COM
5	11 W	Italy	Messine, Sicily	10° W, 32° N	11COM
6	10 I	Libya	Cyrenaique	27° E, 39° N	2CE1, 8COM
7	9 W	Morocco	Tamanar, Essaouira	9°45′ W, 36°N	9COM
8	10 E	Turkey	Urla, Izmir	35° E, 32°45′N	10CE1
8	10 E	Syria	Harem, Oronte Valley	34° E–36° 20′ N	10CE1
9	10 E	Israel	Mont Carmel, Haifa	35°45′E–33° 30′N	10CE1
10	4 I	Tunisia	Zaghouan	10°E–36 N	3CCK, 1COM
10	2 I	Tunisia	El Fath	10°E–36 N	2CCK

Areas are shown in Fig. 1. *n* is the sample size in each grove. Locations: W, west; I, intermediate; E, east in the Mediterranean basin. Lat, latitude; Long, longitude.

*Number of individuals with each chlorotype: CE1 and COM from eastern and western Mediterranean, respectively; CCK from Kabylia, Algeria.

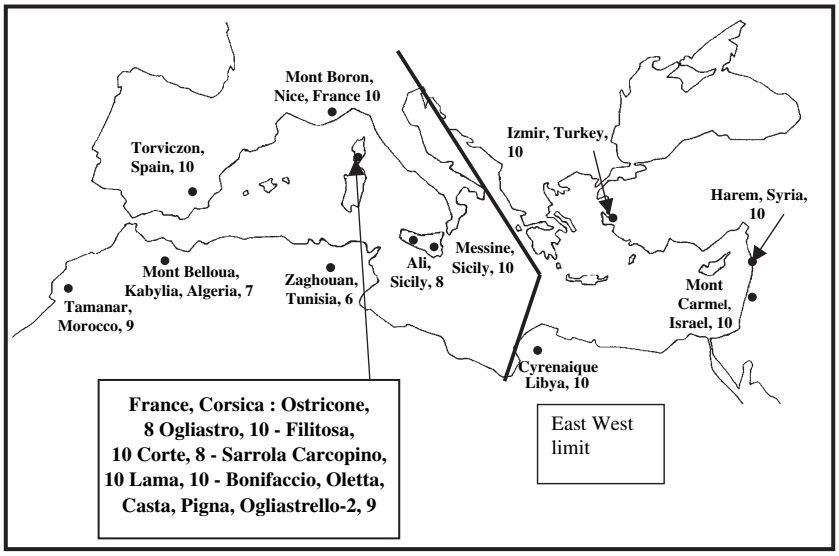

Figure 1 Map showing the approximate distribution of oleaster groves, detailed in Table 1, around the Mediterranean basin. Numbers indicate the sampling size. The straight line indicates the limit between the eastern and western sides.

Table 2 List of SSRs coded according to Sefc *et al.* (2000), and some characteristics of polymorphisms through diversity analyses. Total allele numbers are indicated for the 166 oleasters and the 40 cultivars

SSR code	N-Cultivars*	N-Sefc *et al.*†	Size shortest‡	Size longest**	N-Oleasters§
ssrOeUA-DCA1	7	4	200	230	8
ssrOeUA-DCA3	7	9	230	270	9
ssrOeUA-DCA4	16	11	120	183	26
ssrOeUA-DCA5	8	6	196	240	12
ssrOeUA-DCA7	12	9	130	210	18
ssrOeUA-DCA8	9	7	117	176	20
ssrOeUA-DCA9	15	11	164	220	19
ssrOeUA-DCA11	10	9	130	196	17
ssrOeUA-DCA13	11	5	116	158	12
ssrOeUA-DCA14	9	4	170	194	13
ssrOeUA-DCA15	4	4	244	268	4
ssrOeUA-DCA18	9	10	160	188	12
Total alleles	117				161

*N-Cultivars: mean number of cultivars (this study).
†N-Sefc *et al.*: number of cultivars found by Sefc *et al.* (2000).
‡The range of variation of the polymorphisms is indicated by the shortest and **the longest allele at each locus. SSR size is in bp.
§N-Oleasters: number of cultivars found oleasters.

Data analyses

Multivariate analyses

Chlorotypes were analysed separately from nuclear markers due to maternal inheritance (Besnard *et al.*, 2000). To compute microsatellite genetic data we applied factorial correspondence analysis (FCA) performed with GENETIX4 (Belkhir *et al.*, 1996–98) with rare alleles (frequency below 2%) considered as missing data. All structural parameters (allele frequencies, heterozygosity observed, expected and non-biased, as well as differentiation indices) were computed using the GENETIX software. Computations were also performed with ARLEQUIN (Schneider *et al.*, 2000). To combine the nuclear and cytoplasmic data we introduced the chlorotype as the thirteenth locus in diversity analysis to verify information with those of the 12 nuclear SSR loci.

To assess population structure we used a model-based Bayesian procedure, implemented in the program STRUCTURE (Pritchard *et al.*, 2000). This model enables identification of the K (unknown) RPOPs of individuals, and the assignment of each individual to one or several RPOPs. We therefore considered individuals only, without grove or country of origin information in the case of cultivars. Assignation of one individual in an RPOP was provided by a probability of membership qI chosen at 80%. However, a probability under the threshold means that this individual may have several parental RPOPs, and consequently is admixed via hybridization between RPOPs. STRUCTURE was run with the admixture model and at least five repetitions of 1,000,000 iterations following a burn-in period (initialization) of 30,000 iterations. Lastly, we cross-checked the results to verify clustering and admixture with the program GENECLASS (Piry *et al.*, 2004). The conclusions were the same as with STRUCTURE.

Determination of K (number of RPOPs)

In the first step for estimating K, population structure was assessed using only oleasters ($n = 166$) assuming that sampled oleasters were anonymous trees. We also computed K with all individuals anonymously (166 oleasters and 40 cultivars). Posterior probability values for K (log-likelihood) were estimated, assigning K from 3 to 16. The proportion of membership (qI) of each individual in the remaining seven RPOPs was estimated. In a second step, STRUCTURE was used to compute oleaster and cultivar ancestry without individual and population information (we used the options

usepopinfo = 0, popflag = 0). We retained only oleaster individuals that have ancestors in one RPOP (proportion of membership in each RPOP ≥ 80%) and then pre-defined seven RPOPs with those individuals only. All remaining individuals were not assigned to an RPOP and we computed their proportion of membership from different RPOPs including the population information option (1,000,000 iterations, usepopinfo = 1, popflag = 1, initiation run or burning 30,000). Five repetitions were performed, providing average *qI* values (Table 3). We verified that most runs converged to a similar *qI* and eliminated the aberrant runs.

To estimate the diversity parameters (Φ_{is}, Φ_{st}, F_{it} and F_{st}) we used population differentiation indices based on the rate of out-crossing, selfing and random mating under Hardy–Weinberg equilibrium, with *P* values among groups and pairwise combinations of groves of the RPOPs. These were identified by STRUCTURE employing FCA, GENETIX and ARLEQUIN, using options analysis for specifying either two (west–east) or three (west–intermediate–east) groups. Isolation of populations by distances for the seven RPOPs was computed through the Mantel procedure using GENETIX and ARLEQUIN geographical distances (km) relative to a central reference longitude and F_{st} matrices for RPOPs.

RESULTS

Differentiation with chloroplast markers

The total F_{st} (Wright) for chlorotype in oleasters is 0.690, indicating the presence of a deep genetic and geographical structure. The chlorotype CCK was found mainly in North Africa, with a few occurrences in Sicily (Italy) and Corsica (France). The chlorotype COM was present throughout the west. In contrast, the chlorotype CE1 was found in both the east and the west, yet its frequency was quite different. All the eastern oleasters carried CE1 without exception, whereas in the west trees carrying CE1 were less frequent while being distributed in almost all RPOPs (Table 1). Thus RPOPs in the east carry CE1 only, two RPOPs in the west carry CCK or COM and two contain mixed chlorotypes CE1, COM or CCK. Only one grove (Mont Boron, France) contains the three chlorotypes.

Multivariate and similarity analyses in olives and oleasters

We found 171 alleles in the 206 trees and an average of 14.25 alleles per locus, with a range of 4 to 26 alleles per locus (Table 2), substantially more than reported by Sefc *et al.* (2000). Multivariate analyses (FCA) computed on all oleaster and cultivar samples displayed three partially overlapping groups (Fig. 2). Three oleaster groups and one cultivar group were revealed. We found 161 alleles in oleasters (94% of the total number of alleles). Eastern ($n = 30$) and western ($n = 120$) oleasters shared 126 markers, whereas 12 markers were specific to the east and 33 were specific to the west (not shown). Corsican oleasters (66) displayed nine specific alleles, whereas North African ($n = 17$) and Sicilian ($n = 19$) oleasters each displayed two specific alleles.

Genetic diversity in oleaster populations

Mean heterozygosity ($H_0 = 0.672$ observed) for oleasters is consistently (chi-square, $P < 0.01$) less than expected (0.720) under Hardy–Weinberg equilibrium (mean $F_{is} = 0.090$), suggesting significant inbreeding, most likely due to population isolation. Inter-population genetic differentiation was moderate between the seven RPOPs (mean $F_{st} = 0.104$, Table 4). The estimation of pollen-mediated, gene flow male parents varies from 2 to 9 migrants (N_m) per population. The Mantel tests show a correlation between F_{st} and geographical distance (slope 0.29770, $P < 0.022$, $r^2 = 0.19641$) suggesting that RPOPs may differentiate according to geographical distances.

Admixture analysis and genetic structure in oleasters

We first searched for the *K* presumed ancestral populations by comparing the distribution of the 166 oleasters into groups using Bayesian analyses of the SSR data. We also considered the distribution of 206 (oleaster and cultivar) trees into groups. We successively introduced in the program *K* from 3 to 16 to check if tree groupings were consistent through 10 runs. After the first computations we chose $K = 8$ for oleasters and some trees consistently grouped into eight groups in both cases. Further computations with $K = 8$ included one cluster comprising individuals from Libya and

Table 3 Range of proportionate membership from 10 runs with structure for the seven reconstructed panmictic oleaster populations (RPOPs). The total number was 107 individuals

GRP size	GRP1	GRP2	GRP3	GRP4	GRP5	GRP6	GRP7
1 GRP-Corsica 10*	0.819–0.932						
2 GRP-Turkey 23*		0.839–0.974					
3 GRP-Sicily 35*			0.855–0.978				
4 GRP-Tunisia 3*				0.825–0.910			
5 GRP-Libya 13*					0.823–0.973		
6 GRP-Spain 20*						0.882–0.949	
7 GRP-Israel 3*							0.950–0.962

*Number of trees used to define each RPOP.

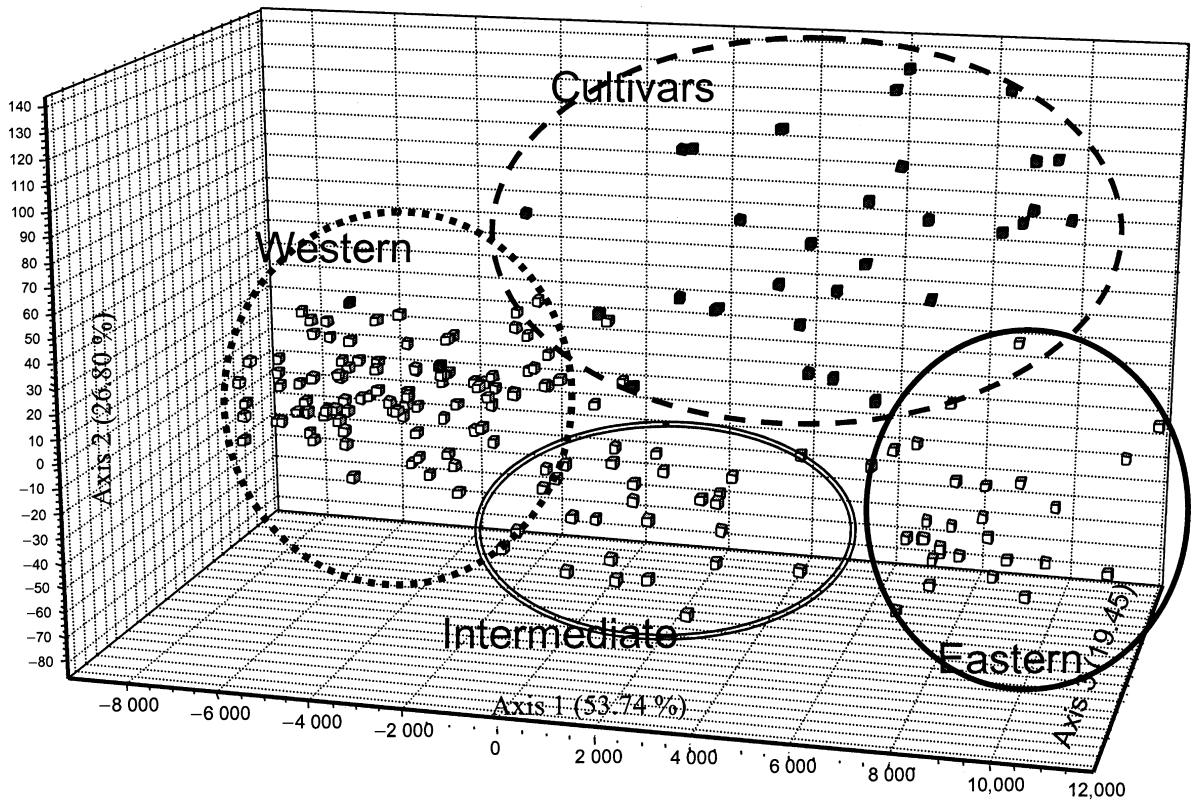

Figure 2 Multivariate analysis (factorial correspondence analysis) on SSR data. Individuals with rare (< 2%) alleles were considered as missing data. Western, eastern and intermediate groups are circled as cultivars, empty and full cubes, respectively.

Table 4 Differentiation indices (F_{st}, Theta; Weir & Cockerham, 1984) between RPOPs. Bold numbers indicate a significant F_{st} ($P < 0.0001$): $0.0 < F_{st} > 0.1$ not differentiated; $0.1009 < F_{st} > 0.15$ fairly differentiated; $0.15009 < F_{st} > 0.20$ very differentiated; $0.2009 < F_{st} > 0.3$ highly differentiated

	GRP2	GRP3	GRP4	GRP5	GRP6	GRP7
1 GRP-Corsica	**0.12117**	**0.10556**	**0.15879**	**0.09393**	**0.08289**	**0.14931**
2 GRP-Turkey		**0.17397**	**0.06856**	**0.10443**	**0.12475**	−0.00387
3 GRP-Sicily			**0.17016**	**0.12575**	**0.06013**	**0.18630**
4 GRP-Tunisia				**0.10900**	**0.10928**	**0.13573**
5 GRP-Libya					**0.08016**	**0.13081**
6 GRP-Spain						**0.12392**
7 GRP-Israel						0

France–Corsica with *qI* probabilities of 0.70 to 0.76, respectively. Since the *qI* were below the minimum threshold of 80%, the group was not accepted. It was assumed that this grove had a complex origin, based on hybridization between two RPOPs, and therefore it was removed. Of a total of 166 oleaster trees, 107 were assigned to one of the seven RPOPs. This minimum threshold was chosen as the number of individuals was high, introducing background noise for *qI* probabilities (Table 3), with most possible membership probabilities above 90%. The seven RPOPs defined by the 107 individuals (Table 3) in most runs showed that $K = 7$ for oleaster, a value which could therefore be used in all further computations. The variation range of *qI* through the 10 repeats for each tree of each RPOP was calculated, which then showed the median values (Table 3). Each RPOP unites individuals either from one location or sometimes from several locations. Sixty-two trees were grouped intto two different RPOPs with a probability *qI* of $0.40 \leq P < 80\%$, and some with probabilities between 0.10 and 0.40 (Table 5). This suggests an admix origin as a hybrid or an advanced hybrid generation. The seven RPOPs showed an east to west cline based on all molecular data. We checked two hypotheses: (1) a strict separation of two groups of RPOPs between the east (three RPOPs) and the west (four RPOPs) and (2) a gradient of RPOPs, with three groups assuming three in the east, two in the west and one in an intermediate position. AMOVA analyses of SSR data show that both are significant (Table 6). The eastern populations are characterized by the combination

Table 5 Proportion of membership (average of five repeats) for individuals from different countries that admixed in one or different RPOPs. A to G correspond to cultivar denomination codes

		Assignation of the referenced tree in RPOPs (1–7)						
Code	Assignation	1	2	3	4	5	6	7
Co_Lam08	3	0.885						
Mo-Ta80k	3	0.871						
Is_Mctv3	7		0.835					
Co_Sac06	1			0.761				
Co_Lam01	1			0.777				
Al_Kab04	6				0.635			
Es_Tor04	6					0.701		
Fr-CoFil12S	3						0.935	
Ly_Lyb03	–*							0.663
Co_Ost05		0.565		0.338				
Fr_MtBo14		0.284		0.437			0.245	
Is_Mc18c	7	0.358						0.619
Is_Mcvh4	7	0.307						0.537
Mo-Ta74k		0.387				0.362	0.214	
Fr-MtBo17		0.364				0.120	0.286	0.106
Ly_Lyb10	5		0.415			0.509		
Tu_Zag02			0.304		0.116			0.325
Fr-MtBo15			0.291				0.278	0.295
Co_Ogl10				0.436		0.227		
A		0.948						
B			0.708					
C				0.943				
D					0.791			
E						0.980		
F							0.845	
G								0.980

*Not assigned.

of the CE1 chlorotype and the nuclear SSRs from the east. In contrast, in the west we found the CE1 chlorotype with a wide range of nuclear markers either unique to the east or unique to the west. The chlorotypes COM and CCK were never found in the east. Finally, FCAs showed that these specific markers provided an efficient basis on which to structure the genetic diversity (Fig. 3a,b). The three methods of clustering: factorial correspondence analyses (FCA, Fig. 2), the Bayesian approach (Table 3 & 4) and population diversity (see Appendix S2 in Supplementary Material) support the same RPOPs (excepting GRPs 2 & 7 from the east; see Appendix S3 in Supplementary Material), suggesting that they correspond only to one population (Table 5 and Fig. 4.).

Oleaster cultivar relationships

The SSR data for 206 trees were computed to verify whether another RPOP could be detected among the cultivars (see Appendix S2 in Supplementary Material). It was evident that all cultivars clustered in one of the areas of origin implied by the seven RPOPs already defined or admixed between two or three of them. No new putative origins were found. Cultivar genotypes were grouped by admixtures to diverse RPOPs, which suggested repeated attempts at domestication for olive and additional crosses between olive cultivars of different origins. The origins of olive cultivars and domestication will be addressed in a companion paper in preparation

DISCUSSION

Methods

For the 206 trees microsatellites displayed on average 14.25 alleles per locus, some of which may be attributed to homoplasy and therefore the marker–phylogeographical relationship may be attenuated. Homoplasy generates confusion between the SSR alleles for a given length that results from different mutation events. Homoplasy in chloroplast and in nuclear SSR could modify our conclusions substantially (Blouin, 2003). STRUCTURE results may be invalid in the presence of population structure. Cytoplasmic markers, which are maternally inherited (Besnard *et al.*, 2000), enabled us to identify a deep structure. The genetic structure for nuclear markers was considerably less evident, probably due to homoplasy inherent to the means of evolution.

We did not observe panmixy for most individual groves, which is required by the software, nor do we know whether this requirement is fulfilled in each RPOP. However, they may

Table 6 Hierarchical analysis of molecular variance (AMOVA) of chlorotype and microsatellite loci allele frequencies among samples of oleasters in the Mediterranean basin. Fixation indices are given for each population. The SE is for separate experiments

(1) Subdivision in west (RPOP1, 3, 5 and 6) and east (RPOP 2, 4 and 7) groups

Comparison	d.f.	Percentage variance	*F* statistics	SE
Among E W within GRPs	1	7.582	0.01369	0.001
Among GRPs	5	7.37	0.00000	< 0.0001
Within GRPs	100	8.71	0.00000	< 0.0001
Within individuals	107	76.33	0.00000	< 0.0001

Fixation indices		*P* values	SE
F_{is}	0.12500	< 0.0001	0.00001
F_{st}	0.19889	< 0.0001	0.00001
F_{it}	0.29736	< 0.0001	0.00001

(2) Subdivision in west (GRP1, 3 and 5), intermediate (GRP4) and east (GRP2 and 7) groups

Comparison	d.f.	Percentage variance	*F* statistics	*P*
Among E I W within GRPs	2	0.60539	0.01760	0.001
Among GRPs	4	0.51922	0.00000	< 0.0001
Within GRPs	100	0.58653	0.00000	< 0.0001
Within individuals	107	4.06542	0.00000	< 0.0001

Fixation indices		*P* values	SE
F_{is}	0.12608	< 0.0001	0.00001
F_{st}	0.19859	< 0.0001	0.00001
F_{it}	0.29622	< 0.0001	0.00001

be considered as multiple subpopulations, and when examined altogether may be considered to represent a metapopulation in Hardy–Weinberg equilibrium. This proposal requires further investigation. The results obtained with STRUCTURE were cross-checked with other methods (FCA, GENECLASS, F_{st} variation and dendrograms, not shown) to verify the conclusions. The methodology used set some geographical limits on the GRPs, as they comprised trees from different groves. A new sampling strategy is being developed to give them a more accurate geographical outline.

Differentiation between the eastern and western Mediterranean basin

The results confirm the significant differences between the eastern and western Mediterranean basin either side of a line from the Adriatic Sea to the Libyan Desert (Fig. 1; Besnard & Bervillé, 2000; Terral *et al.*, 2004). Eastern and western oleasters shared 75.4% of their nuclear alleles. Twelve alleles were specific to the eastern ($n = 30$) and 33 to the western ($n = 120$) Mediterranean. The differentiation parameters (F_{st}, F_{is}) between these two groups were significant ($P < 0.0001$), except for the two eastern samples from groves in RPOPs from Turkey and Israel (see Appendix S3 in Supplementary Material). Consequently, western and eastern Mediterranean trees were differentiated based on nuclear SSR polymorphisms in combination with chlorotypes. Differentiation of RPOPs based on F_{st} for chlorotype was significant (not shown), although it was probably attenuated due to gene flow, namely seed movement via birds or people. For example, it is possible that oleaster seeds may have been carried by humans as a managed pre-domesticate, as has been suggested for other crops, for example in the New World (Smith, 1998; Motamayor *et al.*, 2002). Their genetic contribution may persist in some areas, in some form.

Quézel & Médail (2003; see their Fig. 3.3, p. 62 and Table 3.2, p. 63) have listed 26 GRPs for several woody species in the east or the west of the basin. Petit *et al.* (2003) have shown that for oak the extent of genetic structure is due to their expansion from refuge zones occupied during the last glacial period (*c.* 11,550 years ago) with the climate warming, as documented for several tree species. Some GRPs have sheltered several species, but oleaster requires warmer temperatures than other woody species, which may account for an isolated GRP in Morocco (Quézel & Médail, 2003). For oleasters, all clustering methods together showed that eastern and western oleasters classified into six genetically distinct RPOPs (Table 6; and see Appendix S3 in Supplementary Material), which may coincide with the location of GRPs. The study suggests that several close GRPs could exist for ssp. *europaea* in North Africa and on various islands (Corsica, Sicily) as revealed for *Gentiana ligustica* (Diadema *et al.*, 2005) and *Ficus carica* (Khadari *et al.*, 2005) in the Maritime Alps and Corsica, respectively. Several GRPs may co-exist, as in Corsica, Sicily and North Africa. This means that trees from the different RPOPs in one grove were still genetically isolated, though physically proximate, as they did not cross with each other. It cannot be excluded that the structure pre-dated glaciations and was merely restructured during the last Ice Age, suggesting that oleasters peculiar to the west moved to the east

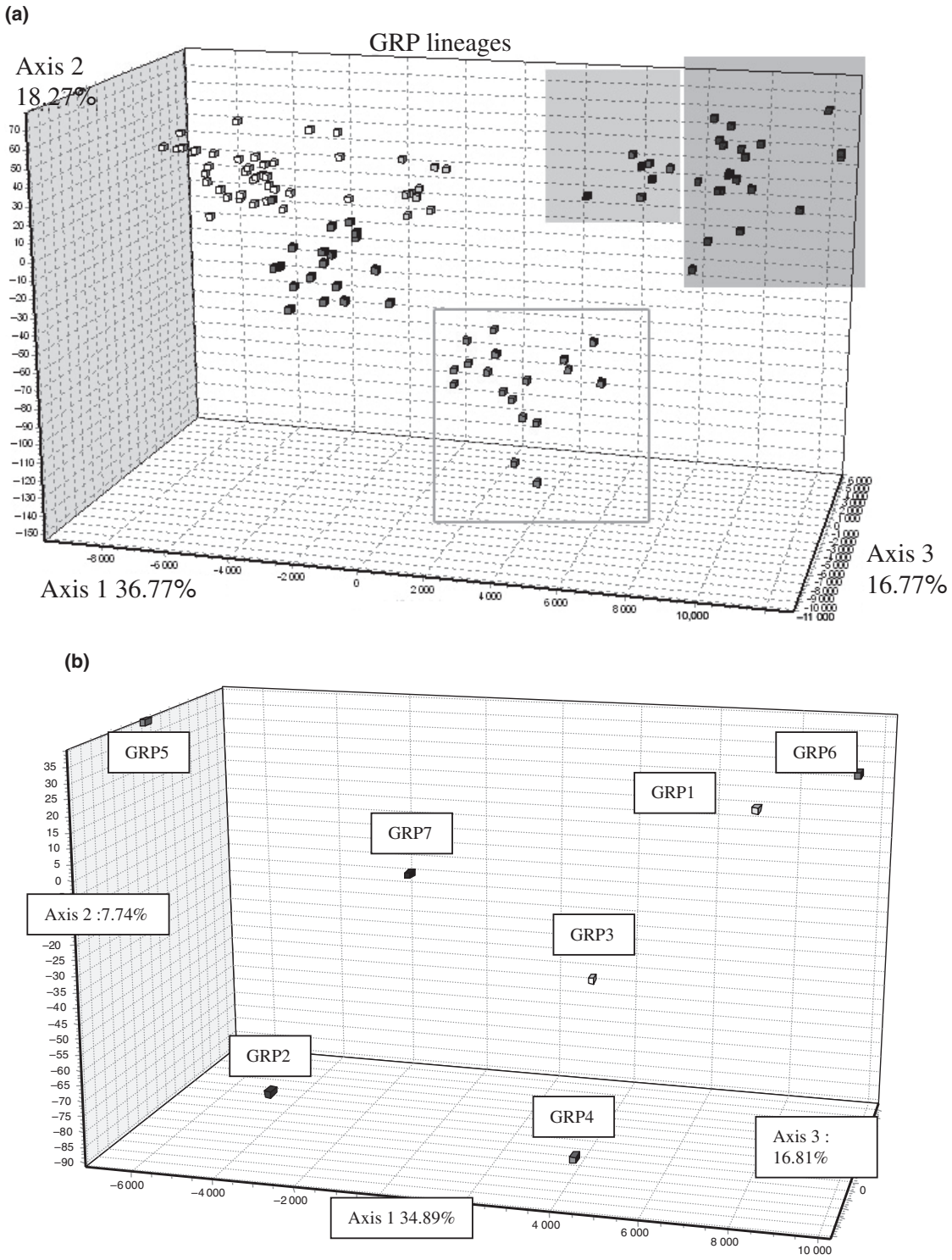

Figure 3 Multivariate analysis (factorial correspondence analysis) on SSR data for the seven reconstructed panmictic oleaster populations (RPOP) using 107 trees. Two orientations of the graphic are shown: (a) front view, (b) front view with populations represented by their barycenter.

at the beginning of the last Ice Age. Its presence at 19,050 yr BP has been established by charred oleaster stones in the Near East (Galili *et al.*, 1997).

Several climatic cycles within a succession of glacial and interglacial periods in the Mediterranean basin have probably imposed strong environmental selection and genetic drift leading to marked differences in both faunal (Hewitt, 1996) and floral diversity between the GRPs (Petit *et al.*, 2003). For oleaster, we assumed that the present geographical structure recognized through molecular diversity and the GRPs matches.

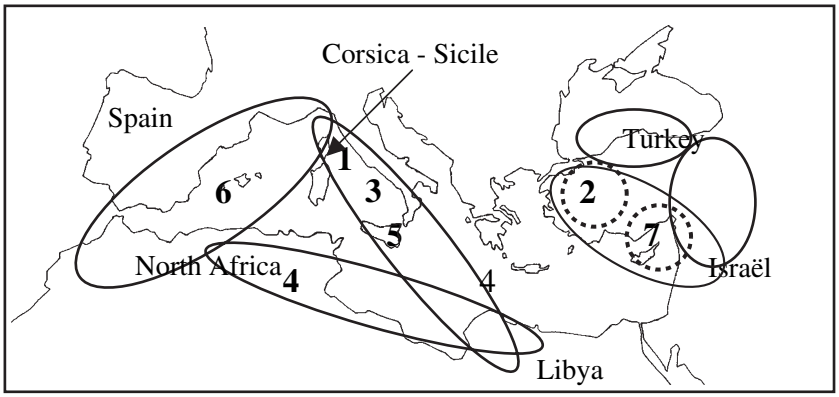

Figure 4 Map of the Mediterranean basin showing the approximate locations of the seven oleaster populations. RPOPs are circled by continuous lines and numbered according to Table 4. Dotted circles indicated that the two RPOPs were not differentiated by all methods.

In the western Mediterranean, five GRPs were identified. GRP1 is nearly unique to Corsica, with the few trees from that GRP found in Morocco requiring further investigation. GRP3 is limited to Sicily and Corsica. Indeed, Sicily has been recognized as a refugium zone for several animal and plant species (Hewitt, 1996). Individuals from Tunisia and Corsica (Lama) constituted GRP4. In other samples from Corsica and Libya (GRP5), we found individuals carrying CE1 that are probably feral (Corte grove). Trees from Spain and North Africa clustered together, making GRP6. Individuals from Syria clustered with those of Turkey (GRP2) and not with those of Israel (GRP7). In the eastern Mediterranean, GRPs 2 and 7 were genetically differentiated with STRUCTURE, though not according to F_{st} distribution using ARLEQUIN. The matrix of P values for F_{st} is not shown since all P values were significant with $P < 0.001$, except for the combination 2*7 ($P < 0.2138$). Moreover, Angiolillo *et al.* (1999) and Besnard *et al.* (2001a,b), using RAPD and AFLP markers, respectively, have found a single homogeneous eastern population.

Gene flow between olive and oleaster

Using admixture methods we detected several mixed groves: Corte (France) suggesting introduction of seeds based on chlorotype only, and in the Mont Boron (France) grove based on SSRs. Individuals from such a mixed grove clustered separately into RPOPs, making it possible to identify their origin. Thus, GRPs appeared efficient and well-defined through admixture analysis. This suggests that either some oleasters have been planted using fruits from other regions or, in contrast, seed-based gene flow from cultivars occurred. It is doubtful whether the Mont Boron population was natural. This grove comprised trees that were assigned ($qI > 0.80$, not shown) to Spain, Libya, Corsica and North Africa, with some trees admixed in different GRPs (Table 5), suggesting that individuals were probably introduced from these regions and produced progenies. Indeed, the website http://www.nissalabella.net/parcmtb.htm shows postcards of Mont Boron bare of trees and states that reafforestation was extensively carried out from 1866 to 1869. Assigning trees to different regions revealed, therefore, that such a reafforestation event had probably occurred in the Mont Boron forest. Corsican oleasters belonged to four different GRPs, but introduction information is not available. These trees were from different localities and isolated geographically, which may indicate that they were survivors from previous populations. The Corte grove may be the product of introduced cultivars with chlorotype CE1 escaping by seed, and probably conserved due to geographical isolation. The dispersal of olive stones by birds has been documented and may be relevant here (Spennemann & Allen, 2000). Wood charcoal artefacts have been identified by Terral & Arnold-Simard (1996), revealing pre-domestication of the oleaster in the western Mediterranean by 8050 years ago. Indeed, Figuieral & Terral (2002) found evidence that western olive domestication probably paralleled eastern events (5850 years ago). In addition, Terral *et al.* (2004) established relationships between charred olive stones from archaeological sites and modern cultivars based on geometrical morphometry.

Existing genetic diversity did not permit the reconstruction of oleaster diversity before the last Ice Age. In oak, diffusion routes from refuge zones to all over Europe have been based on palynological data enabling the location of refugia (Petit *et al.*, 2003) and the reconstruction of recolonization routes (Brewer *et al.*, 2002). This was not possible for oleaster as chlorotypes were mixed in several GRPs, suggesting that ancient taxa carrying the corresponding chlorotypes may have existed in the three refuge zones before the last Ice Age. One was probably in the eastern Mediterranean with CE1. The two others were in the western Mediterranean with CCK in North Africa and COM in Italy (Sicily), France (Corsica) and Spain. Furthermore, microsatellite polymorphisms indicated that refuge zones were deeply structured, but we found trees belonging to one GRP in North Africa, Corsica, Spain and continental France. Gene flow that disturbed the basic tripartite GRP structure, producing the present oleaster distribution, is therefore probably more recent due to human migration and trade. The distribution of these chlorotypes on islands (Sicily, Corsica) and continents (Africa, Europe) suggests their age in these locations and indicates complex seed movement, probably by birds.

Relationship of olive with oleasters

The ages of the trees sampled range between 100 to probably more than 500 years. Leaf morphology, fruit shape and weight did not permit verification of whether trees were oleaster, feral

or cultivar. In some cases, habitat alteration and cultivation traces may have fully disappeared after several centuries of agricultural abandonment. Some cultivars from various countries were added to compare clustering of both types of tree. Since the original oleaster sample did not cover the entire Mediterranean basin, cultivars were chosen from countries not represented by oleasters. It was assumed that if humans had moved olive cultivars long distances, some trees may originate from local oleasters, although animals may have contributed to local spreading. Hybridization would have occurred within local oleasters as well as with introduced cultivars in both directions. This consideration increases the number of possible feral oleasters (oleaster [maternal] × cultivar [paternal] hybrids), since all hybridized trees must be considered feral (see Appendix S3 in Supplementary Material). Admixture analyses also revealed eastward seed gene flow to Israel, Syria and Turkey that was not detected by chloroplast DNA RFLP (all carried CE1) and RAPD markers (Besnard *et al.*, 2002). Those trees appear to be cryptic hybrids between trees from different GRPs, although they are still clustered with the genuine eastern trees. Nevertheless, a cryptic hybrid is an individual that has the morphology of either parent but is the result of hybridization. This can be revealed through a combination of molecular and statistical procedures. Indeed, cryptic hybrids would not be revealed with molecular markers only. Thus, the procedure to determine the status of a tree between genuine oleaster, cultivars and variations of feral hybrids has to include morphological and historical traits, molecular analysis with two or three kind of markers, and statistical analysis such as multivariate or Bayesian analysis.

Model acceptance or rejection: a new model

The Bayesian method is based on several discussed assumptions. The patterns of diversity in eastern and western oleaster groves did not support the stated working hypothesis. The implications of these findings allowed us to better understand the oleaster diversity and the origins of olive from oleasters.

The predictions of our originally stated model on oleaster diffusion in the Mediterranean were not proven: (1) the genetic diversity did not display a bottleneck in the west in comparison to the east; and (2) western oleasters were not derived from eastern cultivars. Rather our results support the possibility that: (3) some western cultivars originated from western oleasters; and (4) diversity is much higher in the west than in the east, thus supporting the hypothesis that presumed western oleasters are genuine. According to these findings a new hypothesis is proposed, namely oleasters originally differentiated in the west where the diversity is much higher.

CONCLUSION

Microsatellite markers bring new insights into oleaster differentiation:

1. they revealed at least six origins for oleasters using three different methods: Bayesian analysis, population differentiation parameters and FCA. We also found support for the argument that marker frequencies displayed a cline between the eastern and western Mediterranean basin (Besnard & Bervillé, 2000, 2002b);

2. the cline can be explained by the recolonization of the Mediterranean basin by oleasters from the six GRPs, which may match Pleistocene refuge zones;

3. with oleaster expansion the populations may overlap in some locations and mix, as we detected with both cytoplasmic and nuclear markers. However, data suggested that fruit displacement, probably due to human movement, has occurred westward;

4. however, the chloroplast diversity was found to be mixed in some of the GRPs, suggesting that the chloroplast polymorphisms differentiated earlier than the last Ice Age;

5. the present analysis of the geographical and genetic divergence of the eastern and western oleaster groups should enable a rational sampling of oleasters for *in situ* conservation of olive genetic resources.

ACKNOWLEDGEMENTS

We thank Daniela Soleri (UC Santa Barbara), Rachid Cheddadi and Jean Maley for many helpful suggestions and Alex J. Chepstow-Lusty for the improvement of the English of our manuscript. We were indebted to Dr Guillaume Besnard who provided numerous oleaster and olive cultivar DNA samples from several countries. Dr Luciana Baldoni and Dr Isabel Trujillo also kindly provided us with oleaster and cultivar samples. This research was conducted at the 'Unité Mixte de Recherche – Diversité et Génome des Plantes Cultivées' (INRA) as a component of a regional programme sponsored by 'The Région Languedoc-Roussillon' focused on understanding the processes of local cultivar adaptation and olive cultivar diversity organization. The study was partly supported within the framework of the DADP2 action, sponsored by the 'Conseil Régional de la Région Languedoc-Roussillon'. Many thanks are due to James Aronson (CEFE-Montpellier), to François Bonhomme and Nicolas Bierne (UMR 5000, CNRS, Montpellier) for many suggestions and their encouragement. This study also benefits from the 'Olive-track' support programme (EC QLK1-2002-02386).

REFERENCES

Angiolillo, A., Mencuccini, M. & Baldoni, L. (1999) Olive genetic diversity assessed using amplified fragment length polymorphisms. *Theoretical and Applied Genetics*, **98,** 411–421.

Belaj, A., Satovic, Z., Rallo, L. & Trujillo, I. (2002) Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. *Theoretical and Applied Genetics*, **105,** 638–644.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L. & Bonhomme, F. (1996–98) *GENETIX, logiciel sous Windows*^{TM} pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Besnard, G. & Bervillé, A. (2000) Multiple origins of the Mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA polymorphisms. *Compte Rendu Académie des Sciences, Sciences de la Vie*, **323,** 173–181.

Besnard, G. & Bervillé, A. (2002a) On chloroplast DNA variations in the olive (*Olea europaea* L.) complex: comparison of RFLP and PCR polymorphisms. *Theoretical and Applied Genetics*, **104,** 1157–1163.

Besnard, G. & Bervillé, A. (2002b) *Les origines de l'olivier (Olea europaea L.) et des oléastres*. AITAE, AEP, Centre d'anthropologie, Archéo-Plantes Hommes et plantes de la préhistoire à nos jours, pp. 29–58. AITAE, Toulouse, France.

Besnard, G., Khadari, B., Villemur, P. & Bervillé, A. (2000) A cytoplasmic male sterility in olive cultivars (*Olea europaea* L.): phenotypic, genetic and molecular approaches. *Theoretical and Applied Genetics*, **100,** 1018–1024.

Besnard, G., Baradat, P., Breton, C., Khadari, B. & Bervillé, A. (2001a) Olive domestication from structure of oleasters and cultivars using RAPDs and mitochondrial RFLP. *Genetics Selection Evolution*, **33** (Suppl. 1)**,** S251–S268.

Besnard, G., Breton, C., Baradat, P., Khadari, B. & Bervillé, A. (2001b) Cultivar identification in the olive (*Olea europaea* L.) based on RAPDs. *Journal of the American Society for Horticultural Science*, **126,** 668–675.

Besnard, G., Khadari, B., Baradat, P. & Bervillé, A. (2002) *Olea europaea* phylogeography based on chloroplast DNA polymorphism. *Theoretical and Applied Genetics*, **104,** 1353–1361.

Blouin, M.S. (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution*, **18,** 503–511.

Breton, C., Besnard, G. & Bervillé, A. (2006) Using multiple types of molecular markers to understand olive phylogeography. *Documenting domestication: new genetic and archaeological paradigms* (ed. by M.A. Zeder, D. Decker-Walters, D. Bradley and B. Smith), pp. 142–152. University of California Press, Berkeley, CA.

Brewer, S., Cheddadi, R., de Beaulieu, J.L. & Reille, M. (2002) The migration of deciduous Quercus throughout Europe since the last glacial period. *Forest Ecology and Management*, **156,** 27–48.

Bronzini de Caraffa, V., Maury, J., Gambotti, C., Breton, C., Bervillé, A. & Giannettini, J. (2002) Mitochondrial DNA variation and RAPD mark oleasters, olive and feral olive from Western and Eastern Mediterranean. *Theoretical and Applied Genetics*, **104,** 1209–1216.

Cipriani, G., Marrazzo, M.T., Marconi, R., Cimato, A. & Testolin, R. (2002) Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. *Theoretical and Applied Genetics*, **104,** 223–228.

Diadema, K., Bretagnolle, F., Affre, L., Yuan, Y.M. & Médail, F. (2005) Geographic structure of molecular variation of *Gentiana ligustica* (Gentianaceae) in the Maritime and Ligurian regional hotspot, inferred from ITS sequences. *Taxon*, **54,** 887–894.

Fabbri, A., Hormaza, J.I. & Polito, V.S. (1995) Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. *Journal of the American Society for Horticultural Science*, **120,** 538–542.

Figueiral, I. & Terral, J.-F. (2002) Late Quaternary refugia of Mediterranean taxa in the Portuguese Estremadura: charcoal based paleovegetation and climatic reconstruction. *Quaternary Science Reviews*, **21,** 549–558.

Galili, E., Stanley, D.J., Sharvit, J. & Weinstein-Evron, M. (1997) Evidence for earliest olive-oil production in submerged groves of the Carmel coast, Israel. *Journal of Archaeological Science*, **24,** 1141–1150.

Green, P.S. (2002) A revision of *Olea* L. (Oleaceae). *Kew Bulletin*, **57,** 91–140.

Hatzopoulos, P., Banilas, G., Gianoulia, K., Gazis, F., Nikoloudakis, N., Milioni, D. & Haralampidis, K. (2002) Breeding, molecular markers and molecular biology of the olive tree. *European Journal Lipid Science Technology*, **104,** 574–586.

Hewitt, G.M. (1996) Some genetic consequence of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnaean Society*, **58,** 247–276.

Khadari, B., Grout, C., Santoni, S. & Kjellberg, F. (2005) Contrasted genetic diversity and differentiation among Mediterranean population of *Ficus carica* L.: a study using mtDNA RFLP. *Genetic Resources and Crop Evolution*, **52,** 97–109.

Lumaret, R. & Ouazzani, N. (2001) Plant genetics. Ancient wild olives in Mediterranean forests. *Nature*, **413,** 700.

Lumaret, R., Ouazzani, N., Michaud, H. & Villemur, P. (1997) Cultivated olive and oleaster: two very closely connected partners of the same species (*Olea europaea* L.). Evidence from enzyme polymorphism. *Bocconea*, **7,** 39–42.

Mekuria, G.T., Collins, G. & Sedgley, M. (2002) Genetic diversity within an isolated olive (*Olea europaea* L.) population in relation to feral spread. *Scientia Horticulturae*, **94,** 91–105.

Motamayor, J.C., Risterucci1, A.M., Lopez, P.A., Ortiz, C.F., Moreno, A. & Lanaud, C. (2002) Cacao domestication I: the origin cacao of cultivated by the Mayas. *Heredity*, **89,** 380–386.

Petit, R.J., Aguinagalde, I., de Beaulieu, J.L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G., Demesure-Musch, B., Palme, A., Martin, R.P., Rendell, S. & Vendramin, G.G. (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300,** 1563–1565.

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L. & Estoup, A. (2004) GeneClass2: a software for genetic assignment and first generation migrants detection. *Journal of Heredity*, **95,** 536–539.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155,** 945–959.

Quézel, P. & Médail, F. (2003) *Ecologie et biogéographie des forêts du bassin méditerranéen*. Elsevier SAS, Paris.

Rallo, P., Tenzer, I., Gessler, C., Baldoni, L., Dorado, G. & Martin, A. (2003) Transferability of olive microsatellite loci across the genus *Olea*. *Theoretical and Applied Genetics*, **107,** 940–946.

Reille, M., Gamisans, J., Andrieu-Ponel, V. & de Beaulieu, J.-L. (1999) The Holocene at Lac de Creno, Corsica, France: a key site for the whole island. *New Phytologist*, **141,** 291–308.

Schneider, C., Roessli, D. & Excoffier, L. (2000) *ARLEQUIN version 2.000. A software for population genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sefc, K.M., Lopes, M.S., Mendonça, D., Rodrigues Dos Santos, M, Da Câmara Machado, M. & Da Câmara Machado, A. (2000) Identification of microsatellites loci in olive (*Olea europaea* L.) and their characterisation in Italian and Iberian olive trees. *Molecular Ecology*, **9,** 1171–1193.

Smith, B.D. (1998) *The emergence of agriculture*. W.H. Freeman & Co., New York, pp. 16–18.

Spennemann, D.H.R. & Allen, L.R. (2000) The avian dispersal of olive (*Olea europaea* L.): implications for Australia. *Emu: Austral Ornithology*, **100,** 264–273.

Terral, J.-F. & Arnold-Simard, G. (1996) Beginnings of olive cultivation in eastern Spain in relation to holocene bioclimatic changes. *Quaternary Research*, **46,** 176–185.

Terral, J.-F., Alonso, N., Capdevila, R.B.I., Chatti, N., Fabre, L., Fiorentino, G., Marinval, P., Perez Jorda, G., Pradat, B., Rovira, N. & Alibert, P. (2004) Historical biogeography of olive domestication (*Olea europaea* L.) as revealed by geometrical morphometry applied to biological and archaeological material. *Journal of Biogeography*, **31,** 63–77.

Villemur, P., Musho, S.U., Delmas, J.M., Maamar, M. & Ouksili, A. (1984) Contribution à l'étude de la biologie florale de l'olivier: stérilité mâle, flux pollinique et période effective de pollinisation. *Fruits*, **39,** 467–473.

Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38,** 1358–1370.

Zohary, D. (1994) The wild genetic resources of the cultivated olive. *Acta Horticulturae*, **356,** 62–65.

Zohary, D. & Spiegel Roy, P. (1975) Beginnings of fruit growing in the Old World. *Science*, **187,** S319–S327.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:

Appendix S1. Identification of olive chlorotypes.

Appendix S2. Values of population parameters for each grove.

Appendix S3. Comparison by permutation of F_{st} between pairs of GRPs.

This material is available as part of the online article from http://www.blackwell-synergy.com

BIOSKETCHES

At the time of this study, **Catherine Breton** was a Masters student studying olive and oleaster relationships. She is currently supported as a PhD student by AFIDOL 'Association Française inter-professionelle de l'Olive' for research on olive, feral olive and oleaster diversity and their relationships in different regions of southern France.

Michel Tersac is an Engineer in the team '*Helianthus* Genetic Resources' and a specialist in genetic diversity analysis methods.

André Bervillé manages the Genetic Resources team on *Helianthus*, which maintains a collection of wild species (700 entries) and cultivated populations + lines (400 entries). He has provided help in the study of olive and oleaster diversity to the Arboriculture Laboratory since 1995.

Editor: Chris Humphries

ERRATUM

Upon original publication Online Early of this article (Breton *et al.*, 2006), the following information was omitted from the author addresses:

The administrative address for Catherine Breton is:

Institut Méditerranéen d'Ecologie et de Paléoécologie (IMEP, CNRS UMR 6116)
Université Paul Cézanne (Aix-Marseille III)
Europole méditerranéen de l'Arbois, Bâtiment Villemin
BP 80. F – 13545 Aix-en-Provence Cedex 04
France
IMEP WebSite: http://www.imep-cnrs.com

REFERENCE

Breton, C., Tersac, M. & Bervillé, A. (2006) Genetic diversity and gene flow between the wild olive (oleaster, *Olea europaea* L.) and the olive: several Plio-Pleistocene refuge zones in the Mediterranean basin suggested by simple sequence repeats analysis. *Journal of Biogeography*, **33,** 1916–1928.