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Olive domestication from structure of oleasters and cultivars using nuclear RAPDs and mitochondrial RFLPs

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Abstract – RAPD profiles of 121 olive cultivars were compared to those of 20 natural oleaster populations from eastern and western parts of the Mediterranean Basin. Considering the proximities of RAPD profiles between cultivars and eastern or western oleaster populations, clear differences appeared between groups of cultivars. Cultivars from Israel, Turkey, Syria, Greece and Sicily were, with very few exceptions, close to the eastern group of oleasters; in contrast, clones from Continental Italy, Continental France, Corsica, Spain and the Maghreb were closer to the western group. This genetic structure is coherent with a local selection of cultivars all around the Mediterranean Basin. The cultivars were also characterised for their mitochondrial cytotype. This information led to the conclusion that a great majority (103 of 121) of the cultivars originated by maternal descent from the eastern populations as they carry the mitotypes ME1 or ME2. However, the selection process, which involved hybridisation by pollen from local populations, could have led to an RAPD profile closer to western than to eastern natural populations. Furthermore, the other cultivars with the western mitotypes MOM or MCK generally kept a nuclear RAPD profile close to the profile of western natural populations. Consequently, they could result from exclusively local material (as for Corsica). Cultivars displaying such mitotypes could also have been selected in hybrids or introgressed genotypes between western local oleasters and the introduced eastern varieties used as male parents, restoring an eastern RAPD type. Therefore, the process of olive domestication appeared as disymmetrical: the western Mediterranean is probably a zone where olive trees from the East, once introduced, have been hybridised and back-crossed with the indigenous olives.

domestication / genetic structure / mitotype / *Olea europaea* / oleaster / olive

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Résumé – Domestication de l'olivier d'après la structure des cultivars et des oléastres basée sur les marqueurs nucléaires RAPD et les RFLP de l'ADN mitochondrial. Les profils RAPD de 121 cultivars d'olivier ont été comparés à ceux de 20 populations naturelles d'oléastres de l'Est et de l'Ouest de la Méditerranée. En considérant les proximités des profils RAPD entre les cultivars de l'Est et de l'Ouest et les populations, des différences nettes apparaissent entre les groupes de cultivars. Les cultivars d'Israël, Turquie, Syrie, Grèce et Sicile sont, avec peu d'exceptions, proches des oléastres de l'Est ; à l'opposé les clones d'Italie continentale, de France continentale de Corse, d'Espagne et du Maghreb sont proches des oléastres de l'Ouest. Cette structure génétique est cohérente avec une sélection locale des cultivars tout autour du Bassin Méditerranéen. Les cultivars ont aussi été caractérisés par le cytotype mitochondrial. Cette information montre que la grande majorité (103 sur 121) des cultivars porte les mitotypes ME1 ou ME2, et aurait donc une origine maternelle dans les populations de l'Est. Cependant, le processus de sélection qui implique l'hybridation par le pollen des populations locales, pourrait mener à avoir un profil RAPD plus proche des populations de l'Ouest que des populations naturelles de l'Est. En outre, les autres cultivars avec les mitotypes de l'Ouest MOM ou MCK, ont généralement conservé un profil RAPD proche des populations naturelles de l'Ouest. En conséquence, elles pourraient résulter de formes exclusivement locales (comme en Corse). Cependant, les cultivars qui portent de tels mitotypes pourraient aussi avoir été sélectionnés à partir d'hybrides ou de génotypes introgressés entre les oléastres de l'Ouest et les variétés, introduites de l'Est, utilisées comme parent mâle, conduisant à un profil RAPD de l'Est. Le processus de domestication de l'olivier apparaît dissymétrique : la Méditerranée de l'Ouest serait une zone où les oliviers de l'Est, une fois introduits, aurait été hybridés et rétro-croisés avec les oliviers indigènes.

domestication / structure génétique / mitotype / *Olea europaea* **/ oléastre / olive**

Abbreviations. ME1 = Mitotype "eastern Mediterranean n°1"; ME2 = Mitotype "eastern Mediterranean n°2"; MOM = Mitotype "western Mediterranean"; MCK = Mitotype characteristic of the cultivar *Chemlal de Kabylie* (western Mediterranean).

1. INTRODUCTION

In fruit trees, knowledge about the structure of genetic diversity is of a great interest for the management of genetic resources. Characterisation of the genetic diversity in both wild and cultivated forms is necessary to study similarities between them and to determine the most likely origins of cultivars. This approach should lead to a better understanding of the domestication process: localisation of the domestication centres and evidence of hybridisation or introgression. From morphological data, it has been supposed that hybridisation between cultivated forms and their wild relatives occurred in several fruit tree species and that this contributed to enhancement of the genetic variability within cultivated species [33]. Molecular studies have supported the existence of hybridisations between differentiated taxa, as in a apple [9,12], in cacao [17], and in coffee [15].

The olive tree is associated with the history of the Mediterranean civilisations. It is widely assumed that it has been exploited for at least nineteen thousand years [14]. Uses of oleasters (wild olive) during the Neolithic period in different parts of the Mediterranean Basin (Near-East, Spain) have been reported by different authors [29, 33]. The domestication of the species, which implied mainly the establishment of orchards and the vegetative multiplication of the agronomically interesting trees, is supposed to have occurred in the Near-East by about 5,700–5,500 years ago [34]. The cultivars could have been disseminated towards the Mediterranean Basin from the Near East during human migrations. Elant [10] has supposed that, in the western part of the Mediterranean, hybridisation between indigenous oleasters and cultivated forms introduced from the Levant could have led to new cultivated forms.

Oleasters are considered as indigenous to the Mediterranean Basin [34] up to Southern France [26]. It is assumed that cultivars have derived from some Mediterranean wild populations, but it is also supposed that some oleasters derived from culture (feral forms) or could be hybrids with cultivated forms. Thus, the study of the genetic structure of oleasters should take into account all information and hypotheses. A close genetic proximity between oleaster and cultivated olive has already been shown with isozymes [18] and RAPDs [4, 6]. We have shown that some cultivars could have been selected from oleaster in the western Mediterranean [5]. Thus, the origin of cultivated olive has been complex and several domestication centres may have existed. Furthermore, using nuclear and mitochondrial DNA markers, a high geographic genetic structure was also shown for oleasters [4]. Two main groups can be distinguished: the Eastern populations characterised by the mitotype ME1, and western populations characterised by the mitotypes MOM and MCK. This structure has probably resulted from the regression of the natural populations during the last glaciations leading to a high genetic drift. In contrast, the feral forms in the western Mediterranean were often characterised by the mitotype ME1 [4].

In the present study, we used both the nuclear RAPD profiles for 57 polymorphic markers and the RFLP mitotypes to obtain complementary information due to the different modes of inheritance (bi-parental or purely maternal). We assessed the genetic proximity between natural populations and groups of cultivars. Since cultivars were related to different populations, this sustained multilocal selection for cultivars. Moreover, we also found cultivars probably derived from hybrids between typical eastern and western populations.

2. MATERIAL AND METHODS

2.1. Vegetal material

Cultivars of reference (codes 1 to 102) and 19 cultivated forms with undetermined denominations (codes 103 to 121) were characterised with RAPDs

(Tab. I) [5]. Twenty oleaster populations were prospected around the Mediterranean Basin (Tab. II) [6]. These populations were sampled as far as possible from present orchards. We assumed that the trees were naturally disseminated because they were not aligned and were usually in areas difficult to access. A total of 235 oleasters was studied representing most of the countries (except some Islands). Due to regression of some populations, the number of trees per population varied from 5 to 22. In this sample, populations from the East carried the ME1 mitotype whereas western populations most frequently carried MOM and MCK mitotypes [4]. In contrast, a weak structure was shown for cultivars [5]. This argues for a wild origin of the sampled oleaster populations. Nevertheless, we cannot exclude the hypothesis that gene flows from the cultivated forms have introgressed oleasters.

2.2. Molecular data

DNA preparation was previously described by Besnard *et al.* [3]. The RAPD amplification and electrophoresis procedures were described by Quillet *et al.* [23]. Eight primers (Bioprobe, France) were used on the DNAs from all individuals: A1, A2, A9, A10, C9, C15, E15, O8. All the cultivars were also characterised for mitochondrial DNA polymorphism using the RFLP method as described by Besnard *et al.* [3]. The trees were classified according to four mitotypes called ME1, ME2, MOM and MCK [3]. Geographic specificity of these mitotypes has been previously discussed by Besnard and Bervillé [4]. Nevertheless, the mitotype ME2 was not found in oleasters. CpDNA and mtDNA polymorphism association has suggested that the cytotype ME2 marks a distinct oleaster population that very probably emerged from the East Mediterranean, in a region which was not studied (*i.e.* North-east Syria, Cyprus and Crete) (Besnard *et al.* unpublished data). We therefore considered this mitotype as originating from the eastern Mediterranean since it was revealed in several cultivars typical of this region (*i.e.*, *Zaity*, *Toffahi* and *Amygdalolia*).

2.3. Statistical analyses

The statistical analyses were performed with the OPEP software [1]. We used a discriminant analysis on qualitative data of the 20 natural populations, following the model described by Saporta [25] and Lebart *et al.* [16] as the DISQUAL procedure. The principle of this method is to compute the axes of a multiple correspondence analysis on the p binary variates recorded for n individuals and then to use the individual coordinates on all the axes or on the most significant of them in a discriminant analysis. If $p < n$ there are $p - 1$ axes; if $p > n$ there are $n - 1$ axes. For this application, with 56 axes from 57 polymorphic RAPDs, we retained the 33 axes for which the F test of population effect was significant at least at the 5% level. The 121 cultivars were used as supplementary data and projected on the axes of the discriminant

Table I. List of the cultivars studied. Country or region of origin: Greece (n° 1-6), Turkey (7-10), Near East (11-17, 103-115), Egypt (18), Yugoslavia (19), Italy (20-38), France (39-82, 118-121), Spain (83-90, 116-117), Portugal (91), Algeria (92-96), Tunisia (97-101), Morocco (102). E-W ratio: East-West ratio based on RAPD similarities. For cultivated forms with unknown denomination (n° 103-121), we attributed them a name referring to the original locality.

N°	Genotype	E-W ratio	N°	Genotype	E-W ratio	N°	Genotype	E-W ratio
1	Kalamon	47.42	42	Olivière	49.05	83	Cornicabra	53.34
2	Vallanolia	37.31	43	Blanquetier d'Antibes	45.48	84	Lechin de Sevilla	64.53
3	Gaïdourolia	42.27	44	Cailletier	46.58	85	Arbequina	51.26
4	Koroneiki	68.12	45	Cayon	62.71	86	Empeltre	58.40
5	Carolia	36.59	46	Salonenque	40.36	87	Picual	62.24
6	Amygdalolia	41.38	47	Verdanel	43.44	88	Villalonga	46.24
7	Uslu	37.85	48	Poumal	41.24	89	Manzanilla	68.67
8	Domat	46.50	49	Redouneil	45.31	90	Sevillenca	50.36
9	Ayvalik	50.77	50	Negrette	54.74	91	Galega	60.33
10	Sofralik	37.99	51	Noirette	40.24	92	Chemlal (Cordoba)	72.32
11	Souri	43.73	52	Grapié	51.53	93	Chemlal	61.12
12	Souri Mansi	46.19	53	Aglandau	46.73	94	Chemlal Mechtrass	61.30
13	Nabali Mohassen	46.57	54	Celounen	46.14	95	Azeradj	47.53
14	Barnea	55.22	55	Courbeil	52.63	96	Taksrit	53.36
15	Kaissy	45.21	56	Coucourelle	58.21	97	Chetoui	60.36
16	Zaity	36.91	57	Cayet Rouge	49.15	98	Zarazi	54.12
17	Merhavia	49.36	58	Rascasset	61.65	99	Meski	37.87
18	Toffahi	38.24	59	Malaussena	62.65	100	Barouni	49.50
19	Oblica	52.08	60	Aubenc	47.07	101	Chemlali	57.03
20	Ascolana Tenera	52.09	61	Reymet	59.73	102	Picholine Marocaine	62.26
21	Pendolino	50.71	62	Curnet	48.15	103	Palmyre 1 (Syria)	48.65
22	Frantoio	48.69	63	Colombale	42.17	104	Palmyre 2 (Syria)	49.57
23	Giarraffa	47.93	64	Poulo	42.45	105	Palmyre 3 (Syria)	35.49
24	Nocellara del Belice	39.08	65	Verdale de l'Hérault	59.41	106	Palmyre 4 (Syria)	43.56
25	Dolce Agogia	57.19	66	Amellau	38.98	107	Palmyre 5 (Syria)	46.86
26	Leccino	51.91	67	Corniale	47.50	108	Palmyre 6 (Syria)	47.28
27	San Felice	53.19	68	Rougette de Pignan	52.08	109	Palmyre 7 (Syria)	45.72
28	Moraiolo	49.72	69	Vermillau	52.07	110	Palmyre 8 (Syria)	49.36
29	Cassanese	62.55	70	Verdelé	45.24	111	Palmyre 9 (Syria)	45.91
30	Leucocarpa	56.45	71	Dorée	55.03	112	Derkouch 1 (Syria)	39.27
31	Zaituna	33.10	72	Pigalle	53.44	113	Derkouch 2 (Syria)	34.87
32	Santagatese	38.52	73	Picholine de Rochefort	38.74	114	Derkouch 3 (Syria)	34.98
33	Tonda Iblea	51.32	74	Tanche	36.65	115	Beth Hakerem (Israel)	45.07
34	Biancolilla	40.77	75	Sauzin	41.05	116	Alaon 1 (Spain)	47.05
35	Passalunara	37.74	76	Blanquetier de Nice	53.54	117	Alaon 2 (Spain)	44.94
36	Moresca	38.08	77	Grossane	49.95	118	Montpellier 1 (France)	47.10
37	Ogliarola Messinese	39.62	78	Filayre Rouge	35.49	119	Montpellier 2 (France)	48.54
38	Nocellara Etnea	46.17	79	Sabina (St Giuliano)	71.51	120	Montpellier 3 (France)	57.51
39	Picholine	51.61	80	Sabina (Corte N° 3)	68.13	121	Montpellier 4 (France)	52.56
40	Lucques	53.63	81	Zinzala	64.79			
41	Bouteillan	47.83	82	Capanacce	72.18			

Table II. List of oleaster populations analysed, location and size of the samples. Geographic groups (East, West or intermediary) were defined according to the genetic structure revealed by Besnard *et al.* [6].

N° pop	Localisation of oleasters	Country	Size	East/West
1	Urla, Izmir	Turkey	6	East
2	Harim, Oronte valley	Syria	13	East
3	Al Ascharinah, El Ghab	Syria	12	East
4	Mont Carmel, Haifa	Israel	18	East
5	Cyrenaique	Libya	15	Intermediate profile
6	Zaghouan	Tunisia	6	West
7	Mont Belloua, Tizi Ouzou	Algeria	7	West
8	Tamanar, Essaouira	Morocco	10	West
9	Immouzzer, High Atlas	Morocco	5	West
10	Torviczon, Andalucia	Spain	15	West
11	Asturias	Spain	5	West
12	Cap des Mèdes, Porquerolles, Var	France	22	West
13	La Repentence, Porquerolles, Var	France	12	West
14	Mont Boron, Nice, Alpes Maritimes	France	22	West
15	Ostricone, Corsica	France	11	West
16	Ogliastro, Corsica	France	10	West
17	Filitosa, Corsica	France	11	West
18	Bonifacio, Corsica	France	6	West
19	Messine, Sicily	Italy	12	West
20	Ali, Sicily	Italy	17	West

analysis only built from the RAPD data of natural populations. Normality of distributions of the coordinates were checked as well as homoscedasticity of the within-population variances (Bartlett's test). The Kullback test of homogeneity of variance-covariance matrices could not be performed because the sizes of the populations were too limited compared with the number of markers.

The cultivars were characterised by their relative distances to the general centroids of eastern and western natural populations. A synthetic new parameter was built for the RAPD profile of each cultivar, to measure its relative average proximity to eastern or western oleasters. This parameter, D_i, was defined as:

$$D_i = 100 \frac{\sqrt{D^2(G_i, \overline{G}_{\text{East}})}}{\sqrt{D^2(G_i, \overline{G}_{\text{East}})} + \sqrt{D^2(G_i, \overline{G}_{\text{West}})}}$$

where $D^2(G_i, \overline{G}_{\text{East}})$ is the Mahalanobis distance, in the multidimensional space, between the ith cultivar, G_i, and the centroid of all the natural individuals from the eastern group.

$\overline{G}$West H $\overline{G}$East

G_i
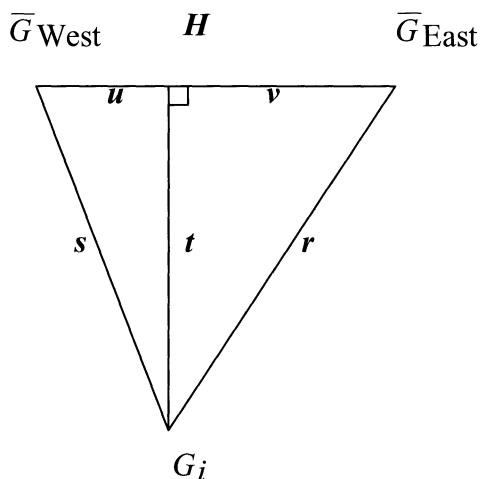
The other parameter: $D^2(G_i, \overline{G}_{\text{West}})$ is the corresponding distance of G_i from the western group. We further name r and s the square roots of the distances of the cultivar from the eastern and western natural populations, respectively. They are the corresponding deviations. As shown by the above drawing, this parameter replaces an unbiased (but not estimable here) expression of the relative deviation from East, $D'_i = 100\frac{v}{u+v}$, where u and v are the deviations from the projection of G_i, H, on the straight line joining $\overline{G}_{\text{West}}$ and $\overline{G}_{\text{East}}$. When the deviation, t, between G_i and H increases $D_i = 100\frac{\sqrt{r^2-t^2}}{\sqrt{s^2-t^2}+\sqrt{r^2-t^2}}$ may become a strongly biased estimate of D'_i because it tends towards the limit value: $D_i = 100\frac{\sqrt{r^2-t^2}}{2\sqrt{r^2-t^2}} = 50$ when t is large, leading to a ratio r/s close to 1; but this parameter, although theoretically less sensitive than D'_i, gives qualitatively the same information as D'_i, with the same critical value of 50 for delineation between two categories of olives according to their genetic proximity to eastern and western natural populations. For D_i, indeed, as for D'_i, the values lower than 50 indicate that the cultivar is closer to the eastern populations and the values greater than 50 characterise the cultivars closer to the western populations, as a value of 50 is expected for the trees exactly intermediate between these two groups. We have excluded the natural Libyan olive trees to define the two centroids, because their RAPD profile was shown to be intermediate between East and West (see below). For the 33 input variates of the discriminant analysis of the 20 natural populations as supplementary data together with individual values of the cultivars, the three parameters s, r, and D, were estimated for each of the 121 RAPD profiles by using the means of the western and eastern populations. These three parameters were then used as new variates to perform a discriminant analysis of country or region effect (with a visualisation of the new cultivar clustering) and the usual additional tests, derived from variance analysis, which will be described further. Thereafter the D parameter will be called further "East-West ratio".

3. RESULTS

3.1. General overview

The discriminant analysis of the 20 natural populations gave 11 axes significantly discriminant at the 5% level (Wilks tests). These axes cumulated 92.6% of the discrimination. All the between-population Mahalanobis distances were significant at least at the 0.1% level except for one comparison, between Mont Boron in France (n° 18) and Mont Belloua in Algeria (n° 7) (results not shown). This difference was however significant at the 10% level. The main feature of the structure of these populations was apparent on the first two axes cumulating 43.38% of the discrimination as displayed by Figure 1A. The first axis clearly separated the eastern group of natural populations (Turkey, Syria and Israel) from the western cluster (Corsica, Sicily, Continental France, Spain, Algeria and Tunisia). The unique Libyan population appeared as intermediate. This fact is coherent with the geographic situation of this country; we should note that, among the 19 other oleaster populations, Tunisia, clustered into the western group, is the closest to Libya.

Consideration of the projections of the 121 cultivars identified by their mitotype showed that the cultivars displaying the mitotypes MOM and MCK were generally far from the four populations representative of the eastern group (Turkey, Syria and Israel) and close to a western oleaster population (Fig. 1A). These mitotypes have been shown to be specific to the western oleasters [4]. The most striking feature was that the mitotypes ME1 and ME2, cumulating 103 trees, appeared as much more homogeneously distributed, with smallest distances as likely from populations of western, eastern or intermediate groups.

Figure 1B shows the clustering of the 121 cultivars relative to their country of origin. The main feature is the non-random distribution of cultivated olives of a given country. The cultivars display a more or less close proximity with the natural populations from their country or region or from a larger geographic area. This is especially the case for Corsican and Algerian varieties within the western countries and for Turkish, Syrian or Israeli olives within the eastern group.

3.2. Further examination of the relation between relatedness of cultivars to natural populations and their country of origin

The second discriminant analysis of country or region effect, performed on s, r and D, used only the 11 countries or regions represented by several cultivars (from 4 to 44 profiles) to build the new axes (the cultivars from Morocco, Lebanon, Egypt, Yugoslavia and Portugal were only used as supplementary data). Only one axis was significantly discriminant at the 0.1% level (Wilks test) with 83.43% of the discrimination. Figure 2A presents the positions of the country or region centroids and of the individual cultivars on the first two axes.
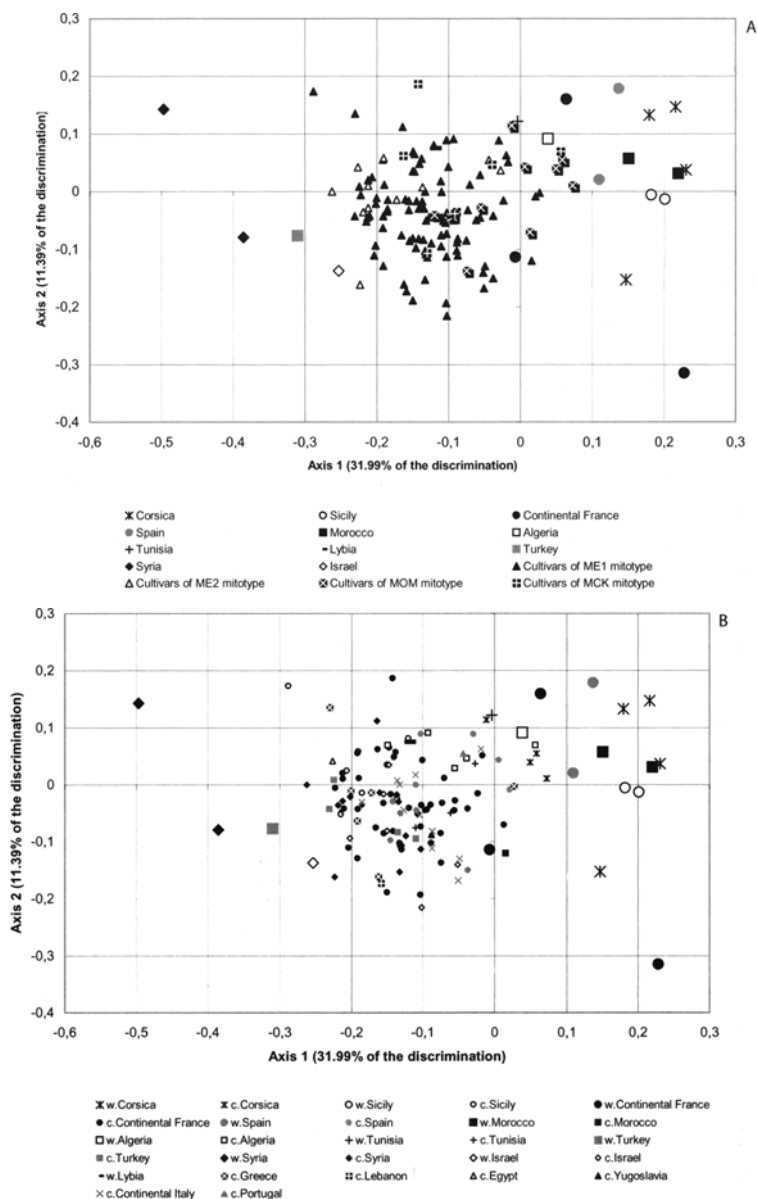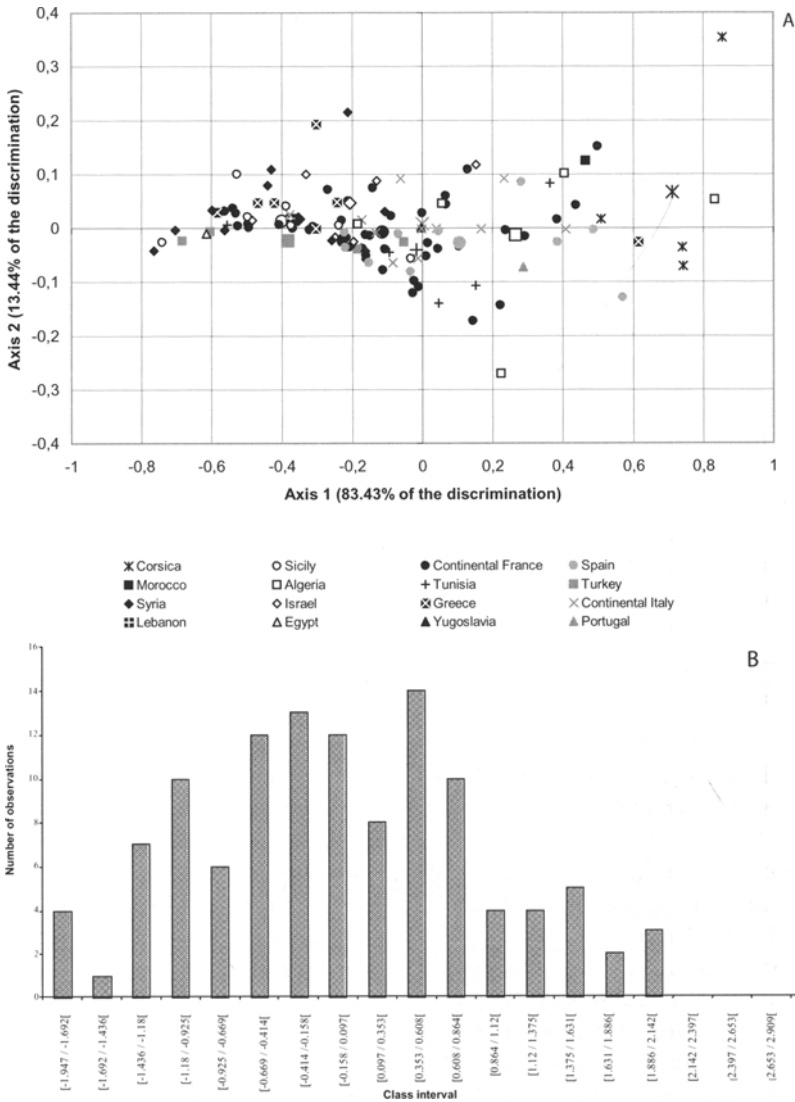
Figure 1. Genetic proximities between 121 cultivars and 20 oleaster populations. **A**. The cultivars are identified by their mitotype. **B**. The cultivars are identified by their country or region of origin. In the legend, the cultivars are prefixed by "c" and the oleaster populations by "w". The centroids of these oleaster populations are represented by a larger symbol.

The most important result is that the only discriminant axis (axis 1) appeared as quite perfectly correlated with the East-West ratio ($r = 0.998^{***}$). The prevailing value of this parameter for cultivar structure per country is confirmed by Table III which displays the relationship between country or region ranking for D and their corresponding distribution on axis 1 of the discriminant analysis with the associated Newman-Keuls tests. This close correspondence was measured by a high value of the Spearman rank correlation ($r = 0.982^{***}$). It means that the only factor to be considered for characterisation of the cultivar proximity to the natural populations of Mediterranean Basin is not the absolute genetic distance to these populations (expressed by the s and r parameters), but the relative proximities to the two groups that we have characterised. This fact has very profound consequences, which will be discussed further.

The validation of the East-West ratio as an adequate measure of the genetic background of oleasters in the cultivars is important to classify the countries and the individual cultivars for predominance of the western or eastern gene pool. Therefore, Table III may be more easily interpreted: cultivars from Israel, Greece, Turkey, Syria and Sicily have on the average a prevailing relatedness with eastern oleasters, whereas cultivars from Corsica, Algeria and Spain are mostly related to western oleasters. Cultivars from Tunisia, Continental Italy and France constitute an intermediate group, which displays equilibrium between western and eastern contributions to the gene pool. The individual values of the East-West ratio for the 121 cultivars are given in Table I.

3.3. Distribution of East-West genetic proximities of cultivars within their country

There is a noticeable dispersion of the cultivars around the corresponding country mean for the East-West ratio; this fact is illustrated by the overlapping groups in the Newman-Keuls test of Table III. On the average, the within-country variances could be considered as homogeneous: Bartlett's homogeneity test was only 9.44 for 10 d.f. (probability of greater chi-square was 0.51). This validated our aim to draw general rules about the between-country pattern of variability for this parameter. Moreover, the distribution of deviations of the cultivars from the means of their countries could be considered as normal, in view of our sample size (insignificant coefficients of skewness and kurtosis, 0.34 and 3.08 for 116 values, respectively). This distribution is displayed in Figure 2B. On the basis of the standardised deviations from the country mean compared to a Student's t distribution with 95 d.f., only two cultivars could be considered as having a "marginal" RAPD profile: *Koroneiki* from Crete (Greece) and *Manzanilla* from Spain, n° 4 and n° 89, respectively (Tab. I). But, as the significance levels increased 1% and 5%, such deviations could be expected by chance in a sample of 116 trees. It is also possible that *Koroneiki* was introduced into Greece from a western area, such as Cyrenaique (Libya).

Figure 2. Illustration of the measurement of the genetic proximity between the cultivars and the eastern or western natural populations. **A**. Clustering of 121 cultivars from 16 countries or regions according to their genetic proximity to the centroids of eastern or western natural populations. The centroid corresponding to a given region is represented by a larger symbol than the elementary cultivars. **B**. Distribution of the standard deviations from the country means for the East-West ratio of 116 cultivars.

Table III. Comparison between the structure of countries for the proximity of their cultivars to oleaster gene pools using a Discriminant Analysis (A) and the synthetic parameter called East-West ratio (B). The significance level of Newman-Keuls tests for comparison of means is 5%.

A. Distribution of the groups of cultivars on the discriminant axis 1

Rank	Country	Axis 1	Standard error	
1	Corsica	0.712	0.138	
2	Algeria	0.266	0.124	|
3	Spain	0.106	0.088	| |
4	C.Italy	0.000	0.083	| | |
5	Tunisia	−0.017	0.124	| | |
6	C.France	−0.115	0.042	| | |
7	Israel	−0.206	0.113	| |
8	Greece	−0.244	0.113	| |
9	Syria	−0.353	0.074	|
10	Turkey	−0.382	0.138	|
11	Sicily	−0.400	0.105	|
Average standard error (harmonic mean):			0.108	

B. Distribution of the groups of cultivars according to the E-W ratio

Rank	Country	E-W ratio	Standard error	
1	Corsica	69.155	3.572	
2	Algeria	59.126	3.195	|
3	Spain	54.703	2.259	| |
4	Tunisia	51.776	3.195	| | |
5	C.Italy	51.774	2.154	| | |
6	C.France	48.996	1.077	| | |
7	Israel	46.823	2.916	| |
8	Greece	45.515	2.916	| |
9	Turkey	43.276	3.572	| |
10	Syria	43.119	1.909	| |
11	Sicily	40.971	2.700	|
Average standard error (harmonic mean):			2.776	

We can then conclude that everything happens as if the proximity of a cultivar from the eastern and western gene pools could be predicted by its country or region of origin, the average deviations from these predictions being the same for every country or region. This is consistent with a process of domestication where the choice of the breeding material was primarily limited by the possibilities of exchange and therefore by the geographic distance. The genetic implications of this conclusion will be discussed further.

3.4. Hybrid status of western cultivars

The results described above do not directly tell anything about the causes of the genetic proximities between the cultivars and the western or eastern natural populations of olives. In particular, taking into account the general mechanism of varietal diffusion from East to West, many cultivars originating from this second zone may be suspected to derive from hybrids between trees from the two main groups.

4. DISCUSSION

It is theoretically clear that the classification of clones is much more complex than a simple dispatching between only two east and west groups, since the present varieties resulted from a long series of migration, hybridisation and introgression processes, which are not easy to unravel. Indeed, most of the steps that led to the present genetic structure are unknown. Individual values of the East-West ratio of cultivars presented in Table I will provide the main elements for an inference about their hybrid status.

The geographic genetic structures observed with mitotypes [4] and with nuclear RAPDs, which correspond to a clear distinction between eastern and western populations, likely reflect the existence of the last refugial zones as shown for other forest trees, insects and animals [27]. Thus, from these data, at least two Mediterranean refugial zones were inferred: one in the East and another in the West (Fig. 3I). Natural barriers to gene flows (Adriatic Sea, Libyan and Egyptian Deserts) have contributed to preserve this structure (Fig. 3II). Palynological and anthracologic data support the existence of O. europaea in the Near East during the last glaciation [2,14,30]. There is also evidence of such a presence in the western Mediterranean during the Upper Pleistocene [22,31], but the number of palynological studies considering the last glaciation (18,000–12,000 BP) is still limited. In contrast, numerous studies about the last 10,000 years have shown the expansion of the olive tree with human activity from the Holocene in all the western European countries [7,8,11,13,19–21,24,26,29,32].

The cultivar classification established on the basis of RAPDs was not congruent with their cytoplasmic classification. On the basis of the distribution of mitotypes in wild and cultivated olives, we clearly shows that the diffusion of more or less selected varieties was oriented from East to West. Furthermore, the genetic background of each cultivar was considered in relation to the genetic diversity and geographical origin of the oleaster. This approach provides objective arguments for classifying the cultivars. We have proved that the RAPD profile of cultivars originating from a limited geographic area (country or region) was not independent of the genotypes of the wild surrounding populations. Of course, this association is not very tight but it is nonetheless
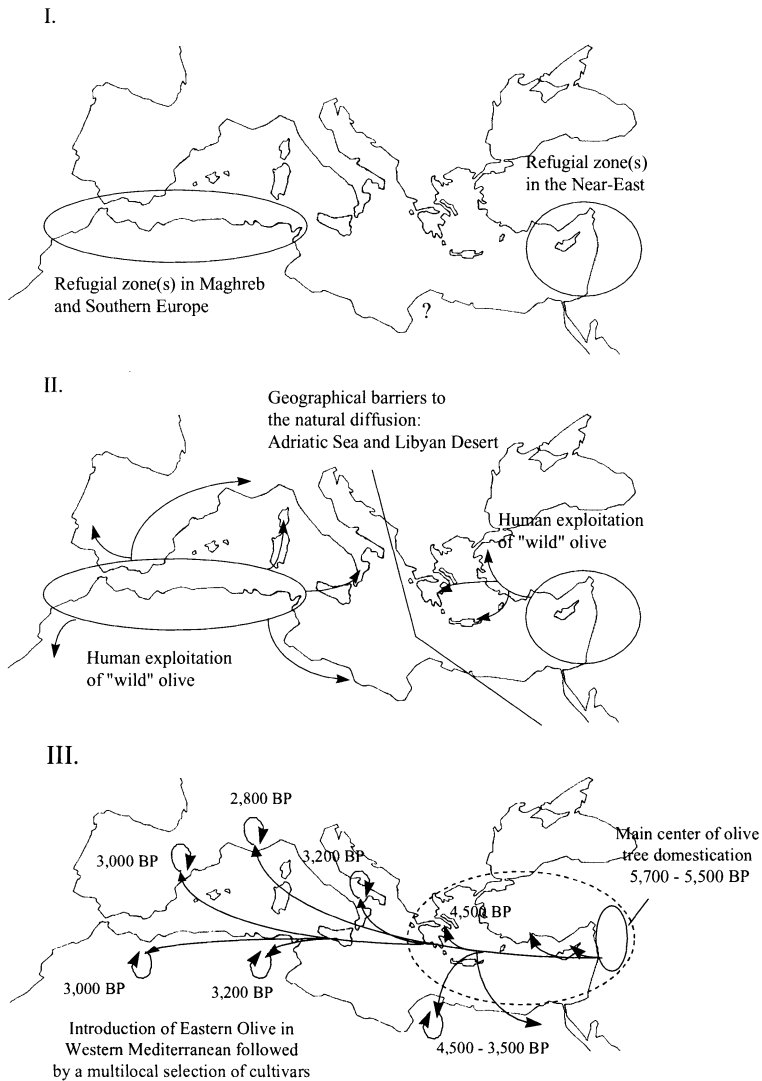
Figure 3. Scenario proposed for explaining diffusion of olive tree inside the Mediterranean Basin since the last glaciation. **I.** Persistence of refugial zones in the West and East of the Mediterranean Basin during the last glaciation (20,000–12,000 BP); II. Natural diffusion of oleaster population with human influence (12,000–6,000 BP); III. Domestication of olive tree in the Near-East and beginning of the diffusion of cultivated olives towards the Mediterranean Basin (since 5,500 BP). Approximate dates of introduction are reported from Terral [28]. ↺: Selection of cultivars from hybridisations between introduced and indigenous olives.

significant. We have also shown that some regions or countries could elaborate their own varieties probably by using only their local genetic resources. The most typical example is given by Corsica, which produced mostly original varieties (*Sabina, Zinzala, Capanacce*). The model that we propose to connect the RAPD profile of cultivars or groups of cultivars to the Eastern or Western groups of oleasters is of course simplified. The sampling of natural populations used in this study did not allow the use of a more diversified set of wild populations. But this model seems realistic, both because there is a great genetic diversity between the two groups of Eastern and Western wild olives (Fig. 1), and also because we have shown that the East-West direction was privileged for the variety transfers.

It is surprising that the classification of cultivars for their proximity to the western and eastern gene pools could be completely achieved by only using the East-West ratio. This efficiency of a simple model (only one parameter) means that the absolute genetic distances between cultivated and oleasters add nothing to a prediction of the origin of the gene pool possessed by the former category only based on the relative distances. The genetic interpretation is that, on average, the points which correspond to the 11 coordinates measuring the RAPD profile of the cultivars are not far from the straight line joining the centroids of the western and eastern oleasters. In other words, t is small compared to r and s. The result is that the bias on D as an estimate of D' is limited. Moreover, if $t = 0$, r and s are on the same straight line and their absolute values add no information to their ratio (which may interpreted as a simple change of units: r and s are normalised by their sum, $r + s$). The last argument is given in Table I where it is shown that the East-West ratio ranges from 33.10 (*Zaituna*, code 31) to 71.51 (*Sabina*, code 79), as with a high value of t, all the values of the parameter should be close to 50. Therefore, when the value of D is really close to 50, as for many Italian or French clones (for instance, *Pendolino*, code 21, *Moraiolo*, code 28, *Picholine*, code 39, *Olivière*, code 42), this may be a strong indication for a hybrid origin of such cultivars between eastern and western natural populations. When more data become available on variability of wild populations, it will be interesting to test other possible models, with an individualisation of more than two groups of oleasters, to characterise the genetic proximities between cultivars and wild gene pools.

5. CONCLUSION: A MODEL FOR OLIVE DOMESTICATION

As the numbers and the sizes of our sampled natural populations were rather limited, we restricted our conclusions to clear general results. First, the domestication of *Olea europaea* in the Mediterranean Basin was a disymmetrical process where the prevailing direction of exchanges of breeding material was from the Near-East towards the occidental countries of Europe and the Maghreb (Fig. 3II). This general pattern of gene flow is clearly established

by the contrast between the general diffusion of the mitotypes ME1 and ME2 in the cultivars from western countries and the absence of a symmetrical dissemination of the mitotypes MOM and MCK in the varieties produced in the eastern Mediterranean. Such a process of old variety transfers from eastern Mediterranean Basin countries is also in agreement with historic data, which indicate that olive breeding began in the Near-East [33]. The privileged maternal lineage among the cultivars, as traced by their mitotype, is also logical because, before the twentieth century, the only practical possibility of long-distance transfer was limited to the seeds and the vegetative propagules.

The second clear conclusion is that the ancient and long-lasting diffusion of more or less improved material has not resulted in a general mix of cultivated genotypes. On the contrary, the present varieties have kept a "memory" of the nuclear genotypes of the surrounding populations of oleasters. The practical consequence of this memory is the persistence of a structure for cultivars. This is in accordance with the genetic resources of their country of origin. This means that long-distance variety transports were likely associated with multiple centres of domestication using the locally available natural populations to produce cultivars adapted to the specific cultural conditions, ecological limitations and traditional habits (Fig. 3III). But more prosaic reasons to use local oleasters could have been a reduced cost and also the difficulty of multiplying quickly and greatly extent the introduced varieties.

As the structure of the RAPD profiles gave clear evidence of the existence of two main groups of oleasters (East and West groups), we built a simplified model to connect the nuclear genotype of the cultivars to these two clusters of oleasters. Due to the limited analysed samples, a more sophisticated modelling would be statistically inefficient. However, although simplified, the model offers an efficient way to provide a rough evaluation of the degree of relatedness of individual cultivars or groups of cultivars (according to the country or region, the geographic area or to some other clustering method) with two main contrasted gene pools. In the future, a more intensive sampling of Mediterranean oleasters and other gene markers as microsatellites will allow a more precise characterisation of the relatedness of cultivars with a series of well-defined groups of oleasters.

REFERENCES

[1] Baradat P., Labbé T., OPEP: Un logiciel intégré pour l'amélioration des plantes pérennes, in: Traitements statistiques des essais de sélection. Stratégies de sélection des plantes pérennes, CIRAD-CP (Ed.), Montpellier, 1995, pp. 303–330.

[2] Baruch U., Bottema S., Palynological evidence for climatic changes in the Levant ca. 17,000-9,000 B.P., in The Natufian Culture in the Levant, Bar-Yosey O., Vall F. (Eds.), International Monographs in Prehistory, Ann Arbor, 1991, pp. 11–20.

[3] Besnard G., Khadari B., Villemur P., Bervillé A., Cytoplasmic male sterility in the olive (*Olea europaea* L.), Theor. Appl. Genet. 100 (2000) 1018–1024.

[4] Besnard G., Bervillé A., Multiple origin of the Mediterranean olive deduced from mitochondrial DNA polymorphism. C. R. Acad. Sci. III 323 (2000) 173–181.

[5] Besnard G., Baradat P., Bervillé A., Genetic relationships in the olive (*Olea europaea* L.) reflect multilocal selection of cultivars, Theor. Appl. Genet. 102 (2001) 251–258.

[6] Besnard G., Baradat P., Chevalier D., Tagmount A., Bervillé A., Genetic differentiation in the olive (*Olea europaea*) complex revealed by RAPDs and RFLPs in the rRNA genes, Genet. Res. Crop Evol. 48 (2001) 165–182.

[7] Brun A., Microflores et Paléovégétations en Afrique du Nord depuis 30 000 ans, Bull. Soc. Géol. France 1 (1989) 25–33.

[8] Carrion J.S., Dupré M., Fumanal M.P., Montes R., A palaeoenvironmental study in semi-arid southeastern Spain: The palynological and sedimentological sequence at Perneras cave (Lorca, Murcia), J. Archaeol. Sci. 22 (1995) 355–367.

[9] Durham R.E., Korban S.S., Evidence of gene introgression in apple using RAPD markers, Euphytica 79 (1994) 109–114.

[10] Elant H., Olive, in Evolution of crop plants, M.W. Simmonds Ed., Edinburgh, Scotland, 1976, pp. 219–221.

[11] Follieri M., Magri D., Sadori L., 250,000-year pollen record from Valle di Castiglione (Roma), Pollen Spores 30 (1988) 329–356.

[12] Kato S., Ishikawa S., Imakawa S., Komori S., Mikami T., Shimamoto Y., Cytoplasmic relatedness of apple landraces and cultivars: a molecular analysis, Euphytica 66 (1993) 99–102.

[13] Kelly M.G., Huntley B., An 11 000-year record of vegetation and environment from Lago di Martignano, Latium, Italy, J. Quat. Sci. 6 (1991) 209–224.

[14] Kislev M.E., Nadel D., Carmi I., Epipalaeolithic (19,000 BP) cereal and fruit diet at Ohalo II, Sea of Galilee, Israel, Rev. Palaeobot. Palynol. 73 (1992) 161–166.

[15] Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot, P., Anthony F., Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.), Theor. Appl. Genet. 100 (2000) 139–146.

[16] Lebart L., Morineau A., Piron M., Statistique exploratoire multidimensionnelle, 2nd edition, Paris, Dunod, 1997.

[17] Lerceteau E., Robert T., Pétiard V., Crouzillat D., Evaluation of the extent of genetic variability among *Theobroma cacao* accessions using RAPD and RFLP markers, Theor. Appl. Genet. 95 (1997) 10–19.

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

[19] Magri D., Late Quaternary vegetation history at Lagaccione near Lagi di Bolsena (central Italy), Rev. Palaeobot. Palynol. 106 (1999) 171–208.

[20] Magri D., Sadori L., Late Pleistocene and Holocene pollen stratigraphy at Lago di Vico (central Italy), Veg. Hist. Archaeobot. 8 (1999) 247–260.

[21] Pérez-Obiol R., Julià R., Climate change on the Iberian Peninsula recorded in a 30,000-Yr pollen record from Lake Banyoles, Quat. Res. 41 (1994) 91–98.

[22] Pons A., Reille M., The Holocene- and upper Pleistocene pollen record from Padul (Granada, Spain): a new study, Palaeogeogr. Palaeoclimatol. Palaeoecol. 66 (1988) 243–263.

[23] Quillet M.C., Madjidian N., Griveau Y., Serieys H., Tersac M., Lorieux M., Bervillé A., Mapping of genetic factors controlling pollen viability in an interspecific cross in *Helianthus* sect. *Helianthus*, Theor. Appl. Genet. 91 (1995) 1195–1202.

[24] Riera-Mora S., Esteban-Amat A., Vegetation history and human activity during the last 6000 years on the central Catalan coast (northeastern Iberian Peninsula), Veget. Hist. Archaeobot. 3 (1994) 7–23.

[25] Saporta G., Probabilité, Analyse des Données et Statistique, Paris, Technip, 1990.

[26] Solari M.E., Vernet J.L., Late glacial and Holocene vegetation of the Corbières based on charcoal analysis at the Cova de L'Espérit (Salses, Pyrénées Orientales, France), Rev. Paleobot. Palynol. 71 (1992) 111–120.

[27] Taberlet P., Fumagalli L., Wust-Saucy A.G., Cosson J.F., Comparative phylogeography and postglacial colonization routes in Europe, Mol. Ecol. 7 (1998) 453–464.

[28] Terral J.F., La domestication de l'olivier (*Olea europaea* L.) en Méditerranée nord-occidentale : Approche morphométrique et implications paléoclimatiques, Thèse, Université Montpellier II, France, 1997.

[29] Terral J.F., Arnold-Simard G., Beginnings of olive cultivation in eastern Spain in relation to Holocene bioclimatic changes, Quat. Res. 46 (1996) 176–185.

[30] Van Zeist W., Woldring H., Holocene vegetation and climate of Northwestern Syria, Palaeohistoria 22 (1980) 111–125.

[31] Watts W.A., Allen J.R.M., Huntley B., Vegetation history and palaeoclimate of the last glacial period at Lago Grande di Monticchio, Southern Italy, Quat. Sci. Rev. 15 (1996) 133–153.

[32] Yll E.I., Perez-Obiol R., Pantaleon-Cano J., Roure J.M., Palynological evidence for climatic change and human activity during Holocene on Minorca (Balearic Islands), Quat. Res. 48 (1997) 339–347.

[33] Zohary D., Hopf M., Domestication of plants in the Old World, 2nd edition, Oxford Clarendon Press, 1994, pp. 137–142.

[34] Zohary D., Spiegel-Roy P., Beginnings of fruit growing in the Old World, Science 187 (1975) 319–327.