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V. Bronzini de Caraffa · J. Maury · C. Gambotti
C. Breton · A. Bervillé · J. Giannettini

Mitochondrial DNA variation and RAPD mark oleasters, olive and feral olive from Western and Eastern Mediterranean

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Abstract The study of genetic diversity within the olive-tree (cultivated and wild forms) may be useful to reveal agronomic traits in the wild germplasm and to try to understand the history of the olive-tree domestication. In this way, a study of nuclear and mitochondrial DNAs of cultivated and wild olives from two Corsican and Sardinian Mediterranean islands was performed using RAPD and RFLP markers. Our results show that most of the varieties and most of the oleasters were separated using the UPGMA dendrogram based on the Nei and Li similarity index. Most of oleasters carried either the MOM or MCK mitotype, characteristic of olives in the Western Mediterranean, whereas most of the varieties carried the ME1 mitotype, characteristic of olives in the East Mediterranean. The results indicate that the combination of mitotype and RAPD markers can be used as a powerful tool for differentiating two groups in the wild forms: the Western true oleasters and the feral forms. The true oleasters are characterized by a Western mitotype and a Western RAPD pattern. Feral forms originate either from varieties or from hybridisation between a variety and an oleaster. Consequently, as expected, some of them aggregated with the varieties from which they were derived. The other feral forms are clustered with the oleasters and were detected only by their mitotype determination. This study has also permitted us to differentiate two populations of cultivated olives in Corsica: one with close relationships with Italian varieties (influenced by the East) and one selected from local oleasters probably due to a better local adaptation than foreign varieties.

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V. Bronzini de Caraffa · J. Maury · C. Gambotti · J. Giannettini (✉)
Centre de Recherche Biodiversité Insulaire Méditerranéenne,
Laboratoire de Biochimie et de Biologie Moléculaire Végétales,
Faculté des Sciences et Techniques, Université de Corse,
BP 52, F20250 Corte, France
e-mail: gianetti@univ-corse.fr
Tel.: +33-4-95-45-01-80, Fax: +33-4-95-45-01-80

C. Breton · A. Bervillé
INRA, UR-Génétique et Amélioration des plantes,
Bat 33, 2 Place Viala, F 34060 Montpellier Cedex 1, France

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Introduction

The olive-tree (*Olea europaea* L.) is one of the most ancient of cultivated trees. Two forms exist: the cultivated form (*O. europaea* subsp. *europaea* var. *sativa*) and the wild form (*O. europaea* subsp. *europaea* var. *oleaster)*. In olive cultivation, the intensive modes of production favour the use of a few varieties with a stable and regular yield over a wide area associated with acceptable organoleptic characteristics. This selection leads to genetic erosion due to the abandonment of numerous locally adapted olive varieties. However, it is necessary to preserve the genetic diversity within the olive and the oleaster in order to develop improvement programs and to determine the relations between the cultivated and wild forms, and in some cases also to reconstitute the olive history. Because of early olive cultivation, the cultivated forms, which have extended considerably over the natural populations, have caused the regression of oleasters. Consequently, at the present time, oleasters are absent from many regions (Ouazzani et al. 1993), and are limited to restricted areas along the shores of the Mediterranean (Zohary and Spiegel-Roy 1975). However, the study of oleasters appears of real interest since it might constitute a gene pool useful for olive improvement programs, i.e. for disease and stress resistance.

Cultivated and wild forms have the same chromosomes number ($2^n = 46$) (Green and Wickens 1989) and are fully inter-fertile. Cultivated olives are propagated vegetatively whereas wild olives only reproduce by sexual means. The cultivated forms have been selected from the wild local forms in several Eastern places (Zohary and Spiegel-Roy 1975). The domestication of the olive-tree started in the Near East about 6,500 years ago and has been disseminated all around the Mediterranean basin (Zohary and Spiegel-Roy 1975; Loukas and Krimbas 1983). Recently, however, Besnard and Bervillé (2000) suggested other

Table 1 Olive varieties studied and their synonyms in corresponding regions: Co = Corsica, Sa = Sardinia, Si = Sicily, It = mainland Italy, Fr = mainland France, IE = Island of Elba

Variety	Synonyms	Variety	Synonyms
Antonina (Co)		Cipresina (It)	
Capanacce (Co)	Raspuluta (Co), Razzola (Co)	Coratina (It)	
Corte variety (Co)		Frantoio (It)	Ghjermana di Balagna (Co), Nieladja (Co), Razzola (Co), Corsicana da Olio (Sa), Casalina (It)
Francardo variety (Co)		Grossa di Cassano (It)	
Migliaciaru variety (Co)		Itrana (It)	Corsicana da Mensa (Sa), Nera di Oliena (Sa), Paschixedda (Sa), Terza Grande (Sa), Terza Piccola (Sa), Tonda di Villacidro (Sa)
Oliese (Co)		Leccino (It)	
Romana a (Co)		Moraiolo (It)	Aliva Nera (Co), Ghjermana (Co)
Romana b (Co)		Pendolino (It)	
Sabina (Co)	Aliva Bianca (Co), Biancaghja (Co)	Piacente (It)	
Zinzala (Co)	Bonifacio (Co), Pinzarole (Co)	San Agostino (It)	
Bosana (Sa)		Biancollila (Si)	
Confetto (Sa)	Maiorca (Sa), Nera di Gonnos (Sa), Sivigliana Sarda (Sa), Tonda di Cagliari (Sa)	Carolea (Si)	
Pizz'è Carroga (Sa)		Giaraffa (Si)	Pezz'è Cuaddu (Sa), Santa Caterrina (It)
Semidana (Sa)	Cariasina (Sa)	Nocellara (Si)	
Sivigliano da Olio (Sa)		Nocellara Belica (Si)	
Ascolana (It)		Picholine (Fr)	

centres of domestication in the Western Mediterranean basin. This suggestion is based on a mitochondrial DNA (mtDNA) study. The mtDNA, generally maternally inherited in dicotyledonous species, has been studied in several plant species in order to determine their geographical origin (Sinclair et al. 1998; Tomaru et al. 1998; Tsumura and Suyama 1998). In the olive, mitochondrial markers have also been shown useful for tracing the maternal line (Besnard and Bervillé 2000). Four mitotypes have been distinguished in the Mediterranean olives: ME1 (the mitotype from the Eastern Mediterranean, no. 1) and ME2 (the mitotype from the Eastern Mediterranean, no. 2) characteristic of olives in the Eastern Mediterranean (Egypt, Greece, Near East and Turkey), MOM (the mitotype from the Western Mediterranean) and MCK (the mitotype characteristic of the cultivar Chemlal de Kabylie from the Western Mediterranean) found in olives from the West (France, Italy, Maghreb, Spain, Yugoslavia). However, an Eastern influence on some Western regions, namely Italy and Libya, but also France and Maghreb, has been shown for cultivated olives (Besnard and Bervillé 2000).

Corsica (France) and Sardinia (Italy) are two islands in the Western Mediterranean, which are only 12-km apart. A long time ago they formed only one block and their separation occurred 24 million years ago according to Gueguen (1995). These two islands have interesting olive and oleaster pools since, in addition to many cultivated varieties, they possess many oleaster forests. Besnard (1999) has previously shown that some varieties were introduced from Italy and others are probably local forms. The presence of feral forms, resulting from cultivars escaping cultivation and also hybridisations between varieties and oleasters, have also been suggested in Corsica and Sardinia using amplified fragment length polymorphisms (AFLP) (Baldoni et al. 2000). Feral olive-trees display an oleaster phenotype and are characterized by very close molecular relationships with varieties and not with oleasters (Angiolillo et al. 1999; Besnard and Bervillé 2000). In the present study, a priori, all wild forms may include feral forms and oleasters and will be compared using RAPDs and RFLPs.

The aim of this work was to determine whether the Corsican and Sardinian olives were subject to an Eastern influence (as in Italy) or if these varieties have a Western origin. Both nuclear markers and mitotypes were employed to determine if Western varieties could have been selected from oleasters. We inferred from the origin of the Corsican varieties that Corsica may have two different olive populations with weak relationships. Moreover, on the basis of both nuclear and cytoplasmic markers we recognized feral forms among the oleasters.

Materials and methods

Plant material

One individual per 32 olive varieties and 99 oleasters were studied. The 32 varieties include the totality of the Corsican (10) and Sardinian (5), 11 from mainland Italy, 5 from Sicily and one from mainland France. Among the 99 oleasters, 91 were Corsican, 7 Sardinian and 1 from the Elba Island (Table 1). Some varieties have several synonymous denominations. To indicate them, we chose the most-widespread denomination in their country of origin.

DNA preparation

Total DNA was extracted from fresh leaves using the method described by Saghai-Maroof et al. (1984) with some modifications. Five grams of fresh leaves were ground in liquid nitrogen and mixed with 20 ml of CTAB buffer (100 mM Tris-HCl pH 8, 1.4 M

Table 2 Mitotype distribution in olives from different origins. **A** In olive varieties. **B** In oleasters. The table does not include the individuals with not determined mitotype

A

Mitotype	Corsican varieties $n = 10$	Sardinian varieties $n = 5$	Italian mainland varieties $n = 10$	Sicilian varieties $n = 5$	French mainland variety $n = 1$	Total varieties $n = 31$
ME1	5	4	10	2	0	21
ME2	0	0	0	3	0	3
EAST	5 (50%)	4 (80%)	10 (100%)	5 (100%)	0	24 (77.4%)
MOM	4	1	0	0	1	6
MCK	1	0	0	0	0	1
WEST	5 (50%)	1 (20%)	0	0	1 (100%)	7 (22.6%)

B

Mitotype	Corsican oleasters $n = 88$	Sardinian oleasters $n = 6$	Elba island oleaster $n = 1$	Total oleasters $n = 95$
ME1	13	0	1	14
ME2	0	0	0	0
EAST	13 (14.8%)	0	1 (100%)	14 (14.7%)
MOM	73	5	0	78
MCK	2	1	0	3
WEST	75 (85.2%)	6 (100%)	0	81 (85.3%)

NaCl, 20 mM EDTA, 2% CTAB), and was then supplemented with 0.2% 2-mercaptoethanol, shaken and incubated at 65 °C for 1 h. Chloroform/Isoamylic alcohol (24:1) was added (10 ml) and mixed vigorously to homogenisation. The aqueous phase was recovered by centrifugation at 7,000 *g* for 20 min, then the process was repeated twice. The upper phase was taken and 2/3 vol of cold isopropanol was added by gently mixing for precipitation. Then the precipitated nucleic acids were transferred to a new tube, rinsed with 2 ml of 76% ethanol, 10 mM of ammonium acetate and resuspended in 500 µl of TE (10 mM Tris-HCl pH 7.6, 1 mM EDTA pH 8). The sample was treated with 50 mg.ml^{-1} of RNAse A (Sigma) for 1 h at 37 °C. Organic extractions were performed by adding an equal volume of Phenol/Chloroform/Isoamylic alcohol, shaken vigorously and centrifuged at 13,000 *g* for 10 min; then the process was repeated with Chloroform/Isoamylic alcohol. DNA was precipitated by adding 0.3 vol of 7.5 M ammonium acetate and 2 vol of cold 76% ethanol. The solution was centrifuged at 13,000 *g* for 10 min. The pellet was rinsed, dried and then resuspended in 500 µl of TE. DNA concentration was determined using a spectrophotometer at 260 nm and diluted to 10 ng. µl^{-1}.

Random amplified polymorphic DNA

The primers used were A01, A02, A09, A10, A16, A19, C09, C15, D03, O08 and O16 (Eurogentec). The 25-µl PCR reaction mixtures contained: 75 mM of Tris-HCl pH 8.8, 20 mM of $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20, 2.5 mM of $MgCl_2$, 50 µM of each of dATP, dCTP, dGTP and dTTP, 40 ng of primer, 30 ng of DNA and 2 units of *Taq* DNA polymerase (Eurogentec). PCR was performed using a Triothermoblock (Biometra). The reaction mixtures were subjected to 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 38 °C for 1 min, 72 °C for 1 min, and then a final 6-min 72 °C extension. Amplification products were analysed by electrophoresis through 1.6% agarose (Eurogentec) followed by ethidium bromide staining. The molecular sizes of the fragments were estimated using a 1-kb DNA ladder (Gibco BRL). The reactions were repeated at least twice and only reproducible, relatively intense, bands were scored.

Restriction fragment length polymorphism of mtDNA

Three micrograms of total DNA were restricted with 24 U of either *Hin*dIII or *Xba*I (Eurogentec) at 37 °C, overnight. Fragments were separated on a 1% agarose gel in 0.5× TBE buffer and blotted onto a Biodyne Nylon membrane (Polylabo) in 0.5 M NaOH. The membrane was rinsed in 2× SSC for 5 min and the DNA fixed under UV light for 3 min. The probe used was the mitochondrial *atp6* gene (Dewey and Tymothy 1986) labelled by random priming using 74 mBq [^{32}P] dCTP (111 TBq/mmol). The membrane was pre-hybridized at 62 °C for 3 h in a buffer containing 0.5% SDS, 6× SSC and 5× Denhardt's solution. The hybridisation was performed in the same buffer after labelled-probe addition at 62 °C for 16 h. The membrane was rinsed in a 1% SDS 40 mM Na_2HPO_4 solution then in a 1% SDS 100 mM Na_2HPO_4 solution at 62 °C. The blots were then exposed at –80 °C to Hyperfilm MP (Amersham Life Science) for a sufficient time depending on the labelling intensity.

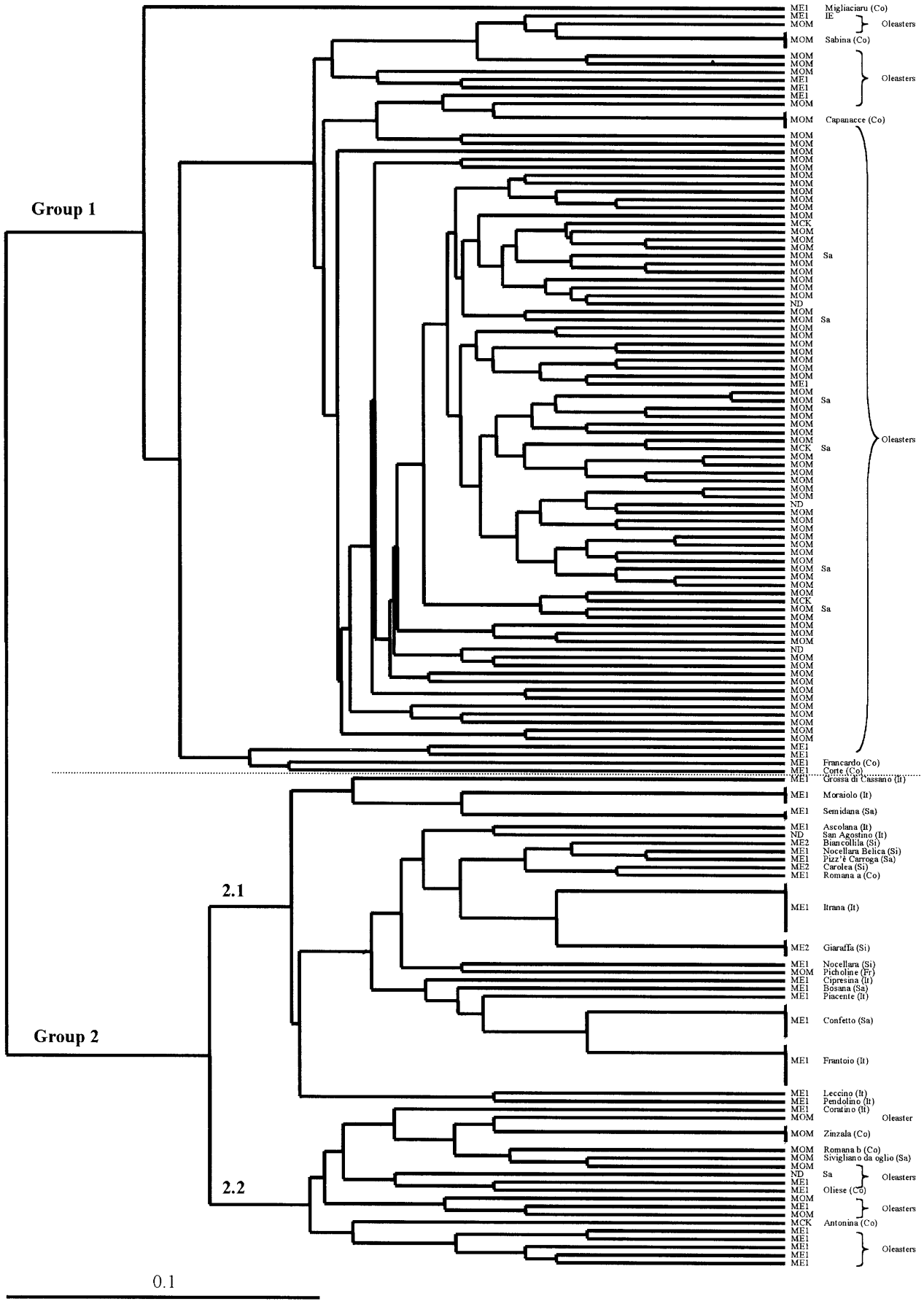
Data treatments

The PCR fragments were scored as present (1) or absent (0). Data were analysed with the PHYLIP program (version 3.5) (Felsenstein 1989) using Nei's similarity index (Nei and Li 1979). A dendrogram was constructed using the UPGMA (unweighted pair group method with arithmetic averages) method (Benzécri 1973). The RAPD data were also analysed by factorial correspondence analysis (FCA) (Benzécri 1982) using the Statgraphics software package (version 3.0).

Results

Mitochondrial DNA variation

Mitochondrial DNA analysis revealed four mitotypes: ME1 and ME2 characteristic of olives in the Eastern Mediterranean, and MOM and MCK characteristic of olives in the West. Among the 131 olives studied, the mitotypes of four oleasters and one variety (San Agostino) were not determined. Table 2 shows the frequency of each mitotype in the cultivated and wild forms. ME1 and ME2 Eastern mitotypes were revealed


Group 1
Group 2
2.1
2.2
0.1
ME1 Migliaciaru (Co)
ME1 IE
MOM
Oleasters
MOM Sabina (Co)
MOM
MOM
MOM
ME1
ME1
ME1
MOM
Oleasters
MOM Capanacce (Co)
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ME1
ME1
ME1 Francardo (Co)
ME1 Corte (Co)
ME1 Grossa di Cassano (It)
ME1 Moraiolo (It)
ME1 Semidana (Sa)
ME1 Ascolana (It)
ND San Agostino (It)
ME2 Biancolilla (Si)
ME1 Nocellara Belica (Si)
ME1 Pizz'è Carroga (Sa)
ME2 Carolea (Si)
ME1 Romana a (Co)
ME1 Itrana (It)
ME2 Giaraffa (Si)
ME1 Nocellara (Si)
MOM Picholine (Fr)
ME1 Cipresina (It)
ME1 Bosana (Sa)
ME1 Piacente (It)
ME1 Confetto (Sa)
ME1 Frantoio (It)
ME1 Leccino (It)
ME1 Pendolino (It)
ME1 Coratino (It)
MOM
Oleaster
MOM Zinzala (Co)
MOM Romana b (Co)
MOM Sivigliano da oglio (Sa)
MOM
ND Sa
Oleasters
ME1
ME1 Oliese (Co)
MOM
ME1
Oleasters
MOM
MCK Antonina (Co)
ME1
ME1
ME1
ME1
ME1
Oleasters

in 77.4% of the varieties and MOM and MCK Western mitotypes were found in 22.6% of the varieties (Table 2A). In Corsica, Western mitotypes were found in five out of ten varieties: Antonina, Capanacce, Romana b, Sabina and Zinzala. The MCK mitotype was exclusively found in the Corsican variety Antonina. In Sardinia, only one variety (Sivigliano da Olio) displayed the MOM Western mitotype. The French variety, Picholine, also carried the MOM mitotype. All of the varieties from mainland Italy and Sicily displayed the ME1 Eastern mitotype. The ME2 mitotype was revealed only in three Sicilian varieties (Biancollila, Carolea and Giaraffa). Out of the 31 varieties studied, only seven carried a Western mitotype: five Corsican, one Sardinian and one from mainland France.

In contrast to the varieties, the majority of oleasters (85.3%) displayed a Western mitotype (Table 2B). The MCK mitotype was revealed in only three oleasters (3.2%): two from Corsica, which account for 2.3% of the Corsican oleasters studied, and one from Sardinia, which accounts for 16.7% of the Sardinian oleasters studied. The ME2 mitotype was not found in any of the studied oleasters.

Table 3 Frequency (in %) of the most discriminating RAPD markers to differentiate olives (both varieties and oleasters) from the East to olives from the West

Marker	East	West
A1-1080	89.3	6.3
A2-1300	75	13.9
A9-680	57.1	8.9
A10-1300	75	6.3
A10-1100	60.7	5.1
A19-450	71.4	2.5
C15-1200	42.9	0
C15-440	78.6	16.5
O8-1200	96.4	21.5
O15-1900	64.3	2.5
O15-430	85.7	8.9
A1-230	32.1	98.7
A16-1150	32.1	89.9
A19-1600	35.7	97.5
O8-340	32.1	94.9

Genetic relationships among individuals and mitotype distribution

The UPGMA dendogram based on the 59 RAPD markers showed two major groups (Fig. 1). Group 1 contains most of the oleasters from both Sardinia and Corsica plus five varieties from Corsica. In contrast, group 2 contains most of the varieties plus a few oleasters.

The mitotype distribution shows that group 1 displays oleasters carrying the MOM (74/84), MCK (3/84) and ME1 (7/84) mitotypes. Among the five Corsican varieties clustered with the oleasters, two carried MOM and three carried ME1 mitotypes, while most of the oleasters displayed a Western mitotype. These three varieties were included in subgroups. The Migliaciaru variety formed a separate branch, and the Corte and Francardo varieties were clustered with two ME1 oleasters (Fig. 1). Group 2 includes varieties carrying ME1 (18/26), ME2 (3/26), MOM (4/26) and MCK (1/26), plus 12 oleasters, seven carrying ME1 and four with MOM. In group 2, subgroup 2.1 contains only varieties carrying the ME1 or ME2 mitotype except for Picholine (MOM). Subgroup 2.2 contains the 12 oleasters plus six varieties carrying ME1 (2/6), MOM (3/6) and MCK (1/6). The individuals with the same mitotype may be far according to genetic distances. However, the results revealed that most of the individuals in group 1 displayed the MOM mitotype and the majority of individuals in group 2 carried the ME1 mitotype. Therefore, individuals in group 1 generally had a nuclear RAPD pattern close to the one of the Western populations, and individuals in group 2 carried a nuclear RAPD pattern close to the one of the Eastern populations.

◀ **Fig. 1** UPGMA dendrogram aggregating RAPD data from all wild and cultivated trees. The geographical origin (except for Corsican oleasters) and mitotype are: Co = Corsica, Sa = Sardinia, Si = Sicily, It = mainland Italy, Fr = mainland France, IE = Island of Elba, ME1 and ME2 = East mitotypes, MOM and MCK = West mitotypes, ND = mitotype not determined

RAPD markers useful to determine olives from the East or the West

In order to determine the most-discriminating RAPD markers to differentiate olives from the East to olives from the West, we considered only olives with a Eastern RAPD pattern and carrying ME1 or ME2 in group 2, or a Western RAPD pattern with a MOM or MCK mitotype in group 1. A factorial correspondence analysis (FCA) was performed using RAPD data (Fig. 2). The first two axes explained 25.3% of the total variability. The FCA revealed a clear differentiation between Eastern and Western olives. Two groups were distinguished (Fig. 2B); the first, on the right, contained most of the Eastern olives and the second, on the left, contained the majority of olives from the West. Some individuals located at the intersection of the two groups are probably hybrids between the Eastern and Western olives. These results showed a correlation between RAPD markers and mitotypes. The FCA also allowed us to determine which of the markers discriminated most clearly between the olives from the East and the olives from the West (Fig. 2A, Table 3); they involve fragments strongly predominant in olives from the East: namely, A1-1080, A2-1300, A9-680, A10-1300, A10-1100, A19-450, C15-1200, C15-440, O8-1200, O15-1900 and O15-430. Those fragments were positively correlated with axis 1 whereas A1-230, A16-1150, A19-1600 and O8-340, which were predominant in olives from the West, were negatively correlated with axis 1. The two markers (A9-740 and A9-760), strongly correlated with axis 2, are not able to determine the olive origin.

Out of the 59 RAPD markers, 32 were associated with the four mitotypes. Six were Western specific,

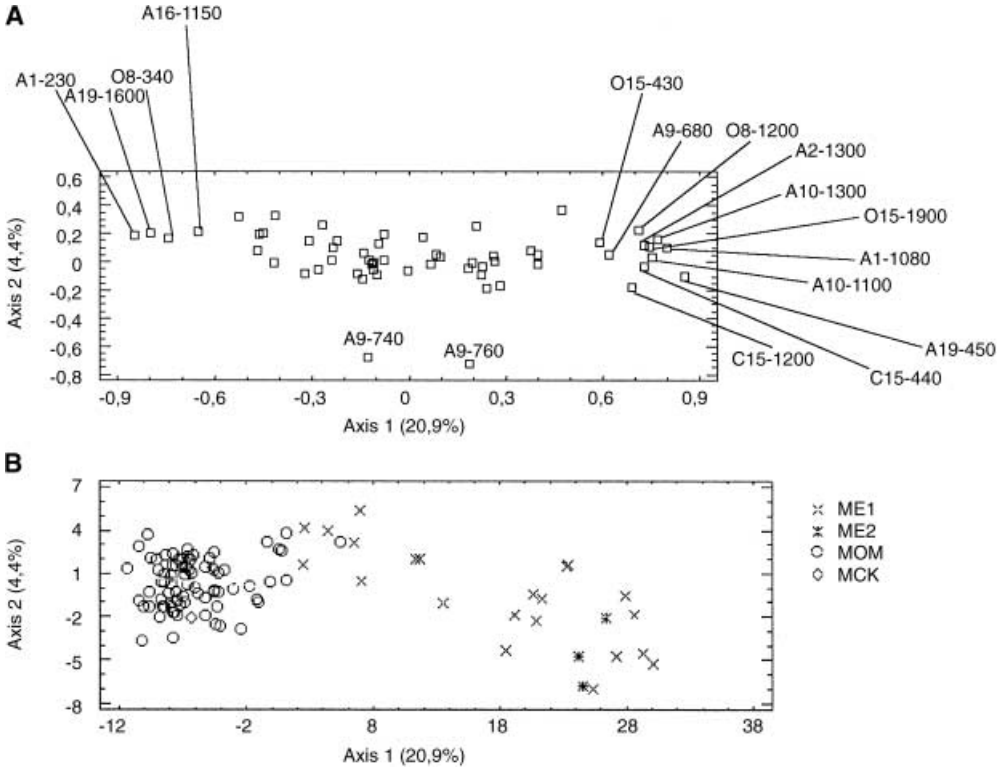

Fig. 2A, B Factorial correspondence analysis (FCA) of RAPD markers. **A** Distribution of the variables. **B** Distribution of the individuals

A1-500 (7.6% of Western olives), A2-1050 (31.6%), C9-1400 (7.6%), C9-410 (19%) and C15-1000 (13.9%), and two were Eastern specific, C9-1200 (14.3% of Eastern olives) and C15-1200 (42.9%).

Discussion

Differentiation of the feral forms from true oleasters of the Western Mediterranean

During the last glaciations (30,000–10,000 BP), at least two Mediterranean refugial zones would have existed: one in the East (Israel, Syria, Turkey) and another in the West (Sicilia, Maghreb) (Zohary and Hopf 1994; Terral and Arnold-Simard 1996; Besnard and Bervillé 2000). Subsequently, the wild olives re-colonized the Mediterranean basin and many crossings between olive-trees of the East and the West probably occurred. It is now assumed that two wild forms exist: the true oleasters (corresponding to the wild forms present in natural areas) and the feral forms (which escaped from cultivation or resulted from hybridisations between the true oleasters and varieties) (Zohary and Hopf 1994; Angiolillo et al. 1999; Baldoni et al. 2000; Besnard and Bervillé 2000). The morphological distinction between the two forms cannot be determined because they have similar characters, such as a smaller fruit size, a lower oil content in the drupe and a typical wild aspect. The identification of the feral forms is done a posteriori with molecular markers since generally they display close relationships with the varieties from which they are derived (Angiolillo et al. 1999; Besnard and Bervillé 2000).

In our study, we revealed that both Corsican and Sardinian oleasters were separated into two groups on the dendrogram. A large majority of the oleasters (87 out of 99) were clustered on group 1 and, except for some individuals, carried a Western mitotype (MOM or MCK). So far, any olive-tree carrying MOM or MCK has been found in the Near East (Besnard and Bervillé 2000). Thus, these oleasters present a Western RAPD pattern and, therefore, would have an origin in the West. This is in agreement with recent studies, which showed that the Corsican oleasters are closely related to the Moroccan and Algerian oleasters (Hess et al. 2000; Besnard et al. 2001). Hence, oleasters characterized by a Western RAPD pattern and an MOM or MCK mitotype, would constitute the true oleaster population of the West.

In addition, the combination of the RAPD markers and the mitotype allowed us to detect seven feral forms in group 1. Indeed, these olive-trees were characterized by an Eastern mitotype and a Western RAPD pattern. They certainly originated from hybridisations between true oleasters of the West used as the male parent and olive-trees originated by maternal descent from the East. The two ME1 individuals belonging to group 1 of the dendrogram (Fig. 1) were closely related to the Corte and Francardo varieties (ME1). Consequently, they are probably feral forms derived from these two varieties.

Some oleasters (12 out of 99) were clustered on group 2 with most of varieties. The majority of these individuals carried the ME1 Eastern mitotype and so present an Eastern RAPD pattern. We have shown that these oleasters were closely related to the varieties. Hence, we suggest that they are feral forms. Furthermore, we detected that

the oleasters characterized by an Eastern RAPD pattern carried an ME1 or MOM mitotype. The detection of the MOM mitotype in these olive-trees confirms that they originated from hybridisations between true oleasters of the West, used as the maternal parent, and Eastern varieties used as the male parent. In the same way, individuals carrying a ME1 mitotype originated from hybridisations between true oleasters of the West, used as the male parent, and Eastern varieties used as the maternal parent.

Determination of two cultivated populations in Corsica

Our results showed that the majority of the varieties displayed an Eastern mitotype. This is in agreement with the history of olive domestication, which suggests an East to a West diffusion of varieties (Loukas and Krimbas 1983; Zohary and Hopf 1994). In mainland Italy and Sicily, the absence of varieties from the West reveals a great influence by the Eastern Mediterranean. In Corsica, where the olive is present since many millennia (Magdeleine and Ottaviani 1984), we observed two populations of cultivated olives: one having close relationships with the oleasters (group 1) and one aggregated with the Italian mainland and Sardinian varieties (group 2). Sardinia was more-influenced by the Eastern Mediterranean, via probably mainland Italy. Such an influence had already been observed by Angiolillo et al. (1998).

Corsican varieties with an Eastern mitotype and an Eastern RAPD pattern (Oliese and Romana a) (group 2) were subject to an Eastern influence in all the Italian mainland varieties studied. In this group, the varieties having a MOM or MCK mitotype would result from hybridisations between olive-trees from the East, used as the male parent, and olive-trees originated by maternal descent from the West. These varieties may be local varieties but showed significant genetic distances with the true oleaster population. The MOM Corsican varieties (Romana b and Zinzala) are clustered with the Sardinian variety carrying a MOM mitotype. The only MCK variety, Antonina, growing in Corsica, probably has an Algerian maternal origin (Besnard et al. 2001).

In group 1, the ME1 varieties (Corte, Francardo and Migliaciaru) probably result from hybridisations between Eastern-originated olive-trees with local oleasters as the male parent. Therefore, these three varieties would be adapted to Corsica. Only two varieties with a Western mitotype were clustered with the oleasters: Capanacce and Sabina. They have probably been directly selected from local oleasters. Both Capanacce and Sabina are late varieties since their olives start to ripen only in February-March. Sabina is the most-commonly grown variety in Corsica. Its characteristics are that the ripe fruits remain a long time on the tree before their abscission and the oil content can reach 50% when olives are very ripe.

Two reasons can explain the presence of local varieties: (1) they may have relevant agronomic adaptable characteristics compared to those introduced, and (2) microregions of the inner island had difficult access and remained isolated for a long time. All Corsican varieties with an Eastern mitotype are minor varieties, which are not exploited any more. These probably derive from Italian varieties introduced into the North of Corsica during the domination of Genoa between the XIVth and XVIIIth centuries.

Conclusion

Results of this study based on mitochondrial DNA variations and RAPD markers provide clear information to differentiate oleasters from feral forms, which is not feasible based on morphology. The presence of primary oleaster populations originating from probably the West refugial zone was found in Corsica and Sardinia. We also show that some Corsican varieties have been selected from this local oleaster population, confirming the multi-origins of the olive.

The study of local varieties is important to understand the extent of gene flow between wild and cultivated forms and to justify the preservation of the biodiversity, and also to warrant controlled names for the local production of olive oil. These varieties may add to the collection and be used in improvement programmes for their adaptable traits.

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