C.R. Acad. Sci. Paris, Sciences de la vie / Life Sciences 323 (2000) 173–181

S0764446900001189/FLA

Multiple origins for Mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA polymorphisms

Guillaume Besnard[a], André Bervillé[a]*

[a] UR-GAP, Inra, 2, place Pierre-Viala, bât. 33, 34060 Montpellier cedex 1, France

Received 5 August 1999; accepted 8 November 1999

Communicated by André Adoutte

Abstract – A study of nuclear and cytoplasmic genetic diversity of cultivated olive, oleaster and other taxa belonging to the complex *O. europaea* was performed. Nuclear DNA polymorphism (RAPDs) in oleaster displays a gradient between the east and west of the Mediterranean Basin. In cultivars, the gradient is less visible owing to their diffusion and selection. Furthermore, three mitotypes (ME1, MOM and MCK) were detected in both cultivated olive and oleaster. A fourth mitotype, ME2, was unique to some cultivars. The preponderant mitotype, ME1, marks the Near Eastern origin of olive in oleaster. In the west of the Mediterranean, another mitotype, MOM, was found in most oleaster and a few cultivars. The third, MCK, was found in a few oleaster from the west and in cultivars originating in Kabylie and Languedoc. We argue that MCK marks an ancestral Mediterranean population. The mitotypes mark independent cultivated olive origins which were not detected with DNA nuclear diversity. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

DNA / olive / oleaster / *Olea europaea* L. / origin / polymorphism / mitotypes

Résumé – **Origines multiples de l'olivier méditerranéen (*Olea europaea* L. ssp *europaea*) établies sur le polymorphisme de l'ADN mitochondrial.** Nous présentons une étude de la diversité génétique nucléaire et cytoplasmique de l'olivier cultivé, de l'oléastre, et de taxa du complexe *O. europaea*. Le polymorphisme de l'ADN nucléaire (RAPD) des oléastres révèle un gradient entre l'est et l'ouest de la Méditerranée. Chez les cultivars, le gradient est moins marqué car le matériel a été diffusé et sélectionné. Par ailleurs, trois mitotypes (ME1, MOM et MCK) ont été détectés à la fois dans l'oléastre et l'olivier cultivé. Un quatrième mitotype, ME2, n'est porté que par quelques cultivars. Le mitotype prépondérant, ME1, marque l'origine de l'olivier cultivé dans l'oléastre du Proche-Orient. À l'ouest de la Méditerranée, le mitotype MOM, se trouve dans la plupart des oléastres et dans quelques cultivars. MCK est présent dans quelques oléastres de l'ouest et des cultivars de Kabylie et du Languedoc. Nous avançons que MCK marquerait une population ancestrale d'oléastres méditerranéens. Ces mitotypes révèlent des origines indépendantes de l'olivier cultivé. La diversité de l'ADN nucléaire ne permet pas de les révéler. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

ADN / olivier / oléastre / *Olea europaea* L. / origine / polymorphisme / mitotypes

* Correspondence and reprints: berville@ensam.inra.fr

Version abrégée

L'origine de l'olivier méditerranéen (*Olea europaea* ssp *europaea*) est mal connue. Les botanistes ont classé l'oléastre et l'olivier cultivé en deux variétés (var. *sylvestris* et var. *europaea*). Certains auteurs considèrent les oléastres comme des formes retournées à l'état sauvage (formes férales). Toutefois, on trouve des oléastres dits « vrais » qui semblent correspondre à des formes sauvages naturelles. La distinction morphologique des deux formes n'est pas stricte. Plusieurs auteurs ont supposé que l'oléastre avait servi de départ à la multiplication des meilleurs arbres pour constituer les premiers cultivars. Nous avons vérifié par le marquage moléculaire que les cultivars sont multipliés par voie végétative et composés généralement d'un seul clone. Nous n'avons pas trouvé de marqueurs spécifiques pour définir les cultivars, les oléastres « vrais » et les formes férales. Néanmoins, une identification des formes férales se fait a posteriori, puisqu'elles se regroupent, dans les dendrogrammes, avec les variétés dont elles dérivent. La domestication aurait été multilocale, ce qui devrait conduire à une structuration analogue de la diversité génétique des oléastres et des cultivars. D'autres auteurs supposent une origine unique au Proche Orient, puis une diffusion des cultivars vers l'ouest (Cyrénaïque, Italie, Maghreb, Espagne, France). Enfin, on a même supposé que les Phéniciens avaient établi des colonies à l'ouest de la Méditerranée pour se pourvoir en oliviers cultivés locaux.

Les analyses du polymorphisme de l'ADN nucléaire avec des marqueurs RAPD révèlent chez l'oléastre un gradient entre l'est et l'ouest (de la Méditerranée), qui est très atténué chez les cultivars. Nous n'avons pas une explication unique pour ce gradient, qui peut provenir : a) de migrations de l'est vers l'ouest, entraînant une forte dérive génétique, en supposant que l'olivier cultivé soit apparu au Proche-Orient, ou b) de deux types d'oléastres à l'est et à l'ouest avec lesquels les cultivars dérivés se seraient croisés.

Pour tenter de reconstituer et suivre la diffusion des cultivars, qui s'est faite par l'homme tout autour de la Méditerranée, nous avons étudié les ADN cytoplasmiques par RFLP. L'ADN du chloroplaste révèle deux types : CNC très prépondérant (95%) et CCK localisé au Maghreb (Algérie, Maroc), en Corse, et au sud de la France. Ces polymorphismes sont insuffisants pour tracer les déplacements. En revanche, le polymorphisme mitochondrial est diversifié en quatre mitotypes. Le mitotype ME1 (73% des cultivars) est présent aussi bien à l'est (Proche-Orient, Grèce, Turquie) qu'à l'ouest (Espagne, Maghreb, France, Italie). Les oléastres de mitotype ME1 sont partout à l'est et peu fréquents à l'ouest. Le mitotype MOM n'est présent que dans certains cultivars de l'ouest (Maghreb, Espagne, France), soit 12% des cultivars, mais il est prépondérant chez les oléastres de l'Ouest. Le mitotype ME2, qui dérive de ME1 par un petit réarrangement, est détecté dans sept cultivars (11%), de l'Égypte au Portugal, mais il n'est pas détecté dans l'oléastre. Enfin, le mitotype MCK n'est trouvé que dans 4% des cultivars et quelques oléastres de l'ouest. Ce mitotype, très différent de tous les autres, est toujours associé au chlorotype CCK. Les mitotypes ME1, MOM et ME2 sont associés au même ADN chloroplastique CNC présent tout autour de la Méditerranée.

Nous considérons que le mitotype MCK provient d'un taxon aujourd'hui disparu, selon le schéma d'hybridation introgressive favorisée par une stérilité mâle cytoplasmique conférée par MCK. Le mitotype MCK représente donc une origine nette et distincte de toutes les autres, dans le bassin méditerranéen. La distribution du mitotype ME1 est en accord avec une étape de domestication à l'est puis une diffusion des cultivars par l'homme vers l'ouest. le mitotype ME1 se retrouve dans un autre taxon du complexe *O. europaea*: *O. laperrinei*, présent au Hoggar, au Jebel Marra, et dans l'est de l'Afrique. De ce fait, l'oléastre de l'Est et *O. laperrinei* ont un ancêtre commun comme parent femelle. Le mitotype MOM, absent à l'est et très fréquent à l'ouest, devrait donc avoir une origine dans les oléastres de cette zone. Les mitotypes MOM, ME2 et MEI sont apparentés. L'importance du réarrangement mitochondrial ne permet pas de dater l'apparition du mitotype MOM relativement à celle du mitotype ME1. Les cultivars portant le mitotype ME2 montrent des déplacements importants, et vraisemblablement une apparition postérieure à la domestication car on ne retrouve pas ce mitotype dans les oléastres.

Chez les oléastres, la distribution des mitotypes ME1, MOM et MCK montre une structure géographique forte. Ces cytoplasmes, présents dans les formes cultivées, suggèrent donc que les cultivars ont trois origines bien distinctes. De plus, les groupes cytoplasmiques cultivés n'étant pas reconnaissables par les marqueurs nucléaires, on en déduit que des croisements entre les différentes origines se sont produits. Le brassage génétique des cultivars n'a pu se réaliser que par leur déplacement lors des migrations et des échanges humains. On ne pressentait pas que ce brassage avait été si intense.

Malgré ces résultats qui précisent l'histoire de l'olivier cultivé, de nombreuses zones d'ombre demeurent, par exemple les origines des mitotypes MOM et MCK. Nous avons des pistes à vérifier. Les analyses moléculaires devraient peu à peu combler les manques. On utilisera d'autres types de marqueurs (SSR en développement) et des séquences (cytoplasmiques et nucléaires), ce qui pourrait bien révéler d'autres surprises.

1. Introduction

Olive origin is still unclear. The Greeks questioned whether the possible origin of cultivated olive trees (*Elaie*) could be found in oleaster (*Cotinos*) [1, 2] and they wondered whether well cared for oleaster could lead to cultivated olive. This reveals that the passage from oleaster to cultivated olive and vice versa was not obvious [3, 4]. A denomination of cultivated olive (= cultivar) usually corresponds to a clone. Each denomination is recognised using about 30 descriptors for trunk, leaf, flower and fruit shape. However, any of these criteria enables a strict differentiation between cultivated olive and oleaster. The location of a tree either in an orchard or close to an orchard or in a forest is an indication of its status: cultivated olive versus feral or oleaster, respectively. Botanists have created two varieties for the Mediterranean olive: one for oleaster (*Olea europaea* ssp. *europaea* var. sylvestris) and one for cultivated olive (*O. e.* ssp *europaea* var. *europaea*) [5] (*table I*). Recently several authors [6, 7] have suggested that cultivated olive originated in the eastern part of the Mediterranean Basin. This is not supported by dendrograms based on RAPD relationships between cultivar/cultivar and cultivar/oleaster [8]. Today, the Mediterranean olive presents phenotypic diversity without any relation to the geographic origin of cultivars. Moreover, archaeological evidence has revealed early domestication in the Near East [9] and logically suggests east to west (east and west refer to the Mediterranean Basin) diffusion of cultivars. However, it has also been suggested that people might have domesticated olive in the west [10, 11, 12] and popular belief was that western cultivars had been brought to the east [13]. In other words, all hypotheses are possible. However, evidence supporting popular belief has not been found.

Olive and oleaster belong to a wide group called the *O. europaea* L. complex which is spread throughout Asia, Africa and Europe (*table I*) [5]. Thus, we inferred that olive might have an origin far from the Mediterranean Basin. We therefore looked at olive diversity using RAPD technology in the *O. europaea* complex (*table I*) to determine whether olive and oleaster might have a common origin, and, if so, which could be the closely related branch in the *O. europaea* species. In addition, we studied cytoplasmic DNA polymorphisms to reconstitute olive cultivar displacements and to compare them with the distribution of cytotypes in present local oleasters.

2. Materials and methods

This study was performed using 121 cultivars and 300 oleasters sampled in 27 populations from all around the Mediterranean Basin, plus 74 trees from other subspecies harvested in Morocco (*O. maroccana*), Algeria (*O. laperrinei*), Canary Islands (*O. e.* ssp. *cerasiformis*), Kenya, South Africa, Zimbabwe (*O. africana*), Yemen (*O. chrysophylla*), Iran, India and China (*O. cuspidata*) (*table I*). The detailed list is available upon request to the authors.

2.1. Molecular analysis

The DNA preparation protocol has been previously described [8]. RAPDs were developed on all the individuals using eight primers (A1, A2, A9, A10, C9, C15, E15 and O8; Bioprobe). The procedure for revelation of RAPD markers has been previously described [14]. To perform RFLP, DNAs were purified. The DNA solution was transferred into an ultracentrifugation tube (Kontron). Ten micrograms of ethidium bromide and Triton (1 % final concentration) were added. Caesium chloride solution was added to obtain a final refractive index $\eta = 1.397$. This solution was centrifuged at 200 000 *g* for 24 h. The DNA band was visualised at 312 nm and recovered with a syringe. The volume was adjusted to 5 mL with water. DNA was precipitated by the addition of ammonium acetate (final concentration 0.2 M) and of cold ethanol (10 mL). The DNA pellet was recovered and washed with 75 % ethanol. The DNA pellet was then dried and dissolved in 0.3 mL of 1X TE. Three micrograms of total DNA were restricted with 24 U of each restriction enzyme (Boehringer) according to provider recommendations. Two restriction enzymes were used separately: *Hind* III and *Xba* I. Restriction fragments were electrophoresed in 1 % agarose gel and were transferred onto a Hybond N^+ Nylon

Table I. The complex *O. europaea* L. [5]: four subspecies with their main taxa and their geographical distribution*.

O. europaea L. subspecies	Geographical origin	*n*
ssp *europaea*		
var *sylvestris* (Miller) Lehr. = Oleasters	Mediterranean Basin	300
var *europaea* = Cultivars	Mediterranean Basin	121
ssp *laperrinei* (Batt. & Trab.) Ciferri		
O. laperrinei Batt. & Trab.	Saharan Mountains	2
O. maroccana Greut. & Burd.	Morocco (Atlas)	8
ssp *cerasiformis* (Webb & Berth.) Kunk. & Sund.	Canary Islands and Madeira	12
ssp *cuspidata* (Wall.) Ciferri		
O. cuspidata Wall.	Asia	7
O. chrysophylla Lam.	Arabia, Abyssinia (Ethiopia)	9
O. africana Mill.	Eastern and Southern Africa	36

* *n* for each taxon is the number of trees analysed in the present study.

membrane (Amersham) with a transblotter. Probes were labelled by random priming using 74 mBq [^{32}P] dCTP (111 TBq/mmole). The membranes were hybridised in a 7 % SDS, 0.25 M Na_2HPO_4 and 1 mM EDTA solution at 65 °C for 18 h and then rinsed in a 1 % SDS and 40 mM Na_2HPO_4 solution, three times at 65 °C for 30 min. Autoradiogram was obtained at –80 °C for a sufficient time depending on the labelling intensity with Hyperfilm MP (Amersham). Two probes corresponding to either the *atp9* mitochondrial gene from maize [15] or to total chloroplast DNA (cpDNA) from *Phillyrea media* L. (provided by P. Saumitou-Laprade) were used to reveal polymorphisms. *Phillyrea* is closely related to *Olea* belonging to the tribe *Oleeae* within the subfamily *Oleideae*. Only a subsample of individuals (210) was analysed for the cytoplasmic diversity.

2.2. Data handling

Geographical distribution of mitotypes was represented on a map of the Mediterranean Basin. Differentiation parameters between oleaster populations were computed: for RAPD and mitotypes, ϕst and Fst were determined, respectively [16, 17]. A factorial correspondence analysis was computed on the data with the procedure 'CORRESP' from SAS.

3. Results

According to RAPD data, the four main taxa of *O. europaea* were recognised (*table I*). *O. maroccana*, which has been considered by some botanists as an oleaster [18], was clearly differentiated from oleaster and forms a distinct taxon. *O. maroccana*, *O. e.* ssp. *cerasiformis* and *O. laperrinei* appeared equally related to Mediterranean olive (not shown) according to genetic distances. Similar relationships between these species were established based on chloroplast polymorphism. Each taxa from eastern and southern Africa (*O. africana*) and Asia (*O. chrysophylla* and *O. cuspidata*) carried a distinct cpDNA, three chlorotypes were found (not shown) [19]. *O. laperrinei* (from Hoggar, Sahara) *O. maroccana* and *O. e.* ssp. *cerasiformis* carried another chlorotype, CCN, also found in most oleasters and cultivars (95 %), whereas other oleasters and cultivars carried the CCK type (found in the Maghreb and in France). Mitochondrial DNA displayed more polymorphisms than chloroplast DNA using the RFLP technology. It displayed 12 mitotypes in the complex *O. europaea*, but only four were present in Mediterranean olive. Based on cytoplasmic markers, we distinguished three groups including both oleaster and cultivated olive. The main group is close to *O. laperrinei*, since both oleaster and cultivated olive share the same chlorotype and mitotype, CCN and ME1, respectively. The second group still carries the chlorotype CCN but associated with the mitotype MOM. The origin of this mitotype is unknown. The third group carries the chlorotype CCK and the mitotype MCK; both still have an unknown origin. However, the MCK mitotype is close to both that of *O. maroccana* and *O. e.* ssp. *cerasiformis* from the Maghreb and Canary Islands, respectively.

Nuclear DNA polymorphisms in oleasters based on 58 RAPDs demonstrated an east to west gradient (*table II*, *figure 1*) although for cultivars this gradient was attenuated, probably owing to cultivar displacements and selection. Moreover, oleaster populations were structured and differentiated as judged by the global ϕst (0.247). Eastern populations were poorly differentiated among themselves ($0.023 < \phi st < 0.093$), as were western populations

Table II. Frequency (in per cent) of the most discriminating markers in 50 oleaster populations from the east and 192 from the west or in 90 cultivars from eastern and 31 cultivars from western Mediterranean*.

Marker	50 trees from five eastern populations	192 trees from 18 western populations	58 trees from four feral western populations	31 cultivars from the east	90 cultivars from the west
A2-475	18	0	0	6	0
A1-1000	100	39	78	100	92
O8-1050	100	48	97	97	99
C9-500	96	43	88	87	97
A1-800	70	16	26	42	27
A2-450	40	96	72	26	46
A1-225	16	87	45	6	27
A1-275	8	64	16	0	24
A2-650	6	91	64	13	50
A1-1200	4	49	36	3	22
A9-275	4	57	34	3	16
A1-825	2	75	43	6	22
C9-450	0	20	5	0	4
C15-675	0	24	26	0	10
A10-625	0	30	14	3	9
A9-950	0	46	17	0	9

* Means without the 58 feral populations from France. Those markers indicate the geographic origin for cultivars or oleasters and those oleaster populations suspected to be feral. Fragment are noted: primer-size in bp.

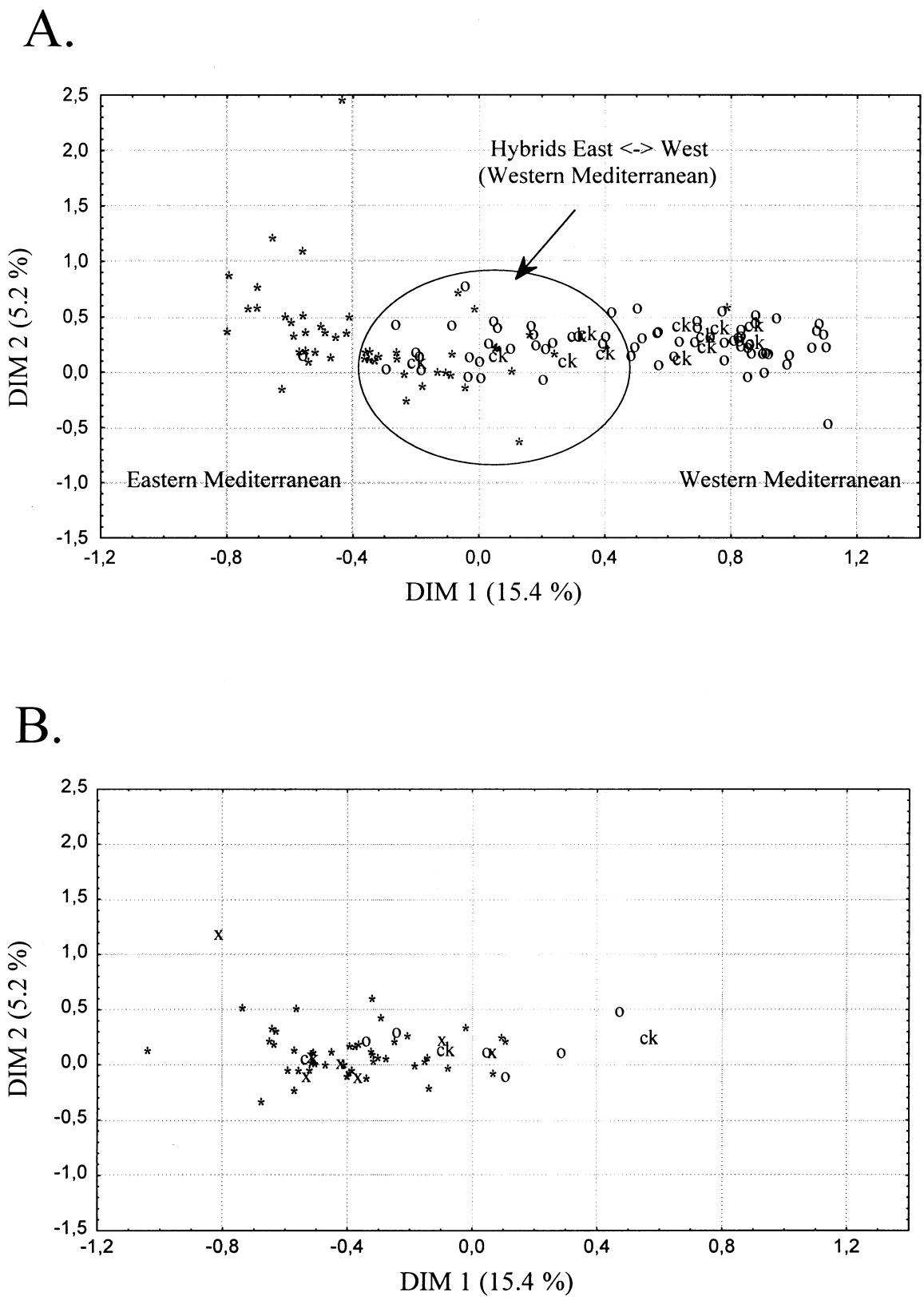

Figure 1. Factorial correspondence analysis (FCA) of the country of origin of the Mediterranean olive computed on RAPD data.
The mitotype of the individuals is reported as additional data: * = ME1; x = ME2; o = MOM; ck = MCK. **A.** Distribution of the oleasters on the first two axes (20.6 %) of the FCA. The circle indicated those oleasters with intermediary RAPDs and carrying either ME1, MOM, or MCK mitotype, which points out that there are hybrids between eastern and western trees. **B.** Distribution of the cultivars on the first two axes (20.6%) of the FCA. Most of cultivars appeared derived from Oleasters from the east or from those hybrids indicated by the arrow.

Table III. ϕst between population couples computed from RAPD data.

	Ostricone (Corsica, France)	Messine (Sicily, Italy)	Torviczon (Andalucia, Spain)	Tamanar (Morocco)	Kabylie (Algeria)	Izmir (Turkey)	Al Ascharinah (Syria)
Messine (Sicily)	0.093 ●●						
Torviczon (Andalucia)	0.146 ●●	0.153 ●●					
Tamanar (Morocco)	–0.008 ns	0.110 ●●	0.118 ●●				
Kabylie (Algeria)	0.155 ●●	0.072 ●	0.037 ns	0.112 ●●			
Izmir (Turkey)	0.492 ●●	0.395 ●●	0.293 ●●	0.444 ●●	0.262 ●●		
Al Ascharinah (Syria)	0.541 ●●	0.457 ●●	0.389 ●●	0.504 ●●	0.344 ●●	0.023 ns	
Mont Carmel (Israel)	0.532 ●●	0.450 ●●	0.379 ●●	0.507 ●●	0.362 ●●	0.068 ●	0.093 ●●

●● significant to 1 %; ● significant to 5 %; ns: not significant. The location is indicated by the place name and the country or island name.

(0 < ϕst < 0.155), but a strong differentiation was shown between eastern and western populations (0.262 < ϕst < 0.541) (*table III*). Some oleaster populations from the east and the west displayed markers not found in cultivars but not all populations contained these markers. However, our cultivar sample from the east (about 30) was too small compared to the number of existing cultivars. Furthermore, most of oleaster populations from the west displayed markers not present in the east, but also found in some cultivars from the west (*table II*). This suggests that these cultivars from the west originated either from oleasters from the west or from hybrids between oleasters from the west and cultivars introduced from the east. Oleasters and cultivars were not differentiated with molecular markers (*figure 1*). Obviously two types of oleasters were observed. Those corresponding to feral forms around current orchards or in old desert orchards and those called 'true' oleasters found in forest or land with apparently no relation to cultivated areas. Feral forms are recognised because they cluster in the dendrogram with the cultivars from which they are derived, whereas 'true' oleasters form several groups more or less related to those of cultivars (data not shown).

Mitochondrial DNA polymorphisms (*figure 2*) in oleasters displayed clear-cut geographic separation: oleasters carrying the ME1 mitotype appeared in the east (Turkey to Near East) and very few were found in the west (*figure 3.B*). The MOM and MCK mitotypes were found in the west (from Libya to the Maghreb and Spain) but were totally absent in the east (*figure 3.B*). In comparison to the nuclear diversity ϕst, the global Fst based on mitochondrial DNA was higher (0.784) and this reflects the fact that cytoplasmic genes were less diffused than nuclear genes. Mitochondrial DNA polymorphisms enabled us to recognise four types of cultivars. According to the country of origin of the cultivars, the ME1 mitotype (73 % of cultivars) was frequent throughout the Mediterranean Basin (*figure 2.A*). The ME2 mitotype (11 % of cultivars) was revealed only in a few cultivars from east to west tracing likely cultivar displacements, and it is likely that they derived from ME1 by a simple rearrangement in the mitochondrial DNA [20]. The MOM mitotype (12 % of cultivars) was revealed in some cultivars from the west' whereas MCK (4 % of cultivars), found also in the Maghreb and France, is associated with cytoplasmic male sterility [20]. MCK mitotype was distinguished from the three others with different couples' enzyme-probe (data not shown). In the west, cultivars carrying the ME1 mitotype were predominant and they

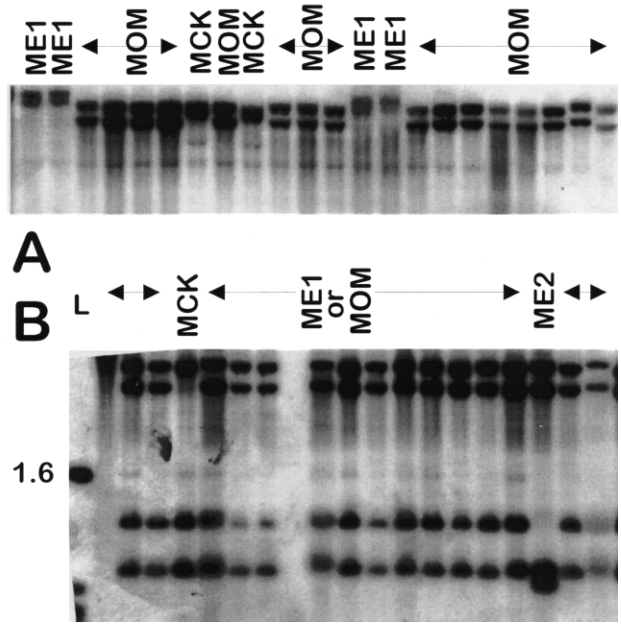

Figure 2. RFLP profiles of total DNA from oleasters or cultivars enabling the four mitotypes ME1, ME2, MCK or MOM to be recognised.
A. Restricted with *XbaI* and hybridising the *atp9* mitochondrial gene from maize, as a probe [15]. **B.** Restricted with *Hind* III and hybridising the *atp9* mitochondrial gene from maize, as a probe [15]. Profiles are coded only as ME1, ME2, MCK or MOM, without the indication of oleaster or cultivar.

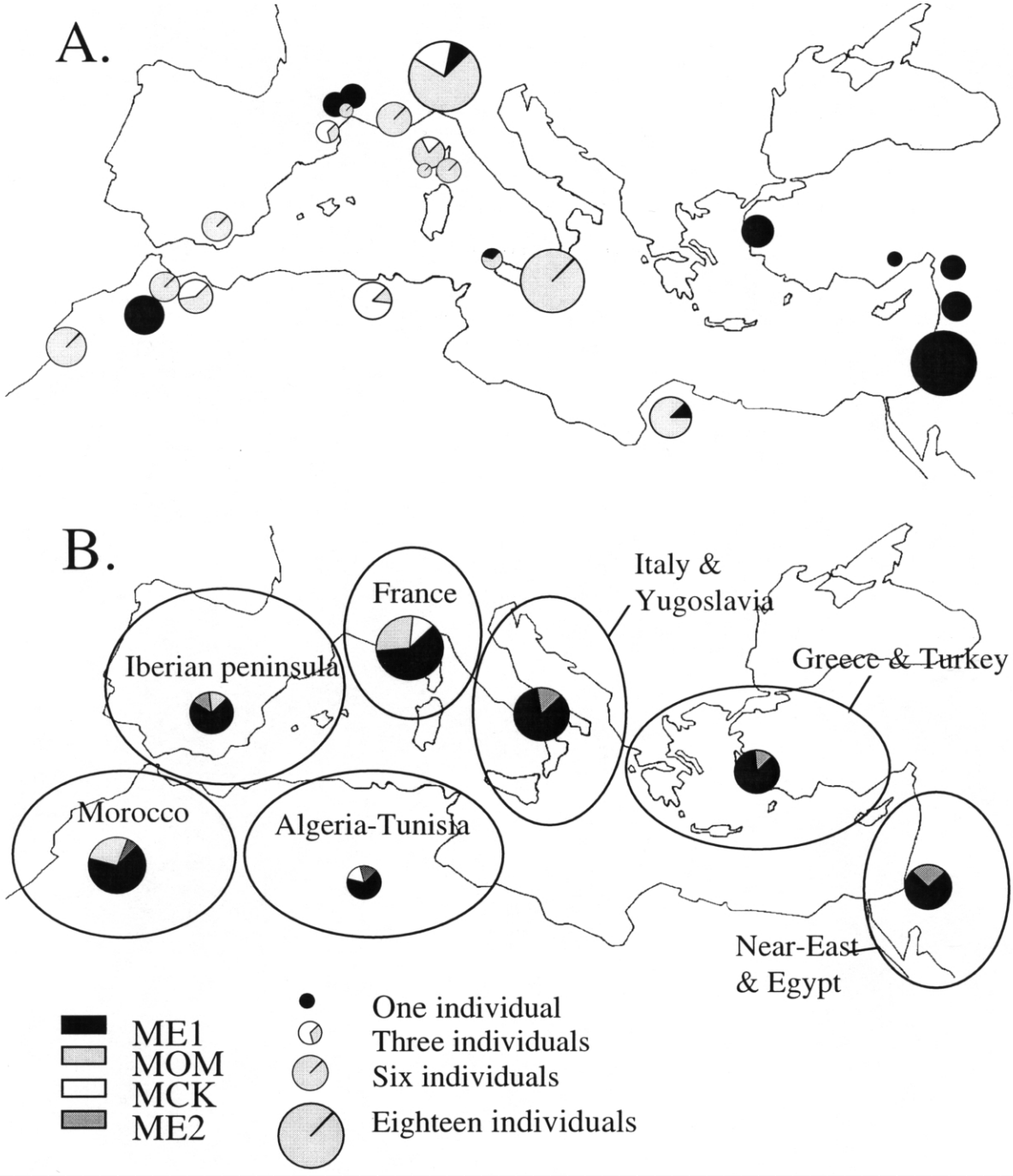

Figure 3. Distribution of the four mitotypes, all around the Mediterranean Basin.
A. For oleasters. **B.** For cultivars. Each pie chart marks the location of the tree origin. Geographic area units are marked by egg-shaped line.

either originated in the east or derived from those cultivars (*figure 3.A*), whereas oleasters in the west carrying the ME1 mitotype probably escaped from cultivation, since their distribution represents a few spots around cultivars carrying the ME1 mitotype (*figure 3.B*).

4. Discussion

Surprisingly, we did not find a close relationship between oleasters in the Near East and *O. cuspidata*, although it was not too far from the Fertile Crescent, about 800 km from the Zagros Mountains (southern Iran). O. africana in East and West Africa, although not distinguished from *O. cuspidata* by Green and Wickens [5] (*table I*), carried different cytoplasmic DNA (Besnard et al., in preparation). The cytoplasmic and nuclear markers did not enable us to find a Mediterranean olive origin in *O. africana*. However, *O. laperrinei* carries the ME1 mitotype as do most oleasters and cultivars. This suggested that this mitotype represents an olive origin in the eastern Mediterranean. *O. la-*

perrinei is now limited to Hoggar and other mountains in Central and East Africa in areas difficult to sample (Jebel Marra, Aïr, Somalian mountains), which explains why our samples are so limited for this study. The ME2 mitotype found only in cultivars might also exist in oleaster but it is probable that our samples of oleasters from the east are insufficient because natural populations of oleasters are decreasing. ME2 could have also appeared after olive domestication. Lastly, MOM and MCK mitotypes are found only in the western Mediterranean. It is unlikely that these mitotypes derived from ME1 along with accompanying recent displacements of cultivated olive. This suggests that MOM and MCK represent two other origins for the Mediterranean olive.

In north-west Africa, *O. maroccana* presents strange features: it carries specific nuclear markers and different cytoplasmic DNAs compared to oleasters and cultivars; it is mixed with them in populations and even in some orchards (near Agadir), and we did not detect *O. maroccana* hybrid either with oleaster or with cultivars. The RFLP pattern of MCK mitotype is related to those of *O. maroccana* and *O. e.* ssp. *cerasiformis*, two relic populations in northern Africa (not shown). Furthermore, the MCK mitotype being associated with another type of chloroplast DNA might correspond to an Olea taxon covering the western part of the Mediterranean Basin from the Tertiary era. These populations were located in refuge zones when glaciation or desertification occurred.

Mitochondrial DNA rearrangements do not enable us to reconstruct the phylogeny of those cytoplasms to determine the chronology of their appearance. Trees with the MCK or MOM mitotypes were mixed in populations and therefore they crossed with each other (*figure 1*). Then, those trees with different cytotypes became indistinguishable on the basis of nuclear markers. Once the ME1 mitotype had been brought to the west from the east, natural crosses probably occurred between cultivars that have also escaped as feral forms. Today, it is not possible to predict the mitotype from the RAPD profile for a cultivar, but the mitotypes and some RAPD markers indicate whether a cultivar has been selected in a western or eastern genetic background (*table II*).

A cultivated olive origin in the eastern Mediterranean has been supported by archaeological artefacts (olive stones and wood fragments, oil industry remains) found in the Near East from 19 000 BP [21]. Exploitation of oleasters occurred (by 8 000 BP), and domestication of cultivars from oleasters had probably taken place by 5 700–5 500 BP [9]. It is likely that these first cultivars later accompanied human displacements to the west.

The presence of *Olea* in the west has been documented by fossil pollen in swamp sites from the Tertiary era [22]. However, *Olea* species cannot be differentiated on the basis of pollen morphology. During the Holocene, the presence of olive in the west is attested by archaeological remains [11, 12]. This olive probably displayed MOM or MCK mitotypes. Subsequently, trees with either the MOM or MCK mitotypes were locally selected in the Maghreb, Spain and France, leading to cultivars. Thus, some cultivars derive from local oleasters. We wondered whether this means that a distinct domestication process occurred in the west, or if western oleasters were picked up and selected after establishment of oleiculture based on eastern cultivars. We now plan to study remains (olive stones and wood) from western archaeological sites in comparison with those of the east, in order to follow and date the appearance of maternal lineages in the west, and to verify their cytotype.

5. Conclusion

Olive origin does not appear to be unique and, therefore, is much more complex than expected. The Near East displayed evidence for the presence of both true oleasters and a domestication site. Oleasters and cultivars carrying the ME1 mitotype point to the fact that domestication occurred in oleasters leading to cultivars. In the west, the MCK mitotype could correspond to remains of *Olea* present since the Tertiary era. The MOM mitotype could be looked for in oleasters from the Maghreb and in *O. laperrinei* from the Sahara and east of Africa. It is clear that west of the Mediterranean Basin there are several origins for olive, either naturally mixed, as shown for forest trees [23], or that have been mixed by human displacements of cultivars. This has also led to the diversity of cultivars which now appears more or less related to the local oleasters [19].

Acknowledgements: We wish to thank F. Dosba, B. Khadari, N. Moutier and M. Chauvet for stimulating discussions. This work was supported by the FAIR program CT 95-0689.

References

[1] Moazzo G.P., Les plantes d'Homère et de quelques autres poètes de l'Antiquité. V. L'olivier (*Elaie*), Ann. Musei Goulandris 9 (1994) 185–223.

[2] Theophraste, Recherches sur les plantes (traduction S. Amigues), Les Belles Lettres, Paris, 1988, 1989, 1993.

[3] Turrill W.B., Wild and cultivated olives, Kew Bull. 3 (1951) 437–442.

[4] Chevalier A., L'origine de l'olivier cultivé et ses variations, Rev. Int. Bot. Appl. Agric. Trop. 28 (1948) 1–25.

[5] Green P.S., Wickens G.E., The *Olea europaea* complex, in: Kit Tan (Ed.), The Davis & Hedge Festschrift, Edinburg University Press, Edinburgh, 1989, pp. 287–299.

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

[7] Angiolillo A., Mencuccini M., Baldoni L., Olive genetic diversity assessed using amplified fragment length polymorphisms, Theoret. Appl. Genet. 98 (1999) 411–421.

[8] Besnard G., Moukhli A., Sommerlatte H., Hosseinpour H., Tersac M., Villemur P., Dosba F., Bervillé A., Origins and domestication of Mediterranean olive tree (*Olea europaea* L.) through RAPD marker analyses, in: Damania A.B., Valkoun J., Willcox G., Qualset C.O. (Eds.),

The Origins of Agriculture and Crop Domestication, ICARDA, Aleppo, 1998, pp. 224–232.

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

[10] Magdelaine J., Ottaviani J.C., L'occupation pré et proto-historique de l'abri de Scaffa Piana près de Saint-Florent, Bull. Soc. Sci. Hist. Nat. Corse 647 (1984) 39–48.

[11] Terral J.F., Débuts de la domestication de l'olivier (*Olea europaea* L.) en Méditerranée nord-occidentale, mise en évidence par l'analyse morphométrique appliquée à du matériel anthracologique, C. R. Acad. Sci. Paris, Série IIa 324 (1997) 417–425.

[12] 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.

[13] Amoureux, Traité de l'olivier, 2nd éd., imprimerie J.F. Picot, Montpellier, 1784, 356 p.

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

[15] Dewey R.E., Schuster W., Levings C.S. III, Tymothy D.H., Nucleotide sequence of Fo-ATPase proteolipid (subunit 9) gene of maize mitochondria, Proc. Natl. Acad. Sci. USA 82 (1985) 1015–1019.

[16] Excoffier L., Smouse P.E., Quattro J.M., Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data, Genetics 131 (1992) 491.

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

[18] Sauvage C., Vindt J., Flore du Maroc analytique, descriptive et illustrée: Spermatophytes, Travaux de l'Institut scientifique chérifien, Éditions Internationales, Tanger, 1952, pp. 95–96.

[19] Besnard G., Étude par des marqueurs moléculaires de la diversité génétique de l'olivier cultivé et des formes sauvages apparentées: applications en identification variétale et pour la gestion des ressources génétiques, thèse, université Montpellier-II, 1999, 321 p.

[20] Besnard G., Khadari B., Villemur P., Bervillé A., A cytoplasmic male sterility in olive (*Olea europaea* L.), Theoret. Appl. Genet. (2000) (in press).

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

[22] Suc J.P., Origin and evolution of the Mediterranean vegetation and climate in Europe, Nature 307 (1984) 429–432.

[23] 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.