Theor Appl Genet (2000) 101:547–553

R. Lumaret · M. Amane · N. Ouazzani · L. Baldoni
C. Debain

Chloroplast DNA variation in the cultivated and wild olive taxa of the genus *Olea* L.

Received: 5 December 1999 / Accepted: 30 December 1999

Abstract Polymorphism in the lengths of restriction fragments of the whole cpDNA molecule were studied in 15 taxa (species or subspecies) of the genus *Olea*. From restriction analysis using nine endonucleases, 28 site mutations and five length polymorphisms were identified, corresponding to 12 distinct chlorotypes. From a phenetic analysis based on a Nei's dissimilarity matrix and a Dollo parsimony cladistic analysis using, as an outgroup, a species of the genus *Phillyrea* close to *Olea*, the ten taxa of section *Olea* were distinguished clearly from the five taxa of section *Ligustroides* which appear to posses more ancestral cpDNA variants. Within the section *Ligustroides*, the tropical species from central-western Africa, *Olea hochtetteri*, showed a chlorotype which differed substantially from those of the other four *Olea* taxa growing in southern Africa, supporting a previous assessment according to which *O. hochtetteri* may have been subjected to a long period of geographical isolation from the other *Olea* taxa. Within the *Olea* section, three phyla were identified corresponding to South and East Africa taxa, Asiatic taxa, and a group including Saharan, Macaronesian and Mediteranean taxa, respectively. On the basis of cpDNA variation, the closest *Olea* taxa to the single Mediterranean species, *Olea europaea*, represented by its very predominant chlorotype, observed in both wild and cultivated olive, were found to be *Olea laperrinei* (from the Sahara), *Olea maroccana* (from Maroccan High Atlas) and *Olea cerasiformis* (from Macaronesia). These three taxa, which all share the same chlorotype, may have a common maternal origin.

Key words CpDNA RFLPs · *Olea* L. taxa · Geographic distribution · Phylogeny

Communicated by P.M.A. Tigerstedt

R. Lumaret (✉)
CEFE, UPR 9056 CNRS, 1919 route de Mende,
F-34293 Montpellier Cedex 5
e-mail: lumaret@cefe.cnrs.mop.fr
Fax: +33-4-67-41-21-38

M. Amane · C. Debain
Centre Louis Emberger-CEFE/CNRS,
F-34293 Montpellier Cedex 05, France

M. Amane · N. Ouazzani
Ecole Nationale d'Agriculture, Département d'Arboriculture,
BP S40, Meknès, Morocco

L. Baldoni
Istituto di Ricerche sulla Olivicoltura, C.N.R., Via Madonna Alta, 128,
I-06128 Perugia, Italy

Present address:
M. Amane, Faculté des Sciences, Université Moulay Ismail,
BP 4010, Meknès, Morocco

Introduction

The genus *Olea* L. includes some 30–35 taxa with uniformly 2n=46 chromosomes (see Green and Wickens 1989 for a review) and which, according to these authors, are considered either as species or as sub-species, or even as varieties. In this genus, *Olea europaea* L. is the only species which has undergone a domestication process [from the wild olive *O. europaea* subsp. *sylvestris* (Miller) Hegi] to produce the crop, *O. europaea* subsp. *europaea* L. (Zohary and Hopf 1993). The other *Olea* taxa include wild forest trees, some of them being used for their hard wood to produce tools, or for their leaves as forage to feed animals (Ciferri 1942; Chevalier 1948). Moreover, *O. europaea* L. which grows around the Mediterranean Basin is the only Mediterranean representative of the genus which otherwise includes mainly tropical taxa distributed over Africa, South Arabia, South and East Asia and Eastern Australia (Turrill 1951). One of the three subgenera identified in the genus *Olea*, the subgenus *Olea*, was divided into two sections by Green and Wickens (1989): (1) *Ligustroides* Benth & Hook. f., which is characterized by taxa showing terminal inflorescences, and (2) *Olea* in which the taxa possess axillary or subterminal inflorescences, as in the case of *O. europaea*. Within each section, the classification of taxa is very confusing. For instance, on the basis of mor-

phological, anatomical, karyological, palynological and biochemical evidence, Green and Wickens (1989) considered the olive taxa from South Asia and from East Africa as an additional subspecies of the European olive, and grouped them all as *O. europaea* L. subsp. *cuspidata* (Wall ex DC.) Ciferri. However, Zohary (1994) objected that the geographical isolation of *Olea africana* and *Olea chrysophylla*, growing in South and East Africa, respectively, and the Asiatic *Olea ferruginea*, together with their morphological differences from *O. europaea*, fully justify their classification as independent species. Furthermore, in a recent study based on amplified fragment length polymorphism in the genus *Olea* (Angiolillo et al. 1999), evidence was obtained that the *Olea* from East Africa and from Asia should indeed be assigned to a different species distinct from the Mediterranean olive, *O. europaea*.

A few additional non-Mediterranean wild taxa are considered to be closely related to the Mediterranean olive tree. Among these, *Olea laperrinei* Batt. and Trabut is a Saharo-montane type considered by Zohary (1994) as "a relict that bridges the Mediterranean forms with their African Savanna relatives." A particular form of *O. laperrinei*, *Olea maroccana*, which occurs in the South of Morocco, is considered either as a subspecies of *O. laperrinei* or as another related species (Quezel 1995). Moreover, *O. europaea* L. subsp. *cerasiformis* (Webb and Berth). Kunkel and Sundig, endemic to Macaronesia (Canary islands, Green Cape islands and Madeira), was reported to be also closely related to the cultivated olive (Zohary 1994).

Restriction-site analysis of chloroplast DNA (cpDNA) has been shown to be a powerful tool for phylogenetic reconstruction at both inter- and intra-specific levels (Palmer 1987). In olives, cpDNA has been confirmed to be maternally inherited (Amane et al. 1999). Low RFLP variation and the occurrence of a very predominant chlorotype have been observed for that molecule in both the cultivated and wild forms of *O. europaea* analysed throughout the Mediterranean Basin (Amane et al. 1999). In the present study, based on a large survey of cpDNA RLFP variation over the whole geographical distribution area of the subgenus *Olea*, we attempt (1) to clarify the maternal phylogenetic relationships between the several taxa, and (2) to assess more particularly the genetic relationships between the cultivated olive and the several related wild *Olea* taxa.

Materials and methods

Plant material

Chloroplast DNA variation was analysed in 15 distinct *Olea* taxa, i.e. in ten and five taxa of the sections *Olea* and *Ligustroides*, respectively. For each taxon, geographical distribution area, collection localities and sample size are indicated in Table 1. For *O. europaea*, chloroplast DNA variation was analysed in 72 olive cultivars, 89 very old trees cultivated locally [*O. europaea* subsp. (or var. *europaea*)] and 101 oleaster trees [*O. europaea* subsp. (or var. *sylvestris*)] from numerous localities throughout the Mediterranean Basin (see Amane et al. 1999 for more detail on sampling design). For each taxon, several localities were sampled except for *O. laperrinei*, *O. chrysophylla* and *Olea capensis* subsp. *capensis*, subsp. *macrocarpa* and subsp. *hochtetteri*. In addition, for the purpose of comparison, two trees of *Phillyrea angustifolia* L., a widespread Mediterranean taxon systematically close to *Olea* (Morettini 1972), were sampled and scored for cpDNA variation. The two trees were growing in two distinct sites, La Gardiole and Le Sambuc, located 100 km apart in the south of France. In both *Olea* and *Phillyrea*, the small sample size per site was consistent with the theoretical prediction of very low variation occurring locally in cpDNA (Pons and Petit 1995).

Extraction and restriction endonuclease analysis of cpDNA

The leafed branches collected on the trees were placed in the dark for 8 days to de-starch the leaves before they were ground in liquid nitrogen and freeze-dried. Chloroplasts were isolated from 2-g aliquots of freeze-dried powder using a non-aqueous procedure, and cpDNA was extracted from chloroplasts as described by Michaud et al. (1995). Aliquots of 20 µg of chloroplast dNA were incubated for 5 h with nine restriction enzymes, *Ava*I, *Bam*HI, *Cfo*I, *Dra*I, *Hae*III, *Hind*III, *Hpa*II, *Nci*I and *Xho*I, according to the recommendation of the suppliers (Boehringer, Appligene). These restriction enzymes regularly provided clear restriction patterns with a large number of fragments (usually over 40). The digestion products were fractionated by electrophoresis on horizontal 0.85% agarose – slab gels which were stained with ethidium bromide and photographed under UV light. Lambda DNA digested with *Hin*dIII and *Eco*RI, 1-kb Ladder DNA and Raoul (Appligene) were used as size standards.

In addition, in the very few cases which needed additional verification because of the occurrence of very close bands on the gels, the mutations were identified and located on the cpDNA molecule by using Southern-blot hybridisation of the cpDNA restriction patterns which were transferred to a nylon membrane (Biodyne A from Pall Filtration technik GmbH). Heterologous probes consisting of nine clones, pTBa1, pTBa2, pTBa5, pTB7, pTB13, pTB18, pTB25, pTB28 and pTX6, from *Nicotiana tabaccum* L. (Sigiura et al. 1986), and three additional clones (ctP4, ctP5 and ctP7) from *Hordeum vulgare* L. (Day and Ellis 1985), were used in order to cover the whole cpDNA molecule. The probes were labelled with dioxigenin-d-UTP (Non-radioactive DNA labelling and Detection Kit, Boehringer Mannheim west Germany) and were hybridized overnight at 68°C. Hybridized olive cpDNA fragments were revealed by immunological detection and chemiluminescence using CSPD from Tropix (Saumitou-Laprade et al. 1993).

Identification of cpDNA mutations

For each cpDNA restriction endonuclease pattern, DNA restriction fragment sizes were determined using the "Bande" computer program (Duggleby et al. 1981). The cpDNA restriction endonuclease patterns of individual trees were scored for fragment-length differences. The cpDNA changes were identified as either length or site mutations. The detection of specific changes, each revealed from an individual olive tree by several restriction enzymes, suggests that alterations in the length of the fragments may be due to DNA length mutations rather than site mutations. By scoring those length mutations arbitrarily as the same mutation (same letter), we avoided counting the same addition/deletion several times and, thereby, overestimating the number of distinct mutations.

Data analysis

The different chlorotypes were scored for presence/absence and pooled to compute a similarity matrix (*F*-values) as proposed by Nei and Li (1979). The distance (1-*F*-values) was then analysed

Table 1 Section, diffusion area, collecting locality, sample size and accession sources of the 15 *Olea* taxa analysed using cpDNA RFLPs; * cultivars and trees cultivated in local sites. ** wild oleaster trees. Source A refers to Amane et al. (1999); B collected in situ by the authors of the present paper; C refers to the list of collectors in the acknowledgement section; D Institute of Olive Research collection, CNR, Perugia, Italy: K RGB Kew Living Collection, London, UK

Section	Species/subspecies	Diffusion area	Origin (Locality)	Sample size	S
Olea	*O. europaea* L.	Mediterranean	Med. Basin		
	Subsp. *europaea*	Basin		*	A
	Subsp. *sylvestris* (Mill.) Lehr.	Mediterranean Basin	Med. Basin	**	A
	O. cerasiformis (Webb & Berth.) Kunk. & Sund.	Macaronesia	Grand Canary:		
			(Tafira Alta)	2	B
			(Valsequillo)	3	B
			Tenerife:		
			(Mt Anaga)	1	B
			La Palma		
			(Santa Rosalia)	4	C
	O. maroccana Gren. & Berth	Morocco	High Atlas: (Immouzzer)		
			(Amskroud)	4	B
				5	B
	O. laperrinei (batt. & Trab.) Ciferri	Sahara	Algeria (La Taessa, Hoggard)	1	C
	O. africana Mill.	Southern Africa	South Africa:		
			(Sun City)	5	B
			(Cape Town)	3	B
			(Boulders)	5	B
			(Pretoria)	1	C
			(Blyde Lake)	2	B
			Reunion island	1	C
		Eastern Africa	Kenya	1	K
	O. chrysophylla Lam.	Eastern Africa Arabia	Ethiopia (Shewa)		C
	O. indica		Kenya	1	K
	O. cuspidata Wall.	Asia	China (unknown loc.)	1	D
			China (unknown loc.)	1	C
	O. ferruginea Royale	Asia	India (unknown loc.)	1	D
			Pakistan (Taxala, Punjab)	4	C
Ligustroides Benth & Hook	*O. capensis*	Southern Africa	South Africa (Cape town)	2	B
	Subsp. *capensis*				
	Subsp. *macrocarpa* (Wright) Verd.	Southern Africa	South Africa (Cape)	4	B
	Subsp. *hochstetteri* (Bak.) Friis & Green	Central Africa	Ivory Coast (Mt. Momi)	2	C
	O. lancea Lam.	Mascareignes, Madagascar	Reunion	1	C
			Mauritius	1	K
	O. exasperata Jacq.	South Africa	South Africa (Boulders)	6	B
			(Cape Peninsula)	3	B

by the Fitch-Margoliash method using the Kitch option of PHYLIP 3.5 (Felsenstein 1993). In addition, considering *P. angustifolia* L. as the outgroup species, the DNA changes observed in that species and in the several chlorotypes identified in *Olea* taxa were scored again for presence/absence and were analysed cladistically by enumeration of the parsimonious rooted trees using the Dollo parsimony method (DOLLOP option of PHYLIP 3.5, Felsenstein 1993). A consensus tree was obtained (CONSENS option of PHYLIP 3.5), and the SEQBOOT option of PHYLIP was also used with 500 bootstrap samples to place confidence limits on branching points in the tree (Felsenstein 1993).

Results

The few chloroplast DNA changes observed previously by Amane et al. (1999) in 2% of the cultivated olive (*O. europaea* L. subsp. *europaea*) and in 14% of the wild olive material *O. europaea* subsp. *sylvestris*) were not detected in the other 13 *Olea* taxa analysed in the present study, and for which the sample size was much more restricted. Therefore, the most common restriction pattern obtained for each endonuclease and the single corresponding majority haplotype (chlorotype) observed in both cultivated and wild Mediterranean olive were considered exclusively. In the 15 *Olea* taxa analysed by digestion with the nine endonucleases, 36 different banding patterns were observed, giving a total of 484 different fragments. A single pattern was obtained for *Hpa*II, *Nci*I and *Xho*I. Overall, the restriction endonucleases *Ava*I, *Bam*HI, *Cfo*I, *Dra*I, *Hae*III, *Hin*dIII, *Hpa*II, *Nci*I and *Xho*I generated an average of 50.0, 42.5, 53.0, 46.5, 58.0, 25.5, 58.0, 54.0 and 26.5 fragments, respectively.

Table 2 Restriction fragment length changes (kb), type of mutation (site, length) in variant restriction patterns of *Olea* taxa compared to the majority pattern observed in *O. europaea*, and identification of the chlorotypes where the changes were observed. Changes attributable to the same mutation are indexed with the same letter

Restriction enzyme	Mutation			Mutation		
	Site		Chlorotype	Length		Chlorotype
	Code	Majority→variant pattern		Code	Majorty→variant pattern	
*Cfo*I	1	4.00+2.23→6.23	IX, X, XI, XII	A	1.87→1.74	XI, XII
	2	3.99+0.37→4.36	VII, VIII			
	3	3.70+x[a]→3.90	IX, X			
	4	3.00+x→3.12	IX			
	5	1.50+x→1.70	IX			
*Bam*HI	6	5.07→3.92+1.15	IX, X, XI, XII	A	3.03→2.90	XI, XII
	7	4.95→4.20+0.75	XI, XII			
	8	3.30→1.80+1.50	XI			
	9	1.90→1.85+x	XI, XII			
	10	1.23+0.87→2.10	VII, VIII			
*Hin*dIII	11	6.80→6.20+0.40	IV	B	4.30→4.27	XII
	12	4.23→3.95+x	IX	C	1.05→1.13	IV, V, VI, IX, X XI, XII
	13	4.22→4.10+x	IX			
	14	2.78→2.39+0.39	VII, VIII			
	15	2.68→1.36+1.32	IX			
	16	2.61→2.49+x	IX			
	17	1.18+x→1.29	VI			
	18	1.18+x→1.27	III			
	19	1.16+x→1.22	II			
*Dra*I	20	12.54+1.06→13.60	XI	B	6.65→6.62	XII
	21	6.65+0.35→7.00	IX	C	7.22→7.30	IV, V, VI, IX, X, XI, XII
	22	2.70→2.55+x	IX, X, XI, XII	D	2.74→2.63	IV, V, VI
	23	2.74+0.76→3.50	VII, VIII	E	4.17→3.45	IX, X, XI, XII
	24	2.35→2.04+0.31	IX, X, XI, XII			
*Hae*III	25	6.80→6.00+0.80	IX	C	2.02→2.10	IV, V, VI, IX, X, XI, XII
				D	2.39→2.28	IV, V, VI
				E	3.42→2.70	IX, X, XI, XII
*Ava*I	26	6.80+5.00→11.80	III, IV, V, VI			
	27	5.00+x→5.20	VII, VIII			
	28	1.30→1.20+x	VIII			

[a] x Fragment not visualised because of size

As compared to the most-common restriction pattern observed in *O. europaea* for each restriction enzyme, the mutations responsible for cpDNA variation in the other *Olea* taxa could be identified and are listed in Table 2. Twenty eight site mutations were found. In addition, five length mutations were identified using from two to three distinct endonucleases for each. The site mutation 26 and the length mutation D were confirmed using probes pTBa1 and pTB25, respectively, and were both located on the large single-copy region of the cpDNA molecule. In addition, as compared to the restriction patterns obtained in *O. europaea*, the four site mutations (6, 9, 22 and 24) and the length mutation (C) present in other *Olea* taxa, and ten additional mutations not observed in *Olea*, were identified in *P. angustifolia.*

From all the mutations obtained in *Olea* with the nine restriction enzymes, 12 distinct haplotypes (chlorotypes) were distinguished. Chlorotype I corresponds to the predominant patterns observed in cultivated and wild Mediterranean olive (*O. europaea*). Chlorotype II differs from chlorotype I by a single site mutation (Table 2) and was observed in all the individuals of *O. laperrinei*, *O. mar-*

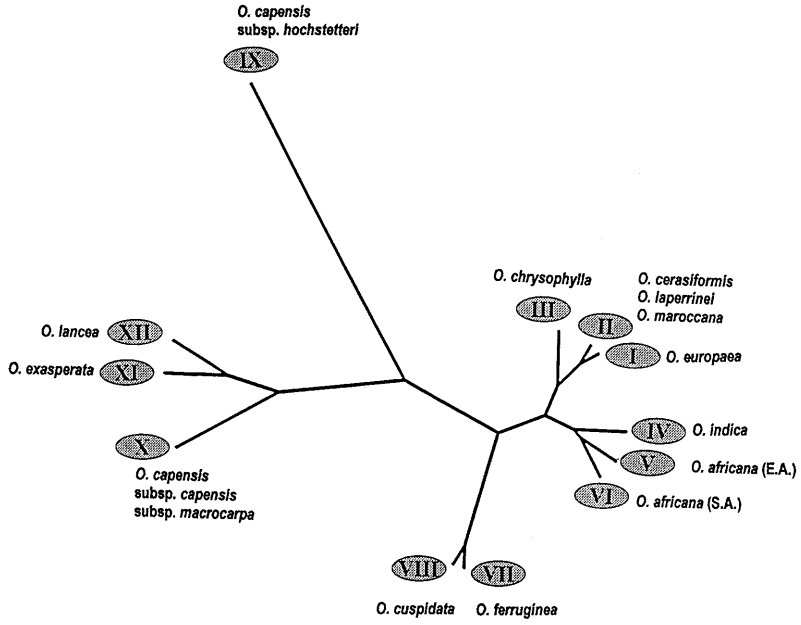

Fig. 1 Fitch-Margoliash phenogram based on the proportion of shared site and length mutations for the 12 chlorotypes observed in the 15 *Olea* taxa scored for nine endonuclease cpDNA RFLPs

occana and *Olea cerasiformis* analysed in this study. Chloroplast III, which also differs from *O. europaea* by a single mutation (Table 2), was restricted to *O. chrysophylla*. Chloroplasts IV, V and VI characterised *Olea indica* from Kenya, *O. africana* from Kenya (Eastern Africa) and *O. africana* from both South Africa and La Réunion island, respectively. Chloroplasts VII and VIII differed from chloroplast I by at least five site mutations (Table 2) and were observed in the Asiatic material of *O. ferruginea* and *Olea cuspidata*, respectively. These two taxa differed by a single mutation. Chlorotypes IX, X, XI and XII were specific to taxa in the section *Ligustroides*, i.e. to *Olea hochtetteri*, to the two other *O. capensis* subspecies, to *Olea exasperata* and to *Olea lancea*, respectively. No cpDNA variation was observed within each collection site or between trees of the same taxon sampled at distinct sites, exept in *O. africana* for which the two chlorotypes V and VI were distributed geographically, in East and southern Africa, respectively. Seventeen of the thirty three mutations observed in the whole *Olea* material were phyletically informative (i.e. observed in at least two chlorotypes).

The similarity index (*F*) values obtained from the whole set of mutations (site and length) ranged from 0.42, between chlorotype IX present in *Olea hochtetteri* and chlorotype VII observed in *O. ferruginea*, to 0.97, between chlorotype I (in *O. europaea*) and chlorotype II present in *O. laperrinei*, *O. maroccana* and *O. cerasiformis*. When the distance matrix obtained from the 1-*F*-values was subject to Fitch-Margoliash analysis, four groups of chlorotypes could be distinguished (Fig. 1). The first group included the chlorotypes X, XI and XII present in the southern African taxa *O. exasperata*, *O. lancea*, and the three subspecies. of *O. capensis*, respectively. Chloroplast IX occurring in *O. hochtetteri* was also connected to that group at a distinct point. The sec-

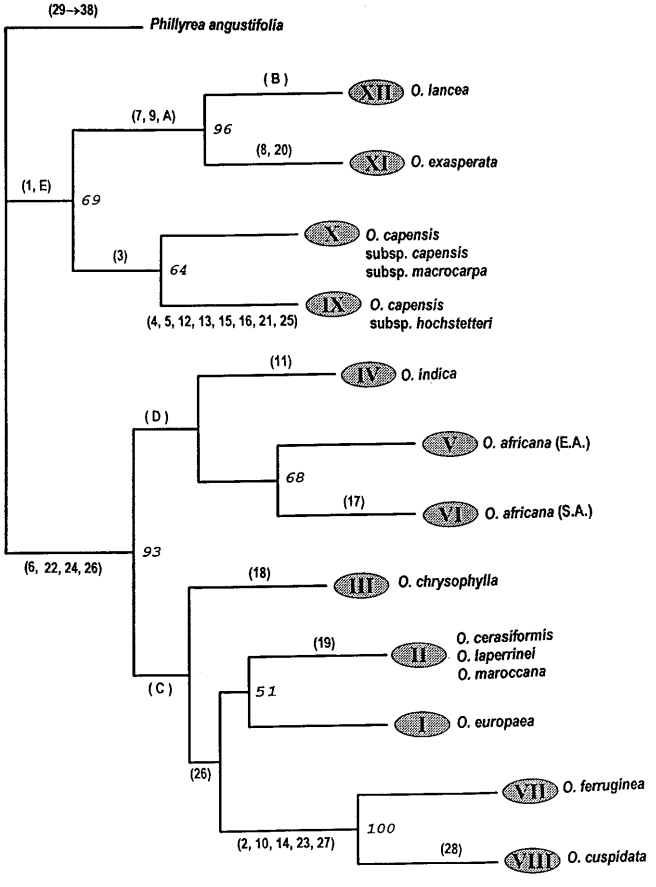

Fig. 2 Consensus tree obtained from the Dollo parsimony method and bootstrap-based confidence limits for the 12 chlorotypes observed in the 15 *Olea* taxa scored for cpDNA variation, using the chlorotype of *P. angustifolia* as an outgroup. The mutations are identified (between parentheses) on the tree branches. For each major branch, the percentage of times that the defined group occurred in the 500 bootstrap samples is indicated

ond group included the chlorotypes VII and VIII, present in the Asiatic taxa O. ferruginea and *O. cuspidata*, respectively. The third group gathered together chlorotypes IV, V and VI present in *Olea indica*, *O. africana* from Kenya and *O. africana* from southern Africa, respectively, and the fourth group included chlorotype I, present in *O. europaea*, chlorotype II, characteristic of the three taxa *O. maroccana*, *O. cerasiformis* and *O. laperrinei*, and chlorotype III present in *O. chrysophylla*.

With the Dollo parsimony method, a similar general clustering pattern was obtained from the analysis of presence/absence of the mutations (with *P. angustifolia* as an outgroup). The most-parsimonious trees required a minimum of 44 steps to account for the 43 mutations. A consensus tree was obtained with confidence values ranging from 50 to 96% of the major branches (Fig. 2).

Discussion

General phyletic organisation in *Olea* based on cpDNA variation

As compared to the cpDNA variation observed using RFLPs in other broad-leaved tree genera with wide geographical distributions, e.g. in *Quercus* (Fagaceae) (Manos et al. 1999) or in *Magnolia* (Magnoliaceae) (Qiu et al. 1995), the cpDNA variation in *Olea* is rather low. However, the data obtained in the present study provide useful information about the phyletic organisation of the genus *Olea*. The cpDNA variation patterns observed between the *Olea* taxa of section *Ligustroides* and those of the section *Olea* support the previous delimitation of these two sections on the basis of morphology. Moreover, our results clearly indicate that, as compared to the taxa of the *Olea* section, those of section *Ligustroides* possess cpDNA molecules more similar to that observed in *P. angustifolia* which, as mentioned, belongs to an Oleaceae genus close to the genus *Olea*. Therefore, the section *Ligustroides*, which comprises mostly tropical taxa growing in southern Africa, may be considered as more ancestral than the other section. This result also supports previous assumptions according to which the genus *Olea* may have been an important component of the Rand flora (indigenous African flora) (Lebrun in Quezel 1978) and may have originated mainly in southern Africa (Ciferri 1942). In the present study, two groups of taxa were distinguished within the *Ligustroides* section on the basis of their cpDNA variation. One comprised the two species *O. lancea* and *O. exasperata*, which possess more-narrow leaves and are endemic to distinct areas of southern Africa where they grow in open and rather dry habitats. The other group includes the three *O. capensis* subspecies, *capsensis* and *macrocarpa* from South Africa and *hochtetteri* from Ivory Coast and Cameroon. These three taxa possess large lanceolate or rounded leaves and grow in humid and often shady tropical environmental conditions. Subsp. *capensis* and *macrocarpa*, which grow in South Africa but are very distinct morphologically, share the same cpDNA chlorotype. Conversely, as shown more particularly by the phenogram based on the distance matrix, and which gives the same weight to any mutation, the marked cpDNA differentiation of subsp. *hochtetteri* with a large number of cpDNA specific mutations, was not expected and suggests that this taxon may be considered as a distinct species in the *capensis* group. However, as was previously noted by Cifferi (1942) in a morphological study of the genus *Olea*, the single *Olea* taxon growing in the Ivory Coast, at the southwestern limit of the genus' distribution, is substantially isolated geographically from any other *Olea* taxon and such a situation may justify further detailed studies.

Within the section *Olea*, three phyla corresponding to (1) South and East African taxa, (2) Asiatic taxa and (3) a group of Mediterranean, Macaronesian and Saharan taxa, were distinguished by both phenetic and cladistic analyses. However, the way the phyla cluster together differs according to the phyletic importance of the mutations which characterise the taxa involved in those clades. For instance, on the phenogram, *O. cuspidata* from China and *O. ferruginea* from India and Pakistan, which both differed from the other *Olea* taxa by five shared mutations and differed from each other by a single cpDNA change, constitute a very distinct phylum. In contrast, on the cladogram these two species clustered with the Mediterranean and Saharan taxa.

The clear cpDNA differentiation between southern and East African taxa and the Asiatic taxa, and the discrimination observed in the present study between each of these two groups and third group of taxa from the Mediterranean Basin, Macaronesia, the Sahara and north-eastern Africa, are not consistent with the previous classification of *Olea* by Green and Wickens (1989). These authors assigned all the southern and East African taxa and the Asiatic taxa to *O. europaea* subspecies *cuspidata*. Moreover, it can be noted that the *O. africana* material analysed from several geographically distant accessions of South Africa, on the one hand, and from Kenya, on the other hand, showed two distinct chlorotypes, suggesting that populations from these two African regions may be isolated genetically and constitute distinct taxa.

CpDNA phyletic relationships between *O. europaea* and the other *Olea* taxa

In both the phenetic and cladistic analyses of cpDNA variation in *Olea*, a particular cluster was constituted by chlorotype I, predominantly and exclusively observed in *O. europaea*, and chlorotype II, shared by three taxa which are distributed in areas geographically close to the Mediterranean Basin. These taxa, i.e. *O. laperrinei* from the central Sahara, *O. maroccana* from the High Atlas in southern Morocco, and *O. cerasiformis* from Macaronesia, may have a common maternal origin and may be distinct from *O. europaea*. In previous studies, these

three taxa were often reported to be closely related to the Mediterranean species and, therefore, to the cultivated olive (Chevalier 1948; Ciferri 1950; Turill 1951; Zohary 1994). Moreover, Chevalier (1948) and Turill (1951) considered that *Olea* wild types from the Sahara, the High Atlas and from Macaronesia were so close morphologically that they should be assigned to the same taxon. Conversely, Green and Wickens (1989) and Zohary (1994) assigned *Olea* wild-types from both Sahara and High Atlas regions, and those from Macaronesia to two morphologically distinct taxa, i.e. subsp. *laperrinei* and *cerasiformis*, respectively. In a recent study based on the AFLP analysis of *Olea* DNA (Angiolillo et al. 1999), *O. laperrinei* and *O. maroccana* were shown to be close but, unfortunately, the Macaronesian wild-type was not analysed. Results obtained in the present study on the basis of cpDNA variation support Chevalier and Turill's assessment, but the possibility that subsp. *laperrinei* and *cerasiformis* may constitute two close taxa with similar or two very close chlorotypes, and more distinct nuclear genomes, cannot be ruled out. Moreover, on the phenetic tree obtained in the present study, chlorotype III, present exclusively in *O. chrysophilla*, was also clustered with chlorotypes I and II from which it differs by very few mutations, one of them (26) being shared with the chlorotypes of *Olea* taxa from East and South Africa. This result suggests that *O. chrysophylla* growing in north-eastern Africa may be considered as an intermediary form between the African and Mediterranean taxa. In the past, several attempts were made to assign a precise origin to *O. europaea* which, according to various authors, was located either in Africa or in Asia (Chevalier 1948; Cifferi 1950). Results of the present study based on cpDNA variation provided clear and useful information to clarify the maternal phyletic relationships among the various *Olea* taxa, but no evidence could be obtained to indicate the precise origin of the Mediterranean Olive. Further studies combining the results from an analysis of multiple nuclear and cytoplasmic genetic characters may be necessary to reach this objective.

Acknowledgements The authors are grateful to G. Beaujean (IRD-Man), M. Dahmani (URBT-Alger), A. Kirk (USDA-Montpellier, J. Li (Univ. Beijin), J.Louarn (IRD-Man), R. Ramon (Santa Cruz), C. Scholtz (Univ. Pretoria), R. Sobhian (USDA-Montpellier) and J. Thompson (CNRS-Mentpellier) for their assistance with the collection of *Olea* material. We also thank J. Aronson (CNRS-Montpellier) for making useful suggestions on how to improve the manuscript. This research was supported by the European Union FAIR Program, Contract no. CT95 0689. The experiments comply with the current laws of France, where the experiments were performed.

References

Amane M, Lumaret R, Hany V, Ouazzani N, Debain C, Vivier G, Duguilloux MF (1999) Chloroplast-DNA variation in cultivated and wild olive (*Olea europaea* L.). Theor Appl Genet 99: 133–139

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

Chevalier A (1948) L'origine de l'olivier cultivé et ses variations. Rev Bot Appl 303–304:1–25

Cifferi R (1942) Recenti progressi degli studi botanico-agrari sull'olivo. In: Proc Convegno di studi Olivicoli, Firenze 1942, Acc Georgofili, Firenze, pp 49–95

Cifferi A (1950) Dati ed ipotesi sull'origine e l'evoluzione dell'olivo. Olearia, pp 114–122

Duggleby RG, Kinns H, Rood J (1981) A computer program for determining the size of DNA restriction fragments. Crit Rev Plant Sci 110:49–55

Day A, Ellis THN (1985) Deleted forms of plastid DNA in albino plants from cereal anther culture. Curr Genet 9:671–678

Felsenstein J (1993) PHYLIP. Phylogeny inference package, version 3.57. Department of Genetics, University of Washington, Seattle, Washington

Green PS, Wickens GE (1989) The *Olea europaea* complex. In: Kit Tan (ed) The Davis and Hedge Festschrift. Edinburgh University Press, Edinburgh, pp 287–299

Manos PS, Doyle JJ, Nixon KC (1999) Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (*Fagaceaea*). Mol Phylog Evol 12:333–349

Michaud H, Lumaret R, Ripoll JPH, Toumi L (1995) A procedure for extraction of chloroplast DNA from broad-leaved tree species. Plant Mol Biol Rep 13:131–137

Morettini A (1972) L'olivicoltura 2nded, Ramo Editoriale degli Agricoltori, Roma

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am Nat 130:6–29

Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. Theor Appl Genet 90:462–470

Quézel P (1978) Analysis of the flora of Mediterranean and Saharan Africa. Ann Missouri Bot Gard 65:479–534

Quézel P (1995) La flore du bassin méditerranéen: origine, mise en place, endémisme. Ecol Med 21:19–39

Qiu YL, Chase MW, Parks CR (1995) A chloroplast DNA phylogenetic study of the Eastern Asia-Eastern North America disjunct section *Rytidospermum* of *Magnolia* (Magnoliaceae). Am J Bot 82:1582–1588

Saumitou-Laprade P, Rouwendal GJA, Cuguen J, Krens FFA, Michaelis G (1993) Different CMS sources found in *Beta vulgaris* ssp. *maritima*: mitochondrial variability in wild populations revealed by a rapid screening procedure. Theor Appl Genet 83:529–535

Sigiura M, Shinozaki K, Zaita N, Kusuda M, Kumano M (1986) Clone bank of the tobacco (*Nicotiana tabacum*) chloroplast genome as a set of overlapping restriction endonuclease fragments: mapping of 11 ribosomal protein genes. Plant Sci 44:211–216

Turrill WB (1951) Wild and cultivated olives. Kew Bull 3:437–442

Zohary D (1994) The wild genetic resources of the cultivated olive. Acta Hort 356:62–65

Zohary D, Hopf M (1993) Domestication of plants in the Old World. Oxford University Press, Oxford