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Genetic relationships in the olive (*Olea europaea* L.) reflect multilocal selection of cultivars

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Abstract One hundred and two olive RAPD profiles were sampled from all around the Mediterranean Basin. Twenty four clusters of RAPD profiles were shown in the dendrogram based on the Ward's minimum variance algorithm using chi-square distances. Factorial discriminant analyses showed that RAPD profiles were correlated with the use of the fruits and the country or region of origin of the cultivars. This suggests that cultivar selection has occurred in different genetic pools and in different areas. Mitochondrial DNA RFLP analyses were also performed. These mitotypes supported the conclusion also that multilocal olive selection has occurred. This prediction for the use of cultivars will help olive growers to choose new foreign cultivars for testing them before an eventual introduction if they are well adapted to local conditions.

Keywords Olive · *Olea europaea* · RAPD · Mitotype · RFLP · Classification

Introduction

The olive tree is one of the first domesticated trees. Its domestication probably occurred in the Near-East some 5500 to 5700 years ago (Zohary and Hopf 1994). It is assumed that cultivars have originated from the wild Mediterranean olive (called oleasters), and have been disseminated all around the Mediterranean following human displacement. It is also presumed that crosses between wild and cultivated olives could have led to new cultivars in different parts of the Mediterranean.

In fruit trees, considering long-term breeding programs, studies dealing with the structure of genetic diversity may give some insights about the selection of cultivated forms, and this may lead to the best management of their diversity. For example, in cacao and grapevine, a correlation between genetic diversity structure and some factors such as the geographic origin or the typical use of the fruits has been demonstrated (N'Goran et al. 1994; Cuisset 1998). For grape, the prevailing breeding process has been based on crosses between cultivars according to microsatellite markers (Bowers et al. 1999). In olive, based on AFLP technology, Angiolillo et al. (1999) have shown that wild olive from the Western Mediterranean and cultivars did not cluster together, but were relatively distant. However, a few oleasters clustered with the cultivars. Another study in olive and oleasters, based on isozymes (Lumaret et al. 1997), did not reveal such a clear-cut difference between cultivars and oleasters. We therefore suspected that the types of molecular markers and the studied samples both for cultivars and oleasters could influence the conclusion. Recently, mitochondrial DNA variations have led us to propose at least two distinct origins of the wild Mediterranean olive, in the Near East and in the Maghreb (Besnard and Bervillé 2000). Moreover, it is likely that from these origins cultivars have been diffused by humans and have been used locally in crosses with local oleasters.

Studying the structure of the genetic diversity of the cultivars should give some insights into the origins of the cultivated olive. Our study was performed using RAPDs and mitochondrial RFLPs on a set of olive cultivars sampled from different countries around the Mediterranean Basin. These markers identified some of the factors (geographic origin and typical use of the fruits) related to their clustering into more or less homogeneous groups.

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Materials and methods

One hundred and two olive genotypes previously identified by Besnard et al. (2000a) were studied (see Table 1). We eliminated all synonymies and kept for cultivars the denomination from the likely country of origin. The countries of origin from the sampled denominations were: Algeria (5), Egypt (1), Continental France

(40), Corsica (4), Greece (6), Continental Italy (12), Sicily (7), Israel (5), Syria (2), Morocco (1), Portugal (1), Spain (8), Tunisia (5), Turkey (4), Yugoslavia (1). For France, we studied both the main denominations and some local ones. More precise details about the material are available on request to the authors. Marker procedures have been described previously for RAPDs (Quillet et al. 1995) and mitochondrial RFLPs (Besnard et al. 2000b). Eight decamers previously screened (Besnard et al. 2000a) were employed: A1, A2, A9, A10, C9, C15, E15 and O8.

Data analysis

The OPEP software (Baradat and Labbé 1995) and SPAD software rel. 3.5 (CISIA 1996) were used for the data computations

Dendrogram construction

The clustering of profiles was performed by a dendrogram following the Ward's minimum variance algorithm (Ward 1963) based on the 44-dimensional space chi-square distances computed by a Multiple Correspondence Analysis. Saporta (1990) and Lebart et al. (1997) have described this algorithm in a generalised form. Its principle is to cluster profiles or groups at each step by keeping a maximum value of the ratio between group sum of squares/total sum of squares.

Geographic differentiation revealed by RAPDs

We used a two-way nested MANOVA model to measure the average differentiation of the profiles in relation to the geographic zone, g_i (East or West), or with the country within each zone (c_{ij}), following the model:

$$y_{ijk} = \mu + g_i + c_{ij} + e_{ijk}$$

with respective variances σ^2_g, $\sigma^2_{c|g}$, and σ^2_e. The three intra-class correlation coefficients are:

$$\theta_1 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_{c|g} + \sigma^2_e},$$

$$\theta_2 = \frac{\sigma^2_{c|g}}{\sigma^2_{c|g} + \sigma^2_e},$$

$$\theta_3 = \frac{\sigma^2_g + \sigma^2_{c|g}}{\sigma^2_g + \sigma^2_{c|g} + \sigma^2_e}.$$

They express respectively the differentiation due to the geographic zone alone among all the countries (θ_1), due to the country within a given geographic zone (θ_2), or due to both the geographic zone and the country within this zone over all the countries (θ_3).

Similarly, we computed the corresponding θ' parameters which are the correlation coefficients between two profiles if the first one is characterised by the marker number n and the second by the marker number n' or vice-versa. These parameters are expressed as:

$$\theta'_1 = \frac{\mathrm{cov}_{c|g(n,n')}}{\sqrt{\sigma^2_{c|g}(n) + \sigma^2_e(n)}\sqrt{\sigma^2_{c|g}(n') + \sigma^2_e(n')}},$$

$$\theta'_2 = \frac{\mathrm{cov}_{g(n,n')}}{\sqrt{\sigma^2_g(n) + \sigma^2_{c|g}(n) + \sigma^2_e(n)}\sqrt{\sigma^2_g(n') + \sigma^2_{c|g}(n') + \sigma^2_e(n')}},$$

$$\theta'_3 = \frac{\mathrm{cov}_{g(n,n')} + \mathrm{cov}_{c|g(n,n')}}{\sqrt{\sigma^2_g(n) + \sigma^2_{c|g}(n) + \sigma^2_e(n)}\sqrt{\sigma^2_g(n') + \sigma^2_{c|g}(n') + \sigma^2_e(n')}}.$$

These models were applied both to each RAPD fragment and to the axes of the Correspondence Analysis. The intra-class correlation coefficients between individual RAPDs and the axes is a measure of their contribution to the differentiation of groups of profiles along each axis. In the case of the fragments, θ' provides a measure of the tendency towards the co-occurrence (+ sign) or of the mutual exclusion (– sign) of two fragments. As usual, θ is a measure of the discriminating accuracy of the considered fragment and provides a substitute of Wright's F_{st} measure of genetic differentiation for dominant markers, in the same way as the ϕ_{st} parameter used by Stewart and Excoffier (1996). The correlation between two alleles (at one locus) sampled for two individuals of the same population is replaced by the correlation between the "phenotypic" values of the two sampled individuals for the dominant marker, assuming panmixis. To avoid ambiguity, we shall further write "one-marker intra-class correlation" for θ, and "two-marker intra-class correlation" for θ'. However, in this analysis, five profiles were not considered because, in our sample, they represented not enough cultivars in a country. Standard errors of the estimates of θ or θ', were computed by the Jackknife method (Shao and Tu 1995; Lebart et al. 1997) with 96 degrees of freedom. The two-marker intra-class correlation θ' is only given for the RAPDs that exhibited significant values for the θ values.

Discriminant analyses

Discriminant analyses on qualitative data were performed with the DISQUAL procedure (Saporta 1990) to test the relationships of RAPD profiles either with the typical olive utilisation (oil, fruits, or oil + fruits), or the country of origin. We selected countries or regions represented in our data by at least four profiles and, in consequence, only 96 profiles were considered. A Multiple Correspondence Analysis performed on the m RAPD fragments in each sample generated the m-1 coordinates of the profiles. The discriminant analyses used these coordinates. The discriminant space finally had p-1 dimensions, p being the number of levels for each factor (two for the kind of use and nine for the country or region of origin). To compute the Mahalanobis distances we used the three first dimensions for countries or regions.

Results

On our cultivar sample, we employed 45 RAPD markers and four mitotypes. From these data, computations were performed.

Cultivar clustering on the dendrogram

Figure 1 displays the dendrogram constructed on the Ward's distances. Twenty four clusters were defined. There is no clear structure with the geographic origin of the cultivars. Nevertheless, a clustering of cultivars of the same region is observed (for instance, the Andalusian cultivars in cluster 17, the Sicilian cultivars in cluster 09). Furthermore, the dendrogram displays two main groups of cultivars: group 1 (clusters 1 to 7) and group 2 (clusters 8 to 24). Group 1 is constituted mainly by Western varieties typically used for oil (except *Galega*, *Cassanese* and *Malaussena* which are used mainly for oil but also for canning), whereas group 2 is constituted by varieties grown for different typical uses. The cultivar clustering obtained with cytoplasmic data was quite different from that obtained with RAPDs (Table 1, Fig. 1).

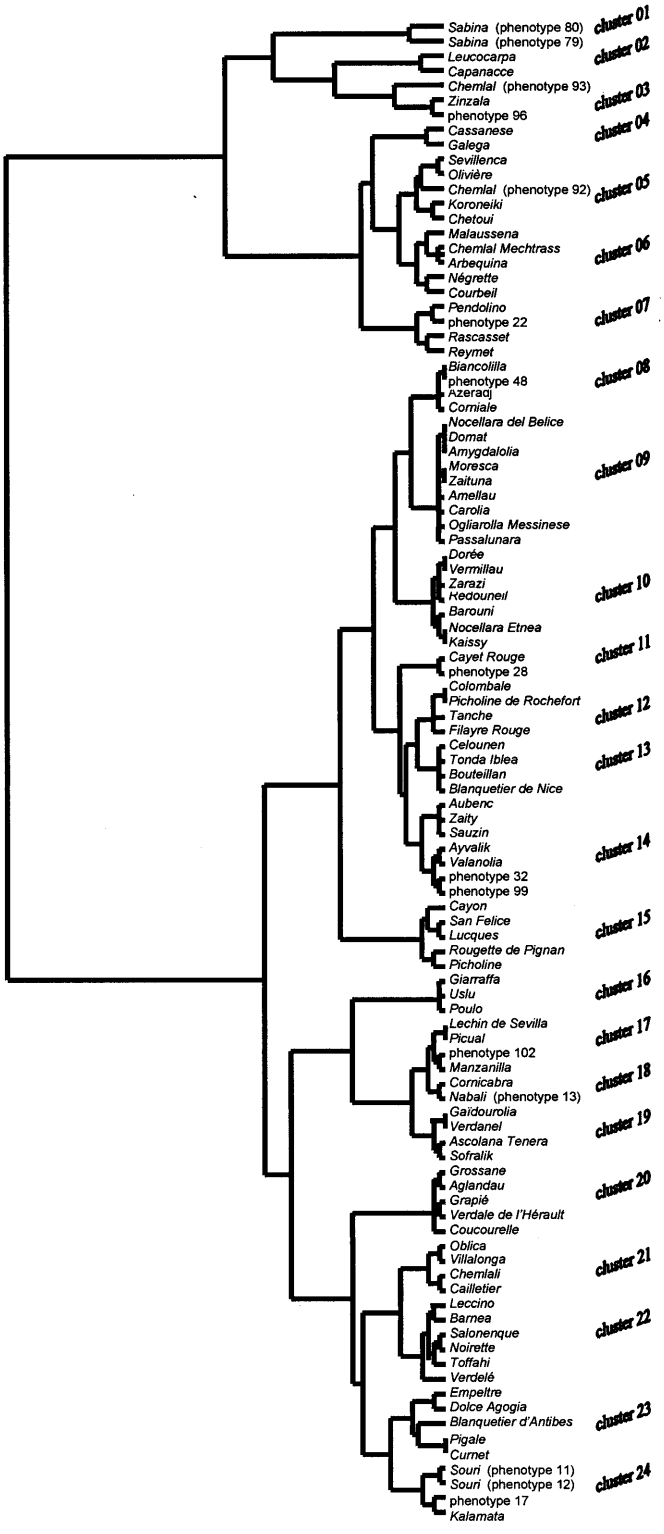

Fig. 1 Dendrogram of the cultivars based on the chi-square distance analysis, constructed with the minimum variance algorithm (Ward 1963)

Geographic structure revealed by individual markers

Table 2 shows that, among the 45 markers, only five play a significant role in explaining the similarity between profiles, and therefore cultivars, in the same country or region. Among this group, only A1–275 is related to an average East-West difference. Nevertheless, the frequency of four of these markers (A1–225, A1–275, A1–1200 and A1–650) is high in the countries for the West and mainly in Corsica (France), Spain and Algeria, whereas it is low in the eastern countries (data not shown). The main part of the genetic variability lies between countries of the same geographic zone. At this level, there are many significant positive or negative associations of RAPDs. Although it is not a direct measure of linkage disequilibrium, the association of "phenotypes" for RAPDs may be interpreted as a likely consequence of this phenomenon.

Only the first two axes of the Correspondence Analysis play a significant role for the structure between countries or regions. They have an obviously greater value for the structure of countries than individual RAPDs as they integrated the information of the 43 polymorphic markers represented into the 11 studied countries or regions.

Factors related with the variability of RAPD profiles

Geographic origin:

Results of the Discriminant Analysis according to the country or region on the same sample of 96 profiles, based on 43 polymorphic RAPDs, are presented in Fig. 2A. The two first axes, which represent 48.41% of the discrimination, were significant at the 1% level (Wilks test), whereas the third axis was significant at the 5% level. On the first two axes, the structure based on the relations between the ten countries or regions is obvious. The coordinates of the 96 profiles and the ten centroids showed a fair individualisation of the clusters containing cultivars from Algeria, Spain, Israel and Corsica. There are two exceptions: the *Empeltre* profile (Balearic islands, Spain), which is clustered with those from continental France, and the Israeli *Nabali* "*Mohassen*" profile, which is close to those from continental France and Italy. There was a non-significant difference between continental Italy, Sicily, Greece and continental France (Table 3). The profiles of cultivars from Israel and those from Tunisia and Turkey are close, although the average distance with this last country is significant (P<5%; Table 3). Finally, we observed a good differentiation of the profiles from Spain, Algeria and Corsica, with highly significant average distances from other countries or regions (Table 3). The French *Olivière* profile was strongly located towards the Algerian profiles (Fig. 2A); its mitotype was MCK, as for *Chemlal*. This suggests a possible common origin. This great variability between countries within the same geographic zone obscures the average East-West difference (Fig. 2A). It also explains why

Table 1 Codes of the clones identified with RAPDs and their mitotype. The country of origin of the denominations are given in parentheses (Gr=Greece; Tk=Turkey; Is=Israel; Sy=Syria; Eg=Egypt; Yu=Yugoslavia; It=Italy; Its=Sicily, Italy; Fr=France; Frc=Corsica, France; Sp=Spain; Pt=Portugal; Tu=Tunisia; Al=Algeria; Mo=Morocco). [1] The cultivars used to produce oil; [2] the cultivars used to produce both oil and canned fruits; [3] the cultivars used to produce canned fruits

Code	Clone and provenance	Mitotype	Code	Clone and provenance	Mitotype
1	*Kalamata* (Gr)[3]	ME1	52	*Grapié* (Fr)[3]	ME1
2	*Vallanolia* (Gr)[1]	ME1	53	*Aglandau* (Fr)[2]	ME1
3	*Gadouralia* (Gr)[3]	ME1	54	*Celounen* (Fr)[1]	ME1
4	*Koroneiki* (Gr)[1]	ME1	55	*Courbeil* (Fr)[1]	MCK
5	*Carolia* (Gr)[3]	ME1	56	*Coucourelle* (Fr)[1]	MOM
6	*Amygdalolia* (Gr)[3]	ME2	57	*Cayet Rouge* (Fr)[1]	ME1
7	*Uslu* (Tk)[3]	ME1	58	*Rascasset* (Fr)[1]	ME1
8	*Domat* (Tk)[3]	ME1	59	*Malaussena* (Fr)[2]	ME1
9	*Ayvalik* (Tk)[1]	ME1	60	*Aubenc* (Fr)[1]	ME1
10	*Sofralik* (Tk)[3]	ME1	61	*Reymet* (Fr)[1]	ME1
11	*Souri* (Is)[2]	ME1	62	*Curnet* (Fr)[1]	MCK
12	*Souri "Mansi"* (Is)[2]	ME1	63	*Colombale* (Fr)[2]	ME1
13	*Nabali "Mohassen"* (Is)[2]	ME1	64	*Poulo* (Fr)[3]	ME2
14	*Barnea* (Is)[2]	ME1	65	*Verdale de l'Hérault* (Fr)[2]	ME1
15	*Kaissy* (Sy)[1]	ME1	66	*Amellau* (Fr)[3]	ME1
16	*Zaity* (Sy)[1]	ME2	67	*Corniale* (Fr)[1]	ME1
17	*Merhavia* (Is)[3]	ME1	68	*Rougette de Pignan* (Fr)[1]	ME1
18	*Toffahi* (Eg)[3]	ME2	69	*Vermillau* (Fr)[1]	ME1
19	*Oblica* (Yu)[2]	ME1	70	*Verdelé* (Fr)[1]	ME1
20	*Ascolana Tenera* (It)[3]	ME1	71	*Dorée* (Fr)[1]	ME1
21	*Pendolino* (It)[1]	ME1	72	*Pigale* (Fr)[1]	MOM
22	*Frantoio* (It)[1]	ME1	73	*Picholine de Rochefort* (Fr)[2]	ME1
23	*Giarraffa* (It)[3]	ME2	74	*Tanche* (Fr)[2]	ME1
24	*Nocellara del Belice* (It)[3]	ME1	75	*Sauzin* (Fr)[2]	ME1
25	*Dolce Agogia* (It)[1]	ME1	76	*Blanquetier de Nice* (Fr)[1]	MOM
26	*Leccino* (It)[1]	ME1	77	*Grossane* (Fr)[3]	ME1
27	*San Felice* (It)[1]	ME1	78	*Filayre Rouge* (Fr)[1]	ME1
28	*Moraiolo* (It)[1]	ME1	79	*Sabina* (Frc)[1]	MOM
29	*Cassanese* (It)[2]	ME1	80	*Sabina* (Frc)[1]	MOM
30	*Leucocarpa* (It)[1]	ME1	81	*Zinzala* (Frc)[1]	MOM
31	*Zaituna* (Its)[2]	ME1	82	*Capanacce* (Frc)[1]	MOM
32	*Santagatese* (Its)[1]	ME1	83	*Cornicabra* (Sp)[1]	ME1
33	*Tonda Iblea* (Its)[2]	ME1	84	*Lechin de Sevilla* (Sp)[1]	MOM
34	*Biancolilla* (Its)[1]	ME2	85	*Arbequina* (Sp)[1]	ME1
35	*Passalunara* (Its)[2]	ME1	86	*Empeltre* (Sp)[2]	ME1
36	*Moresca* (Its)[2]	ME1	87	*Picual* (Sp)[1]	ME1
37	*Ogliarola Messinese* (Its)[1]	ME1	88	*Villalonga* (Sp)[1]	ME1
38	*Nocellara Etnea* (Its)[3]	ME1	89	*Manzanilla* (Sp)[2]	ME1
39	*Picholine* (Fr)[2]	MOM	90	*Sevillenca* (Sp)[1]	ME1
40	*Lucques* (Fr)[3]	ME1	91	*Galega* (Pt)[2]	ME2
41	*Bouteillan* (Fr)[1]	ME1	92	*Chemlal* (Al)[1]	MCK
42	*Olivière* (Fr)[1]	MCK	93	*Chemlal* (Al)[1]	MCK
43	*Blanquetier d'Antibes* (Fr)[1]	MCK	94	*Chemlal "Mechtrass"* (Al)[1]	ME1
44	*Cailletier* (Fr)[2]	ME1	95	*Azeradj* (Al)[1]	ME1
45	*Cayon* (Fr)[1]	MOM	96	*Taksrit* (Al)[1]	ME1
46	*Salonenque* (Fr)[3]	ME1	97	*Chetoui* (Tu)[1]	ME2
47	*Verdanel* (Fr)[1]	ME1	98	*Zarazi* (Tu)[2]	MCK
48	*Berdaneil* (Fr)[3]	ME1	99	*Meski* (Tu)[3]	ME1
49	*Redouneil* (Fr)[1]	ME1	100	*Barouni* (Tu)[3]	ME1
50	*Négrette* (Fr)[1]	ME1	101	*Chemlali* (Tu)[1]	ME1
51	*Noirette* (Fr)[1]	ME1	102	*Picholine Marocaine* (Mo)[2]	ME1

characterisation of the structure by intra-class correlation coefficients leads to the conclusion of a prevailing proportion of the variability 'between-countries' within a geographic zone.

Typical olive use

Results of the Discriminant Analysis according to the type of use of fruits (oil, canning or both) are presented in Fig. 2B. The coordinates on the two axes, which describe the complete discriminant space, correspond to the three kinds of use and to the individual values of the 96 retained profiles. The first axis separates the profiles of the trees typically used for oil from those only used for canning, in spite of a slight overlap. The profiles of the cultivars with a mixed use were not well separated from those used for canning but were, on the average, intermediate between those for single-uses, as shown by the coordinates of the centroids. On the basis of Newman-

Table 2 Matrix of intra-class correlation coefficients (θ and θ') following a two-way nested ANOVA for five RAPDs and two axes of Correspondence Analysis highly discriminant at the country or region level. θ=a one-marker intra-class correlation coefficient (in bold characters), and θ'=a two-marker intra-class correlation coefficient. * Significant at the 5% level; ** significant at the 1% level ***; significant at the 0.1% level

Item	A1–225	A1–275	A1–1200	A2–650	A10–1250	Axis 1	Axis 2
	Between geographic zones (θ_1 and θ_1')						
A1–225	**0.000**						
A1–275	0.000	**0.105***					
A1–1200	0.000	0.066	**0.003**				
A2–650	0.000	0.090	0.082	**0.035**			
A10–1250	0.000	–0.131	–0.061	–0.139	**0.032**		
Axis 1	0.000	0.086	0.033	0.082	–0.116	**–0.021**	
Axis 2	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
	Between countries within geographic zone (θ_2 and θ_2')						
A1–225	**0.241***						
A1–275	0.248*	**0.188**					
A1–1200	0.262**	0.221*	**0.221***				
A2–650	0.171*	0.219**	0.079	**0.246***			
A10–1250	–0.114	–0.045	–0.160*	0.061	**0.216****		
Axis 1	0.385**	0.347**	0.328	0.244**	–0.126	**0.570*****	
Axis 2	0.032	0.070	0.091	0.079	–0.261*	0.009	**0.668***
	Between geographic zones and between countries within geographic zone (θ_3 and θ_3')						
A1–225	**0.241***						
A1–275	0.235	**0.273****					
A1–1200	0.262**	0.274	**0.214**				
A2–650	0.168	0.294**	0.159	**0.273***			
A10–1250	–0.112	–0.173	–0.218	–0.080	**0.241***		
Axis 1	0.382**	0.411*	0.357*	0.319	–0.239	**0.577*****	
Axis 2	0.032	0.066	0.091	0.078	–0.256	0.009	**0.668***

Table 3 Relationships between RAPD profile groups defined by the geographic zone (country or region) of cultivars. Within each cell: [a] is the Mahalanobis distance between the two modalities of the factor; [b] is the *F* test with 3 and 84 *df*; [c] indicates the significance level (***: significant at the 0.1% level, **: significant at the 1% level, *: significant at the 5% level, and ns: not significant at the 10% level)

Zone	Greece	Turkey	Israel	Contin. Italy	Corsica	Contin. France	Spain	Tunisia	Algeria
Turkey	3.24[a] 2.53[b] ns[c]								
Israel	6.70 5.94 **	4.17 3.02 *							
Continental Italy	0.68 0.86 ns	1.56 1.49 ns	6.50 7.27 ***						
Corsica	30.33 23.69 ***	34.15 22.23 ***	45.24 32.72 ***	25.91 24.73 ***					
Continental France	0.43 0.72 ns	5.45 6.45 ***	10.43 15.09 ***	1.55 4.36 **	29.56 34.98 ***				
Spain	7.94 8.86 ***	17.61 15.28 ***	11.71 11.73 ***	11.85 17.87 ***	40.70 35.33 ***	8.45 18.33 ***			
Tunisia	7.89 7.01 **	6.66 4.82 ns	0.77 0.62 ns	7.84 8.77 ***	40.58 29.35 ***	11.67 16.89 ***	9.30 9.31 ***		
Algeria	20.54 18.23 ***	39.19 28.35 ***	42.98 34.97 ***	25.41 28.43 ***	33.13 23.96 ***	16.45 23.80 ***	13.95 13.98 ***	39.54 32.17 ***	
Sicily	0.81 0.86 ns	4.66 3.85 *	11.52 10.93 ***	1.40 1.95 ns	30.32 25.12 ***	0.33 0.63 ns	12.02 14.60 ***	13.59 12.90 ***	19.73 18.73 ***

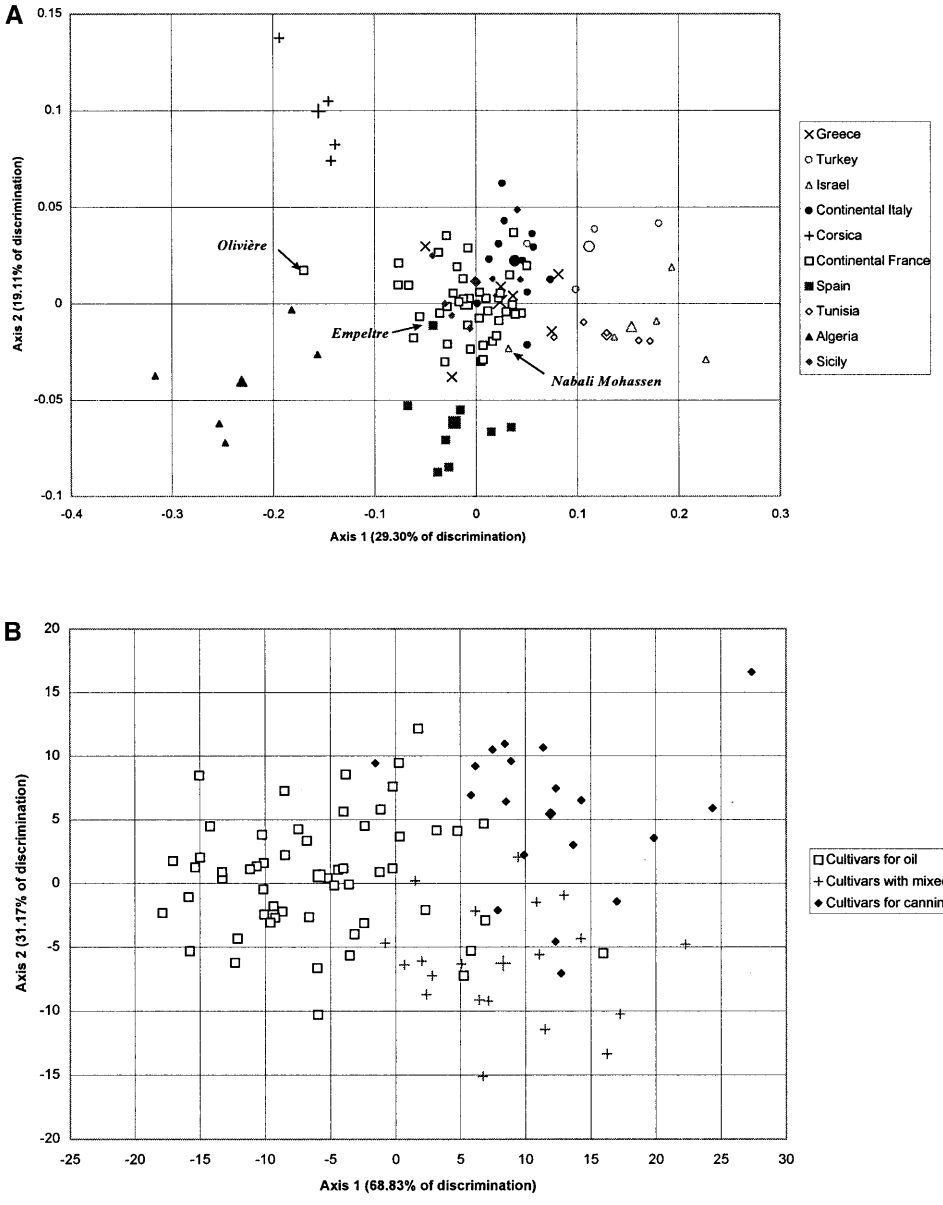

Fig. 2 A Relationship between the RAPD profiles for 96 cultivars, and their ten countries or regions of origin on the first two axes of a Discriminant Analysis on qualitative data. The centroid of a given category is represented by the same symbol as the corresponding individuals, but is bigger. **B** Relationship between the RAPD profiles of 96 cultivars and the three typical uses of their olives (oil, canning or mixed), on the two axes of a Discriminant Analysis on qualitative data. The centroid of a given category is represented by the same symbol as the corresponding individuals, but is bigger

Table 4 Relationships between the RAPD profile groups defined by the typical use of cultivars. Within each cell: [a] is the Mahalanobis distance between the two modalities of the factor; [b] the second value is the *F* test with 2 and 92 *df*; [c] indicates the significance level for all comparisons (***: significant at the 0.1% level). CANN.=group of cultivars used to produce canned fruits; OIL=group of cultivars used to produce oil; and MIXED=group of cultivars both used to produce canned fruits and oil

	OIL	MIXED
MIXED	3.41[a] 24.87[b] ***[c]	
CANN.	4.24 29.76 ***	3.32 15.98 ***

Keuls tests, differences between the centroids of the cultivar profiles for oil and those for canning or mixed uses were significant at the 1% level on the first axis, whereas differences were not significant between the profiles for canning and mixed uses. On the second axis, all the differences are significant at least at the 5% level. Table 4 shows that for the Mahalanobis distances computed on these two axes, the three classes of olive use were significantly different ($P<0.1\%$). Moreover, the hierarchy of these two distances is well in agreement with an intermediate position of the profiles for mixed uses. Furthermore, we can note that the 17 cultivars displaying a Western mitotype are grown to produce oil, except for *Picholine* and *Zarazi* which are used to produce both canned fruits and oil.

Discussion

Origin of cultivated olive

A clustering of profiles from the same region was observed in the dendrogram. For example, the profiles of Andalusian cultivars were all grouped (*Picual*, *Lechin de Sevilla*, *Cornicabra*, *Manzanilla*, and *Picholine Marocaine* called *Canivano Blanco* in Andalusia) suggesting a common genetic basis for these cultivars. FDA revealed also a strong individualisation of the profiles from Algeria, from Israel, from Spain, and from Corsica. This structure of the genetic diversity in comparison with the geographic origin of the cultivars most likely reflects a multilocal selection of the olive in to different genetic pools.

In cultivated olive, the most frequent mitotype is ME1. For wild olive (oleaster) populations, this mitotype is the only one present in the Near East (Besnard and Bervillé 2000). The prevailing occurrence of ME1 within French cultivars resulted therefore from human displacement of genotypes from the Eastern to the Western Mediterranean Basin. Furthermore, the mitotypes MOM and MCK were found only in the Western Mediterranean. This suggests an independent domestication in the Western Mediterranean zone, as argued by Terral and Arnold-Simard (1996), in addition to cultivar creation in the Eastern Mediterranean Basin (Zohary and Hopf 1994). Since the Holocene, and up to the Roman period, human displacement for colonisation has yet been shown to occur from the East Mediterranean to the West Mediterranean countries and this probably explains why the mitotypes MOM and MCK have not been introduced back in the East (Italy, Greece and Near-East).

Mitochondrial DNA and RAPDs (nuclear DNA) led to a different hierarchy of the profiles. This probably reflects the complex origin of the Mediterranean cultivated olive. The mitotypes label four different maternal lineages, which came from different wild populations or taxa (Besnard and Bervillé 2000). Regarding the mitotype, closely related RAPD profiles belong either to the eastern (ME1 or ME2) or to the western (MOM or MCK) group of the Mediterranean Basin (Besnard and Bervillé 2000). For instance, this is the case for the cluster 2 (Fig. 1) between *Leucocarpa* (ME1) and *Capanacce* (MOM), and for the cluster 3 between *Zinzala* (MOM) and *Taksrit* (ME1). Nuclear and cytoplasmic DNAs have therefore most likely been combined due to crosses between trees from different origins.

The non-random association of the nuclear markers at the country or region level may be either: (1) a reflection of multi-allelism at a given locus – in that case, one should expect a negative value of the two-marker intraclass correlations; or (2) of linkage disequilibrium (positive or negative values) due to their common or distinct origins. The five markers related to geographic origin have been shown in wild olive to be correlated with mitotypes (Besnard 1999). Consequently, it is likely than the association between these markers is due to their common origin in a wild population.

The relationship between RAPD profiles and the common utilisation of fruits (oil, canning, or both uses) may be due either to a single origin of varieties with big fruits or to their less-close proximity with wild populations due to a stronger or longer selection towards fruit size. Testing these hypotheses would require a comparison of profiles (RAPDs and mitotypes) between cultivars and wild populations. This integrated work is currently being undertaken (Besnard et al., in preparation). Nevertheless, our results support the proposal that selection in the western genetic pool has been performed mainly to improve oil production.

Conclusion

These profiles exhibited significant relationships with the two following factors: (1) typical use of the fruits, and (2) geographic origin. These relationships suggest that the species domestication, which lasted several thousands years, retained the memory of moves which occurred through the Mediterranean countries. For a better understanding of the mechanisms involved, more research is required, involving a genetic comparison between cultivars and wild populations of eastern and western parts of the Mediterranean Basin. The distribution of cultivar mitotypes has shown that the prevailing displacement of cultivars occurred from East to West (Besnard and Bervillé 2000). But these cultivar transfers did not exclude an original breeding effort within the western countries, which has occasionally led to novel cultivars, such as *Picholine*, *Lechin de Sevilla* and *Zinzala*, from the locally available wild olive trees. Lastly, the non-random association of RAPDs is most likely the reflection of linkage disequilibrium which one might expect after the many ”founder” effects which occurred during the various steps of olive tree domestication in diverse countries.

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References

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

Baradat P, Labbé T (1995) OPEP: un logiciel intégré pour l’amélioration des plantes pérennes. In: Traitements statistiques des essais de sélection. Stratégies de sélection des plantes pérennes. CIRAD-CP, Montpellier, pp 303–330

Besnard G (1999) Etude de la diversité génétique de l’olivier cultivé et de ses formes sauvages apparentées à l’aide de marqueurs moléculaires: applications pour l’identification variétale et pour la gestion des ressources génétiques. Thèse Université Montpellier II, France

Besnard G, Bervillé A (2000) Multiple origins of the Mediterranean Olive deduced from mitochondrial DNA polymorphisms. C R Acad Sci, Série III 323:173–181

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

Besnard G, Baradat P, Bervillé A (2000b) Olive cultivar identification using nuclear RAPDs and mitochondrial RFLPs. (in press) Acta Hort

Bowers J, Boursiquot JM, This P, Chu K, Johansson H, Meredith C (1999) Historical genetics: the parentage of Chardonnay, Gamay and other wine grapes of Northeastern France. Science 285:1562–1565

CISIA (1996) *Logiciel* SPAD, version 3 for Windows. The methods and algorithms used by this software are described in the volume by Lebart et al. (1997) in this reference list

Cuisset C (1998) Étude de la diversité génétique de la vigne (*Vitis vinifera* L.) par les marqueurs morphologiques et moléculaires. Thèse de doctorat de l'ENSA Montpellier, France

Lebart L, Morineau A, Piron M (1997) Statistique exploratoire multidimensionnelle, 2nd edn. Dunod, Paris, France

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

N'Goran JAK, Laurent V, Risterucci AM, Lanaud C (1994) Comparative genetic diversity studies of *Theobroma cacao* L using RFLP and RAPD markers. Heredity 73:589–597

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

Saporta G (1990) Probabilité, analyse des données et statistique. TECHNIP, Paris, France

Shao J, Tu D (1995) The jackknife and the bootstrap. Springer, New York, USA

Stewart CN, Excoffier L (1996) Assessing population genetic structure and variability with RAPD data; application to *Vaccinium macrocarpon* (American Cranberry). J Evol Biol 9:153–171

Terral JF, Arnold-Simard G (1996) Beginnings of olive cultivation in eastern Spain in relation to holocene bioclimatic changes. Quat Res 46:176–185

Ward JHJr (1963) Hierarchical grouping to optimize an objective function. J Am Sat Assoc 58:236–244

Zohary D, Hopf M (1994) Domestication of plants in the Old World, 2nd edn. Clarendon Press, Oxford, UK