Using Multiple Types of Molecular Markers to Understand Olive Phylogeography

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Olive and Its History

The olive *(Olea europaea* ssp. *europaea* var. *europaea)* has played an important role in the Mediterranean Basin for hundreds of years (Figure 11.1). It is not only a key economic crop; the olive's hardiness, hardness, and longevity have come to symbolize values that Mediterranean cultures hold central. The wild form known as oleaster *(Olea europaea* ssp. *europaea* var. *sylvestris)*, with its ability to survive in harsh environments, is also a key component of the countryside across the region.

Olive production is difficult and the harvest is challenging because of drought, frost, biology of flowering, and pruning. As an outcrossed wind-pollinated species, a majority of olive cultivars are self-incompatible, and some are male-sterile (Besnard et al. 2000). Despite these difficulties, olive cropping continues to increase in acreage, largely because of the high potential return to olive growers from the production of olive oil. Although there has been growing competition from seed oils in recent years, olive oil, with its well known health benefits, continues to be a highly valued product both within and outside the Mediterranean Basin. As a result, most olive cultivars are grown for oil (90% of production), a few are grown for table fruit, and some are grown for mixed uses. Oil olives are usually harvested when they overripen, or turn dark red to black. Commercial olive oil labeled "extra virgin" is obtained by crushing fresh olives at about 20° C and then separating the clear oil from the pulp and water. Table olives, which are usually bigger than oil olives, are either canned for extended storage or rapidly preserved with various spices. Green olives are harvested before turning color (before maturation), whereas black and red olives are fully ripe.

Thousands of cultivars have been described based on fruit, pit, and leaf shapes and colors; tree architecture; and phenology (i.e., flowering time). However, some synonymies among olive cultivar names have been revealed with molecular markers (Besnard et al. 2001d). The species *Olea europaea* belongs to a widespread genus that is mostly subtropical or subequatorial in distribution. The Mediterranean olive (subspecies *europaea*) is one of various subspecies, the others of which are distributed in Africa and Asia (Green 2002). Olive trees are known for their frost sensitivity, and in some Mediterranean areas they are periodically destroyed by exceptional frosts (below −15° C). Consequently, the traditional inference has been that the ssp. *europaea* diffused from warmer African regions north into the Mediterranean Basin.

Scenarios regarding the early history of the olive are abundant in the literature. In Greek mythology, the olive was brought to Greece by Athena (Estin and Laporte 1987), its fruit ripening when no other fruits were available during the winter. Botanists once thought that domesticated olive (var. *europaea)* was introduced to the Mediterranean Basin and that self-sustaining plants called oleasters (var. *sylvestris)* were feral forms escaped from the cultivars (Chevalier 1948; Green 2002). Furthermore, historians (e.g., Amoureux 1784) proposed that Phoenicians, Greeks, and Romans not only introduced domesticated olive from the eastern to the western Mediterranean, but that these travelers later brought back locally selected western cultivars to the East.

Feral forms of cultivated olive are able to sustain themselves in woodland areas. Morphologically, they appear similar to the truly wild oleasters. Only the relatively large size of the fruit reveals their origins as cultivar escapes. However, some cultivars have small fruits, which makes it difficult to distinguish their escapes (i.e., feral oleasters) from wild oleasters. Moreover, juvenile trees and those that have been severely pruned or grazed exhibit distinct leaf shapes and tree architectures, which often confound identification.

Olive *(O. e. europaea)* occurs at the northern limit of *Olea* L. taxa, which mostly have tropical or subtropical distributions. Within the genus *Olea*, the subgenus *Olea* is divided into two sections—section *Ligustroides* Benth. & Hook. and section *Olea.* Species belonging to section *Ligustroides* occur in central and southern Africa (Green and Wickens 1989). Section *Olea* contains the *O. europaea* complex, in which six subspecies have been recognized (Green and Wickens 1989; Vargas et al. 2001; Green 2002). Whereas *O. e. europaea* occurs in the Mediterranean region, the other five *O. europaea* subspecies are distributed in Africa and Asia. Some of these subspecies are closely related to olive and therefore have been considered candidates for progenitor to the crop. Those in greatest proximity to the Mediterranean are in northern Africa (Besnard et al. 2002a). They include *O. e. laperrinei* (Batt. & Trab.) Ciferri, which is present in the Saharan mountains (Wickens 1976; Quézel 1978); *O. e. maroccana* (Greut. & Burd.) Vargas et al., which occurs in southwestern Morocco (Médail et al. 2001); *O. e. cerasiformis* (Webb & Berth.) Kunk. & Sund., an endemic in Madeira; and *O. e. guanchica* Vargas et al., an endemic in the Canary Islands (Hess et al. 2000).

According to pollen grain remains (Quézel 1978), a wild *Olea* taxon existed in the Mediterranean Basin at the end of

FIGURE 11.1 Oleasters near Tamanar, Morocco.

the Tertiary Era. During the Quaternary Era, the situation was similar, but glaciating periods greatly disturbed the distribution of the taxon. As early as 19,000 BP, oleaster was present in the eastern Mediterranean (Kislev et al. 1992), where its fruits were gathered, presumably for food. Several botanists and historians think that oleaster occurred in this region much earlier (Zohary and Spiegel-Roy 1975). For example, palynological data suggest the presence of O. europaea in the Near East around 30,000 BP (Van Zeist and Woldring 1980; Neef 1990), although other less thermophilic tree species, such as oak, were absent (Brewer et al. 2002). Domestication of O. europaea in this region occurred by 6000 BP (Galili et al. 1997).

Scientists have traditionally believed that (1) wild oleasters are a homogenous group confined to the eastern Mediterranean; (2) cultivars were derived from a single oleaster source in the Near East and then carried westward to the rest of the Mediterranean Basin; and (3) oleasters in the West are all of feral origin. These hypotheses are rooted in the long-held belief that feral oleasters can be identified simply by morphology. However, we now know that identification of feral oleasters can be confounded by the age of the tree, as well as by the effects of grazing and pruning. Lumaret and Ouazzani (2001) claimed that they could recognize wild oleasters using nuclear markers correlated with domestication traits, and that their results suggested that oleaster was present in the western Mediterranean before the arrival of cultivated olive. Other recent studies (e.g., Terral and Arnold-Simard 1996; Terral 2000; Besnard and Berville 2000; Besnard et al. 2001a, 2001b) have also put some of the traditional hypotheses in question. For example, additional olive domestication sites have been documented based on archaeological remains for Spain (6000 BP) by Terral (2000) and for Corsica (at least 5000 BP) by Magdelaine and Ottaviani (1984).

New Tools for Studying Olive Origins

The utility of molecular tools for evolutionary studies arises from the insensitivity of the genetic markers to environmental factors. Several markers based on DNA amplification technology have been used to explore various independent portions of the olive genome (e.g., Besnard and Berville 2000, 2002; Besnard et al. 2002b, 2002c; Bronzini de Caraffa et al. 2002), including DNA from the nucleus, chloroplast (cpDNA), and mitochondria (mtDNA).

The technique of random amplified polymorphic DNA (RAPD) consists of the amplification of short nuclear DNA fragments (200 base pairs (bp) to 2.5 kb) adjacent to a 10-bp-long primer. Fragments that differ in length within and between samples are identified by the differential mobilities of the fragments (which appear as bands) in an electrified, porous gel. A single primer often produces multiple bands for a single sample. Consequently, these data are treated as band presence/absence characters.

Simple sequence repeats (SSRs) consist of one-, two- or three-bp motifs, repeated a variable number of times (e.g., about ten). Specific repeats are identified by long (ca. 20 bp) flanking primer pairs that delimit a very short DNA fragment (100–250 bp). Again, fragment length polymorphisms among samples are identified by their mobilities on a gel. However, given the specificity of the long primers, usually only a single region within a genome is amplified. Thus, in the case of nuclear SSRs in a diploid taxon, the single- or double-banded patterns are interpreted as one or two alleles at a single locus for homozygous and heterozygous individuals, respectively.

As with SSRs, restriction fragment length polymorphisms (RFLPs) are typically used to compare DNA fragment length polymorphisms in organelles (e.g., chloroplasts or mitochondria). Although the RFLP flanking sequences (i.e., hybridization probes) are much longer than SSR primer sequences, and are therefore quite specific, the procedure is more costly than RAPDs or SSRs. The RFLP data from multiple probe and restriction enzyme combinations can be combined to recognize distinct genetic patterns, called chlorotypes or mitotypes. Cytoplasmic markers such as mitochondrial RFLPs and chloroplastic SSRs trace maternal lineages only, since these organelles are usually passed to offspring from the female parent.

In this chapter we examine nuclear, chloroplastic, and mitochondrial DNA-based molecular diversity of populations and cultivars of O. e. europaea from around the Mediterranean Basin. With these data, we address questions concerning the early distribution of oleaster and the origins and spread of domesticated olive.

Materials and Methods

Plant Material

From the Mediterranean Basin, 235 trees representing 27 oleaster populations were sampled for molecular markers (Table 11.1). Population size varied from 5 to 22 individuals per population, except for Corsica, where it varied from 1 to 10. For geographic analyses, populations were scored as "East" or "West" (Table 11.1). The north-south dividing line between these Mediterranean Basin regions follows natural

TABLE II.I

List of Oleaster Populations Analyzed, Their Locations, the Markers Tested, and the Mitotype Results

Pop No.	Population Location[a]	Country	RAPD[b]	SSR[b]	Mitotypes[c]
1	Urla, Izmir*	Turkey	+	+	7 ME1
2	Harem, Oronte Valley*	Syria	+	+	13 ME1
3	Al Ascharinah, El Ghab*	Syria	+		12 ME1
4	Mont Carmel, Haifa*	Israel	+	+	18 ME1
5	Cyrenaique	Libya	+	+	4 ME1; 11 MOM
6	Zaghouan	Tunisia	+	+	6 MCK; 1 MOM
7	Mont Belloua, Kabylia	Algeria	+	+	6 MCK; 1 MOM
8	Tamanar, Essaouira	Morocco	+	+	10 MOM
9	Immouzzer, High Atlas	Morocco	+		3 MCK; 2 MOM
10	Torviczon, Andalucia	Spain	+	+	4 ME1; 13 MOM
11	Asturias	Spain	+		3 MCK; 2ME1; 2 MOM
12	Cap des Mèdes, Porquerolles, Var	France	+		1 MCK; 3 ME1; 18 MOM
13	La Repentence, Porquerolles, Var	France	+		1 MCK; 10 MOM
14	Mont Boron, Nice, Alpes Maritimes	France	+	+	4 MCK; 2 ME1; 16 MOM
15	Ostricone, Corsica	France	+	+	2 MCK; 9 MOM
16	Ogliastro, Corsica	France	+	+	10 ME1
17	Filitosa, Corsica	France	+	+	2 MCK; 9 MOM
18	Bonifacio, Corsica	France	+	+	5 MOM
19	Messine, Sicily	Italy	+	+	17 MOM
20	Ali, Sicily	Italy	+	+	2 ME1; 10 MOM
21	Corte, Corsica	France		+	8 ME1
22	Sarrola Carcopino, Corsica	France		+	5 ME1; 5 MOM
23	Lama, Corsica	France		+	3 ME1; 7 MOM
24	Oletta, Corsica	France	+	+	1 MOM
25	Casta, Corsica	France	+	+	1 MOM
26	Pigna, Corsica	France	+	+	1 MOM
27	Ogliastrello 2, Corsica	France	+	+	1 MOM

[a] Locations with an asterisk are from the eastern Mediterranean Basin.
[b] All populations were tested for mitotypes; populations tested for RAPDs or SSRs are marked with a "+" in the appropriate column.
[c] Number of individuals with each mitotype (MCK, ME1, MOM).

geographic barriers to geneflow, namely the Adriatic Sea and the Libyan and Egyptian deserts.

Reference cultivars (#1–102 in Table 11.2) and 19 cultivated forms of undetermined cultivar affiliation (#103–121) that had been initially sampled for RAPD variation (Besnard et al. 2001b) were included in this study. Cultivars were chosen to represent maximum diversity in olive. We added to the dataset additional domesticated types from Corsica (#122–127) because of the potential importance of this island in early human migrations. One or more individuals were tested per cultivar. In all, 122 cultivars were tested for nuclear RAPDs, 40 were tested for chloroplastic SSRs, and 127 were tested for mitotypes (Table11.2). For geographic analyses, cultivars were assigned to country or region based on the origin of the collection or as inferred from the cultivar's name (Table 11.2).

One to two samples each of three other *Olea europaea* subspecies (ssp. *laperrinei, maroccana,* and *guanchica*) were also examined to help clarify the origins of ssp. *europaea*.

Molecular Data

Total DNA preparation was performed as previously described in Besnard and Bervillé (2000). The RAPD amplification and electrophoresis procedures are described by Quillet et al. (1995). Eight RAPD primers were used: A1, A2, A9, A10, C9, C15, E15, and O8. Chloroplast SSR procedures followed those described previously by the authors (Besnard et al. 2002c), with primers (OeUA-DCA 01, 03–09, 11, 13–15, and 18) used according to Sefc et al. (2000). Individuals were characterized for mitochondrial DNA polymorphism using the RFLP methods described in Besnard and Bervillé (2000).

Data Analyses

In this chapter we present the geographic distributions of mitotypes and a discussion of a previously published dendrogram based on the RAPD data (Besnard et al. 2001a, 2001d). More detailed analyses of the mitotype and RAPD datasets are given in Besnard et al. (2001b, 2001c, 2001d) and Besnard and Bervillé (2000).

TABLE II.2
List of the Cultivars Studied[a]

#	Cultivar or Landrace Name	RAPD	Mt DNA	SSR	#	Cultivar or Landrace Name	RAPD	Mt DNA	SSR	#	Cultivar or Landrace Name	RAPD	Mt DNA	SSR
1	Kalamon	+	+		44	Cailletier	+	+	+	87	Picual	+	+	+
2	Vallanolia	+	+		45	Cayon	+	+		88	Villalonga	+	+	
3	Gaïdourolia	+	+		46	Salonenque	+	+		89	Manzanilla	+	+	
4	Koroneiki	+	+		47	Verdanel	+	+		90	Sevillenca	+	+	
5	Carolia	+	+		48	Poumal	+	+		91	Galega	+	+	
6	Amygdalolia	+	+		49	Redouneil	+	+		92	Chemlal (Cordoba)	+	+	+
7	Uslu	+	+		50	Négrette	+	+	+	93	Chemlal	+	+	
8	Domat	+	+		51	Noirette	+	+		94	Chemlal Mechtrass	+	+	
9	Ayvalik	+	+		52	Grapié	+	+		95	Azeradj	+	+	
10	Sofralik	+	+		53	Aglandau	+	+		96	Taksrit	+	+	
11	Souri	+	+	+	54	Celounen	+	+		97	Chetoui	+	+	
12	Souri Mansi	+	+		55	Courbeil	+	+		98	Zarazi	+	+	+
13	Nabali Mohassen	+	+		56	Coucourelle	+	+	+	99	Meski	+	+	
14	Barnea	+	+		57	Cayet Rouge	+	+	+	100	Barouni	+	+	
15	Kaissy	+	+		58	Rascasset	+	+	+	101	Chemlali	+	+	
16	Zaity	+	+	+	59	Malaussena	+	+		102	Picholine Marocaine	+	+	+
17	Merhavia	+	+		60	Aubenc	+	+		103	Palmyre 1 (Syria)	+	+	
18	Toffahi	+	+		61	Reymet	+	+		104	Palmyre 2 (Syria)	+	+	
19	Oblica	+	+		62	Curnet	+	+	+	105	Palmyre 3 (Syria)	+	+	
20	Ascolana Tenera	+	+	+	63	Colombale	+	+		106	Palmyre 4 (Syria)	+	+	
21	Pendolino	+	+		64	Poulo	+	+		107	Palmyre 5 (Syria)	+	+	
22	Frantoio	+	+	+	65	Verdale de l'Hérault	+	+		108	Palmyre 6 (Syria)	+	+	
23	Giarraffa	+	+	+	66	Amellau	+	+	+	109	Palmyre 7 (Syria)	+	+	
24	Nocellara del Belice	+	+		67	Corniale	+	+	+	110	Palmyre 8 (Syria)	+	+	
25	Dolce Agogia	+	+	+	68	Rougette de Pignan	+	+		111	Palmyre 9 (Syria)	+	+	
26	Leccino	+	+	+	69	Vermillau	+	+		112	Derkouch 1 (Syria)	+	+	
27	San Felice	+	+		70	Verdelé	+	+		113	Derkouch 2 (Syria)	+	+	
28	Moraiolo	+	+	+	71	Dorée	+	+		114	Derkouch 3 (Syria)	+	+	
29	Cassanese	+	+	+	72	Pigale	+	+		115	Beth Hakerem (Israel)	+	+	
30	Leucocarpa	+	+		73	Picholine Rochefort	+	+		116	Alaon 1 (Spain)	+	+	
31	Zaituna	+	+		74	Tanche	+	+		117	Alaon 2 (Spain)	+	+	
32	Santagatese	+	+		75	Sauzin	+	+	+	118	Montpellier 1 (France)	+	+	
33	Tonda Iblea	+	+	+	76	Blanquetier de Nice	+	+		119	Monptellier 2 (France)	+	+	
34	Biancolilla	+			77	Grossane	+	+	+	120	Monptellier 3 (France)	+	+	
35	Passalunara	+			78	Filayre Rouge	+	+	+	121	Montpellier 4 (France)	+	+	
36	Moresca	+	+	+	79	Sabina (St Giuliano)	+	+	+	122	Corse S (France)		+	+
37	Ogliarola Messinese	+	+		80	Sabina 300	+	+	+	123	Corse G (France)		+	+
38	Nocellara Etnea	+	+	+	81	Zinzala 302	+	+	+	124	Zinzala Sud (France)		+	+
39	Picholine	+	+	+	82	Capanacce	+	+	+	125	Filitosa 12 (France)		+	+
40	Lucques	+			83	Cornicabra	+	+	+	126	Bonifacio 9 (France)		+	+
41	Bouteillan	+			84	Lechin de Sevilla	+	+		127	Bonifacio (France)		+	+
42	Olivière	+	+	+	85	Arbequina	+	+						
43	Blanquetier d'Antibes	+	+		86	Empeltre	+	+						

[a] Country or region of origin: Greece (#1–6), Turkey (7–10), Near East (11–17, 103–115), Egypt (18), Yugoslavia (19), Italy (20–38), continental France (39–78, 118–121), Corsica (79–82, 122–127), Spain (83–90, 116–117), Portugal (91), Algeria (92–96), Tunisia (97–101), Morocco (102). Cultivated forms with unknown names (#103–121) are named with reference to the original locality from which they were collected. A "+" in a column means that the cultivar was tested with the corresponding marker.
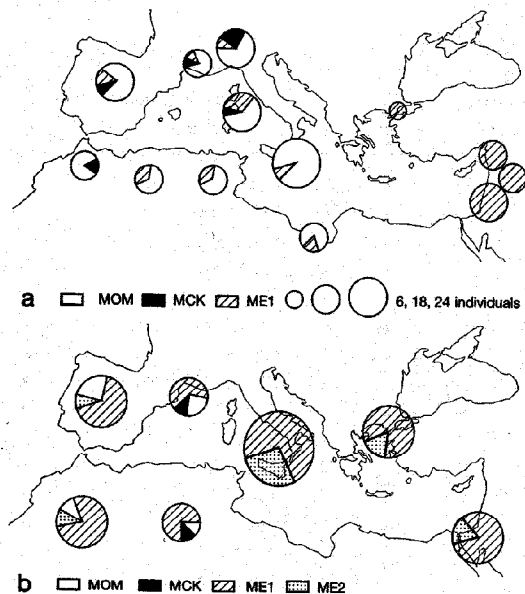
FIGURE 11.2 Map of oleaster and cultivar mitotypes in the Mediterranean Basin. (a) Distribution of mitotypes in oleasters. From small to large, the three circle sizes represent 6, 18, and 24 sampled individuals, respectively. (b) Regional distribution of mitotypes in cultivars.

From the SSR dataset, the centroid values of Nei's genetic distances (Nei 1978) were used to create a dendrogram of oleaster populations. Clustering was performed using Ward's algorithm (Ward 1963). Other analyses of the SSR data included multiple correspondence analyses (MCA), which were performed on two subsets of the SSR dataset—all individuals and the cultivars only.

Results

Oleasters

Distinct mitotypes were revealed in ssp. *laperrinei* (mitotype = ME1), ssp. *guanchica* (MCE), and ssp. *maroccana* (MMA). Three mitotypes were found in oleaster (ssp. *europaea* var. *sylvestris*)—MCK, ME1, and MOM. ME1 was found in all eastern Mediterranean Basin populations and in about half of the western populations (Figure 11.2a; Table 11.1). MOM and MCK were found exclusively in the West, specifically in northwestern Africa and western Europe. Eastern oleaster populations typically appeared homogeneous, possessing only ME1. In contrast, western populations were often heterogeneous. Most western populations possessed MOM; almost half of those populations also possessed MCK; and three populations (#11, 12, and 14) possessed all three mitotypes (Figure 11.2a; Table 11.1). Among the 13

Type of Marker	Oleasters				
	Number Observed	Total	Specific to East[a]	Specific to West[a]	Cultivars
Mitotypes[b]	4	3	1	2	4
RAPD Bands[c]	57	57	6	12	45
SSR Alleles[d]	173	167	12	33	99

[a] "West" and "East" refer to the Mediterranean Basin.
[b] Besnard et al. (2002b).
[c] Besnard et al. (2001a, 2001c).
[d] Breton & Bervillé unpublished data.

populations from Corsica and Sicily, 11 possessed MOM, 2 possessed MCK, and 5 possessed ME1 (Table 11.1).

RAPDs and SSRs also revealed an "East vs. West" structure in oleaster diversity. We found a gradient between the east and the west in the frequencies of some RAPD and SSR markers (Besnard et al. 2001b; Table 11.3). Also, some markers were unique to the east, while others were unique to the west (Table 11.3).

In the SSR-based dendrogram of oleaster populations, we obtained three main groupings corresponding to the eastern Mediterranean, the western Mediterranean, and Sicily and Corsica (Figure 11.3). In the plot produced by the MCA of all individuals (cultivars and oleasters), we found that eastern (e.g., #1, from Turkey) and centrally located (#5, from Libya) populations clustered near the top and right side of the plot (Figures 11.4a, c, and e); Sicilian (#20), Corsican (#17), and western populations clustered on the left side of the plot (Figures 11.4b, d, and e); and the cultivars clustered primarily in the lower right corner of the plot (Figure 11.4e).

Olive Cultivars

In cultivars, four mitotypes were revealed, including ME2, which was not found in oleaster (Figure 11.2b). Most of the cultivars possessed ME1 (74%), whereas 10%, 8%, and 8% possessed ME2, MOM, and MCK, respectively. Correlation between geography and genetic structure appeared weaker for cultivars than it did for oleasters. For example, ME1 was found in cultivars from throughout the Mediterranean Basin. However, most cultivars containing MOM and MCK were from western regions (Figure 11.2b), whereas ME2 was most frequent in eastern countries (Besnard et al. 2001d).

A subset of the RAPD bands found in oleasters was found in olive cultivars (Table 11.3). A previously constructed dendrogram based on RAPD data placed cultivars into 24 small groups within two major groupings (Figure 2B in Besnard et al. 2001d). Although the major groupings did not
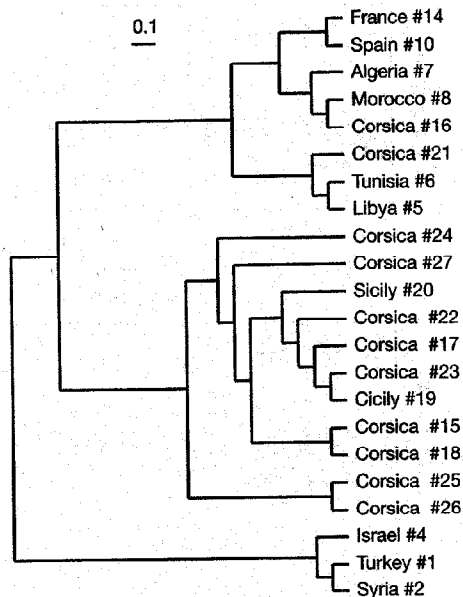
```
        0.1                    ┌─ France #14
         ─                   ┌─┤
                           ┌─┤ └─ Spain #10
                           │ │
                           │ └─── Algeria #7
                         ┌─┤     ┌─ Morocco #8
                         │ │   ┌─┤
                         │ └───┤ └─ Corsica #16
                       ┌─┤     └─── Corsica #21
                       │ │       ┌─ Tunisia #6
                       │ └───────┤
                       │         └─ Libya #5
                     ┌─┤     ┌─────── Corsica #24
                     │ │   ┌─┤
                     │ │   │ └─────── Corsica #27
                     │ └───┤     ┌─── Sicily #20
                     │     │   ┌─┤
                     │     │   │ └─┬─ Corsica #22
                     │     └───┤   └─ Corsica #17
                     │         │   ┌─ Corsica #23
                     │         └─┬─┤
                     │           │ └─ Cicily #19
                     │           │ ┌─ Corsica #15
                     │           ├─┤
                     │           │ └─ Corsica #18
                     │           │ ┌─ Corsica #25
                     │           └─┤
                     │             └─ Corsica #26
                     │           ┌─── Israel #4
                     └───────────┤ ┌─ Turkey #1
                                 └─┤
                                   └─ Syria #2
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FIGURE 11.3 Dendrogram based on the SSR dataset for oleasters. Population codes are in Table 11.1. The bar indicates scale.

have a clear geographic structure, the smaller groups corresponded partially to country or region of origin. The larger of the two major groupings contained cultivars from all countries and included all four mitotypes. The minor group, which also included all four mitotypes, consisted primarily of cultivars from the West, including Corsican cultivars such as "Sabina" (#79 and 80) and "Zinzala" (#81). The only cultivar in this group presumed to be from the East was "Koroneiki" (#4) from the island of Crete, in Greece. However, a cultivar's name may not be an accurate indicator of the cultivar's region of origin.

The SSR-based MCA plot of cultivars placed all cultivars, except for most of those from Corsica, together on the left side of the plot (Figure 11.4f).

Comparison of Oleaster and Olive

Genetic diversity with respect to RAPDs and SSRs was found to be higher in oleasters than in the cultivars (Table 11.3). However, the cultivars possessed all three oleaster mitotypes, plus a unique mitotype: ME2.

Looking across both cultivars and oleasters, we see that ME1 and ME2 are characteristic of the eastern Mediterranean, and MOM and MCK are essentially western mitotypes (Figures 11.2a and b). Some RAPD and SSR markers are also region-specific (Table 11.3). For example, C15-1200 is an eastern-specific RAPD band, whereas A1-1080 is a western-specific RAPD band. Some cultivars possess western-specific oleaster RAPD bands as well as western-specific mitotypes, suggesting

that these cultivars were selected directly from oleasters in the western Mediterranean.

In the SSR-based MCA plot of all individuals, most cultivars were separated from oleasters, the former being restricted to the lower right corner of the plot (Figure 11.4e). Thus, the SSR alleles are fairly good indicators of domestication status. However, many of those Corsican cultivars that clustered distinctly in Figure 11.4f clustered among the western oleasters in Figure 11.4e. These cultivars include "Zinzala" (#81 and 124) and "Sabina" (#80), which also share uncommon western-type RAPD and SSR markers, as well as MOM, with two oleaster populations (#25 and 26). Thus, these cultivars may have been directly selected from these populations. Other oleaster trees that clustered near to cultivars in Figure 11.4e (in the lower right corner) may represent escapes from cultivation. Such oleasters typically possessed eastern-specific RAPD and mitotype markers, as well as SSR alleles typical of the cultivars.

Discussion: Molecular Diversity

The Ancestor(s) of Oleaster

In addition to the SSR data presented here, ongoing research on chloroplastic genetic variation has recently included the RFLP technique, which produced chlorotype data (Besnard et al. 2002c) similar to the mitotype data described in this chapter. The chlorotype data indicate that *O. e. europaea* possesses chlorotypes from two distinct lineages, and therefore one of the lineages may be of hybrid origin. One maternal lineage is probably *O. e. laperrinei*, which not only shares a chlorotype with ssp. *europaea*, but also shares the mitotype ME1.

The oleaster mitotypes MOM and MCK were not found in the other subspecies. However, ssp. *maroccana* possessed MMA, which is similar to MCK; perhaps the latter mitotype evolved from the former (Médail et al. 2001). The origin of MOM is still unclear. It could have evolved within oleaster or it could have been transferred from another, possibly lost, infraspecific taxon of *Olea europaea*.

Oleaster Diversity

Genetic diversity in oleaster, as indicated by RAPDs, SSRs, and mitotypes, appears highly correlated with geography, with a clear differentiation between populations in the eastern Mediterranean and those in the West. This disjunction suggests that oleasters survived in separate refugia during Quaternary glaciations. Three of these refugia are characterized by specific mitotypes: (1) Northwestern Africa, where MCK was preserved; (2) The western Mediterranean, where MOM was preserved; and (3) the Near East, where ME1 was preserved. The relatively ubiquitous ME1 may have been preserved in one or more of the western refugia as well. RAPD and SSR data indicate that there could be a fourth refuge zone encompassing Corsica and Sicily, as discussed in the next section. RAPD variation suggests that
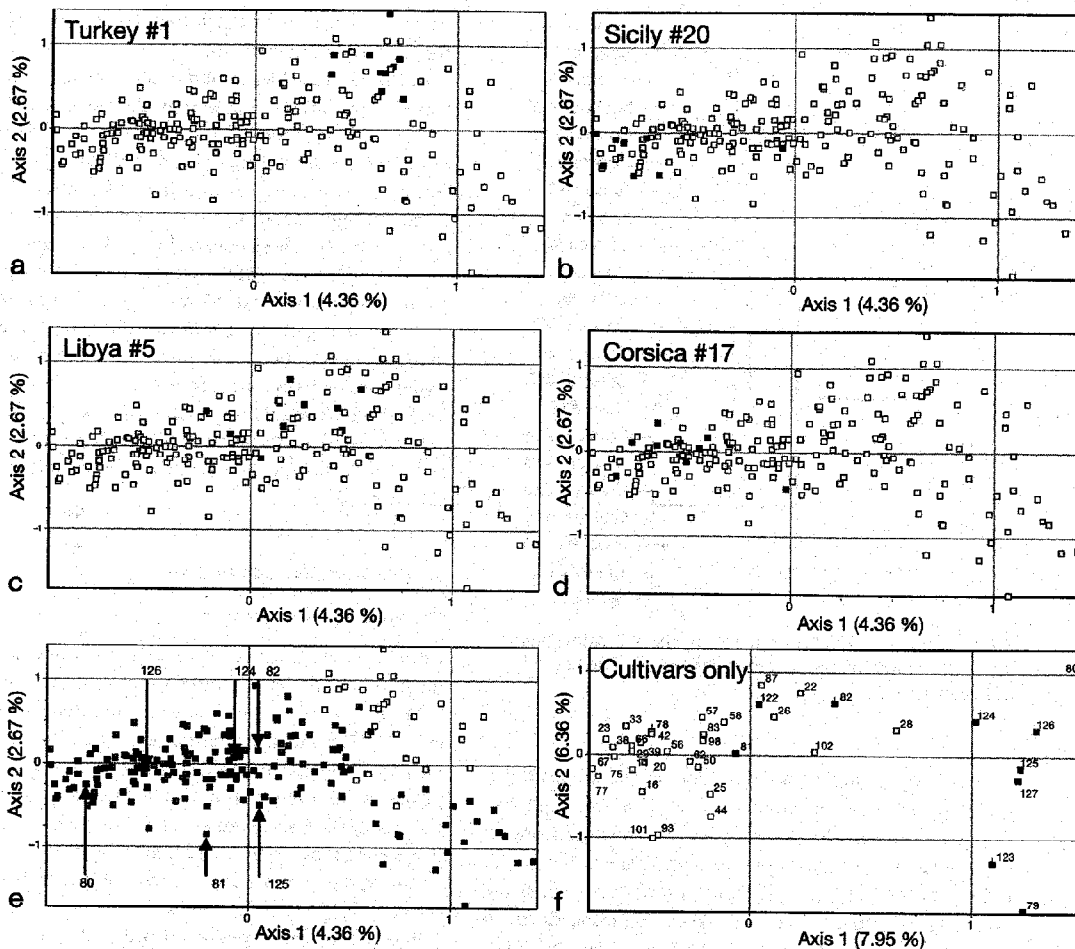
FIGURE 11.4 Results from multiple correspondence analyses based on SSR data. Analysis of 214 individuals representing oleaster populations and olive cultivars. (a) Oleaster population #1 (from Turkey), highlighted in black. (b) Oleaster population #20 (Sicily), highlighted in black. (c) Oleaster population #5 (Libya), highlighted in black. (d) Oleaster population #17 (Corsica), highlighted in black. (e) Western oleasters in gray, eastern oleasters in white, and cultivars in black. Arrows indicate the position of the Corsican cultivars, referenced by the cultivar codes in Table 11.2. (f) Cultivars only. Corsican cultivars are shown in black. The numbers correspond to the cultivar codes in Table 11.2.

only weak geneflow has occurred among these refugia since the glaciations.

Genetic diversity is higher in the West than in the East, suggesting that oleaster diversified in the West. We believe the data best support the hypothesis that oleaster arose and diversified in southern Morocco, where it could have been in contact with ssp. *maroccana* (and hence received a MMA/MCK mitotype relative), and later migrated eastward, with only the ME1 mitotype arriving in the Near East (Figure 11.5a).

Corsican Oleasters and Cultivars

Near the end of the Quaternary glaciations, Corsica was in contact with continental Italy, Sardinia, and Sicily (Vigne 1992). Sicily has been proposed as a refuge for other tree species during this period (Quézel 1995). Migration of endemic oleasters from Sicily to Corsica could have occurred early during Quaternary recolonization, when sea levels were still low (Taberlet at al. 1998). Our RAPD and SSR data indicate distinct genetic profiles for and close relationships among some Corsican oleasters and cultivars (e.g., "Sabina" and "Zinzala"). A recent RAPD study performed by Bronzini de Caraffa et al. (2002) on a larger sample of wild populations from Corsica and Sicily revealed a similar association among oleasters and cultivars of this region. Consequently, it appears that some olive cultivars may have been selected directly from ancient oleasters indigenous to these islands. In fact, this region may have been a separate center of olive domestication, as suggested by archaeological evidence of olive domestication by 5000 BP in Corsica (Magdelaine and Ottaviani 1984).
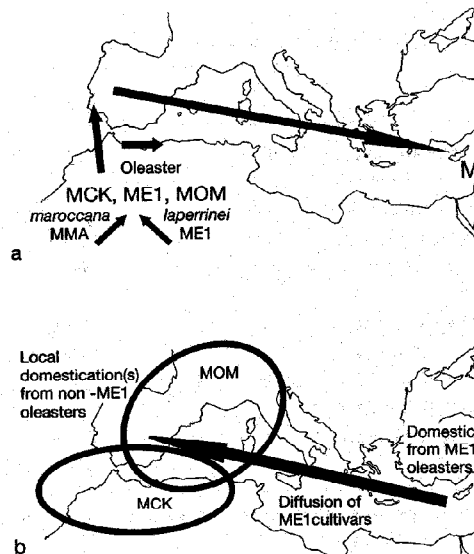
FIGURE 11.5 Maps of possible routes followed by oleasters and cultivars in the Mediterranean Basin. (a) Possible routes (arrows) followed by oleaster mitotypes from the West to the eastern Mediterranean Basin. (b) Westward diffusion of cultivars from the primary center of olive domestication in the East.

The genetic distinctiveness of oleasters in Corsica was probably enhanced by the isolation of the populations on an island (Bronzini de Caraffa et al. 2002). In time, this isolation would have been diminished by human migrations as cultivars were exchanged throughout the Mediterranean. Historically, Corsica has been invaded by Phoenician, Roman, Genoese, Italian, and French peoples. Thus, its importance as a migratory crossroads as well as its intermediate position in the Mediterranean Basin could account for the high levels of genetic diversity observed on this island (Bronzini de Caraffa et al. 2002).

Cultivar Diversity

Most cultivars are clearly of eastern oleaster origin, possessing eastern-type RAPDs and mitotypes. These cultivars occurred in the larger of two branches in the dendrogram based on the RAPD data. The smaller branch contained some Corsican cultivars that were also revealed as distinct by the SSR data (Figure 11.4f). The genetic profile of these cultivars—with oleaster-type SSRs and western-type RAPDs and mitotypes—suggests that they were selected directly from western oleasters.

Although cultivars possessing only western-type RAPDs and mitotypes were most likely selected from western oleasters, cultivars with western-type RAPDs, but also containing the ubiquitous ME1, were probably also selected in the West. ME1 could have been preserved in western as well as eastern oleaster refugia during the Quaternary. Or, the presence of

ME1 in western cultivars could be the result of geneflow with eastern cultivars that were carried by humans from the eastern Mediterranean to the West (Figure 11.5b).

The Advantage of Using Multiple Molecular Markers for Studying Domestication

Not all genetic markers tell the same story. Nuclear DNA, which exists as two copies contributed to progeny by both parents, undergoes recombination. In contrast, the cytoplasmic genomes (i.e., of chloroplasts and mitochondria), which are passed on to progeny by a single parent (usually the maternal parent), do not experience this type of gene shuffling. Nuclear DNA markers, such as the RAPDs examined in this study, often exhibit more variation at a particular taxon level than do cytoplasmic markers (e.g., chloroplastic SSRs and mitotypes).

Also, there are differences in specificity for the various marker types. Of the marker types used in this study, RAPD primers were the least specific in targeting DNA sequences, whereas the primers producing the RFLP-based mitotypes were the most specific. This means that one would expect to find less variation in a taxon for the mitotypes than for the SSRs or RAPDs.

The different rates of evolution for these various markers in the three genomes allow us to reconstruct an evolutionary history for a taxon at multiple levels—for example, between the taxon and its closest relatives, and within the taxon at various levels. For olive (O. e. europaea), we were able to distinguish: (1) most cultivated (var. europaea) from wild (var. sylvestris) germplasm with SSRs; (2) a geographic divergence of East vs. West within the subspecies with RAPDs, and to some extent mitotypes; and (3) the relationships with other subspecies and the geographic origins of ssp. europaea with mitotypes. Consequently, we could piece together a history for olive that would not have been possible with just one type of marker.

Also, with the combined data we can now more clearly distinguish wild from feral oleasters, the latter typically lacking oleaster-type SSR and RAPD markers (Table 11.4). The feral forms include escapes from cultivation, as well as hybrids between cultivars and wild oleasters.

Summary

Oleasters originated and spread in the western Mediterranean, becoming more diversified (e.g., obtaining MOM and a MMA/MCK relative) as a result of contact with other subspecies (e.g., O. e. laperrinei and O. e. maroccana). From a western center of diversity, one lineage of oleasters, which possessed ME1, spread to the Near East. Thus, by the time of the Quaternary glaciations, several mitotypes were scattered throughout the Mediterranean Basin. Some of these genetically differentiated populations survived in refugia until the end of the glaciations. Subsequent expansion of oleasters was probably limited by natural geographic barriers (e.g.,

TABLE II.4
Key for Prediction of the Wild versus Feral Status of Any Oleaster Tree[a]

Information on the Tree			Predicted Status	Observed
Location	Mitotype	RAPD Profile and Specific Markers	Wild/Feral	Yes/No
East	ME1	East, with oleaster-specific markers	Wild	Yes
East	ME1	East, no oleaster-specific markers	Feral[b]	Yes
East	ME1	East-West, with or without oleaster-specific markers	Feral[c]	Yes
East	ME2	—	—	No
West	MOM,MCK	West, with oleaster-specific markers	Wild	Yes
West	MOM,MCK	West, no oleaster-specific markers	Feral[d]	Yes
West	MOM,MCK	East-West, with or without oleaster-specific markers	Feral[c]	Yes
West	ME1	East, no oleaster-specific markers	Feral[b]	Yes
West	ME1	West, with oleaster-specific markers	Wild	Yes
West	ME2	—	—	No

[a] Prediction based on the location of the tree, its mitotype, its RAPD profile (East vs. West), and its specific SSR and RAPD markers (i.e., whether or not it possesses markers specific to oleasters).
[b] Escaped from eastern cultivars.
[c] Hybrids between cultivars and oleasters.
[d] Escaped from western cultivars.

the Adriatic Sea, and the Libyan and Egyptian deserts), which served to maintain a strong, geographically based genetic structure in oleasters until human migrations.

Olive domestication occurred in the Near East, where it is well documented by 6000 BP (Zohary and Spiegel-Roy 1975). The first cultivars, which possessed ME1, were carried by humans to western portions of the Mediterranean Basin, where geneflow increased the diversity in both western oleasters and in western cultivar selections. Another source of genetic diversity in cultivars may stem from additional centers of olive domestication from genetically differentiated oleasters. Recently, archaeological evidence has been found of olive domestication by 6000 BP in Spain (Terral 2000) and by 5000 BP in Corsica (Magdelaine and Ottaviani 1984). Consequently, we think that at least two domestications occurred more or less simultaneously on both sides of the Mediterranean Basin.

The mitochondrial DNA polymorphisms, in combination with chloroplast SSRs and nuclear RAPDs, were an excellent set of molecular markers with which to unravel olive phylogeography.

Future Research Needs

To determine exactly when and where olive cultivars originated and spread in the Mediterranean Basin will require combined data from archaeology, genetics, history, and palynology. The present diversity and distribution of cultivars have undoubtedly been perturbed by the movement of seeds and cuttings by humans who practiced coastal trade around the Mediterranean Sea during both prehistoric and historic times. Knowing cultivar age is important for establishing genealogical relationships among these cultivars. We plan, therefore, to establish a chronology of mitotype movements,

using seeds found in archaeological sites with known dates. Such seeds are usually found charred or in amphora, conditions leading to highly altered nuclear DNA that is difficult to amplify. However, we believe that mitochondrial DNA, which is much more abundant in remains than nuclear DNA, could probably be amplified and compared.

Various herbarium samples of olive have been collected during the two last centuries. Some of those from eastern Africa were collected by Italian, English, and French botanists, who each named their specimens independently. Consequently, a review of these specimens could reveal cultivar synonymies. Fruit color for many specimens may have faded, however, making these determinations more difficult (Green 2002).

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