Chapter 10
Genetic Diversity and Conservation of Olive Genetic Resources

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Abstract The olive (*Olea europaea* subsp. *europaea*) is indigenous to the Mediterranean Basin and is the most economically important oil tree crop in temperate areas. Olive cultivars (*Olea europaea* subsp. *europaea* var. *sativa*) have been empirically selected and vegetatively propagated in all the traditional olive-growing countries. However, the domestication history of the olive and its relationship with its wild ancestor (*Olea europaea* subsp. *europaea* var. *sylvestris*) remain puzzling. The knowledge of the relationship between cultivars and wild olives is critically important for conservation purposes, for breeding programs, for the design of genome association studies, and to untangle the population history. In this chapter, we examine the characterization of olive genetic resources (wild and cultivated) in the main olive-growing regions of Spain using microsatellite (SSR) markers. We observed significant differentiation between the cultivars from south and northeast Spain, which possibly indicate independent selection processes. In addition, our results revealed differential relationships and admixture

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events between the wild and cultivated olives depending on their region of origin. Finally, we describe how the new olive-growing systems, which are more intensive and mechanically harvested, are leading to a reduction in the number of cultivars used in new plantations. Coordinated efforts involving the application of ex situ and in situ conservation approaches are needed to evaluate and preserve the wealth of genetic legacy present in both the wild and cultivated olive. These actions are urgent, given the rapid expansion of new olive plantations and the severe effects of climate change that are predicted for the Mediterranean Basin.

Keywords *Olea europaea* L. · Domestication · Traditional cultivars · Microsatellites · Genetic erosion · Germplasm banks

10.1 Introduction

The olive tree (*Olea europaea* subsp. *europaea*) and its main products, oil and table olives, are deeply rooted in the history of Mediterranean societies due to their economic and cultural importance. Since ancient times, commercial shipping has extended olive-growing westward across the Mediterranean Basin. The olive remains an important species worldwide because it is the most economically important oil tree crop in temperate areas, with 10.2 million ha under cultivation (FAO 2012). Reflecting the historical importance of olive cultivation in the Mediterranean Basin, the leading producers of olives are Spain, Italy and Greece (Vossen 2007). However, olive is also a crop that is increasingly being cultivated in Argentina, Australia, Chile, China, and the United States (FAO 2012).

Cultivated olive (*Olea europaea* subsp. *europaea* var. *sativa*) consists of a broad diversity of clonally propagated cultivars (Fig. 10.1) (Rallo 2005; Haouane et al. 2011; Trujillo et al. 2013). Olive cultivars often grow near its wild ancestor (*Olea europaea* subsp. *europaea* var. *sylvestris*), called "oleaster," which is indigenous to most areas of the humid and subhumid thermo-Mediterranean with low occurrences of frost (Rivas-Martinez and Gandullo 1987; Carrión et al. 2010).

Despite the close geographic link between cultivated and wild olives, the genetic relationship between the two is somewhat puzzling. According to archeological remains, olive was first grown in the eastern Mediterranean Basin—approximately 6000 years ago (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). The analysis of chloroplast DNA also indicated the Syrian-Turkish border as the primary domestication center (Besnard et al. 2013). Soon after domestication, the discovery of clonal propagation techniques may have boosted the expansion of olive in the Mediterranean Basin, along with other long-lived perennial crops such as grape and fig (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). Clonal propagation was remarkably effective, because approximately 90 % of the olive cultivars across the Mediterranean basin share the same "eastern-like" chlorotype (Besnard et al. 2013). Under this "single domestication" scenario, it is possible that local wild olives acted as pollen donors to the primary domesticated

Fig. 10.1 Olive cultivars present a large diversity. As an example, we can appreciate the variety of fruit morphologies and phenological stages showed by a handful of olive cultivars, collected the same day (24th of October), in the World Olive Germplasm Bank of Cordoba, Spain (Picture courtesy of D. Barranco)

cultivars, thus reducing the possible deleterious effects associated with inbreeding and most likely producing better locally adapted cultivars.

An alternative or complementary scenario for olive domestication posits the existence of several primary domestication centers throughout the Mediterranean Basin; these centers may coincide with quaternary long-term refugia (Breton et al. 2009; Besnard et al. 2013). Two observations support the multilocal domestication hypothesis. First, putative quaternary refugia show the highest plastid DNA (ptDNA) diversity for wild olives, suggesting that they could have been an essential foundation for cultivated olive (Besnard et al. 2013). Second, two minor haplotypes, E2, and E3 occur only in wild germplasm and cultivars from the central and western Mediterranean Basin, implying that they arose separately from any putative site of single domestication (Besnard et al. 2013).

Regardless of the primary origin of olive cultivars, once the superior genotypes were propagated clonally, they were able to spread via migration. The migration history of olives is particularly complex; the Phoenicians, Greeks, and Romans were thought to have expanded olive cultivation from east to west through both the northern and southern coasts of the Mediterranean Basin (Zohary and Spiegel-Roy 1975; Kaniewski et al. 2012). The migration of clones has led to confusion in the cultivar identity and nomenclature, such that most of the ~1200 (Bartolini et al. 1998) Mediterranean cultivars are of uncertain pedigree. Moreover, each traditional olive-growing country has its own cultivars, and these cultivars are typically only shared in border areas (Rallo 2005; Trujillo et al. 2013).

Identification of existing cultivars represents the first step in their cataloging. Only morphological descriptors were used for identification purposes until the 1980s. The main shortcoming for the use of these characters is the influence of environment on the expression of morphological traits (Rallo 2014).

In Andalusia, Spain, a systematic pomological characterization, including 55 morphological qualitative descriptors from tree (3), shoot (3), leaf (11), inflorescence (4), fruit (16), and stone (18) from 511 trees sampled in 83 localities found

out 197 different denominations, allowed the discrimination of 156 different cultivars and the establishment of synonyms, homonyms, and wrong denominations. This work (Barranco et al. 1984) provided a general elaiography of the most important olive region in the world and evidenced the usefulness of a morphological schedule for cataloging cultivars. This schedule was the base of the descriptors adopted by the International Union for the Protection of New Varieties of Plants (UPOV) for the olive. A simplified morphological schedule with only 27 descriptors have been used for cataloging the main 139 cultivars of the world for the IOC (Barranco et al. 2000), 262 cultivars from Spain (Barranco et al. 2005), 91 cultivars from France (Moutier et al. 2004), 202 in Italy (Muzzalupo 2012), and 56 cultivars in Tunisia (Trigui and Msallem 2002). Therefore, a systematic and simplified morphological schedule carried out by trained workers appears as a useful tool for cataloging olive cultivars (Rallo 2014).

The use of molecular markers for genotyping olive cultivars started with isozymes in the 1980s (Pontikis et al. 1980). The advent of DNA markers and their use for genotyping olive started in the mid of 1990s. Since that time, genotyping and studies on variability of olive cultivars increased exponentially. Critical review of the numerous elaiographical lists and the modern research tools used, particularly DNA and molecular markers, lead to a final exhaustive report on characters used for olive classification (Ganino et al. 2006).

A strategy based on a consensus list of minimum morphological characters (Barranco et al. 2000, 2005) and simple sequence repeat (SSR) (Baldoni et al. 2009) is in development. Works carried out in the Germplasm Banks of Marrakech (Haouane et al. 2011) and Córdoba (Trujillo et al. 2013) illustrate on the power of this strategy to identify in a short delay the accessions of cultivars' collections.

10.2 The Characterization of Olive Genetic Diversity: The Case of Cultivated and Wild Olives in Spain

10.2.1 Background

The diversity of cultivars in olive-growing countries is progressively changing. The clonal propagation of olive was performed by farmers using large propagules such as hardwood cuttings, suckers, or spheroplasts. Currently, olive is propagated by the nursery industry using small semi-hardwood leafy cuttings. This change has facilitated the movement of cultivars to areas that are far from their traditional growing regions. However, the nursery industry is only propagating selecting outstanding traditional cultivars and some newly bred cultivars. For example, in Spain, only six oil cultivars (Arbequina, Arbosana, Frantoio, Hojiblanca, Koroneiki, and Picual) and four table olive cultivars (Gordal Sevillana, Hojiblanca, Manzanilla Cacereña, and Manzanilla de Sevilla) represent more than 90 % of the commercialized nursery plants (Rallo and Muñoz-Díez 2010). Similar trends have been reported in most countries.

This reduction in the number of olive cultivars used in the new plantations might lead to progressive genetic erosion phenomena. Genetic erosion is defined as "*the permanent reduction in richness or evenness of common local alleles or the loss of combination of alleles over time in a defined area*" (Maxted and Guarino 2006).

A major emphasis on the exploration, cataloging, conservation, and evaluation of olive genetic resources is necessary to counteract possible genetic erosion phenomena. These types of studies are being carried out in Spain (Barranco and Rallo 2000; Barranco et al. 2005) and other countries (Khadari et al. 2003; Gemas et al. 2004; Bracci et al. 2009; Haouane et al. 2011; Yoruk and Taskin 2014), thus increasing the worldwide cultivar germplasm banks and accessions (Bartolini and Cerreti 2008).

Among the olive genetic resources, very little attention has been paid to oleasters, despite their importance as a source of genetic variability. In recent years, various studies have focused on the genetic variation of wild olive populations and their relationships with cultivars using different molecular markers (Lumaret and. Ouazzani 2001; Besnard et al. 2002, 2007; Lumaret et al. 2004; Breton et al. 2006; Belaj et al. 2007). Detailed analyses at a smaller scale may produce new insights in olive domestication and provide a better understanding of the distribution of genetic diversity at regional levels (Baldoni et al. 2006). In addition, the comparison of the genetic diversity between the wild and cultivated forms in specific areas might allow us to evaluate the potential loss of genetic variability as a consequence of domestication and the posterior intensification of agricultural systems.

In this chapter, we illustrate the characterization of olive genetic resources (wild and cultivated) in the main olive-growing regions of Spain using SSR markers and extending the study previously carried out by Belaj et al. (2010). Spain is the first olive oil producing country in the world and offers optimal conditions to perform this study for two main reasons. First, there is a rich diversity of traditional cultivars that have been systematically surveyed and characterized by morphological descriptors and molecular markers (Barranco et al. 2000, 2005; Trujillo et al. 2013). Second, Spain includes the most important reservoir of genetic variability for wild olive (Rubio de Casas et al. 2006; Carrión et al. 2010; Besnard et al. 2013).

The comparison between cultivated and wild populations at a regional scale may shed light on the following: (1) the genetic diversity of wild and cultivated olives; (2) their genetic differentiation and relationships; (3) the occurrence of gene flow between wild and cultivated olives, and (4) the genetic structure of wild and cultivated forms.

10.2.2 Sampling and Methodological Approach

We included wild and cultivated olives from the six main olive-growing regions of Spain (Barranco et al. 2005): west (W), southwest (SW), south central (SC), southeast (SE), east (E), and northeast (NE) (Fig. 10.2). In total, we analyzed 331 samples, of which 93 were traditional cultivars and 238 were wild olives (Table 10.1).

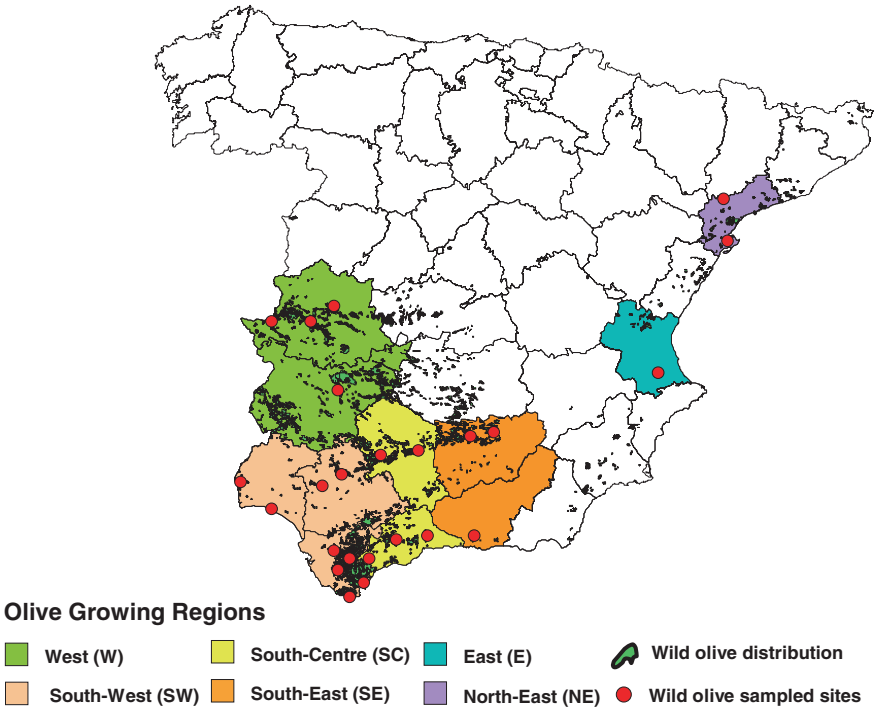

Fig. 10.2 Geographical regions and wild olive populations sampled in this study

Table 10.1 Status, origin, and genetic variability parameters for the wild and cultivated olives included in this study

	Group	Status[a]	Origin	*n*	Am	Ar	Au	Ho	He	Fis
(a)	C	C		93	9.14	9.12	8	0.73	0.69	−0.06
	W	W		238	15.93	13.32	103	0.65	0.77	0.16
(b)	W	C	West	15	4.79	3.83	2	0.71	0.64	−0.11
	SW	C	Southwest	22	6	4.08	0	0.76	0.67	−0.14
	SC	C	South-Center	12	4.64	3.84	0	0.73	0.65	−0.12
	SE	C	Southeast	22	4.71	3.6	1	0.73	0.64	−0.15
	E	C	East	8	5.21	4.71	0	0.7	0.68	−0.02
	NE	C	Northeast	14	6.36	4.93	0	0.75	0.73	−0.03
	W	W	West	43	9.43	4.87	1	0.64	0.72	0.11
	SW	W	Southwest	85	13.43	5.6	23	0.66	0.77	0.14
	SC	W	South-Center	53	11.14	5.47	8	0.65	0.77	0.16
	SE	W	Southeast	22	9.57	5.58	4	0.66	0.76	0.13
	E	W	East	11	6.79	5.35	8	0.66	0.76	0.13
	NE	W	Northeast	24	8.36	5.05	1	0.67	0.73	0.08

[a]*C* Cultivated, *W* Wild , *n* sample size, *Am* average number of alleles per locus, *Ar* allelic richness, *Au* number of unique alleles; *Ho* observed heterozygosity, *He* expected heterozygosity, *Fis* inbreeding coefficient

Table 10.2 Diversity parameters of the 14 SSR markers used in this study

Marker	Na	Ho	He	An	PIC
ssrOeUA-DCA3	15	0.767	0.757	−0.008	0.740
ssrOeUA-DCA9	23	0.921	0.909	−0.007	0.904
ssrOeUA-DCA11	16	0.703	0.741	0.026	0.716
ssrOeUA-DCA13	8	0.580	0.748	0.140	0.713
ssrOeUA-DCA15	7	0.253	0.682	0.467	0.623
ssrOeUA-DCA16	39	0.912	0.922	0.005	0.918
ssrOeUA-DCA18	13	0.918	0.877	−0.024	0.867
GAPU59	15	0.718	0.737	0.011	0.700
GAPU71B	6	0.724	0.716	−0.002	0.670
UDO99-011	16	0.827	0.838	0.011	0.823
UDO99-019	8	0.327	0.552	0.254	0.503
UDO99-024	17	0.602	0.799	0.147	0.775
UDO99-039	24	0.494	0.875	0.279	0.865
UDO99-043	24	0.729	0.908	0.109	0.903
Mean	16.5	0.677	0.790		0.766

Number of alleles (*Na*), expected (*He*) and observed (*Ho*) heterozygosity, null allele frequency (*An*), and Polymorphic Information Content (*PIC*)

Total DNA was extracted from young leaves and genetically characterized using 14 SSR markers (Table 10.2). These markers had previously been used to distinguish among cultivars in the World Olive Germplasm Bank of Cordoba (WOGBC), Spain due to their high resolution. They were also used in previous studies to describe the genetic patterns between wild and cultivated olives (Erre et al. 2009; Belaj et al. 2010; Diez et al. 2011, 2012). The SSR amplification was performed in a total volume of 20 µl, containing 2 ng of genomic DNA, 1X supplied PCR buffer (Biotools, Spain), 200 µM of each dNTP (Roche), 0.25 units of Taq DNA polymerase (Biotools, Spain), and 0.2 µM of forward (fluorescently labeled) and reverse primers. The PCR reactions were carried out in a thermal cycler (Perkin-Elmer-9600) using the following program: denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 50 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 7 min. The detection of amplification products was performed with an ABI 3130 Genetic Analyzer using the internal standard GeneScan 400 HD-Rox. Two cultivars, Arbequina and Frantoio, were used as controls in all runs.

We characterized the overall genetic diversity of our samples by calculating the number of parameters per microsatellite locus using the PowerMarker V3.23 software package (Liu and Muse 2005). The parameters were as follows: average number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and Polymorphism Information Content (PIC) (Botstein et al. 1980). The presence of null alleles (An) was estimated using the Cervus software package (Marshall et al. 1998).

We also evaluated the genetic diversity of the groups of samples by comparing the average number of alleles per locus (Am), allelic richness (Ar) (Petit et al. 1998), observed (Ho) and expected (He) heterozygosity, and inbreeding coefficient (Fis) with the software Fstat v2.9.3.2 (Goudet 1995). The unique alleles (Au) (alleles present only in a group) were determined using Microsat (Minch et al. 1996). We applied two-way AMOVA and calculated pairwise Fst values to study the distribution of the molecular variance in our set of samples using Arlequin 3.5.1.3 software (Excoffier et al. 2005).

In addition, we studied the relationship among genotypes that were grouped according to their status and geographical origin. To study this relationship, we built an unrooted phylogenetic tree based on the Cavalli-Sforza and Edwards chord distance (CS), and the Fitch-Margoliash least squares algorithm implemented in the FITCH program of the PHYLIP 3.6b software package (Felsenstein 1989). The robustness of the tree nodes was evaluated using 10,000 bootstrap (BS) replications.

To detect clusters of genetically similar genotypes and to estimate the individual coefficients of admixture with regard to the detected clusters, we used a Bayesian clustering method described in Corander et al. (2003) implemented in the software Bayesian analysis of population structure (BAPS) (Corander et al. 2008). BAPS uses a stochastic optimization algorithm for analyzing Bayesian models of population structure, which greatly improves the speed of the analysis compared to traditional MCMC-based algorithms. Furthermore, comparison tests have shown that BAPS has comparable statistical power to STRUCTURE software and increased power over small geographical distances (Corander and Marttinen 2006; Latch et al. 2006). When testing for population clusters, we ran 10 replicates for every level of K (K is the maximum number of clusters), up to $K = 12$. When estimating individual ancestry coefficients via admixture analysis, we utilized only clusters that had at least 10 individuals present within them. In addition, we used the recommended number of reference individuals (200) and 100 iterations to estimate the admixture coefficients of the reference individuals.

10.2.3 Genetic Diversity of Wild and Cultivated Olives in Spain

Our study uncovered abundant allelic variation and high overall genetic diversity in both cultivated and wild olives. A total of 231 alleles were found across the 14 SSR markers. The average number of alleles per locus was 16.5, with a maximum of 39 alleles (ssrOeUA-DCA16) and a minimum of six alleles (GAPU71B). The average PIC value was high (0.766), which was similar to the values of other studies that used these markers (Díez et al. 2011; Trujillo et al. 2013). The expected heterozygosity (He) was larger than the observed heterozygosity (Ho), possibly due to the presence of null alleles in some of the markers (Table 10.2).

Because domestication involves the selection of individuals with outstanding agronomical performance, much of the genetic diversity present in the wild ancestors of the crops was lost. For instance, some annuals such as soybean, maize, and wheat have lost 34, 38, and 70–90 % of the genetic diversity that was present in their wild ancestors, respectively (Tenaillon et al. 2004; Hyten et al. 2006; Haudry et al. 2007). The following results of this study were in agreement with this premise: (i) both forms, wild and cultivated olives, shared only ~52 % of the alleles; (ii) the wild olives presented 10 times more unique alleles than the cultivars (103 vs. 8; Table 10.1a); and (iii) the allelic richness (Ar), which allows the comparison between groups independent of their sample size, was higher in the wild than in the cultivated olives, although this latter comparison was not significant (Wilcoxon rank test; $p = 0.064 > 0.05$). This lack of significance may most likely be related to the fact that the transition from wild to cultivated forms appears to be smoother in long-lived perennials than in annual plants. For example, no genetic bottleneck was detected between traditional cherry cultivars and wild cherries (Mariette et al. 2010). Similarly, traditional cultivars of grape and apple showed as much genetic variation as their wild relatives (Myles et al. 2011; Cornille et al. 2012). Two distinctive features of perennial plants may contribute to lessen their domestication bottlenecks. First, long-lived plants are generally open-pollinator species, a characteristic that might have favored the gene flow between wild and cultivars with the consequent maintenance of high levels of genetic diversity (Miller and Gross 2011). Second, perennial crops are typically clonally propagated, and this technique decreases the number of generations between the cultivars and their wild ancestors, and consequently, the differences between them (Mckey and Elias 2010; Miller and Gross 2011). Moreover, clonal propagation facilitates the existence of overlapping generations, which also contributes to this slight differentiation.

In our study, approximately 11 % of the molecular variance was due to differences between the cultivated and wild forms (Table 10.3).

Notably, the Fst values were significant for all the cultivated and wild comparisons except for the pairs of groups from the E and the NE (Table 10.4). As an additional distinctive feature between the cultivated and wild forms, the cultivars showed a negative Fis value, indicating an excess of heterozygotes; by contrast, the wild groups favored homozygosity, with Fis > 0.0 (Table 10.1a, b). Using SSR markers, several authors also reported the same trend (Breton et al. 2006; Belaj et al. 2007, 2010; Erre et al. 2009), but others found similar Fis values for both wild and cultivated olives (Yoruk and Taskin 2014). While the pervasive character of this opposite trend in Fis values still needs further confirmation, it might be the outcome of several processes. First, the indirect selection of highly heterozygous genotypes during domestication may occur because it is possible that they exhibit better agronomical performance or hybrid vigor. However, the existence of this phenomenon in olive remains unclear (Biton et al. 2012). Second, the accumulation of somatic mutations may occur during myriads of generations of clonal reproduction in cultivars, especially in highly variable and neutrally evolving genomic regions, such as SSRs. These regions might accumulate mutations

Table 10.3 AMOVA considering the variation at three hierarchical levels, groups (wild versus cultivated), geographical populations within groups and among individuals within geographical populations

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	*p*-value
Between groups (wild vs. cultivated)	1	191.06	0.655 = Va	11.20	$p < 0.001$
Among geographical populations, within groups	10	150.90	0.195 = Vb	3.33	$p < 0.001$
Among individuals within geographical populations	650	3249.26	4.998 = Vc	85.47	$p < 0.001$
Total	661	3591.23	5.848		

Table 10.4 Pairwise Cavalli-Sforza (1967) distance (upper diagonal) and Fst values (lower diagonal) between olive groups. Cultivated and Wild groups are identified with red and green color, respectively

	W	SW	SC	SE	E	NE	W	SW	SC	SE	E	NE
W		0.019	0.020	0.016	0.042	0.082	0.107	0.100	0.092	0.103	0.077	0.092
SW	0.003		0.018	0.017	0.038	0.062	0.095	0.081	0.073	0.086	0.059	0.078
SC	0.009	0.011		0.012	0.033	0.066	0.113	0.098	0.089	0.096	0.072	0.082
SE	−0.003	0.009	−0.003		0.041	0.067	0.114	0.102	0.094	0.100	0.073	0.088
E	**0.032***	0.047	0.019	0.038		0.056	0.100	0.094	0.083	0.089	0.061	0.064
NE	**0.118**	**0.113**	**0.098**	**0.109**	**0.043**		0.092	0.077	0.071	0.081	0.052	0.030
W	**0.186**	**0.176**	**0.192**	**0.198**	**0.166**	**0.147**		0.018	0.020	0.032	0.056	0.066
SW	**0.157**	**0.143**	**0.155**	**0.165**	**0.138**	**0.119**	**0.011**		0.014	0.025	0.048	0.054
SC	**0.152**	**0.135**	**0.152**	**0.162**	**0.129**	**0.117**	**0.011**	**0.007**		0.022	0.040	0.050
SE	**0.148**	**0.135**	**0.141**	**0.155**	**0.116**	**0.117**	**0.031**	**0.024**	0.01		0.053	0.054
E	**0.089**	**0.081**	**0.085**	**0.089**	0.046	**0.038**	**0.064**	**0.049**	**0.037**	**0.031**		0.052
NE	**0.131**	**0.134**	**0.115**	**0.133**	**0.057**	0.018	**0.114**	**0.092**	**0.090**	**0.077**	**0.032**	

*Significant values in bold

without necessary phenotypic consequences in crop morphology and agronomic performance (Mckey and Elias 2010; Miller and Gross 2011; Díez et al. 2011). Finally, differential autogamy rates may occur in the wild and cultivated olive forms.

Despite the cultivated olive being considered as almost a strict out-crosser (Diaz et al. 2006), certain self-compatibility rates have been found for some cultivars (Guerriero and Bartolini 1995; Koubouris et al. 2014). Although higher self-compatibility rates in the wild progenitor than in the crops are not frequent in long-lived perennials (Miller and Gross 2011), this possibility has never been studied in olive; further, its possible relationship with the domestication process has also not been explored.

10.2.4 Genetic Relationships Between Wild and Cultivated Olives at a Regional Level

Although most of the molecular variance was due to differences between the wild and cultivated forms (~11 %) and between samples (~85 %), a subtle but significant proportion (3.3 %) of the molecular variance was due to differences among samples arranged according to their areas of origin (Table 10.3). This geographical differentiation pattern was clear among the wild groups but not in the cultivated groups. For example, the Fst values were significant between the wild olive groups and were more important for those not geographically adjacent (Table 10.4). By contrast, no significant Fst values were found between most of the cultivated populations. The recent movement of cultivars linked to human migration might have blurred the geographical fingerprint that was once present in the traditional cultivars (Baldoni et al. 2006). Only the cultivars from the east and northeast regions showed significant Fst values compared to all the other cultivars. Previous studies based on RAPD markers that analyzed Spanish cultivars reported the distinctiveness of the olive cultivars from the east and northeast compared to those from the rest of the country, suggesting they might be derived from different domestication processes (Belaj et al. 2004, 2010).

The dendrogram and the Bayesian analyses demonstrated the differences between the wild and cultivated samples. Again, the only pairs of cultivated and wild groups that were closely related were mostly those from the east and northeast regions (E and NE). These samples had an intermediate position between the wild and cultivated olives in the dendrogram (Fig. 10.3), and formed a distinctive genetic cluster in the Bayesian analysis (Fig. 10.4). Thus, summarizing the results from the Bayesian analysis and the dendrogram, our samples clustered into the following three well-supported (BS values > 95 %) groups: (i) cultivars from the western and southern regions; (ii) cultivars and wild populations from the east and northeast; and (iii) wild populations from the western and southern regions of Spain.

The genetic similarity between local cultivars and wild olives has been previously used as a proxy to support or reject the local domestication of these cultivars (De Caraffa et al. 2002; Baldoni et al. 2006). According to the dendrogram and the Bayesian analysis, the cultivars from the south (SW, SE, and SC) and west of Spain were minimally related to their local wild olives. Díez et al. (2011) found similar patterns when analyzing ancient olives from southern Spain, suggesting that the beginnings of olive growing in some areas of the west Mediterranean Basin could be based on the grafting of not necessarily autochthonous cultivars over local oleasters (Díez et al. 2011). In line with this hypothesis, almost all the cultivars from southern Spain presented the same haplotype (E1.1), which is broadly represented in wild and cultivated olives from the eastern Mediterranean Basin, where olive was likely primarily domesticated (Besnard et al. 2013).

Conversely, most of the cultivars from east (E) and northeast (NE) Spain were closely related to the local wild forms. This finding might suggest that these

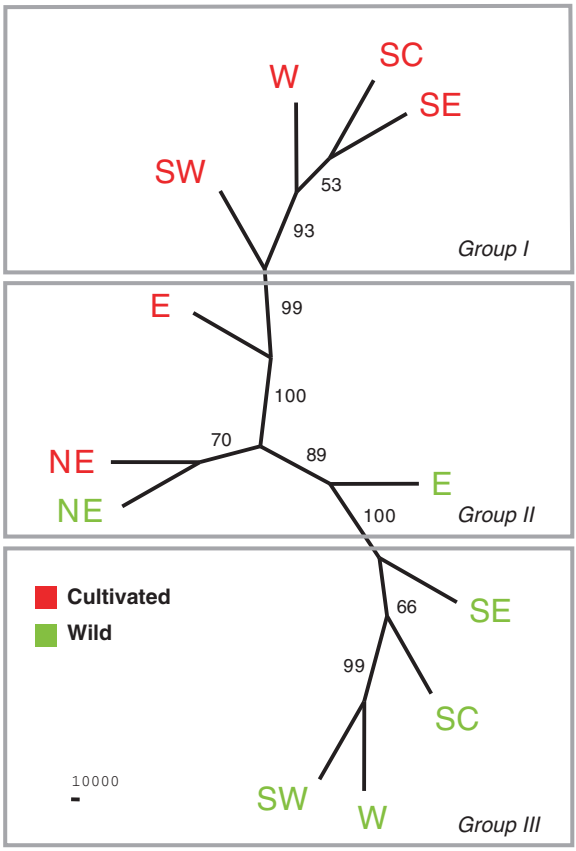

Fig. 10.3 Dendrogram showing the relationships between cultivated and wild olive samples arranged according to their geographical origin; West (*W*), Southwest (*SW*), South-Center (*SC*), Southeast (*SE*), East (*E*), and Northeast (*NE*). Bootstrap values are given in percentages over 10,000 replicates

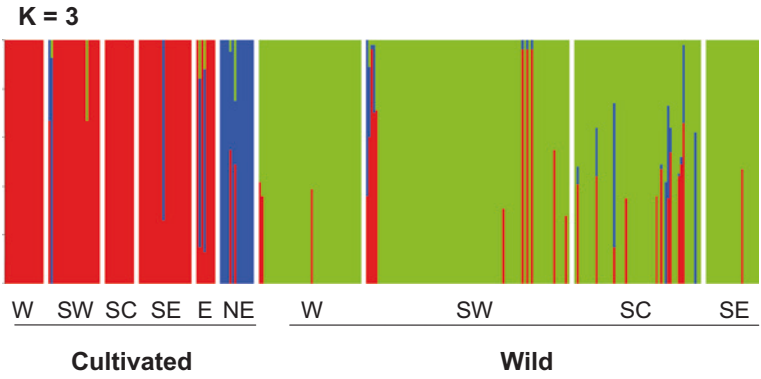

Fig. 10.4 Proportions of ancestry of the wild and cultivated olive samples (N = 331) based on $K = 3$ subdivisions. The geographical origins as well as the putative status of the samples (cultivated or wild) are specified

cultivars were derived from an alternative domestication or diversification process, possibly involving the direct selection from local oleasters or the admixture between them and not local cultivars. In agreement with this concept, Belaj et al. (2010) suggested the possibility of admixture events gave rise to the olive cultivars in northeast but not in south Spain (Belaj et al. 2010). However, the similarity between cultivated and wild E and NE samples could be due to the feral status of our putatively wild samples. Indeed, oleasters and feral forms are sometimes hard to distinguish morphologically; moreover, in the east and northeast, wild olive populations are scarce and fragmented compared to those in the south and west.

However, presuming the wild status of the samples, our data suggest that the genetic diversity within olives has been shaped by hybridization with wild oleasters mostly in E and NE Spain. Conversely, this process has been absent or very subtle in the south and west, where wild and cultivated populations were grouped in homogeneous and distinctive genetic clusters.

10.3 The Loss of Genetic Variability: Conservation Strategies in Olive

The knowledge about the relationships between cultivars and wild olives is critically important for conservation purposes, breeding programs, the design of genome association studies, and to untangle the population history. In addition, these studies allow us to track the evolution of genetic diversity and its potential loss in crops as a consequence of domestication and the posterior intensification of growing systems. This phenomenon has not been well documented despite its crucial importance for the sustainability of agriculture and food security (van de Wouw et al. 2009).

Regardless of the primary origin of olive cultivars, our dataset provides two snapshots of olive genetic diversity in the main olive-growing regions of Spain. First, the wild olives depict the genetic diversity of the species as part of the spontaneous Mediterranean vegetation; and second, the traditional cultivars maintain the genetic diversity that has served as a foundation for the solid and extensive rainfed olive-growing system over centuries.

The wild olive populations showed an outstanding allelic variability, most of which was not present in the cultivars. This wild germplasm represent an uncharacterized source of genetic resources for breeding; moreover, as suggested by our results, the highly diverse wild olives from south Spain played a minor role in the domestication of olive. The key to combat devastating diseases with no sources of complete resistance within the cultivated olive, such as Verticillium wilt that is caused by the fungus *Verticillium dahliae* Kleb., might be provided by wild germplasm (Colella et al. 2008; Trapero et al. 2015), as observed in other perennial

crops such as pistachio (Morgan et al. 1992). For these reasons, the characterization and preservation of wild olive germplasm is of outstanding importance.

Conservation efforts should also focus on traditional olive cultivars. The intensification of olive-growing systems is triggering both the standardization of cultivars in new plantations and the development of breeding programs to search for cultivars adapted to new planting systems. For example, high-density hedgerow systems in both rainfed (>1000 olives per ha) and irrigated (>1500 olives per ha) conditions are spreading worldwide (Rallo 2014). Only a handful of cultivars fit the requirements needed for this new system. Among them, Arbequina is the cultivar of choice, which is planted worldwide. A wave of newly bred olive cultivars, a product of the crossing between cvs. Arbequina and Picual, will soon be released to complement the availability of cultivars for intensive planting systems (Rallo 2014).

This substitution process may mimic the transition between pre-cultivated forms and the current traditional cultivars in the past. However, its geographical scale is quite different. In the past, olive growing had different characteristics even between regions from small geographical areas (e.g., south and northeast Spain). Currently, the new olive-growing systems are global. The same five cultivars, Arbequina, Arbosana, Frantoio, Koroneiki, and Picual, are being used in most new olive plantations worldwide. This trend might lead to a genetic erosion process where the traditional local cultivars could be progressively substituted and finally lost unless conservation plans are implemented.

In addition, the outbreaks of epidemic diseases can seriously affect the maintenance of local cultivars. For example, a devastating disease, denoted as "Olive Quick Decline Syndrome," affected olive trees in the Apulia region of southern Italy in October 2013. This syndrome, which is generally associated with the quarantine bacterium *Xylella fastidiosa*, several fungal species of the genus *Phaeoacremonium* and *Phaemoniella*, and the moth *Zeuzera pirina* (Saponari et al. 2013), mainly killed 200–300-year-old olives—most of them local cultivars.

In this scenario, ex situ and in situ conservation efforts are required to avoid the irreversible loss of traditional cultivars. Ex situ field collections of trees have been the typical method for the conservation of olive cultivars. In 1994, the International Olive Council (IOC) promoted a Network of National Banks of Germplasm. This network also includes two international repositories, the Olive World Germplasm Banks of Córdoba (Spain) and Marrakech (Morocco). A third repository is under development in Izmir (Turkey). Despite the existence of this network, the exploration and conservation of olive genetic resources is still incomplete and requires further efforts in all the olive-growing countries. For instance, a review by FAO reported the existence of 107 collections of olive cultivars worldwide; however, even in these institutions, approximately 20 % of the accessions were labeled as "unknown" (Bartolini and Cerreti 2008). One of the main advantages of ex situ conservation is that it allows the evaluation of the cultivars for many traits in the same environment. Recent efforts have been paid to the development of core collections in olive (Haouane et al. 2011; Belaj et al. 2011; Díez et al. 2012; El Bakkali et al. 2013). These core collections, which consist of a limited

number of the accessions, were chosen to cover the genetic spectrum of the entire collection (Brown 1989). Core collections represent an efficient strategy for studying the interaction of genotypes and environments to reduce the effort in the evaluation of agronomic characters.

In situ conservation permits the coevolution of genotypes in their original environment. It appears as a valuable tool not only for the preservation of wild olive populations but also for monumental olives. The long life span of olive results in the existence of both centennial and millennial trees across the Mediterranean Basin. The study of ancient olives has been fruitful for both germplasm collection and to increase the knowledge regarding olive domestication (Erre et al. 2009; Diez et al. 2011; Cicatelli et al. 2013; Salimonti et al. 2013; Barazani et al. 2014). An international network of in situ monumental and wild olives appears to be a strategic initiative for the future of this crop (Rallo 2014).

Thus, knowledge about the local genetic variation of olive germplasm, including wild and cultivated forms, is the first and necessary step for the sustainability of olive growing. The sustainability of olive-growing systems is particularly important when considering the forecast for climate change in the Mediterranean Basin and its possible effects on olive growth (Ponti et al. 2014). More frequent extreme weather is predicted by most climate change models, along with a significant increase in the summer air temperature and water stress, mainly for Mediterranean regions (Tubiello et al. 2000). In particular, shifts in precipitation patterns will affect most European regions, with increased risks of drought; given this scenario, the consequences would be most dramatic for the Mediterranean coast of Europe (Lung et al. 2014). Under these circumstances, the evaluation of the potential adaptation of the olive cultivars to different climatic conditions is crucial. To do so, it is necessary to examine the phenological characterization of the genotypes under different climatic conditions, as well as to evaluate their tolerance to biotic and abiotic stresses. The establishment of several core collections, managed by the IOC network of Germplasm Banks composed of 23 banks, may provide an ideal opportunity to achieve this goal.

10.4 Conclusions and Prospects

The new olive-growing systems, which are more intensive and mechanically harvested, are leading to the progressive reduction in the number of traditional olive cultivars used in new plantations. This phenomenon might cause the irreparable loss of genetic variation in olive. In this context, the exploration, identification, and conservation of olive genetic resources, both cultivated and wild, is an urgent task. The phenotypical characterization of olive germplasm is crucial for identification purposes, breeding programs and to examine the impact of climate change on olive-growing systems. Wild olives represent an unexplored source of genetic variability, which also require further characterization and conservation efforts. The characterization of wild and cultivated germplasm at a regional level is

necessary for conservation purposes, as well as for olive breeding and to untangle the domestication history of this crop. Global and coordinated ex situ and in situ conservation programs should be designed to evaluate and preserve the wealthy genetic legacy present in olive germplasm.

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