

Compendium of Plant Genomes

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The Olive Tree Genome

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The Olive Tree Genome

 Springer

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Preface

Olive (*Olea europaea* L., subsp. *europaea*, var. *europaea*), a multifunctional long-living tree crop, is relevant not only for table olive and oil production, but also for shaping and protecting the landscape and for its impact on human nutrition and rural lifestyle.

It is usually accepted that olive has been primarily domesticated in the Levant. Then, three main clusters of the var. *europaea* inside the primary gene pools have been identified for the cultivated olive in Eastern, Central, and Western Mediterranean. These centers of diversity likely reflect crop diversification from East to West, but could also result from independent domestications.

Gene exchanges between wild (*Olea europaea* L., subsp. *europaea*, var. *sylvestris*, named as oleaster) and cultivated olive have played a major role in the diversification of the crop. In the Mediterranean area, where minimum winter temperatures do not usually fall below -7°C , olive cultivation occupies 12 million hectares, representing about 95 % of total world olive cultivated area. Recently, its cultivation has spread to non-traditionally olive-growing countries, i.e., USA, Argentina, Chile, South Africa, and Australia, with intensive and super high-density systems, for which high productive, high oil producing, and low vigor varieties are required.

The *Olea* species belongs to the Oleaceae family that comprises nearly 25 genera and 600 species distributed in the temperate and tropical regions. In this family, plants are mostly evergreen trees, bushes, and vines, many of them producing essential oils in their flowers and fruits. The olive has a medium-sized genome (about 1.4 Gb), but the high number of chromosomes ($n = 23$), the large amount of the repetitive component ($>70\%$, made up by 30 % of tandem repeat sequences and 40 % of transposable elements), and the high level of heterozygosity have made very difficult the sequencing tasks and only a first draft of the olive genome sequence has recently been released.

The molecular bases underlying the phenotypic differences among cultivars still remain poorly understood. Nowadays, the acknowledged beneficial health properties of the extra-virgin olive oil and the ability of the species to produce under harsh conditions (e.g., drought stress) have provided new impulses for introducing innovation through olive genomics and breeding, leading to a deeper understanding of the biological processes underlying oil accumulation, polyphenol synthesis, adaptation to environmental constraints,

and response to threatening epidemics by biological agents. The ‘omics’ studies have particularly been useful to unravel the intricacy of main biochemical pathways and to characterize genes involved in the expression of complex traits.

Information about olive phylogeny, domestication, and relationships with related wild forms represents a fundamental prerequisite for the genetic improvement of the species, allowing for the introgression of important alleles from oleaster or from other *O. europaea* subspecies. The intercompatibility between cultivated olive and related forms has been analyzed for numerous subspecies, resulting compatible with the subsp. *cuspidata*, *laperrinei* and tetraploid *cerasiformis*, while a pre- or post-zygotic incompatibility has been observed in other cases (e.g., *ferruginea* and *Olea capensis*, respectively). The in vitro techniques now available may overcome these intercross limitations, opening the road toward new hybridization approaches.

Although the poor knowledge available on the genetic basis of the main olive characters, the lack of sound QTL markers, the limited experience on gene-transfer technologies, and the long generation interval, significant programs of genetic improvement may be undertaken profiting of the new information rising from biotechnology and genomics research. Harnessing innovations in these two research fields will help the development of fast-track breeding procedures, to improve important economical and agronomical traits, shorten the prefruiting period, and increase the selection efficiency of the designed new olive varieties through the cloning and genotyping of in vitro germinated embryos or developed seedlings.

Topics of this book cover the description of olive genetic resources, the classical and modern breeding methods for releasing new cultivars, the genotype/environment interactions determining the response to biotic and abiotic stresses, the fruit metabolism related to oil production and synthesis of health beneficial molecules, the mapping of genes and QTLs, the genome sequencing, and the transcriptomic and proteomic strategies pertinent to the development of molecular platforms and templates amenable to the precise and rapid genetic modifications using the recently developed genome-editing tools.

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Abbreviations

ABA	Abscisic acid
AChE	Acetylcholinesterase
ADH	Alcohol dehydrogenase
AFLP	Amplified fragment length polymorphism
ArMV	Arabidopsis mosaic virus
ATT	Alcohol acetyl transferases
BCA	Biological control agent
BSseq	Bisulfite sequencing
CAP	Common Agrarian Policy
CDD	Cumulated degree day
cDNA	Complementary DNA
ChIPseq	Chromatin immunoprecipitation sequencing
CLRV	Cherry leaf roll virus
cM	centiMorgan
CMV	Cucumber mosaic virus
CoDiRO	Complesso del disseccamento rapido dell'Olivo
cpDNA	Chloroplast DNA
DAF	Days after flowering
DArT	Diversity arrays technology
dsRNAs	Double-stranded RNA
Ece	Saturated paste extract
Eco-TILLING	Targeting-induced local lesions in genomes
EFSA	Commission of the European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EST	Expressed sequence tag
EU	European Union
EVOO	Extra-virgin olive oil
FAD	Fatty acid desaturase
FAO	Food and Agriculture Organization
FsRIDL	Female-specific release of insects carrying a 'Dominant Lethal'
GBS	Genotyping by sequencing
GP	Gene pool
GSI	Gametophytic self-incompatibility
GWAS	Genome-wide association study
HD	High density
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid

IOGC	International olive genome consortium
IOC	International olive council
ISSR	Inter-simple sequence repeat
JERF	Jasmonate and ethylene response factor
LAI	Leaf area index
LA	Linoleic acid
LDR-UA	Ligation detection reaction-universal array
LG	Linkage group
LnA	Linolenic acid
LN	Liquid nitrogen
LOX	Lipoxygenase
MAS	Marker-assisted selection
MFO	Mixed function oxidase
MS	Murashige and Skoog (medium)
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NGS	Next-generation sequencing
nsSNPs	Non-synonymous single nucleotide polymorphisms
OCWE	Olive crop wild relatives
OLF	Olive fly
OLRV	Olive latent ring spot virus
OLS	Olive leaf spot
OLV-3	Olive latent virus
OLYaV	Olive leaf yellowing-associated virus
OM	Olive medium-Rugini olive medium
OMMV	Olive mild mosaic virus
Ops	Organophosphates
OQDS	Olive quick decline virus
OSLV	Olive semi-latent virus
OWGB	Olive World Germplasm Bank
OYMDaV	Olive yellow mottle and decline-associated virus
PCD	Programmed cell death
PCR	Polymerase chain reaction
PDO	Protected designation of origin
PEG	Polyethylene glycol
PGIP	Polygalacturonase-inhibiting protein
PGI	Protected geographic identification
PPM	Plant preservative mixture
PR	Pathogen-related protein
PrsS	Pistil S determinant
PSII	Photosystem II
PVS1	Plant vitrification solution 1
PVS2	Plant vitrification solution 2
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIDL	Release of insects carrying a 'Dominant Lethal'
RNAseq	RNA sequencing
ROS	Reactive oxygen species

RWC	Relative water content
SA	Salicylic acid
SCAR	Sequence characterized amplified region
SCR	S-locus cysteine rich protein
SHD	Super high density
SIT	Sterile insect technique
SLG	S-locus glycoprotein
SLRSV	Strawberry latent ring spot virus
SNP	Single nucleotide polymorphism
SRK	S-locus receptor kinase
SSH	Suppression subtractive hybridization
SSR	Simple sequence repeat
TDZ	Thidiazuron
TF	Transcription factor
TG	Taxon group
TNA	Total nucleic acid
TNV-D	Tobacco necrosis virus D
UNESCO	United Nations Educational, Scientific and Cultural Organization
VOCs	Volatile organic compounds
VOO	Virgin olive oil
VWO	Verticillium wilt of olive
Wt	Wild type
WUE	Water use efficiency
Xf	<i>Xylella fastidiosa</i>

Guillaume Besnard

Abstract

The olive is the most iconic Mediterranean tree. The multiple uses of wild and cultivated olives make this species economically significant and a keystone of traditional Mediterranean agrosystems. The literature on its domestication is reviewed here, with a focus on the recent results on population, archaeobotanical, and genetic studies. Since the Late Tertiary, the olive distribution has been shaped by past climatic and geological changes as well as humans during prehistoric and historic times. It is usually accepted that olive has been primarily domesticated in the Levant. Three main gene pools are, however, identified for the cultivated olive in eastern, Central, and western Mediterranean. These centers of diversity likely reflect crop diversification from East to West but could also result from independent domestications. The breeding process is still ongoing, including areas outside of the native range where cultivated olives and wild relatives were introduced into the same regions. Gene exchanges between wild and cultivated olives have played a major role in the diversification of the crop. In the future, the *in situ* conservation of wild populations, locally endangered, should be essential to preserve the evolutionary potential of the cultivated olive.

1 Introduction

The cultivated olive (*Olea europaea* L. subsp. *europaea* var. *europaea*; Oleaceae) is the most iconic tree of the Mediterranean basin, and

its omnipresence in agrosystems makes this species economically significant and a keystone of the traditional Mediterranean agriculture (Loumou and Giourga 2003). Today, hundreds of cultivated olive varieties are reported to produce both oil and/or table fruits (Bartolini et al. 2005), but a few major cultivars are usually exploited at a regional scale (e.g., Khadari et al. 2008). The first use of the olive is still hotly debated (e.g., Vossen 2007; Margaritis 2013). At the Copper and Bronze Ages, the primary utilization of olive oil is

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reported for light and body ointment with ritual significance, but its culinary use was found later and widespread during the early Roman epoch (Tardi 2014). The Mediterranean wild olive tree—usually named oleaster—[*O. europaea* subsp. *europaea* var. *sylvestris* (Mill.) Lehr] is also a source of wood and fodder for cattle (Margaritis 2013). The double nature of the olive as a wild element of the vegetation of the Mediterranean basin and as a crop was confounding for researchers addressing its domestication. It has been long supposed that the olive was not native to the Mediterranean basin and cultivars were introduced from adjacent regions [all Mediterranean spontaneous trees being seen as feral olives (e.g., Oliver 1868; Newberry 1937; Ciferri and Breviglieri 1942; Chevalier 1948; Turrill 1951)], but today this idea is categorically refuted and an autochthonous Mediterranean origin has been definitely demonstrated (e.g., Angiolillo et al. 1999; Besnard et al. 2001b; Terral et al. 2004; Carrión et al. 2010). Palaeobotanical, archaeological, historical, and molecular data have recently been accumulated, and a critical evaluation of this evidence allowed reconsidering the biogeography of the wild olive and the history of its cultivation (e.g., Terral et al. 2004; Carrión et al. 2010; Kaniewski et al. 2012; Besnard et al. 2013b; Díez et al. 2015). In this chapter, and based on a literature review, I propose a sequential history of the olive during the Quaternary, from the Late Pliocene to historical times.

2 Long Persistence and Diversification of Oleaster Populations in the Mediterranean Basin During the Pleistocene

Wild olives belong to the so-called olive complex in which six subspecies are recognized (Médail et al. 2001; Green 2002). These taxa are naturally distributed from South Africa to South Asia, in Saharan mountains, Macaronesia, and Mediterranean countries (Fig. 1). According to phylogenetic dating analyses, the most recent common ancestor of olive subspecies dates back to the Late Miocene or Early Pliocene (Besnard

et al. 2009). An aridification of the Saharan region from the Late Miocene until present (De Menocal 1995; Schuster et al. 2006) may have contributed to reduce gene flow between North African and Tropical African olive populations. This may explain the early split in phylogenetic reconstructions between subsp. *cuspidata* and other subspecies (Besnard et al. 2007, 2009). The ancestor of the Mediterranean olive was thus probably present in the Mediterranean area during the Messinian Salinity Crisis about five to six million years ago (Gautier et al. 1994). Three distantly related plastid DNA lineages (namely E1, E2, and E3) were revealed in the Mediterranean olive (Fig. 2; Besnard et al. 2007, 2013b). Based on a fossil-calibrated dating, it was shown that their divergence may have started during the Late Pliocene or Early Pleistocene (Besnard et al. 2013b) with the establishment of the Mediterranean climate (Suc 1984).

The Pleistocene was characterized by climatic fluctuations punctuated by glacial and dry periods. In response to these climatic shifts, oleaster populations have experienced successive contractions and expansions. Today, eastern and western Mediterranean wild olive populations are genetically differentiated as a result of gene exchange limitations due to geographic distance and natural barriers (deserts, seas, or mountains) over long periods of time (e.g., Angiolillo et al. 1999; Besnard et al. 2001b, 2007, 2013a, b; Lumaret et al. 2004; Rubio de Casas et al. 2006; Breton et al. 2008; Belaj et al. 2010; Besnard and El Bakkali 2014; Díez et al. 2015). Based on nuclear, biparentally inherited markers, two main gene pools have thus been recognized in the eastern and Western/central Mediterranean basin. The initial pattern of genetic differentiation has, however, been considerably blurred due to gene flow from cultivated to wild olives. Phylogenetic patterns were also investigated on a few single copy genes that revealed divergent lineages of alleles (Besnard and El Bakkali 2014). Interestingly, these allelic lineages are mixed in oleasters, both in the eastern and western Mediterranean basin, suggesting that ancient admixture events have also occurred, possibly before historical times with recurrent gene flow breaks and

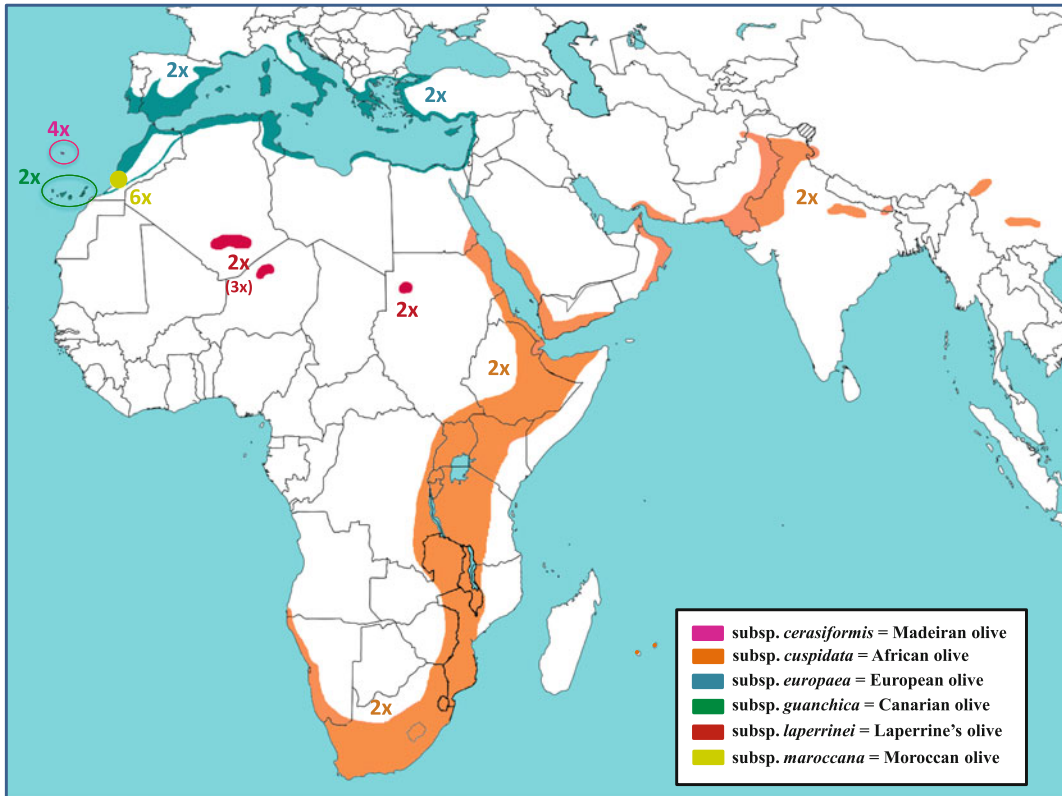


Fig. 1 Native distribution of the olive relatives (*Olea europaea* L.; according to Rubio de Casas et al. 2006). Six subspecies are currently recognized in the olive complex (Médail et al. 2001; Green 2002). They are usually considered as the primary genetic resources of the cultivated olive (Zohary 1994; Green 2002), but to date cross-compatibility has been reported only between

diploids (e.g., Besnard et al. 2008, 2013a, 2014; Hannachi et al. 2009; Cáceres et al. 2015). Polyploidy level is indicated for each subspecies according to Besnard et al. (2008). Hexaploidy and tetraploidy were reported in subspecies *maroccana* and *cerasiformis*, respectively. A few triploids (ca. 3%) have also been revealed in the Lapperine's olive (Besnard and Baali-Cherif 2009)

reconnections due to past climatic changes. Phylogeographic patterns have been more deeply investigated using strictly maternally inherited genomes (i.e., mitochondria and plastids; Besnard et al. 2002). These organellar genomes are uniparentally transmitted and consequently more prone to genetic drift than nuclear genes (e.g., Schaal and Olsen 2000). Furthermore, polymorphism of the organellar DNA is disseminated only by seeds, and hence at shorter distance than nuclear DNA polymorphisms, which are also dispersed by pollen. Organellar DNAs are therefore very useful to reveal genetic patterns of strong differentiation and to study phylogeographic processes. In oleasters, most of plastid

haplotypes (or chlorotypes) are confined to limited areas, while a few (also detected in cultivars: E1.1, E1.2, E1.3, E2.1, E2.2, E3.1, and E3.2; Fig. 2) have spread throughout the Mediterranean basin (Besnard et al. 2013b). Prior to the human spread of both oleasters and cultivated olives, the plastid lineage E1 was probably restricted to the East, from Greece to the Levant, while the plastid lineages E2 and E3 were specific to the West and Central parts. Today, three regional hot spots of plastid DNA diversity are identified in oleasters, namely the Levant (lineage E1), the Aegean region (lineage E1), and the Gibraltar Strait (lineages E2 and E3). The high genetic diversity found in these three areas might indicate that they

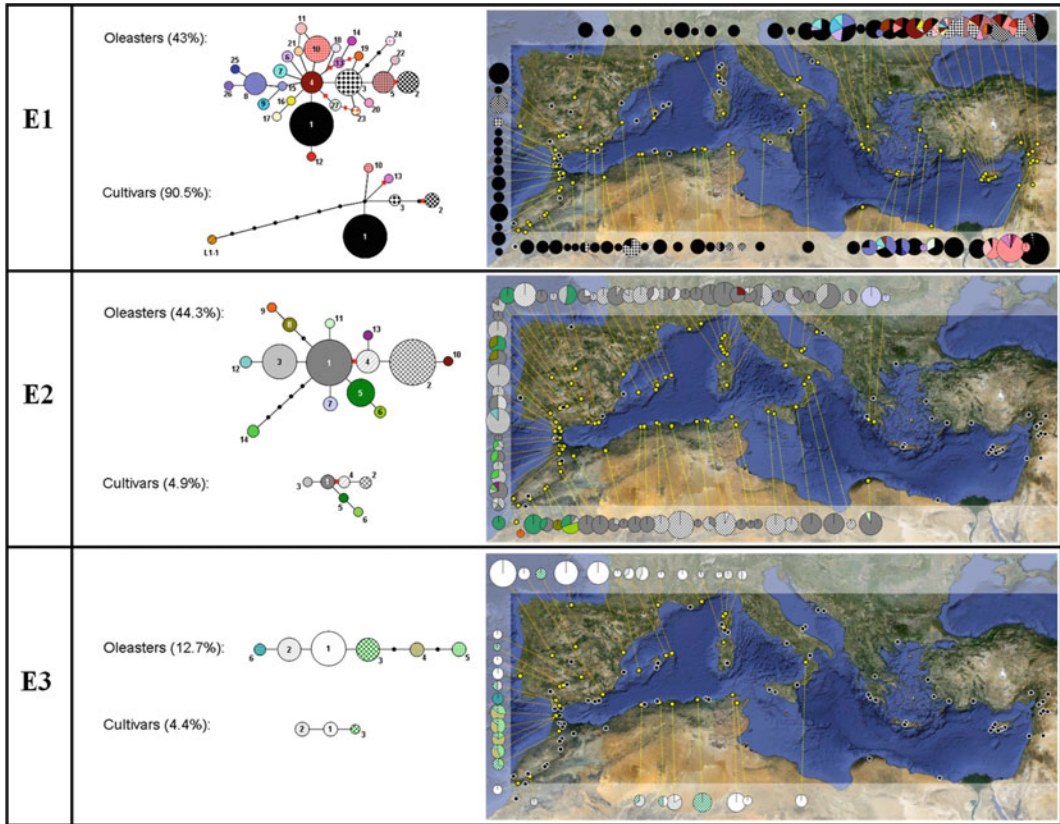


Fig. 2 Diversity of the three Mediterranean olive plastid lineages (namely E1, E2, and E3) reproduced from Besnard et al. (2013b). A total of 1797 trees (1253 oleasters and 534 cultivars) were characterized with 61 polymorphic plastid loci, especially multistate microsatellites (i.e., mononucleotide stretches) that are variable and informative in the olive (Besnard and Bervillé 2002). On the left, reduced median haplotype networks (Bandelt et al. 1999) for each lineage and for both wild and cultivated gene pools are shown. Each chlorotype is numbered and represented by a symbol with a definite color and/or motif. Chlorotype frequencies are proportional to symbol diameter. The missing, intermediate

nodes are indicated by *small black points*. The frequency of each lineage in oleasters and cultivars is indicated in *brackets*. On the right, the geographical distribution of chlorotypes in oleaster populations is given. The size of pie charts is relative to the number of trees analyzed per location. For more details on the analyses, see Besnard et al. (2013b). In lineage E1, note that chlorotypes E1.1, E1.2, and E1.3 (the most frequent chlorotypes of cultivated olives) have spread on the whole Mediterranean basin. Their presence in non-cultivated olives from the western Mediterranean area is interpreted as an evidence of ferality (i.e., trees escaped from cultivation; Besnard et al. 2013b).

have acted as long-term refugia for the oleaster (Besnard et al. 2013b). Barriers to dispersal (e.g., Libyan Desert, Adriatic Sea, and Rechinger's Line) have probably limited long-distance dispersal of these lineages and prevented their complete admixture during post-glacial recolo-

nization. A coalescent-based Bayesian approach indicated that the present diversification of the three Mediterranean lineages has started during the Middle Pleistocene or Early Late Pleistocene, long before the Last Glacial Maximum (Besnard et al. 2013b).

3 First Uses of the Oleaster During the Holocene and Early Evidence of Domestication in the Levant

Human exploitation of oleasters is attested by archaeobotanical data since the Upper Paleolithic and Early Neolithic (Kislev et al. 1992; Terral 1997; Terral et al. 2004; Carrión et al. 2010; Kaniewski et al. 2012; Zohary et al. 2012). The fossil record also shows that wild olive populations have progressively recolonized the Mediterranean area during the post-glacial period (Carrión et al. 2010). Notably, olive abundance in palynological records increased at the Holocene with human activities both in the East and West of the Mediterranean basin indicating that the expansion was associated with green oak deforestation (Carrión et al. 2010). This early use and further spread could have been mostly linked to an exploitation of oleasters for wood and/or fodder, which is usually regarded as a pre-domestication stage (Renfrew 1972; Margaritis 2013). Indeed, olive fruit production was very likely favored by a pruning effect, offering to humans the possibility to select trees with the highest agronomic values.

As the olive has been exploited by humans since prehistoric times, it is important to identify the reasons of its cultivation and to define the process of its domestication (i.e., that aims to optimize fruit production). Sedentary human communities probably established the first orchards of selected olive genotypes (in particular, with higher fruit set, bigger fruits, and higher oil content) to optimize olive production and meet a sudden increase of the local or regional demand due to human population growth. Olive domestication is also characterized by vegetative propagation of the most valuable individuals (Zohary et al. 2012). Such genotypes were probably also selected for their ability to grow in anthropogenic environments and their propensity to be multiplied (i.e., grafting and cuttings). It is, however, very likely that olive domestication has been a continuous process involving the selection of trees propagated using both vegetative and sexual reproduction as well as the reiterated

cultivation of wild trees that presented the most interesting agronomic traits. Such practices still occur in some places, and the traditional exploitation of spontaneous forms can be observed in different places of the Mediterranean basin (e.g., Monastery of Stavrovouni, Cyprus; Rif Mountains, Morocco; Andalusian Mountains, Spain; G. Besnard, B. Khadari, and R. Rubio de Casas, pers. observ.).

Although the use of wild olives has been documented since the Late Paleolithic, it is commonly believed that cultivation of the tree postdates Neolithic grain agriculture (Galili et al. 1997; Carrión et al. 2010; Kaniewski et al. 2012; Zohary et al. 2012). Phylogeographic and population genetic studies demonstrated that cultivated olive mainly derives from the eastern oleaster gene pool (e.g., Besnard et al. 2001b, 2013a, b; Lumaret et al. 2004; Baldoni et al. 2006; Breton et al. 2008; Díez et al. 2015). In particular, both plastid and nuclear data sustain a major origin in the Near East (Fig. 3a). Three chlorotypes belonging to lineage E1 (i.e., E1.1, E1.2, and E1.3) characterize ca. 90 % of cultivars and are now observed in feral olives in the whole Mediterranean basin (Fig. 2; Besnard et al. 2013b). Based on the present distribution of E1 chlorotypes in oleasters and their phylogenetic relationships, Besnard et al. (2013b) have argued that the main chlorotypes of cultivated olives (i.e., E1.1, E1.2, and E1.3) originated in the northwest of the Fertile Crescent.

The olive oil trade has been developed during the Chalcolithic period in the Near East (Kaniewski et al. 2012). Based on this archaeological evidence, olive domestication is usually considered to have started then (Liphschitz et al. 1991; Galili et al. 1997), but an earlier cultivation cannot be excluded. In the northwestern Fertile Crescent, major human civilizations have emerged during the Neolithic and in particular the Pre-Pottery Neolithic B (PPNB; Edwards et al. 2004) that domesticated many crops and animals (Zeder 2011). It was hypothesized that these sedentary cultures might have also started domesticating the olive (Kaniewski et al. 2012; Besnard et al. 2013b).

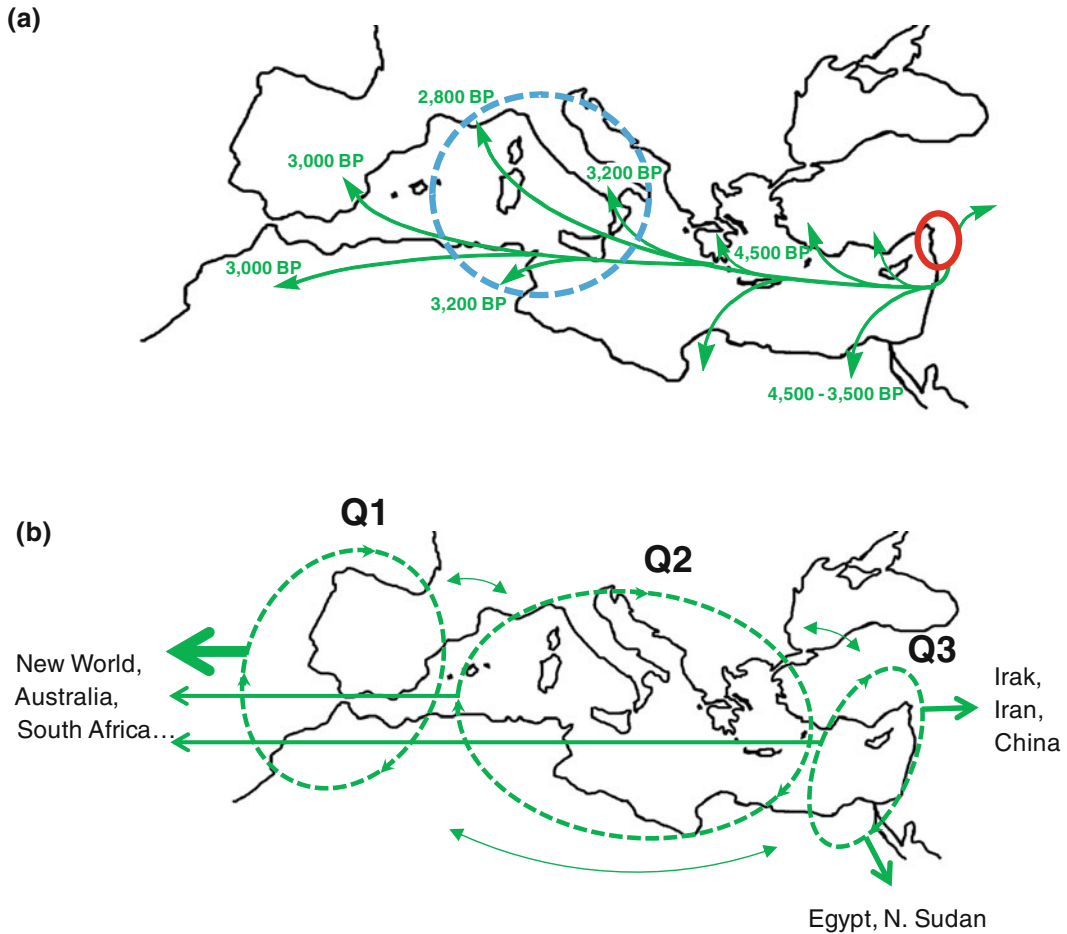


Fig. 3 Scenario on the primary domestication and secondary diversification of the olive [modified from Besnard and Rubio de Casas (2016)]. **a** The red circle indicates the region of initial domestication in the northern Levant during the Holocene, maybe during the Pre-Pottery Neolithic B period (Kaniewski et al. 2012; Besnard et al. 2013b). Green arrows indicate the subsequent human-mediated diffusion of the crop throughout the whole Mediterranean basin (approximate dates are given and deduced from archaeological data that attested for the development of oleiculture and olive oil trade; from Terral 1997). The dotted blue circle indicates a putative independent domestication in the Central Mediterranean

as posited by Díez et al. (2015). **b** Three main regions (dotted circles) of cultivated olive diversification (with possible, but limited admixture) are recognized as inferred by genetic analyses (Haouane et al. 2011; Belaj et al. 2012; Díez et al. 2012, 2015; Besnard et al. 2013a). The three gene pools (Q1, Q2, and Q3) are named according to Díez et al. (2015). Arrows indicate the spread of each gene pool and notably out of the native area. A possible new diversification has occurred or is ongoing in these new areas (Hosseini-Mazinani et al. 2014), particularly in contact with other wild relatives (subsp. *cuspidata*) in Africa, Asia, or Australia (Besnard et al. 2014)

4 Secondary Diversification of the Crop Across the Mediterranean Basin Versus Multiple Independent Domestications

The olive oil trade was first developed in the Near and Middle East before becoming widespread across the whole Mediterranean basin (e.g., Kaniewski et al. 2012; Newton et al. 2014; Fig. 3a). Studies on the genetic diversity of cultivated olive revealed a structure in relation to the geographic origin of varieties and their different uses, i.e., oil or table fruits (Claros et al. 2000; Belaj et al. 2001; Besnard et al. 2001a; Owen et al. 2005; Marra et al. 2013; Linos et al. 2014; Yoruk and Taskin 2014; Biton et al. 2015). Based on comprehensive samplings, independent research teams also revealed that present olive cultivars belong to three main genetic pools that approximately match three geographic areas corresponding to the West (namely Q1), Center (Q2), and East (Q3) of the Mediterranean basin (Fig. 3b; Haouane et al. 2011; Belaj et al. 2012; Díez et al. 2012, 2015; Besnard et al. 2013a). Other studies have additionally reported structural details at a regional scale that could reflect the clustering of very closely related individuals that were selected locally (e.g., Khadari et al. 2003; Breton et al. 2008; Muzzalupo et al. 2014). From these results and studies, there is clear and unanimous evidence for multiple centers of diversity of the cultivated olive tree.

Several authors have argued that cultivated olive diversification occurred in westernmost regions not as the result of local independent domestication but as the consequence of hybridization among local oleasters or pre-domesticated forms and introduced cultivars (Besnard et al. 2001b, 2013a, b; Díez et al. 2015). Biton et al. (2012) reported hybrid vigor F1 in olive progenies, which might suggest that admixture between genetic pools may indeed potentially generate superior new genotypes. This scenario of a primary domestication event in the Levant followed by secondary diversification has recently been challenged by Díez et al. (2015), who suggested that an independent

domestication (of Q2) could have also occurred in central Mediterranean. Besnard et al. (2013a) however showed, based on nuclear markers (microsatellites), that most Mediterranean cultivars were mainly assigned to the eastern oleaster genetic pool, while no cultivar was unambiguously assigned to the western one, even those with plastid lineages that originated from the western Mediterranean basin. This result supports that present elite cultivars either belong to the eastern genetic pool or are admixed forms. In addition, several teams have reported a significant excess of heterozygosity in cultivated olive (Díez et al. 2011; Besnard et al. 2014), which is congruent with the hypothesis of admixture-mediated diversification of the crop (i.e., of a single initial domestication followed by secondary domestication events). It is, however, important to note that other authors have reported an excess of homozygosity (e.g., Lumaret et al. 2004). This apparent incongruence could be explained by differences in the plant sampling and genetic markers used by different authors. Indeed, excessive homozygosity can be caused not only by the presence of null alleles on some loci, but also by the selection on some alleles (for instance, on isozyme loci; Lumaret and Ouazzani 2001). Conversely, the most likely cause for a global excess of heterozygosity such as the one revealed by studies using nuclear microsatellites (Díez et al. 2011; Besnard et al. 2014) is the maintenance of early generation admixed individuals but it could also be partly due to an accumulation of mutations on highly mutable loci in ancient genotypes (e.g., Baali-Cherif and Besnard 2005; Barazani et al. 2014).

Another relevant result reported by Díez et al. (2015) concerns the South Iberian group of cultivars (namely group Q1), for which they demonstrated a relatively recent origin following a strong genetic bottleneck. Using co-ancestry analyses, they identified two ancient varieties that could be the main progenitors of Q1. This means that the selection of the Q1 cultivar group was initially based on a very limited number of genotypes. This also demonstrates that the genetic basis of the current elite olive material can be locally reduced and that the selection of

cultivars could have been sometimes constrained by available genetic resources and not necessarily involved a major contribution of autochthonous oleasters.

5 New Opportunities for Crop Diversification Out of the Mediterranean Range

Wild diploid olive subspecies can be easily crossed with the Mediterranean olive and these taxa can thus be considered as primary genetic resources of the olive (e.g., Zohary 1994; Besnard et al. 2008; Hannachi et al. 2009; Klepo et al. 2013; Arias-Calderón et al. 2015; Cáceres et al. 2015). The long evolutionary history of the olive complex in contrasted environments over three continents (Fig. 1; Médail et al. 2001; Green 2002) makes that all wild olive taxa can be considered as a putative source of genes for the improvement of the cultivated olive (e.g., Lavee and Zohary 2011), notably for adaptations to new habitats and pathogen resistance (e.g., Arias-Calderón et al. 2015; Trapero et al. 2015). Non-natural contacts between the cultivated olive and non-Mediterranean wild relatives have been favored by humans with the diffusion of cultivars. Admixture between different olive subspecies has been observed both in the native and introduced ranges of *O. europaea* (Besnard et al. 2013a, 2014).

Within its native range, the cultivated olive historically spreads beyond the boundaries of the Mediterranean area, in particular in the Middle to Far East (from Iraq to SW China), but also in Saharan oases, the Canary Islands, and the Central Saharan mountains (Besnard et al. 2013a; Noormohammadi et al. 2014; Mousavi et al. 2014; Hosseini-Mazinani et al. 2014; Zhan et al. 2015). Contacts between the cultivated olive and the wild subspecies *cuspidata*, *guanchica*, or *laperrinei* have therefore potentially occurred. Diversification of crops by admixture with different closely related taxa has been already documented in fruit trees such as apples and date palms (Cornille et al. 2012; Zehdi-Azouzi et al. 2015). In olive, early generation hybrids are rare

but have been detected with nuclear microsatellites. In particular, the ‘Dohkar’ variety showed that hybridizations between Laperrine’s and Mediterranean olives have occurred and contributed to cultivar diversification in the Maghreb (Besnard et al. 2013a).

During the last five centuries, the cultivated olive has been introduced into new regions, from the New World to Australia and New Zealand (e.g., Hobman 1993; Koehmstedt et al. 2011; Beghé et al. 2015). During crop diffusion, new genotypes were selected after supposedly uncontrolled crosses between cultivars and/or feral olives (e.g., Beghé et al. 2015). The wild African olive has also been introduced during the nineteenth century to Australia and New Zealand, and latter to Hawaii. Mediterranean and African subspecies have both naturalized in southeast Australia and have admixed in different places near Sydney and Adelaide (Cuneo and Leishman 2006; Besnard et al. 2014; Cornuault et al. 2015). In Southern Australia, promising genotypes were selected among naturalized olives (Sedgley 2004), and the possibility that these trees have been introgressed by subspecies *cuspidata* remains to be tested.

6 Concluding Remarks

The contribution of several disciplines was necessary to depict the processes of olive domestication, spread, and diversification. The olive now represents a case study of fruit tree domestication but its history is complex and several issues still need to be investigated. As mentioned above, a great part of the cultivated olive’s genetic background came from the eastern Mediterranean. Such a situation could reflect that the primary domesticated gene pool harbors major alleles of domestication at some loci that have been maintained during the secondary diversification. The identity of major traits under selection is, however, not yet clearly identified and usually relates to fruit or vegetative traits but also to increased adaptation to cultivation. Deciphering this complex process of olive cultivar selection still represents an important challenge with

potential applications in the breeding of new varieties. Furthermore, the possibility of gene exchange between cultivars and local uncultivated olives (wild or feral) was an important feature of cultivated olive diversification that potentially allowed and allows the preservation of a high evolutionary potential in the crop (e.g., McKey et al. 2010). This might facilitate its adaptation to new environments and climates, as well as the breeding for specific agronomic traits (e.g., oil quality or disease resistance; Klepo et al. 2013; Arias-Calderón et al. 2015). This important link between wild and cultivated gene pools should be preserved in the Mediterranean basin (e.g., Díez et al. 2016). In situ and ex situ conservation strategies should thus be considered for wild olive populations, especially in the Near East where genuine oleasters have been reported to be rare and endangered (Lumaret and Ouazzani 2001).

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