

Article

The Shape Diversity of Olive Stones Resulting from Domestication and Diversification Unveils Traits of the Oldest Known 6500-Years-Old Table Olives from Hishuley Carmel Site (Israel)

Jean-Frédéric Terral ^{1,2,*} , Vincent Bonhomme ^{1,2}, Clémence Pagnoux ^{2,3}, Sarah Ivorra ^{1,2}, Claire Newton ⁴, Laure Paradis ^{1,2} , Mohammed Ater ^{2,5} , Jalal Kassout ^{2,5} , Bertrand Limier ^{1,2,6}, Laurent Bouby ^{1,2} , Fiona Cornet ¹, Oz Barazani ⁷, Arnon Dag ⁸  and Ehud Galili ^{9,10}



Citation: Terral, J.-F.; Bonhomme, V.; Pagnoux, C.; Ivorra, S.; Newton, C.; Paradis, L.; Ater, M.; Kassout, J.; Limier, B.; Bouby, L.; et al. The Shape Diversity of Olive Stones Resulting from Domestication and Diversification Unveils Traits of the Oldest Known 6500-Years-Old Table Olives from Hishuley Carmel Site (Israel). *Agronomy* **2021**, *11*, 2187. <https://doi.org/10.3390/agronomy11112187>

Academic Editor: Giovanni Caruso

Received: 25 September 2021

Accepted: 25 October 2021

Published: 29 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

- ¹ ISEM, Institut des Sciences de l'Evolution-Montpellier, Université de Montpellier/CNRS/IRD/EPHE, Place Eugène Bataillon, 34090 Montpellier, France; bonhomme.vincent@gmail.com (V.B.); sarah.ivorra@umontpellier.fr (S.I.); laure.paradis@umontpellier.fr (L.P.); bertrand.limier@umontpellier.fr (B.L.); laurent.bouby@umontpellier.fr (L.B.); fionacornet@gmail.com (F.C.)
 - ² Laboratoire International Associé/International Research Project EVOLEA, INEE-CNRS, France-Morocco, Montpellier, France; clemence.pagnoux@protonmail.com (C.P.); mohammed.ater@gmail.com (M.A.); jalalkassout@gmail.com (J.K.)
 - ³ Archéozoologie, Archéobotanique: Sociétés, Pratiques et Environnements (AASPE), CNRS, MNHN, CP56, 43 Rue Buffon, 75005 Paris, France
 - ⁴ 18, Avenue de Saint-Valérien, Rimouski, QC G0L 1B0, Canada; clairenewton@gmail.com
 - ⁵ Laboratoire Botanique Appliquée, Equipe Bio-Agrodiversité, Département de Biologie, Faculté des Sciences, Université Abdelmalek Essaâdi, B.P. 2062, 93 030 Tetouan, Morocco
 - ⁶ INRAE, Centre Occitanie-Montpellier, 2 Place Pierre Viala, 34000 Montpellier, France
 - ⁷ Institute of Plant Sciences, Agricultural Research Organization, Rishon LeZion 7505101, Israel; barazani@agri.gov.il
 - ⁸ Gilat Research Center, Agricultural Research Organization, M.P. Negev, Gilat 85280, Israel; arnondag@volcani.agri.gov.il
 - ⁹ Zinman Institute of Archaeology, University of Haifa, Mount Carmel, Aba Khoushy Ave. 199, Mount Carmel, Haifa 3498838, Israel; galilish@netvision.net.il
 - ¹⁰ Leon Recanati Institute for Maritime Studies, University of Haifa, Mount Carmel, Aba Khoushy Ave. 199, Mount Carmel, Haifa 3498838, Israel
- * Correspondence: jean-frederic.terral@umontpellier.fr

Abstract: The first exploited and domesticated olive forms are still unknown. The exceptionally well-preserved stones from the submerged Hishuley Carmel site (Israel), dating from the middle of the 7th millennium BP, offer us the opportunity to study the oldest table olives discovered so far. We apply a geometrical morphometric analysis in reference to a collection of modern stones from supposed wild populations and traditional varieties of various origins, genetic lineages and uses. Analyses carried out on modern material allow the characterization of the extent of stone morphological variation in the olive tree and the differentiation of distinct morphotypes. They also allow to discuss the status of supposed wild populations and the divergence between groups of varieties and their wild progenitors, interpreted from evolutionary and biogeographical perspectives. The shape of archaeological stones compared to the differentiation model unveils morphological traits of olives most likely belonging to both wild olive trees and domesticated forms, with some of them showing a notable domestication syndrome. These forms at the early stages of domestication, some of which are surprisingly morphologically close to modern varieties, were probably used for dual use (production of olive oil and table olives), and possibly contributed to the dispersion of the olive tree throughout the Mediterranean Basin and to its subsequent diversification.

Keywords: archaeobiology; domestication and diversification; morphometrics; *Olea europaea* L. subsp. *europaea* (olive tree); perennial crop history; shape diversity; table olives

1. Introduction

The olive tree (*Olea europaea* L. subsp. *europaea*.) is undoubtedly the iconic fruit tree of the Mediterranean Basin. Its origins are enrooted in a palaeogeographical and palaeo-ecological history of several million years and linked with the Mediterranean climate [1–3]. The wild olive tree or oleaster (*O. e.* subsp. *e.* var. *sylvestris*), the ancestor of all the cultivated varieties (*O. e.* subsp. *e.* var. *europaea*) [4], is a characteristic element of the Mediterranean vegetation, in particular of the meso-thermomediterranean bioclimatic stages of which it is one of its main markers [5]. It can be found, often mixed with numerous feral individuals escaped from cultivation [6], in the matorrals and woodlands of xerophytic Mediterranean areas. It is currently present in the Levant, Turkey, the Peloponnese and coasts of mainland Greece, the Maghreb, the Southern Iberian Peninsula, Southern Italy, Cyrenaica (Lybia), the Mediterranean islands, and more sporadically on the Northern Mediterranean coast [7]. Its distribution area is less extensive than that of the cultivated olive tree. Nowadays, more than 1200 olive varieties are cultivated all around the Mediterranean which accounts for 90% of the cultivated area globally [8], to produce oil and table olives (<https://www.internationaloliveoil.org/> (accessed on 25 October 2021)).

Palaeobotanical and archaeobotanical findings show that the oleaster persisted in the Mediterranean, even at the height of the pleniglacial cooling [9,10]. Very scarce carpological data are related to Palaeolithic and Mesolithic hunter-gatherer populations that lived on the shores of the Mediterranean during the last glacial period and the early Holocene. The occasional recordings of olive stones, however, show that wild olives were sometimes consumed. As such, the example of the underwater site of Ohalo II, in the Sea of Galilee, is quite exceptional because of the discovery of thousands of charred fruits and seeds, including olive stones, which are evidence of the diet of the Epipaleolithic hunter-gatherers who lived in this habitat around 21,000–18,000 BP [11,12]. The existence of refuges during the Last Glacial Maximum, in the Near East, in the south of the Iberian Peninsula, as well as probably in North Africa and Sicily, would have favored a rapid expansion of the oleaster on the shores of the Mediterranean with the Holocene warming. The rapid Holocene expansion of olive populations was driven first by climatic factors and then by human activity, as shown by *Olea* palynological records, increasing over time in both the Eastern and Western Mediterranean Basin [10,13–15]. Olive macro-remains became more common between 11,000 and 5000 BP and penetrated new regions, particular in Western Mediterranean areas [16,17].

Recent genetic studies based on nuclear microsatellite and plastid markers revealed an East-West differentiation of oleaster populations [18–22]. A coalescent-based Bayesian approach further specified the geographic structure of the oleaster genetic diversity in the Mediterranean, consisting of 3 distinct lineages [21–23]: E1, from the Peloponnese (Greece) to the Levant; E2 and E3 in the western part of the Mediterranean Basin.

Olive domestication is considered to have begun in the Levantine region during the Chalcolithic period (6000–5500 BP) [7,13,24–27]. The beginnings of oleiculture in the Near East are already well documented, both by archaeology and archaeobotany, but also by written sources [7,28,29]. Rock-hewn structures observed at several sites in the Jordanian highlands were interpreted as olive mills dating back to the Chalcolithic period [30]. Around the same period, archaeobotanical remnants (stones, charcoal) of olive trees are more frequent. Stones found in archaeological excavations are often fragmented, which is generally regarded as a marker of oil extraction. In reference to the Oleaster distribution range assessed by a genetic model [21], some of these sites located outside of the bioclimatic zone where the oleaster can grow today were interpreted as a reliable sign of cultivation and agronomical developments (crop irrigation, seedling transplantation and probably grafting) [7,27,31,32]. Olive remains from Kfar Samir, a submerged site off the Carmel Coast located some 1800 m north of Hishuley Carmel site, provide early evidence (around 7000 BP) of oil extraction [25,33]. Their morphometric patterns were studied using traditional morphometry [34]. The results showed new evidence of olive exploitation, probably oleaster, supporting the hypothesis that olive exploitation and management emerged

centuries before domestication. A similar pattern of pre-domestication was recorded in Spain between the 3rd and 2nd millennium BP [35,36]. At Ebla (Syria), during the Bronze Age (5th millennium BP), administrative records engraved on clay tablets showed vast olive plantations under royal control, with tax royalties paid by peasants in the form of oil [28].

According to archaeological and archaeobotanical data, from the Levantine primary domestication centre, selected olive forms, slowly and gradually diffused, probably by vegetative propagation through cuttings or grafting, and so is the associated agronomic knowledge and techniques. They first reached the Aegean around 4500 BP, then the Central and Western Mediterranean where domesticated olives were found in Italy and Spain in the Late Bronze Age, around 3200–3000 BP [26,37,38]. Finally, they reached Southern France around 2800–2600 BP.

In many occidental areas, domesticated olive forms introduced from the Eastern Mediterranean crossed with local wild or domesticated varieties [39]. These secondary domestication centres have played a fundamental role in the adaptation of non-native varieties and the diversification of the olive tree. Hence, selection events took place independently of the primary centre, such as in Southwestern Spain, 1500–1000 years before the introduction of new varieties, probably by the Phoenicians [40]. Subsequently massive and repeated arrival phases of new domesticated forms totally blurred the original local genetic diversity. Later, the Roman oleiculture has left in all Mediterranean countries numerous and varied archaeological remains providing valuable documentation of the development of production over space and time [28,41]. Olive oil production sites can be identified by the presence of mills or grinders, presses, vats, and cellars with storage jars, as well as crushed stones, representing extraction waist. Amphora manufacturing workshops are generally located in the production areas. These containers, often characteristic of a particular content (e.g., oil, wine, fish sauces) and intended for exchanges and trades transport for exchange, also make it possible to follow commercial interactions in the ancient world [42].

In spite of this abundance of archaeological, bioarchaeological, historical and genetic data acquired over the last 20 years, the characteristics and identity of the first domesticated varieties, especially those that were used for the production of table olives, are still unknown. Moreover, the ancient texts and treatises mentioning different types of olive trees do not seem to be useful in identifying ancient varieties [43]. These texts mainly mention the methods, recipes, and preparation processes, which may vary according to the degree of fruit maturity (green and black olives) such as debittering, the use of condiments to improve the taste (seasoning) and preservation processes for commercial purposes and transport.

The discovery of numerous waterlogged stones in Hishuley Carmel (Figure 1), a submerged site off the Carmel Coast (Israel) in the heart of the olive domestication cradle, offers an unprecedented opportunity to provide new insights into the possible first olive varieties. Some of these stones have been analyzed in a preliminary manner using a comparative approach of traditional morphometry [33]. The sizes of these archaeological stones were compared to stones from two current local varieties (Souri, a traditional local variety and Barnea, a modern variety developed in the 1970s in Israel). This previous analysis study, based on a narrow modern reference collection and morphological characters greatly influenced by environmental conditions [40,44,45], suggested that the stones from the Hishuley Carmel site could belong to wild forms ('undomesticated'), without excluding the hypothesis that they could be derived from domesticated olive trees [33].

The present study focuses on the morphological changes of olive stones associated with their domestication and the identification of shape changes that accompanied, the selection pressures occurring during the domestication process (domestication syndrome). It uses geometric morphometry applied to archaeological stones, which are further compared to a modern reference collection of stones from wild olives, feral forms and modern varieties.

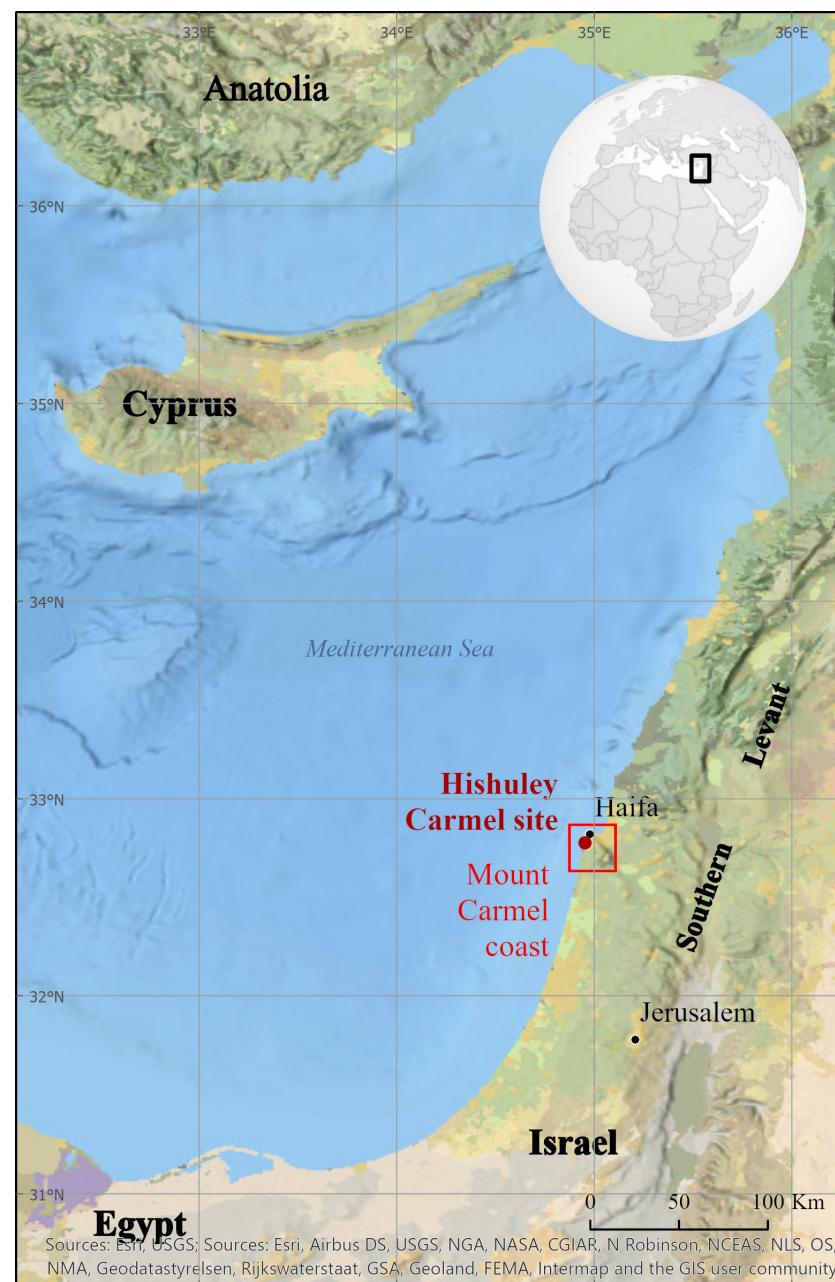


Figure 1. Location of Hishuley Carmel site (Israel).

Firstly, we aim to decipher the morphological diversity of olive stones and identify the relationships between stone shape, uses of different varieties, and biogeographical, biological and genetic traits of the modern olives. Then, a comparison of the morphological signatures of waterlogged olive stones recovered from two distinct structures at the Hishuley Carmel site with this discriminant model is performed for the first time. We expect to reveal the features of some of the oldest domesticated forms in the Levantine cradle of olive domestication, whose fruits were used to produce table olives, as shown by archaeology. The results are placed with a more general context, relating to the evolutionary and biogeographical history of the olive tree in the Mediterranean.

2. Materials and Methods

2.1. Plant Material

Archeological stones were recovered in 2011 from the submerged prehistoric site of Hishuley Carmel, on the Mount Carmel coast (Israel) (Lat. 32.77714, Long. 34.95371),

during the course of underwater surveys (Figure 2). Stones were located in 2 distinct constructed structures (A and B), located 3 m apart, which were interpreted as installations used for table olives production [33]. Archaeological data and experiments demonstrated that the stones were issued from fruits prepared (debitting, pickling or dry salting) to be consumed whole (table olive).



Figure 2. Olive stones from the Hishuley Carmel site during cleaning, sorting and recovery (photo taken by Sarah Ivorra).

Numerous stones were collected from these two structures and have been carefully examined. Ninety-nine stones were collected from structure A and 148 from structure B, which were apparently undeformed, unbroken, and well-preserved. These were sampled and analyzed using the geometric morphometric method presented below. A subsample of few olive stones from each structure was dated using ^{14}C .

The modern consisted of 319 stones from 17 supposed wild olive populations (Table S1) and 1641 stones from 55 varieties (Table S2) from various geographical origins in the Mediterranean Basin. Some of these were synonymic varieties such as Koroneiki and Psilolia (Crete), as well as Athalassa and Lefkara (Cyprus). The varieties correspond to the same cultivar, thus to the same genotype, but are named differently according to geographical and/or cultural factors. Three varieties from different countries (Souri (Israel), Sourani (Syria), and Istambuli (Turkey)) are suspected to be the same cultivar. Thus, the shape of these varieties is supposed to be identical or very similar.

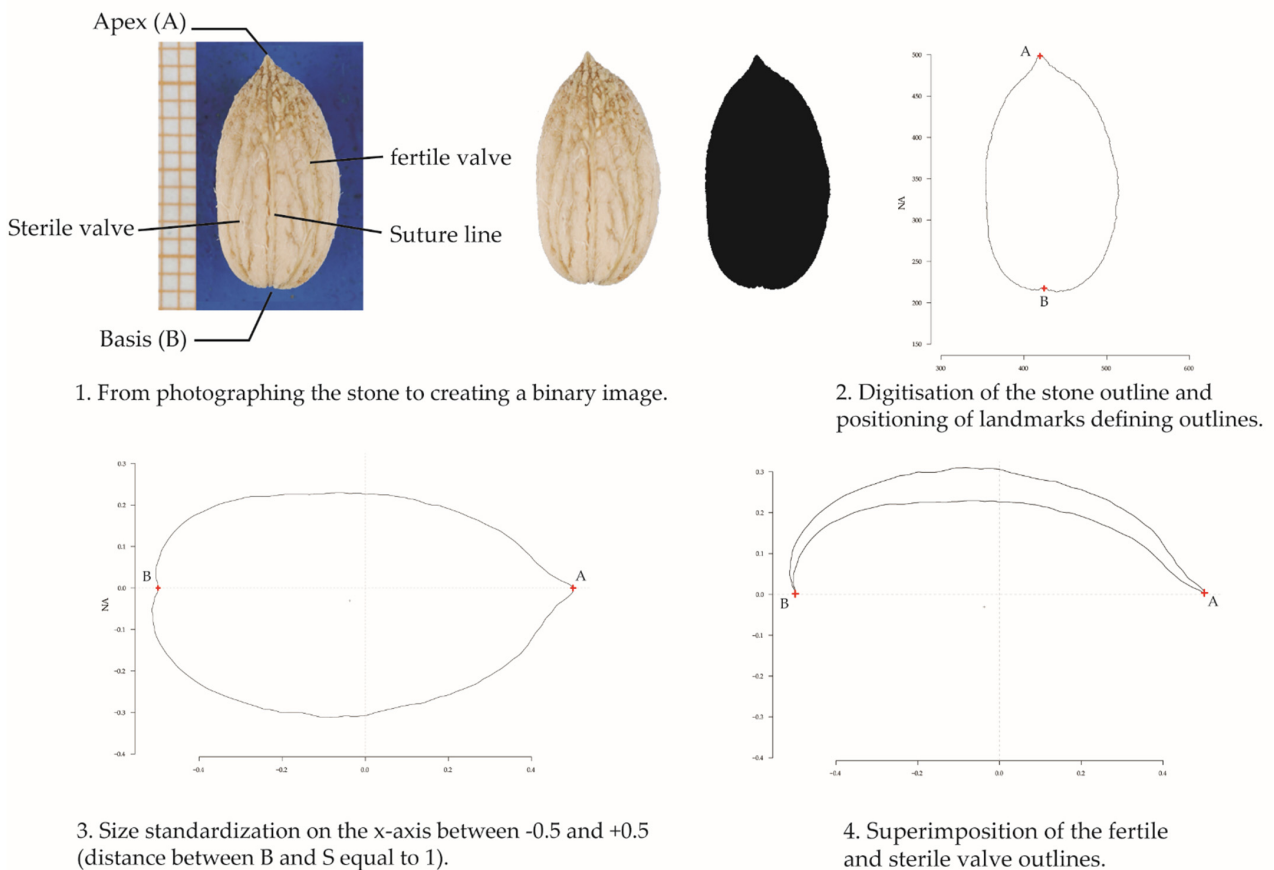
Stones were collected between 1994 and 2019 during the course of field surveys, either by our team or through collaborations. All the stones of these varieties were collected in the 'Conservatoire Botanique National Méditerranéen de Porquerolles' (Porquerolles Island, France); the Melgueil INRAE collection (Maugio, France); the experimental station of Tassaout (WOGB-Marrakech, INRA-Morocco); olive groves in the Peloponnese, Phocis, and Crete (Greece); and orchards in Galilee (Israel) in order to provide a representative sample of the current diversity of olives cultivated around the Mediterranean Basin.

2.2. Stone Shape Analysis

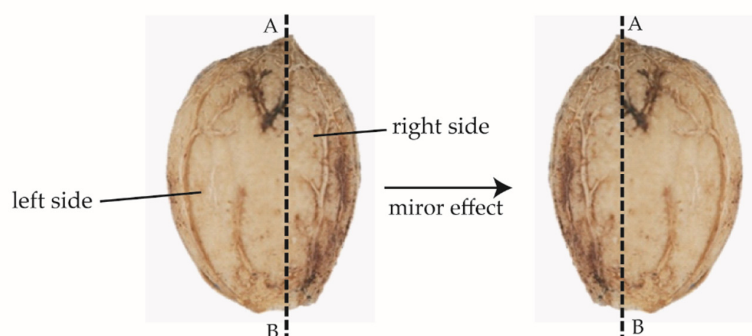
All analyses were performed in the R 4.0.0 [46] with the *Momocs* 1.3.1 package for morphometrics [47,48], using *MASS* 7.3.51.6 [49] for discriminant analyses and cross-validation, hierarchical clustering analysis and *ape* for unrooted tree representation [50].

The olive stone is a sclerified endocarp whose shape varies from subspherical to fusiform, according to its genetic origin (Figure 3). It is composed of two merged asymmetrical valves (fertile and sterile) protecting one seed and merged at the level of the suture line. Each valve is a carpellar leaf, itself asymmetrical when observed in dorsal view. In order to remove the potential effect of asymmetry, the outlines are positioned so that size (right side) > size (left side) (Figure 3).

A. Olive stone in lateral view (example of one stone from the Cyressino variety):



B. Fertile valve of the olive stone in dorsal view (example of one stone from a wild tree):



Before the digitisation step, stone is positioned using a mirror effect such that Size (right side) > Size (right side) in order to remove the fluctuating asymmetry effect.

Figure 3. The morphometric protocol applied to olive stones described step by step (Sarah Ivorra and Jean-Frédéric Terral, CNRS/UM—ISEM).

The 4 open outlines (fertile and sterile valves in lateral view; right and left sides of the sclerified carpellar leaf in dorsal view) were defined in a first session by 20 and in a second session by 120 landmarks (x ; y), including the 2 homologous points [basis (B) and apex (A) of the stone]. Our objective was to test the descriptive power of open outlines in relation to the number of landmarks used; in other words, to test whether the use of a large number of landmarks allowed for better capture of the morphology of open outlines (Figure 3).

In previous studies, such open outlines were fitted using a polynomial regression [40,51], while third-polynomial curves were used to characterize olive valves. This fitting appears to be a suitable compromise between quadratic polynomial curves (x^2 —parabolic) which are too imprecise, and fourth-degree polynomial curves which tend to exaggerate local irregularities in the outlines. Unfortunately, the use of the third-polynomial curve meant it was not possible to record the acuminate or pointed apex, characteristics of stones of certain varieties, such as ‘Olivière’.

Finally, each stone was defined using 2 equations: (1) fertile valve: $y_F = b_0 + b_1x + b_2x^2 + b_3x^3$; (2) sterile valve: $y_S = b'_0 + b'_1x + b'_2x^2 + b'_3x^3$.

The b_i coefficients, including the intercept b_0 , were used as quantitative variables in further statistical analyses. In such natural polynomial equations, b_i coefficients are correlated and change along increasing fitting degrees. This is why we used the orthogonal polynomials, also called Legendre’s polynomials, as a method of fitting a least-squares curve along each valve outline and providing uncorrelated coefficients.

Legendre polynomials are the simplest of the orthogonal polynomials because their weight function is equal to 1. For n -Legendre polynomials, $P(x)$, which are recursive [52], and n -Legendre coefficients, c , which are used as shape descriptors, the expansion of the width function, $W(x)$, is:

$$W(x) = \sum_1^N C_n P_n(x)$$

The width function $W(x)$ is a linear combination of Legendre polynomials of degree n degree in x , and each Legendre coefficient, C_n , is an independent shape descriptor due to orthogonality. Legendre polynomials form a complete orthogonal system based on the interval $[-1, 1]$ and a weight function (ρ) of 1. Expansion of the width function represents the expression of Legendre polynomials as a series. From (x , y) coordinates of olive stone valve outline, uncorrelated coefficients from the orthogonal polynomial regression were used as numerical shape descriptors. Practically, they were obtained using the *opoly* function in the *Momocs* package [47].

Each stone (modern and archaeological) generated 16 quantitative parameters (8 for the lateral side—4 for the fertile valve and 4 for the sterile valve; 8 for the dorsal side—4 for the right side and 4 for the left). These 16 quantitative parameters of shape are used for multivariate statistical analyses.

2.3. Statistical Analyses

The descriptive power of the two digitalization approaches (open outlines defined by 20 and 120 landmarks) was tested using the RV test by comparing the 2 morphological distance matrices expressing morphological disparity between both wild populations and varieties. Potential differences in shape among the 2 archaeological stone sets were tested using a multivariate analysis of variance (MANOVA) on the polynomial coefficients. Linear discriminant analysis (LDA) was performed on the modern reference collection of 1940 olive stones in order to test the morphological discrimination between 71 accessions of olive trees defined as 15 supposed wild populations with 20 stones per population except for Dor (19 stones) and 55 varieties with 30 stones per variety, except ‘Djlot Shami’ (27 stones), ‘Gaidouriola’ (29), ‘Kortbi (29), ‘Souri’ (27) and ‘Tanche’ (29). Indeed, some stones with slight malformations caused by parasite attacks were excluded. A hierarchical ascendant clustering using the Ward method on the distance between each supposed wild population and variety allowed us to highlight the underlying grouping structure among accessions. Each cluster corresponds to a morphotype including the wild population, with varieties or

mixtures whose mean shape may be calculated. The discrimination of each morphotype was recalculated at different levels of aggregation of the clustering hierarchical clustering tree using the confusion matrix established by the LDA. Different levels of aggregation are possible and we chose a 75% of discrimination accuracy threshold as being robust enough for further used for archaeobotanical inference, and to stop refining.

The archaeological olive stones were then compared to the retained morphotypes. These were included in the LDA as predicted individuals and then assigned to a morphotype with a probability of identification which corresponded to the sum of the assigned probabilities of inferred wild and feral populations and/or varieties that made up the morphotype. Stones with a posterior probability of assignment <0.75 were filtered out.

3. Results

3.1. Number of Landmarks Used to Define the Stone Outline

Regarding the entire available material, the open outlines of all olive stones (4 curves: fertile and sterile valves in lateral view; large and small sides of the fertile valve in dorsal view) were defined by 20 and 120 landmarks in two measurement sessions, aimed at testing the descriptive power of open outlines in relation to the number of landmarks used. The polynomial coefficients were synthetic shape variables, which were treated as quantitative variables in a linear discriminant analysis (LDA) whose explanatory variable included 72 modalities corresponding to supposed wild populations and cultivars. The morphological disparity between wild populations and cultivars was summarized within two Mahalanobis distance matrices between each population or cultivar centroid (for 20 and 120 points per outline, respectively). The two distance matrices were compared using the RV test. The results of the test ($RV = 0.97$, $p < 0.0001$) indicated high similarity between distance matrices. Therefore, defining the contours using 120 points did not provide a more precise description of the morphology of the olive stones, than when using 20 points. The following analyses were thus performed using 20 landmarks per outline for both modern material and archaeological stones. Shape descriptors of the modern and archaeological stones are presented in Tables S3 and S4, respectively.

3.2. Morphological Differentiation between Current Wild Populations and Varieties

The hierarchical clustering was calculated using the Ward approach on the Mahalanobis distance matrix calculated on the accession (supposed wild populations and varieties) centroid. A typology based on morphological relationships among accessions and distinct morphological groups was established (Figure 4). With this classification method, increasing members of olive populations and cultivars were linked together and aggregated in larger clusters of increasingly dissimilar elements. The robustness of the clusters was assessed through their accuracy: that is the correct proportion of accessions correctly classified in this cluster versus all others.

The cluster analysis and linear discriminant analysis (LDA) carried out using the morphometric data emphasized 4 main sets or morphological clusters of modern reference olive stones, within which several morphotypes were distinguished. A total of 12 main morphotypes were evidenced with discrimination rates greater than or equal to 75% (Figure 4; Table S5).

The first set included two morphotypes (MT1 and MT2). MT1, mainly composed of stones from supposed wild populations, was characterized by rounded and rather symmetrical stones. MT2 was characterized by slightly asymmetrical stones in lateral view (the sterile valve was less voluminous than the fertile valve) with a rounded base and a slightly narrowed apex. The second set (MT3 and MT4) comprised more tapered, elliptical, non-acuminate, and slightly asymmetric stone morphologies in lateral view for MT3, and slightly acuminate stones with relatively pronounced asymmetry in lateral view for MT4. The third set consisted of 5 morphotypes that were distinguished by a very tapered stone (decreased thickness toward one end). The MT5 stones were weakly acuminate and asymmetrical, essentially in lateral view. The MT6 stones resembled the

MT5 stones, although the apical tip was slightly more pronounced and the centre of gravity of the stone was shifted towards the apex. MT7 was characterized by very tapered and asymmetrical stones, especially in lateral view, with a centre of gravity located in the middle part of the stone or even slightly below. MT8 had stone features with the same characteristics as MT7, but with the centre of gravity located at the upper part of the stone, which were asymmetrical in lateral view. MT9 was similar to MT8 but its stones were much more tapered. The fourth and last set was defined by 3 distinct morphotypes. MT10 was characterized by elliptical stones that were slightly asymmetrical in lateral view. MT11 was distinguished from MT10 by its slightly acuminate apex. For MT12, the stone apex was more pronounced, meaning the centre of gravity shifted slightly towards the base of the stone.

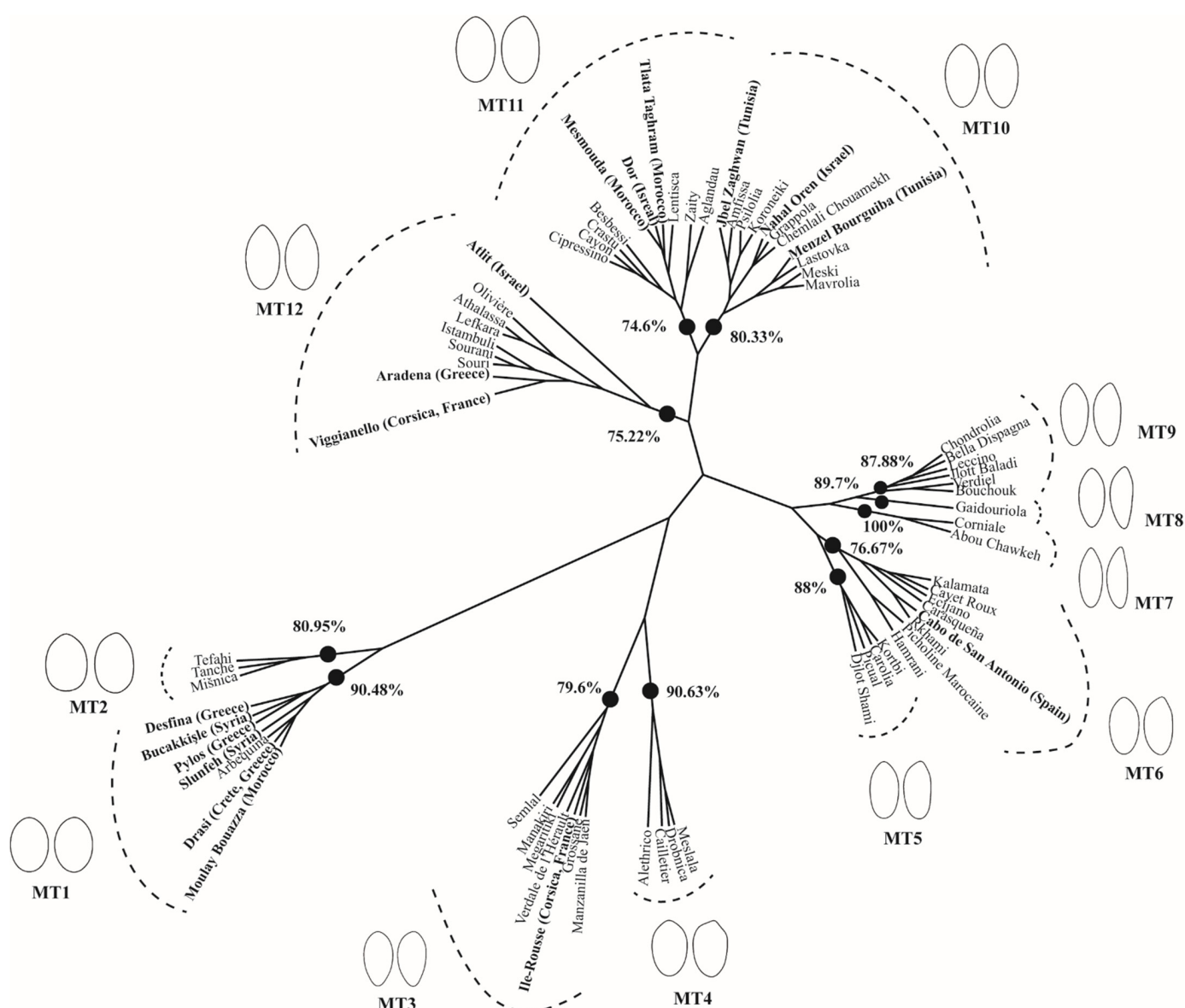


Figure 4. Hierarchical clustering obtained for the supposed wild olive populations and varieties. Discriminant rates (%) and reconstructed size-standardized stone shapes in dorsal (left side) and lateral views (right side) for the 12 identified morphotypes are shown. Supposed wild populations are written in boldface.

The varieties constituting these morphotypes were compared to: (1) the origins of populations and varieties according to the biogeographical context in the Mediterranean basin [53] (Tables S1 and S2; Figure S1A); (2) the main use of the varieties (Table S2; Figure S1B);

(3) their geographical origin inferred by DNA nuclear markers (gene pool) [21,54,55] (Table S2; Figure S1C); (4) their affiliation of a specific maternal lineage (cpDNA) [20,21,55] (Table S2; Figure S1D).

3.3. Morphological Variability of the Two Sets of Archaeological Stones (A and B) from Two Distinct Structures in the Hishuley Carmel Site

In order to compare the shape of stones from the two archaeological sets sampled from the two distinct structures of olive processing structures, the MANOVA carried out on the 16 orthogonal polynomial coefficients to compare the morphologies of the two stone sets showed that there were no significant differences between them (Wilks' lambda = 0.922, $p = 0.262$). For further analyses such as a comparison of the archaeological stone shape to current morphotypes, the two sets were treated together as a single entity. The results of morphometric analysis performed on the archaeological stones are presented in Table S4.

3.4. Dating of Archaeological Olive Stones

Olive stones from the two sets corresponding to two distinct archaeological structures were dated using radiocarbon (Figure 5). They were both dated in the mid-7th millennium BP (A: cal. 6638–6449 BP and B: cal. 6679–6498 BP) and from a cultural point of view in the Chalcolithic period [33].

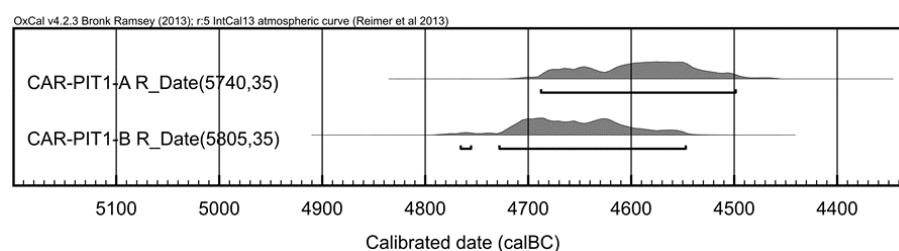


Figure 5. Report on C-14 dating of olive from structures A and B in the Hishuley Carmel site, carried out in the Poznań Radiocarbon Laboratory, Poland [33].

3.5. Identification of Morphotypes in the Archaeological Material

The LDA trained on the modern material was used to predict the domestication status of the 247 archaeological stones. The LDA was then assigned to a morphotype with a probability of identification which corresponded to the sum of posterior probabilities of assignment of wild populations and/or varieties that made up the 12 morphotypes. Identifications associated with a posterior probability greater than or equal to 0.75 were retained; those below were filtered out.

Ninety stones (36.8%) could not be classified, probably due to deformations not detected during their initial examination or because these archaeological stones did not have any current analogue in our reference collection. Among the morphotypes distinguished in the current reference material, 8 morphotypes were highlighted in the archaeological material but with a different relative frequency. While some morphotypes were represented by only a few stones (MTs 3, 4), 5 main morphotypes dominate (Figure 6):

- MT1 (40 stones—25.5% of the classified archaeological material), consisting of stones from supposed wild populations and one variety with a ‘primitive’ morphology (Arbequina) differing from wild forms by its larger size, although this trait was not considered in this study. Arbequina was distinguished from wild populations with a rate of 86.7%;
- MT2 (12 stones—7.6%) defined by stones of varieties (all for table or mixed use) from the Eastern Mediterranean and one French variety. All varieties were allocated to the Eastern gene pool;
- MT10 (15 stones—9.6%), composed of varieties of the Eastern maternal lineage, which were relatively different in terms of geographical origin (cultivation area and nuclear genetic data) and use of the fruit;

- MT11 (25 stones—15.9%) composed of supposed wild populations and varieties with diverse origins, uses and maternal lineages;
- MT12 (15 stones—9.6%) including mainly oriental varieties used primarily for oil or mixed use.

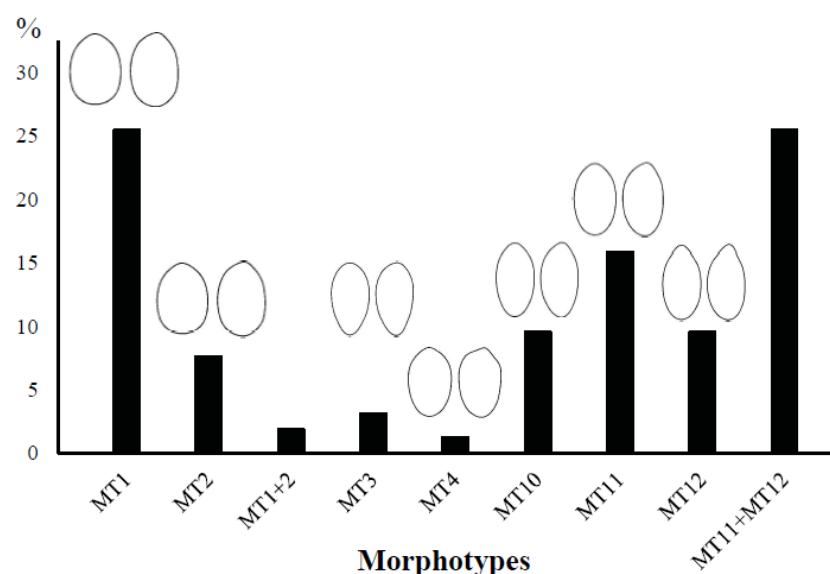


Figure 6. Percentage of archaeological stones affiliated with LDA-defined morphotypes.

Finally, 3 stones (1.9%) and 40 stones (25.5%) were classified in MT1 + 2 and MT11 + 12, respectively, at a higher level of aggregation, respectively.

4. Discussion

It is known that the Levant region is the cradle of olive domestication [13,28,39]. Although this issue has long been debated, studies across various domains (archaeology, archaeobotany and genetics) agree that the Eastern genetic resources provided the bulk of the cultivated pool, which today are very diversified and widespread around the Mediterranean Basin, and beyond. However, this primary domestication model does not challenge the existence during glacial periods of refuges located south of the Iberian and Italian peninsulas, which enabled the oleaster, the ancestor of the cultivated olive, to progressively recolonize north Northern European areas. Prehistoric and protohistoric cultures exploited the local oleasters, and whether, unconsciously or not, probably selected interesting variants that further resulted in morphological types [36,56,57]. However, these likely punctual and minor events of human selection are nowadays almost totally masked by the diversity of oriental forms that were introduced and spread all over the Mediterranean throughout ancient and medieval times. The main areas where eastern varieties were introduced, which probably vegetatively propagated and then crossed with local clones, constituted secondary domestication and diversification centres favoring olive adaptation. The Picholine Marocaine variety is a very demonstrative example. In fact, it belongs to the Near-Eastern maternal lineage (E1) but is characterized by Western nuclear markers [21,54,55]. Its genetical duality shows that its ancestors are of Eastern origin, but since they were introduced further west, they have been progressively admixed with Western forms involving the substitution of the former nuclear genetic material by a ‘Western genome’.

While palaeogenomics has opened up promising perspectives on the evolutionary history and genealogy of ancient cultivated forms, morphometric tools remain essential in deciphering morphology of bioarchaeological material in a non-destructive way and in characterizing the status and relationships of modern populations. Even if the use of morphometrics approaches, such as palaeogenomics, involves post-depositional constraints

that may partially or totally degrade the material, the acuity and resolution power are not dismantled. The quantification of shapes by means of mathematical and statistical approaches is both descriptive and decisional by providing very efficient biosystematic criteria. Regarding grapevine, the validation of geometric morphometric results related to the history and identity of Roman French grape varieties [44] using palaeogenomics [58] has illustrated the level of resolution achieved by shape analysis, which is often at the infraspecific level [59]. Measuring the phenotype also means integrating genotype expression through the filter of development. This is modulated by the environment and by abiotic (climatic or cultivation practices in the case of cultivated plants) and biotic (extrinsic and intrinsic, such as inter-individual or intra-individual competition) factors. Competition between seeds developing within a closed structure, such as the pips in a grape berry, illustrates the role played by developmental and endogenous factors within the same individual [45]. The use of the geometric component of morphology (shape), independently of size (by size standardization), will allow studies on conservative criteria as demonstrated by previous studies showing that environmental factors do not significantly influence the stone geometry [26,40]. Moreover, geometric morphometrics allow to overcome a number of interpretative barriers related to changing environmental pressures to be overcome. This technique also limits or annihilates phenotypic plasticity (i.e., size changes across an environmental gradient), and finally reveals variations of genetic origin such as those related to artificial selection pressures and domestication (see [60,61]). The use of traditional measurements provides size variations that have been developed over long chronological periods, meaning the reproducibility of the trait measurements has been tested previously.

In this study, the quantified morphological variations, ranging from rounded stones to more elliptical, tapering and asymmetrical shapes, highlight the diversity of spontaneous forms growing today in Mediterranean plant communities. The supposedly wild forms are distributed across several distinct morphotypes (MTs: 1, 3, 6, 10, 11 and 12) (Figure 4). To interpret such morphological diversity, Terral et al. [40] invoked different geographical origins, arguing that the morphology of the oleaster stones have differentiated as a result of the Quaternary fragmentation of the former distribution area. This fragmentation resulted in the rupture of gene flows between regions during ice ages, especially between the Eastern and Western Mediterranean. This hypothesis was supported by genetic data [18]. However, more recent studies have shown that this geographical differentiation is actually indirect, through the feral status of these populations [26,62,63]. Indeed, these populations were morphologically differentiated because they derived from varieties of different morphological features and distinct geographical origins. Therefore, in order to infer the status of these populations, it is necessary to refer directly to the morphology. Morphotype 1 (MT1) corresponds to round-shaped stones, a simple and minimalist morphology that is also found in other wild relatives or ancestors of cultivated perennial species such as grapevine (*Vitis vinifera* subsp. *sylvestris*) [44,45,59,64] or the palm genus (*Phoenix* spp.) [60,61,65]. The single variety enclosed in MT1 would be one variety with a 'primitive' (*sensu* plesiomorphic) morphology that human breeding pressures have barely impacted. In contrast, other morphological types of supposed wild populations would most likely represent feral forms, as noticed for Israeli populations [6]. Feral olive trees are omnipresent in the Mediterranean region, and are exploited in some areas for different purposes (rootstock, food, cosmetics, medicinal uses) [66,67]. The issue of feral olives in Mediterranean plant communities raises the question of the future of genuine wild populations subject to increasing human disturbances and genetic pollution (gene flows between cultivated orchards and local wild populations), especially since olive plants are wind-pollinated. Finally, it is important to stress that MT1 is not geographically structured. Stones of this morphotype may be considered as the ancestral morphotype showing a robust phenotype (canalization process according to the theoretical model of Waddington [68] which was revisited more recently by Siegal and Bergman [69], i.e., an inexistent or very low phenotypic plasticity, despite genetic variations and heterogeneous environmental

constraints). The same trend has been demonstrated in other species mentioned above, namely in grapevine and date palm [44,60].

Unlike oleasters, any morphological deviation from the genuine wild morphotype may be considered as the result of human selection pressures, although the stone geometry was certainly not the target of domestication. The morphological deviation from the wild rounded morphotype may be considered as a tenuous but real domestication syndrome.

Numerous morphotypes have been found and distinguished, ranging from elliptical with rounded extremities to more tapered, apex-pointed and asymmetrical shapes (Figure 4; Table S4). These result from a complex history and evolution processes related to human-associated migration and the spread of olive forms in the Mediterranean Basin over millennia. Moreover, it is important to notice that morphotypes characterized by a very pronounced domestication syndromes such as MT5, 6, 7, 8, or 9, are discriminated at a low level of aggregation in the clustering. On the other hand, the other morphotypes whose morphological divergence from MT1 is lower, are distinguished at a higher level of aggregation. This is the case for MT10, 11 and 12.

The results of the identifications of the archaeological stones clearly show that oleasters (probably genuine wild olive) have long been exploited for fruit to be treated (dry salting, debittering and pickling) for human consumption [33] as in the context of Hishuley Carmel site or to be used to produce oil, as recently demonstrated in Roman Andalusia (Spain) [63]. In the middle of the 7th millennium BP on the Carmel coast, the olive tree was probably at an early stage of domestication. This may explain why only stones characterized by a weak (low morphological deviation from the wild morphotype) but real domestication syndrome (MT2, 3, 4, 10, 11, 12) were identified in the archaeological material (Figure 7).

Therefore, it is not surprising that we found the wild morphotype to be associated with domesticated forms that are morphologically different, such as stones from morphotypes 2, 10 and 11, which were the most numerous. These distinct morphotypes evidenced in the archaeological material seem to characterize two different stages of domestication. MT2 appears to be relatively close to MT1 given the results of the cluster analysis (Figure 4). However, it is defined by table olive varieties of Eastern origin from both geographical and genetic points of view (Table S4). These results are in agreement with the geographical (Israel) and archaeological contexts (devices used for table olive production) of the site. MT11 shows a more accentuated morphological divergence (domestication syndrome), suggesting that varieties of this group from mainly Eastern Mediterranean areas, are at a more advanced stage of domestication. Although the following features were deliberately not the focus of our investigations for reasons mentioned above, fruits of this group are bigger and offer the consumer a larger pulp but not necessarily higher oil content. Fruit size, fruit production and oil content are certainly traits that, originally and empirically, were selected (for the first time in the Levant) from wild morphological variants and then maintained by vegetative means (cuttings and grafting). However, the archaeological stones are mostly affiliated with groups of varieties used for the production of oil or as table olive (Table S4). Thus, we suggest that the olive tree was first domesticated for oil production. Indeed, the treatment of the fruit to remove bitterness and lead the initiation of the fermentation process, required to reduce the pH and allow the preservation of olives for long time involves a more complex process. Later, the fruit was used for different purposes and variants with larger fruit selected for the production of table olives.

These morphotypes did not remain confined to the Mount Carmel area but contributed to the spread of the olive tree in the Mediterranean basin. Indeed, the morphotypes recognized in the archaeological material are constituted by varieties regarded today as having various origins, even if the Eastern of admixed lineages dominates (Table S2; Figure S1), testifying to the complexity of exchanges and agrobiodiversity around the Mediterranean Basin. Within the current research context, MT2 was recognized in Egypt during the 7th century BC (Persian period) [32]. MT10 and 11 were evidenced for the first time, in continental Greece at the transition of the 2nd and 1st millennia BC (Iron Age) [70],

in Northwestern and Southern Spain, in the 5th century BC (Iberic period) [40] and since the 1st century BC (Roman period) [63], respectively.



Figure 7. Photographs of archaeological stones from the Hishuley Carmel site allocated to modern olive morphotypes from which a representative was photographed and trimmed and the stone was masked before the extraction of open outline coordinates (photos taken by Sarah Ivorra and Clémence Pagnoux).

5. Conclusions

This study provides new insights and knowledge on the history of olive exploitation and domestication from archaeological and evolutionary perspectives. Based on geometrical descriptors of stone outlines, traits weakly influenced by environmental parameters, especially climatic and cultivation practices, this study allows the characterization of the phenotypic features of exceptionally preserved stones of the oldest table olives uncovered so far. Using a reference model of stone shape diversity and divergence based on a modern collection of stones of supposed wild populations and varieties, originating from different regions of the Mediterranean, the analysis of archaeological stones revealed a surprising shape diversity. These range from a round morphotype, considered ancestral and typical of genuine wild forms, to more complex morphologies, testifying to the strong selection pressures (asymmetrical, tapered stones that can be pointed at the apex). The main morphotypes found at Hishuley Carmel illustrated in Figure 7, show how close the shape of the stones is to some modern varieties. Since the emergence of olive domestication, some selected shapes would not have changed significantly over time, considering that for some varieties today represented by heritage trees that are several centuries or even millennia old, few generations have succeeded one another until today.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112187/s1>, Figure S1. Clustering of the obtained morphotypes, with cross-validation values on branches. For varieties, the panels represent the same tree with tips colored according to the different biogeographical origin (A), use (B), origin inferred by DNA nuclear markers (C) and maternal lineage (D). Table S1. Supposed wild olive (oleaster) populations. Table S2. Studied cultivated varieties. Table S3. Geometrical parameters (*bi*) for each supposed wild population and variety from morphometric analysis of modern reference olive stones. These parameters were calculated using 20 landmarks per outline. (A) dorsal view; (B) lateral view. Table S4. Morphotypes differentiated by cluster analysis, constituted by modern supposed wild populations and varieties whose area of cultivation and belonging to a gene pool according to genetic data [21,54,55] are presented. Table S5. Geometrical parameters (*bi*) from morphometric analysis performed on archaeological pits from Hishuley Carmel site.

Author Contributions: Conceptualization, J.-F.T. and E.G.; methodology, J.-F.T., C.P., V.B. and S.I.; resources, J.-F.T., A.D., O.B., C.P., S.I., L.P., M.A., J.K., C.N., B.L., L.B. and E.G.; investigations, J.-F.T., V.B., C.P., F.C. and S.I.; data curation, J.-F.T. and S.I.; writing—original draft preparation, J.-F.T., writing—review and editing, J.-F.T.; visualization, J.-F.T., V.B. and S.I.; supervision, J.-F.T.; project administration, J.-F.T.; funding acquisition, J.-F.T. and M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the LIA/IRP EVOLEA (PI: J.-F. Terral and M. Ater) and supported by the OleaD CEMEB Labex project (Montpellier, France) (PI: Jean-Frédéric Terral and Catherine Roumet, UMR CEFV).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable to this article.

Acknowledgments: We thank all those who have enabled us to establish a modern reference collection: Jean-Christophe Auffray, Laurent Fabre, Mecit Vural (Gazi University, Turkey), Ashraf Tubeileh (ICARDA, Aleppo, Syria), Anwar Al Ibrahim and Malek Sheik Abdeen (Idleb Agricultural Research Center, GCSAR), Abelmajid Moukhli and Hayet Zaher (INRA-Morocco), Christian Pinatel (Centre Technique de l'Olivier, Aix-en-Provence), André Martre (known as the 'French pope of the olive tree', now deceased), Nathalie Moutier (INRAE), Jean-Paul Roger, Bouchaib Khadari and Sylvia Lochon-Menseau (CBNMP), and Bruno Bernazeau (INRAE—CBNMP). We are grateful to Allowen Evin (ISEM) for her advice regarding morphometry and Marie-Rose Mazel, David Mazel and Fabienne Chassefeyre for their help with logistical aspects.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Suc, J.-P. Origin and evolution of the Mediterranean vegetation and climate in Europe. *Nat. Cell Biol.* **1984**, *307*, 429–432. [CrossRef]
2. Palamarev, E. Paleobotanical evidences of the Tertiary history and origin of the Mediterranean sclerophyll dendroflora. *Plant Syst. Evol.* **1989**, *162*, 93–107. [CrossRef]
3. Terral, J.-F.; Badal, E.; Heinz, C.; Roiron, P.; Thiebault, S.; Figueiral, I. A hydraulic conductivity model points to post-neogene survival of the mediterranean olive. *Ecology* **2004**, *85*, 3158–3165. [CrossRef]
4. Green, P.S. A Revision of *Olea* L. (Oleaceae). *Kew Bull.* **2002**, *57*, 91. [CrossRef]
5. Martinez, S.R. *Memoria del Mapa de Series de Vegetacion de España*. 1: 400000; Icona: Madrid, Spain, 1987.
6. Barazani, O.; Keren-Keiserman, A.; Westberg, E.; Hanin, N.; Dag, A.; Ben-Ari, G.; Fragman-Sapir, O.; Tugendhaft, Y.; Kerem, Z.; Kadereit, J.W. Genetic variation of naturally growing olive trees in Israel: From abandoned groves to feral and wild? *BMC Plant Biol.* **2016**, *16*, 1–11. [CrossRef]
7. Zohary, D.; Hopf, M.; Weiss, E. *Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in Southwest Asia, Europe, and the Mediterranean Basin*; Oxford University Press: London, UK, 2012.
8. FAO. FAOSTAT. 2019. Available online: <http://www.fao.org/faostat/en/#home> (accessed on 26 December 2019).
9. Figueiral, I.; Terral, J.-F. Late Quaternary refugia of Mediterranean taxa in the Portuguese Estremadura: Charcoal based palaeovegetation and climatic reconstruction. *Quat. Sci. Rev.* **2002**, *21*, 549–558. [CrossRef]
10. Carrión, Y.; Ntinou, M.; Badal, E. *Olea europaea* L. in the North Mediterranean Basin during the Pleniglacial and the Early–Middle Holocene. *Quat. Sci. Rev.* **2010**, *29*, 952–968. [CrossRef]
11. Kislev, M.; Nadel, D.; Carmi, I. Epipalaeolithic (19,000 BP) cereal and fruit diet at Ohalo II, Sea of Galilee, Israel. *Rev. Palaeobot. Palynol.* **1992**, *73*, 161–166. [CrossRef]
12. Snir, A.; Nadel, D.; Groman-Yaroslavski, I.; Melamed, Y.; Sternberg, M.; Bar-Yosef, O.; Weiss, E. The Origin of Cultivation and Proto-Weeds, Long Before Neolithic Farming. *PLoS ONE* **2015**, *10*, e0131422. [CrossRef]
13. Kaniewski, D.; Van Campo, E.; Boiy, T.; Terral, J.-F.; Khadari, B.; Besnard, G. Primary domestication and early uses of the emblematic olive tree: Palaeobotanical, historical and molecular evidence from the Middle East. *Biol. Rev.* **2012**, *87*, 885–899. [CrossRef]
14. Langgut, D.; Adams, M.J.; Finkelstein, I. Climate, settlement patterns and olive horticulture in the southern Levant during the Early Bronze and Intermediate Bronze Ages (c.3600–1950 BC). *Levant* **2016**, *48*, 117–134. [CrossRef]
15. Langgut, D.; Cheddadi, R.; Carrión, J.S.; Cavanagh, M.; Colombaroli, D.; Eastwood, W.J.; Greenberg, R.; Litt, T.; Mercuri, A.M.; Miebach, A.; et al. The origin and spread of olive cultivation in the Mediterranean Basin: The fossil pollen evidence. *Holocene* **2019**, *29*, 902–922. [CrossRef]
16. Badal, E.; Bernabeu, J.; Vernet, J.; García, E.B. Vegetation changes and human action from the Neolithic to the Bronze Age (7000–4000 B.P.) in Alicante, Spain, based on charcoal analysis. *Veg. Hist. Archaeobotany* **1994**, *3*, 155–166. [CrossRef]
17. Heinz, C.; Figueira, I.; Terral, J.J.-F.; Claustre, F. Holocene vegetation changes in the northwestern Mediterranean: New palaeoecological data from charcoal analysis and quantitative eco-anatomy. *Holocene* **2004**, *14*, 621–627. [CrossRef]
18. Breton, C.; Terral, J.-F.; Pinatel, C.; Médail, F.; Bonhomme, F.; Bervillé, A. The origins of the domestication of the olive tree. *Comptes Rendus Biol.* **2009**, *332*, 1059–1064. [CrossRef]
19. Besnard, G.; Baradat, P.; Breton, C.; Khadari, B.; Bervillé, A. Olive domestication from structure of oleasters and cultivars using nuclear RAPDs and mitochondrial RFLPs. *Genet. Sel. Evol.* **2001**, *33*, S251–S268. [CrossRef]
20. Besnard, G.; Bakkali, A.E.; Haouane, H.; Baali-Cherif, D.; Moukhli, A.; Khadari, B. Population genetics of Mediterranean and Saharan olives: Geographic patterns of differentiation and evidence for early generations of admixture. *Ann. Bot.* **2013**, *112*, 1293–1302. [CrossRef]
21. Besnard, G.; Khadari, B.; Navascués, M.; Fernández-Mazuecos, M.; Bakkali, A.E.; Arrigo, N.; Baali-Cherif, D.; De Caraffa, V.B.-B.; Santoni, S.; Vargas, P.; et al. The complex history of the olive tree: From Late Quaternary diversification of Mediterranean lineages to primary domestication in the northern Levant. *Proc. R. Soc. B Biol. Sci.* **2013**, *280*, 20122833. [CrossRef]
22. Díez, C.M.; Trujillo, I.; Martínez-Urdiroz, N.; Barranco, D.; Rallo, L.; Marfil, P.; Gaut, B.S.; Ruiz, P.F.M. Olive domestication and diversification in the Mediterranean Basin. *N. Phytol.* **2015**, *206*, 436–447. [CrossRef]
23. Besnard, G.; Terral, J.-F.; Cornille, A. On the origins and domestication of the olive: A review and perspectives. *Ann. Bot.* **2017**, *121*, 385–403. [CrossRef]
24. Liphshitz, N.; Gophna, R.; Hartman, M.; Biger, G. The beginning of olive (*Olea europaea*) cultivation in the old world: A reassessment. *J. Archaeol. Sci.* **1991**, *18*, 441–453. [CrossRef]
25. Galili, E.; Stanley, D.J.; Sharvit, J.; Weinstein-Evron, M. Evidence for Earliest Olive-Oil Production in Submerged Settlements off the Carmel Coast, Israel. *J. Archaeol. Sci.* **1997**, *24*, 1141–1150. [CrossRef]
26. Newton, C.; Lorre, C.; Sauvage, C.; Ivorra, S.; Terral, J.-F. On the origins and spread of *Olea europaea* L. (olive) domestication: Evidence for shape variation of olive stones at Ugarit, Late Bronze Age, Syria—A window on the Mediterranean Basin and on the westward diffusion of olive varieties. *Veg. Hist. Archaeobotany* **2014**, *23*, 567–575. [CrossRef]
27. Dighton, A.; Fairbairn, A.S.; Bourke, S.; Faith, J.T.; Habgood, P. Bronze Age olive domestication in the north Jordan valley: New morphological evidence for regional complexity in early arboricultural practice from Pella in Jordan. *Veg. Hist. Archaeobotany* **2017**, *26*, 403–413. [CrossRef]

28. Brun, J.-P. *Archéologie du vin et de L'huile. De la Préhistoire à L'époque Hellénistique*; Errance: Paris, France, 2004.
29. Salavert, A. Olive cultivation and oil production in Palestine during the early Bronze Age (3500–2000 b.c.): The case of Tel Yarmouth, Israel. *Veg. Hist. Archaeobotany* **2008**, *17*, 53–61. [[CrossRef](#)]
30. Lovell, J.L.; Meadows, J.; Jacobsen, G. Upland Olive Domestication in the Chalcolithic Period: New 14C Determinations from El-Khawarij (Ajlun), Jordan. *Radiocarbon* **2010**, *52*, 364–371. [[CrossRef](#)]
31. Neef, R. Introduction, Development and Environmental Implications of Olive Culture: The Evidence from Jordan. In *Man's Role in the Shaping of the Eastern Mediterranean Landscape*; Bottema, S., Entjes-Nieborg, G., van Zeist, W., Eds.; Balkema Publishers: Rotterdam, The Netherlands, 1990; pp. 295–306.
32. Newton, C.; Terral, J.-F.; Ivorra, S. The Egyptian olive (*Olea europaea* subsp. *europaea*) in the later first millennium BC: Origins and history using the morphometric analysis of olive stones. *Antiquity* **2006**, *80*, 405–414. [[CrossRef](#)]
33. Galili, E.; Langgut, D.; Terral, J.F.; Barazani, O.; Dag, A.; Horwitz, L.K.; Ramirez, I.O.; Rosen, B.; Weinstein-Evron, M.; Chaim, S.; et al. Early production of table olives at a mid-7th millennium BP submerged site off the Carmel coast (Israel). *Sci. Rep.* **2021**, *11*, 1–15. [[CrossRef](#)]
34. Kislev, M.E. Wild olive endocarp at submerged Chalcolithic Kfar Samir, Haifa, Israel. *J. Israel Prehist. Soc.* **1995**, *26*, 134–145.
35. Terral, J.-F.; Arnold-Simard, G. Beginnings of Olive Cultivation in Eastern Spain in Relation to Holocene Bioclimatic Changes. *Quat. Res.* **1996**, *46*, 176–185. [[CrossRef](#)]
36. Terral, J.-F. Exploitation and Management of the Olive Tree During Prehistoric Times in Mediterranean France and Spain. *J. Archaeol. Sci.* **2000**, *27*, 127–133. [[CrossRef](#)]
37. Rowan, E. Olive Oil Pressing Waste as a Fuel Source in Antiquity. *Am. J. Archaeol.* **2015**, *119*, 465–482. [[CrossRef](#)]
38. Tanasi, D.; Greco, E.; Noor, R.E.; Feola, S.; Kumar, V.; Crispino, A.; Gelis, I. 1H NMR, 1H–1H 2D TOCSY and GC-MS analyses for the identification of olive oil in Early Bronze Age pottery from Castelluccio (Noto, Italy). *Anal. Methods* **2018**, *10*, 2756–2763. [[CrossRef](#)]
39. Gros-Balthazard, M.; Besnard, G.; Sarah, G.; Holtz, Y.; Leclercq, J.; Santoni, S.; Wegmann, D.; Glémin, S.; Khadari, B. Evolutionary transcriptomics reveals the origins of olives and the genomic changes associated with their domestication. *Plant J.* **2019**, *100*, 143–157. [[CrossRef](#)] [[PubMed](#)]
40. Terral, J.-F.; Alonso, N.; Capdevila, R.B.I.; Chatti, N.; Fabre, L.; Fiorentino, G.; Marinval, P.; Jordà, G.P.; Pradat, B.; Rovira, N.; et al. Historical biogeography of olive domestication (*Olea europaea* L.) as revealed by geometrical morphometry applied to biological and archaeological material. *J. Biogeogr.* **2003**, *31*, 63–77. [[CrossRef](#)]
41. Brun, J.-P. *Archéologie du vin et de L'huile Dans l'Empire Romain*; Errance: Paris, France, 2004.
42. Laubenheimer, F. *Le Temps des Amphores en Gaule. Vins, Huiles et Sauces*; Errance: Paris, France, 1990.
43. André, J. *L'Alimentation et la Cuisine à Rome*; Les Belles Lettres: Paris, France, 1981.
44. Terral, J.-F.; Tabard, E.; Bouby, L.; Ivorra, S.; Pastor, T.; Figueiral, I.; Picq, S.; Chevance, J.-B.; Jung, C.; Fabre, L.; et al. Evolution and history of grapevine (*Vitis vinifera*) under domestication: New morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Ann. Bot.* **2009**, *105*, 443–455. [[CrossRef](#)]
45. Bonhomme, V.; Picq, S.; Ivorra, S.; Evin, A.; Pastor, T.; Bacilieri, R.; Lacombe, T.; Figueiral, I.; Terral, J.-F.; Bouby, L. Eco-evo-devo implications and archaeobiological perspectives of trait covariance in fruits of wild and domesticated grapevines. *PLoS ONE* **2020**, *15*, e0239863. [[CrossRef](#)] [[PubMed](#)]
46. R. R: The R Project for Statistical Computing. R. 2021. Available online: <https://www.r-project.org/> (accessed on 16 September 2021).
47. Bonhomme, V.; Picq, S.; Gaucherel, C.; Claude, J. Momocs: Outline Analysis Using R. *J. Stat. Softw.* **2014**, *56*, 1–24. [[CrossRef](#)]
48. Bonhomme, V.; Picq, S.; Claude, J. Momocs Package 2020. Available online: <https://cran.r-project.org> (accessed on 16 September 2021).
49. Venables, V.N.; Ripley, B.D. *Modern Applied Statistics with R*; Springer: New York, NY, USA, 2002.
50. Paradis, E.; Schliep, K. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **2018**, *35*, 526–528. [[CrossRef](#)]
51. Burger, P.; Terral, J.-F.; Ruas, M.-P.; Ivorra, S.; Picq, S. Assessing past agrobiodiversity of *Prunus avium* L. (Rosaceae): A morphometric approach focussed on the stones from the archaeological site Hôtel-Dieu (16th century, Tours, France). *Veg. Hist. Archaeobotany* **2011**, *20*, 447–458. [[CrossRef](#)]
52. Szokefalvi-Nagy, B. *Introduction to Real Functions and Orthogonal Expansions. University Texts in the Mathematical Sciences*; Oxford University Press: Oxford, UK, 1965.
53. Blondel, J.; Aronson, J. Biodiversity and ecosystem function in the Mediterranean Basin: Human and non-human determinants. In *The Function of Biodiversity*; David, G.X., Richardson, D.M., Eds.; Springer: New York, NY, USA, 1995; pp. 43–119.
54. Bakkali, A.E.; Haouane, H.; Moukhli, A.; Costes, E.; Van Damme, P.; Khadari, B. Construction of Core Collections Suitable for Association Mapping to Optimize Use of Mediterranean Olive (*Olea europaea* L.) Genetic Resources. *PLoS ONE* **2013**, *8*, e61265. [[CrossRef](#)]
55. Khadari, B.; Bakkali, A.E.; Essalouh, L.; Tollon, C.; Pinatel, C.; Besnard, G. Cultivated Olive Diversification at Local and Regional Scales: Evidence From the Genetic Characterization of French Genetic Resources. *Front. Plant Sci.* **2019**, *10*, 1593. [[CrossRef](#)] [[PubMed](#)]
56. Terral, J.-F. La Domestication de L'olivier (*Olea europaea* L.) en Méditerranée Nord-Occidentale: Approches Morphométriques et Implications Paléoclimatiques. Ph.D. Thesis, Université de Montpellier 2, Montpellier, France, 1997.

57. Margaritis, E. Distinguishing exploitation, domestication, cultivation and production: The olive in the third millennium Aegean. *Antiquity* **2013**, *87*, 746–757. [[CrossRef](#)]
58. Ramos-Madrigal, J.; Runge, A.K.W.; Bouby, L.; Lacombe, T.; Castruita, J.A.S.; Adam-Blondon, A.-F.; Figueiral, I.; Hallavant, C.; Martínez-Zapater, J.M.; Schaal, C.; et al. Palaeogenomic insights into the origins of French grapevine diversity. *Nat. Plants* **2019**, *5*, 595–603. [[CrossRef](#)] [[PubMed](#)]
59. Bonhomme, V.; Terral, J.-F.; Zech-Matterne, V.; Ivorra, S.; Lacombe, T.; Deborde, G.; Kuchler, P.; Limier, B.; Pastor, T.; Rollet, P.; et al. Seed morphology uncovers 1500 years of vine agrobiodiversity before the advent of the Champagne wine. *Sci. Rep.* **2021**, *11*, 1–14. [[CrossRef](#)]
60. Terral, J.-F.; Newton, C.; Ivorra, S.; Gros-Balthazard, M.; de Morais, C.T.; Picq, S.; Tengberg, M.; Pintaud, J.-C. Insights into the historical biogeography of the date palm (*Phoenix dactylifera* L.) using geometric morphometry of modern and ancient seeds. *J. Biogeogr.* **2012**, *39*, 929–941. [[CrossRef](#)]
61. Gros-Balthazard, M.; Newton, C.; Ivorra, S.; Pierre, M.-H.; Pintaud, J.-C.; Terral, J.-F. The Domestication Syndrome in *Phoenix dactylifera* Seeds: Toward the Identification of Wild Date Palm Populations. *PLoS ONE* **2016**, *11*, e0152394. [[CrossRef](#)]
62. Besnard, G.; De Casas, R.R. Single vs multiple independent olive domestications: The jury is (still) out. *New Phytol.* **2016**, *209*, 466–470. [[CrossRef](#)]
63. Bourgeon, O.; Pagnoux, C.; Mauné, S.; Vargas, E.G.; Ivorra, S.; Bonhomme, V.; Ater, M.; Moukhli, A.; Terral, J.-F. Olive tree varieties cultivated for the great Baetican oil trade between the 1st and the 4th centuries ad: Morphometric analysis of olive stones from Las Delicias (Ecija, Province of Seville, Spain). *Veg. Hist. Archaeobotany* **2017**, *27*, 1–14. [[CrossRef](#)]
64. Bouby, L.; Figueiral, I.; Bouchette, A.; Rovira, N.; Ivorra, S.; Lacombe, T.; Pastor, T.; Picq, S.; Marínval, P.; Terral, J.-F. Bioarchaeological Insights into the Process of Domestication of Grapevine (*Vitis vinifera* L.) during Roman Times in Southern France. *PLoS ONE* **2013**, *8*, e63195. [[CrossRef](#)]
65. Gros-Balthazard, M.; Galimberti, M.; Kousathanas, A.; Newton, C.; Ivorra, S.; Paradis, L.; Vigouroux, Y.; Carter, R.; Tengberg, M.; Battesti, V.; et al. The Discovery of Wild Date Palms in Oman Reveals a Complex Domestication History Involving Centers in the Middle East and Africa. *Curr. Biol.* **2017**, *27*, 2211–2218. [[CrossRef](#)] [[PubMed](#)]
66. Ater, M.; Barbara, H.; Kassout, J. Importance des variétés locales, de l'oléastre et des pratiques traditionnelles de l'oléiculture dans la région de Chefchaouen (Nord du Maroc). In *L'oléiculture au Maroc de la Préhistoire à nos Jours: Pratiques, Diversité, Adaptation, Usages, Commerce et Politiques*; Ater, M., Essalouh, L., Ilbert, H., Moukhli, A., Khadari, B., Eds.; Options Méditerranéennes, CIHEAM: Montpellier, France, 2016; pp. 109–121.
67. Aumeeruddy-Thomas, Y.; Moukhli, A.; Haouane, H.; Khadari, B. Ongoing domestication and diversification in grafted olive-oleaster agroecosystems in Northern Morocco. *Reg. Environ. Chang.* **2017**, *17*, 1315–1328. [[CrossRef](#)]
68. Waddington, C.H. Canalization of Development and The Inheritance of Acquired Characters. *Nat. Cell Biol.* **1942**, *150*, 563–565. [[CrossRef](#)]
69. Siegal, M.; Bergman, A. Waddington's canalization revisited: Developmental stability and evolution. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10528–10532. [[CrossRef](#)] [[PubMed](#)]
70. Pagnoux, C. Emergence, Développement et Diversification de L'arboriculture en Grèce du Néolithique à L'époque Romaine. Confrontation des Données Archéobotaniques, Morphométriques, Epigraphiques et Littéraires. Ph.D. Thesis, Université Paris 1—Panthéon-Sorbonne, Paris, France, 2016.