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Eric Garnier, Estelle Leca, Karim Barkaoui. Leaf and pit traits of 35 olive varieties from different genetic groups growing in the French germplasm collection of the Porquerolles Island (Provence, France). Scientific Reports of the Port-Cros National Park, 2021, 35, pp.235-260. hal-03324266

HAL Id: hal-03324266

<https://hal.science/hal-03324266>

Submitted on 23 Aug 2021

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Leaf and pit traits of 35 olive varieties from different genetic groups growing in the French germplasm collection of the Porquerolles Island (Provence, France)

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Abstract. The olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*) is currently one of the most widely cultivated temperate fruit trees in the world. It is characterised by a large genetic diversity, as well as a large variation in phenotypic traits. Across the Mediterranean Basin, the nuclear genetic diversity of olive is organized into three main groups: 'West', 'Central', 'East', with an additional group ('Mosaic' or 'admixed') corresponding to varieties resulting from the hybridization of varieties belonging to at least two of these three groups. The first aim of the present study is to test whether the phenotypic traits of the cultivated varieties differ among these four genetic groups. To do so, nine leaf traits and five pit traits were measured on 35 varieties belonging to these four groups. The second aim of this study is to use this combination of traits to assess whether some trait covariations previously observed across and/or within species hold within olive, a sclerophyllous species characteristic of the Mediterranean Basin. We found that:

- (1) Trait values span a wide range of variation across varieties, from approximately 7-fold for pit volume, the most variable trait, to 1.3 fold for leaf dry matter content, the least variable one;
- (2) For most traits, there was no significant difference among the four genetic groups recognized across the Mediterranean Basin;
- (3) Leaf size was positively related to leaf mass *per area*, a trait describing the morpho-anatomy of the leaf; this is the consequence of leaf mass increasing more rapidly than leaf area, and agrees with the hypothesis that bigger leaves require to be stiffer to compensate for mechanical damage and gravity;
- (4) The two underlying components of leaf mass *per area*, leaf thickness and density, contributed equally to its determination;
- (5) Leaf and pit sizes were positively related. A structural equation modelling approach allowed us to show that this relationship was mediated through leaf mass *per area*, which is interpreted in the context of the recently proposed "Seed-Phytometer-Leaf" model.

Perspectives to this work include: (a) further testing of the lack of phenotypic differences among genetic groups by increasing the number of varieties studied; (b) collecting data on fruits and twigs to test the underlying allometric model linking pit and leaf size, and (c) assessing how several leaf traits determined in this study - leaf area, leaf width, leaf mass *per area* - relate to gas exchange and plant water economy, so as

to better understand how the different olive varieties cope with the contrasting climatic conditions from which they originate. This work would strengthen the functional bases for the selection of olive varieties.

Keywords: allometry, biomechanics, genotypic group, functional traits, olive tree, phenotypic variation, germplasm collection.

Résumé. Traits des feuilles et des noyaux de 35 variétés d'oliviers appartenant à des groupes génétiques différents, cultivés dans la collection variétale de référence de Porquerolles (Provence, France). L'olivier (*Olea europaea* L. subsp. *europaea* var. *europaea*) est l'un des arbres fruitiers tempérés les plus largement cultivés dans le monde. Il se caractérise par une forte diversité génétique, et présente une importante variation pour de nombreux traits phénotypiques. Autour du Bassin Méditerranéen, la diversité génétique de l'olivier s'organise en trois groupes principaux : 'Ouest', 'Central' et 'Est', auquel s'ajoute un groupe ('Mosaïque' ou 'admixé') qui correspond à des variétés issues de l'hybridation de variétés appartenant à au moins deux des groupes précédents. Le premier objectif de la présente étude est de tester si les traits phénotypiques des variétés cultivées diffèrent entre ces quatre groupes génétiques. Ceci a été réalisé en quantifiant neuf traits des feuilles et cinq traits des noyaux pour 35 variétés réparties dans ces différents groupes. Le second objectif est d'utiliser cette combinaison de traits pour tester si certaines covariations entre traits observées dans des études précédentes entre espèces et/ou au sein d'une espèce, sont également trouvées chez l'olivier, espèce sclérophylle caractéristique du Bassin méditerranéen. Nos résultats montrent que :

- (1) Il existe une forte gamme de variation entre variétés pour tous les traits étudiés ; cette gamme est la plus large pour le volume du noyau (facteur ~7 entre les deux extrêmes) et la plus étroite pour la teneur en matière sèche des feuilles (facteur 1,3 entre les deux extrêmes) ;
- (2) Pour la plupart des traits mesurés, on n'observe pas de différence significative entre les quatre groupes génétiques distribués autour du Bassin méditerranéen ;
- (3) La taille des feuilles est corrélée positivement à leur masse surfacique - un trait relatif à la morpho-anatomie des feuilles -, reflétant le fait que la masse des feuilles augmente plus rapidement que leur surface. Ceci est en accord avec l'hypothèse stipulant que des feuilles plus grandes doivent être plus rigides, afin de pouvoir supporter les dommages mécaniques et les effets de la gravité ;
- (4) L'épaisseur des feuilles et la densité des tissus contribuent de façon équivalente à la détermination de la masse surfacique des feuilles ;
- (5) La taille des feuilles est positivement corrélée à celle des noyaux. Une approche de modélisation en équations structurelles a permis de montrer que la masse surfacique des feuilles jouait un rôle central dans cette relation, ce qui est interprété dans le contexte du modèle « Graine-Phytomètre-Feuille » récemment proposé.

Les perspectives envisagées pour ce travail comprennent : (a) un test plus général de l'absence de différences phénotypiques entre groupes génétiques, en augmentant le nombre de variétés étudiées ; (b) la collecte de données sur les fruits et les branches, afin de tester le modèle allométrique sous-jacent reliant la taille des feuilles à celle des noyaux, et (c) l'établissement des relations entre certains traits foliaires déterminés dans la présente étude - surface, largeur et masse surfacique -, les échanges gazeux et l'économie de l'eau, afin de mieux comprendre l'adéquation entre la physiologie des différentes variétés d'oliviers et les conditions climatiques de leur aire géographique d'origine. Ce travail permettrait de consolider les bases fonctionnelles pour la sélection des variétés d'oliviers.

Mots-clés : allométrie, biomécanique, collection variétale, groupe génétique, olivier, traits fonctionnels, variation phénotypique.

Introduction

The olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*) is currently one of the most widely cultivated temperate fruit trees in the world and is characterised by a large genetic diversity, with an estimation of 1 200 cultivated varieties described (Bartolini *et al.*, 2005, IOC 2019). This large genetic diversity goes with a large variation in phenotypic traits, which has long been used to classify and identify olive varieties (Ganino *et al.*, 2006 for a review).

Across the Mediterranean Basin, the genetic diversity of olive is organized into three main gene pools (Breton *et al.*, 2006; Diez *et al.*, 2015; Khadari and El Bakkali, 2018): (i) one in the East, including wild (*O. europaea* subsp. *europaea* var. *sylvestris* (Mill.) Leh.) and domesticated olive, (ii) one in the Center and the West with only wild olive, and (iii) one in the Center and the West with only cultivated olive, which can be further divided into two subclusters, the West, and the Center. An additional group, defined as 'Mosaic' or 'admixed', corresponds to genotypes resulting from the hybridization of varieties either among varieties and/or between varieties and wild olives of different origins (Khadari and El Bakkali, 2018). Although several studies have combined molecular and morphological markers to improve the identification of olive varieties (e.g. Cantini *et al.*, 2008; Trujillo *et al.*, 2014), to our knowledge, there is no evaluation to date on how the genetic organization of olive across the Mediterranean maps onto phenotypic variations.

The first aim of the present study is to test whether the phenotypic traits of the cultivated varieties differ among the 'East', 'Central', 'West', and 'Mosaic' genetic groups described above. To do so, we measured 14 traits (nine leaf traits and five pit traits: cf. Table II) on 35 varieties belonging to these four groups as identified through genetic data (Diez *et al.*, 2015; Khadari and El Bakkali, 2018). Beyond some of the major traits traditionally used for the identification of olive varieties such as leaf length and width, leaf size, pit length and width, pit mass (cf. Ganino *et al.*, 2006), we determined leaf traits known to play a key role in leaf and plant functioning (Garnier *et al.*, 2016): leaf mass *per area* (LMA, the ratio of leaf dry mass to leaf area), leaf dry matter content (LDMC, the ratio of leaf dry mass to water-saturated fresh mass) and leaf thickness (LT). The second aim of this study is to use this combination of traits to assess whether some trait covariations previously observed across and/or within species also hold within the sclerophyllous olive species. Covariations were evaluated both within leaves and between organs. In leaves, we assessed: (1) whether LMA was related to leaf size in olive, reflecting higher costs of light interception in larger leaves (Milla and Reich, 2007), and (2) the relative roles of leaf dry matter content - a surrogate of leaf tissue density (Garnier and Laurent, 1994) - and leaf thickness in the determination

of LMA (Witkowski and Lamont, 1991; Poorter and Garnier, 2007). Between organs, we tested whether there was a coordination between leaf and pit traits with detectable relationships between leaf and pit size resulting from allometric constraints (the so-called Corner's rules: Corner, 1949), and/or whether leaf morpho-anatomy was related to pit size as predicted by the Seed-Phytometer-Leaf model (Hodgson *et al.*, 2017).

To address these questions, we determined leaf traits on olive trees grown in the germplasm collection managed by the “Conservatoire Botanique National Méditerranéen de Porquerolles” (CBNMed hereafter) in Porquerolles (Var, Provence, France). This collection hosts a major part of the French national germplasm as well as several varieties originating from various places around the Mediterranean Basin (Lochon-Menseau and Khadari, 2014). Genotypes have been previously characterized using molecular markers (cf. Khadari and El Bakkali, 2018), allowing one to assign each tree safely to a recognized variety. For pit traits, we used a collection built progressively over time by various members of the CBNMed, stored in the premises of the Port-Cros National Park in Porquerolles.

To summarize, we use the data collected from these two collections to: (1) assess whether there are significant differences among the four genetic groups identified across the Mediterranean Basin for the 14 traits measured, and (2) test covariations among these traits using a combination of univariate and multivariate methods, followed by a structural equation modelling approach.

Material and Methods

Study site and selection of varieties

The olive germplasm collection is located on the Porquerolles island (42°59'29"N, 6°12'14"E; 18 m a.s.l.). Mean annual temperature and rainfall are 16.8 °C and 615 mm, respectively (data over the 1990-2019 period from the nearest meteorological station, 2 km North-East of the study site). The bioclimate is classified as mesomediterranean characterised by sub-humid conditions according to Daget (1977) and Ozenda (1981).

The “Mediterranean collection” consists in a 1.7 ha plot (~ 145 m length x 118 m width) planted with 457 trees (7 m distance between rows; 5 m between lines) belonging to 100 cultivated varieties (cf. Khadari *et al.*, 2019). Most trees were planted between 1979 and 1991. The phenotypic characterization was conducted on 35 varieties belonging to the four nuclear genetic groups identified across the Mediterranean

Basin using molecular markers (Khadari and El Bakkali, 2018), 21 mainly cultivated in France and 14 cultivated in other Mediterranean countries (Table I). These were selected to represent the largest possible range of geographical origins as available in the collection (Fig. 1).

Table I. Name, origin, use and genetic group of the 35 olive varieties screened in the present study. The names, origins and uses are taken from Moutier *et al.* (2004, 2011) for varieties mainly cultivated in France and from the OLEA databases (<http://www.oleadb.it/>) for the others. Genetic groups are taken from Khadari and El Bakkali (2018).

Name of variety	Country of origin	Use	Genetic group
Aglandau	France	Oil, table	Mosaic
Amygdalolia	Greece	Oil, table	Center
Arbequina	Spain	Oil	Center
Ascolana tenera	Italy	Table, oil	Mosaic
Aubenc	France	Oil	Unknown
Baguet	France	Oil	Mosaic
Barouni	Tunisia	Table, oil	West
Blanc de Payzac	France	Oil	East
Cayet roux	France	Oil, table	Mosaic
Cayon	France	Oil, table	Mosaic
Chemlali	Tunisia	Oil, table	Mosaic
Colombale	France	Oil	Mosaic
Courbeil	France	Oil	Mosaic
Ecijano	Spain	Oil	Unknown
Grapié	France	Oil	Mosaic
Grappola	Italy	Oil, table	Center
Grossane	France	Table, oil	Mosaic
Koroneiki	Greece	Oil	Mosaic
Lucques	France	Table, oil	Mosaic
Manzanilla	Spain	Table, oil	West
Meski	Tunisia	Table, oil	East
Montaurounenque	France	Oil	Mosaic
Négrette	France	Oil	Mosaic
Oblonga	Italy	Oil	Center
Oliviére	France	Table, oil	Mosaic
Petit Ribier	France	Oil	Center
Picholine du Languedoc	France	Table, oil	Mosaic
Picholine marocaine	Morocco	Table, oil	West
Picual	Spain	Oil, table	West
Reymet	France	Oil	Center
Rougette de l'Ardèche	France	Oil	Mosaic
Tanche	France	Table, oil	East
Verdale de l'Hérault	France	Table, oil	Unknown
Verdanel	France	Oil	Mosaic
Zard	Iran	Table, oil	East



Figure 1. Geographical area of origin of the 35 varieties studied (Moutier *et al.*, 2004, 2011 for French varieties and the OLEA databases [<http://www.oleadb.it/>] for the others). The color code of points corresponds to the genetic group ('East', 'Center', 'West', 'Mosaic' and 'Unknown') as identified by Breton *et al.* (2006) and Khadari and El Bakkali (2018). The data sources for the elaboration of the background map are taken from NASA SRTM (www2.jpl.nasa.gov/srtm/) and GEBCO (www.gebco.net/data_and_products/gridded_bathymetry_data/).

Measurement of phenotypic traits

A total of 14 phenotypic traits was assessed on leaves (nine traits) and pits (five traits) of the different varieties (Table II).

Table II. List of the 14 phenotypic traits assessed in the present study, with their abbreviations and units.

Organ	Trait	Abbreviation	Unit
Leaf	Leaf length	LL	cm
	Leaf width	LW	cm
	Leaf length/leaf width	LL/LW	cm/cm
	Leaf area	LA	cm ²
	Leaf fresh mass	LFM	g
	Leaf dry mass	LDM	g
	Leaf mass <i>per area</i>	LMA	g m ⁻²
	Leaf dry matter content	LDMC	mg g ⁻¹
	Leaf thickness	LT	μm
Pit	Pit length	PL	cm
	Pit width	PW	cm
	Pit length/pit width	PL/PW	cm/cm
	Pit dry mass	PDM	g
	Pit volume	PV	cm ³

Leaf traits were measured in October 2019 on leaves collected from trees of the Mediterranean collection, produced between March and May of the same year; these leaves were thus approximately 6 to 7 month-old at the time of measurement. Ten intact leaves (*i.e.* free from herbivore or pathogen damage), mostly taken from 3 trees *per* variety (*i.e.* 3 to 4 leaves *per* tree), were collected from the eastern, southern, and western sides of the outer tree canopy. As soon as they were cut off from the branch, leaves were put into a test tube with the petiole sunken in deionized water, and placed in a cool box until further processing in the lab. Within two to three hours following collection, test tubes containing the leaves were placed into a cool room for at least 12 hours to ensure full hydration (Garnier *et al.*, 2001). Leaf saturated fresh mass (LFM) and thickness were measured on these fully hydrated leaves, using a precision scale and a digimatic micrometer (Mitutoyo), respectively. Leaves were then scanned, and their length (LL), width (LW), and area (LA) were determined using the ImageJ software (<https://imagej.nih.gov/ij/>). Samples were then oven-dried at 60°C for at least two days, and their dry mass (LDM) was measured. Leaf length/leaf width (LL/LW), leaf mass *per* area, and leaf dry matter content were derived from these measurements (see Garnier *et al.*, 2001 for LMA and LDMC). Leaves were assigned to one of three leaf shape categories according to the LL/LW ratio as (cf. Moutier *et al.*, 2004): LL/LW < 4: elliptical; 4 < LL/LW < 6: elliptical-lanceolate; LL/LW > 6: lanceolate.

Pit traits were assessed from 20 intact dry pits *per* variety, randomly chosen from the collection preserved in the premises of the Port-Cros National Park. Pit length (PL) and width (PW) were measured with a digital calliper, from which the pit length/pit width ratio (PL/PW) was derived. Pits were assigned to one of four pit shape categories according to the PL/PW ratio as (Moutier *et al.*, 2004): PL/PW < 1.4: spherical; 1.4 < PL/PW < 1.8: ovoid; 1.8 < PL/PW < 2.2: elliptical; PL/PW > 2.2: elongate. Pit volume (PV) was measured with a method based on Archimede's principle using the Sartorius density determination kit (Sartorius YDK01LP, Göttingen, Germany), which involves the determination of pit dry mass (PDM) and pit density (data not reported here) during the process. Pit data are missing for four cultivars - Ecijano, Grappola, Picholine marocaine and Zard - which were not represented in the collection.

The average trait values for all varieties are given in Appendices I (leaf traits) and II (pit traits).

Statistical analyses

We compared leaf and pit trait values of varieties with one-way ANOVAs, and tested the differences between genetic groups with nested ANOVA considering 'varieties' as a random factor. Varieties

with an ‘Unknown’ genetic group were removed from the analyses. In the case of significant ANOVA, we conducted a post-hoc Tukey test to compare the genetic groups to each other. We performed a principal component analysis (PCA) to analyze the multivariate relationships between five leaf (LA, LL/LW, LMA, LDMC, LT) and two pit (PV, PL/PW) traits, and tested differences in coordinates of genetic groups along the first three axes of the PCA. The seven other traits were not included as variables in the PCA to avoid too much redundancy. However, we plotted both LDM and PDM as illustrative variables in the PCA to visualize how organ mass relates to the principal components determined by these traits.

We then used simple linear regressions to test the bivariate relationships between LMA, LDM, and PDM. As a confirmatory step, we used structural equation models (SEMs) to test the causal relationships between leaf traits and organ sizes. We compared two baseline models : (1) a ‘biomechanical’ model, which assumes that big organs (high LDM, high PDM) require stiff leaves (high LMA) for biomechanical reasons; and (2) an ‘allometric’ model, which assumes that the mass of organs are inter-correlated following allometric rules, implying that varieties with big leaves (LDM) also have big pits (PDM). We evaluated the models using maximum likelihood estimates (χ^2 -test), the Akaike information criteria (AIC), the goodness-of-fit index (GFI). Good models have low χ^2 (non-significant test), low AIC, GFI > 0.90. We calculated the standardized path coefficients and the explained variance (R^2) for all the dependent traits in both models.

All statistical analyses were performed with the R software version 4.0.3 (R Development Core Team, 2020), using the packages *tidyverse*, *FactoMineR* (PCA) and *lavaan* (SEM).

Results

Leaf and pit trait variation across olive varieties

The olive varieties spanned a wide range of variation for both leaf and pit morphological traits (Fig. 2 and 3; Appendices III and IV). LA varied up to threefold (Fig. 2a), opposing the small leaves of varieties like *Verdale de l'Hérault* or *Koroneiki* to the larger leaves of *Amygdalolia* or *Reymet*. Leaf shape, determined by LL/LW (Fig. 2b), were either elliptical (9 varieties), elliptical-lanceolate (24 varieties) or lanceolate (2 varieties). Similarly, PV varied more than seven-fold (Fig. 2c), opposing the small pits of varieties like *Chemlali* or *Koroneiki* to the big pits of *Barouni*. Pit shape, determined by PL/PW (Fig. 2d) were either spherical (2 varieties), ovoid (10 varieties), elliptical (16 varieties) or elongated (3 varieties). In comparison, LMA, LDMC, LT varied within a narrower yet significant range of values (Fig. 3). Despite significant differences

between varieties for all the traits ($p < 0.001$), only LL/LW ($p = 0.045$) and PV ($p = 0.044$) differed significantly between the genetic groups. Varieties from the ‘East’ group had typical elliptical leaves while the varieties from the ‘Center’, ‘Mosaic’ and then the ‘West’ groups had a range of elliptical-lanceolate leaves. Varieties from the ‘West’ group had the biggest pits, followed by the varieties from the ‘East’, then the ‘Mosaic’, and finally the ‘Center’ groups.

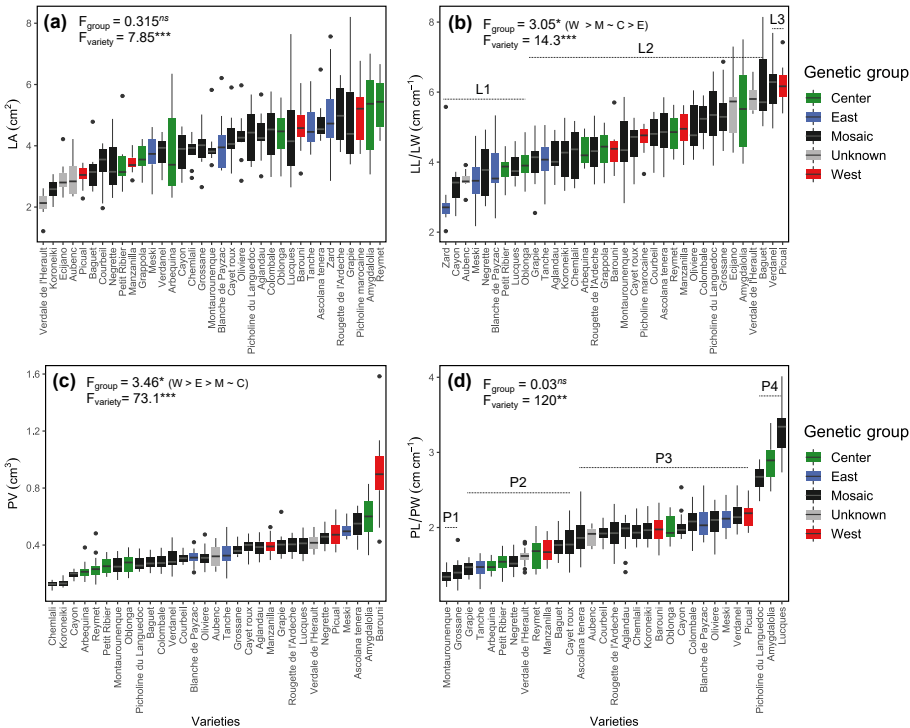


Figure 2. Distribution of (a) leaf area (LA), and (b) leaf length to leaf width ratio (LL/LW), for 35 olive varieties, and of (c) pit volume (PV), and (d) pit length to pit width ratio (PL/PW), for 31 varieties of the ‘Mediterranean collection’ of Porquerolles. Boxplots show median (horizontal line), inter-quartiles (boxes), and non-outlier ranges (vertical lines); outliers are plotted as points. Varieties are sorted by their mean trait values and colored according to their genetic group. The dashed horizontal lines on graph (b) and (d) indicate the leaf shape (L1: ‘elliptical’; L2: ‘elliptical-lanceolate’; L3: ‘lanceolate’) and the pit shape (P1: ‘spherical’; P2: ‘ovoid’; P3: ‘elliptical’; P4: ‘elongated’) category according to reference values of LL/LW and PL/PW, respectively. The differences between genetic groups were tested using nested ANOVAs without the ‘Unknown’ group. Significance levels are : ^{ns} not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

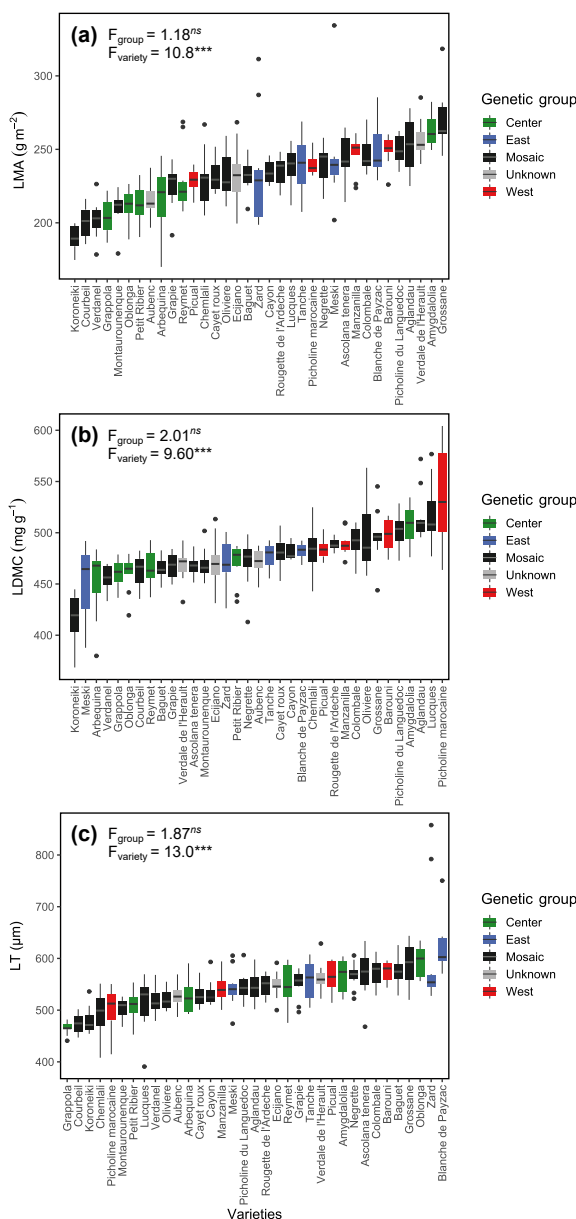


Figure 3. Distribution of **(a)** leaf mass *per area* (LMA), **(b)** leaf dry matter content (LDMC), **(c)** leaf thickness (LT), for 35 olive varieties of the ‘Mediterranean collection’ of Porquerolles. Varieties are sorted by their mean trait values and colored according to their genetic group. Boxplots show median (horizontal line), inter-quartiles (boxes), and non-outlier ranges (vertical lines); outliers are plotted as points. The differences between genetic groups were tested using nested ANOVAs without the ‘Unknown’ group. Significance levels are : *ns* not significant ; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Covariations of leaf and pit traits

Leaf and pit traits co-varied significantly across the 35 olive varieties. The first three components of the PCA involving seven traits explained 73.4 % of the total variation in traits (Fig. 4, Table III).

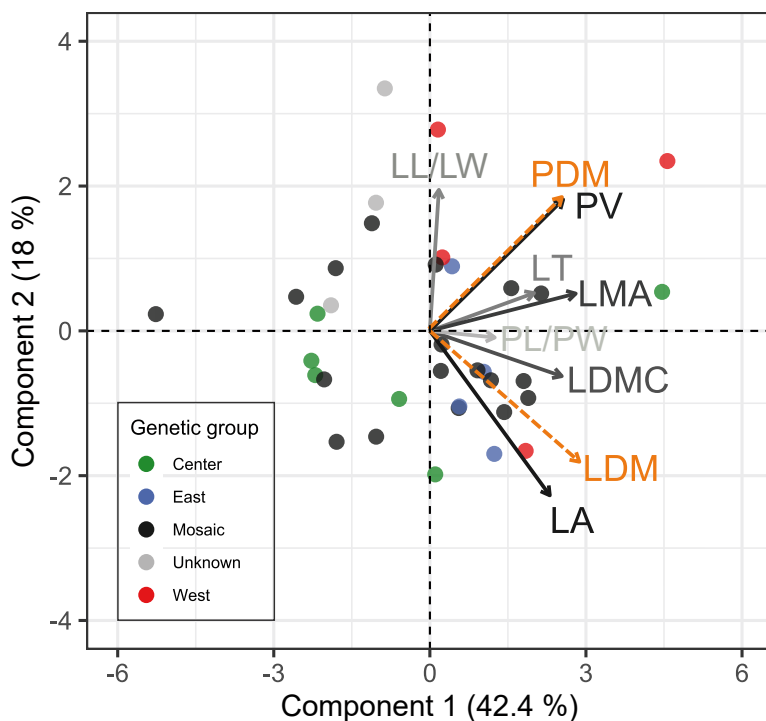


Figure 4. Principal component analysis (PCA) showing the relationships between the morphological leaf and pit traits for 35 olive varieties of the ‘Mediterranean collection’ of Porquerolles. The first two components accounted for 60.4 % of the total variance (the third component accounted for 13 %, see Table III). The solid grey arrows represent the active traits used in the PCA. Their contributions to the first two components are indicated using a shade of grey: dark grey indicates the most significant traits. The orange dashed arrows represent the illustrative variables. The colored data points represent the varieties and their genetic group.

Table III. Trait loadings for the PCA performed across the 35 olive varieties. The percentage of variance explained by each of the first three components is shown in brackets. Significant trait loadings are shown in bold. Abbreviations : LA, leaf area; LDMC, leaf dry matter content; LL/LW, leaf length/leaf width; LMA, leaf mass *per* area; LT, leaf thickness; PL/PW, pit length/pit width; PV, pit volume; LDM, leaf dry mass; PDM, pit dry mass.

Traits	PC 1 (42.4 %)	PC 2 (18 %)	PC 3 (13 %)
<i>Active variables</i>			
LA	0.66	- 0.65	- 0.06
LDMC	0.73	- 0.18	0.41
LL/LW	0.05	0.59	0.40
LMA	0.80	0.15	- 0.06
LT	0.57	0.15	- 0.51
PL/PW	0.36	- 0.03	0.75
PV	0.73	0.52	- 0.12
<i>Illusrative variables</i>			
LDM	0.82	- 0.52	- 0.06
PDM	0.73	0.52	- 0.10

The first component (42.4 %) was determined by leaf resource-use traits (LMA, LDMC, LT) and leaf and pit sizes (LA, PV), the second component (18 %) by leaf and pit size and shape (LA, LL/LW, PV), and the third component (13 %) essentially by pit shape (PL/PW) and LT. LDM was positively correlated to the first component ($r = 0.82$, $p < 0.001$), negatively to the second one ($r = -0.52$, $p = 0.001$) but not correlated to the third component (Table III). PDM was also positively correlated to the first ($r = 0.73$, $p < 0.001$) and second ($r = 0.52$, $p = 0.001$) components, but not to the third one (Table III). There was no significant difference among genetic groups along any of the three components, although the 'West' group had marginally higher coordinates along the second component, reflecting its larger pit size and LL/LW compared to the other groups (Fig. 5).

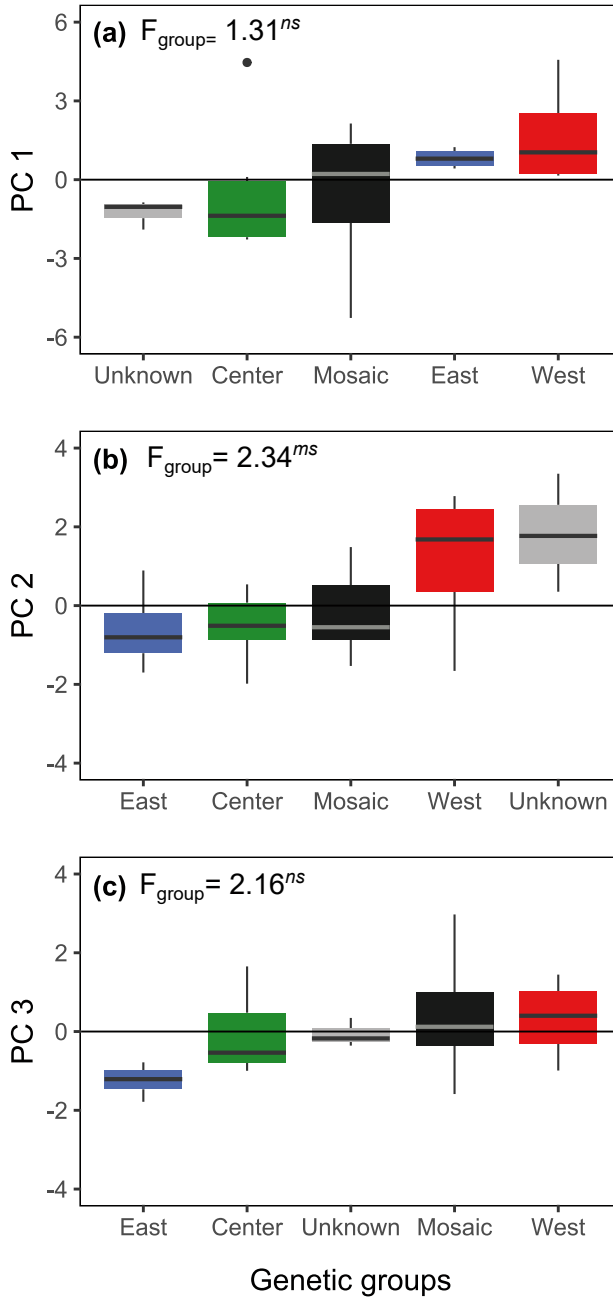


Figure 5. Coordinates of olive genetic groups along **(a)** the first (PC1), **(b)** the second (PC2), and **(c)** the third (PC3) principal components of PCA. Genetic groups are sorted by their mean coordinates. Boxplots show median (horizontal line), inter-quartiles (boxes), and non-outlier ranges (vertical lines); outliers are plotted as points. The differences between genetic groups were tested using nested ANOVAs without the 'Unknown' group. Significance levels are : *ns* not significant ; *ms* $p < 0.1$.

Determinants of LDM and PDM

LMA was significantly related to both LDM (Fig. 6a) and PDM (Fig. 6b) across the olive varieties.

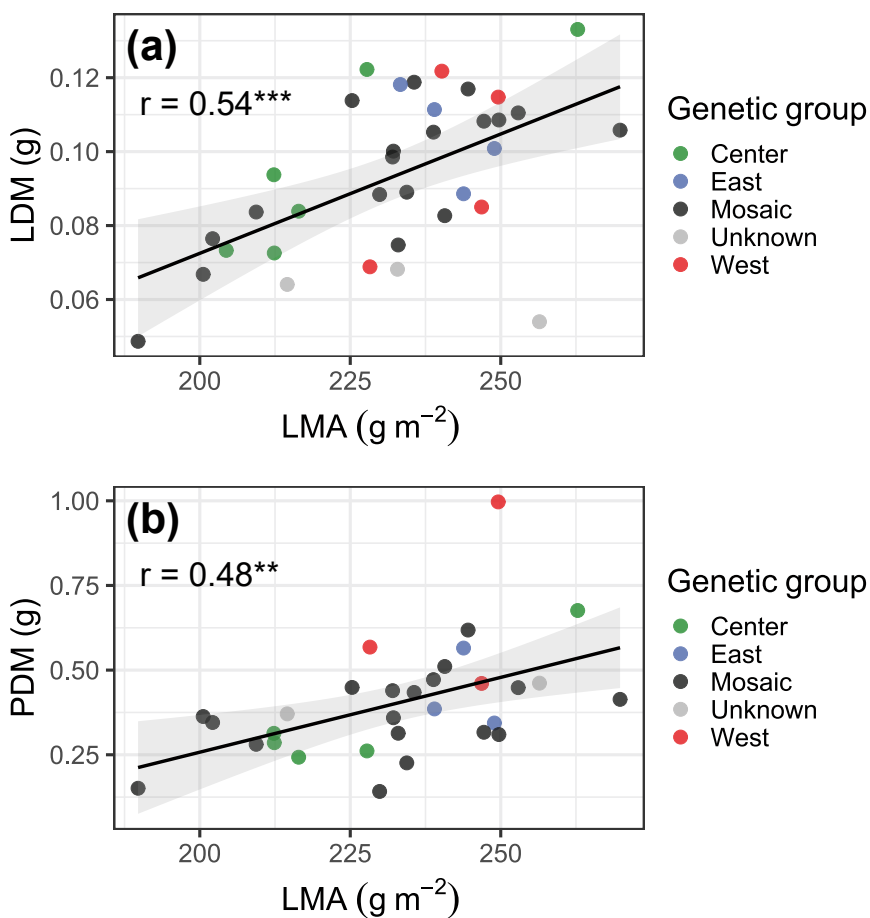


Figure 6. Bivariate relationships between leaf mass per area (LMA) and **(a)** leaf dry mass (LDM), and **(b)** pit dry mass (PDM). Pearson correlation coefficients are indicated on each graph. The regression line is in black; the 95 % - confidence interval is in light grey. Data points are the means of each olive variety ($n = 35$ for LDM ; $n = 31$ for PDM). Colors indicate the genetic group of the varieties. Significance levels are : $^{**} p < 0.01$; $^{***} p < 0.001$.

In the two SEMs tested (Fig. 7), LMA was positively determined by both LDMC (path coef. = 0.55) and LT (path coef. = 0.50). Both models explained approximately three-quarters of the variation in LMA across olive varieties ($R^2 = 0.71$). Overall, the “biomechanical” model (Fig. 7a) better explained the causal relationships between leaf traits and organ sizes than the “allometric” model (Fig. 7b). The “biomechanical” model (Fig. 7a) had the lowest AIC (AIC = 49.44, $\Delta AIC = -2.91$), non-significant χ^2 test ($\chi^2 = 6.01$, $df = 9$, $p = 0.199$), and high goodness-of-fit (GFI = 0.96). In comparison, the ‘allometric’ model (Fig. 7b) had a higher AIC (AIC = 52.35, $\Delta AIC = + 2.91$), a significant χ^2 test ($\chi^2 = 10.9$, $df = 4$, $p = 0.05$), and a lower goodness-of-fit (GFI = 0.93). In the “biomechanical” model, LMA directly determined LDM (path coef. = 0.56) and PDM (path coef. = 0.50), with no significant co-variation between LDM and PDM, explaining one-third and one-quarter of the variation in LDM ($R^2 = 0.32$) and PDM ($R^2 = 0.25$), respectively. By contrast, although LMA directly determined LDM as well in the “allometric” model (path coef. = 0.56), LDM also significantly determined PDM (path coef. = 0.37), but explained a lower part of the variation of PDM ($R^2 = 0.14$) than LMA did in the “biomechanical” model.

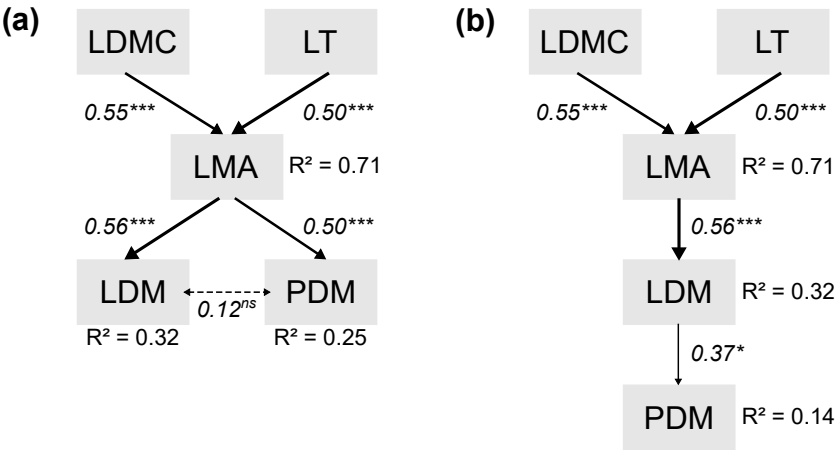


Figure 7. Structural equation models showing the causal relationships between olive leaf and pit traits under either **(a)** the ‘bio-mechanic’ hypothesis (GFI index = 0.961) and **(b)** the ‘allometric’ hypothesis (GFI index = 0.933). The arrows represent relationships between the traits (boxes). Standardized path coefficients give the strength of the relationships. R^2 values indicate the total explained variation of dependent traits. Significance levels are : * $p < 0.05$; *** $p < 0.001$.

Discussion

All traits screened in the present study display a wide range of variation among olive varieties. Since all trees are grown under the same environmental conditions and samples collected to minimize variations in environmental conditions across the tree canopy, such variation is largely attributable to intrinsic genotypic differences among these varieties. However, this phenotypic variation was only hardly associated with the genetic structuration of olive across the Mediterranean Basin. The range of variation in trait values was large enough to (i) detect significant patterns of trait covariations within and between organs, and (ii) suggest a model of causal relationships among leaf traits and pit size. These different points are discussed below.

Trait variation across olive varieties

The traits values found here are in the range of what is usually observed for field-grown cultivated olive trees, whether for leaf shape and dimensions (e.g. Moutier *et al.*, 2004, 2011; Trentacoste and Puertas, 2011; Koubouris *et al.*, 2018), leaf mass *per area* and its components (e.g. Proietti and Famiani, 2002; Bacelar *et al.*, 2004), or pit size and shape (e.g. Moutier *et al.*, 2004, 2011; Trentacoste and Puertas, 2011). The average LMA value across varieties (233 g m^{-2}) - taken as a coarse index of sclerophylly (Salleo and Nardini, 2000) -, is slightly higher than the average value found for Mediterranean sclerophylls (185 g m^{-2} ; Flexas *et al.*, 2014) and close to the average value found in 79 populations of *Quercus ilex* (230 g m^{-2}), another sclerophyllous species iconic of the central and western Mediterranean vegetation (Niinemets, 2015).

Trait values spanned a substantial range of variation across varieties (~ 7-fold for PV, the most variable trait, to 1.3 fold for LDMC, the least variable one), and significant differences among varieties were found for all traits. Significant differences among genetic groups were found only for pit volume and leaf shape (LL/LW). Interestingly, the LL/LW ratio is the most discriminant leaf trait for the identification of olive varieties (cf. Moutier *et al.*, 2004). However, although most pit traits are also considered discriminant, we did not find any significant differences among genetic groups for the three other pit traits (PL, PW and PL/PW). The tendency remained similar when we assessed differences among groups using the multivariate trait space instead of individual traits: only the 'West' group was significantly - though marginally - different from the three other groups, as a consequence of the bigger pits and more lanceolate-shaped leaves (higher LL/LW ratio) of varieties belonging to this group. We thus conclude that the morphological traits we used - some of which with a high discriminatory power to identify olive varieties - have only a limited ability to discriminate among the

genetic groups identified across the Mediterranean Basin. Assessing the robustness of this finding would require to increase the number of varieties tested and reach a more balanced distribution of varieties among groups (cf. Table I). Alternatively, other genetic groupings might also be tested, as the assignment of varieties to different clusters is still under debate (Diez *et al.*, 2015; Besnard and Rubio de Casas, 2016). Whatever the case, these further testings could be conducted in the context of germplasm collections with a wider genetic coverage such as the Worldwide Olive Germplasm Banks of Tassaout (close to Marrakech, Morocco: Haouane *et al.*, 2011) or Córdoba (Spain: Caballero *et al.*, 2006).

Covariations among traits

Multi- and bi-variate relationships show significant patterns of trait covariation within leaves on the one hand, and between leaves and pits on the other hand.

First, we found a positive relationship between LMA and leaf size, as assessed with leaf dry mass. This is the consequence of leaf mass increasing more rapidly than leaf area in olive, as found for many other species (Milla and Reich, 2007 and references therein). Assuming that the relationship between LA and LDM can be written as $LDM = \alpha LA^\beta$ (Milla and Reich, 2007), we found a value of 1.11 (± 0.06 SE) for the scaling coefficient β . This value is very close to the central tendency found within-species ($\beta = 1.10$) for the 157 species included in the study by Milla and Reich (2007) and is in line with broad trends found among species (Niklas *et al.*, 2007). This finding agrees with the hypothesis that bigger leaves require to be stiffer to compensate for mechanical damage and gravity (Milla and Reich, 2007 and references therein). We show here that this also holds in the case of olive, a sclerophyllous species with inherently small leaves with high LMA values.

Second, both structural equation models show that LMA is determined almost equally by its two underlying components, leaf thickness and density (Witkowski and Lamont, 1991; Poorter and Garnier, 2007), the latter approximated using LDMC (Garnier and Laurent, 1994). Across the range of variation of LMA (from 189 to 270 g m⁻²), LT varied between 465 and 622 μ m and LDMC varied between 414 and 537 mg g⁻¹, independently from one another. These results are qualitatively comparable to what Shipley (1995) found on 34 species of angiosperms, except that LDMC and LT were positively - albeit weakly - related in this study. We are aware of only three studies in which LMA and its components have been simultaneously determined in leaves of olive trees. In the first one, conducted on five varieties (Bacelar *et al.*, 2004), LMA varied between 184 and 234 g m⁻² and was positively related to LT (range of variation: 431-554 μ m), with only little

variation in tissue density among varieties (460-482 mg g⁻¹). The two other ones (Guerfel *et al.*, 2009; Ennajeh *et al.*, 2010) involved only two varieties each, and showed that the high LMA varieties had higher leaf tissue density and thicker leaves than the low LMA varieties, in line with our results. In these three studies, differences in lamina thickness were attributable to the different proportions of mesophyll components in leaves (e.g. upper palisade layer vs. spongy parenchyma), with different combinations among varieties. Interpreting differences in tissue density requires the determination of the proportions of light (*i.e.* mesophyll, epidermis) and dense (*i.e.* vascular bundles, sclerenchyma) tissues in the leaves (Garnier and Laurent, 1994; van Arendonk and Poorter, 1994), which was not done in these studies. Further insights into the underlying causes of variation in LMA components among varieties would therefore require a detailed anatomical description of the different leaf tissues and their chemical composition (John *et al.*, 2017).

Finally, we found that varieties with large leaves (high leaf dry mass) also have big pits (high pit dry mass), pointing to a certain degree of coordination between vegetative and reproductive organs, as hypothesized and shown for several genera by Primack (1987). According to Corner's rules of allometry in terrestrial plants (Corner, 1949), this relationship might be mediated through the size of twigs, as large twigs are necessary to bear big vegetative and reproductive appendages (e.g. leaves, inflorescences, fruits). Not all data support this hypothesis however (Hiura *et al.*, 1996; Cornelissen, 1999; Hodgson *et al.*, 2017), and the comparison of the two structural equation models tested with our data showed that this allometric model had a lower explanatory power than the alternative model in which the relationship between leaf and pit mass is mediated through LMA. This finding agrees with the Seed-Phytomer-Leaf theoretical model (SPL hereafter) proposed by Hodgson *et al.* (2017), which predicts a positive relationship between leaf stiffness and the mass of reproductive organs. In this model, based on four key variables, the size and growth of vegetative organs are linked to the size of reproductive structures. A simplified version of the SPL model uses LMA as a surrogate of growth (cf. Garnier *et al.*, 2016 for a discussion), and predicts that slowly-growing species with stiff leaves (high LMA, high LDMC leaves) also tend to have big seeds. Given that seed and pit sizes are strongly related in olive ($r = 0.69$, $p < 0.001$, $n = 99$: data from Ruby 1918), our results are in line with this prediction. Although the data used by Hodgson *et al.* (2017) also support this model across a wide range of species, a positive association between LMA (or its underlying components) and seed mass has not always been found in other studies (see Segrestin *et al.*, 2020). Further testing of this model is thus required both across and within species. The case of fruit trees appears particularly interesting in this context, since in these species, the fruit but not the leaf, has been the target of human selection.

Conclusions and perspectives

Our study shows that leaf and pit traits span a large range of variation across olive varieties originating from geographic regions spread over the Mediterranean Basin. For most traits, this variation does not relate to the genetic groups previously recognized across the Basin, which needs to be further assessed on a wider range of varieties belonging to the different genetic groups. Variation in leaf mass *per* area, a key plant functional trait, was equally explained by its two underlying components, leaf thickness and density, and was pivotal to explain the relationship between leaf and pit mass.

The pit data collected here will be further enriched with data on fruits and twigs to test whether Corner's allometric model in which twig size plays a central role holds in the specific case of olive. Further studies will also be conducted to assess how several leaf traits determined in this study - leaf area, leaf width, leaf mass *per* area - relate to chemical composition, gas exchange and plant water economy, so as to better understand how the different olive varieties cope with the contrasting climatic conditions from which they originate. This work would strengthen the physiological bases for the selection of olive varieties in the context of climatic changes currently occurring in the Mediterranean Basin.

Acknowledgements. We thank Bouchaib Khadari and Sylvia Lochon-Menseau, from the Conservatoire Botanique National Méditerranéen de Porquerolles, for providing us with a wealth of information on the Porquerolles germplasm collection, and for their constant support during this work. Bruno Bernazeau (Sauvegarde des Forêts Varoises) is warmly thanked for his welcome and continuous help, and his key role in the maintenance of the collection. Marc Duncombe, the Director of the Port-Cros National Park, is thanked for making premises available to conduct this work. Cyril Bernard (CEFE) elaborated the background map used in Figure 1, and Benoît Garnier helped to design the final layout of this figure. Finally, we thank the reviewers, André Bervillé and Jean-Frédéric Terral, for valuable suggestions to improve the manuscript. This study was partially supported by a grant from the French National Research Agency under the "Investissements d'avenir" program (Labex Agro: ANR-10-LABX-001-01, coordinated by Agropolis Fondation) to KB, and by the European Research Council (ERC) ('CONSTRAINTS': grant ERC-StG-2014-639706-CONSTRAINTS to Cyrille Violle, at CEFE).

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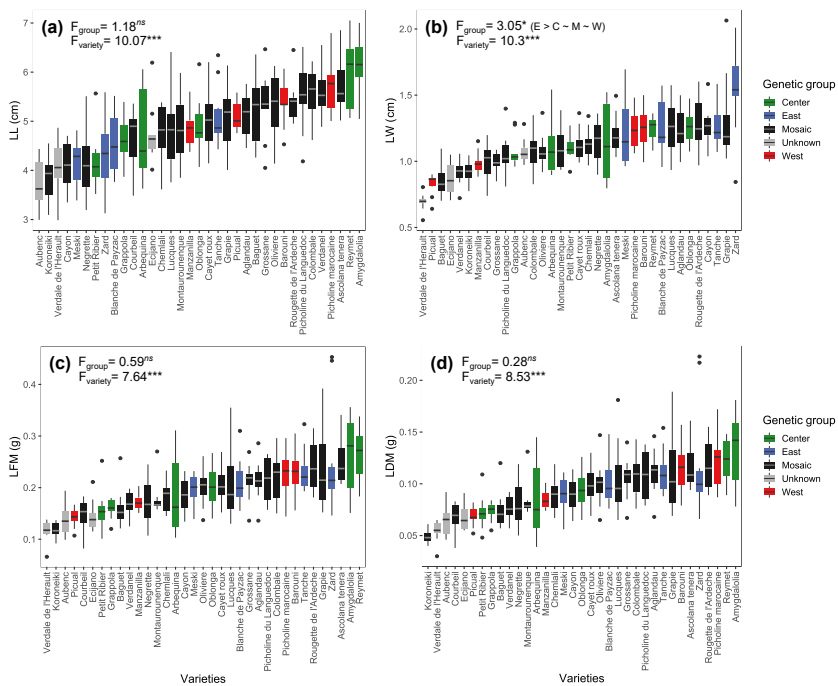
Appendix I. Average values of the 9 leaf traits measured on samples taken from trees of the Porquerolles germplasm collection. Abbreviations: LL, leaf length; LW, leaf width; LL/LW, leaf length to leaf width ratio; LA, leaf area; LFM, leaf fresh mass; LDM, leaf dry mass; LMA, leaf mass *per* area; LDMC, lead dry matter content; LT, leaf thickness

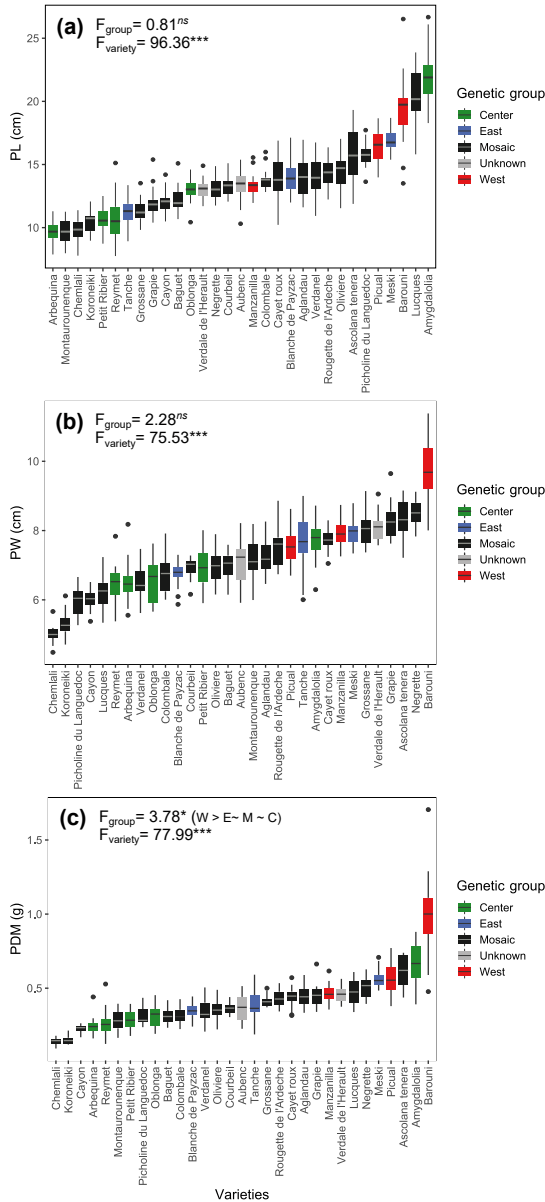
Variety	LL (cm)	LW (cm)	LL/LW (cm cm ⁻¹)	LA (cm²)	LFM (g)	LDM (g)	LMA (g m ⁻²)	LDMC (mg g ⁻¹)	LT (µm)
Aglandau	5.13	1.25	4.14	4.36	0.214	0.110	253	516	548
Amygdalolia	6.09	1.15	5.56	5.09	0.263	0.133	263	508	567
Arbequina	4.73	1.09	4.33	3.79	0.184	0.084	216	452	527
Ascolana tenera	5.74	1.21	4.82	4.76	0.250	0.117	245	468	570
Aubenc	3.75	1.07	3.49	2.98	0.136	0.064	215	472	525
Baguet	5.21	0.85	6.18	3.19	0.161	0.075	233	465	577
Barouni	5.38	1.24	4.39	4.59	0.230	0.114	250	498	575
Blanc de Payzac	4.60	1.25	3.78	4.07	0.209	0.101	249	482	622
Cayet roux	5.04	1.13	4.50	4.25	0.205	0.099	232	481	530
Cayon	4.11	1.27	3.25	3.79	0.185	0.089	234	482	532
Chemlali	4.79	1.13	4.27	3.83	0.183	0.088	230	483	495
Colombale	5.57	1.08	5.21	4.38	0.221	0.108	247	490	574
Courbeil	4.66	0.99	4.75	3.33	0.144	0.067	201	464	474
Ecojano	4.74	0.87	5.53	2.93	0.145	0.068	233	470	548
Grapié	5.10	1.32	3.99	5.04	0.243	0.114	225	467	550
Grappola	4.64	1.07	4.37	3.59	0.159	0.073	204	460	465
Grossane	5.33	1.00	5.38	3.91	0.213	0.106	270	497	589
Koroneiki	3.84	0.92	4.22	2.57	0.118	0.049	190	414	481
Lucques	4.80	1.25	3.86	4.42	0.205	0.105	239	517	515
Manzanilla	4.84	0.99	4.93	3.44	0.173	0.085	247	490	542
Meski	4.15	1.22	3.53	3.65	0.194	0.089	244	451	544
Montaurounenque	4.82	1.10	4.48	3.99	0.178	0.084	209	470	503
Négrette	4.17	1.14	3.72	3.41	0.175	0.083	241	471	568
Oblonga	4.92	1.25	3.95	4.40	0.203	0.094	212	460	595
Olivière	5.34	1.08	4.98	4.30	0.202	0.100	232	495	520
Petit Ribier	4.18	1.11	3.79	3.43	0.155	0.073	212	471	511
Picholine du Languedoc	5.52	1.05	5.34	4.34	0.216	0.109	250	502	547
Picholine marocaine	5.73	1.23	4.67	5.05	0.227	0.122	240	537	500
Pical	5.10	0.83	6.21	3.01	0.142	0.069	228	484	566
Reymet	6.01	1.25	4.82	5.38	0.264	0.122	228	465	549
Rougette de l'Ardèche	5.41	1.27	4.34	5.03	0.243	0.119	236	489	548
Tanche	5.09	1.28	4.03	4.65	0.233	0.111	239	477	558
Verdale de l'Hérault	4.07	0.69	5.87	2.11	0.115	0.054	256	468	564
Verdanel	5.62	0.92	6.19	3.78	0.167	0.076	202	456	518
Zard	4.36	1.56	2.93	4.93	0.249	0.118	233	471	604

Appendix II. Average values of the 5 pit traits measured on samples preserved in the pit collection in Porquerolles. Abbreviations: PL, pit length; PW, pit width; PL/PW: pit length to pit width ratio; PDM, pit dry mass; PV, pit volume; NA, data not available.

Variety	PL (cm)	PW (cm)	PL/PW (cm cm ⁻¹)	PDM (g)	PV (cm ³)
Aglandau	1.40	0.725	1.94	0.448	0.383
Amigdalolia	2.21	0.770	2.87	0.676	0.612
Arbequine	0.97	0.652	1.48	0.243	0.214
Ascolana tenera	1.57	0.837	1.88	0.618	0.542
Aubenc	1.33	0.708	1.89	0.371	0.323
Baguet	1.22	0.698	1.76	0.314	0.281
Barouni	1.92	0.973	1.97	0.997	0.896
Blanche de Paysac	1.40	0.674	2.08	0.344	0.312
Cayet roux	1.39	0.773	1.80	0.439	0.382
Cayon	1.21	0.602	2.01	0.226	0.196
Chemlali	0.98	0.501	1.96	0.142	0.124
Colombale	1.38	0.674	2.06	0.316	0.282
Courbeil	1.33	0.692	1.92	0.363	0.310
Ecijano	NA	NA	NA	NA	NA
Grapié	1.20	0.826	1.45	0.449	0.399
Grappola	NA	NA	NA	NA	NA
Grossane	1.13	0.806	1.41	0.414	0.365
Koroneiki	1.04	0.530	1.97	0.151	0.134
Lucques	2.05	0.623	3.30	0.472	0.404
Manzanilla	1.34	0.790	1.69	0.461	0.396
Meski	1.69	0.795	2.13	0.565	0.509
Montaurounenque	0.97	0.718	1.35	0.281	0.254
Négrette des Vans	1.31	0.851	1.54	0.511	0.453
Oblonga	1.30	0.656	1.99	0.313	0.270
Olivière	1.45	0.697	2.08	0.359	0.312
Petit Ribier	1.06	0.697	1.53	0.286	0.254
Picholine du Languedoc	1.58	0.596	2.66	0.310	0.271
Picholine marocaine	NA	NA	NA	NA	NA
Pical	1.64	0.757	2.17	0.568	0.479
Reymet	1.07	0.642	1.66	0.261	0.235
Rougette de l'Ardèche	1.44	0.752	1.92	0.434	0.400
Tanche	1.12	0.769	1.46	0.385	0.338
Verdale de l'Hérault	1.31	0.810	1.61	0.461	0.424
Verdanel	1.41	0.653	2.16	0.345	0.305
Zard	NA	NA	NA	NA	NA

Appendix III. Distribution of **(a)** leaf length (LL), **(b)** leaf width (LW), **(c)** leaf saturated fresh mass (LFM), and **(d)** leaf dry mass (LDM) for 35 olive varieties of the ‘Mediterranean collection’ of Porquerolles. Boxplots show median (horizontal line), inter-quartiles (boxes), non-outlier ranges (vertical lines) and outliers (points). Varieties are sorted by their mean trait values and colored according to their genetic group. The differences between genetic groups were tested using nested ANOVAs, excluding the ‘Unknown’ group. Letters indicate which groups (C: Center, E: East, M: Mosaic, W: West) differed from the others according to *post-hoc* Tukey tests. Significance levels are : ^{ns} not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$





Appendix IV. Distribution of **(a)** pit length (PL), **(b)** pit width (PW), **(c)** pit dry mass (PDM) for 31 olive varieties of the ‘Mediterranean collection’ of Porquerolles. Boxplots show median (horizontal line), inter-quartiles (boxes), non-outlier ranges (vertical lines) and outliers (points). Varieties are sorted by their mean trait values and colored according to their genetic group. The differences between genetic groups were tested using nested ANOVAs without the ‘Unknown’ group. Letters indicate which groups (C: Center, E: East, M: Mosaic, W: West) differed from the others according to *post-hoc* Tukey tests. Significance levels are : *ns* not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.