Geometric Morphometrics use in the examination of subgenus *Quercus* leaf shape variation in Algeria

Abdeldjalil Aissi*, Yassine Beghami

LAPAPEZA, Université Batna 1, ISVSA, Batna, Algeria

Abstract

Abdeldjalil, A., Beghami, Y., 2022. Geometric Morphometrics use in the examination of subgenus *Quercus* leaf shape variation in Algeria. *Folia Oecologica*, 49 (2): 175–181.

The latest findings on the taxonomic review of *Quercus faginea* Lam. complex using 'traditional morphometrics', demonstrating that the species is represented in Algeria by both *Q. faginea* and *Q. canariensis* Willd. Significant variations of the leaf form were also discernible among both species. In this study, the landmark-based geometric morphometrics analysis was used to assess the shape variation of the leaves found on oak stands. 2,600 leaves per 13 stands were collected and scanned, and then using Tps range and MorphoJ software, 11 landmarks—that represent the leaf morphological features—were recorded on leaf images. Shape components and non-forms variations were obtained through a full Procrustes fit followed by creating a leaf-superimposed configuration. Principal component analysis, canonical variate analysis, and discriminate analysis were used to statistically evaluate the leaf shape variability. The results revealed no clear distinction between the two species based on leaf shape. Climate change and environmental factors also appear to have possibly caused a divergent morphological evolution; a reduced leaf size with enduring indumentum—among other *Q. faginea* traits—could be an efficient mean of adapting to Mediterranean xeric conditions.

Keywords

adaptation traits, *Q. canariensis* Willd., *Q. faginea* Lam., landmarks, morphological evolution

Introduction

The systematic classification of taxa of the genus *Quercus* (Fagaceae) is usually presented using morphological and morpho-anatomical analyses of floral organs and leaves (e.g., Camus, 1938), and micro-morphological analyses (e.g., Tschan and Denk, 2012). This "traditional" morphological method (see Dean et al., 2004) involves measuring the linear distances between different points to analyze the variations in shape within and among the populations under study, thus conducting descriptive multivariate statistical analyses (Blackith and Reyment, 1971). However, the strong correlation between linear distances and sample size (Bookstein, 1986) makes it difficult to distinguish between taxa (Jensen, 1990; Kremer et al., 2002). This indicates that the linear points do not precisely correspond to the geometry of the subject under study, and therefore a graphical representation of the subject cannot be produced (Dean et al., 2004).

To overcome these issues, new morphometric techniques—now known as "Geometric Morphometrics (GM)"—have been developed to study, quantitatively compare the morphological variations between each species, and effectively visualize the shape/form deformations, while maintaining the complete geometric data of the subject (Rohlf and Marcus, 1993; Kendall, 1989; Bookstein, 1996). Using a combination of both previous and present morphometric techniques (two recognized styles: landmark-based and outline) will provide greater examination of the existing relationships between different species and will lead to a better understanding of the morphological variations within and among species, thus improving future taxonomic, phylogenetic, and eco-physiological studies (Viscosi et al., 2009a).

Bearing this in mind, studies on the white oaks of the sub-genus *Quercus* show the significance of these "revolutionary" methods (Rohlf and Marcus, 1993). Not only do these studies highlight the substantial differences between

*Corresponding author:
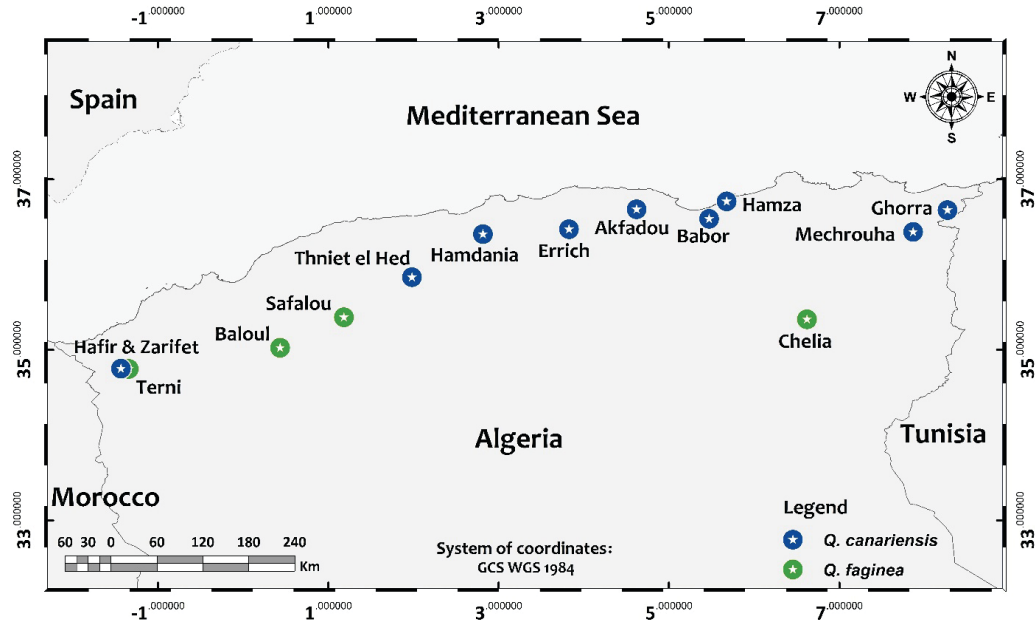e-mail: aissi.abdedjalil@gmail.com

Fig. 1. Map of the studied stands (represented by *Q. canariensis* and *Q. faginea*) in Algeria.

the analyzed species, but also demonstrate the intermediate morphological status of hybrid leaves (Viscosi et al., 2009a; 2009b; Viscosi et al., 2010; Albarrán-Lara et al., 2010; Peñaloza-Ramírez et al., 2010). These studies were carried out without considering qualitative traits, which have been regarded in some studies as necessary for interspecific distinction (Bruschi et al., 2000; Kremer et al., 2002).

In Algeria, the oak stands of the *Quercus* subgenus are represented by the *Quercus faginea* Lam. Complex, comprising of forms (species, subspecies and variety) that are difficult to distinguish and whose taxonomic history is extremely complex (see Aïssi et al., 2021). The results from the morphological and micromorphological study of this complex group found in Aïssi et al. (2021) reveal that the analyzed populations (13 stands spread out over the entire distribution area; Fig. 1) belong to two species: *Quercus canariensis* Willd. and *Q. faginea* Lam. (Fig. 1). The latter is represented by two of its subspecies: *faginea* and *broteroi*. Thus, intermediate leaf type and significant form variation within and between the two groups were discernible. Therefore, these quantitative traits alone do not constitute a practical criterion that can distinguish between both species. However, qualitative traits related to the indumentum type on the abaxial side of leaves seem to be effective, i.e. deciduous for *Q. canariensis* and persistent for *Q. faginea*. Furthermore, at the intraspecific scale, leaf margins, namely toothed leaves differs *faginea* subspecies from *broteroi*, characterized by lobed leaves (Aïssi et al., 2021).

The purpose of this study is to apply landmark-based geometric morphometrics to the systematics of the sub-genus *Quercus* found in Algeria, examine the morphometric relationship existing between *Q. canariensis* and *Q. faginea*, and find intermediate forms between these species.

Materials and methods

Sampling procedures

Throughout the entire distribution area of the two oak stands (Fig. 1), 2,600 mature leaves were collected from 100 mature

Table 1. Study stands main ecological factors

Stands	Abbreviation	Rainfall (mm)	Elevation (m)	Bioclimate
Terni	Trn	688	1,300–1,350	Cool sub-humid
Hafir and Zarifet	Hfr	600–750	1,100–1,300	Cool sub-humid
Balloul	Bll	430	850	Cool semi-arid
Safalou	Sfl	620	950–1,100	Cool sub-humid
Thniet el Hed	Teh	630–870	1,300–1.600	Cool sub-humid
El hamdania	Hmd	1,100–1,300	1,150–1,600	Cool humid
Errich	Err	630	500–600	Cool sub-humid
Akfadou	Akf	1,078–1,132	750–1,300	Cool humid
Babor	Bbr	1,200–1,500	1,200–1,650	Cool humid
Hamza	Hza	922	450-500	Mild Humid
Ghorra	Gra	950	700–850	Cool humid
Machrouha	Mch	625	850–1,000	Cool sub-humid
Chelia	Chl	360–523	1,200–1,700	Cold semi-arid

Table 2. Description of landmarks recorded on the right half of *Q. canariensis* and *Q. faginea* leaves (Viscosi et al., 2009a)

Landmark	Description
1	The start of the petiole
2	Beginning at the petiole, the midrib intersects with vein of the first basal lobe (referred to landmark 1)
3	At the widest point of the leaf blade, the midrib meets the vein of the lobe (referred to landmark 8)
4	Immediately above the apex of the leaf blade, the midrib meets the vein of the first lobe (referred to landmark 7)
5	The leaf blade's apex
6	The sinus's base is directly above the leaf blade's apex.
7	The tip of the lobe just above the leaf blade's apex.
8	At the widest point of the leaf blade, the tip of the lobe.
9	Just above the lobe of landmark 8, the base of the sinus
10	Starting from the petiole, the tip of the first lobe
11	Petiole-blade junction

trees (20 leaves per tree) that were found on 13 stands (same sample localities from the morphological and micromorphological study by Aïssi et al., 2021) with different environmental conditions (Table 1) (Aïssi et al., 2021). The data revealed that both leaf species exhibits dissimilar distributions (cf. Fig. 1). *Q. canariensis* covers a large area and forms large stands stretching across the far eastern and central parts of Algeria (Maire, 1961; Aïssi et al., 2021), with a smaller population found in the far West along the Tlemcen Mountains (Hafir and Zarifet forests). *Q. faginea* appears in isolated stands alongside other species in four separate locations. Aside from the close distribution found in Tlemcen region, the two species stands do not share the same biogeographical distribution (Aïssi et al., 2021). As per the prior source, the type of indumentum on the leaves was used to identify the two species and appropriately allocate the population samples, with *Q. canariensis* leaves having a deciduous indumentum and *Q. faginea* leaves having a persistent indumentum.

Further assortment of the leaves helped to eliminate those bearing morphological abnormalities, thus obtaining 10 leavers per tree. The remaining leaves were scanned and saved on a tps (.tps) file using tpsUtil software v1.67 (Tps Software series). Using tpsDig2 v.2.26, 11 landmarks were digitized (Viscosi and Cardini, 2012) (Table 2). For further description of these landmarks, see Viscosi et al. (2009a). Before adopting these procedures, several combinations were tested to determine the best represented morphometry of the two analyzed species (Viscosi et al., 2009a; 2009b

and further combinations). At the end of the digitization, a file (.tps) was created containing all the geometric information (raw coordinates) of the leaves. An approximation of the Procrustes space was created by using tpsSmall software v1.33. A full Procrustes fit (Dryden and Mardia, 1998) via MorphoJ software v1.06d (Klingenberg, 2011) was achieved to eliminate any non-form variations and thus identify shape components. Using Mahalanobis distance, a final check was made to detect and eliminate specimens that were well beyond average or that contained any anomalies (repetition and mispositioning of the landmarks) created during digitization (Klingenberg and Monteiro, 2005).

Statistical analyses

With the use of MorphoJ software, a principal component analysis (PCA) was performed to inspect trends in the stand shape and determine any variability between the two taxa and stands. To resolve the issue with intra-tree morphological variability (Jensen, 1990), the leaves of each tree were further processed to create a mean leaf configuration (Viscosi et al., 2009a). In this new configuration, the number of leaves was reduced to 130 (one leaf per tree). Based on this configuration, a second PCA was performed. In addition, canonical variate analysis (CVA) was used to compare the two taxa and shape variations of the leaves in the stands. Discriminate analysis (DA) through cross-validation function was also used to test the significant differences of the leaf shapes between the two taxa.

Table 3. Procrustes distances within-group matrices from canonical variate analysis (CVA) of leaf shape of 13 stands of *Q. canariensis* and *Q. faginea* (9 and 4 stands, respectively)

|	Hfr	Trn	Bll	Sfl	Teh	Hmd	Err	Akf	Bbr	Hza	Gra	Mch
Trn	0.04											
Bll	0.09	0.07										
Sfl	0.06	0.06	0.08									
Teh	0.06	0.07	0.12	0.06								
Hmd	0.03	0.04	0.08	0.05	0.05							
Err	0.09	0.10	0.11	0.05	0.07	0.07						
Akf	0.12	0.11	0.07	0.10	0.15	0.12	0.14					
Bbr	0.10	0.09	0.04	0.08	0.12	0.09	0.10	0.05				
Hza	0.08	0.07	0.08	0.05	0.07	0.06	0.07	0.09	0.06			
Gra	0.09	0.07	0.06	0.06	0.10	0.07	0.08	0.08	0.04	0.05		
Mch	0.15	0.14	0.11	0.13	0.16	0.14	0.15	0.07	0.09	0.11	0.09	
Chl	0.05	0.03	0.07	0.06	0.08	0.04	0.10	0.10	0.08	0.06	0.06	0.12
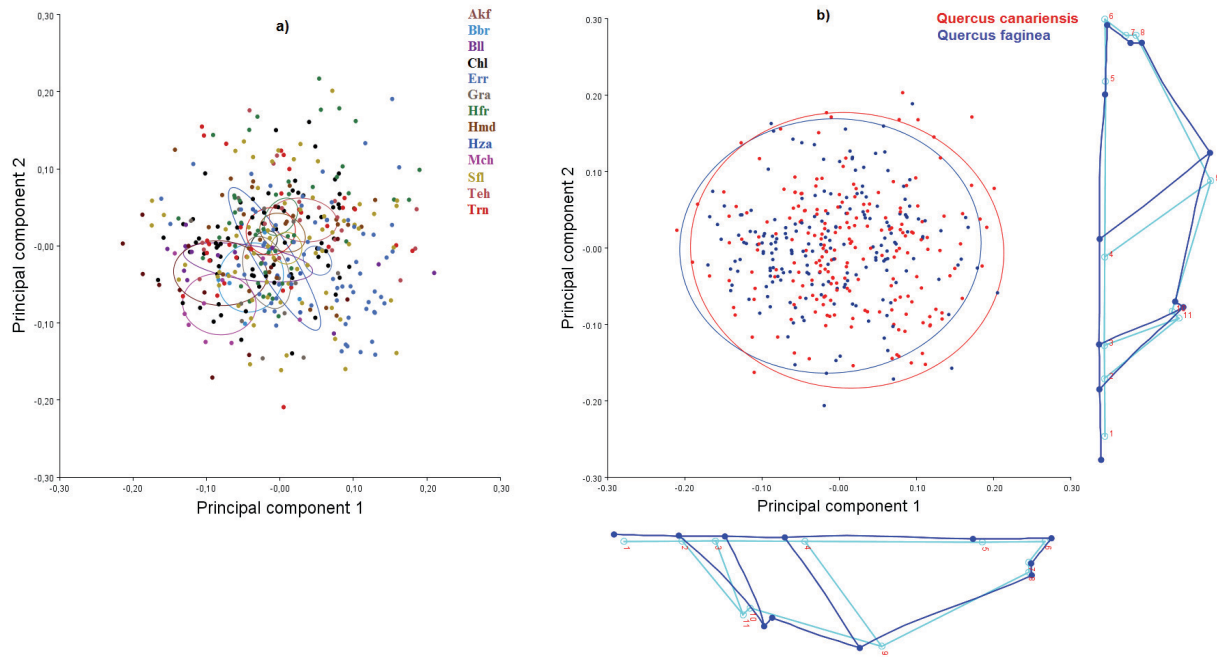
Fig. 2. Principal component analysis (PCA) (axes 1 and 2) plot performed on all leaves, showing the leaf shape of 13 stands of *Q. canariensis* and *Q. faginea* (9 and 4 stands, respectively), with 95% confidence ellipses for stands means (a) and taxa means (b). The conformation graphs (Wireframe Graph) show the morphological features of the leaves along both axes.
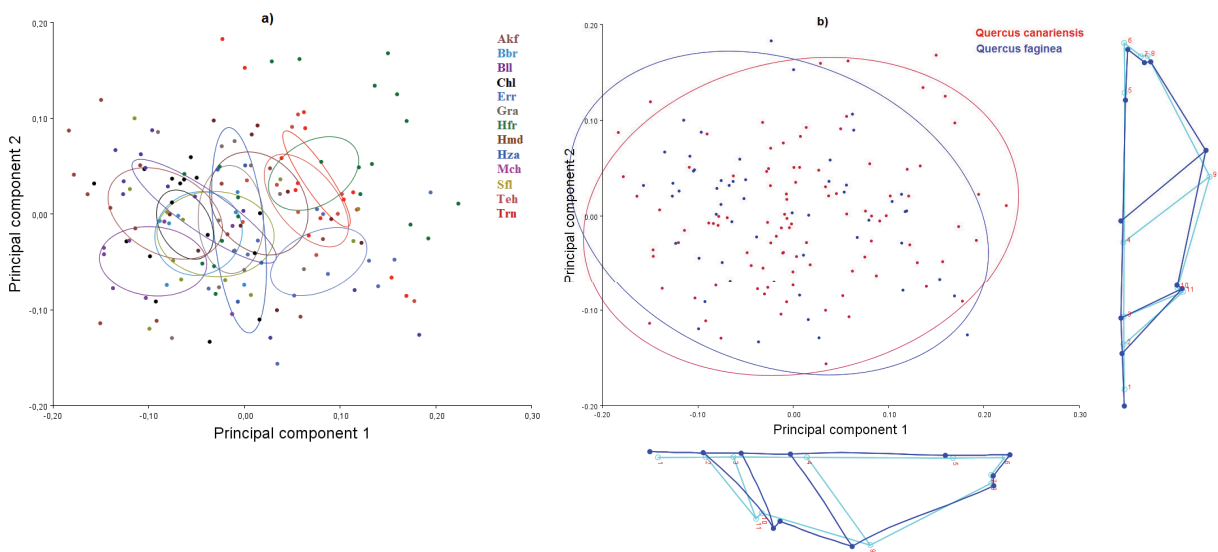

Fig. 3. Principal component analysis (PCA) (axes 1 and 2) plot performed based on the mean leaf configuration (130 leaves), showing the leaf shape of 13 stands of *Q. canariensis* and *Q. faginea* (9 and 4 stands, respectively), with 95% confidence ellipses for stands means (a) and taxa means (b). The conformation graphs (Wireframe Graph) show the morphological features of the leaves along both axes.

Results

Multivariate analysis

Figures 2 and 3 show, respectively, the results of the first two components of the PCA performed for each leaf, on both stands and taxa levels (axes one and two) showing variations of 36.60% and 28.95% for the first PCA (a), and of 37.36% and 28.42% for the second PCA (b), as well as the first two components of the PCA performed and based on the mean leaf configuration. Axes one and two showing a variation of 26.40% and 15.24% for the first PCA, (a), and 46.16% and 25.33% for the second PCA (b). In all four diagrams, the dot cluster trend shows strong correlations between the different examined stands. In fact, the leaves of the stands pre-classified as *Q. faginea* completely overlap those of *Q. canariensis*. This similarity is also indicated by conformation graphs (Wireframe Graph) which show no morphological shape differentiation among the different populations and species (Fig. 2 and 3). Similarly, confidence ellipses of the mean
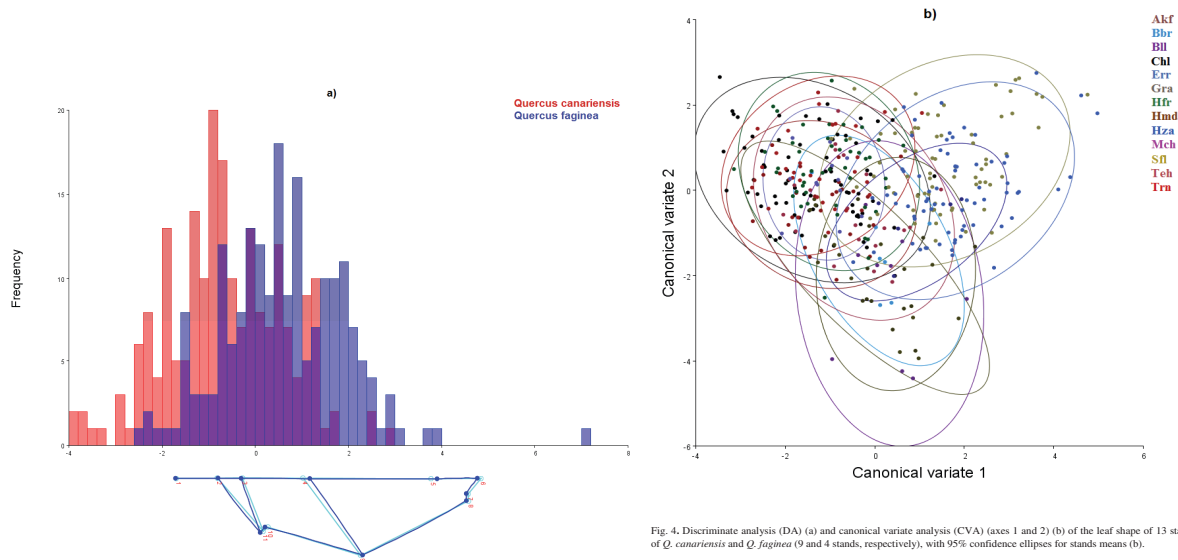
178

Fig. 4. Discriminate analysis (DA) (a) and canonical variate analysis (CVA) (axes 1 and 2) (b) of the leaf shape of 13 stands of *Q. canariensis* and *Q. faginea* (9 and 4 stands, respectively), with 95% confidence ellipses for stands means (b).

of each stand widely overlap and therefore do not provide a sufficiently clear distinction among and between the two oaks (Fig. 2 and 3).

The DA between the means of the two oaks turned out to be significant (Fig. 4a, $T^2 = 143.70$, $p = > 0.0001$). However, a cross-validation test revealed that 68.58% of *Q. canariensis* individuals and 67.78% of *Q. faginea* individuals were correctly classified. This indicates that unambiguously identifying a given leaf (regardless of other morphological traits) seems unfeasible. Moreover, the results of the CVA computed with Mahalanobis distance (sequential Benferroni significance) demonstrated insignificant inter-population differences (Table 3, Fig. 4a). The confidence ellipses of the mean of each stand similarly implies a scarce differentiation based on leaf shape that does not allow for a clear distinction between *Q. canariensis* and *Q. faginea* (Fig. 4b).

Discussion

The approximation of our data in the tangent space shows that the gradient of the regression line is practically equal to one ($r^2 = 0.999996$ with a correlation of 1.000000). In previous studies, several authors have stated that the distance between the Procrustes and tangent space is generally small (ROHLF, 1999). Although the regression line for the data approximation is close to 1 (DEAN et al., 2004), checking the variation between the two spaces is essential and ensures that the approximation of the tangent space can be used (VISCOSI and CARDINI, 2012).

Contrary to previous morphometric studies, the aforementioned results showed no clear morphological distinction between the two analyzed species. It is important to note the frequency in previous studies to compare the differences between different species of red oak in North America (JENSEN et al., 1993), white oak in Europe (VISCOSI et al., 2009a; 2009b; VISCOSI et al., 2010; VISCOSI 2015), several types of oak in Mexico (ALBARRÁN-LARA et al., 2010; PEÑALOZA-RAMÍREZ et al., 2010), two sympatric oak found in China (LIU et al., 2018), and four sympatric Mediterranean oaks and hybrids in

Algeria (AKLI et al., 2022). However, the results of the analyses highlighted a strong correlation between the two species and drew attention to a strong morphological similarity of leaf shape between *Q. faginea* and *Q. canariensis*. This would explain why botanists previously encountered difficulties when designating the taxonomy of *Q. faginea* Lam. complex (cf. AISSI et al., 2021). Our results thus conflict with those obtained by VISCOSI et al. (2009b), showing that the implementation of the mean leaf configuration can provide a solution for the lack of significant differences within and between the analyzed species.

By acknowledging the efficient combination of landmarks that were chosen, the morphological variability between the stands of the two species is mainly due to a variation in the size of their leaves. In fact, these stands show a gradual reduction in leaf size, which is a characteristic trend from east to west and from north to south. Environmental and climatic variations are most likely the main causes of this phenomenon (TRABUT, 1892; AISSI et al., 2021). Drought is also commonly known to cause a reduction in the leaf size of several plants (SCHIMPER, 1903), and it would be no different for the leaves of the *Quercus* species. The results from CORCUERA et al. (2002) found that oaks in the Mediterranean have leaves with a smaller leaf surface area compared to those found in nemoral and temperate zones. In addition, intense solar radiation and high temperatures appears to affect the leaf size, by producing either small-sized or widely-lobed leaves (MCDONALD et al., 2003). However, MAHEBI BIJARPASI et al. (2019) noted that *Fagus orientalis* Lipksy morphological and physiological traits could be affected differently along elevation gradients, reflecting a specific adaptation to local conditions. A comparative study between three oak species (*Q. robur* L., *Q. pyrenaica* Willd. and *Q. faginea*) revealed a correlation between reduced leaf size and shape. This included lobed margins on one side and leaf temperature on the other, thus suggesting that these morphological traits contribute to a lower leaf temperature (GIL-PELEGRÍN et al., 2017). The reduction in leaf size is associated with a decrease in surface area, which should increase the specific hydraulic conductivity and make the species more resistant to Mediterranean climate conditions (PEGUERO-PINA et al.,

179

2016; Gil-Pelegrín et al., 2017).

The qualitative characteristics of the leaves are known for their numerous functions in several species of the genus *Quercus* (Tschan and Denk, 2012; Gil-Pelegrín et al., 2017), especially the indumentum. This includes interspecific separation (Bruschi et al., 2000; Kremer et al., 2002) and the role of the indumentum on the abaxial side of the leaf in xeric environmental conditions (He et al., 2014). The results found in Gil-Pelegrín et al. (2017) demonstrated that the pubescence of the abaxial side of the leaves of the *Quercus* genus is related to the climatic conditions of their habitats, especially during drought. This signifies that pubescent leaves characterize the species that thrive in extremely dry conditions. This study also found that evergreen oaks from arid and Mediterranean areas tend to have the thickest pubescence on the abaxial side of their leaves. Hardin in (1979) made a link between environmental conditions and leaf pubescence, noting that the density of leaf pubescence increases at the intraspecific level as a result of drought. In fact, the reduced size and durability of the leaf indumentum of *Q. faginea*, among other traits, may be a mean of adapting themselves to xeric environmental conditions (Aissi et al., 2021). In contrast, Tschan and Denk (2012) suggest that the deciduous appearance of the indumentum of *Q. canariensis* leaves is likely related to the fact that this species has developed under mesic conditions. Larger leaves with deciduous indumentum appear to be linked to the ecological conditions of the stands in Algeria, especially the higher average rainfall and preferential growth of *Q. canariensis* on more developed soils and on sandstone, schist and siliceous substrates. On the contrary, *Q. faginea* thrives within specific habitat parameters and thus generally grows in less developed soils (Aissi et al., 2021).

Considering the results, *Q. faginea* and *Q. canariensis* appears to share most of the same biometric characteristics, excluding their size. The climatic and environmental changes in Algeria may have caused a divergent morphological evolution. Furthermore, the genetic diversity studied at sequenced microsatellites of 11 Algerian *Q. faginea* and *Q. canariensis* stands exhibited a continuous pattern of genetic differentiation between the two main genetic clusters representing the two species. This pattern was also found with the eight specific clusters that have been identified, and therefore does not unambiguously define any limits between the two species (Lepais et al., 2022). The intermediate and genetic characteristics of the populations—especially the smallest and most isolated (Lepais et al., 2022)—and the morphological similarities among stands and species may be supported by maintaining ancestral polymorphism or ancient hybridization. These makes a significant contribution to the overall genetic diversity of *Q. faginea* (s.l), thus creating an urgency to take action and preserve its vulnerable status, and more specifically that of *Q. faginea* (s.s) (cf. Lepais et al., 2022).

Conclusion

The landmark-based geometric morphometrics analysis of the stands reveal no clear distinction between the two species in terms of form. The analysis thus demonstrated a strong morphological similarity between the different stands examined. These findings strongly support that the morphological evolution of both species could be attributed to climatic change and stand conditions. *Q. faginea* is likely the result of an adaptation to the xeric conditions of the Mediterranean climate. Further genetic analyses is ongoing to study the potential links

between genetics and size variation of the leaves found on stands across species distribution ranges and to better understand their evolutionary history.

References

Aissi, A., Beghami, Y., Lepais, O. Vela, E., 2021. Analyse morphologique et taxonomique du complexe Quercus faginea (Fagaceae) en Algérie (Morphological and taxonomic analysis of Quercus faginea (Fagaceae) complex in Algeria). *Botany*, 99: 99–113. https://doi.org/10.1139/cjb-2020-0075

Akli, A., Lorenzo, Z., Alia, R., Rabhi, K., Torres, E., 2022. Morphometric analyses of leaf shapes in four sympatric Mediterranean oaks and hybrids in the Algerian Kabylie forest. *Forests*, 13, 508: 1–11. https://doi.org/10.3390/f13040508

Albarrán-Lara, A.L., Mendoza-Cuenca, L., Valencia-Alvados, S., Gonzales-Rodríguez, A., Oyama, K., 2010. Leaf fluctuating asymmetry increases with hybridization and introgression between Quercus magnoliifolia and Quercus resinosa (Fagaceae) through an altitudinal gradient in Mexico. *International Journal of Plant Sciences*, 171: 310–322. https://doi.org/10.1086/650317

Blackith, R., Reyment, R.A. (eds), 1971. *Multivariate morphometrics*. New York: Academic Press. 412 p.

Bookstein, F.L., 1986. Size and shape spaces for landmark data in two dimensions. *Statistical Science*, 1: 181–222. https://www.jstor.org/stable/2245441

Bookstein, F.L., 1996. Combining the tools of Geometric Morphometrics. In Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P., Slice, D.E. (eds). *Advances in morphometrics*. NATO ASI Series, Series A, Life Sciences, v. 284. Boston: Springer, p. 131–151. https://doi org/10.1007/978-1-4757-9083-2_12

Bruschi, P., Vendramin, G.G., Bussotti, F., Grossoni, P., 2000. Morphological and molecular differentiation between Quercus petraea (Matt.) Liebl. and Quercus pubescens Willd. (Fagaceae) in northern and central Italy. *Annals of Botany*, 85: 325–333. https://doi.org/10.1006/anbo.1999.1046

Camus, A., 1938. *Les Chênes : Monographie du genre Quercus. Volume 2. Atlas* [Oaks: monograph of the genus Quercus. Volume 2. Atlas]. Paris: Paul Lechevalier & Fils. 830 p.

Corcuera, L., Camarero, J.J., Gil-Pelegrín, E., 2002. Functional groups in Quercus species derived from the analysis of pressure-volume curves. *Trees*, 16: 465–472. https://doi.org/10.1007/s00468-002-0187-1

Dean, C., Adams, F., Rohlf, J., Dennis, E.S., 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, 71: 5–16. https://doi.org/10.1080/11250000409356545

Dryden, I.L., Mardia, K.V., 1998. *Statistical shape analysis*. Chichester, UK: John Wiley and Sons.

Gil-Pelegrín, E., Ángel, S.M., María, C.J., Peguero-Pina, J.J., Sancho-Knapik, D., 2017. Oaks under Mediterranean

-type climates: functional response to summer aridity. In GIL-PELEGRÍN, E., PEGUERO-PINA, J.J., & SANCHO-KNAPIK, D. (eds). *Oaks physiological ecology. Exploring the functional diversity of genus Quercus L. Volume 7*. Spain: Springer International Publishing, 2017, p. 137–177. https://doi.org/10.1007/978-3-319-69099-5_5

HARDIN, J.W., 1979. Patterns of variation in foliar trichomes of eastern North American Quercus. *American Journal of Botany*, 66: 576–585. https://doi.org/10.1002/j.1537-2197.1979.tb06260.x

HE, Y., LI, N., WANG, Z., WANG, H., YANG, G., XIAO, L., WU, J., SUN, B., 2014. Quercus yangyiensis sp. nov. from the Late Pliocene of Baoshan, Yunnan and its paleoclimatic significance. *Acta Geologica Sinica*, 88: 738–747. https://doi.org/10.1111/1755-6724.12234

JENSEN, R.J., 1990. Detecting shape variation in oak leaf morphology: a comparison of rotational-fit methods. *American Journal of Botany*, 77: 1279–1293. https://doi.org/10.2307/2444589

JENSEN, R.J., HOKANSON, S.C., ISEBRANDS, J.G., HANCOCK, J.F., 1993. Morphometric variation in oaks of the Apostle Islands in Wisconsin: evidence of hybridization between Quercus rubra and Q. ellipsoidalis (Fagaceae). *American Journal of Botany*, 80: 1358–1366. https://doi.org/10.1002/j.1537-2197.1993.tb15375.x

KENDALL, D.G., 1989. A survey of the Statistical Theory of Shape. *Statistical Science*, 4: 116–120. https://doi.org/10.1214/ss/1177012589

KLINGENBERG, C.P., MONTEIRO, L.R., 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Systematic Biology*, 54: 678–688. https://doi.org/10.1080/10635150590947258

KLINGENBERG, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11: 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

KREMER, A., DUPOUEY J.L.J., DEANS, D., COTTRELL, J., CSAIKL, U., FINKELDEY, R., ESPINEL, S., JENSEN, J., KLEINSCHMIT, J., BARBARA, V.D., DUCOUSSO, A., FORREST, I., DE-HEREDIA, U.L., LOWE, A.J., TUTKOVA, M., MUNRO, R.C., BADEAU, S.S.V., 2002. Leaf morphological differentiation between Quercus robur and Quercus petraea is stable across western European mixed oak stands. *Annals of Forest Science*, 59: 777–787. https://doi.org/10.1051/forest:2002065

LEPAIS, O., AISSI, A., VÉLA, E., BEGHAMI, Y., 2022. Joint analysis of microsatellites and flanking sequences enlightens complex demographic history of interspecific gene flow and vicariance in rear-edge oak populations. *Heredity*. https://doi.org/10.1101/2021.07.12.452011

LIU, Y., LI, Y., SONG, J., ZHANG, R., YAN, Y., WANG, Y., DU, F.K., 2018. Geometric morphometric analyses of leaf shapes in two sympatric Chinese oaks: Quercus dentata Thunberg and Quercus aliena Blume (Fagaceae). *Annals of Forest Science*, 75: 90. https://doi.org/10.1007/s13595-018-0770-2

MAIRE, R., 1961. *Flore de l'Afrique du Nord. Volume 7*. Paris, France: Paul Lechevalier & Fils, p. 97–105.

McDONALD, P.G., FONSECA, C.R., OVERTON, J.M., WESTOBY, M., 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Functionnal Ecology*, 17: 50–57. https://doi.org/10.1046/j.1365-2436.2003.00698.x

MOHEBI BIJARPASI, M., ROSTAMI SHAHRAJI, T., SAMIZADEH LAHIJI, H., 2019. Genetic variability and heritability of some morphological and physiological traits in Fagus orientalis Lipsky along an elevation gradient in Hyrcanian forests. *Folia Oecologica*, 46: 45–53. https://doi.org/10.2478/foecol-2019-0007

PEGUERO-PINA, J.J., SISÓ, S., SANCHO-KNAPIK, D., DÍAZ-ESPEJO, A., FLEXAS, J., GALMÉS, J., GIL-PELEGRÍN, E., 2016. Leaf morphological and physiological adaptations of a deciduous oak (Quercus faginea Lam.) to the Mediterranean climate: a comparison with a closely related temperate species (Quercus robur L.). *Tree Physiology,* 36: 287–299. https://doi.org/10.1093/treephys/tpv107

PEÑALOZA-RAMÍREZ, J.M., GONZÁLES-RODRÍGUEZ, A., MENDOZA-CUENCA, L., CARON, H., KREMER, A., OYAMA, K., 2010. Interspecific gene flow in a multispecies oak hybrid zone in the Sierra Tarahumara of Mexico. *Annals of Botany*, 105: 389–399. https://doi.org/10.1093/aob/mcp301

ROHLF, F.J., MARCUS, L.F., 1993. A revolution morphometrics. *Trends in Ecology and Evolution*, 8: 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

ROHLF, J., 1999. Shape Statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification*, 16: 197–223. https://doi.org/10.1007/s003579900054

SCHIMPER, A.F.W., 1903. *Plant-geography upon a physiological basis*. Oxford, UK: Clarendon Press. https://doi.org/10.5962/bhl.title.8099

TPS software series, Morphometrics at SUNY Stony Brook, 2016. [cit. 2016-12-26]. https://life.bio.synsb.edu/morpho/

TRABUT, L., 1892. Sur les variations du Quercus Mirbeckii. Durieu en Algérie [About Quercus Mirbeckii. Durieu variations in Algeria]. *Revue Générale de Botanique*, 4: 1–6.

TSCHAN, G.F., DENK, T., 2012. Trichome types, foliar indumentum and epicuticular wax in the Mediterranean gall oaks, Quercus subsection Galliferae (Fagaceae): implications for taxonomy, ecology and evolution. *Botanical Journal of Linnean Society*, 169: 611–644. https://doi.org/10.1111/j.1095-8339.2012.01233.x

VISCOSI, V., FORTINI, P., SLICE, D.E., LOY, A., BLASI, C., 2009a. Geometric morphometric analyses of leaf variation in four oak species of subgenus Quercus (Fagaceae). *Plant Biosystems*, 143: 575–587. https://doi.org/10.1080/11263500902775277

VISCOSI, V., LEPAIS, O., GERBER, S., FORTINI, P., 2009b. Leaf morphological analysis in four European oak species (Quercus) and their hybrids: a comparison of traditional and new morphometric methods. *Plant Biosystems*, 143: 564–574. https://doi.org/10.1080/11263500902723129

VISCOSI, V., LOY, A., FORTINI, P., 2010. Geometric morphometric analysis as a tool to explore covariation between shape and other quantitative leaf traits in European white oaks. In NIMIS, P.L., VIGINES, L.R. (eds). *Tools for identifying biodiversity: progress and problems*. Trieste, Italy: EUT Edizioni Università di Trieste, 2010, p. 257–261.

VISCOSI, V., CARDINI, A., 2012. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PloS One*, 7: 1–20. https://doi.org/10.1371/annotation/bc347abe-8d03-4553-8754-83f41a9d51ae

VISCOSI, V., 2015. Geometric morphometrics and leaf phenotypic plasticity: assessing fluctuating asymmetry and allometry in European white oaks (Quercus). *Botanical Journal of Linnean Society*, 179: 335–348. https://doi.org/10.1111/boj.12323