Leaf heteroblasty and morphotypes of *Acer monspessulanum* (Djurdjura, Algeria) as revealed by traditional and geometric morphometrics

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Abstract. The genus *Acer* which is largely distributed in the Northern hemisphere is represented in Algeria by four species of which *Acer monspessulanum* is the most common. These four species and their putative hybrids are coexisting in many parts of their Algerian distribution range but their leaf morphology has not been the subject of quantitative analyses despite the interest of such kind of data in taxon delineation particularly in the case of interspecific hybridization. The present work is the first step towards a quantitative analysis of leaf morphology in *Acer* species in Algeria. We presently relied on traditional and geometric morphometrics methods in the study of *Acer monspessulanum* subsp. *monspessulanum* leaf morphology in two sites of the Djurdjura Mountain with consideration of tree and within-shoot effects. The results showed congruence between the two methods which both highlighted the presence of a marked heteroblasty. Basal leaves are twice as large as apical ones for all measured leaf features. The petiole is longer than the blade in basal leaves and inversely in apical ones. The median leaves have intermediate values. The results revealed also the coexistence of two contrasted leaf morphotypes on distinct trees of both sites.

Keywords: *Acer monspessulanum,* Djurdjura, heteroblasty, leaf, morphotypes.

Introduction

The Maples (*Acer* L.; *Sapindaceae*) consist of over 150 species with a large distribution in the Northern hemisphere (Van Gelderen *et al.*, 1994). Numerous authors identified Maple species based on foliar and reproductive traits (De Jong, 1976), bud morphology (Ogata, 1967), vein architecture (Tanai, 1978) and more recently with molecular data and phylogeny (Ackerly and Donoghue, 1998; Grimm *et al.*, 2007).

The genus *Acer* is well-known for its polymorphic leaves rendering the taxonomical identification difficult and controversial even actually (De Jong, 2019). In addition to the variability among species, leaf morphology may change under the influence of environmental factors (Gratani, 2014). For example, colder temperatures induced an increasing leaf dissection and tooth number in *A. rubrum* (Royer *et al.*, 2008; Royer *et al*., 2009). In *Ziziphus jujuba*, the density of leaf venation increased while leaf area and leaf perimeters decreased in arid areas inversely in humid areas (Li *et al.*, 2015). Furthermore, leaf morphology may also vary within the same individual. This trend is studied in many woody genera such as *Acer* (Critchfield, 1971; Powell *et al*., 1982; Steingraeber, 1982), *Crataegus* (Dickinson and Phipps, 1985), *Eucalyptus* (Vlasveld *et al.*, 2018), *Populus* (Eckenwalder, 1980; Eckenwalder, 1996; Slavov and Zhelev, 2011), *Quercus* (Blue and Jensen, 1988; Bruschi *et al.*, 2003; Kusi and Karsai, 2020). Kusi and Karsai (2020) demonstrated that the branch position was the main source of variation in leaf morphology of *Quercus* species. Indeed, outermost leaves of the canopy are smaller, more lobed and have higher LMA contrary to innermost leaves. Leaf form may vary even from node to node during the growing season of a given individual. This pattern is called seasonal heteroblasty (Herrera, 2009). It is present in Maples (Critchfield, 1971) whose shoots bear leaves of different shapes depending on their position on shoot nodes (either proximal or distal). The leaves formed on the basal nodes are supposed to have initiated their development in early spring and rest in the overwinter buds as leaf primordia, while the leaves of the distal nodes are formed in the same season in which they are initiated (Critchfield, 1971; Eckenwalder, 1980; Herrera, 2009). However, the intensity of seasonal heteroblasty expression differs in Maple species. It is marked on some species such *Acer pensylvanucum* and *A. rubrum* and uncommon or poorly expressed on others such as *A. spicatum* (Critchfield, 1971).

Leaf morphology may be analyzed by traditional and by geometric morphometrics methods. Traditional morphometrics consists of analysing different morphological variables (such as linear distance measurement, counts, ratio, angles, etc.) with the uni- or multivariate statistical analyses (Rohlf, 1990; Rohlf and Marcus, 1993; Adams *et al.*, 2004). It is an interesting method which

is used until recently in plant systematic studies (Marcysiak, 2012; Morel *et al.*, 2021). The second used method is the geometric morphometrics comprising the landmark approach, among others, and which has been increasingly used since thirty years (Viscosi and Cardini, 2011). It consists of collecting 2 or 3-dimensional coordinates of biologically definable landmarks (Adams *et al.*, 2004). These points must be homologous because landmark-based methods operate only with the coordinates of these reference points, so-that the objects studied should be directly comparable (Pavlinov, 2001). This method has proved its effectiveness in several studies and fields (Klingenberg, 2010; Viscosi and Cardini, 2011). In botany, Jensen *et al.* (2002) were the first authors who used geometric morphometrics in analysis of leaf shape variability. Since then numerous studies were performed (Viscosi *et al.,* 2009; Klingenberg *et al.,* 2012; Chitwood and Otoni, 2017). There are also authors who combined traditional and geometric morphometric methods in their studies of leaf shape variation (Viscosi *et al.,* 2009; Proietti *et al.,* 2021).

The genus *Acer* is represented in North Africa by 5 taxa including species and subspecies. Algeria records the greatest diversity of this genus in this area. According to flora books (Battandier and Trabbut, 1888; Lapie and Maige, 1916; Quezel and Santa, 1963) four species are naturally occurring in Algeria: *Acer monspessulanum* L. subsp. *monspessulanum, A. opalus* subsp*. opalus, A. opalus* subsp*. obtusatum* (ex. *A. obtusatum* K. & W.)*,* and *A. campestre*. Quezel and Santa (1963) mentioned also the presence of *A. monspessulanum* subsp. *martinii* and *A. x hyrcanum* a putative hybrid between *A. opalus* and *A. monspessulanum*, and they mentionned the commonness of the later in Chelia forest (Aurès Mountain). All these *Acer* species occur either sparsely or as clumps of variable size and occupy the understory of oak and Atlas cedar forests in Algeria (Mediouni and Azira, 1992; Yahi *et al.,* 2008). In Morocco, two species are present; *A. monspessulanum* L. subsp. *monspessulanum* and *A. opalus* subsp. *granatense* (Boiss.) Font Quer & Rothm (Fennane *et al.,* 2014), mainly as understory species of Pinapo fir, Atlas cedar and oak forests (Benabid, 2000; Navarro-Cerrillo *et al.*, 2013; Navarro-Cerrillo *et al.*, 2020). While in Tunisia, only *A. monspessulanum* L. subsp. *monspessulanum* is found in a single site i.e. at Jebel Serej National Park in an Aleppo pine matorral (Le Floc'h *et al.,* 2010; Mechergui *et al.,* 2018; Jaouadi *et al.,* 2020).

The range of these Maples is variable: Mediterranean and Euro-Sibirian (*A. campestre*), North african and Southern West European (*A. opalus* subspecies), or Mediterranean (*A. monspessulanum*). Nord Africa is therefore the southernmost or the westernmost distribution limits of some of these maples. Hybrids could occur between species of the same section, especially between *A. monspessulanum* and *A. opalus* (Van Gelderen *et al.,* 1994; Grimm *et al.,* 2007) increasing the complexity of species identification.

A. monspessulanum is the most common Maple in Algeria. It has also a wide distribution throughout the Mediterranean area until Iran and occurs in the central to the northern latitude of Europe. This large distribution allows the presence of many geographical variants (Van Gelderen *et al.,* 1994). However, their classification is controversial. In Turkey and Iran for example, 5 and 8 subspecies are identified respectively (Amini et al. 2016; Seki 2019). In Algeria, Quezel and Santa (1963) mentioned two subspecies of A. *monspessulanum*: subsp. *monspessulanum* and subsp. *martinii.* However, A. *monspessulanum* subsp. *martinii* Jordan is recognized as a hybrid between *A. monspessulanum* and *A. opalus* (Van Gelderen *et al.,* 1994; Bottacci, 2014), while in Europe only the typical subspecies is recognized (Van Gelderen *et al.,* 1994). Nonetheless, these taxa have not been the subject of comprehensive studies in North Africa and more specifically in Algeria where the species reaches the southernmost limit of their range. Indeed, if we except flora books (authors op cit.) mentioning these taxa, quantitative data on morphological traits of Algerian Maples are lacking.

In this study, we are focusing on variability of leaf morphology in *A. monspessulanum* subsp*. monspessulanum* in the Djurdjura Mountains (northern Algeria) using traditional and geometric morphometric methods as a first step towards a quantitative analysis of the whole co-existing *Acer* taxa in this Mountain including specimens of intermediate leaf morphology between typical Maples. And because lack of knowledge on heteroblasty pattern could lead to taxonomical confusions (Steingraeber, 1982; Eckenwalder, 1996), we sought to analyze the contribution of this variable to leaf morphology variation. As a whole, we addressed the following questions: which amount of leaf morphological variation may be explained by factors such as site, tree within-site and leaf position within-shoot? Is the trend of variation revealed by the traditional morphometric method comparable to that revealed by the geometric- morphometric method?

Materials and methods

Study species

Acer monspessulanum L. is a deciduous tree shrub up to 12 m tall, usually bush (Lapie and Maige, 1916), occasionally taller with a rounded crown (Van Gelderen et al. 1994), characterized by a grey-yellow smooth barks (Lapie and Maige, 1916). The leaves are extremely variable (Van Gelderen *et al.*, 1994), lobed to three short and subequal lobes 3-6 cm, coriaceous, green on abaxial and glaucuous on adaxial side. The flowers are greenish-yellow, in corymbs, erect at first and pendent later, with long and slender pedicel (Tutin *et al.,* 1978). The samara is glabrous, with dressed and subparallel wings, shrinks at the base and a very convex dorsal margin (Quezel and Santa, 1963).

Study site

The study was done on samples collected in two sites of the Djurjdura Mountain which comprises both pure and mixed patches of holm-oak and Atlas cedar and tree species such as Maples participate to the forest composition. Such forests patches have been the subject of floristic studies mentioning the existence of *Acer* species (Mediouni and Azira, 1992; Yahi *et al.,* 2008). *A. monspessulanum* is actually the most present Maple on this mountain occupying both open and relatively dense stands either as scarce individuals or as more or less dense clumps.

The two investigated sites are located at the most distant parts of the Djurdjura Atlas cedar-holm-oak forests in order to maximize expression of the potential leaf shape variability (Fig. 1). The first one, i.e. Tala Guilef (hereafter referred to as TG) is located in the Djurdjura National Park (36°28'14.4"N, 03°59'55.48"E) and the second one, i.e. Azro n'Thor (hereafter referred to as AT) is located in its periphery (36°29'35.42"N, 04°23'44.15" E).


Figure 1. Location of two harvested *Acer monspessulanum* sites in the Djurdjura (Google Earth, 2022).

Both sites occupy the same altitudinal range (1290 m a.s.l.) on North-eastern exposures and are characterized by a fresh perhumid bioclimate. They correspond to the open parts of the forest stands with the presence of sparse *Cedrus atlantica* and *Quercus ilex* trees as well as *Crataegus monogyna* and some shrubs such as *Prunus prostrata*, *Juniperus oxycedrus* and *Rubus ulmifolius*.

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Sampling and measurements

We harvested 15 mature trees in each site and collected several shoots per tree. The collected shoots have an average of 3 leaf nodes and are 4 cm long. They may reach a maximum of 8 leaf nodes (unpublished data). For the homogenization purpose, we only retained shoots with 3 nodes (Fig. 2). Leaves were removed from the shoots with respect of their insertion level. They were dried using a standard method and scanned on the abaxial surface using a scanner Epson stylus SX100 at 300 dpi resolution. Hereafter we refer to the leaves of the first node as basal leaves (BL); to those of the second node as median leaves (ML) and to those of the third node as apical leaves (AL).
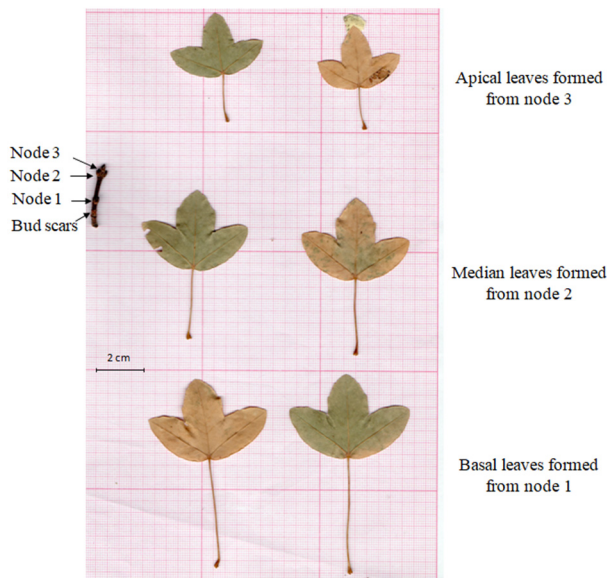
Figure 2. Leaves of *A. monspessulanum* and their position on the shoot.

Measurements with the traditional morphometrics method

Ten parameters were measured on a total of 597 leaves representing 30 trees and two sites (Fig. 3) with the Digimizer software (version 3.7.). Eight quantitative variables were directly measured in addition to two synthetic ones (i.e. ratios between variables). Then all measurements were analyzed with R statistical software v. 4.0.3. First, we performed a descriptive analysis, then we computed a Principal Component Analysis on average values of leaf traits according to their insertion within shoots.Values obtained on median and apical leaves were divided on those of basal leaves and expressed in percent of basal leaf values.

N°	Morphological characters	Units	
	Parameters measured:		
1	Leaf length		
2	Blade width	cm	
3	Blade length	cm	
4	Petiole length	cm	
5	Apical lobe width	cm	
6	Apical lobe length	cm	
7	Angle between nervures of the lateral lobes	cm	
8	Leaf area	°	
	Ratios :	cm²	
9	Blade / petiole length ratio		
10	Apical lobe length/ blade length ratio		

Figure 3. Morphological leaf traits computed by traditional morphometrics on *A. monpessulanum* leaves.

Measurements with the geometric morphometrics method

For this method we retained a total of 251 leaves with intact margins to avoid measurement errors during landmarks digitalization. Based on several authors (Jensen *et al.,* 2002; Viscosi *et al.,* 2009; Viscosi and Cardini, 2011), we recorded seven landmarks on each leaf surface with the TPS package (Rohlf, 2015) (Fig. 4). We repeated the procedure twice in order to estimate measurement error as mentioned by Viscosi and Cardini (2011). We performed a geometric analysis with MorphoJ 1.06d software (Klingenberg, 2011) available on the website (MorphoJ (morphometrics.uk)).

Landmark	Description	
1	Beginning of the petiole	
2	Junction of the blade and petiole	
3	Tip of the right lateral lobe nerv	
4	Right base of the apical lobe sinus	
5	Tip of the apical lobe	
6	Left base of the apical lobe sinus	
7	Tip of the left lateral lobe nerv	

Figure 4. Landmarks configuration recorded on the entire leaf of *A. monspessulanum*

We carried out Procrustes Anova analysis of leaf shape variation at the following hierarchical levels: Between sites, between trees within site, between nodes within shoot, then we calculated an average of the effect that induces the largest share of the total variance of the leaf shape and performed a Principal Component Analysis (PCA) and a Discriminate Analysis (DA). The shape variation along each axis was visualized using wireframe diagrams.

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Results

Traditional morphometrics
Descriptive statistics

The mean and coefficient of variation of measured traits are showed in Tab. 1. The basal leaves revealed the greatest values compared to median and apical ones for all measured leaf traits. The total leaf length, the blade length, the blade width, the petiole length, and leaf area were found as 6.56 cm, 3.19 cm, 4.42 cm, 3.99 cm, and 7.14 cm², respectively. These values were twice as long as those of apical leaves which correspond to 3.65 cm, 2.33 cm, 2.96 cm and 1.82 cm and 3.29 cm² for the same leaf traits, respectively. In contrast, the *blade to petiole length ratio* showed lower values in basal leaves (0.83), comparatively to apical ones (1.82).

Table 1. Overall mean and coefficient of variation of *Acer monspessulanum* leaf morphological traits according to within-shoot leaf insertion level.

Leaf characteristics		Basal leaf	Median leaf	Apical leaf
Total leaf length (cm)	Mean	6.56 (22.6)	5.31 (23.0)	3.65 (33.9)
	Relative growth (%)	100%	80.9%	55.6%
Blade width (cm)	Mean	4.42 (17.2)	3.98 (20.3)	2.96 (30.1)
	Relative growth (%)	100%	90.0%	66.9%
Blade length (cm)	Mean	3.19 (19.4)	2.97 (23.2)	2.33 (31.6)
	Relative growth (%)	100%	93.1%	73.0%
Petiole length (cm)	Mean	3.99 (29.7)	2.89 (32.9)	1.82 (47.2)
	Relative growth (%)	100%	72.4%	45.6%
Apical lobe width (cm)	Mean	1.79 (32.0)	1.68 (36.8)	1.30 (42.9)
	Relative growth (%)	100%	93.8%	72.6%
Apical lobe length (cm)	Mean	1.65 (35.1)	1.60 (37.4)	1.32 (40.6)
	Relative growth (%)	100%	96.9%	80%
Angle between lateral lobes (°)	Mean	117 (13.9)	117 (12.6)	125 (12.4)
	Relative growth (%)	100%	100%	106.8%
Area (cm²)	Mean	7.14 (25.2)	5.80(30.8)	3.29 (50.1)
	Relative growth (%)	100%	80.8%	46.0%
Blade length/petiole length	Mean	0.83 (20.1)	1.08 (20.6)	1.39 (21.8)
Apical lobe length/blade length	Mean	0.50 (19.1)	0.52 (18.5)	0.56 (15.7)

The numbers in the parentheses are coefficients of variation (CV %).

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The angle between lateral lobes was similar in basal and median leaves (i.e. 117°) while it was slightly wider in apical ones (i.e. 125°). Overall, the *apical lobe length to blade length ratio* seemed not to vary along the shoot with mean values ranging between 0.50 and 0.56 respectively on basal and apical leaves.

As reported in Tab. 1, there is a gradual decrease in values of leaf traits along the shoot from the basal to the apical node. Values of apical leaves represented between 46 and 80% of those of the basal leaves depending on traits while those of the median leaves represented between 72 and 96.9% of those of the basal ones. This indicates more expressed differences between the basal and apical leaves than between the basal and median ones. Mean values of some leaf traits were slightly higher at Tala Guilef site comparatively to Azro n'thor one (Tab. 2) with leaf area as the most variable feature between these two sites (i.e. 7.64 cm² at TG versus 6.49 cm² at AT).

Table 2. Mean and coefficient of variation of *Acer monspessulanum* leaf morphological traits according to site and within-shoot leaf insertion level.

Leaf characteristics	TG			AT		
	BL	ML	AL	BL	ML	AL
Total leaf length (cm)	6.80	5.51	3.72	6.25	5.09	3.55
	(20.5)	(20.5)	(31.6)	(24.6)	(25.5)	(37.0)
Blade width (cm)	4.57	4.16	3.10	4.24	3.78	2.77
	(16.8)	(19.7)	(29.7)	(17.0)	(19.9)	(29.4)
Blade length (cm)	3.26	3.07	2.42	3.11	2.87	2.21
	(21.6)	(23.7)	(33.1)	(15.7)	(21.9)	(28.2)
Petiole length (cm)	4.13	2.96	1.79	3.82	2.80	1.86
	(28.3)	(31.2)	(45.5)	(31.2)	(34.8)	(49.4)
Blade length/petiole length	0.81	1.08	1.45	0.86	1.08	1.30
	(17.1)	(20.3)	(20.8)	(22.6)	(21.1)	(21.9)
Apical lobe length (cm)	1.61	1.60	1.37	1.69	1.59	1.27
	(41.0)	(42.4)	(44.6)	(26.8)	(30.9)	(33.2)
Apical lobe width (cm)	1.90	1.80	1.37	1.66	1.54	1.21
	(32.3)	(36.8)	(44.7)	(29.5)	(34.5)	(38.6)
Apical lobe length/blade length	0.47	0.50	0.55	0.53	0.54	0.56
	(19.1)	(20.3)	(16.0)	(17.3)	(15.5)	(15.1)
Angle (°)	118.	119.	127.	115.	115.	121.
	(13.1)	(12.7)	(11.8)	(14.9)	(12.3)	(12.8)
Area (cm²)	7.64	6.44	3.57	6.49	5.08	2.92
	(21.4)	(24.7)	(44.8)	(27.8)	(34.0)	(56.5)

The numbers in the parentheses are coefficients of variation (CV %)

Principal component analysis (PCA)

The two first axes of the PCA explained 82.8% of the total variance of which 65.1% captured by the first principal component (PC1) which clearly separates leaves according to their position on the shoot (Fig. 5). The positive
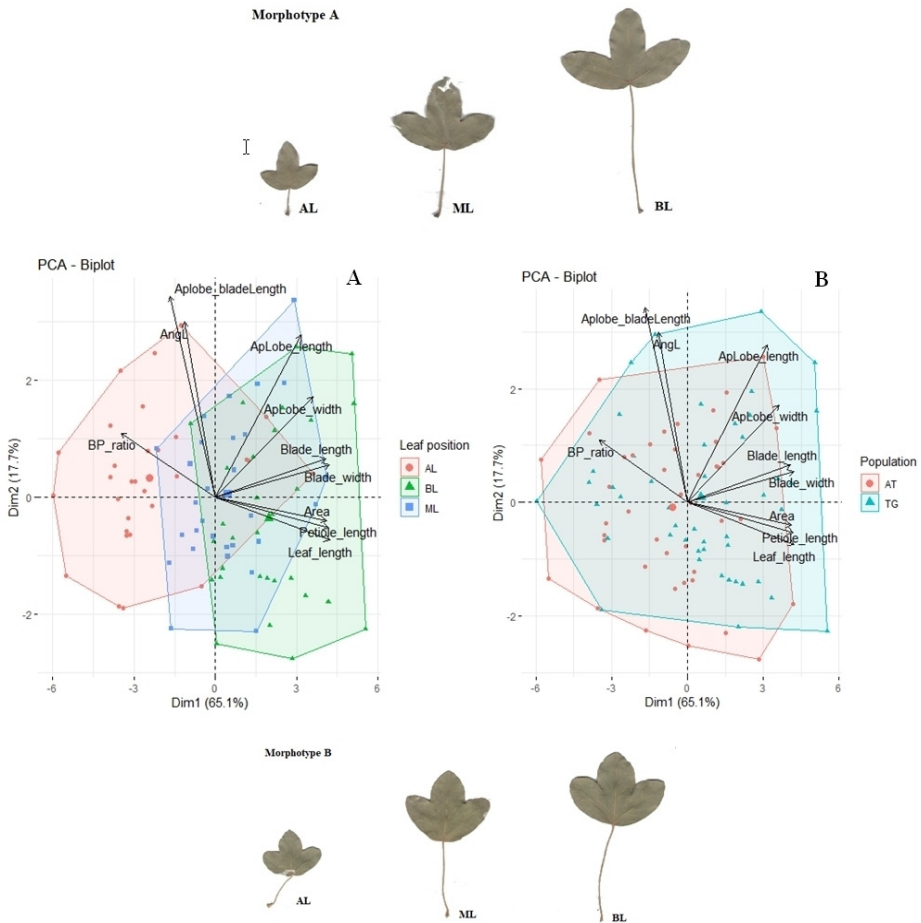
Figure 5. PCA Bitplot showing the distribution of leaf individuals and a vector plot of 10 variables. **A**: Leaf mean values are coloured according to their position on the shoot: Red circles (apical leaves); green triangles (basal leaves); blue squares (median leaves). **B:** leaf mean values are coloured according to their site: red circles at Azro n'Thor (A) and blue triangles at Tala Guilef (TG). The representative leaf images corresponding to the traits illustrated by the PC1: from right to left: basal (BL), median (ML) and apical leaves (AL); and PC2 from the top to bottom: morphotype A and morphotype B.

side of the PC1 is characterized by leaves which are longer, with a bigger area but with a blade relatively shorter than the petiole. These features are associated to basal leaves (BL). Conversely, the negative side of the PC1 is characterized by leaves which are shorter, with a smaller area but with a blade

longer than the petiole. These characteristics are associated to apical leaves (AL). The median leaves (ML) are located in the centre of the graph and are overlapping with the previous groups. The PC2 is related to the angle between lateral lobes from a hand and apical lobe to blade length ratio from another hand. This axis is related to the variability between trees as shown on Fig. 5. The positive side of the PC2 is characterized by a wide angle between lateral lobes (AngL) and high apical lobe to blade length ratio (ApLobe_bladeLength), which corresponds to the morphotype "A". In the opposite side of the PC2, the leaves have a narrower angle between lateral lobes and a small apical to blade length ratio which corresponds to the morphotype "B".

Geometric morphometrics method
Procrustes ANOVA

The analysis of variance (Tab. 3) showed that leaf size did not vary significantly with site and tree but varied significantly with leaf position within shoot. Indeed, leaf position explained 70.1% of the total variance. Leaf effect was highly significant (p<0.001), accounting for about 14% of the total variance.

Table 3. Results of the Procruste ANOVA computed on the whole sample of leaves.

Centroide size variation						
Effect	Explained SS (%)	SS	MS	Df	F	P
Site	0.07	1,058	1,058	1	0.15	0.70136
Trees	14.93	203,897	7,031	29	0.645	0.909
Leaf position	70.15	958,144	10,888	88	7,09	<.0001
Leaves	14.83	202,629	1,535	132	8519,46	<.0001
Measurement error	0.003	0,0452	0,0001	251		
Total	100	1365.773				
Shape variation						
Site	2.39	0,255	0,0255264797	10	2.72	0.00329
Trees	25.46	2,713	0,0093553007	290	1.54	<.0001
Leaf position	49.68	5,293	0,0060156850	880	3,35	<.0001
Leaves	22.23	2,369	0,0017953602	1320	188,32	<.0001
Measurement error	0.22	0,024	0,0000095335	2510		
Total	100	10.654				

SS: sum of squares; MS: mean sum of squares; df: degree of freedom.

The Procrustes shape showed that the factors taken into account (i.e. site, tree, leaf position and leaves) contributed significantly to leaf shape variability of Montpellier Maple (Tab. 3). Variation driven by site effect was slight

(explaining only 2.4% of the total variance) but statistically significant. Trees and insertion level explained about 25.4 and 49.6 % of the total variance, respectively. Leaves for their part, explained 22.2% of the total variance.

These results indicate that leaf position (i.e. insertion level) is the main source of variation in leaf size and shape. Therefore we conducted a subsequent Principal Component Analysis (PCA) using data pooled by leaf position (i.e. 3 means per tree and a total of 90 on the whole sample of trees).

Principal component analysis (PCA)

To study leaf shape variability, we conducted a PCA on data averaged by insertion level. The results showed that 91.9% of the total variance was explained by the three first PCs. In detail, the main part of total variance was explained by the PC1 (76.26%), followed by the PC2 and PC3 which explained respectively 9.12 and 6.16% of the total variance.
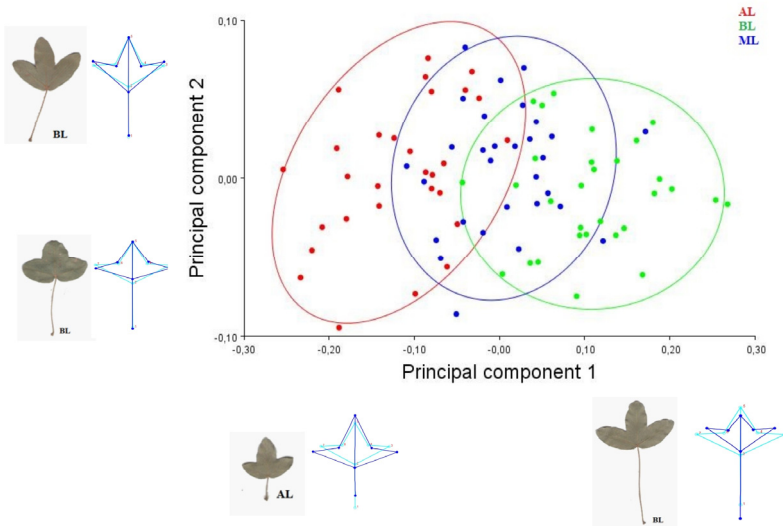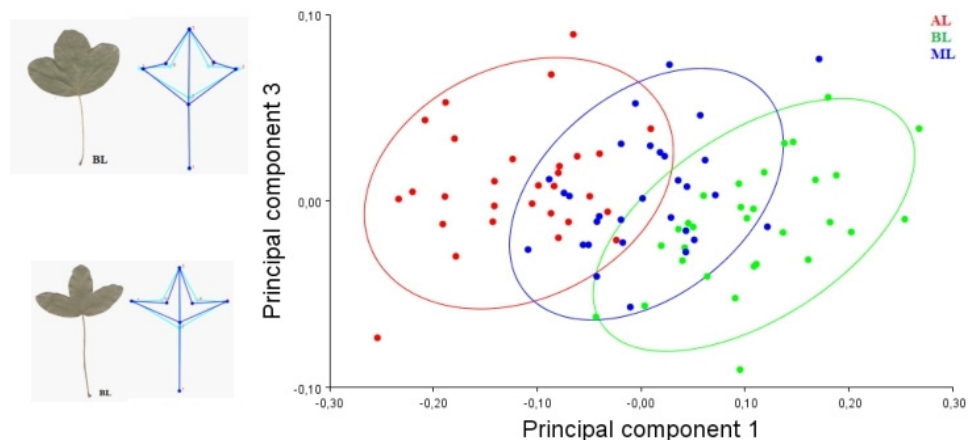

Figure 6. Scatter plot of the PC1 and PC2 scores derived from landmarks data. Wireframe graph and its corresponding leaf images showing major features correlated with the extremes of variation along the axis.

The scatter plot of PC1 and PC2 scores (Fig. 6) showed that the three leaf positions were separated with a partial overlap along the PC1, which was mainly related to change in leaf shape along the shoot. The positive values were characterized by a contracted apical lobe (lm5), a narrow angle between lateral

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lobes (lm3, lm2, lm7), a narrower blade (lm3, lm4, lm6, lm7) and a long petiole (lm1). The negative values were characterized by an elongated apical lobe, a wider angle between lateral lobes, an expanded blade and a short petiole.

The PC2 discriminated the trees according to the angle between lateral lobes (lm3, lm2, lm7), lateral lobe tips (lm3, lm7), and to a lesser extent, according to apical lobe width (lm4, lm6). The angle between lateral lobes, lateral lobe tips and consequently the blade width and the apical lobe base were narrower in positive values and wider in negative ones.

The PC3 explained only 6.16% of the total variance (Fig. 7), which was related to leaf base (lm2), apical lobe width (lm4 and lm6) and the importance of apical lobe to blade ratio. Leaves characterized by a wide apical lobe base, a short apical lobe compared to blade length and a tronquate leaf base were found on the positive side of the PC3, while those with a narrow apical lobe, a long apical lobe compared to blade length and a cordate leaf base, were localised in the negative side of the PC3.


Figure 7. Scatterplot of the PC1 and PC3 scores derived from landmarks data. Deformation grids and wireframe graph showing major features correlated with the extremes of variation along the axes.

Discriminant analysis (DA)

The cross-validation DA showed the important differences in leaf shape between basal and apical position (Fig. 8a), and a small overlap in shapes of basal vs median leaves (Fig. 8b), and median vs apical ones (Fig. 8c). The pairwise comparison showed T^2 constantly significant (P<0.0001); the values were 324.64, 160.79 and 107.70 for basal vs apical, basal vs median and median

vs apical leaves, respectively. In addition to these comparisons, we obtained a correct classification for 100 and 93.10% of the basal and apical leaves, 93.33 and 90% of the basal and median leaves and 90 and 82.75% of the median and apical leaves.
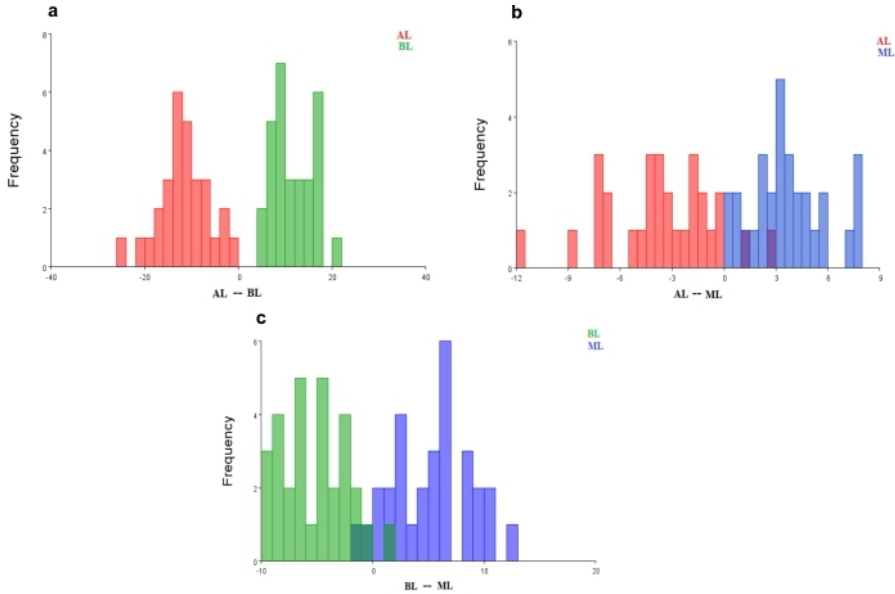
Figure 8. Discriminate analysis of the leaf shape of the three positions on shoot of *A. monspessulanum*. Green bars: Basal leaves (BL); Blue bars: Median leaves (ML); Red bars: Apical leaves (AL).

Discussion

This paper represents the first contribution to the quantitative study of foliar morphological traits of Montpellier Maple at its southernmost limit in North Africa, and especially in Algeria. Here, we used both traditional and geometric morphometrics methods in order to analyze leaf morphology of *Acer monspessulanum* subsp. *monspessulanum* harvested in the Djurdjura Mountain (Algeria north-centre) at three hierarchical levels: sites, trees within site and nodes within shoot.

Both traditional and geometric morphometrics revealed significant differences in leaf morphology according to its position on the shoot despite the smaller sample size retained for the geometric morphometrics analysis (251 leaves compared to 597 for traditional morphometrics). The basal leaves

showed higher values for all measured morphological traits, followed by median leaves with intermediate attributes while apical leaves displayed the smallest values. Although leaf position on tree may cause variation of some leaf traits (Blue and Jensen, 1988; Bruschi *et al.,* 2003), we consider leaf variation recorded in this study as rather driven by heteroblasty. Firstly, because we sampled only small shoots (average of 4 cm long) discarding the bias which may result from shoot length variation, secondly, because we collected the shoots from small and well spaced trees with a full access to sunlight discarding the bias which may result from a differential access to sunlight.

Heteroblasty is previously reported on some *Acer* species such as *A. pensylvanicum* and *A. rubrum* (Critchfield, 1971), *A. saccharum* (Powell *et al.,* 1982; Steingraeber, 1982) and other genera such as *Berberis* (Pabón-Mora and González, 2012), *Populus* (Eckenwalder, 1996; Slavov and Zhelev, 2011), with within-shoot nodes bearing leaves of different morphologies, a trend linked to leaf ontogeny, since basal leaves of a given shoot initiate their development in the previous season, overwinter as embryonic leaves and leaf primordia, and reach their maturity rapidly after leaf unfolding in the following spring, while distal leaves initiate their development during the growing season (Steingraeber, 1982). Furthermore, Spriggs *et al.,* (2018) suggested that heteroblasty in *Viburnum* is closely related to growth architecture and is linked to leaf position along the shoot rather than to the exact timing of leaf emergence.

This node to node variability is not limited to leaf shape but concerns also leaf nitrogen content in *Olea* europaea which declines from basal to apical leaves, monoterpene composition of essential oil in *Mentha piperita* which increases from the base to the tip and is also found in other organs like flowers and fruits (Herrera, 2009).

Critchfield (1971) stated that heterophylly is marked in *A. campestre*, *A. orientale* and *A. monspessulanum* (ex. *Campestria* Pax. section) and these species have 1 to 3 pairs of leaves formed completely in buds and may be reinforced by additional pairs of leaves (i.e. late leaves) on longer shoots. He reported that the most obvious distinction between the two kinds of leaves (early vs. late) in *A. monspessulanum* is the presence of more lobes and blunt teeth in late leaves. However, data of the present study and additional observations (data not shown) don't corroborate this author's statement about the distinctive trait between leaves of the basal and distal nodes. Indeed, we observed that shoots of *A. monspessulanum* bear an average of 3 pairs of leaves regardless of shoot types (long or short) and may reach rarely a maximum of 8 pairs of leaves. Although we considered only those shoots bearing three pairs of leaves in this study, the results showed evident differences in leaf morphology from the base to the tip of the shoot. We didn't study the morphology of leaf

margins in this paper, though, based on our observations, the apical leaves did not have systematically more lobes and teeth and some of them have an entire margin like basal leaves.

The Procrustes ANOVA results (Tab. 3) showed measurement errors which are lower to all main effects (0.003% and 0.2% for centroide size and shape respectively), therefore we may consider the error as negligible and the digitalization well carried out as stated by Viscosi and Cardini (2011). The main source of variation (70 and 49 % for centroide size and shape respectively) was induced by leaf position on the shoot. The first principal component explained 65 and 71.6 % of the total variance in traditional and geometric morphometrics respectively, and separated clearly the basal and the apical leaves into two groups, while the median leaves overlapped with apical ones from a hand and basal ones from another hand. The basal leaves may be characterized by a long petiole compared to blade length, and a contracted apical lob while apical leaves seem to have a short petiole, a large blade and an elongated apical lobe. The median leaves, for their part, have intermediate attributes.

The traditional morphometrics (Tab. 1) showed that the relative development of median leaves reached 72 to 96% of that of basal ones. The contrast was more evident for the apical leaves which showed a development representing between 45.6 and 80% of that of basal leaves depending on the trait. This trend was evidenced by the discriminate analysis (*DA*) which showed that 100 % of basal and 93% of apical leaves were well classified. This is similar to results of Spriggs et al. (2018) on *Viburnum sp.* who found that 87 to 97% of early and late leaves were well distinguished on the basis of shape only.

Variation of the apical lobe to blade length ratio and of the angle between lateral lobes along the shoot was more clearly evidenced by the geometric morphometrics method than by the traditional one. And such trend of variation is in concordance with Critchfield (1971), who reported, on other *Acer* species, a larger apical lobe and a wider angle in apical leaves, comparatively to basal ones.

According to our results, this variability of lateral lobes' angle is more related to variability among trees as revealed in the PC2 of both traditional and geometric morphometrics. Therefore, we suggest, that mean values calculated on the whole sample of leaves (Tab. 1) are hiding a certain amount of among-tree variability. Indeed, as depicted in Figure 5 and 6 both the traditional and the geometric morphometrics methods showed that apical lobe to blade length ratio and the angle between lateral lobes are rather related to variability among trees. Despite the major part of variation being due to leaf position on the shoot, the second principal component of the PCA with the classic morphometrics method, and the second and the third principal components of the PCA with the

geometric morphometrics, revealed the presence of two leaf morphotypes regardless of leaf node position. This discrimination was related to lateral lobes' angle and the apical lobe to blade length ratio according to the traditional morphometrics, and to the apical lobe features according to the geometric morphometrics.

Among the variety of leaf morphotypes observed in this study, we retained the two most contrasted ones named as morphotypes A and B. The morphotype A is characterized by elongated and triangular lobes with a long apical lobe, reaching 2/3 of the blade length and a wide angle between lateral lobes ($\approx$ 120°). The morphotype B is characterized by obtuse lobes, with a short apical lobe to blade length ratio of about 1/3 and a narrow angle between lateral lobes ($\approx$ 90°). The great variability of *Acer monspessulanum* leaf shape is reported in the literature, the lobes can be rounded, cordate, triangulate, ovoid, oblong or toothed. Indeed, the apical lobes may represent ½ to 2/3 of the blade length (Pignatti, 1982; Van Gelderen *et al.,* 1994; Seki, 2019). However, to our knowledge, there are not published works about the coexistence of these morphotypes as observed in the present study.

The PCA computed with both traditional and geometric morphometrics data didn't discriminate between sites regarding leaf morphotype and didn't support the relatively greater values revealed by the descriptive statistics for Tala-Guilef site. The coexistence of the two leaf morphotypes in the two investigated sites may be due to their location within the same latitudinal and altitudinal range (i.e. distant by only 35 kms from east to west). Investigating a larger area of Montpellier Maple in Algeria could reveal additional morphotypes and trends of variation since Nikzat-Siahkolaee *et al.,* (2021), for example, found a relationship between geographical gradients and the three leaf morphotypes recognized in *A. cappadociccum* of Iran.

Flora books suggested studying only mature leaves in order to avoid the leaf heteroblasty, which there are sometimes referred as "typical" or "adult" leaves (Critchfield, 1971). However, basing on the precedent studies on leaf maple heteroblasty such as Critchfield (1971), Steingraeber (1982) and Powell *et al.* (1982), the leaves were categorized into two kinds according to their morphology: the preformed (early) and neoformed (late) ones. Nevertheless, in this study we showed that basal leaves are bigger than median ones, particularly in leaf area and petiole length, eventhough those latter were fully expanded and considered as mature. On the other hand, Fennane *et al.,* (2014) suggested considering only mature leaves of fructified shoots. However, there may be an inconvenient in this case due to the complexity of *Acer* reproductive system (De Jong, 1976; Van Gelderen *et al.,* 1994). In addition, a large interannual fluctuation in seed production was recorded in *Acer* species (Houle, 1999), with zero production

in some years for *A. pseudoplatanus* (Wesołowski *et al.,* 2015), *A. monspessulanum* and *A. opalus* subsp. *obtusatum* (personal observations). Consequently, we suggest taking into account only leaves of the first node within a given shoot.

Conclusions

The present morphological study of *Acer monspessulanum* subsp. *monspessulanum* leaves, using a classic method and a geometric-morphometrics one, revealed concordant trends. Among the following factors: site, tree, and leaf insertion within shoot; the latter revealed to be the main driver of leaf shape and size variation. And since leaf shape is used as a principal characteristic in species identification, we suggest considering only the basal leaves in future morphological investigations in order to ovoid confusions. In addition to the heteroblasty, the study revealed also the coexistence of two leaf morphotypes on distinct trees of both sites. To complete this first quantitative study of *A. monspessulanum* leaf morphology, it would be interesting to include samples harvested on a larger range of the species in Algeria.

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