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Variations in leaf morphological and functional traits of *Quercus castaneifolia* C.A.Mey. (*Fagaceae*) in Azerbaijan

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Abstract

The variations of leaf morphology and functional traits of *Quercus castaneifolia* C.A.Mey. in various regions of Azerbaijan with different gradients of water availability, elevation, mean annual precipitation and mean annual temperature have been studied. The differentiation in morphological and functional traits of 300 leaves from 30 trees from 3 populations were measured and analyzed using univariate and multivariate analyses. The aim of the study was to evaluate inter- and intra-population differentiation. As a result, we determined that morphological characteristics such as leaf size, leaf area, leaf width, and leaf perimeter decreased with increasing precipitation. The foliar moisture parameters, leaf mass per unit area, water content, relative water content, and succulence, positively correlated with annual precipitation and elevation but negatively correlated with mean annual temperature. This study provides evidence for the adaptive plasticity-polymorphism of leaves in response to environmental changes.
Keywords: leaf functional traits, leaf morphology, population differentiation, Quercus, variation

Introduction

Forest trees, like oaks, rely on high levels of genetic variation to adapt to varying environmental conditions. Thus, genetic variation and its distribution are important for the long-term survival and adaptability of oak populations under a changing environment. As species encounter new stresses, such as climate change and pollution, management measures are required to conserve their gene pool (Lind-Riehl, 2014). Genetic variation and high levels of phenotypic plasticity contribute to the success of the genus *Quercus* L. However, these characteristics pose difficulties when estimating the genetic architecture of populations (Kashani and Dodd, 2002). Leaves of some oak species under environmental changes and habitat factors such as an elevation change or altitudinal gradients show different morphological forms; therefore, several dichotomous keys based on morphological characteristics have been developed to describe species and sections within *Quercus* (Jensen, 1990).

Leaves are important organs for photosynthesis and play an important role in the survival and growth of a plant. Leaves are organs sensitive to environmental changes in the process of evolution and may exhibit phenotypic plasticity as a response to environmental changes. The plasticity of leaf morphological traits across habitats has long been of interest to ecologists because these traits are considered good predictors of plant performance and adaptation (Lind-Riehl, 2014). Climatic parameters are the main environmental factors affecting tree growth (Filippo *et al*., 2002). Leaf variations are mainly shaped by fluctuations in environmental factors (Körner, 2007; Jump *et al*., 2009). The leaf morphological traits of many species vary with elevation (Cordell *et al*., 1998; Bresson *et al*., 2011). For example, the lower temperatures found at higher elevations can restrict the extension of leaves and reduce their size (Magnani and Borghetti, 1995). However, the affirmation of leaf morphological plasticity and its regulations in different environments are still required.

Despite the ecological and economic importance of *Quercus*, natural populations are rapidly disappearing, due to changing climatic conditions, global warming, illegal logging, forest fires and the deforestation associated with conservation of lands for agriculture, fruticulture and pasture (Saenz-Romero *et al*., 2003). Long generations, inadequate seed production relative to demand, the impossibility of storing seeds for long periods, and hardly any vegetative propagation pose difficulties in forestry and *Quercus* improvement programs. The high morphological diversity of oaks has been of great interest in protection and conservation studies analyzing the patterns of variation in morphological traits. (Bruschi *et al*., 2000; Ardi *et al*., 2012; Ekhvaia *et al*., 2018; Reyha *et al*., 2020).

The Caucasus region is one of the 25 global biodiversity hotspots and constitutes a shelter area for Neogene relict species as well as a center of ongoing radiation (Ekhvaia *et al*., 2018). The oak tree has a special symbolic, ecological, and economical value in Azerbaijan. *Q. castaneifolia* C.A.Mey. was first described in 1831 and belongs to section *Cerris* Loudon (1838: 1846), a placement within the genus that has remained stable since Camus' monograph (1936–1954). However, the taxonomy within *Q. castaneifolia* has been debated, with at least eight intraspecific botanical taxa (subspecies, variety, and form) described based on differences in leaf, acorn, trichome, foliar epidermis, and pollen morphology (Panahi *et al*., 2011, Rix and Kirkham, 2009) and there are no currently accepted infraspecific names, in the *World Checklist of Vascular Plants* (WCVP, 2021). It has yet to be analyzed for Red Listing to determine its conservation status (Oldfield and Eastwood, 2017). Oaks (*Quercus* spp.) are the most important tree species for forestry in Azerbaijan. National IUCN Status: Refers to the category of "those close to danger" NT. It is a rare species of Azerbaijan (Red Book Azerbaijan Republic). The Chestnut-leaved Oak occurs only in Azerbaijan and Iran. In Azerbaijan, it grows in the Talysh Mountain regions as well as in isolated sites of the Ismayilli Region in the eastern parts of the

Greater Caucasus Mountains (Bandin and Prilipko, 1964; Menitsky, 2005; Qurbanov, 2004; Askarov, 2010; Mammadov, 2016). In Iran, it inhabits the northern slopes of the Elburz Mountains in the provinces of Gilan, Mazandaran and Golestan. In the Talysh region, its elevational distribution extends from the Caspian lowlands to over 1600 m above sea level. In the Elburz Mountains, it is found even at a height of 2400 m, with a peak in the submontane and montane deciduous forests between 700 and 1400 m (Menitsky, 2005; Kozlovski and Gerber, 2014; Gurbanov and Isgandar, 2015; Mammadov, 2016).

Protection and conservation of high value forest genetic resources requires information on the patterns of genetic and morphological variation among and within populations (Saenz-Romero *et al*., 2003). The main aims of the presented study were to determine whether chestnut-leaved oaks growing under contrasting environmental conditions show different sensitivity to climatic parameters and to evaluate inter- and intra-population differentiation.

Material and methods

Study areas. This study was carried out on chestnut-leaved oak trees (*Quercus castaneifolia*) growng in three stands located in different regions of Azerbaijan, under contrasting environmental conditions. The study areas were Hirkan National Park (HNP) (natural population), Lankaran Lowland (LLd) ((natural population), and Mardakan Arboretum (MA) (planted population). The area of Hirkan National Park is 99% covered by forests in a primarily mountainous region (altitude: 534 m; coordinates: 38°37'50″N, 48°42'42″E). Soils of the study area were meadow-chestnut. Lankaran is a city in Azerbaijan, on the coast of the Caspian Sea, near the southern border with Iran (altitude: 28-200m; coordinates: 38°45'13"N, 48°51'04"E). Lankaran has a borderline humid-subtropical climate and a hot-summer Mediterranean climate since only two summer months have precipitation levels below the 40 mm threshold. Lankaran has cool, wet winters and very warm, partially dry/highly humid summers (Figs. 1–2). The precipitation in our research areas (Lankaran, Astara and Baku) is quite different varying between 216 mm and 1280 mm **(**Mammadov *et al*., 2010). Soils of the study area were yellow-podzol-gley. Mardakan arboretum (MA) is 4.5 km from the shore of the Caspian Sea (altitude: 8 m; coordinates: 40°29'32"N, 50°08'20"E). The soil cover is represented by various soil combinations, 80-85% of the arboretum is covered with limestone (Museyibov, 1998; Mammadov *et al*., 2010). Soils of the study area were gray-earth and gray-brown. A population was selected from each of these regions by various soil combinations, 80-85% of the arboretum is covered with limestone. (Museyibov, 1998; Mammadov *et al*., 2010). Soils of the study area were gray-earth and gray-brown. A population was selected from each of these regions.

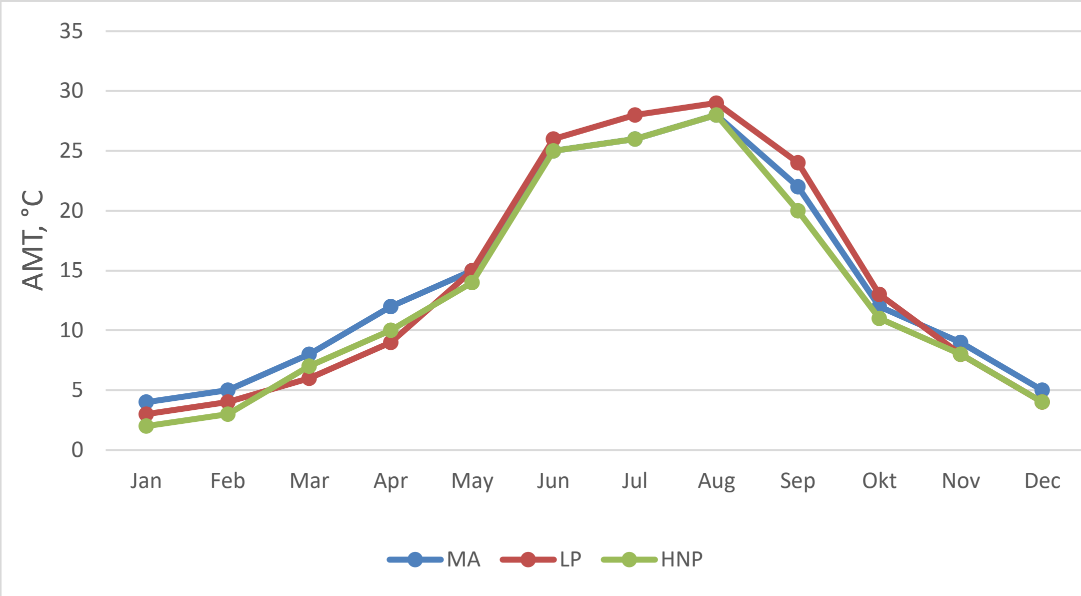

Figure 1. Average Monthly Temperature (°C) for each population. (Museyibov, 1998, Mammadov *et al*., 2010) MA - Mardakan Arboretum, LP - Lankaran Lowland, HNP -Hirkan National Park

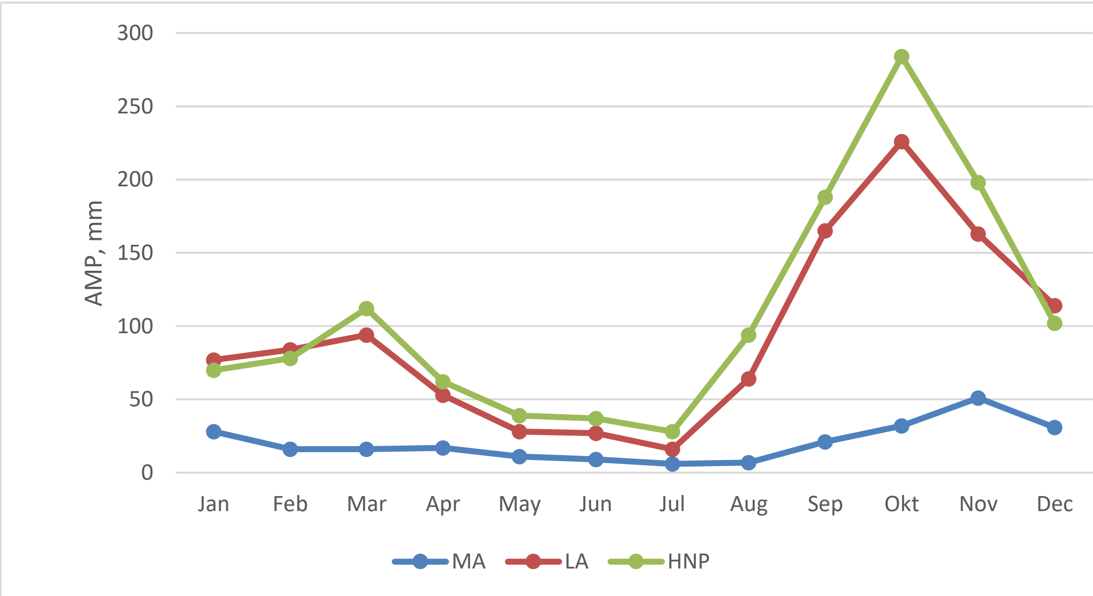

Figure 2. Average monthly precipitation (mm) for each population. (Museyibov, 1998, Mammadov *et al*., 2010) MA - Mardakan Arboretum, LA - Lenkaran Lowland, HNP - Hirkan National Park

We collected leaf samples based on Viscosi and Cardini (2011) and Jensen (1990) protocols. The same sampling design and methods were applied for each population. Ten mature trees in a small area (0.5–1 ha) of homogeneous open oak forest were selected. Tall trees, 8–10 m, were chosen and four outermost branches (light subsample) and four innermost branches (shade subsample) of each tree crown were randomly selected. To avoid seasonal and positional variations, samples were collected from different branches at approximately the same height and location, where leaf growth had stopped. Branches were collected from the four cardinal compass directions. Leaf age was practically the same, although there was a small variation in budburst among and within trees. In experimental design, only the branch position was considered (Jensen, 1990; Viscosi and Cardini, 2011). The most important factor for within-plant variation was inner vs outer position of branch regardless of compass direction or height.

Morphometric analysis. Ten leaf samples per tree were collected from 30 trees of all populations. A total of 300 leaves were morphometrically measured by CI-202 LESER AREA METER, USA (Jensen, 1990; Viscosi and Cardini, 2011). Sampling was done at midday and only mature leaves were considered. The following parameters were examined: leaf area (LA) (cm), leaf length (LL) (cm), leaf width (LW) (cm), leaf perimeter (LP) (cm), the ratio of leaf length to width (LL/LW) and shape factor (F). Leaf traits were analyzed in the laboratory of the Dendrological Arboretum, ANAS.

The sclerophylly indices were calculated after certain measurements on the leaves with the accuracy of 0.1 mg using EK-610i electronic scales: leaf mass per area (LMA), fresh weight (FW); fresh weight at full turgor (saturated weight (SW) after immersion of leaf petioles in demineralized water for 24 h in the dark); dry weight (DW) after drying in an oven at 70 °C for 72 h (Filippo *et al*., 2002). The foliar moisture parameters were calculated according to the following formula:

$$\text{Leaf Mass per Area (LMA; mg cm}^{-2}\text{)} = DW/LA$$

$$\text{Water Content (WC; \%)} = [1 - (DW/FW)]100\%$$

$$\text{Relative Water Content (RWC; \%)} = [(FW-DW)/(SW-DW)]100\%$$

$$\text{Succulence: (S mg cm}^{-2}\text{)} = (FW-DW)/LA$$

Statistical analysis. The variation analysis of the studied traits was performed using the RCBD method (Randomized Complete Block Design). The value of the variance coefficient of all morphological traits, which we get using RCBD method, represents the high reliability of the variance analysis. In turn, this result is the beginning of the performance of other statistical analyses of the studied parameters. Thus, if there is no statistical significance of any trait during ANOVA, it means that statistical analysis for this trait discontinues. A variance analysis was also performed with Least Significant Difference (ANOVA– LSD) test. All statistical routines were calculated with the program Statistical Product and Service Solutions16 (SPSS 16**)**.

Results and discussion

In this study, samples from 3 populations over Azerbaijan belonging to *Quercus castaneifolia* were studied. We found that leaf morphological traits of *Q. castaneifolia* varied significantly across our 3 study sites (Fig. 3). The results of the ANOVA analysis showed differences among populations (Tables 1–2). The value of the variance coefficient of all morphological traits, which we get using the RCBD method, represents the high reliability of the variance analysis (Table 2). The significance of parameters for population identification is presented in Table 3. As seen in Table 3, the leaf area in HNP (53.88±2.53 cm^2) is smaller

Figure 3. Morphological variation under different ecological conditions in *Quercus castaneifolia* C.A.Mey. leaves. 1 - Hirkan National Park, 2 - Mardakan Arboretum, 3 - Lankaran Lowland

compared with that in LLd (65.68±2.07 cm^2) and MA (66.26±2.18 cm^2). ANOVA showed a statistically significant differentiation of 5% for LA. [LCD=3.99% (leaf significant differentiation) (Table 2)]. The longest leaves were collected from the LLd population (19.23±0.91 cm) and leaves of the other populations were relatively shorter (HNP (16.30±0.81 cm) and MA (18.98±0.92 cm). There is a slight difference in the leaf perimeter between the HNP (103.98±2.11cm) and LLd (115.94±2.54 cm) populations. However, a sharp increase was observed in this parameter in the MA (139.5 ±2.73 cm) population, CV=10.16% and distribution of this trait was intermediate. The smallest value for the ratio LL/LW (2.63±0.36) was observed in the MA population. There is a slight difference in this ratio between the HNP (4.82±0.29) and LL (5.01 ±0.26) populations. The highest leaf shape factor was found in the HNP (0.08±0.001) population. Whereas the smallest value for this trait was observed in the MA population (0.04±0.001), CV=45.43%, the distribution was normal. It was found that most of the studied morphological characters have statistical significance for populations (Table 3).

Table 1. Interpopulation values of statistical parameters for the studied characters in *Quercus castaneifolia* C.A.Mey.

	LA (cm)	LL (cm)	LW (cm)	LP (cm)	R	F
Min	46,95	13,68	5,59	95,87	1,68	0.01
Max	93.62	22.46	8.18	187.78	5.23	0.132
Average rate	62.46	18.44	7.36	119.92	4.34	0.01
Standard error	±2.21	±0.85	±0.16	±2.44	±0.29	±0.001
Variation	271.15	3.18	1.82	290.95	9.43	0.0004
Standard discriminant	16.68	2.10	1.2	17.53	2.73	0.024
Median	58.48	17.25	7.47	118.38	4.05	0.062
CV	35.20	12.25	19.23	10.16	57.67	45.43

Table 2. Results of variation analysis based on RCDP method for the studied characters

Leaf characters	Repeat	Genotype	Error	LSD %5	C.V %
df	1	30	30	-	-
LA (cm^2)	2.38^{*}	153.77^{**}	0.29	3.99	9.48
LL (cm)	$0.05^{n.s}$	1.325^{**}	0.459	2.09	8.17
LW (cm)	$0.031^{n.s}$	0.978^{**}	0.341	1.32	4.75
LP (cm)	1.43^{**}	1.12^{**}	0.251	2.98	4.57
R	0.170^{*}	99.2^{**}	0.91	4.93	3.75
F	0.382^{**}	45.67^{**}	1.20	1.77	11.35

* indicates significance at the 0.05 level (2-tailed) ** indicates significance at the 0.01 level (2-tailed).

Table 3. Intrapopulation changes in the morphological parameters of the *Quercus castaneifolia* C.A.Mey. leaf under different environmental conditions. HNP - Hirkan National Park, LLd - Lankaran Lowland, MA - Mardakan Arboretum, CV - coefficient of variation (%)

	LA (cm)	LL (cm)	LW (cm)	LP (cm)	R	F
HNP	53.88±2.53	16.3±0.81	7.01±0.17	103.98±2.11	4.82±0.29	0.08±0.001
LLd	65.68±2.07	19.23±0.91	7.28±0.16	115.94±2.54	5.01±0.26	0.07±0.001
MA	66.26±2.18	18.98±0.92	7.9±0.15	139.5±2.73	2.63±0.36	0.04±0.001

Table 4. Foliar moisture (water content - WC, relative water content - RWC and succulence - S) for *Quercus castaneifolia* C.A.Mey. across 3 collection sites. HNP - Hirkan National Park, LLd - Lankaran Lowland, MA - Mardakan Arberetum

	LMA(mg cm^{-2})	WC (%)	RWC (%)	S mg cm^{-2}
HNP	7.42±0.61	58.03±1.62	71.39±1.82	7.79±0.56
LL	6.24±0.52	57.73±1.58	73.34±1.95	8.83±0.66
MA	3.77±0.11	40.32±1.05	70.39±1.82	5.85±0.38

As seen in Table 4, the lowest values of the foliar moisture indices were observed in the MA population (LMA=3.77±0.11 mg cm^{-2}, WC=40.32±1.05%, RWC= 70.34±1.94%, S=5.85±0.38 mg cm^{-2}). The highest value of relative water content (RWC=73.34%±1.95) and succulence (S=8.83 mg cm^{-2}) of chestnut-leaved oak leaves were in the LLd population. However, the highest values of LMA (7.79 mg cm^{-2}) and WC (58.03±1.62) were found in the HNP population samples. This indicates that plants in the Mardakan population are exposed to water stress compared to other populations.

The amount of precipitation varies sharply, but there aren't important differences between temperature and altitude in our study fields (Figs. 1–2). Variation analysis revealed that the HNP population differs from other populations due to the small leaf area, high leaf mass per area, and a highwater content of *Quercus castaneifolia* leaves. In addition, the Mardakan population could be differentiated from the remaining two populations by their smaller values of water content, relative water content and succulence. Water is a key determinant of leaf size, whereas temperature is relatively less critical (Peppe *et al*. 2011; Michal *et al*., 2016). The higher leaf mass per area and leaf density may be advantageous to reduce water loss by transpiration during the summer period (Kozlowski and Gerber, 2014).
The numerical values that were highly varied amongst the populations were leaf area (CV=35.20%), the ratio of leaf length to width (CV=57.67%), the shape factor (CV=45.43%) (Table 2). The difference between populations in the width of the leaf is moderate, but there is a marked difference in the length of the leaf although it is not significant. Only leaf perimeter parameter did not differ significantly amongst the populations, it had the least variations (CV=10.16%). Among the six traits investigated here, the ratio of leaf length to width (R) indicates the greatest variability in inter-population and it had the maximum coefficient of variation (CV=57.67%). With increasing precipitation, LA, LW and LP decreased gradually; LL, and LL/LW ratio were variable, although the shape factor (F) increased with changing precipitation. This implied that trees from the lower precipitation had higher leaf dimensions

(Table 3) Variation of morphometric parameters indicated that those traits had significantly adaptive plasticity under different environmental conditions (Givnish *et al*., 2004; Sun *et al*., 2016; Jump *et al,* 2009), which could potentially facilitate the high adaptive ability of *Quercus castaneifolia* in different environmental conditions.

Trees with low LA can reduce the excessive loss of water by evaporation and make water use more efficient, generating an important mechanism to address the scarcity of water resources. On the other hand, we identified that the longest leaves with the highest leaf areas were not necessarily located in areas with high precipitation, as reported in some studies (Gouveia and Freitas, 2009; Moles *et al*., 2014).

Studies of leaf ecophysiological properties explain a relationship between ecosystems and global climate change (Sabate *et al*., 1999; Thomas, 2000; Moles *et al*., 2014). The higher leaf mass per area and leaf density may be advantageous to reduce water loss by transpiration during the summer period (Sabate *et al*., 1999). The higher LMA protects the plant leaf from wear and tear and deters them from herbivores. Data from many habitats reveal a negative correlation between LMA and leaf size (Bilgin *et al*., 2004; Yalchin, 2018). This is consistent with the results of our study. As can be seen from Tables 2 and 3, the smallest LA and the highest LMA were recorded in the HNP population, but the highest LA and smallest LMA were recorded in the MA population.

We found that leaf moisture indices (LMA, WC, RWC, S) increased with increasing annual precipitation (Table 4). Probably, the conditions occurring during leaf differentiation and/or sprouting play a more crucial role (Bilgin *et al*., 2004; Moles *et al*., 2014). Foliar moisture parameters were positively correlated with mean annual precipitation and elevation but negatively correlated with mean annual temperature. The smallest value of leaf mass per area, water content, relative water content, and succulents in MA population are indicators of drought stress factor (Tab. 4). According to the results of our research, chestnut leaved oaks are tolerant to drought. The chestnut-leaved oak is principally a mesophilic species, preferring moderate temperatures and humidities. It thrives best on fertile, moist but not too wet and relatively deep soils, such as in the Ashagi Duzen Forest, a preserved forest area within Hyrcanian National Park in the lowlands of Lankaran. At higher elevations in the Talysh Mountains, *Q. castaneifolia* is found only on southern exposures; this fact is evidence of its high tolerance for long-lasting periods of drought (Kozlowski and Gerber, 2014; Zale, 2018).

Our study highlighted that leaf size wasn't obviously restricted with a short supply of water. It indicates chestnut leaved oaks are tolerant to drought. So, variations in leaf size along climatic gradients may result from greater evaporative demand of larger leaves due to enhanced thickness of the boundary layer for energy and gaseous exchange (Busotti *et al*., 2002). However, leaf size may also decline due to overall resources limitation in stressful environments, making the construction of large leaves with extensive vascular and cell-wall fractions overly expensive (Sun *et al*., 2016).

Given their significant plasticity, the morphological traits examined here should contribute to the adaptations of these plants to different environmental conditions. It has been documented that the leaf morphological variability of species along elevational gradients is related to environmental factors. There was a strong relationship between morphological features and environmental factors, mostly precipitation.

Conclusions

This paper presents the variation data on the response of morphological and moisture indices of leaves of *Quercus castaneifolia* from three provenances under different environmental conditions. The value of the variance coefficient of all morphological traits, which we have got using the RCBD method, represents the high reliability of the variance analysis. The most varied morphometric traits are the ratio of leaf length to width (R) and the shape factor (F) in chestnut oak populations. We found that leaf moisture indices (i.e., LMA, WC, RWC, S) increased with increasing elevation and annual precipitation. Data from many habitats revealed a negative correlation between LMA and leaf size. It has been documented that the leaf morphological variability of species along elevational gradients is related to environmental factors. A strong relationship between morphological features and environmental factors (mostly precipitation) has been identified. Such variation in morphological and moisture traits can provide evidence of adaptation of the species to changing environmental conditions for a wide distribution and it also indicates the evolutionary potential and protection of species.

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