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# Inter- and intrapopulation variations in leaf morphological and functional traits of *Quercus petraea* ssp. *iberica* under ecological factors in Azerbaijan

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***Abstract:*** Variation in leaf morphology is an important indicator of how plants respond to different environmental conditions. We analyzed the variation of morphological and functional traits of *Q. petraea* ssp. *iberica* leaf and its relationships with environmental and geographical variables across its distribution of this species to evaluate populations differentiation. The leaves of the tree were collected in diverse forest types (Ismailli, Qabala) and in arboretum (Baku) from -28 m to 900 m a.s.l., in 2017. We measured and analyzed the differentiation in morphological and functional traits of 300 leaves from 30 trees belong to three populations using univariate and multivariate methods. The morphological characteristics such as leaf size, area, widgth of leaves were measured. The results indicated that the leaf peremeter was decreased with increasing precipitation, and the values of morphological traits varied along elevation. Foliar moisture parameters (leaf mass per area, water content, relative water content, succulence) were positively correlated with annual precipitation. The precipitation was the most significant environmental factors in the variations of foliar moisture parameters and morphological traits. This study provided evidence for the adaptive plasticity-polymorphism of leaves in response to environmental changes.

***Key Words:*** *ANOVA, morphological variation, population, RWC, taxon*

## INTRODUCTION

The morphological and functional traits of plants explain their species response to changes in gradients [Salgado-Negrete et al., 2013; Valladares et al., 2014], which can be used to predict the responses of communities to environmental changes [Lohbeck et al., 2013]. Differences in phenotypic and physiological responses are associated with the geographical locations of populations at local or regional scales [Fajardo, Piper, 2011].

Leaves are organs exposed to different environmental factors, and it is reasonable to expect that their morphology and structure represent the responses of the plants to environmental conditions, such as water availability or light intensity, as well as intra- and interspecific interactions [Bruschi et al., 2000; Castro-Díez et al., 1997; Cuevas-Reyes et al., 2018]. 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 [Díaz et al., 2001]. Elevational gradients represent a "natural laboratory" within which we can better understand how such traits fluctuate in response to growing conditions. This is because of environmental factors vary along an elevation gradient, even in some cases within a small geographic area [Hovenden, Brodribb, 2000; Rezende et al., 2015]. Moreover, when one considers their close associations with environmental factors, these morphological traits can be used to predict the consequences of climate changes. The leaf morphological traits of many species vary with elevation. Those variations are mainly shaped by fluctuations in environmental factors. For example, the lower temperatures found at higher elevations can restrict the extension of leaves and reduce their size [Yin et al., 2004; Körner, 2007; Jump et al., 2009]. Different ecological and evolutionary studies have suggested that temperature and precipitation are the main determinants of plant morphological features on a global scale [Moles et al., 2014]. However, leaf phenotypic variability can also be explained by latitudinal and elevational gradients [Tang, Ohsawa, 1999].

Previous studies have documented the importance of geographic and environmental factors as regulators of plant structure, morphology, species colonization and

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establishment in different habitats [Bruschi et al., 2000; Díaz, Cabido, 2001]. The high morphological diversity of oaks has been of great interest in taxonomic and ecological studies analyzing the patterns of variation in morphological traits in relation to environmental gradients [Bruschi et al., 2000; 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 values in Azerbaijan. Georgian oak − *Q. petraea* ssp. *iberica* Steven ex. M. Bieb. is highly drought- and cold-resistant mountain species (Syn.: *Q. petraea* (Matt.) Liebl.) and occur in the same geographical regions in the Caucasus, with the durmast oak occupying lower altitudes and being gradually replaced by the large-anthered oak − at higher altitudes [Menitsky, 2005].

Studies of Azerbaijani oaks have been carried out in a traditional way until now [Bandin, Prilipko, 1964; Menitsky, 2005; Qurbanov, 2004; Asgerov, 2010; Mammadov, 2016]. The altitude of the research areas (Baku, Ismailli, Qabala) was quite different. These regions were ideal for studying the plasticity of leaf structure and function because of their higher elevational gradients and environmental variations. With a preferred elevation range between 600 m and 1000 m a.s.l., this species is distributed more widely than most other broad-leaved species. However, studies on the comparative morphology of this species are still rare. Although previous findings have improved our understanding of how *Q. petraea* ssp. *iberica* spread along elevations [Bandin, 1964; Mammadov, Khalilov, 2002; Asgarov, 2010; Mammadov, 2016], but no reports have described the morphological trait variations through an entire elevation range of the distribution. Moreover, details about potential correlations between elevation and traits, such as leaf shape and leaf moisture indices are still lacking. It is a part of a larger study on the ecological, morphological, and molecular characterization of some oak species in Azerbaijan. In this study, we collected leaves of *Q. petraea* ssp. *iberica* at three high-elevation sites, and applied multiple-regression models to explore: (1) how leaf morphological traits change with increasing elevation? (2) the key environmental factor(s) that contribute to those variations? (3) the ecological implications of altered leaf morphology for plant adaptability?

## MATERIAL AND METHODS

The purpose of the research, performed in August 2017, was the evaluation of the effects of environmental factors and irrigation conditions on morphological traits of leaves of oak trees.

*Plant materials.* This study was carried out on Georgian oak. The trees were grown in three stands located in the different regions of Azerbaijan, under different environmental conditions. The study areas were Baku, Ismailli and Qabala. The ecological characteristics of the populations were given in table 1. The soils of the study area in Qabala were mountain-forest, typical brown; in Ismailli meadow-cinnamon, partially alkaline and salty in Baku.

*Morphological and functional traits.* 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; Vincenzo, Andrea, 2011]. Sampling was done at midday and only mature leaves were considered. The following parameters were examined: some morphometric traits (leaf area (LA), leaf length (LL), leaf width (LW), the ratio of leaf length to width (LL/LW) and shape factor (F). Leaf traits were analysed in the laboratory of 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.). The foliar moisture parameters were calculated according to the following formula [Flippo et al., 2002]:

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

Water Content: (WC; %)=[1− (DW/FW)]100%;

Relative Water Content:
(RWC; %)=[(FW−DW) (SW−DW)]100%;

Succulence: (S mg, $cm^{-2}$)=(FW−DW)/LA.

*Statistical analysis.* The variation of the studied traits was performed using the Randomized Complete Block Design (RCBD) method. 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 was the beginning of the performance of other statistical analyses based on studied parameters. Thus, if there is

**Table 1.** Geographic location and climate conditions of the sampled populations of *Q. petraea* ssp. *iberica*.

| Locality | Geographic coordinates | Altitude (m) | *Pa* (mm) | *T* (°C) |
|---|---|---|---|---|
| Baku | 40°23'43"N 49°52'56"E | -28 | 216 | 14.2 |
| Ismailli | 40"47'24"N 48"09'07"E | 500-800 | 500-1000 | 11-13 |
| Qabala | 40"58'53"N 47"50'45"E | 900 | 800-850 | 10-12 |

***Note:*** *Pa*: mean annual precipitation (mm), *T*: mean annual temperature (°C) [Mammadov et al., 2010; Museyibov, 1998].

no significance difference of any trait during ANOVA, it means that statistical analysis for this trait discontinues. Least Significant Difference (ANOVA–LSD) test was used to compare the mean traits. All statistical analyses were conducted using the program Statistical Product and Service Solutions16 (SPSS 16).

## RESULTS AND DISCUSSION

All studied leaf morphological traits varied considerably among three sampling sites (Figure). The ANOVA showed that there were significant differences among the populations. The significance of parameters for populations identification was presented in the tables 2 and 3. The numerical values that were highly varied amongst the populations, they presented in the first three rows (i.e., leaf area, perimeter and shape factors). The leaf width parameter among the populations differed on the average, whereas the leaf length among the populations was significant difference, but was not high. Only ratio leaf lenth to leaf width parameter presented last in the table did not differ significantly amongst the populations. Among the six traits investigated here, leaf area (LA) indicate that the greatest variability in inter-population and it had the maximum coefficient of variation (CV=34.6%). The longest leaves were collected from the Ismailli population (27.62 cm) and the shortest leaves from Qabala population (9.00 cm) (Tab. 2). By contrast, the ratio of leaf length to width (LL/LW) had the least variations (CV=11.3%). With increasing elevation, LW, LP were decreased significantly; LL, LA and LL/LW ratio were variable, however, the shape factor (F) was generally stable with changing elevation. This implied that trees from the lower altitudes had higher leaf dimensions (Tab. 3).

Qabala population variation analysis revealed that differentiated from the other posts, as they were

**Table 2.** Interpopulation values of statistical parameters for the studied characters in *Q. petraea* ssp. *iberica.*

| Statistic indicators \ Traits | Area ($cm^2$) | Length (cm) | Width (cm) | Perimeter (cm) | Ratio | Factor |
|---|---|---|---|---|---|---|
| Min | 38.87 | 9.00 | 5.53 | 92.56 | 1.67 | 0.01 |
| Max | 106.59 | 27.62 | 10.08 | 171.76 | 3.87 | 0.08 |
| Average rate | 48.76 | 16.64 | 6.73 | 131.27 | 2.95 | 0.038 |
| Standard error | ±2.43 | ±0.91 | ±0.16 | ±2.54 | ±0.36 | ±0.002 |
| Variation | 285.15 | 3.28 | 1.98 | 320.15 | 10.33 | 0.0004 |
| Standard discriminant | 15.67 | 2.00 | 1.51 | 18.43 | 2.61 | 0.022 |
| Median | 45.45 | 12.27 | 6.47 | 134.38 | 1.05 | 0.032 |
| CV (%) | 34.6 | 15.78 | 21.72 | 28.42 | 11.03 | 32.23 |

**Table 3.** Intrapopulation changes in the morphological parameters of the *Q. petraea* ssp. *iberica* leaf in the different environmental conditions.

| Locality | LA | LL | LW | P | R | F |
|---|---|---|---|---|---|---|
| Qabala | 42.23±2.53 | 11.39±0.81 | 7.12±0.17 | 134.98±2.41 | 2.62±0.29 | 0.04±0.001 |
| Ismailli | 92.56±2.07 | 13.31±0.91 | 7.68±0.16 | 142±2.64 | 2.71±0.36 | 0.03±0.001 |
| Baku | 67.26±2.18 | 10.25±0.92 | 8.24±0.15 | 148.5±2.43 | 2.23±0.38 | 0.03±0.001 |


**Figure.** Morphological variation under different ecological conditions in *Q. petraea* subsp. *iberica*: 1-Baku, 2-Qabala, 3- Ismailli.

smaller leaf area, the highest leaf mass per area and the water content. In addition, Qabala population could be differentiated from the remaining two populations by their smaller values of leaf width, leaf perimeter. But, Ismailli population variation analysis showed that leaves of *Q. patraea* ssp. *iberica* were differentiated from the other populations, as they were the highest value for all studied characters (Tab. 3). The longest leaves were collected from the Ismailli population (27.62 cm) and the shortest leaves were collected from the Qabala population (9.00 cm) (Tab. 2).

As seen in table 4, the lowest values of the foliar moisture indices were found in Baku's population (LMA=1.46±0.10mg cm2, WC=8.41±1.15%, RWC= 37.46±1.82%, S=0.13±0.01mg cm2). The highest value of relative water content (RWC=84.15±2.05) of Georgian oak leaves was obtained in Ismailli's population. However, the highest values of LMA (7.16±1.14 mg cm2 ),WC (62.23±1.82) and S (8.24±1.08 mg cm2) were found in Qabala's population.

We found that leaf morphological traits of *Q. petraea* ssp. *iberica* varied significantly across in the three studied sites (Tab. 1). This indicated that those traits had significantly adaptive plasticity under different environmental conditions, which could potentially facilitate the high adaptive ability of *Q. petraea* ssp. *iberica* along high elevations. This species is distributed across a wide elevational range, and leaves in higher elevations exhibited lower leaf area, length, and width; lower shape factor and ratio of leaf length to width. Abiotic conditions are the major factors that shape plant traits along elevational gradients [Körner, 2007]. Annual temperature decreased with increasing annual precipitation and elevation in the studied sites (Tab. 1). High LA values were found in trees at localities with a high rainfall. These 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, Freitas, 2009; Moles et al., 2014].

As the elevation increased, values for LW and LP decreased significantly and all were negatively correlated with mean annual precipitation. It has been observed that LA and leaf size are directly proportional to the decreasing amount of rainfall and soil nutrients [Bilgin et al., 2004; Eda, 2015; Yalchin, 2018]. The marked morphological differences recorded between the three populations might therefore reflect isolation and strong ecological adaptation to different environments.

**Table 4.** Elevation-associated variations in foliar moisture (LMA leaf mass per area, water content, WC, relative water content, RWC and succulence, S) for *Q. petraea* ssp. *iberica* across three collection sites.

| Locality | LMA (mg cm$^2$) | WC (%) | RWC (%) | S mg cm$^2$ |
|---|---|---|---|---|
| Baku | 1.46±0.10 | 8.41±1.15 | 37.46±1.82 | 0.13±0.01 |
| Ismailli | 6.81±0.91 | 54.26±1.65 | 84.15±2.03 | 8.08±1.01 |
| Qabala | 7.16±1.14 | 62.23±1.82 | 83.38±2.13 | 8.24±1.08 |

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 deter 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 it can be seen from tables 3 and 4, the smallest LA and the highest LMA were recorded in the Qabala population.

Water is a key determinant of leaf size, whereas temperature is relatively less critical [Michal, 2016; Peppe et al., 2011]. Drought imposed during leaf ontogeny may result in an alteration in LMA. An elevation in LMA is sometimes associated with increased water-use efficiency. In *Q. petraea* ssp. *iberica*, severe drought resulted in a decrease in the following: the biomass of leaves [Frank, Thomas, 2000]. We found that leaf moisture indices (i.e., LMA, WC, RWC, S) enhanced with increasing elevation and annual precipitation (Tab. 3). 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 Baku's population are indicators of drought stress factor (Tab. 4). Georgian oaks are highly drought- and cold-resistant mountain oaks [Ekhvaia et al., 2018; Menitsky, 2005]. In the genus *Quercus*, a deep root system helps to avoid dehydration and to tolerate a decreased water supply [Frank, Thomas, 2000]. The results of this study revealed that precipitation was the key environmental factor in variation of foliar moisture parameters and morphological traits.

The significant variability in the leaf traits reflects their plasticity, and it demonstrates their important roles in the adaptability of species to the environmental changes. 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). Likely, since the Late Miocene, two main regions corresponding to the Euxinian (Western Caucasus) and Caucasian (Eastern Caucasus) provinces played major roles as a linkage crossroad and in both preserving and incresing diversity. More extensive investigations with additional markers (e.g., the nuclear genome), bio-ecological descriptors, further populations, and species from border regions are needed to fully understand the true extent of the oak diversity in the Caucasus and assist conservation of this important species.

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## Azərbaycanda ekoloji faktorların təsirindən *Q. petraea* ssp. *iberica* yarpaqlarının morfoloji və fizioloji xüsusiyyətlərinin populyasiyadaxili və polpulyasiyalararası variasiyası

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Yarpaq morfologiyasındakı variasiya bitkilərin müxtəlif ətraf mühit şəraitinə necə uyğunlaşdığını əks etdirən əsas göstəricidir. *Q. petraea* ssp. *iberica* populyasiyalarında müxtəlifliyi qiymətləndirmək üçün növün yayıldığı ərazilərdə yarpaqlarının morfoloji və funksional əlamətlərinin variasiyasını və bu variasiyanın ətraf mühit şəraitinin, eləcə də coğrafi şəraitin dəyişməsi ilə əlaqəsini analiz etdik. Yarpaq nümunələri 2017-ci ildə -28 - 900 m yüksəkliklərdən müxtəlif meşə formasiyalarından (İsmayıllı və Qəbələ) və dendraridən (Bakı) toplanmışdır. Təkölçülü və çoxölçülü analizlərdən istifadə edərək 3 populyasiyadan 30 ağacın 300 yarpağının morfoloji və funksional fərqini ölçdük və analiz etdik. Nəticədə, yağıntının miqdarı artdıqca yarpağın bəzi morfoloji əlamətlərinin (yarpaq sahəsi, yarpağın eni və perimetri) ölçüsünün azalmasını müəyyən etdik. Hündürlük boyunca morfoloji əlamətlərin göstəriciləri dəyişmişdir. Yarpağın nəmlik göstəriciləri (sahəyə görə yarpağın kütləsi, su tutumu, nisbi su tutumu, şirəlilik) illik yağıntının miqdarı ilə müsbət korrelyasiya yaradır. Yarpağın nəmlik göstəricilərinin və morfoloji əlamətlərinin dəyişməsində yağıntı açar ekoloji amildir. Bu iş yarpaqların dəyişən mühit şəraitinə cavab olaraq yarpaq plastikliyinin adaptiv polimorfizmini ifadə edir.

***Açar sözlər:*** *ANOVA, morfoloji variasiyalar, populyasiya, RWC, takson*

## Внутрипопуляционная и межпопуляционная вариация морфологических и физиологических особенностей листьев *Q. petraea* ssp. *iberica* под влиянием экологиеских факторов в Азербайджане

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Вариация в морфологии листьев - важный индикатор того, как растения реагируют на различные условия окружающей среды. Для оценки дифференциации популяции проанализирована изменчивость морфологических и функциональных признаков листьев *Q. petraea* ssp. *iberica* и его взаимосвязь с экологическими и географическими переменными в распределении этого вида Растения собирали в различных типах лесов (Исмаиллы и Габала) и в дендрарии (Баку) на высоте от -28 до 900 м в течение 2017 года. С использованием одномерного и многомерного анализа по морфологическим и функциональным признакам измерена и проанализирована дифференциация 300 листьев 30 деревьев из 3-х популяций. В результате установлено, что с увеличением количества осадков некоторые морфологические характеристики, такие как размер листа, площадь листа, ширина листа, периметр листа уменьшаются. С высотой показатели морфологических признаков изменялись. Параметры влажности листьев (масса листьев соответственно площади, влагоемкость, относительная влажность, сочность) положительно коррелировали с годовым количеством осадков. Ключевым фактором окружающей среды в изменении параметров влажности и морфологических характеристик листвы являются осадки. Это исследование свидетельствует об адаптивном полиморфизме пластичности листьев в ответ на изменения окружающей среды.

***Ключевые слова:*** *ANOVA, морфологическая вариация, популяция, RWC, таксон*