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MORPHOLOGICAL VARIABILITY OF ACORNS AND ITS TAXONOMIC SIGNIFICANCE IN *QUERCUS* L. FROM TURKEY

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Abstract

Morphological variations of acorn among and within the groups of *Quercus* species were studied. A total of 617 acorns belonging to 14 species representing all 3 sections of *Quercus* L. (Fagaceae) in Turkey were examined in this study. Specimens were collected from 47 different populations over both Anatolian and Thrace part of Turkey. Principal component analysis was used to analyze the morphological characteristics of acorns. Results obtained from this study demonstrate the use of morphological characters in differentiating the taxa of *Quercus* and *Cerris* sections studied. Another important finding is the introgression among the acorns of species within *Quercus* section.

Introduction

Turkey has long been blessed by the diversity, abundance, and beauty of oaks. Great variety of oaks present in both Anatolian and European parts of Turkey ranging from Mediterranean, Aegean, and Black Sea coastal regions to towering coastal and interior mountains. The group occupies 25 per cent of the forest area of Turkey (Ertaş 1995). The *Quercus* in Turkey is divided into three sections according to Flora of Turkey and East Aegean Islands (Davis *et al.* 1965-1985).

An acorn is the fruit of the oak, which is an oval nut growing in a woody cup or cupule. Nut consists of a hard and indehiscent shell inclosing a kernel. Cupule is a massive, robust and often compound envelope of fruits (Fey and Endress 1983).

Acorns vary greatly in size between species, subspecies and even within the same population depending on the taxa and its environment (Bonito *et al.* 2011, Dufour-Dror and Ertaş 2002, Galván *et al.* 2012, Ramírez-Valientea *et al.* 2009). Within the genus *Quercus*, *Q. ithaburensis* probably has the largest acorns, while *Q. petraea* has the smallest (Pavlik 1991). The fruits of the species within section *Cerris* are matured in two years, and contain a large amount of fat, while the species of *Quercus* section produce small fruits without excess amount of fat. The acorn maturation as a character is commonly used to differentiate major groups within *Quercus* in Flora of Turkey and the East Aegean Islands (Davis and Cullen 1979).

Quercus has attracted the attention of the evolutionists for its very poor reproductive development (Bacilieri *et al.* 1996). Among-tree relationships based on fruit/bud characters were found to be significantly different from among-tree relationships based on leaf characters of the genus (Jensen 1989, 1992).

Vegetative characters are often viewed as having less taxonomic utility than reproductive characters (Ornduff 1978, Sivarajan and Robson 1991). However, in the subgenus *Quercus*, the leaf alone may be sufficient for discrimination purposes since it varies significantly from species to species with respect to shape, size and hairiness (Viscosia *et al.* 2009). Morphological studies based on acorn characters are incredibly few. In a few recent studies on acorn morphology (Jensen 1992, Jensen and Tomlinson 2003) some fruit/bud characters were found to be useful in identifying

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oak species with multivariate analysis. Several acorn characters like length, width, diameter and weight were preferred by some other studies (Barzdajn 2002; Galván *et al.* 2012, Ivanković *et al.* 2011, Major 2002, Nikolić and Orlović 2002).

Presence of a rich and diverse flora of oaks ranging from coastal regions to top of high mountains, diversity in shape and size of acorns (Jensen 1980, 1989), and availability of various analyzing techniques motivated us to perform this study. Can morphology of acorns be distinctive for at least some groups of *Quercus*? To answer that, this study has aimed to evaluate the morphological variations among acorns of different groups of *Quercus* species as well as acorns of closely related species. Present authors have tried to extract principal components and plot the specimens onto a two dimensional graph. By this way it would be possible to show whether they are morphologically different.

Materials and Methods

Acorns were picked up directly from trees. Acorn samples were chosen randomly among the mature and normal shaped ones during sampling process. Authors paid attention to select acorns with cupules, since characters of coupling cupule would also be useful.

Samples were collected from the different locations with respect to phytogeographical regions when possible (Table 1). Each location represents an oak population of one or more species. Each species were preferably represented by 4 populations, each population by 3 trees, and each tree by 5 acorns. A number of 47 populations belonging to 14 species were sampled from 43 distinct locations. Information on nomenclature, populations, plant samples and locations are represented in Table 1. Acorns were treated as operational taxonomic units (OTUs). Measurements of 13 characters were taken from a total of 617 acorns. These characters are summarized in Table 2 and described in Fig. 1. All characters were quantitative continuous. Longest cupule scale was measured for cupule scale length character. Since only regularly shaped acorns were included in the study, authors assumed that acorn has radial symmetry.

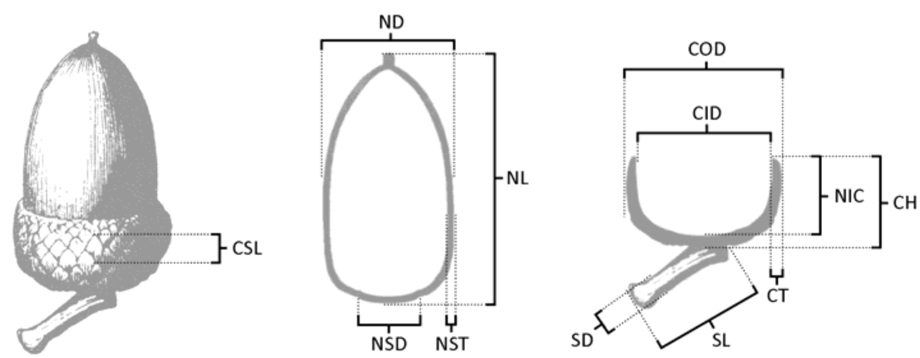

Fig. 1. Morphological characters of the acorn (NL = Nut length, ND = Nut diameter, NSD = Nut scar diameter, NST = Nut shell thickness, NOC = Nut out of cupule, NIC = Nut in cupule (Cupule depth), CH = Cupule height, CID = Cupule inner diameter, COD = Cupule outer diameter, CT = Cupule thickness, CSL = Cupule scale length, SL = Stalk length, SD = Stalk diameter).

In order to view the groupings or the clusters for the classification of acorns, the morphological characteristics of 617 acorns belonging to 14 oak species from Turkey were analyzed by Principal Component Analysis (PCA) module using STATISTICA version 8.0. A matrix of raw data with quantitative variables was needed to be treated by PCA. Therefore, a data

Table 1. Samples with taxonomy and locality information.

Taxon	Population	(n)	Station	Altitude (m)	Coordinates N	E
Section *Ilex* Loudon						
Q. aucheri Jaub. & Spach	AUC114	3	114	90	37°44.967	029°16.360
	AUC117	3	117	180	37°33.558	028°04.047
	AUC118	2	118	300	37°32.889	028°05.310
Q. coccifera L.	COC128	3	128	140	39°29.722	026°21.148
	COC166	3	166	1415	36°51.407	033°16.702
	COC176	3	176	710	37°26.291	037°12.226
	COC209	3	209	100	41°16.197	028°40.196
Q. ilex L.	ILX113	3	113	110	37°41.905	027°11.334
	ILX206	3	206	60	41°09.136	031°23.627
Section *Cerris* Loudon						
Q. brantii Lindl.	BRA156	3	156	1025	39°11.541	039°42.114
	BRA158	3	158	1135	39°03.650	038°30.024
	BRA081	1	81	1470	38°13.157	041°52.131
	BRA172	3	172	500	36°28.514	036°16.735
Q. cerris L.	CER137	3	137	1065	40°09.950	035°07.235
	CER181	3	181	1470	38°12.485	035°53.293
	CER210	3	210	40	41°52.450	027°57.408
	CER200	3	200	500	39°15.279	026°04.446
Q. ithaburensis Decne. ex Decne.	ITH216	2	216	50	40°38.848	026°14.970
	ITH186	2	186	1140	39°04.457	029°27.465
	ITH169	1	169	1110	36°33.774	033°54.779
	ITH124	3	124	70	39°03.309	026°53.490
Q. libani Olivier	LIB151	3	151	1310	39°33.334	040°02.531
	LIB146	1	146	1500	37°54.411	042°56.096
	LIB159	3	159	920	39°12.550	038°35.229
	LIB087	1	87	1490	38°58.110	041°05.685
Q. trojana Webb.	TRO163	3	163	1110	37°09.275	033°25.859
	TRO193	3	193	245	39°48.725	027°37.757
	TRO220	3	220	20	37°25.358	027°13.200
	TRO185	3	185	970	39°21.732	030°02.756
Section *Quercus* (Endl.) Örsted						
Q. frainetto Ten.	FRA208	3	208	80	41°12.036	029°00.825
	FRA191	3	191	340	39°49.444	027°48.992
	FRA187	2	187	700	39°08.550	028°43.917
Q. infectoria Olivier	INF163	3	163	1110	37°09.275	033°25.859
	INF145	3	145	1500	37°54.411	042°56.096
	INF199	3	199	40	39°04.500	026°56.805
	INF183	3	183	650	40°27.452	030°21.736
Q. macranthera Fisch. & C.A. Mey. ex Hohen.	MAC140	3	140	1535	39°57.091	039°37.896
	MAC150	3	150	1975	39°51.962	040°37.616
Q. petraea (Matt.) Liebl.	PET115	3	115	290	37°53.422	027°21.973
	PET087	2	87	1490	38°58.110	041°05.685
	PET204	3	204	700	40°18.258	032°25.869
Q. pubescens Willd.	PUB133	2	133	1080	40°43.991	034°34.512
	PUB102	3	102	1050	38°58.078	030°06.433
	PUB182	3	182	660	40°31.450	031°04.973
Q. robur L.	ROB084	2	84	1480	38°33.442	042°05.492
	ROB140	3	140	1535	39°57.091	039°37.896
	ROB208	2	208	80	41°12.036	029°00.825
	ROB189	3	189	365	39°14.093	028°06.501

matrix was compiled from original measurements. For each character the average of 5 acorns were calculated. Each acorn (as OTUs) corresponded to a row and each character (variable) to a column within the data matrix. All 617 OTUs were used for the first analysis. Secondary data matrices were derived from the main matrix by dividing it into three parts according to the OTUs of three sections (*Quercus*, *Ilex* and *Cerris*). To show the variation within the sections, analysis was also performed for smaller groups of OTUs grouped by 3 sections. All 13 variables were chosen as 'variables for analyses'. Section names were used as 'grouping variables' when defining plot markers in the analysis of all OTUs. Species names were used in the analysis of *Quercus*, *Ilex* and *Cerris* sections for 'grouping variables'. Two dimensional projections of all OTUs, and *Quercus*, *Ilex* and *Cerris* sections were generated and shown in Fig. 2. Analyses were plotted by two factors with the highest percentages, for explaining the most of the data. Four PCA plots were calculated based on measurements of morphological characters. The plots of four analyses were represented in Fig. 2. To show the relationships among the characters, correlations were calculated.

Results and Discussion

In this study the components - also known as factors - in PCA plots, showed approximately 69% of the total variance is accounted for all by components 1 and 2 combined and 63% in the *Ilex* plot (Fig. 2.B). In the *Quercus* and *Cerris* plots, the coverage of the accounted variance was less than 60%. In PCA of morphological measurements, first principal component is generally

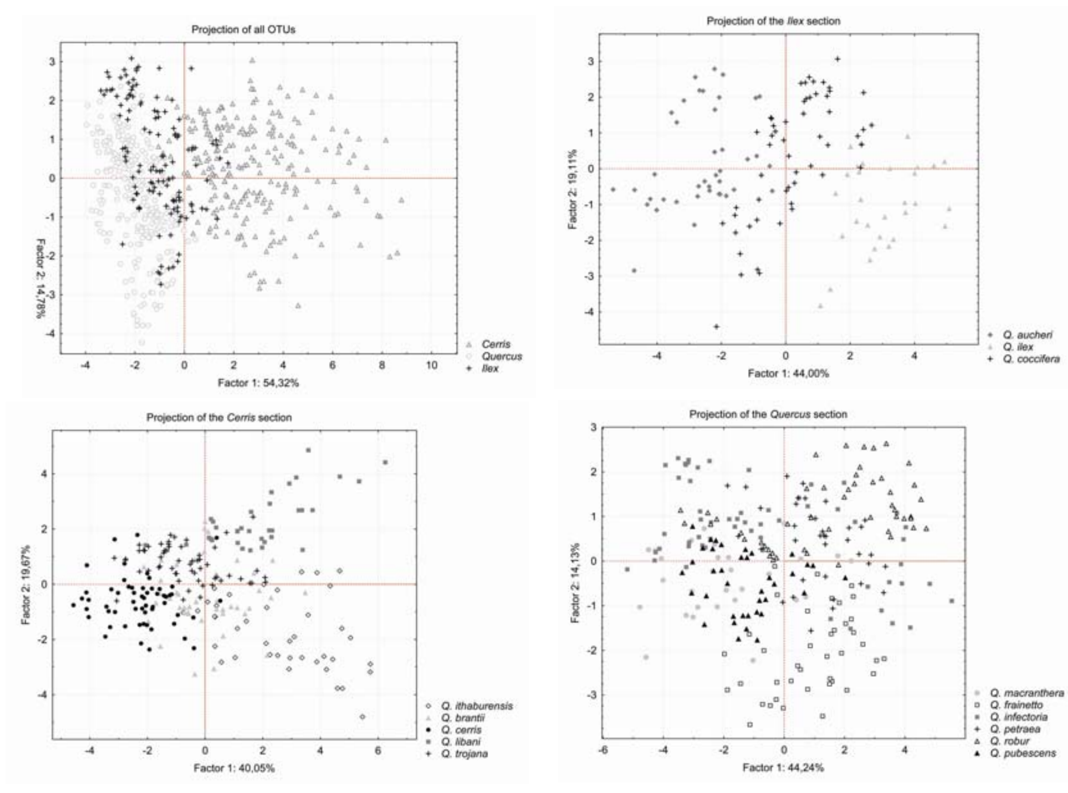

Fig. 2. PCA Projections of all sections (A), of *Ilex* section (B), of *Cerris* section (C) and of *Quercus* section (D) according to first two factors (principal components).

taken as a measure of size, and subsequent principal components are then describing various aspects of shape (Cadima and Jolliffe 1996). Size dependent characters especially nut lengths and widths were generally used to estimate acorn volume (Aizen and Patterson 1990). On the other hand, it is not possible to predict the resolution of the other components since the percentage values of these components are very close to each other.

In this study, two bigger sections, *Quercus* and *Cerris*, were separated by the first factor which ordinates acorns by their sizes. Within the *Cerris* section, *Q. ithaburensis* and *Q. libani*, the two species with biggest acorns were scattered on the opposite site of *Quercus* and *Ilex* sections. This is because the fruits of species within section *Cerris* are matured in two years, and contains a quantity of fat, while *Quercus* section is represented with small fruits without excess amount of fat. Therefore, this study supports the view that first component generally ordinates OTUs according to their size. The first component accounts for 69% of the total variation and has the highest values of coefficients (not included in the study) among the cupule characters. Thus, PCA finds variations in cupule characters most significant (Fig. 2).

Principal component analysis plots, by the use of highest two factors, were explained more than half of the data which is an acceptable value for most cases. However, *Quercus* and *Cerris* plots were found to be approximately 10% less than complete plot. This is basically caused by the variation within *Cerris* and *Quercus* sections. *Cerris* section is particularly known by their reproductive strategy, and biennial maturation. Biennial maturation specialize acorns to survive under different climatic conditions. This simply causes a variety of morphological changes in characteristics of acorns to adapt to variable and unpredictable climatic conditions, characteristic of the Mediterranean climate (Elena-Rossello *et al.* 1993). Beside the complexity and variation in morphological features of its acorns, *Cerris* section is the simplest to discriminate from other sections by their acorn size and biennial maturation. Only some acorns belonging to *Ilex* section might penetrate into the *Cerris* group. They were the most common acorns of *Q. aucheri* whose inclusion in *Ilex* section was seemed to us suspicious. By their appearance, *Q. aucheri* acorns resemble those of the *Cerris* section substantially. Differentiation and grouping of acorns of different species in the results of PCA is simpler than those of the *Quercus* section. OTUs show a triangular distribution over the plot (Fig. 2A). *Q. ithaburensis*, *Q. libani* and *Q. cerris* are on the corners of the triangle, and *Q. trojana* is between the two corners of *Q. libani* and *Q. cerris*. Only the exception is *Q. brantii*, which is scattered widely in the middle and overlaps with three other species.

Ilex section, with its three species, is the least complex section by number. Each of the three species are scattered almost equally and parallel to each other in the PCA plot (Fig. 2B). In the same plot, *Q. coccifera* spreads in the two other species. *Q. aucheri* scattered at one side with biggest acorns in the section and *Q. ilex* at the other side with smallest acorns.

Quercus section is the most problematic group in the genus. Hybridization takes place among the species of this section more frequently (Borazan and Babac 2003, Viscosia *et al.* 2009). In taxonomic discrimination, the biggest problems occur especially in section *Quercus* (Bruschi *et al.* 2000). Although PCA separated some species distinctly, most are overlapping with other species. However, the sample size is not sufficient and excessive number of species is covered to state something about hybridization among these species. Although PCA analyses could not separate the six taxa belonging to the section *Quercus* clearly, the results support the group of *Q. robur*, *Q. macranthera* and *Q. frainetto* and that of *Q. infectoria* and *Q. robur* and isolated position of *Q. petreae* from the section. Both *Q. infectoria* and *Q. robur* have two subspecies. *Q. petraea* has 3 three subspecies, but these are not differentiated in the plot. Overlapping positions of these three species (*Q. infectoria*, *Q. robur* and *Q. petraea*) is remarkable. Comparing to Borazan and Babac

(2003) in which morphological analysis of oak leaves were issued, we can say that acorn characters might also be useful in the separation of oak species.

Correlations among characters also give remarkable results. ND is found to be correlated with CID and COD. COD in *Cerris* section only the exception is which is below the level of significance. This is because certain species in this section have very thick and long scales while some other species with the same size have not (Borgardt and Nixon 2003). The same situation also occurs in NSD since ND and NSD are highly correlated. Correlation between CID and COD is as low as on the significance limit and CT is highly correlated with CSL because of the same thick scale reason. SL shows a negative correlation with most of the length and diameter characters in *Ilex* section. *Q. aucheri* acorn has the largest size without a stalk, *Q. coccifera* has short or no stalk at all and *Q. ilex* has the longest stalks.

Results obtained in this study demonstrate the usefulness of acorn morphology in delimitation of some *Quercus*. Acorn shape characteristics are found valuable taxonomically especially at the section level and also for small groups of species. Analysis of acorn shape variation might be most successfully applied in *Cerris* section for taxonomic delimitation.

This study has proved the taxonomic utility of fruit characters in Fagaceae and also states that relationships among the characters can be explained by the use of geometric morphometric methods.

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