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Cytogenetic Relationships of Turkish Oaks

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Abstract

The genus *Quercus* L. represented by 18 species belonging to three sections (*Quercus* L., *Ilex* Loudon and *Cerris* Loudon) in Turkey has a problematic taxonomy especially because of widespread hybridization observed frequently among oak species. In the present chapter, detailed karyotypic investigation of the 16 oak species from three sections in Turkey, their taxonomic relationships with each other and European oaks and finally situations that increase the variation and taxonomic problems among oak species in Turkey were evaluated to contribute to the taxonomic relationships of the genus. The somatic chromosome number in all taxa examined confirmed that the chromosome number of the genus is consistent with $2n = 24$. However, some differences were determined among species, although similar results were observed in many oak species in Turkey contrary to European oaks.

Keywords: *Quercus*, *Ilex*, *Cerris*, Turkey

1. Introduction

The genus *Quercus* L. belonging to the Fagaceae family is one of the most abundant and economically important genera of woody plants in temperate and tropical areas of the northern hemisphere. The genus contains over 500 species and is distributed across the North and Central America, Asia, Europe and North Africa [1].

Turkey has a large land area covered by forest with 21.2 million ha. The oaks in Turkey are ecologically and economically very important trees and make up 26% of the total forest area. However, a large part of this is not so productive due to especially excessive cutting and grazing. Turkey is one of the richest countries in species number, diversity and endemic species [2]. The genus is currently represented by 23 taxa and 3 endemic species such as *Q. aucheri*, *Q. vulcanica* and *Q. macranthera* subsp. *syspirensis* [2, 3].

The most important reason which influences species number and diversity is the location and geomorphological structure of Turkey [4]. Turkey is composed of Anatolian and Thrace peninsulas where three phytogeographic regions are crossed such as Irano-Turanian, Mediterranean and Euro-Siberian regions. Its geomorphological structure reveals numerous climatic regions which influence the flora. The main climatic changes in Turkey occurred especially at the beginning of the Holocene, and these climatic conditions have affected the topography and distribution of today's plant vegetation [5]. Today's plant zones occurred in Anatolia, about 8000 years ago [5–11]. During the last glacial period, coastal plants, such as *Pinus sylvestris* and *Picea* sp., which were grown in cold climate in East Black Sea, have been taken up to higher places up gradually, and their places have been occupied by broad-leaved forests, such as *Quercus* spp., *Fagus*, and so on, living in the shaded coastal areas [5].

Another important factor affecting species diversity and distribution is the location of Turkey between the Asian and European continents. As a result, Anatolia has served as a migration route and refuge regions for many plants and animals from Asia into Southeast Europe [12].

Besides its location and phytogeographical regions, Anatolian Diagonal which divides Anatolia as eastern and western parts is another factor affecting species diversity, number and distribution [4, 12–15]. Anatolian Diagonal separates many plant and animal species into eastern and western Anatolia.

Turkey is a rich country in the aspect of oak variation with 18 species belonging to three sections (*Quercus* L., *Ilex* Loudon, and *Cerris* Loudon).

Section *Quercus* L. is characterized by the greatest number of species among the three sections in Turkey: *Q. pontica* C. Koch., *Q. robur* L., *Q. hartwissiana* Steven., *Q. macranthera* subsp. *suspirans* (C. Koch.) Menitsky, *Q. frainetto* Ten., *Q. petraea* (Mattuschka) Lieb., *Q. vulcanica* (Boiss. Heldr. ex) Kotschy, *Q. infectoria* Oliver, *Q. pubescens* Willd and *Q. virgiliana* Ten. [2].

Section *Ilex* Loudon is characterized by the evergreen trees and shrubs: *Q. ilex* L., *Q. coccifera* L. and *Q. aucheri* Jaub. et Spach. [2].

Section *Cerris* Loudon is the second largest section and includes five species; *Q. libani* Olivier, *Q. trojana* Webb, *Q. cerris* L., *Q. brantii* Lindl. and *Q. ithaburensis* subsp. *macrolepis* (Kotschy) Hedge et Yalt. [2].

Vegetative characters are preferred instead of reproductive characters in situations which are not helpful in the classification [16]. Leaves are good indicators of putative hybridization and very useful for identification of oaks. Individuals that exhibit intermediate morphological characters can be seen widely because of interspecific hybridization and even sometimes exhibit high morphological variation, and it is not possible to identify an oak tree to a species. In this case, acorns are secondary important materials in the separation of oak species [17–19]. Although vegetative characters are crucial to differentiating species within the genus and are frequently preferred in the identification of oaks, these are insufficient and risky because of hybridization behaviors caused by weak reproductive barriers between oak species. Oaks are wind-pollinated species and they can spread across wide geographic regions [20–22]. As a result, many oak species grow in mixed populations that increase the hybridization in the same or different sections. In addition to wind-pollination and weak reproductive barriers

between species [14, 22–25], insufficient diagnostic morphological characters [24–25] and the lack of investigations for each taxon such as ecological, historical and genetic descriptors [25] make problematic the genus *Quercus* in Turkey and similarly in the world.

Scientific interest has recently moved from classification of the species with classic descriptors to understanding of oak evolution with molecular markers [24–27]. However, oak taxonomy is still problematic and under debate. Although cytologic studies are very important and useful for determining taxonomy and polyploidy, studies on the genus *Quercus* are still insufficient because of the small size of chromosomes and difficulties in the germination of acorns.

2. Materials and methods for karyotype analyses of Turkish oaks

In our previous studies, acorns belonging to 16 oak species from three different sections were collected in different times from various locations in Turkey. The species, sections and locations are presented in **Table 1**. Acorns of three species (*Q. libani*, *Q. petraea* subsp. *iberica* and *Q. infectoria* subsp. *infectoria*) were germinated in plastic cups filled with water in room temperature [28]. The other 14 *Quercus* taxa studied (*Q. robur*, *Q. hartwissiana*, *Q. macranthera* subsp. *syspirensis*, *Q. frainetto*, *Q. vulcanica*, *Q. infectoria* subsp. *boissieri*, *Q. pubescens*, *Q. virgiliana*,

Species	Section	Locations
<i>Q. coccifera</i>	Ilex	Uşak, Hatay
<i>Q. ilex</i>	Ilex	Zonguldak, Düzce
<i>Q. aucheri</i>	Ilex	Aydın, İzmir, Muğla
<i>Q. cerris</i>	Cerris	Balıkesir
<i>Q. ithaburensis</i>	Cerris	Çanakkale, Balıkesir
<i>Q. libani</i>	Cerris	Between Erzincan-Tercan
<i>Q. trojana</i>	Cerris	Uşak University/1 Eylül Campus/Uşak
<i>Q. petraea</i> subsp. <i>iberica</i>	Quercus	Samsun: 2–3 km on the Ladik road after Havza
<i>Q. infectoria</i> subsp. <i>infectoria</i>	Quercus	Sakarya:Bilecik road to Taraklı from Geyve
<i>Q. infectoria</i> subsp. <i>boissieri</i>	Quercus	Between Dikili-Candarlı/İzmir
<i>Q. pubescens</i>	Quercus	Bayat/Afyon
<i>Q. robur</i>	Quercus	Uşak University/1 Eylül Campus/Uşak
<i>Q. vulcanica</i>	Quercus	Sultan Mountain/Afyonkarahisar
<i>Q. hartwissiana</i>	Quercus	Between Bursa-Yalova/Güney village
<i>Q. frainetto</i>	Quercus	Between Bursa-Yalova/Güney village
<i>Q. macranthera</i> subsp. <i>syspirensis</i>	Quercus	Between Abant-Mudurnu/Bolu
<i>Q. virgiliana</i>	Quercus	Abant lake/Bolu

Table 1. Species, sections and localities of studied species.

Q. ilex, *Q. coccifera*, *Q. aucheri*, *Q. trojana*, *Q. cerris* and *Q. ithaburensis* subsp. *macrolepis*) were germinated in the refrigerator at 4°C [29–31]. It can be stated that the method giving the best results for the germination of acorns was in the refrigerator at 4°C. As a first treatment, germinated roots in the lengths of 2–10 mm for each studied species were pretreated in α -monobromonaphthalene for about 16 h at 4°C. After first treatment, root tip meristems were fixed overnight with 3:1 absolute alcohol-glacial acetic acid mixture. Fixed root tips were stored in 70% alcohol at 4°C until analyses. Prior to staining, hydrolysis was carried out with 1 N HCl solution at 60°C for 13 min and 30 min depending on the species [28–31]. Root tips were then washed with distilled water. Finally, the root tips were stained with freshly prepared Feulgen or Orcein for 2 h. Squashes were made with 2% aceto orcein for Feulgen staining and 45% acetic acid for Orcein staining. The best metaphase plates were frozen in liquid nitrogen to make permanent using Entellan and then photographed 10 × 100. For all studied taxa, at least five plates of metaphase chromosomes were measured on the basis of long arm, short arm and arm ratio. Homologous chromosome pairs were identified and arranged. Chromosome pairs for all taxa were classified according to the nomenclature of Levan et al. [32] and Stebbins [33]. The karyotype asymmetry parameters like intrachromosomal asymmetric index (A_1) and interchromosomal asymmetric index (A_2) were calculated following Zarco [34].

3. Karyotype analyses of Turkish oaks

In the present chapter, karyotype analyses of the 16 oak species completed in previous studies from three sections in Turkey [28–31] and their taxonomic relationships with each other and European oaks were evaluated.

Section *Ilex* containing evergreen oaks in Turkey is represented by three species such as *Q. coccifera*, *Q. ilex* and endemic species *Q. aucheri* which are distributed only in Turkey and in some East Aegean islands of Greece. In this chapter, detailed chromosome measurements of all species from section *Ilex* are stated and compared with each other.

Section *Quercus* has the greatest number of species and widest distribution in the world. Similarly, in Turkey, most species belonging to the genus *Quercus* are in section *Quercus*: *Q. pontica*, *Q. robur*, *Q. hartwissiana*, *Q. macranthera* subsp. *sysprensensis*, *Q. frainetto*, *Q. petraea*, *Q. vulcanica*, *Q. infectoria*, *Q. pubescens* and *Q. virgiliana* [2]. Chromosome analyses of all species from the section *Quercus* in Turkey have been completed except *Q. pontica*. In this chapter, all studied species are evaluated and compared with each other according to chromosomal parameters.

Section *Cerris* includes five species in Turkey: *Q. libani*, *Q. trojana*, *Q. cerris*, *Q. brantii* and *Q. ithaburensis* subsp. *macrolepis* [2]. All species examined except *Q. brantii* are evaluated in detail.

3.1. Comparisons of Turkish oaks on the basis of sections

3.1.1. Karyotype analyses and relations of oak species from section *Ilex*

Acorns as plant materials for each species were obtained from different locations in Turkey (Table 1). Analyzed somatic metaphase plates show that chromosomes of three taxa, namely *Q. coccifera*, *Q. ilex* and *Q. aucheri*, were very small and similar with diploid chromosome number $2n = 24$ (Table 2 and Figure 1).

Species	Somatic chromosome number	Karyotypic description	Length range (μm)	Haploid complement (μm)	A_1	A_2
Section: <i>Ilex</i>						
<i>Q. coccifera</i>	$2n = 24$	24m	(0.80–1.98)	14.61	0.19	0.27
<i>Q. ilex</i>	$2n = 24$	24m	(1.07–2.05)	17.47	0.21	0.20
<i>Q. aucheri</i>	$2n = 24$	24m	(1.12–2.56)	19.76	0.22	0.24
Section: <i>Cerris</i>						
<i>Q. cerris</i>	$2n = 24$	24m	(0.99–2.11)	17.33	0.18	0.23
<i>Q. ithaburensis</i>	$2n = 24$	24m	(0.90–2.06)	15.66	0.17	0.28
<i>Q. libani</i>	$2n = 24$	24m	(0.81–2.18)	16.53	0.19	0.29
<i>Q. trojana</i>	$2n = 24$	14m + 10sm	(2.29–6.65)	49.62	0.28	0.30
Section: <i>Quercus</i>						
<i>Q. petraea</i> subsp. <i>iberica</i>	$2n = 24$	24m	(0.86–1.66)	14.33	0.15	0.19
<i>Q. infectoria</i> subsp. <i>infectoria</i>	$2n = 24$	24m	(0.91–1.96)	16.17		0.22
<i>Q. infectoria</i> subsp. <i>boissieri</i>	$2n = 24$	24m	(1.02–2.35)	17.89	0.21	0.24
<i>Q. pubescens</i>	$2n = 24$	24m	(1.01–2.01)	16.89	0.19	0.21
<i>Q. robur</i>	$2n = 24$	24m	(1.75–3.92)	31.78	0.22	0.22
<i>Q. vulcanica</i>	$2n = 24$	24m	(1.25–3.13)	22.63	0.18	0.28
<i>Q. hartwissiana</i>	$2n = 24$	22m + 2sm	(0.85–1.83)	15.22	0.22	0.23
<i>Q. frainetto</i>	$2n = 24$	22m + 2sm	(0.76–1.80)	14.50	0.25	0.24
<i>Q. macranthera</i> subsp. <i>sypirensis</i>	$2n = 24$	22m + 2sm	(0.88–1.99)	16.04	0.22	0.21
<i>Q. virgiliana</i>	$2n = 24$	24m	(0.85–2.16)	15.84	0.22	0.27

Table 2. Species, somatic chromosome numbers, karyotypic descriptions and other morphometric parameters of previous analyses on the Turkish *Quercus* taxa.

Averages of chromosomal lengths of investigated species ranged from 0.80 to 2.56 μm . Among these, *Q. coccifera* has the smallest chromosome set (0.80–1.98) and haploid complement value with 14.61 μm . On the contrary, the highest chromosome set and haploid complement value were observed in *Q. aucheri* with 1.12–2.56 and 19.76, respectively. Among the studied taxa, the lowest intrachromosomal asymmetry index (A_1) and the highest interchromosomal asymmetry index (A_2) were observed in *Q. coccifera*. However, all investigated species showed very similar intrachromosomal asymmetry index (A_1).

Finally, it can be concluded that the members of *Ilex* section are similar in chromosomal parameters such as small chromosome set and haploid complement, $2n = 24$ chromosomes, all metacentric chromosomes and very close A_1 values. The main reason of the similarity between

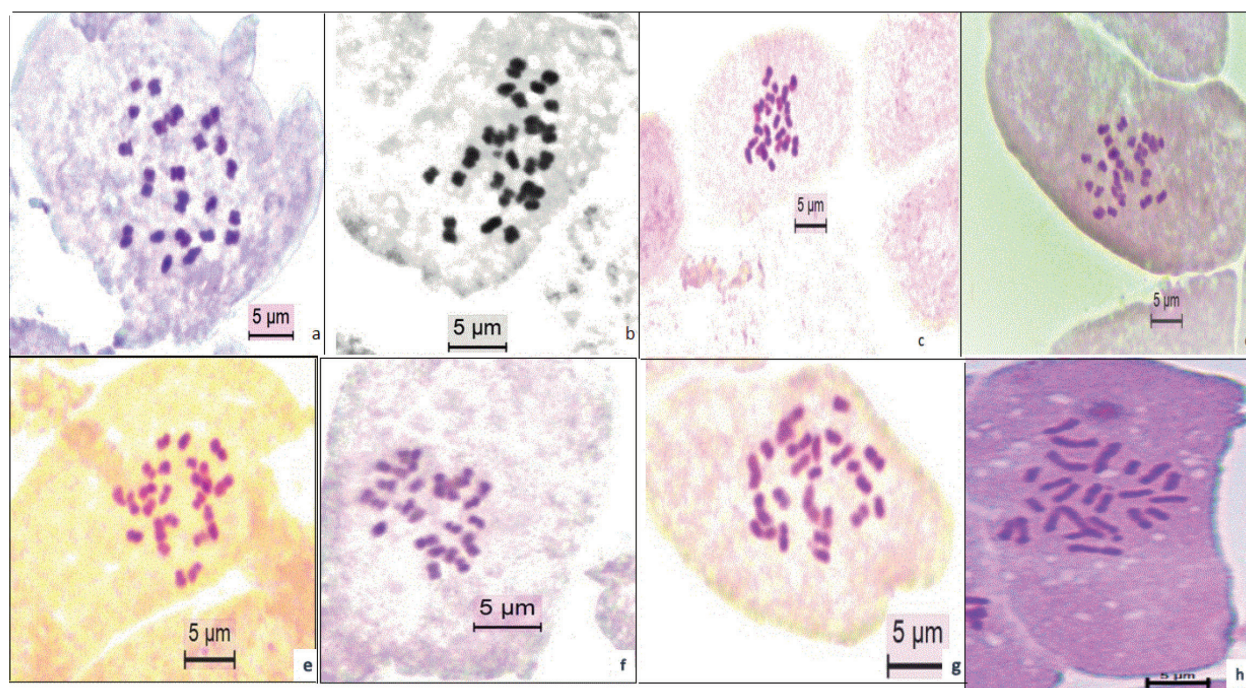


Figure 1. Somatic chromosomes of some Turkish oak species (a) *Q. hartwissiana*, (b) *Q. frainetto*, (c) *Q. macranthera* subsp. *sympirensis*, (d) *Q. virgiliana*, (e) *Q. infectoria* subsp. *boissieri*, (f) *Q. pubescens*, (g) *Q. vulcanica*, and (h) *Q. trojana* [30, 31].

species belonging to same section may be caused by the gene flow and genetic similarity. Besides that, in comparison between studied three taxa, it can be stated that *Q. ilex* and *Q. aucherii* show more similarity than *Q. coccifera* in all chromosomal parameters such as length range, haploid complement, A_1 and A_2 values. Similarly, Yılmaz et al. [35] stated in previous report on DNA comparison of three species belonging to *Ilex* section that *Q. ilex* and *Q. aucherii* were observed as close two separate groups and populations of *Q. coccifera* showed more differences than populations of *Q. ilex* and *Q. aucherii*.

Q. coccifera and *Q. ilex* analyzed before by D'emerico et al. [36, 37] show clearly differences with the presence of submetacentric chromosome pairs, while these two taxa examined in Turkey consist of all metacentric chromosomes and the less parametric values in all chromosomal morphometric measurements in comparison.

When we evaluate geographical distribution of these three taxa in Turkey.

Q. coccifera which is a Mediterranean element has the wide distribution area in comparison with *Q. ilex* and *Q. aucherii*. *Q. coccifera* is distributed along the coastal regions of the Mediterranean Sea, the Aegean Sea, the Marmara Sea and rarely the Black Sea.

Q. ilex which is another species of Mediterranean origin is limited to coastal regions of the Black Sea, the Marmara Sea and the Aegean Sea.

Q. aucherii which is an endemic species has not shown wide distribution and restricted to south-west Anatolia in Turkey.

Similarity observed between *Q. ilex* and *Q. aucherii* could be caused by distribution in more restricted area and more isolated habitats in comparison with *Q. coccifera*. Furthermore, hybridization behavior is mostly observed in oaks, especially in habitats where two or more species overlap [38, 39]. This situation may be reason of variation of *Q. coccifera*.

3.1.2. Karyotype analyses and relations of oak species from section *Cerris*

Plant materials for *Q. cerris*, *Q. ithaburensis* and *Q. trojana* were collected from West Anatolia in Turkey (**Table 1**). On the contrary of these three taxa, *Q. libani* was collected from East Anatolia region of Turkey (**Table 1**).

Detailed karyotype analyses of *Q. cerris*, *Q. ithaburensis*, *Q. libani* and *Q. trojana* from section *Cerris* were examined and determined the chromosome number for each taxon as $2n = 24$ [28, 29, 31]. Chromosome length ranges of *Q. cerris*, *Q. ithaburensis* and *Q. libani* were very similar with (0.99–2.11), (0.90–2.06) and (0.81–2.18), respectively. Similarly, haploid complement values for these three taxa were quite close and compatible values with (17.33), (15.66) and (16.53), respectively. Karyotypic description of all taxa analyzed except *Q. trojana* consists of metacentric chromosomes. Furthermore, chromosomal asymmetry index, A_1 and A_2 , were compatible for these three taxa like other chromosomal parameters. Finally, it can be stated that all taxa analyzed from section *Cerris* showed similar and low parametric values except *Q. trojana* (**Table 2**).

Q. trojana, contrary to other species, showed the highest values in all morphometric parameters such as length range, haploid complement, karyotypic description and A_1 and A_2 values. In other words, among the studied taxa, the biggest variation was determined in *Q. trojana*. Chromosome length range and haploid complement value of this taxon have the highest values with 2.29–6.65 and 49.62 μm , respectively, in comparison with other studied taxa. *Q. trojana* approximatively show equal sum of metacentric and submetacentric chromosomes. Karyotypic description of this taxon consists of 14 metacentric and 10 submetacentric chromosomes (14m + 10sm) and shows similarity with the results provided from D'emerico et al. [36] (8m + 4mSC + 10sm + 2smSC). Chromosomal asymmetry index, A_1 and A_2 , have the highest value among the examined species with 0.28 and 0.30, respectively.

The most important reasons that affect the species number, variation and distribution in different regions are their location, geomorphologic structure and climatic effects. Examined *Q. trojana* is a species belonging to the Uşak/Uşak University-Campus location. The location of *Q. trojana* is quite rich on account of species diversity. This taxon is located as mixed oak populations with *Q. cerris*, *Q. ithaburensis*, *Q. robur*, *Q. infectoria* and *Q. coccifera* in the same location. Hybridization is mostly observed in restricted zones where the habitats of two or more species overlap [38, 39]. Many oak species are located at the same region or even at the same location due to the factors mentioned earlier and this is one of the most important factors that increases the hybridization especially between species belonging to the same section. This situation may be a reason for the high variation in this taxa.

Especially north-west Turkey is one of the regions having the highest species diversity and distribution for oaks. Northwest Turkey contains oak species which range from 13 to 15 [4]. The main reason why this region is rich in oak variation is that it is a transitional zone between Asia and Europe. Anatolia has served as a migration route facilitating the penetration of Asiatic plant elements into Southeast Europe [12]. Turkey has been under the influence of numerous climatic regions and three phytogeographic regions (Euro-Siberian, Irano-Turanian and Mediterranean regions) due to its geomorphologic structure [4]. Another reason of the high species diversity for northwest Turkey is that it is the place where the two different phytogeographical regions (Euro-Siberian and Mediterranean regions) overlap. Locations of *Q. cerris* and *Q. ithaburensis* examined in this study and rich species diversity belong to the same region in northwest Turkey (**Table 1**). The main reason for the similarity between

Q. cerris and *Q. ithaburensis* belonging to same section may be because of the gene flow and genetic similarity. Furthermore, it can be stated that the reason for the similarity in their chromosomal parameters may be probably caused by hybridization between *Q. ithaburensis* and *Q. cerris* which is a very common oak in Turkey.

In comparison with European oaks, all parametric values provided from *Q. cerris* by D'emerico et al. [36] are higher than examined taxon in Turkey except chromosome number ($2n = 24$). These differences can be caused by different geographical regions having different oak populations and environmental conditions.

Q. libani is distributed along and in the eastern part of the Anatolian Diagonal. The eastern part of Anatolian Diagonal is known for its high mountains. Observations by Uslu and Bakış [4] have supported that the number of samplings is decreased in high mountain region of 1100–1200 m. Furthermore, human impact over the vegetation such as heavy grazing and forest destruction may be the other reason for the least diversity in this region. Distribution of species belonging to Section *Cerris* has a relation with Anatolian Diagonal. *Q. cerris*, *Q. ithaburensis* and *Q. trojana* have distribution in the western part of the Diagonal. On the contrary, *Q. libani* and the last species of the section, *Q. brantii*, have distribution in the eastern part of the Diagonal. Cytogenetic study made on *Q. brantii* can be very useful in understanding the relations between *Q. libani* and *Q. brantii*.

3.1.3. Karyotype analyses and relations of oak species from section *Quercus*

Section *Quercus* is characterized by 10 species in Turkey, and detailed karyotype analyses have been completed in all species such as *Q. robur*, *Q. hartwissiana*, *Q. macranthera* subsp. *sypirensis*, *Q. frainetto*, *Q. petraea*, *Q. vulcanica*, *Q. infectoria*, *Q. pubescens* and *Q. virgiliana* except *Q. pontica* [28, 30, 31]. *Q. infectoria* is represented by two subspecies known as *Q. infectoria* subsp. *infectoria* and *Q. infectoria* subsp. *boissieri* in this study. In other words, the section *Quercus* was represented by 10 taxa for cytogenetic comparison.

The chromosome number of 10 taxa analyzed from section *Quercus* support that the basic chromosome number of the genus is $n = 12$ (Table 2 and Figure 1). Majority of the examined taxa in the section *Quercus* have karyotypes with predominance of metacentric chromosomes. However, it is observed that *Q. hartwissiana*, *Q. frainetto* and *Q. macranthera* subsp. *sypirensis* have two submetacentric chromosomes.

Plant samples of *Q. hartwissiana* and *Q. frainetto* were collected from the same location. Among the examined taxa, the karyotypes of *Q. hartwissiana* and *Q. frainetto* show close similarity in terms of chromosome number ($2n = 24$), chromosome morphology ($22m + 2sm$), small chromosome sets (0.85–1.83 and 0.76–1.80) and small haploid complement values with 15.22 and 14.50, respectively.

Q. hartwissiana, *Q. frainetto*, *Q. macranthera* subsp. *sypirensis* that are endemic taxa and *Q. virgiliana* were collected from the north-west region of Turkey having the highest species diversity and distribution due to its geomorphologic structure and climatic effects. The main reason for the similarity between these taxa may be because of the gene flow and genetic similarity due to the mixed oak population in restricted area and hybridization because of weak reproductive barrier between oak species especially belonging to the same section.

It was previously stated by D'emerico et al. [36] that chromosome number of *Q. frainetto* is $2n = 24$ and karyotypic description of this taxon was $14m + 2mSC + 6sm + 2smSC$. Although chromosome number of examined species showed the consistency with $2n = 24$, other parameters such as chromosome morphology, haploid complement, A_1 and A_2 showed less values and differences according to D'emerico et al. [36].

Detailed chromosome measurements of *Q. virgiliana* were previously reported by D'emerico et al. [36]. It was stated by D'emerico et al. [36] that karyotypic description and A_1 value of *Q. virgiliana* were $10m + 4mSC + 8sm + 2smSC$ and 0.35, respectively. While the similarity in terms of chromosome number ($2n = 24$) in comparisons with karyotypes was observed, chromosome morphologies differentiated according to total lengths of chromosomes and karyotypic description. In this study, *Q. virgiliana* has the smaller chromosome set, all metacentric chromosomes and the less parametric value for A_1 .

The less values for many measured parameters were observed in *Q. frainetto* and *Q. virgiliana* in comparison with D'emerico et al. [36]. These differences can be caused by oak species living in different geographical regions, hybridization and gene flow between oak species distributed in this area.

Q. pubescens has a wide distribution range in the northern, western, southern and central parts of Turkey. However, it is known as a species that has not crossed the eastern border of the Anatolian Diagonal. The *Q. pubescens* chromosome number was observed as $2n = 24$ with all metacentric chromosomes. *Q. pubescens* has the small chromosome set, $1.01\text{--}2.01\text{ }\mu\text{m}$, and haploid complement value, $16.89\text{ }\mu\text{m}$. Chromosomal asymmetric index values, A_1 and A_2 , show low value among the studied taxa with 0.19 and 0.21, respectively. Previously, chromosome numbers and morphometric parameters of *Q. pubescens* were reported by D'emerico et al. [37]. The chromosome number of this taxon was reported as $2n = 24$ with 18 metacentric and 6 submetacentric chromosomes by D'emerico et al. [37]. In comparison with D'emerico et al. [37], chromosome number is compatible with $2n = 24$ but chromosome morphologies showed differences with all metacentric chromosomes. However, as a result of a small chromosome set ($1.01\text{--}2.01\text{ }\mu\text{m}$), there were also differences in haploid complement value in comparison with the value reported (27.28). In Turkey, there are many hybrids of *Q. pubescens*, especially with *Q. petraea*, *Q. infectoria* and *Q. macranthera* subsp. *sypirensis* [2]. These differences can be caused by oaks living in different geographical regions and hybridization behaviors seen commonly between oak species living in mixed populations.

Quercus infectoria containing two subspecies such as *Q. infectoria* subsp. *infectoria* and *Q. infectoria* subsp. *boissieri* in Turkey has the widest distribution area, especially in the west, south and south-east regions of Turkey. *Q. infectoria* subsp. *infectoria* has a more limited distribution area compared to *Q. infectoria* subsp. *boissieri* in Turkey. In the comparison with these two subspecies, very similar results are observed with chromosome number and all metacentric chromosomes. Furthermore, it was observed that the chromosome sets of *Q. infectoria* subsp. *boissieri* and *Q. infectoria* subsp. *infectoria* were quite small and similar, $1.02\text{--}2.35$ and $0.91\text{--}1.96$, respectively.

Finally, it can be stated that all taxa analyzed belonging to section *Quercus* in Turkey are compatible with each other according to many chromosomal parameters such as length range, haploid complement, A_1 and A_2 value except *Q. vulcanica* and *Q. robur* (Table 2).

Q. robur together with *Q. vulcanica* show differences in chromosome lengths and haploid complement compared to the other examined taxa (**Table 2**). The haploid complement value of *Q. robur* is the highest with 31.78 and chromosome lengths range from 1.75 to 3.92 μm . The total chromosome number and karyotypic description for this taxon are compatible with other taxa. *Q. robur* is a species belonging to the Uşak/Uşak University-Campus location (**Table 1**). This taxon is located as mixed oak populations with *Q. cerris*, *Q. ithaburensis*, *Q. trojana* and *Q. coccifera* in the same location. Hybridization in restricted zones, where there is quite rich species diversity, was frequently observed. The reason for high variation may be because of its distribution with other taxa.

Q. vulcanica is an endemic taxon distributed in restricted areas such as Isparta/Eğirdir and Afyon/Sultan Mountains in Turkey. The haploid complement has the second highest value with 22.63 μm after *Q. robur* and chromosomal lengths ranged from 1.25 to 3.13 μm . Among the studied taxa, *Q. vulcanica* has the second lowest A_1 value (0.18) and the highest A_2 value (0.28) (**Table 2**). In other words, it can be stated that the endemic species *Q. vulcanica* exhibited high variation among the studied taxa in chromosome lengths, haploid complement value and asymmetric index A_1 and A_2 .

This species is naturally distributed from 1200 to 2000 m altitude in restricted areas such as Kutahya-Turkmen Mountains, Konya-Sultan Mountains and Isparta-Eğirdir (Yukari Gokdere village). This high variation could be caused by the geographical distribution in this restricted area and more isolated habitats when compared with other oak species. *Quercus vulcanica* has been faced with the threat of extinction because of over exploitation for wooden home appliances, veneer and furniture. To protect this valuable resource, 1300.5 ha area near the Eğirdir Yukari Gökdere village was declared as a Nature Reserve Area for this endemic species. Eğirdir-Yukarıgökdere location is accepted as the best location of the species.

High variation observed in *Q. vulcanica* could be caused by distribution in restricted area and more isolated habitats in comparison with other oak species.

3.2. Situations that increase the variation and taxonomic problems among oak species in Turkey

- Hybridization and introgression are an important process in evolution, diversification and speciation of many plants [40, 41]. Oaks have long been considered a group with high frequency of widespread hybridization. Many morphological studies on *Quercus* species show the presence of hybridization with hybrid individuals that exhibit intermediate morphological features between parent taxa and support interspecific gene flow [14]. Furthermore, recently, many molecular techniques have been used to understand the relations between *Quercus* taxa and the determining of hybridization. Especially in Europe, species that dominate forests such as *Q. petraea*, *Q. robur* and *Q. pubescens* have been studied extensively using different molecular techniques [42–46]. However, oak taxonomy is still problematic and under debate often due to insufficient diagnostic morphological characters [24–25], weak reproductive barriers between species, wind-pollination [14, 22–25] and the lack of investigations for each taxon such as ecological, historical and genetic descriptors [25]. All these factors that is stated increase the taxonomic problems and make problematic the genus. In addition to these factors, identification of oak species

can be difficult due to species boundaries being fuzzy as a result of interspecific gene flow and variation within species. The most important reason for hybridization in oaks is the presence of hybrid zones that are frequently observed when species separated by weak reproductive barriers come into geographical contact [47]. In other words, sympatry creates high opportunity for hybridization in oaks. Nevertheless, the climate selection for species that create the hybrid zones is very important because they determine the extent to which hybrids persist in a given locality. In Turkey, these factors that increase the hybridization and gene flow between oak species are frequently observed and cause taxonomic problems.

- Another situation affecting variation, hybridization, speciation and taxonomic problems in oaks is Anatolian Diagonal which separates Turkey into West and East (**Figure 2**). It can be stated that distributions of some *Quercus* species at the subspecies level is related to phytogeography and Anatolian Diagonal [4, 12, 48]. For example, while *Q. petraea* subsp. *pinatiloba* has a distribution area in the eastern part of the Anatolian Diagonal, the other two subspecies of *Q. petraea*, *Q. petraea* subsp. *iberica* and *Q. petraea* subsp. *Petraea*, show distribution in the western part of the Diagonal. Similarly, *Q. robur* subsp. *robur* shows distribution in the western part of the Diagonal whereas *Q. robur* subsp. *pedunculiflora* has distribution in the eastern part of Diagonal. This separation at the subspecies level shows the importance of the Anatolian Diagonal in the evolution, diversification and speciation of many plants in Turkey. Furthermore, distributions of some oak species have considerable relation with Anatolian Diagonal in species level. Especially, distributions of species belonging to the Section *Cerris* show high relation with Anatolian Diagonal. While *Quercus brantii* and *Q. libani* have a distribution area in the eastern part of the Diagonal, *Q. ithaburensis* subsp. *macrolepis*, *Q. cerris* and *Q. trojana* show distribution in the west part of the Diagonal. However, some taxa from Section *Quercus* show distribution tendency with respect to the Diagonal. This is a case showing the influence on some oak species distribution of Anatolian Diagonal and as a result of this, gene flow is affected in intraspecific and interspecific level.
- Geomorphological structure and location of Turkey have an important effect on oak distribution and diversity. One of the main reasons why Turkey is rich in oak variation and distribution is that it is a transitional zone between Asia and Europe [4, 31]. Anatolia has served as a migration route facilitating the penetration of Asiatic plant elements into Southeast Europe [12]. Furthermore, Turkey has been under the influence of three different phytogeographic regions known as Euro-Siberian, Irano-Turanian and Mediterranean regions due to its geomorphologic structure [4, 31]. These phytogeographic regions that are caused by geomorphologic structure create different climatic regions that have an effect on species distribution, variation and the extent of hybrid zones.
- The changes that started at the beginning of the neotectonic period in the middle of the Miocene [49] affected the paleogeography of Turkey and have changed the geomorphology of Anatolia. Furthermore, the formation of today's topography together with climatic changes affecting the distribution of plant flora in Turkey has occurred in the Quaternary, especially at the beginning of the Holocene. In Turkey, the main reason for oak richness, variation and distribution that is creating the hybrid zones is the geomorphology and climatic structure affected by geologic history.

- Another important reason that makes it difficult to understand the relationships among the oaks and increases the taxonomic problems in Turkey is the lack of adequate conservation programs for the use of oak trees. Oak trees have high economic value and have been used for many purposes, such as ornaments, wood, fuel wood, nonwood products, in the timber industry, and so on. Beside this, large parts of the forests in Turkey are being degraded by villagers due to excessive cutting and grazing. However, there is not enough protection strategies and management plans for oak species except one national park for *Quercus vulcanica*.
- The lack of comprehensive studies on the genus in Turkey is the other situation that is causing taxonomic problems and difficulties in understanding the relations between oak species.
- All studies on cytogenetic of Turkish oaks show that average chromosome lengths of oak species analyzed are below 2 μm . The effects on the chromosome lengths of chemicals used to obtain metaphase chromosomes during cytological studies considerably complicate cytological comparison in species with very small chromosomes such as oaks.

3.3. General comparisons between Turkish and European oaks on the basis of chromosome structures

Chromosome analyses of 16 *Quercus* species in Turkey have been completed [28–31] and confirmed that the chromosome number of the genus is consistent with $2n = 24$ (Table 2). These results are in agreement with previous researches based on chromosome number of the *Quercus* species from different parts of the world [36, 37, 50–52]. However, some exceptions on different chromosome number are reported, contrary to results provided from studies on Turkish and European oaks [51, 53, 54]. Furthermore, occasionally ploidy variation may

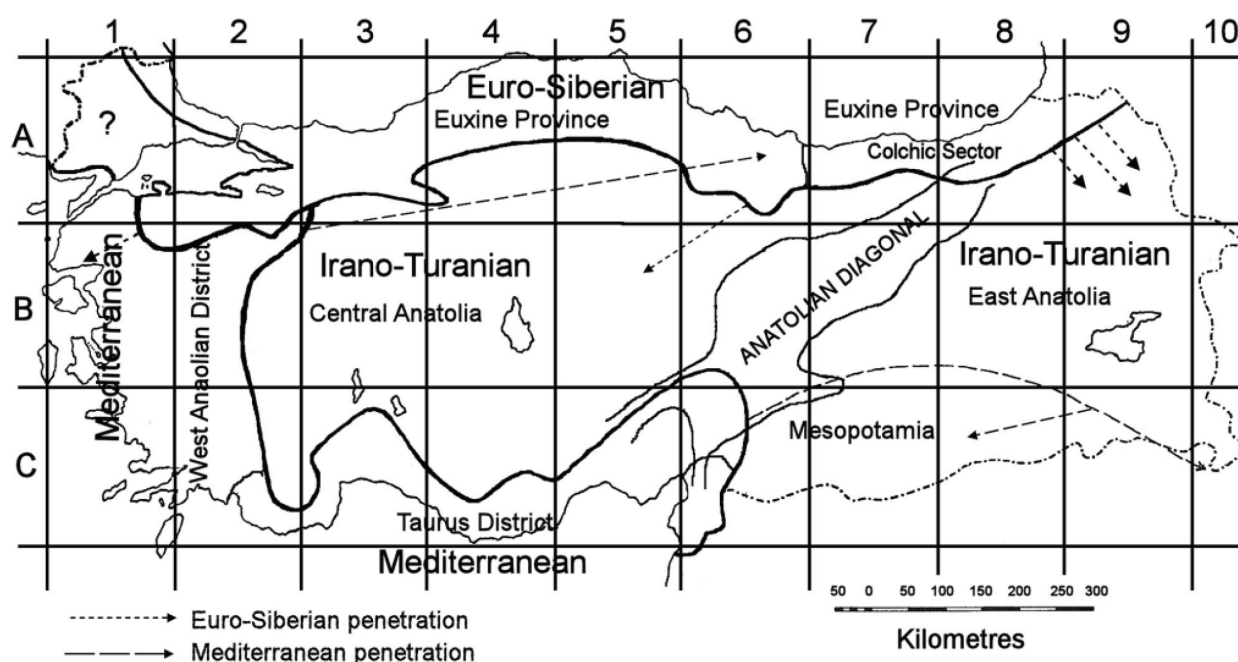


Figure 2. Anatolian Diagonal and phytogeographical regions of Turkey after Davis [4, 12].

be observed in individual trees such as triploid samples of *Q. robur* with $2n = 3x = 36$ [55]. Similarly, the presence of endopolyploid cells ($2n = 4x = 48$) together with diploid cells is reported in an individual tree of *Q. frainetto* [36].

If the chromosome lengths in the evaluation of European and Turkey oaks are taken into consideration, it can be said that European oak species clearly have a bigger chromosome set than Turkish oak species. In addition to haploid complements and length ranges of species examined, it is observed that other morphometric parameters of karyotypes such as A_1 and A_2 show bigger values in opposition to Turkish oaks. Also, while the *Quercus* taxa analyzed in Turkey have predominant karyotypes with metacentric chromosomes, karyotypes having approximately equal sum of metacentric and submetacentric chromosomes are observed in European oaks. Consequently, higher values for many chromosomal parameters in European oaks than in Turkish oaks were observed. This situation may be caused by gene flow between different oak species because of weak reproductive barriers in different geographical regions.

Mixed populations that are composed of different oak species in different geographical regions and gene flow between these may be the cause of these differences between Turkish and European oaks. Also, differences of environmental and climatic factors affecting species variation and distribution in different geographical regions may be another important reason of this situation.

4. Conclusion

This study contributes to understanding the relations on the basis of sections among 16 Turkish oak species belonging to three sections and contributes to the comparisons between Turkish oaks and European oaks. Additionally, results obtained in this study provide useful knowledge on the cytogenetic of the genus *Quercus*. However, lack of adequate conservation programs for the use of oak trees in Turkey is the main reason that makes it difficult to understand the relationships among the oaks and increases the taxonomic problems. For example, endemic species *Q. vulcanica* has been faced with the threat of extinction because of overexploitation for wooden home appliances, veneer and furniture. To protect this valuable resource, 1300.5 ha near the Eğirdir Yukari Gokdere village was declared as a Nature Reserve Area for *Q. vulcanica*. Nevertheless, there is not enough protection for oak species except Nature Reserve Area for this endemic species. Furthermore, conservation of oak biodiversity should be considered not only in protected areas but also in managed forests as well.

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