GENETIC DIVERSITY PATTERN IN ***POPULUS NIGRA*** POPULATIONS OF TWO MAJOR RIVER SYSTEMS IN TURKEY

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ABSTRACT

GENETIC DIVERSITY PATTERN IN *Populus nigra* POPULATIONS OF TWO MAJOR RIVER SYSTEMS IN TURKEY

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European black poplar (*Populus nigra* L.) is an important tree species in terms of social, economic and ecological interest in Turkey. Although, poplar plantations in large areas meet the needs of the economy, the natural genetic resources of the species have been highly degraded due to anthropogenic effects and natural biological events. To assess genetic diversity structure of natural populations, 124 naturally distributed European black poplar trees from two major rivers (Kızılırmak and Göksu) in Turkey were sampled and screened using 20 nuclear microsatellite DNA loci. To detect the possibility of natural hybridization, 10 reference *Populus deltoides* L. trees were also studied with six microsatellite loci. The results from hybrid detection analysis indicated that the majority of individuals from two river systems was found as pure European black poplar. Four trees in Göksu and one tree in Kızılırmak river populations were determined as F_2 hybrids according to with high probability calculations with diagnostic and informative alleles. A reduced level of expected genetic diversity (Mean He= 0.55) and the excess of heterozygosity (Mean Ho= 0.80) were detected in the studied populations. The results suggested that all populations experienced a recent bottleneck event which reduced allelic diversity caused to excess of heterozygosity. Kızılırmak river populations were not differentiated significantly from Göksu river population (F_{ST} = 0.06). There was little differentiation among four populations of Kızılrımak river (F_{ST} = 0.03) due to high level of vegetative material

(polen, seed and branches) circulation within the river system. Human impact, biological events such as, bottleneck and natural hybridization events caused the eroded genetic diversity and extensive genetic material circulation from *P.deltoides* polluted local genetic resources. To prevent further genetic degradation and to protect genetic resources of the species, efficient conservation and breeding strategies should be implemented as soon as possible. This study provides the necessary genetic information to develop such strategies.

Keywords: European Black Poplar, Hybrid, Bottleneck, Genetic diversity, Genetic Erosion, Genetic Pollution

ÖZ

TÜRKİYE DEKİ İKİ TEMEL NEHİR SİSTEMİNDEKİ *Populus nigra* POPULASYONLARININ GENETİK ÇEŞİTLİLİK YAPISI

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Avrupa karakavağı (*Populus nigra* L.), Türkiye'de sosyal, ekonomik ve ekolojik açıdan önemli bir ağaç türüdür. Her ne kadar, büyük alanlarda yapılan kavak plantasyonları ekonominin ihtiyaçlarını karşılasa da, türün doğal genetik kaynakları antropojenik etkiler ve doğal biyolojik olaylar nedeniyle büyük ölçüde zarar görmüştür. Mevcut popülasyonların genetik çeşitlilik yapısını değerlendirmek için, Türkiye'deki iki büyük nehirde (Kızılırmak ve Göksu) doğal olarak yayılış gösteren 124 Avrupa kara kavak ağacı örneklenip, 20 nükleer mikrosatellite DNA lokusu kullanılarak taranmıştır. Doğal melezleme olasılığını saptamak için, 10 referans *Populus deltoides* L. ağacı altı mikrosatellit lokusu ile çalışılmıştır. Melez olasılığı analiz sonuçları, iki nehir sisteminden örneklenen bireylerin çoğunluğunun saf Avrupa kara kavağı olduğunu göstermiştir. Göksu nehrinden dört ağaç ve Kızılırmak nehri popülasyonlarından bir ağaç tanısal ve bilgilendirici alellerle yapılan yüksek olasılık hesaplarına göre F_2 melezi olarak belirlenmiştir. Çalışılan popülasyonlarda azalan beklenen genetik çeşitlilik (Ortalama He = 0.55) ve heterozigotluk fazlalığı tespit edilmiştir (Ortalama Ho = 0.80). Tüm popülasyonların, düşük miktarda allelik çeşitliliğe ve heterozigot fazlalığına neden olan yakın bir darboğaz olayından geçtiği görülmektedir. Kızılırmak nehri popülasyonları, Göksu nehri popülasyonundan önemli oranda farklılaşmamıştır (F_{ST}=0.06). Kızılırmak nehrinin dört popülasyonu arasında nehir içindeki vejetative materyal (Polen, tohum, çelik) transferinin yüksek

olmasından dolayı çok az genetik farklılaşma görülmektedir (F_{ST}=0.03). İnsan etkisi, darboğaz ve doğal melezleme olayları gibi biyolojik etmenlerde azalmış genetik çeşitliliğe ve yoğun genetik materyal transferi ise lokal gen kaynaklarının kirlenmesine neden olmuştur. Daha fazla genetik bozulmayı önlemek ve türün genetik kaynaklarını korumak için etkili koruma ve ıslah stratejilerinin biran evvel geliştirilmesi gerekmektedir. Bu çalışma bu tür stratejilerin geliştirilmesi için gerekli genetik bilgiyi sağlamaktadır.

Anahtar Kelimeler: Avrupa Kara Kavağı, Melez, Dar Boğaz, Genetik Çeşitlilik, Genetik Erozyon, Genetik Kirlilik

To my family…

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ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
CTAB	Cetyl Trimethyl Ammonium Bromide
DAPC	Discriminant Analysis of Principal Components
DNA	Deoxyribonucleic Acid
dNTP	Deoxy ribonucleotide triphosphate
EDTA	Ethylenediaminetetraaceticacid disodium salt
GDA	Genetic Data Analysis
He	Expected Heterozygosity
Ho	Observed Heterozygosity
HWE	Hardy–Weinberg Equilibrium
MCMC	Markov Chain Monte Carlo
MSN	Minimum Spanning Network Analysis
Ne	Effective number of alleles per locus
PCR	Polymerase Chain Reaction
SSR	Simple Sequence Repeats
TBE	Tris-Borate-EDTA
TE	Tris EDTA
TÜBİTAK	The Scientific and Technological Research Council of Turkey
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean

CHAPTER 1

INTRODUCTION

Humans have become the dominating species with in the last milenia. Large-scale habitat destruction and fragmentation by humans have wiped out various animal and plant populations. Human impact on the environment is considered as one of the main causes of recent extinction events (Haddad et al. 2015). Biological diversity has decreased accordingly, as the loss of species diversity was no longer balanced by speciation. Genetic diversity is essential for the long-term survival of endangered species and for all species to evolve in their changing environment. Species that lack adequate genetic variation are at greater risk of extinction. Because of demographic and environmental stochasticity, habitat deterioration in the remaining habitat fragments and loss of genetic variation and inbreeding in small and isolated populations, many species are under the being extinct (Hanski 2011).

European black poplar (*Populus nigra* L.) is a native European tree species which sustain viability, stability and specificity of ecosystems along river ecosystems. Once widespread, European black poplar is now considered extremely threatened due to mainly anthropogenic effects and biological processes (bottleneck, hybridization). In terms of conservation, species deserves special attention to maintain its natural populations with a broad genetic diversity.

1.1. Genus *Populus*

The *Populus* and *Salix* form two separate genus in the Salicaceae family based on the phylogenetic structure (Leskinen and Alstrm-Rapaport 1999; Cervera et al. 2005;

Hamzeh et al. 2006). Based on relative morphological similarity and crossability (Eckenwalder 1996), the genus *Populus* consists of 29 species placed into six sections (Section Abaso Eckenwalder, Section Turanga Bunge, Section Leucoides Spach, Section Aigeiros Duby, Section Tacamahaca Spach, and Section Populus Duby). Extensive phenotypic variation observed within broadly distributed *Populus* species and existence of many hybrids result in the difficulties in taxonomy of the genus. It is believed that extensive natural hybridization within and among sections played an important role in the evolution of extant species of *Populus* (Eckenwalder 1996).

The species of *Populus* are known as most commercially exploited groups of forest trees for industrial wood and are suitable for clonal forestry (Hamzeh et al. 2006). It is known to be the most abundant woody plant genus distributed in the temperate and subtropical zones of the northern hemisphere from 20° to 70° N latitude and many other parts of the world (Taylor 2002). Due to availability of physical and molecular genetic maps of the member of genus, their potential to grow fast, easy production and small genome size, poplar species are used extensively in physiology, biochemistry, agronomy, biotechnology, genetic engineering and genomics studies of tree species around the world (Siler et al. 2014).

1.2. *Populus nigra* L.

1.2.1. Taxonomy of *Populus nigra* L.

Populus nigra L. had been probably evolved in fluvial corridors at least 58 million years ago (Eckenwalder 1996). According to the results of Cottrell et al. (2005), during the last ice age, populations of European black poplar remained in southern Spain, southern Italy and the Balkans. Recolonization of the species occurred along the North and Central European fluvial corridors during the Holocene. European black poplar is characterized with diploid chromosome number of 2n = 38. Based on nuclear DNA

and morphology, the species is clearly placed in the section Aigeiros (Eckenwalder 1996; Hamzeh et al. 2006; Cervera et al. 2005).

1.2.2. Ecology and Distribution of *Populus nigra* L.

European black poplar is one of the most representative tree species of riparian ecosystems which are known as among the most diverse and important ecosystems in Europe (Vietto et al. 2008). As center of biodiversity, European black poplar populations provide habitats of many threatened and common animals, such as insects, bird and small mammals (Vanden Broeck 2003; Rathmacher et al. 2010). The species is naturally distributed mostly along rivers and streams with a great diversity of population types, from individual trees to pure or mixed tree stands (Siler et al. 2014). Populations of the species contribute to the natural control of flooding and water quality. European black poplars, one of the fastest growing temperate trees, forms metapopulations (Zsuffa 1974) because of its heliophily and plasticity characteristics (Cagelli and Lefevre 1995). It is a fast growing and opportunistic tree species with a good tolerance to high water level. After disturbances like floods and fire, the species recolonizes affected sites (Romme et al. 1997; Rood et al. 2007).

Because of its widespread and ancient cultivation, the exact limits of the original natural range of the species have become unclear. Naturally grown European black poplar stands mainly exist along rivers and streams and their distribution is sometimes very scattered and extended (Guilloy-Froget et al. 2002). The species has been distributed from UK in the north west to Portugal in the south west to Turkey in southeast and Latvia in the northeast. In addition, its distribution reaches as far as eastern China taking in much of the Middle East.

1.2.3. Biology of *Populus nigra* L.

Populus nigra is a deciduous tree species with single-stemmed rounded outline. It can grow up to 30 m in height and 1.5 m in diameter. The species is dioecious, which male and female flowers are clustered in pendulous catkins on different male and female trees. The female catkins carry seeds covered by cottony hairs. The cottony structure promotes the dispersal of seeds over long distances with help of wind and increases rates of migration and gene flow. Male and female trees flower in early spring for 1 to 2 weeks. European black poplar can reach at reproductive maturity within 10–15 years under favorable conditions in natural populations (Stanton and Villar 1996). In addition to its dioecious nature, it has an efficient dissemination of pollens and seeds via wind and water (Lexer et al. 2005; Rathmacher et al. 2010). It can also reproduce by vegetative propagation naturally (root suckers of mature trees) and human assistance (Arens et al. 1998; Barsoum et al. 2004). Fallen trees, broken roots, branches and abscised short shoots carried by rivers and human can root very easily when vegetative materials partly planted in suitable environment. Because of the vegetative propagation, genotypes of the species can persist on sites for long periods beyond the longevity of single trees (Legionnet et al. 1997; Arens et al. 1998; Jelic et al. 2015).

1.2.4. Scientific and Economic Considerations on *Populus nigra* L.

The European black poplar is known to be a keystone species and center of biodiversity in the riparian ecosystems throughout Europe (Vanden Broeck 2003). It has an important role in the initial phase of the development of riparian forests and in the natural control of flooding and water quality. As a result of easy vegetative propagation, rapid juvenile growth, high biomass yields and high plasticity in response to environmental changes, the species have become one of the most extensively cultivated trees in temperate regions (Jelic et al. 2015). Poplars have been used as windbreaks, shelterbelts and used for reforestation of lowlands in temperate regions

of the world (Confalonieri et al. 2003). In addition to their potential to be used for pollution, mitigation and microclimate regulation, natural and cultivated poplar plantations have played an important role for erosion control near streams and rivers (De Rigo et al. 2016).

The European black poplar wood is used extensively as a feedstock for pulp and paper production because of low lignin and high carbohydrate components. Because of its low density, ease of flaking, low cost and availability, the wood has been used for production of furniture, particleboard, flake and strand-based composite boards. In addition to its economic value, European black poplar is used as model organism for tree molecular biology and biotechnology studies and used as a parent pool in poplar breeding programs in many parts of the world (Frison et al. 1995).

1.2.5. Threats to Genetic Resources

Although, *Populus nigra* is becoming increasingly threatened by human activity and genetic pollution with cultivated trees and hybrids, in the IUCN red list, the species is classed as *Data Deficient* due to inadequate information on threat, population size and decline (Harvey-Brown 2017). It is recommended that related data should be collected to determine the conservation category of the species. Because of habitat fragmentation and loss, a continuing decline in both population size and in number of mature individuals has been found around the world. Although natural populations in Spain, Portugal and Albania are thought to be stable, the situation of the European black poplar in other Western Europe countries is considered close to extinction (De Rigo et al. 2016). The species is considered as one of the rarest native tree species in Belgium (Vanden Broeck et al. 2005) and considered rare in Switzerland with at least 900 individuals remaining (Csencsics et al. 2009). There is no information for population structure and threats to species for Asian and African populations due to a

lack of research. Recently, Çiftçi et al. (2017) reported that the condition of European black poplar in Turkey have been threatened by similar biological and human actions.

One important threat to natural populations of the species is the alteration of riparian ecosystems by human disturbance, such as drainage of rivers, management of riverbanks, agricultural land acquisition and urbanization. Constructions of dams, dykes, roads, highways, bridges have altered the regeneration capacity of the species. In addition, autochthonous European black poplar resources have been over-exploited for wood industry and daily needs of rural environment for years. *P. nigra* can make hybrids with *P. deltoides*, *P. trichocarpa*, *P. maximowiczii* and *P. laurifolia* naturally and artificially by breeders (Cottrell et al. 2005). Gene exchanges from pure exotic species, their hybrids or cultivated trees possessing a very narrow genetic base could lower the effective population size and reduce the overall fitness of seedlings of native European black poplar (Cagelli and Lefevre 1995; Rhymer and Simberloff 2016). It is known that the establishment of poplar hybrids occurs spontaneously in addition to human activity. The cultivated hybrid trees can produce viable seeds and propagate sexually along several river systems in Europe (Toplu 2005; Smulders et al. 2008b; Heinze 2008). Native European black poplar populations have been replaced by widespread cultivated hybrid poplar plantations because cultivated *P.* × *canadensis* hybrids have fast growth, good wood quality, frost resistance and easy propagation by cuttings. Overexploitation, habitat fragmentation, reduction and competition for new habitats with seedlings of cultivated or naturally growing hybrid poplars are considered as serious threats to remaining natural poplar populations.

1.2.6. Conservation of the Genetic Resources of *Populus nigra* L.

1.2.6.1. Conservation Strategies

Although, the species has most recently been classified as "Data Deficient" in the European Red List (Harvey-Brown 2017), its populations have been considered "Critically Endangered" in Hungary (Denes 2001) and "Endangered" in Czechia (Holub and Prochazka 2000). Restoring the habitat of the species habitats and maintaining the genetic diversity, several *in situ* and *ex situ* conservation programs have been implemented within Europe. Jelic et al. (2015) listed the main questions in conservation of European black poplar as

• How to identify species?

• What are the relevant life-history traits and the reproductive biology of the species?

• Which populations and/or individuals to conserve?

To accurately determine the conservation status of the *Populus nigra*, more information about population size, demographic status and genetic structure of the species should be determined to answer these listed questions.

For maintaining the genetic variability of the species, *in situ* genetic reserves have to be set up for conserving genetic resources in the wild by using planting material originating from *in situ* stands or *ex situ* collections. Trees from natural populations should be selected for the establishment of *exsitu* germplasm collections, and development of breeding programs with *exsitu* collections. To perform an effective conservation program, a clear taxonomic treatment is needed, based on natural hybridization and backcrosses of hybrids with European black poplar. Basic knowledge on the biology of poplars should be considered when planning and conducting conservation practices. Several European countries (Turkey, Austria, Belgium, Great Britain, France, Germany, Italy, Netherlands, Spain and Hungary)

have independently set up clone banks with the perspective of *ex situ* conservation approaches to conserve the genetic diversity of this threatened species.

1.2.6.2. Evaluation of Genetic Diversity and Genetic Improvement

For an effective protection and use of the remaining genetic resources of European black poplar, molecular genetic studies are regarded as key. As conservation targets, the number and distribution of alleles and genotypes within and between populations should be determined to identify threatened populations and conservation of individual genotypes with favorable characteristics. There are a plenty of genetic studies dealing with determination of the remaining genetic diversity and characterize the genetic structure of European black poplar populations in Europe by using biochemical and nuclear markers (Rajora 1989; D'Ovidio et al. 1990; Storme et al. 2004; Cortan et al. 2016)

Isozyme and random amplified polymorphic DNA (RAPD) markers have been successfully used for identification of clones and determination of the interrelationships among various species (Rajora 1989; Castiglione et al. 1993; Janssen 1997). AFLP markers are considered appropriate for hybrid detection because of the large number of loci sampled across the whole genome (Arens et al. 1998). Several molecular techniques such as ribosomal DNA (D'Ovidio et al. 1990, 1991; Faivre-Rampan et al. 1992a, b), mitochondrial DNA (Barrett et al. 1993), chloroplast DNA (Smith and Sytsma 1990) and RFLP of genomic DNA (Keim et al. 1989) have been applied for identification and differentiation of poplar species since the late 1980s.

Microsatellites or simple sequence repeats (SSRs) are DNA region of a variable number of tandem repeats with a core repeat of two to six base pairs. These co-dominant markers can be amplified by using the unique flanking sequences obtained by sequencing genomic DNA (Smulders et al. 1997). Microsatellite markers are accepted to be keystone in population genetic studies as a result of high level of polymorphism, reproducibility, automated and high throughput genotyping (Parida et al. 2009). These markers have been used extensively for population genetic and conservation studies of poplar species (Storme et al. 2004; Smulders et al. 2008a; Liesebach et al. 2010; DeWoody et al. 2015; Jiang et al. 2015; Jelic et al. 2015, Lewandowski 2016, Cortan et al. 2016; Çiftçi et al. 2017). Clone, cultivar and hybrid identifications in poplars can be detected by help of some microsatellite loci containing species-specific alleles (Fossati et al. 2003). These markers also have been used effectively for genome mapping studies (Gaudet et al. 2008). Currently, genome-wide SNP discovery and SNP related gene expression studies are progressing for population genetic studies and identification of QTLs through natural-population based genetic association studies in *P. nigra* (Chu et al. 2014; Faivre-Rampant et al. 2016).

1.3. Importance of *Populus nigra* L. Populations inTurkey

Turkey is a rich country in terms of wide forest ecosystem diversity due to the variable climatic and topographic features. Many forest tree species in different ecosystems are found naturally throughout the country. Natural poplar stands in different forest regions of Turkey cover about 260,681 ha area (142,322 ha pure stand, 118,359 ha mixed stand with other forest trees). *Populus tremula* is the main species of the natural poplar stands. Some natural stands of *Populus euphratica* occur in South and South-east Anatolia. *Populus alba* occurs naturally at different sites of Turkey as small groups (Velioğlu and Akgül 2016).

Populus nigra L. is one of the main tree species in central and eastern part of Turkey and naturally distributed mostly along rivers and streams of country with a great diversity of population types, from individual to pure or mixed stands (Velioğlu and Akgül 2016). The species is one of the many cultivated plants brought together by nomadic Turks during migration from Central Asia, the homeland of Turkish people, to Anatolia at the 12th century (Yaltırık 1973). In addition, rural people carried poplar and willow trees with them and planted whenever they moved in new settlement areas. Anatolian villagers have made poplar breeding in a primitive manner from ancient times to today by using traditional methods to meet the need of wood used as the building material for their homes, vehicles and energy resources. Varieties and cultivars of poplar in Anatolia have been developed as a result of natural and artificial selection for centuries. *Populus nigra* var. *italica*, *Populus usbekistanica* var. *afghanica* and *Populus nigra* var. *caudina* are the most widely cultivated varieties of the species in different regions of Turkey (Toplu 2005).

European black poplar is a highly valuable species in terms of social, economic and ecological interest. European black poplar constitutes an important place in Anatolian culture and history. There are many villages, towns and districts named after "Kavak" meaning poplar in Turkish. Planting poplar tree has been a tradition and sacred work in Anatolian people's daily life (Kahraman et al. 2014; Yaltırık 1973) that contributed greatly to dispersal of clonal materials. It has considerable contributions to rural and national economies of Turkey. It is the most widely studied and commercialized forest tree in Europe. Turkey has approximately 145000 hectares of poplar plantations. 68000 ha of these plantations are composed of various European black poplar clones, the remaining are covered by *P x canadensis* hybrids and *Populus deltoides*. In Turkey, annually 1.4 million m^3 poplar wood is produced by using *P. nigra* clones (Ministry of Forest and Water Affairs 2012). European black poplar is being used extensively in wood industry for obtaining furniture, particleboard, plywood and round-wood for rural construction has considerable contributions to rural and national

economies of Turkey (Vanden Broeck 2004; Toplu 2005). To meet the need for high amount of wood raw materials, row plantations have been established on river and stream banks and edges of farm fields in forest poor areas with commercially registered European black poplar clones (Gazi (TR- 56/52), Anadolu (TR-56/75), Behicbey (TR62/154), Geyve (TR-67/1), and Kocabey (TR-77/10)) (Velioğlu and Akgül 2016). To increase the wood quantity and to improve wood quality, hybridization studies between *P. deltoides* which is not naturally distributed in Turkey, and native *P. nigra* have been undertaken in Turkey since 1965 (Toplu 2005). In addition to registered European black poplar clones, *P. deltoides* and *P. x canadensis* hybrid clones have been used substantially for poplar row plantations in the coastal regions of Turkey (Tunçtaner 1991).

Eventhough, plantations meet the needs of the economy, they can affect negatively the genetic integrity of native poplar trees by reducing their distribution range. Hybridization and interbreeding between cultivated (*P. nigra, P. deltoides* and *P.x canadensis*) and natural trees cause to genetic "swamping" of native trees. As a result of disruption of natural distribution areas due to urban extension, construction of hydroelectrical power plants and irrigation dams and introgression of exotic poplar genes into natural populations, European black poplar has been one of the most threatened tree species in Turkey and Europe (Vanden Broeck et al. 2004; Ciftci et al. 2017).

Conservation and restoration of natural ecosystems of European black poplar are being given high priority in many European countries (Lefevre et al. 2001; Cooper 2006). The Poplar and Fast Growing Forest Trees Research Institute in Izmit have performed conservation studies since 1962. A wider conservation program was started in 1990 throughout Turkey. A national project named as “Genetic characterization of Turkish black poplar genetic resources and development of molecular black poplar breeding

program" was completed at 2013 with collaboration of The Poplar and Fast Growing Forest Trees Research Institute and Middle East Technical University (Kaya et al. 2014). Genetic diversity of the important and major genetic resources of the species collected from six regions of Turkey archived in a clone bank in Ankara were determined and also compared with two newly found natural populations by Çiftçi et al. (2017). According to obtained results, natural populations are significantly differentiated from clone collections whereas little differentiation was observed among clone bank populations. Existing genetic structure of clone bank populations of *P. nigra* for *ex situ* conservation purpose has not been studied adequately because of phenotypic selection, high level of admixture and clonal duplication which caused by human activity.

To investigate genetic diversity and structure of natural European black poplar populations in highly fragmented Göksu and Kızılırmak river systems in Turkey and to understand factors that shaping the genetic structure of these populations, five populations from these rivers were sampled and studied with polymorphic nuclear microsatellite markers. To test if there is genetic pollution in these river systems from hybrid *P. x canadensis* and *P. deltoides* plantations, several loci including species specific alleles for *P. deltoides* were also examined. By considering the results of the study, it is recommended that related data should be used to determine the conservation category of the species and to design effective conservation and breeding strategies for preventing further genetic degradation and maintaining existing genetic diversity of the species in its natural habitats. In addition, obtained information about the impact of long cultural practices on genetic architecture of river systems could help to understand the reproductive systems of trees, population dynamics in natural ecosystems, and genetic integrity of the native *Populus nigra*.

CHAPTER 2

OBJECTIVES OF THE STUDY

Main objective of the study is to understand magnitude of genetic diversity and population structure of European black poplar populations from its natural distribution areas for evaluation of conservation strategies and genetic improvement of the species in Turkey.

Further objectives can be listed as;

- Estimating the potential hybrid categories and pure European black poplar individuals of the natural populations based on the allele frequencies.

- Characterizing the genetic resources of European black poplar from Göksu and Kızılırmak rivers in Turkey.

- Detecting possible genetic groups to understand the amount of gene flow and genetic differentation of populations between two different rivers.

-Generating genetic data for efficient breeding and conservation strategies with cooperation between scientists and responsible government institutions at national level in Turkey.

CHAPTER 3

MATERIAL AND METHODS

3.1. Field Sampling and Plant Material

Total of 124 European black poplar genotypes (trees) from two river systems (Four populations from Kızılırmak and one population from Göksu river) for DNA extraction and further molecular analysis were sampled by considering geography, topography, climate and river dynamics. To detect the putative hybridization with *P. deltoides*, 10 reference *P. deltoides* trees obtained from Poplar and Fast Growing Forest Trees Research Institute (Kocaeli, Turkey) were also included in the study.

The Kızılırmak is the longest river system (1,355 km long) flowing entirely within Turkey. It starts from Imranlı at 2000 meters from the sea level in Kızıldağ, Sivas province, goes through many provinces of Central Anatolia (Sivas, Kayseri, Nevşehir, Kırşehir, Kırıkkale, Ankara, Aksaray, Çankırı and Çorum) and meets in the Blacksea in Samsun province. This river basin includes great portion of European black poplar natural range in Turkey. The watersheds of the Kızılırmak river are located at mostly high plateau without forest cover where intensive human assisted clonal material circulation occurs. Sampling of trees was carried out at four locations representing upstream, middle and downstream parts of the Kızılırmak river. The Göksu river (260 km long) flows into the Mediterranean Sea. The watersheds of Göksu river system are located in forested Mediterranean region where poplar wood is not in demand by locals so almost no clonal material circulation occurs except for the hybrid poplar plantations. Due to loss of old and native trees in their fragmented natural range in Göksu river, sampling limited to only one location in central part of the river and composed of mostly clonally propagated trees and in lesser extent from seeds. Samples from Göksu river can give valuable information about the genetic structure of populations because of geographic isolation from other rivers by Taurus Mountain which hinder gene flow.

When considering distribution range of the species, samples from Kızılırmak and Göksu river represent mainly great majority of genetic resources of the European black poplar populations from Turkey. Corresponding locations of sampled populations and number of sampled trees were illustrated on Table 3.1 and Figure 3.1. In the Göksu river system, it was difficult to find continuous large population due to overexploitation and fragmentation in some cases (Figure 3.2). Collection of fresh leaf samples was made from trees, which were at least 200 meter apart to prevent genetic relatedness and sampling clones from the same cohort. Collected leaf samples were kept in silica gel until DNA extraction in the laboratory.

Table 3.1. Information on studied European black poplar populations from Kızılırmak and Göksu rivers in Turkey

River	Population ID	Altitude Range	Number of Sampled individuals	Distinct genotypes	Number of hybrid trees
Kızılırmak	Kayseri (UP-KAY)	789-1113m	28	20	-
	Kırşehir (MID-KIR)	640-816m	22	22	1
	Kırıkkale (MID-KRK)	730-1269m	28	28	-
	Çorum (DOWN-COR)	358-424m	21	14	-
Göksu	Göksu (GKS)	91-104m	25	25	4

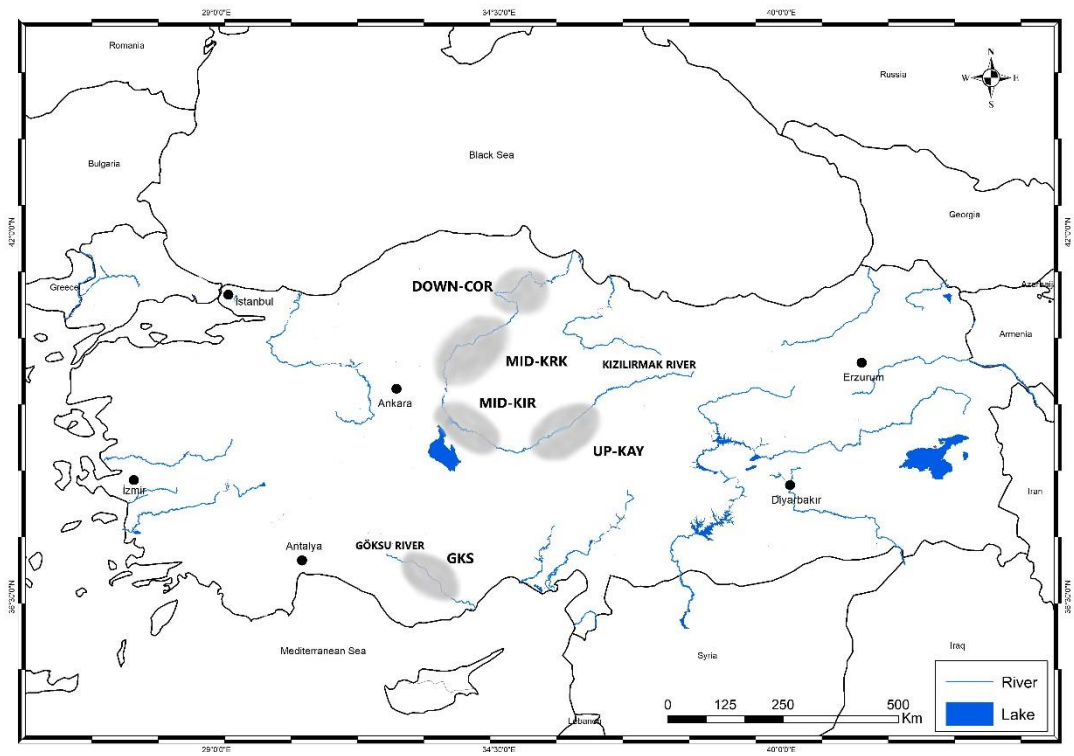


Figure 3.1 Sampling locations of the studied populations from Göksu and Kızılırmak rivers

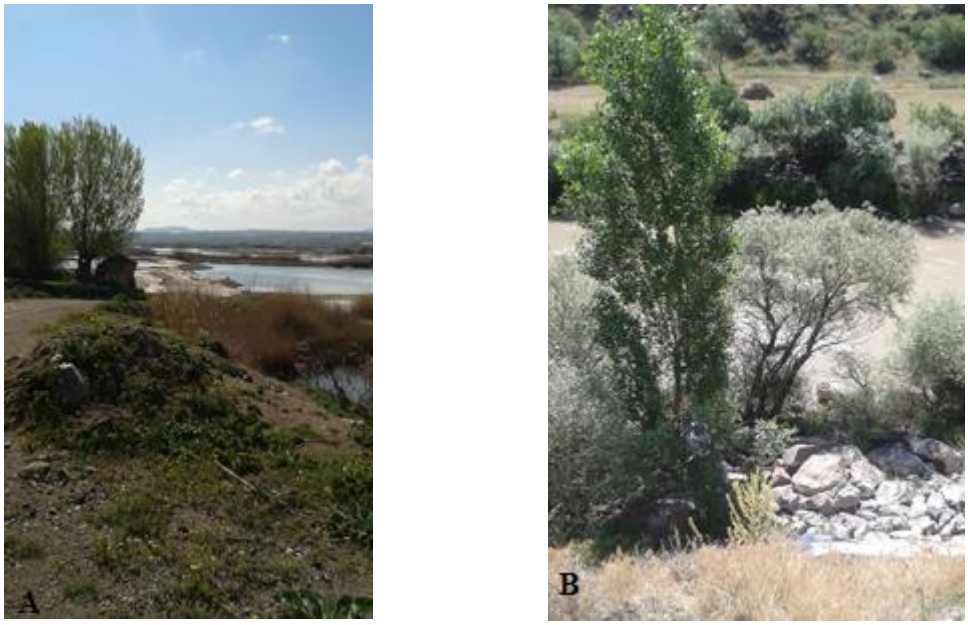


Figure 3.2. View from the study area in Kızılırmak (A) and Göksu river (B)

3.2. DNA Extraction

Sampled leaves were grinded with sterile mortar and pestle using liquid nitrogen (-196°C). Nuclear DNA extraction from young leaf samples was performed using Doyle and Doyle (1990) CTAB extraction protocol with some modifications. Instead of isoamyl alcohol, octanol was used in the extraction step of isolation with chloroform (Appendix A). Concentration and quality of the isolated DNA samples were measured by using NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific, Wilmington, USA) and checked by amplifying obtained DNA with a suitable SSR primer in Polymerase Chain Reactions (PCR).

3.3. Molecular Markers and Polymerase Chain Reactions

To determine genetic diversity and structure of European black poplar populations from two major river systems, 20 nuclear microsatellite markers were selected from the studies of Van Der Schoot et al. (2000), Smulders et al. (2001), Wu et al. (2008) and the International *Populus* Genome Consortium (IPGC) SSR Resource (International *Populus* Genome Consortium 2016, http: //www.ornl.gov/sci/ipgc/ssr_resouce. htm) (Table 3.2). After successful amplification of the corresponding primers successfully, forward primers were synthesized with different fluorescent dyes (FAM, HEX, and TAMR) to distinguish multiplex PCR products during the DNA fragment analysis. The polymerase chain reaction for all loci was performed in a 25 µl total volume composed of 5 µl 5x HOT FIREPol Blend Master Mix (Solis Biodyne, Estonia), 0.5 µl each primer pair, 10ng template DNA (5 µl diluted DNA) and 14 µl sterile PCR water. The same PCR protocols described by Van Der Schoot et al. (2000) and Smulders et al. (2001) with different annealing temperature between 52-60^{o}C were applied for all primer pairs (Table 3.3). Obtained PCR products were screened on the 3% agarose gel to confirm amplification of the studied microsatellite region.

Table 3.2. Information on the studied 20 microsatellite loci

Locus ID	Repeat Motif	Left Primer (5′−3′)	Right Primer (5′−3′)	Product size in literature	Ta°C
WPMS03	GT	TTCTTTTTCAACTGCCTAACTT	TGATCCAATAACAGACAGAACA	280	53
WPMS04	GT	ACACGGGTCTTTTATTCTCT	TGCCGACATCCTGCGTTCC	270	55
WPMS05	GT	TTCTTTTTCAACTGCCTAACTT	TGATCCAATAACAGACAGAACA	280	55
WPMS07	GT	ACTAAGGAGAATTGTTGACTAC	TATCTGGTTTCCTCTTATGTG	230	55
WPMS09	(GT)(GA)	CTGCTTGCTACCGTGGAACA	AAGCAATTTGGGTCTGAGTATCTG	260	60
WPMS10	GT	GATGAGAAACAGTGAATAGTAAAGA	GATTCCCAACAAGCCAAGATAAAA	250	53
WPMS12	GT	TTTTTCGTATTCTTATCTATCC	CACTACTCTGACAAAACCATC	170	52
WPMS14	CGT	CAGCCGCAGCCACTGAGAAATC	GCCTGCTGAGAAGACTGCCTTGAC	245	60
WPMS15	CCT	CAACAAACCATCAATGAAGAAGAC	AGAGGGTGTTGGGGGTGACTA	193	60
WPMS16		CTCGTACTATTTCCGATGATGACC	AGATTATTAGGTGGGCCAAGGACT	150	55
WPMS18	GTG	CTTCACATAGGACATAGCAGCATC	CACCAGAGTCATCACCAGTTATTG	245	57
WPMS20	TTCTGG	GTGCGCACATCTATGACTATCG	ATCTTGTAATTCTCCGGGCATCT	252	60
PMGC14	CTT	TTCAGAATGTGCATGATGG	GTGATGATCTCACCGTTTG	210	55
PMGC2163	GA	CAATCGAAGGTAAGGTTAGTG	CGTTGGACATAGATCACACG	220	55
PMGC27	GA	ATTGTAATTATTGAACACATGCC	GTGCAGTTCAGAGTATTGTTG	210	55
PMGC2889	GA	CCCAAGATCCGATTTTTGGG	CACAATGTACAAATCGCTGTC	207	57
PMGC93	CTT	ATCATGCGTTCGGCTACAGC	CTCAAACTCCAACTGTTATAAC	350	55
Pe5	TC	ACCCACCCAATGTGCAGCCCTGCAA	CTCGCCCTCTATATATCTCTATGAA	164–206	57
Pe13	$(CT)_6(GT)_5$	TTCAACTTGACTAGTTGTAACTCTC	CACTTTCCCAGCTATCCCTTTCTAA	121–137	57
Pe14	CT	TCGAAATGGGAGATCTGTAGAGGTG	CACCACAACAGCGTACAGAAATGAA	137–178	60

Table 3.3. PCR mixture composition and cycling parameters

SSR Loci	Ingredients	Volume (µL)	PCR Cycling Parameters		
WPMS03, WPMS04,WPMS5, WPMS7, WPMS09, WPMS10, WMPS12, WPMS14, WPMS15, WPMS16,WPMS18, WPMS20 PMGC14, PMGC2168, PMGC27, PMGC2889, PMGC93	dH2O	14	Initial Denaturation 3min.		94°C
	Master Mix	5	25 cycle	Denaturation 30 sec.	94°C
	Primers (10µM)	0.5+ 0.5		Annealing 45 sec.	Ta
	DNA (10ng/µL)	5		Extension 1 min.	72°C
	Total	25	Final Extension 20 min.		72°C
Pe5, Pe13,Pe14	dH2O	14	Initial Denaturation 3min.		94°C
	Master Mix	5	25 cycle	Denaturation 30 sec.	94°C
	Primers (10µM)	0.5+ 0.5		Annealing 30 sec.	Ta
	DNA (10ng/µL)	5		Extension 40 sec.	72°C
	Total	25	Final Extension 20 min.		72°C

3.4. DNA Fragment Analysis

Microsatellite genotyping for the European black poplar trees was performed by Capillary Electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in BM Laboratory Systems Facilities, Ankara. Amplified fragments of loci with help of SSR markers labelled with a fluorescent dye were separated by considering the relative size of each fragment in the sample and comparing fragments with the standard curve. Electropherograms for each individual tree were checked to determine the allele sizes of the studied microsatellite loci by implementing Peak Scanner v2.0 (Applied Biosystems) with the GeneScan 400 size standard (Figure 3.3).

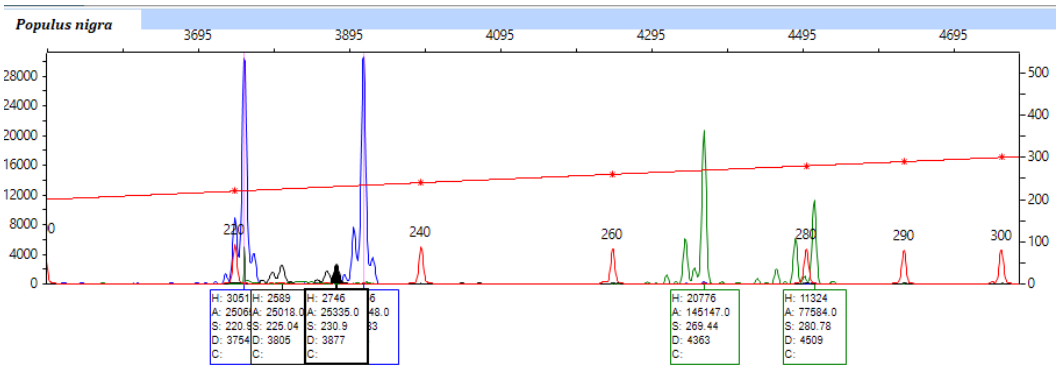


Figure 3.3. An electrophenogram of three studied microsatellite loci (Blue, black and green peaks represent alleles belonging to WPMS03, WPMS18 and WPMS20 loci, respectively)

3.5. Genetic Analyses for Populations

3.5.1. Genetic Identification of Duplicated Trees

Like other members of the Salicaceae family, European black can propagate vegetatively as an alternative to sexual reproduction in highly dynamic

hydrogeomorphic conditions, despite having effective sexual reproduction system giving high fecundity and very high production of germinable, wind-dispersed seeds (Barsoum et al. 2004). Vegetative propagation of European black poplar occurs with help of propagules (branches, roots, root-borne suckers, broken stems) disseminated by water and human activities. Accurate understanding of the genetic diversity structure of studied European black poplar populations, duplicated genotypes were determined by GenClone 2.0 software and removed from further genetic analysis. GenClone 2.0 identified distinct multilocus genotypes by using permutation and re-sampling approaches to test for the reliability of sets of loci and computing statistics to test for clonal identity of trees. (Arnaud-Haond and Belkhir 2007)

3.5.2. Null Allele Frequency Estimation

Analysis of microsatellite locus may be sometimes hampered by apparent heterozygote deficiency caused by the occurrence of null alleles and undetected alleles for a particular locus. Any mutation on the annealing site of the flanking region of one allele cause to prevention of amplification (Pemberton et al. 1995). Amplification of only one allele present at the other homologous chromosome will cause to excess of homozygosity. Short allele dominance also leads to failure of long allele to amplify during PCR amplification (Chapuis and Estoup 2007; Shinde et al. 2003). The presence of null alleles leading to deviations from Hardy-Weinberg equilibrium may cause to overestimation of genetic diversity parameters of studied populations (de Sousa et al. 2005; Chapuis and Estoup 2007). To estimate null allele frequency, to check scoring errors and allele dropouts, MICRO-CHECKER 2.2.3 programme (Van Oosterhout et al. 2004) was implemented. Chakraborty et al. (1992) and Brookfield (1996) methods were used to estimate the null allele frequency (r) (Appendix E).

3.5.3. Hybrid Detection

To detect the possible occurrence of hybrid individuals among the collected trees and intogression from *P. deltoides*, 10 reference *P. deltoides* trees were analyzed with six polymorphic SSR markers. Species specific alleles for *P. deltoides* at WPMS09, WPMS18, WPMS16, PMGC14 and PMGC2163 loci from previous studies (Fossati et al. 2003; Smulders et al. 2008b; Khasa et al. 2005; Jelic et al. 2015) and PMGC2709 in the current study were used to screen studied European black poplar populations.

These previous studies determined 158 species specific alleles by considering allele frequencies in *P. nigra* and *P. deltoides* in a subset of the samples from both species. Informative alleles based on the likelihood of the sample belonging to a certain species or hybrids were determined. The NEWHYBRIDS 1.1beta software (Anderson and Thompson 2002) was implemented to estimate the potential hybrid categories of the sampled individuals based on the allele frequencies of WPMS09, WPMS16, WPMS18, PMGC14, PMGC2163 and PMGC2709 loci. The NEWHYBRIDS program uses an inheritance model defined in terms of genotype frequencies for the various hybrid categories. The software calculates allele frequencies of all loci for parental species and progenies based on parental species specific alleles. Potential hybrid trees from the hybrid detection analysis are assigned to six categories (*P. nigra*, *P. deltoides*, F1 hybrid cross of the two parental species, F2 cross, backcross of F1 hybrid to *P. nigra*, and backcross of F1 hybrid to *P. deltoides*) according to their posterior probabilities of ancestry using all information in frequency differences between alleles in *P. nigra* and *P. deltoides*. The program gives a histogram of estimated frequencies of all alleles changing between 0 and 1 for all six loci of each tree to be used in posterior probabilities of six categories given above.

3.5.4. Genetic Diversity Parameter for Loci and Populations

Magnitude and structure of genetic diversity in the studied river systems were determined by GENALEX 6.503 (Peakall and Smouse 2012) program for each population within the river systems by determining the number of alleles, mean effective number of alleles (Ne), proportion of polymorphic loci (%P), observed (Ho) and expected (He) heterozygosity and private alleles number were assessed by GENALEX for each locus and population (Peakall and Smouse 2012). Allelic richness (Ar) based on a minimal sample size was computed by FSTAT version 2.9.3.2 (Goudet 1995). Polymorphism information content (PIC) at each locus was calculated by CERVUS version 3.0.7 (Kalinowski et al. 2007). The Garza-Williamson Index (Garza and Williamson 2001) was implemented in software ARLEQUIN version 3.5.1.2 (Excoffier and Lischer 2010) to detect whether studied populations experienced a recent bottleneck event and had a decline in effective population size (Appendix E).

F statistics (F_{IS}, F_{IT}, and F_{ST}) for the studied populations within the river systems were calculated with GENALEX program to detect the variation in gene frequency among those subpopulations and the degree of genetic differentiation among subpopulations (Appendix E). To test whether there is any deviation from Hardy-Weinberg Equilibrium, exact tests of GENEPOP program (Rousset 2008) were performed by the Markov chain method with dememorization number 1000, the number of batches 100, and the number of iterations per batch 1000. Also to check the deviation, CERVUS program was implemented. Analysis of Molecular Variance (AMOVA) is a methodology, which gives result of how much of the genetic variability is attributable to variability within populations, and how much to variability between populations. To estimate components of the variation (Partition of the variation) among river systems, among and within populations within the river systems, analyses of

molecular variance (AMOVA) were carried out with the number of different alleles based (the infinite allele model) option in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010).

3.5.5. Population Genetic Structure

Genetic differentiation among studied populations within river systems was examined by pairwise F_{ST} values. Pairwise F_{ST} matrix and their corresponding number of migrants (Nm values) were obtained by using ARLEQUIN program. To show the genetic differences across populations regardless of river systems, the pairwise F_{ST} values were used to generate a principal coordinate analysis (PCoA) based on the covariance matrix with data standardization as implemented in GENALEX. As a result of absence of coordinate information about all trees, centroids of sampling locations were calculated by considering latitude and longitude values and these locations were used for producing pairwise geographic distance matrix. Mantel test with 9999 randomizations was applied to detect correlation between geographic and genetic distances based on the pairwise geographic distance matrix and the pairwise F_{ST} matrix by *ade4* package in R program (Thioulouse et al. 1997).

To detect the presence or absence of genetic barrier between populations of two rivers, R-package *adegenet* (Jombart 2008) using monmonier algorithm was implemented with some point coordinates (from a point layer) and a matrix displaying genetic distance between the points. The phenetic relationships between the populations were assessed with a phenogram based on Nei‘s (1978) coancestry identities with GDA (Genetic data analysis) software (Lewis and Zaykin 2002) with the Neighbour-joining distance method.

To identify the genetic structure of populations, a Bayesian iterative algorithm was used to assign individuals to clusters as implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). Admixture model was assumed and run settings were as follows: a burn-in of 50,000 and 250,000 Markov chain Monte Carlo iterations, possible cluster numbers (K) tested from K=1 to K=5, 10 replications were performed for each K. The web-based tool STRUCTURE HARVESTER (Earl Dent and Vonholdt 2012) was used to assess the most likely value of K from STRUCTURE run results for the detection of the number of genetic groups present in the data by implementing EVANNO method (Evanno et al. 2005). Multiple runs for the true K value were analyzed with the CLUMPP software (Jakobsson and Rosenberg 2007) to identify the best alignment to the replicated results of the cluster analysis. Lastly, POPHELPER (Francis 2016) software was used to visualize the output generated from the admixture analysis.

Discriminant analysis of principle components (DAPC) was performed to group the populations by using R package of *adegenet* (Jombart 2008). DAPC is a multivariate method designed to identify and describe clusters of genetically related individuals. Principal coordinate analysis identifies genetic structures by using rich information from genetic data and Discriminant Analysis provides assignment of individuals to groups. DAPC analysis gives a visual assessment of population differentiation (Jombart et al. 2010). Additionally, to understand and visualize the distance between populations and individuals of two rivers, Minimum Spanning Network (MSN) analyses were carried out with the microsatellite data by using *poppr* and *magrittr* packages of R (Kamvar et al. 2014). MSN analysis groups multilocus genotypes by considering genetic distances between them. MSN is constructed by using genetic distance matrix based on calculated number of allelic differences between two samples.

CHAPTER 4

RESULTS

4.1. Detection of Clones

Several trees in the populations of Kızılırmak river were found as clones of five genotypes. Those genotypes were repeatedly sampled in different number from UP-KAY (Upstream population of Kızılırmak, Kayseri) and DOWN-COR (Downstream population of Kızılırmak, Çorum) populations. GKS (Göksu population), MID-KIR (Midstream population of Kızılırmak, Kırşehir) and MID-KRK (Midstream population of the Kızılırmak, Kırıkkale) populations did not include any clones of the sampled trees. Fifteen sampled clones which duplicates of 5 genotypes were excluded from further genetic diversity and structures analyses of populations.

4.2. Null Allele Presence

After null allele checking, it was seen that some loci showed moderate level of null allele frequency, especially for particular populations (WPMS04, WPMS10 in GKS, WPMS05, WPMS07 in MID-KRK and WPMS05 in DOWN-COR). Locus WPMS05 has been found to possess null allele (Table 4.1) for all populations except UP-KAY. No important changes in the diversity values were examined when the loci showing moderate null allele frequency have been discarded from the analysis. So all studied loci were included in all-genetic diversity and structure analyses.

Table 4.1. Null allele frequency for each locus

Loci	Null allele frequency				
	GKS	UP-KAY	MID-KIR	MID-KRK	DOWN-COR
WPMS20	-0.15	-0.23	-0.17	-0.18	-0.18
WPMS04 *	0.10	-0.00	0.00	-0.01	-0.01
WPMS05*	0.01	-0.01	0.06	0.09	0.14
WPMS07 *	-0.00	-0.09	-0.22	0.08	-0.19
WPMS09	-0.09	-0.17	-0.22	-0.16	-0.25
WPMS10 *	0.07	-0.00	-0.00	-0.00	0.04
WPMS12	-0.14	-0.23	-0.21	-0.18	-0.30
WPMS14	-0.14	-0.16	-0.24	-0.14	-0.23
WPMS15	-0.25	-0.31	-0.29	-0.23	-0.27
WPMS16	-0.23	-0.30	-0.25	-0.23	-0.30
WPMS18	0.06	-0.22	-0.19	-0.01	-0.27
WPMS03	-0.14	-0.11	-0.19	-0.09	-0.18
PMGC14	-0.08	-0.23	-0.24	-0.16	-0.27
PMGC21	-0.17	-0.20	-0.24	-0.10	-0.26
PMGC27	-0.19	-0.21	-0.19	-0.17	-0.26
PMGC28	-0.13	-0.22	-0.25	-0.04	-0.27
PMGC93	-0.17	-0.19	0.04	-0.20	-0.17
PE5	-0.24	-0.00	-0.16	-0.21	-0.18
PE13	-0.33	-0.17	-0.24	-0.07	-0.22
PE14	-0.20	-0.23	-0.28	-0.19	-0.27

*Possible null allele possessing loci

4.3. Hybrid Detection

In reference *P. deltoides* trees, the PMGC2163 and WPMS18 loci were found to be monomorphic with species-specific alleles at 186 bp for PGMC2163 and 220 bp for WPMS18 loci. The WPMS16 locus contained three alleles found only *P. deltoides* at 129, 135 and 147bp. The WMPS09 locus comprised five (218, 220, 230, 232 and 236 bp) and PMGC2709 locus had seven alleles (202, 210, 214, 216, 220, 222 and 224 bp) which were found only in *P. deltoides* trees with low frequency. The locus PMGC14 had the allele of 190 bp in size specific to *P. deltoides* trees (Table 4.3). The results from hybrid analysis using NEWHYBRIDS software indicated that the majority of individuals from two river systems was found to be as pure *P. nigra* and the studied 10 *P. deltoides* samples were also confirmed as pure with posterior probability higher than 0.99. However, five trees morphologically sampled as *P. nigra* were determined to be F2 hybrids with high probability (0.61–0.99) with the use of diagnostic and informative alleles (Figure 4.1). Allele combinations of these hybrid trees at six informative loci were given at Table 4.2. Five hybrid individuals possessing alleles specific to *P. deltoides* in the current study were excluded from further genetic diversity analyses.

Table 4.2. Allele combination and genotype class of hybrid trees (Bold alleles were only found in *P. deltoides* trees)

Tree name-Population	WPMS09	WPMS16	WPMS18	PMGC14	PMGC21	PMGC27	Genotype class with probability
G7-GKS	246/274	**135**/144	225/231	**190**/208	224/242	**202/216**	F2(0.99)
G8-GKS	**236**/252	144/150	**220**/228	**190**/208	242/242	190/194	F2(0.99)
G16-GKS	246/260	**135**/141	**220**/231	217/217	224/228	**202/216**	F2(0.99)
G25-GKS	**236**/252	144/150	**220**/231	**190**/208	**186**/242	**202/216**	F2(0.99)
47-UPKAY	246/274	141/144	225/231	196/202	224/240	190/**210**	F2/bxnigra (0.61/035)

Table 4.3. List of alleles which are specific to *Populus deltoides* in the current and previous studies

Locus name	Smulders et al. 2008b	Fossati et al. 2003	Khasa et al. 2005	Jelic et al. 2015	Current study
WPMS09	234 base pairs (bp)	234 (bp)	Not studied	232 (bp)	218, 220, 230, 232 236 (bp)
WPMS16	Alleles not reported by authors	Alleles not reported by authors	Not studied	Alleles not reported by authors	129, 135, 147 (bp)
WPMS18	220 (bp)	220 (bp)	Not studied	213 (bp)	220 (bp)
PMGC14	268 (bp)	193, 196 (bp)	Not studied	186 (bp) 189 (bp)	190 (bp)
PMGC2163	Not studied	Not studied	185, 187 (bp)	Not studied	186 (bp)
PMGC2709	Not studied	Not studied	Not studied	Not studied	202, 210, 214, 216 220, 222, 224 (bp)

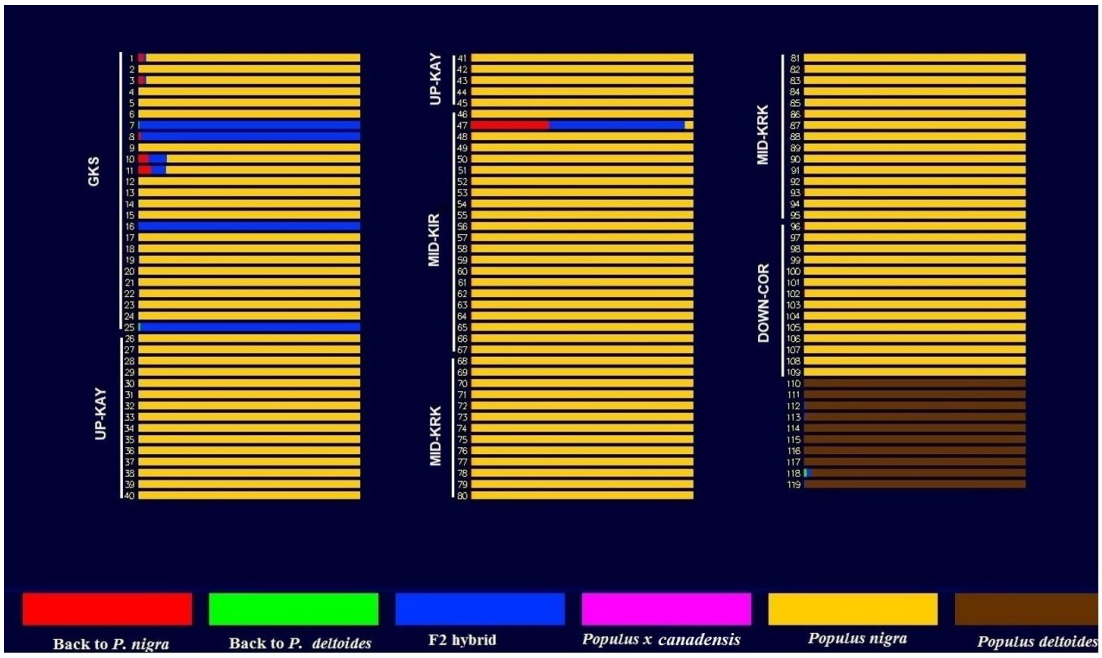


Figure 4.1. Genotype class of the studied *Populus nigra* and *Populus deltoides* trees based on posterior probabilities of genotype frequency classes obtained from NEWHYBRIDS software

4.4. Genetic Diversity Characterization

4.4.1. Descriptive Statistics by Loci

All of the studied 20 loci are found to be polymorphic. The locus Pe14 displayed the lowest allele number as 4, while WPMS07, WPMS05 loci had the highest allele number with 14. WPMS05 locus has the highest private allele number (Eight). The detected alleles for each locus were given at Table 4.4. Except WPMS04, WPMS05 and WPMS010, all SSR loci had higher He and PIC values with high Ar values. The number of effective alleles per locus (Ne) ranged from 1.26 in WPMS10 to 3.07 in PMGC28. Thirteen SSR loci were highly informative with PIC values higher than 0.5. The PIC values changed from 0.20 (WPMS10) to 0.74 (PMGC14). The value of allelic richness for each locus ranged from 3.07 in WPMS03 to 6.92 in PMGC14 with a mean of 4.25 (Table 4.5).

The mean observed and expected heterozygosity values were found to be 0.80 and 0 .56, respectively. The observed and expected levels of heterozygosities were high, but they were in close range in all loci except for the WPMS04, WPMS05 and WPMS10 (Table 4.5). Seventeen loci showed excess of heterozygotes in accordance with the negative inbreeding coefficient (F_{IS}). According to the results of the Hardy-Weinberg equilibrium test except for the WPMS04 and WPMS12, the other loci showed significant departures from HWE ($p<0.001$) in the natural populations. There is no evidence of linkage disequilibrium between the pairs of loci. F_{ST} values ranged between 0.01 in WPMS12, WPMS14, WPMS15, WPMS16 and 0.30 in WPMS07 with a mean of 0.06 when all loci and all populations are considered (Table 4.6).

Table 4.4. Observed alleles and their length as base pairs in the studied 20 loci

Microsatellite	Observed Alleles
WPMGC03	216,220, 226,228,234
WPMGC04	246,248,254,256,260,264,274,286
WPMGC05	224,226,232,236,246,262,268,274,278,280,282,284, 288,290
WPMGC07	214,218,220,224,226,230,232,236,246,250,254,258,262
WPMGC09	208,236,246,250,252,256,258,260,268,274
WPMGC10	210,220,236,238,244,246,248,254,268
WPMGC12	156,164,166,174,178
WPMGC14	210,213,216,225,231,234,237,243,249,252,261
WPMGC15	183,195,204,210,213,216,219
WPMGC16	135,141,144,150,153,156,159
WPMGC18	220,225,228,231,234,237,246,249
WPMGC20	263,269,272,275,281
PMGC14	189,192,195,198,201,204,207,210,213,216,219,225
PMGC2163	186,206,216,220,222,224,228,234,240,242,246,252,256,260
PMGC27	190,194,198,200,202,204,208,210,216
PMGC28	180,184,190,192,198,200,202,208,212,216
PMGC93	348,351,354,357,363
Pe 5	134,136,138,152, 160,162,168,174
Pe 13	126,128,130,134,136,138,140,142,158
Pe 14	144,148,150,160

Table 4.5. Genetic diversity parameters of 20 nuclear SSR loci studied in *Populus nigra* populations

Loci	N	Na	Ne	Ar	PA	PIC	Ho	He	F	P
WPMS03	21.80±0.11	5	2.13±0.11	3.07	1	0.44	0.75±0.06	0.54±0.03	-0.43	***
WPMS04	21.60±2.31	8	1.55±0.08	3.63	4	0.35	0.32±0.04	0.35±0.04	0.06	NS
WPMS05	21.40±2.27	14	1.95±0.45	5.47	8	0.46	0.32±0.10	0.42±0.10	0.20	***
WPMS07	21.80±2.38	14	2.22±0.29	5.82	7	0.68	0.64±0.15	0.52±0.10	-0.23	***
WPMS09	21.60±2.32	10	2.69±0.25	4.42	4	0.58	0.91±0.02	0.63±0.03	-0.47	***
WPMS10	21.20±2.18	9	1.26±0.07	3.25	4	0.20	0.17±0.04	0.20±0.05	0.14	***
WPMS12	20.60±1.94	5	2.53±0.10	3.31	1	0.53	0.95±0.03	0.62±0.02	-0.57	ND
WPMS14	21.80±2.38	11	2.99±0.19	4.83	4	0.62	0.97±0.01	0.68±0.02	-0.46	***
WPMS15	21.60±2.32	7	2.27±0.06	3.37	1	0.46	0.99±0.01	0.57±0.01	-0.76	***
WPMS16	21.80±2.38	7	2.20±0.11	3.11	3	0.44	0.95±0.02	0.55±0.02	-0.75	***
WPMS18	21.80±2.38	8	2.39±0.14	4.49	3	0.56	0.78±0.02	0.59±0.03	-0.35	***
WPMS20	21.80±2.38	5	2.53±0.08	3.39	2	0.54	0.91±0.03	0.62±0.01	-0.50	***

N= Mean number of individuals with amplification, Na=mean number of different alleles, Ne=mean number of effective allele Ar=allelic richness, PA= Private allele, PIC=polymorphic information content, Ho=observed heterozygosity, He=expected Heterozygosity, F =inbreeding coefficient, P= Deviation from Hardy-Weinberg equilibrium, *** : P<0.001, NS: Non-significant, ND: Non-deviating.

Table 4.5. (Continued)

Loci	N	Na	Ne	Ar	PA	PIC	Ho	He	F_{IS}	P
PMGC14	21.80±2.38	12	2.78±0.19	6.92	5	0.74	0.96±0.03	0.65±0.02	-0.51	***
PMGC21	21.40±2.18	13	2.54±0.13	5.35	6	0.63	0.93±0.04	0.62±0.02	-0.53	***
PMGC27	21.20±2.13	9	2.74±0.17	4.22	5	0.58	0.97±0.01	0.64±0.03	-0.54	***
PMGC28	21.80±2.38	9	3.07±0.37	5.33	3	0.65	0.97±0.04	0.67±0.04	-0.46	***
PMGC93	21.60±2.38	5	2.85±0.13	3.78	1	0.59	0.89±0.08	0.66±0.02	-0.38	***
PE5	21.20±2.31	8	2.52±0.15	4.45	4	0.64	0.86±0.08	0.61±0.03	-0.43	***
PE13	21.00±2.49	9	2.25±0.15	3.65	3	0.47	0.87±0.07	0.56±0.03	-0.59	***
PE14	21.60±2.54	4	2.55±0.11	3.10	1	0.54	0.98±0.01	0.62±0.02	-0.62	***
	21.52±0.47	4.27	2.40±0.06	4.25	3.5	0.54	0.80±0.03	0.56±0.01	-0.41	

N= Mean number of individuals with amplification, Na=mean number of different alleles, Ne=mean number of effective allele Ar=allelic richness, PA= Private allele, PIC=polymorphic information content, Ho=observed heterozygosity, He=expected Heterozygosity, F =inbreeding coefficient, P= Deviation from Hardy-Weinberg equilibrium ***: P<0.001, NS: Non-significant, ND: Non-deviating.

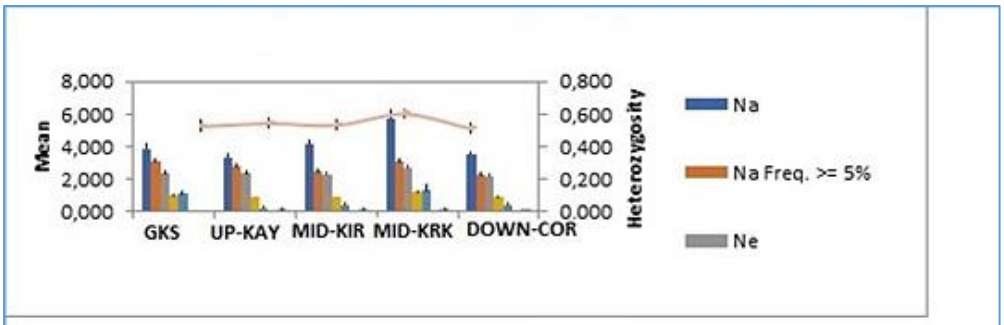
Table 4.6. F-statistics (F_{IS}, F_{IT} and F_{ST}) and number of migrant (N_M) for each locus (HWE deviations *** p<0,001,

**p<0, 01, *p<0, 05)

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
WPMS03	-0.43***	-0.39	0.02	8.36
WPMS04	0.06**	0.10	0.04	5.57
WPMS05	0.20***	0.27	0.08	2.55
WPMS07	-0.27***	0.10	0.30	0.58
WPMS09	-0.47***	-0.43	0.02	8.84
WPMS10	0.14**	0.16	0.02	9.40
WPMS12	-0.57***	-0.54	0.01	14.28
WPMS14	-0.46***	-0.44	0.01	13.47
WPMS15	-0.76***	-0.76	0.01	73.27
WPMS16	-0.75***	-0.74	0.01	31.69
WPMS18	-0.35***	-0.24	0.08	2.76
WPMS20	-0.50***	-0.47	0.02	16.83
PMGC14	-0.51***	-0.27	0.15	1.33
PMGC21	-0.53***	-0.38	0.09	2.38
PMGC27	-0.54***	-0.52	0.02	16.80
PMGC28	-0.46***	-0.40	0.04	5.27
PMGC93	-0.38***	-0.34	0.02	10.96
PE5	-0.43***	-0.22	0.14	1.49
PE13	-0.59***	-0.53	0.03	7.12
PE14	-0.62***	-0.59	0.02	12.99
Mean	-0.41***	-0.33	0.06	12.30

4.4.2. Descriptive Statistics for Population Genetic Diversity

All studied populations from the river systems were found to be polymorphic with 100% of the loci being polymorphic. Average number of alleles per locus in studied populations varied between 3.35 in UP-KAY and 5.75 in MID-KRK populations. The effective allele number was ranged from 2.18 for DOWN-COR to 2.68 for MID-KRK populations. Although the effective numbers of alleles were low, they followed a similar pattern with respect to the average number of alleles per locus. Similarly, the highest mean allelic richness (Ar=4.34) was observed in MID-KRK population, which indicates that this population shows the highest genetic diversity, while the lowest (Ar=3.10) was estimated in UP-KAY population (Table 4.7). Göksu and MID-KRK populations have very high private allele numbers (21 and 25, respectively). The mean allelic patterns across studied populations were provided in Figure 4.2.



Mean values Population	GKS	UP-KAY	MID-KIR	MID-KRK	DOWN-COR
Na	4.45	3.35	4.25	5.75	3 55
Na Freq. >= 5%	3.05	2.75	2.45	3.05	2.25
Ne	2.51	2.35	2.28	2.68	2·18
I	0.94	0.92	0.93	1.15	0.86
No. Private Alleles	1.05	0.20	0.40	1.30	0.40
No. LComm Alleles (<=25%)	0.00	0.00	0.00	0.00	0.00
No. LComm Alleles (<=50%)	0.00	0.10	0.15	0.15	0.05
He	0.56	0.55	0.54	0.60	0.51
uHe	0.54	0.56	0.54	0.61	0.53

Figure 4.2. Schematic representation of mean allelic patterns and expected heterozygosity across populations (The explanations for population codes were given at Table 3.1.)

Observed heterozygosity values of the populations were high and close in magnitudes, ranging from 0.76 to 0.83 with the mean of 0.80, while expected heterozygosity values were found at moderate level with the mean of 0.55. It was ranged from 0.51 in DOWN-COR and 0.60 in MID-KRK populations. F_{IS} values were estimated to be negative for all populations of the river systems, indicating an excess of heterozygosity. To test whether there was a past bottleneck in the populations, Garza-Williamson indices (M value) were calculated for all populations. M values for each polymorphic locus were found to be smaller than the critical value 0.68. As matter of fact the M values were low, ranged between 0.20 and 0.22 (Table 4.7, Figure 4.3) indicating occurrence of past bottleneck events in all populations.

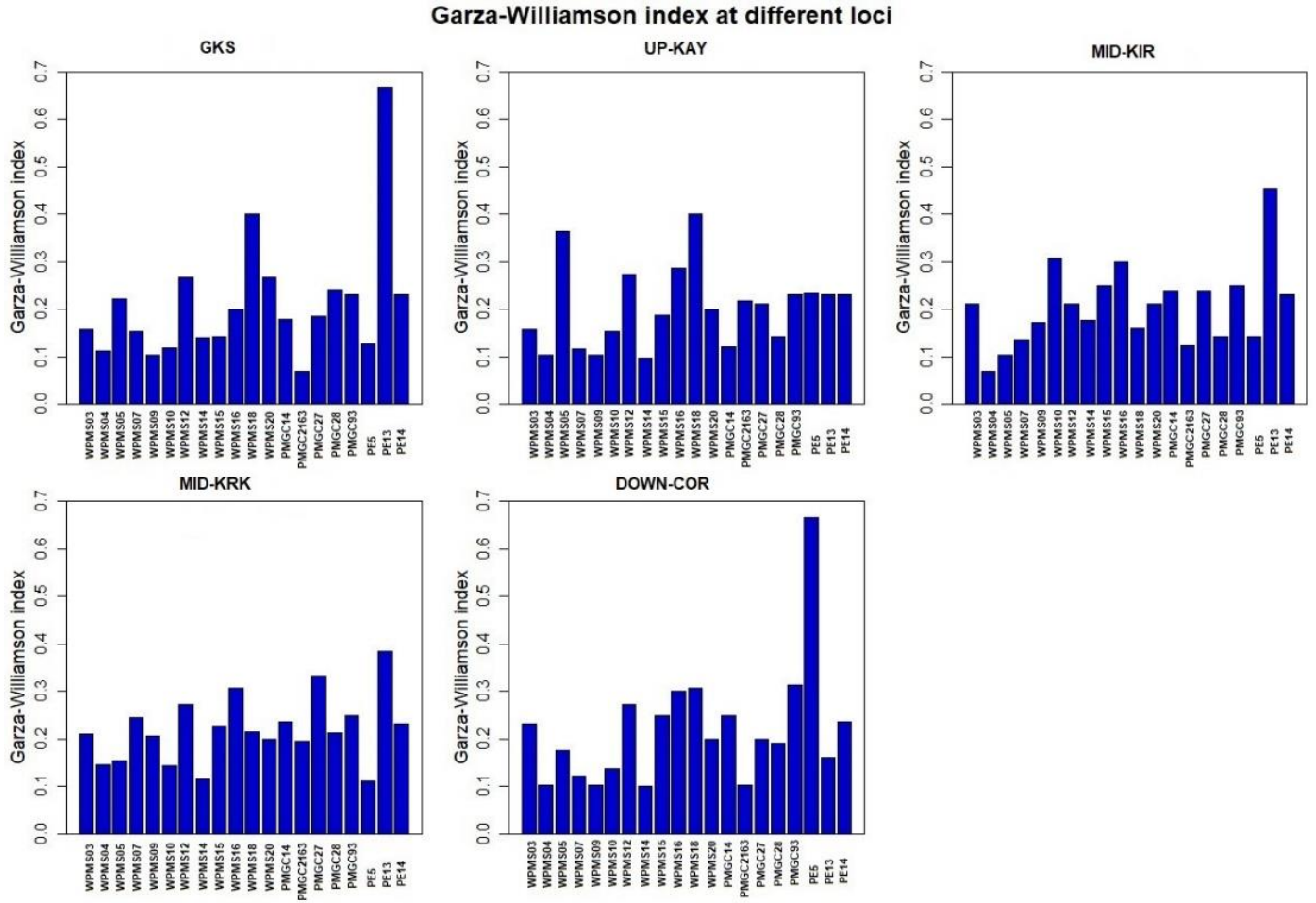


Figure 4.3. The Garza-Williamson index values indicating whether studied populations experienced a recent bottleneck at 20 microsatellite loci in five *Populus nigra* populations (See for description of populations and for loci at Table 3.1 and Table 4.5).

Table 4.7. Genetic diversity estimates for the European black poplar populations studied in Göksu and Kızılırmak rivers

Pop	N	Na	Ne	Ar	PA	%P	Ho	He	M	F_{IS}	F_{ST}	P
GKS	20.35±0.23	4.45±0.35	2.51±0.17	3.85	21	100%	0.76±0.07	0.56±0.04	0.21±0.13	-0.32±0.08	0.03	***
UP-KAY	20.00±0.00	3.35±0.18	2.35±0.12	3.10	4	100%	0.82±0.06	0.55±0.03	0.20±0.08	-0.46±0.06		***
MID-KIR	20.90±0.07	4.25±0.23	2.28±0.10	3.48	9	100%	0.82±0.06	0.54±0.03	0.21±0.09	-0.48±0.07		***
MID-KRK	27.55±0.17	5.75±0.57	2.68±0.13	4.34	25	100%	0.79±0.05	0.60±0.02	0.22±0.07	-0.30±0.06		***
DOWN-COR	13.80±0.12	3.55±0.17	2.18±0.11	3.34	8	100%	0.83±0.07	0.51±0.3	0.22±0.13	-0.53±0.09		
Total	20.52±0.47	4.27±0.17	2.40±0.06	3.64		100%	0.80±0.3	0.55±0.01	0.21±0.10	-0.42±0.03	0.06	

N= Sample size, Na= Mean allele number, Ne= Effective number of alleles, %P= Percentage of Polymorphic loci, Ho= Observed heterozygosity, He= Expected heterozygosity, M= Garza-Wiliamson index, F= Inbreeding coefficient, FST= Fixation index, P= Deviation from Hardy-Weinberg equilibrium *** P<0.001 (Average deviation for all studied locus)

4.5. Genetic Differentation

Low level of genetic differentation was found between populations of Göksu and Kızılırmak rivers (F_{ST}=0.06). The F_{ST} value among four Kızılırmak populations was calculated as 0.03 (Table 4.7).

4.5.1. Analysis of Molecular Variance (AMOVA)

To figure out the partition of total genetic diversity with respect to the between and within the river systems, AMOVA was carried out with F_{ST}-based on the infinite allele model. The variation attributed to differentiation between two river systems was 8.76 % of the total variation while only 1.78% of the total variation was attributed to differences among populations within the river systems. Of the total genetic variance, 89.45 % was explained significantly by the differences among individual trees within populations with F_{ST} = 0.10 value (Table 4.8).

Table 4.8. Analysis of molecular variance (AMOVA) of European black poplar populations from two river systems.

Source of variation	Sum of squares	Variance components	Percantage of total variation
Among rivers	54.19	0.56	8.76
Among populations within rivers	30.98	0.11	1.78
Within Populations	1202.29	5.72	89.45
Total	1287.47	6.39	100
Fixation index	F_{ST}=0.10		

4.5.2. Pairwise F_{ST} matrix

Pairwise F_{ST} values between Göksu and four populations of Kızılırmak river were changed from 0.06 for GKS and MID-KIR / MID-KRK and to 0.07 for GKS and UP-KAY / DOWN-COR populations. On the other hand, four populations of the Kızılırmak River did not differentiate significantly from each other. This was evident from F_{ST} values (Figure 4.4, Table 4.9). However, high level of gene flow was present between midstream (MID-KIR) and downstream (DOWN-COR) populations of Kızılırmak river (Number of migrants: 20.79).

Table 4.9. Pairwise F_{ST} values between population pairs. The numbers in parenthesis following F_{ST} are number of migrants

	GKS	UP-KAY	MID-KIR	MID-KRK	DOWN-COR
GKS	-	-	-	-	-
UP-KAY	0.07(3.38)	-	-	-	-
MID-KIR	0.06(4.14)	0,02(11.39)	-	-	-
MID-KRK	0.06(3.87)	0.02(12.36)	0.02(12.20)	-	-
DOWN-COR	0.07(3.46)	0.03(8.68)	0.01(20.79)	0.03(8.01)	-

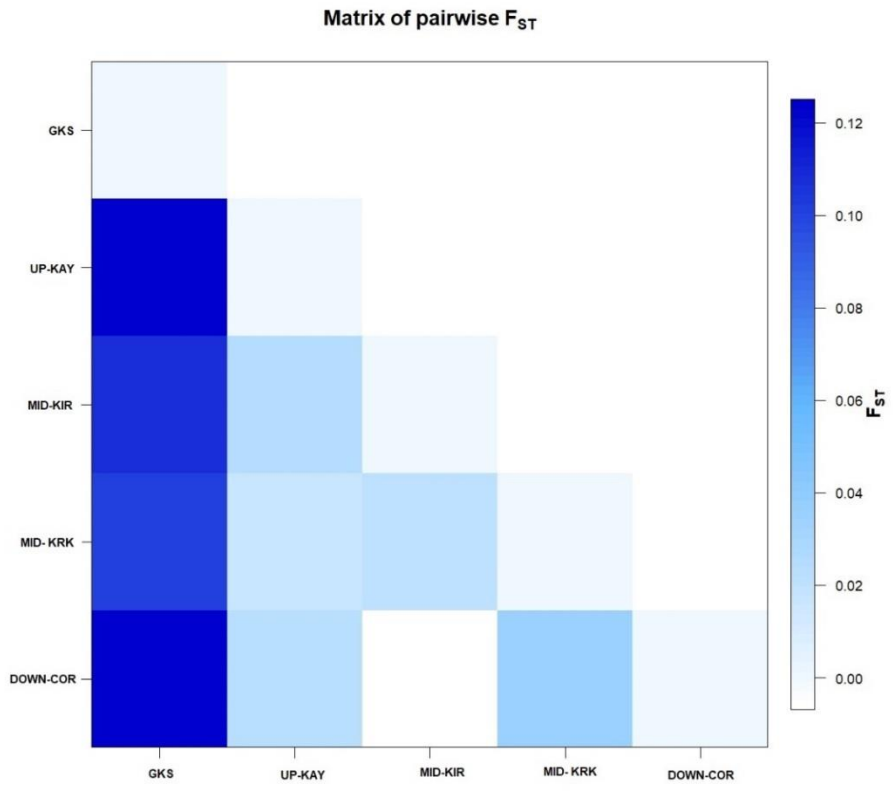


Figure 4.4. Schematic representation of pairwise F_{ST} matrix of populations. The darker the color indicates, the higher the pairwise F_{ST} values

4.5.3. Principal Coordinate Analysis

Based on the Pairwise F_{ST} matrix, Principal Coordinates analysis showed overall differences among populations of two rivers. According to the first principal component explained 56.88% of the variance, GKS population was clearly separated from populations of Kızılırmak river (Figure 4.5, Table 4.9). However, four populations from different locations of Kızılırmak river were not clearly separated from each other (Figure 4.5).

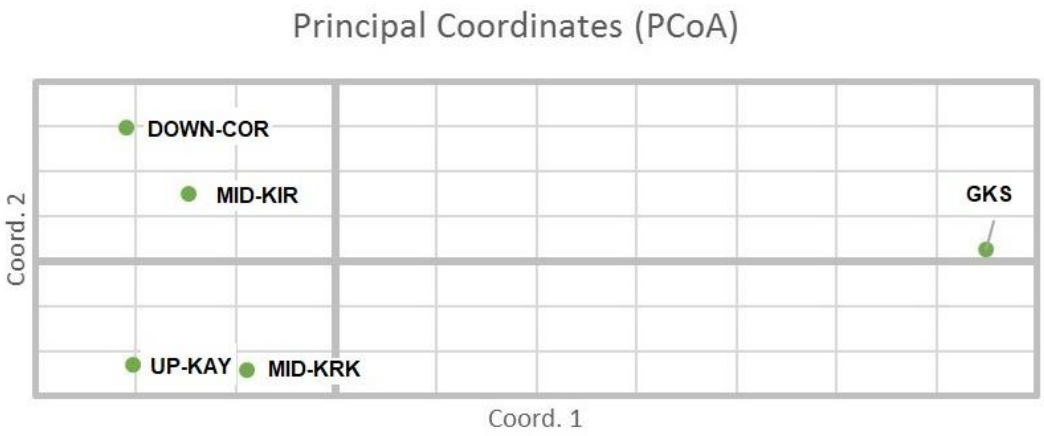


Figure 4.5. The differentiation of *Populus nigra* populations based on Principal Component analysis using population pairwise F_{ST} values

The results of Mantel test indicated that differentiation between population pairs is not the result of isolation by distance since low levels of correlations (P value= 0.0428) between genetic and geographic distances were found (Table 4.9, Table 4.10). Barrier detection analysis suggested that there is one possible barrier between Göksu and Kızılırmak river populations (Figure 4.6).

Table 4.10. Pairwise matrix of geographical distance between population pairs of Göksu and Kızılırmak rivers

	GKS	UP-KAY	MID-KIR	MID-KRK	DOWN-COR
GKS	-	-	-	-	-
UP-KAY	2.92	-	-	-	-
MID-KIR	2.62	1.39	-	-	-
MID-KRK	3.20	2.27	0.95	-	-
DOWN-COR	4.20	1.90	1.61	1.62	-

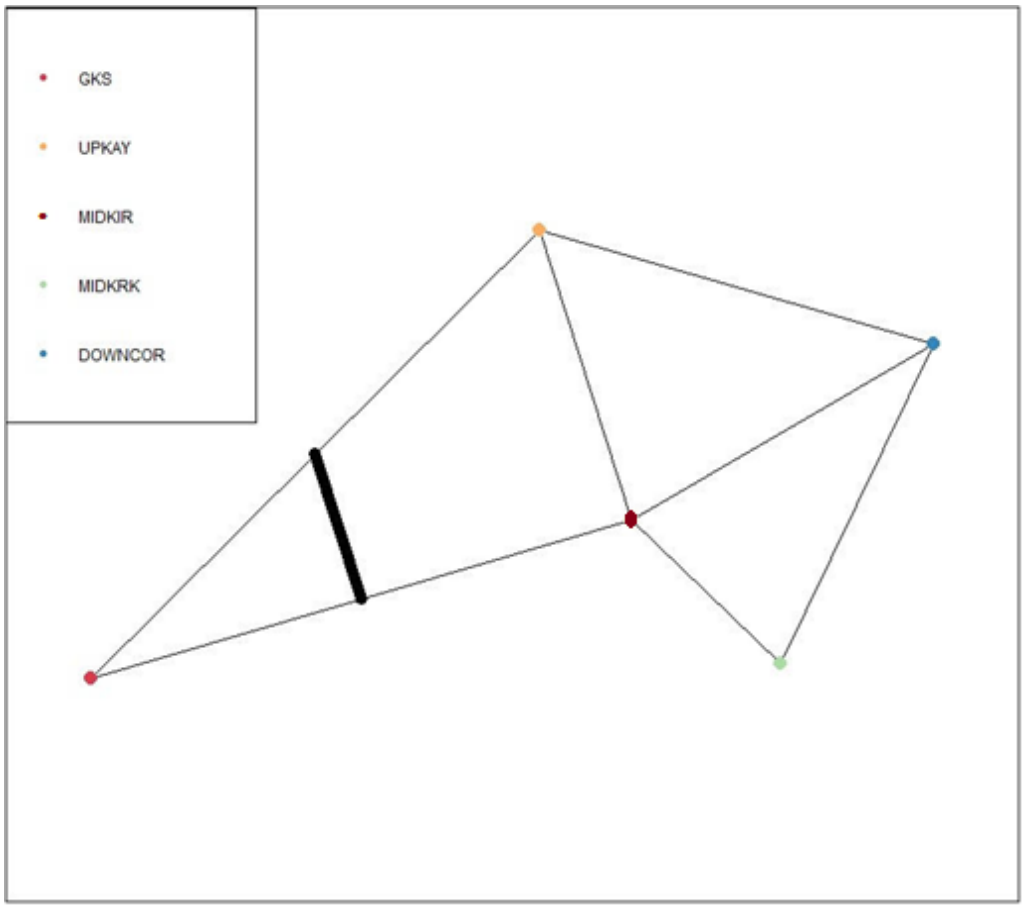


Figure 4.6. The placement of detected geographic barrier by adegenet R package using Monmonier's algorithm (Solid bold line represents the barrier between Göksu and Kızılırmak river populations).

4.5.4. Phenetic Relationships among Populations

According to the constructed dendogram, Göksu and Kızılırmak river populations were placed into two major clusters based on coancestry identity with Neighbour-joining clustering method (Figure 4.7). The Kızılırmak populations constituted two subclusters. One of the subclusters was composed of UP-KAY and MID-KRK populations and other one included MID-KIR and DOWN-COR populations.

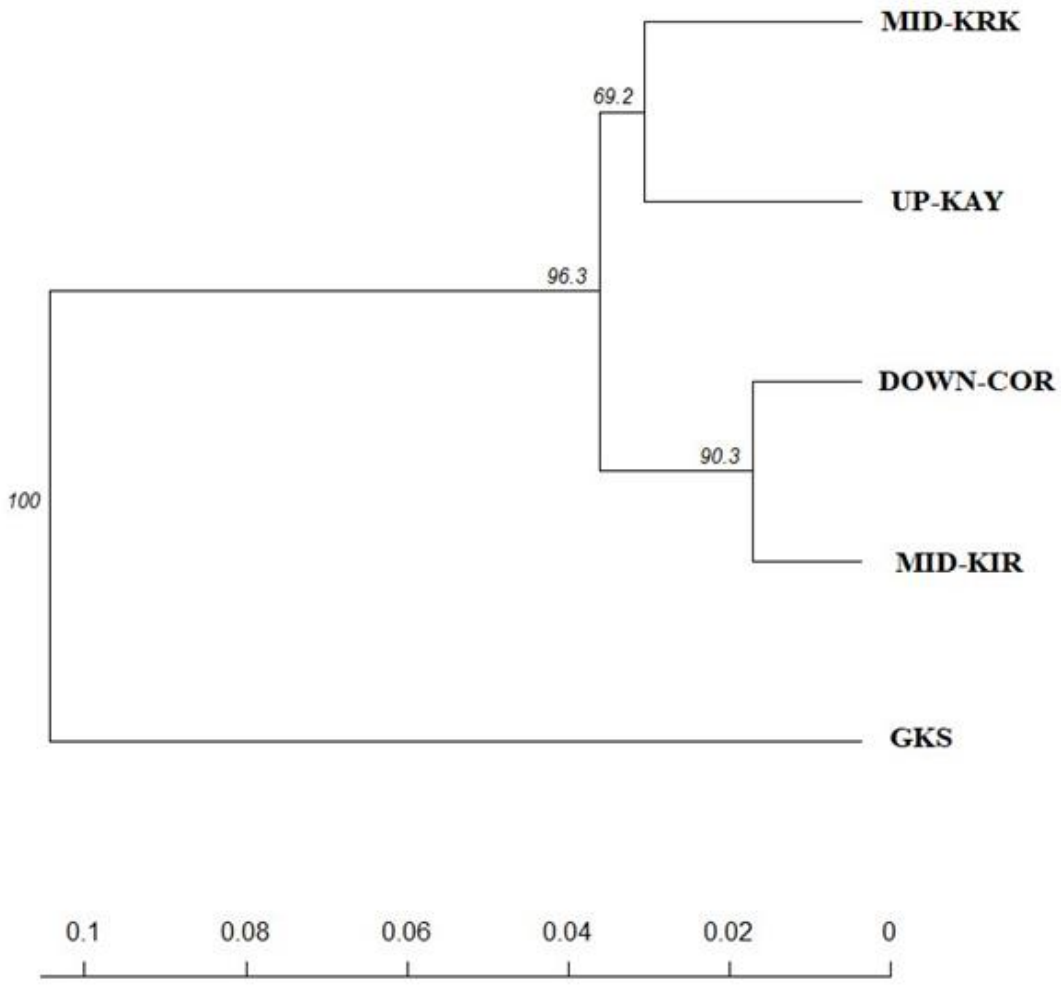


Figure 4.7. Neighbour-joining dendogram based on coancestry identity for *Populus nigra* populations from Göksu and Kızılırmak rivers (The numbers on branches are bootstrap values).

4.5.5. Genetic Grouping of Populations

The numbers of genetic clusters represented in the samples of the European black poplar in Turkey were assessed without prior information to understand the actual population structure. The STRUCTURE HARVESTER program illustrated K as 2 in accordance with Evanno's ΔK estimate, which selects the highest ΔK and lowest standard deviation (Figure 4.8, Table 4.11).

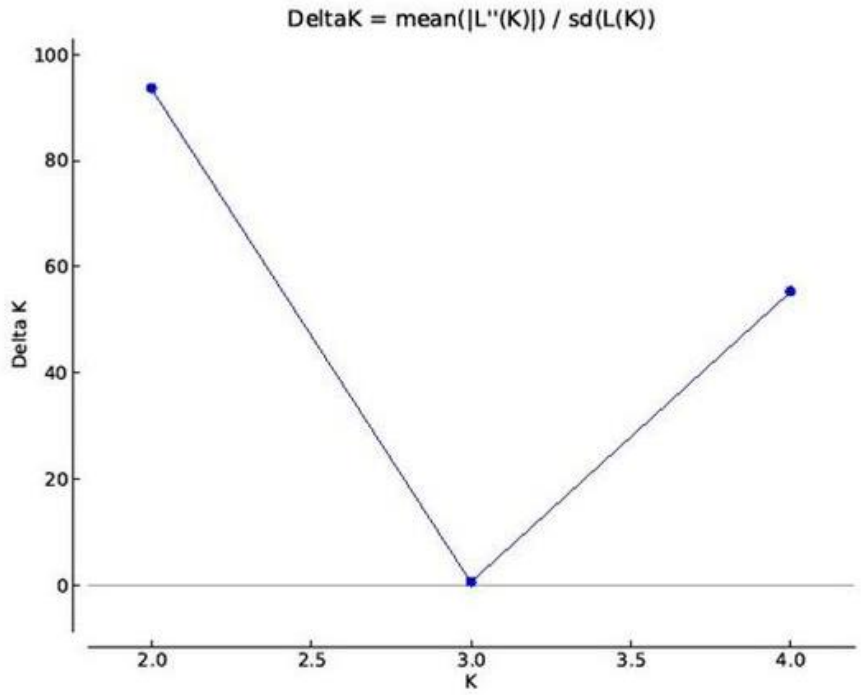


Figure 4.8. Graph of ΔK value for Göksu and Kızılırmak river populations determined by Evanno's method

Table 4.11. Estimated ΔK values for Göksu and Kızılırmak populations

K	Reps	Mean LnP(K)	St. Dev Ln	P(K) Ln'(K)	|Ln''(K)|	Delta K
1	10	-5205.45	0.0972	NA	NA	NA
2	**10**	**-4718.81**	**2.7098**	**486.64**	**253.8**	**93.65873**
3	10	-4485.97	29.6579	232.84	22.46	0.757302
4	**10**	**-4275.59**	**3.2157**	**210.38**	**178.09**	**55.38068**
5	10	-4243.3	10.1108	32.29	NA	NA

Figure 4.9 shows the two inferred clusters with the associated populations. Each color represents a different cluster and black segments separate the preassumed populations. Eventhough, all studied populations appeared to belong to first inferred cluster with higher membership values, each population recruited trees from the assumed two clusters. The DOWN-COR population had the highest estimated membership values (0.87), whereas the UP-KAY population had the low estimated membership values (0.56) in their respected cluster (Table 4.12).

Table 4.12. The proportion of estimated membership to each of the two inferred genetic groups of Göksu and Kızılırmak populations

Given population	Inferred cluster 1	Inferred cluster 2	Number of individuals
GKS	0.61	0.39	21
UP-KAY	0.56	0.44	20
MID-KIR	0.80	0.20	21
MID-KRK	0.57	0.43	28
DOWN-COR	0.87	0.13	14

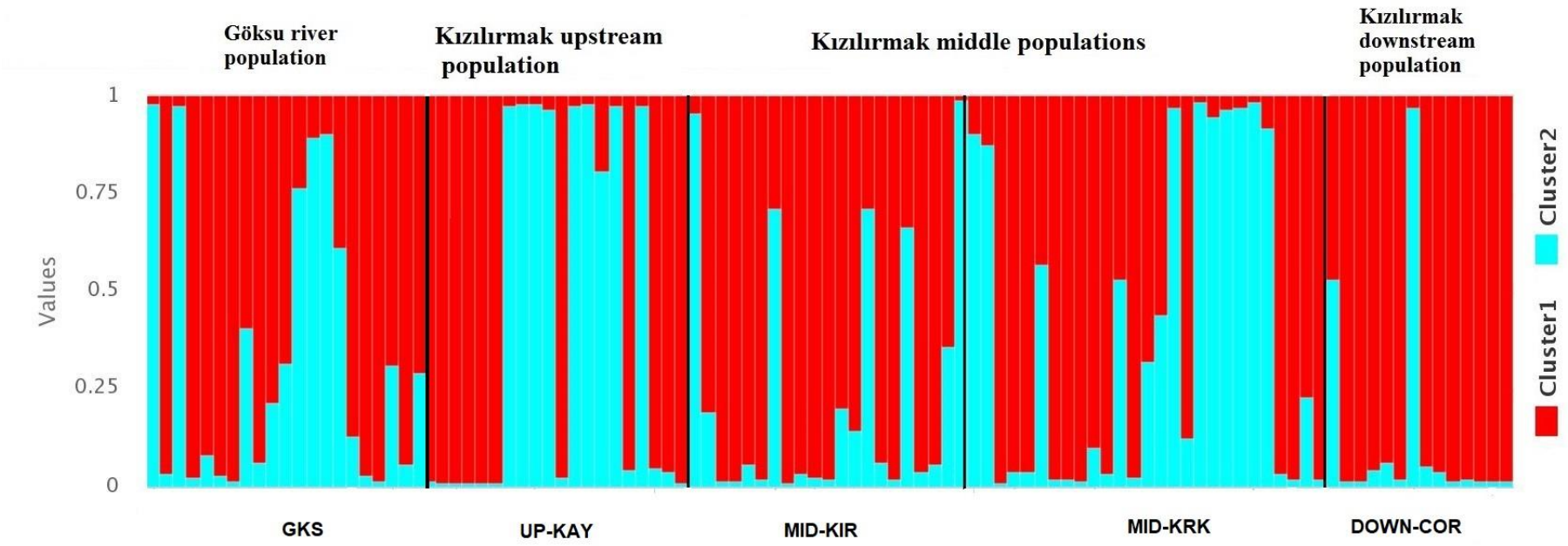


Figure 4.9. Clustering pattern (based on STRUCTURE analysis) of *Populus nigra* populations from Kızılırmak and Göksu rivers with the highest ΔK value at K=2 (Red and turquoise colors represent cluster 1 and cluster 2, respectively). Black vertical lines separate populations.

A clear peak in the second largest value of ΔK was obtained at K = 4 (Figure 4.8). All individual of GKS belonged to third genetic cluster, which showed strong ancestry values with an average 0.86. The second genetic cluster, which included 55 individuals of Kızılırmak river, showed 0.51 mean inferred membership values for MID-KRK and 0.85 for DOWN-COR populations. Ten and eighteen individuals from Kızılırmak river were assigned to first and fourth genetic clusters with high membership values, respectively (Figure 4.10, Table 4.13).

Table 4.13. The proportion of estimated membership to each of the four inferred genetic groups of Göksu and Kızılırmak populations

Population	Inferred cluster 1	Inferred cluster 2	Inferred cluster 3	Inferred cluster 4	Number of individual
GKS	0.04 (0)	0.02 (0)	0.86(21)	0.09 (0)	21
UP-KAY	0.05 (1)	0.54 (11)	0.01 (0)	0.40 (8)	20
MID-KIR	0.11 (2)	0.77 (17)	0.01 (0)	0.11 (2)	21
MID-KRK	0.20 (5)	0.51 (15)	0.02 (0)	0.26 (8)	28
DOWN-COR	0.12 (2)	0.85 (12)	0.02 (0)	0.01 (0)	14

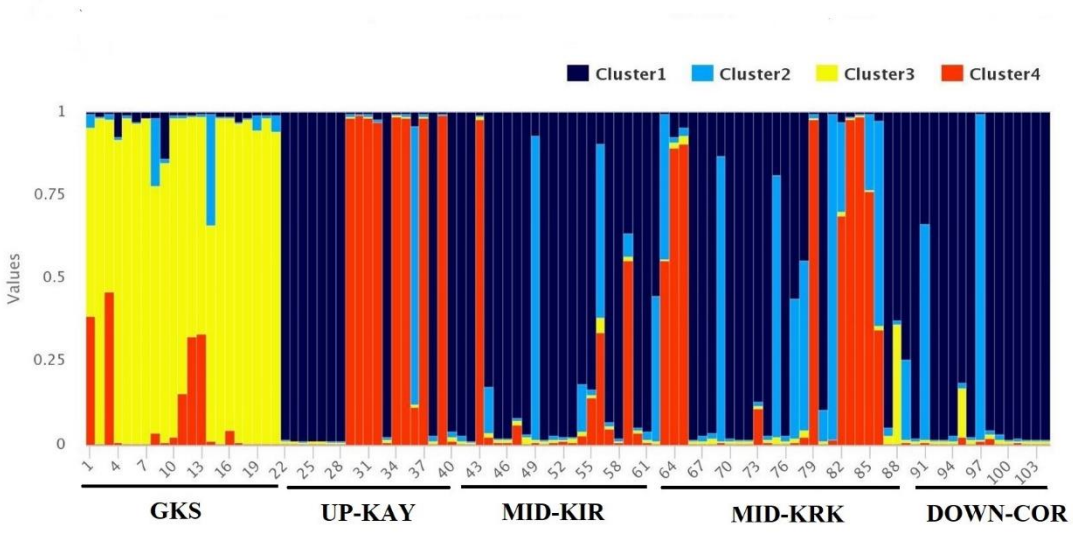


Figure 4.10. Clustering pattern (based on STRUCTURE analysis) of *Populus nigra* populations from Kızılırmak and Göksu rivers with the second highest ΔK value at K=4 (Navy blue, turquoise, yellow and red colors represent cluster 1, cluster 2, cluster 3 and cluster 4, respectively)

Several runs with and without prior information and different number of burn-in and Markov chain Monte Carlo iterations have been performed to interpret and check the STRUCTURE results with possible cluster numbers from K=1 to 5 and K=1 to 10. The largest ΔK value was found at K=4 with lowest standard deviation for each run (Table 4.14, Figure 4.11).

Table 4.14. ΔK estimates for Göksu and Kızılırmak river populations (Run with prior information and 50000 of burn-in and 150000 of Markov chain Monte Carlo iterations)

K	Replication	Mean LnP(K)	Stdev Ln	P(K) Ln'(K)	|Ln"(K)|	Delta K
1	10	-4800.78	0.1033	NA	NA	NA
2	10	-4363.37	15.0868	437.41	251.35	16.66027
3	10	-4177.31	55.1307	186.06	47.01	0.852701
4	**10**	**-3944.24**	**2.3095**	**233.07**	**253.22**	**109.6429**
5	10	-3964.39	5.8072	-20.15	NA	NA

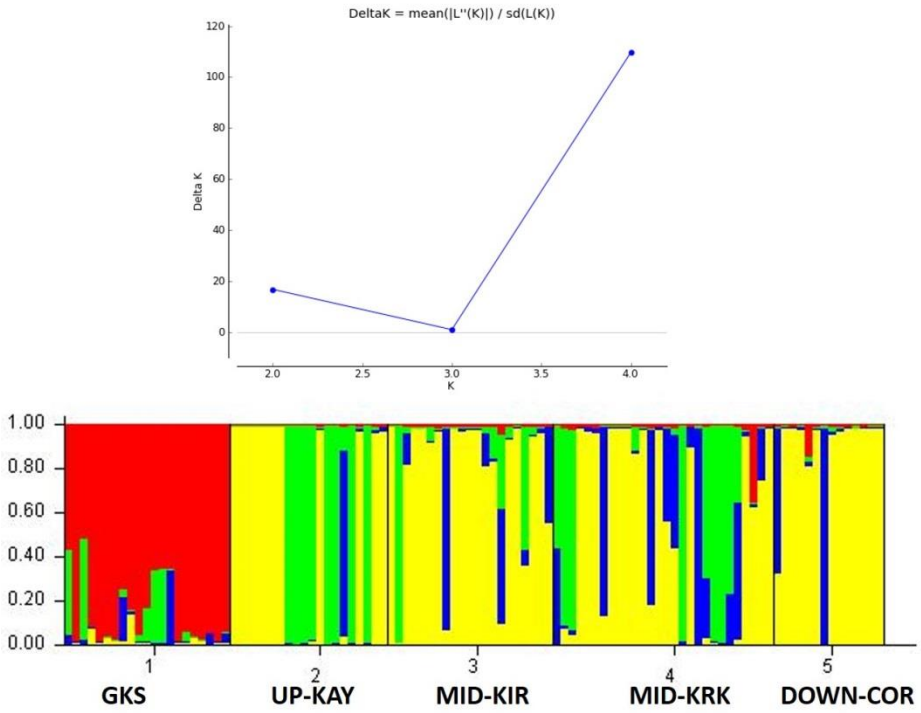


Figure 4.11. Clustering pattern (based on STRUCTURE analysis) of *Populus nigra* populations from Kızılırmak and Göksu rivers with prior information and 50000 of burn-in and 150000 of Markov chain Monte Carlo iterations (The highest ΔK value at K=4. Red, yellow, green and blue colors represent cluster 1, cluster 2, cluster 3 and cluster 4, respectively. Black vertical lines separate populations).

To detect the genetic structure of the Kızılırmak river populations, a new STRUCTURE analysis was implemented with four populations. Kızılırmak river seemed to be originated from three different genetic groups of the European black poplar (Figure 4.12, Figure 4.13). Eventhough, three genetic groups were present, one genetic group with higher membership values was dominant (Table 4.15). When K value accepted as 4 for Göksu and Kızılırmak populations, individuals from Kızılırmak river represent the same genetic structure with similar membership values for K= 3.

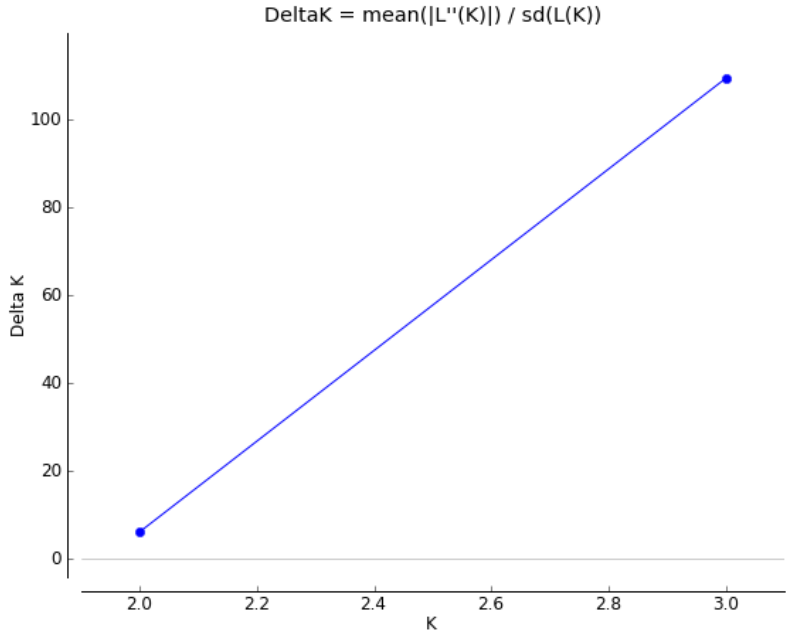


Figure 4.12. Graph of ΔK value for Kızılırmak populations determined by Evanno's method

Table 4.15. The proportion of estimated membership to each of the three inferred genetic groups of Kızılırmak river populations

Given population	Inferred cluster 1	Inferred cluster 2	Inferred cluster 3	Number of individuals
UP-KAY	0.55	0. 05	0.40	20
MID-KIR	0.78	0.11	0.11	21
MID-KRK	0.53	0.21	0.26	28
DOWN-COR	0.86	0.13	0.01	14

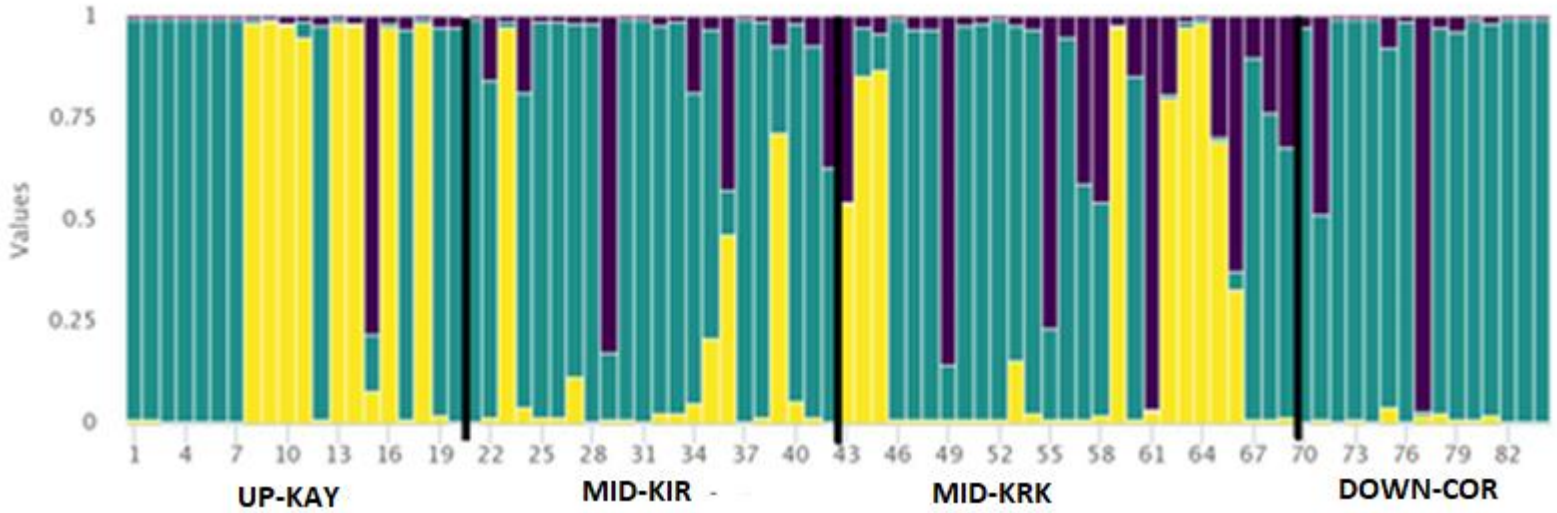


Figure 4.13. Clustering pattern (based on STRUCTURE analysis) of *Populus nigra* populations from Kızılırmak river (Green, purple and yellow colors represent cluster 1, cluster 2 and cluster 3, respectively. Black vertical lines separate populations).

4.5.6. Discriminant Analysis of Principle Components (DAPC) and Minimum Spanning Network analysis (MSN)

DAPC analysis was performed to group the studied European black poplar populations of the two river systems. As parallel to the obtained results from PCoA, the DAPC showed that Göksu river population was clearly separated from Kızılırmak populations (Figure 4.14). The separation of Göksu population from Kızılırmak river populations by STRUCTURE analysis (when K value accepted as 4) was also confirmed by DAPC analysis, which estimated cluster numbers for Göksu and Kızılırmak river populations as 6.

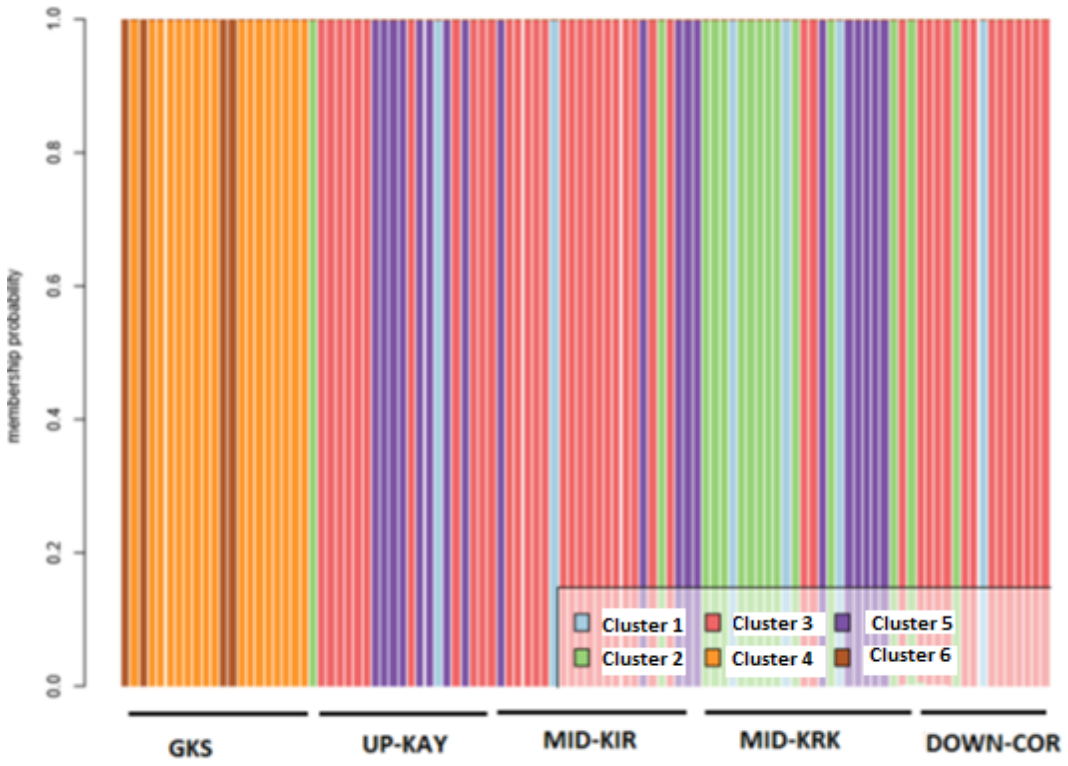


Figure 4.14. Estimated clusters for Göksu and Kızılırmak river populations by Discriminant Analysis of Principle Components

The result of MSN analysis indicated that individuals of the Göksu river were closely grouped together and clearly separated from individuals of Kızılırmak river. The members of Kızılırmak river were not found to be related with their originated upstream, middle and downstream populations (Figure 4.15). They were mainly found to be genetically close to each other.

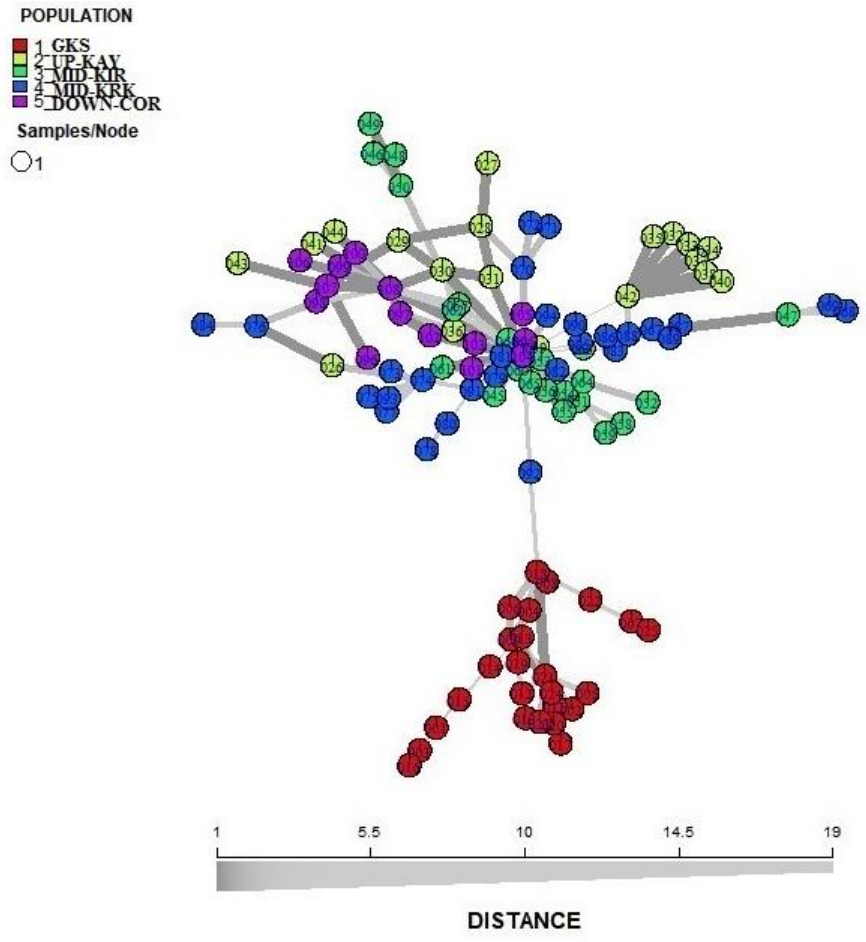


Figure 4.15. Minimum Spanning Network analysis (MSN) of 104 *Populus nigra* trees from Göksu and Kızılırmak rivers.

CHAPTER 5

DISCUSSION

In a previous study, in order to reveal existing genetic structure of *Populus nigra* populations in Turkey, about three hundred European black poplar clones sampled from all over Turkey for *ex situ* conservation were screened with SSR markers for breeding purposes (Çiftçi et al. 2017). Due to phenotypic selection and extensive circulations of clonal materials, accurate level of genetic diversity of the populations could not be investigated. To determine the level of genetic diversity and structure of European black poplar populations in riparian ecosystems, natural populations from two major river systems in Turkey were studied with 20 nuclear microsatellite markers.

5.1. Clonality of Populations

The results of present study revealed that there were clones which were widely moved around the Kızılırmak river system. Although all precautions were taken during the sampling of trees, five trees which were repeatedly duplicated in different numbers were sampled in UP-KAY and DOWN-COR populations of Kızılırmak river system. Previous studies reported either no clonal duplication (Smulders et al. 2008a; Jelic et al. 2015) or variable degree of duplication in their study materials (Pospiskova and Bartokova 2004; Legionnet et al. 1997) depending on their sampling area and set up. The high amount of clonal duplications found in populations of the Kızılırmak river could be explained by results of reduced natural dynamics of the river due to constructed dams and high level of human activity. In addition, ability to undergone vegetative propagation and dispersal of seeds and propagules easily by water and wind increase the level of clonality within the river system. High occurrence of identical

ramets in upstream and downstream populations could be due to clonal propagation from root suckers and rooting of branches and twigs carried from upstream and middle parts of the river to downstream by river flow (Barsoum et al. 2004).

5.2. Hybridization and Intogression Event

To be sure about the integrity of *P. nigra* trees and detect any intogression and hybridization events, NEWHYBRID program was implemented with Bayesian probability calculations by using different allele combinations and allele frequencies at multiple loci. The analysis on integrity of *P. nigra*, existence of introgression and hybridization showed that except for one tree from Kızılırmak and four trees from Göksu river systems, the remaining trees were classified as pure *P. nigra* with 99.9% integrity. Hybrid trees were placed into F2 hybrid class with 0.99 probability for Göksu and 0.61 for Kızılırmak river. Hybrids seem to be frequent in European black poplar populations. For example, Smulders et al. (2008b) reported that 14 of 42 young poplar trees sampled along the Rhine river in Netherlands to be assigned as F2 or backcross hybrid.

Compared to a previous study (Jelic et al. 2015) which concerns the presence of hybrids with *Populus deltoides*, the number of hybrids in the current study is very low especially in the Kızılırmak river. This is most likely to occur due to lack of suitable climate and plantation sites available for hybrid poplar. Relatively high presence of hybrids in Göksu river can be explained by existence of some commercial plantations with hybrid poplar (*P. ×canadensis*) which provide raw materials for a Paper Mill located in Tasucu town in the Göksu Delta. The detected hybrids (F2) were found to be derived from a direct mating between cultivated *P. ×canadensis* clones. This result indicates that hybrid trees in the commercial plantations produce pollen and seeds. Eventhough, Heinze and Lickl (2002) doubted whether the genomes of *P. deltoides* and *P. nigra* were sufficiently congruent to allow F2 and BC in all directions, it was

reported that there are no apparent crossing barriers for gene flow between cultivated hybrid poplars and native European black poplar (Vanden Broeck et al. 2004; Smulders et al. 2008 b). It is observed that hybrid seedlings are grown naturally in high number around the Göksu river banks. Similar to the results of Smulders et al. (2008 b), occurrence of low quantity of *P. nigra* seedlings along the Göksu river could be explained that high numbers of hybrid seedlings outcompete and suppress *P. nigra* seedlings to be established in the same habitat along the river ecosystems (Heinze 2008).

The length of species specific alleles for the studied loci was slightly different from those reported in the previous studies. Fossati et al. (2003) and Smulders et al. (2008b) found the *P. deltoides*-specific allele as 234 bp at the locus WPMS09. In the current study, the length of this allele was 232 bp which was similar to the findings of Jelic et al. (2015). Also, four alleles at the WPMS09 were detected as specific to in *P. deltoides* (218, 220, 230, and 236 bp). Even though, Jelic et al. (2015) reported the length of diagnostic allele for the locus WPMS18 as 213 bp, the result of Fossati et al. (2003), Smulders et al. (2008b) and current study showed as of 220 bp for *P. deltoides*. Smulders et al. (2008b) and Fossati et al. (2003) also reported specific alleles of *P. deltoides* in the PMGC14 locus as 193 and 196 bp, and 193, 199 bp, respectively. In contrast, these three alleles were not specific to *P. deltoides*, and observed with variable frequencies ranging between low and high in *P. nigra* populations in the current study. Allele with 190 bp length corresponding to the alleles 189 bp in Jelic et al. (2015) was detected only in *P. deltoides* trees (Table 3). For the locus PMGC2163, 186 bp allele was found to be as diagnostic for *P. deltoides* in the present study. However, Khasa et al (2005) reported two diagnostic alleles as 185 and 187.

In the current study, WPMS16 and PMGC2709 contain alleles found in only *P. deltoides* at 129, 135 and 147 bp and at 202, 210, 214, 216, 220, 222 and 224 bp,

respectively with low frequencies. For the WPMS16 locus, there is an overlapping allelic range for *P. nigra* and *P. deltoides*. While Fossati et al. (2003) did not find any specific allele for the WPMS16 locus, Smulders et al. (2008b) and Jelic et al. (2015) revealed some specific alleles in *P. deltoides*. Even though the frequencies of these informative alleles were low to be used as diagnostic marker, they were analyzed to increase power of estimating probability of hybrid status of individuals in these two previous studies and in the current study. To understand the reliability of WPMS16 and PMGC2709 loci to detect *P. deltoides* contributions, a more reliable background from different black poplar and *P. deltoides* populations from different countries should be considered in future studies. Species specific and diagnostic alleles found for *P. deltoides* in the present study could be useful to make inferences on the genetic background of European black poplar trees in the natural distribution area and to identify hybrid and pure trees in natural stands.

5.3. Genetic Diversity Pattern for Loci

There were considerable differences between European black poplar populations of Turkey and other European countries with regard to allelic diversity, observed and expected heterozygosity values as a result of population size, demographic history of populations and different study area in terms of climate, geography and topography. All loci which are polymorphic have a good ability to infer differentiation among populations of two rivers. Based on allelic richness values, assessing populations' long-term potential more effectively than heterozygosity (Allendorf 1986), locus PMGC14 was the most informative with the highest allelic richness and PIC values. The number of effective and observed alleles and allelic richness for all loci were slightly lower compared to the study of Jelic et al. (2015), Cortan et al. (2016), except WPMS16, and similar compared to Ratcmatcher et al. (2010).

High levels of observed heterozygosity and low levels of expected heterozygosity for the studied loci were found as compared to the previous studies which used several common SSR markers (Lewandowski et al. 2017 (WPMS03, WPMS04, WPMS05, WPMS07, WPMS09, WPMS10, WPMS12), Cortan et al. 2016 (WPMS09, WPMS14, WPMS16, WPMS18, WPMS20, PMGC14, PMGC2163), Jelic et al. 2015 (WPMS03, WPMS05, WPMS09, WPMS12, WPMS16, WPMS18,PMGC14), Rathmacher et al. 2010 (WPMS05, WPMS09, WPMS14, WPMS18, WPMS20, PMGC14, PMGC2163)). WPMS04, WMPS05 and WPMS10 loci indicated positive F_{IS} values due to excess of homozygote individuals derived from inbreeding of relevant trees. Eighteen of 20 SSR loci showed significant deviation from HWE proportions in Turkish natural populations due to the excess of heterozygosity known as a cause of deviation from the Hardy-Weinberg Equilibrium frequencies (Allendorf and Luikart 2007). Studied loci generally have common heterozygote patterns for majority of European black poplar trees and several homozygotes with rare alleles. Departures from HWE for 18 loci could be a result of reduced effective population sizes due to a recent bottleneck event, high level of gene flow, nonrandom mating and phenotypic selection and frequent dispersion of trees by human in the studied populations. In the studied European black poplar populations, assumptions of the Hardy-Weinberg (Random mating, no gene flow and large population size) were not in action. When comparing similar studies dealing with same loci, only one locus (PMGC14) showed deviation from HWE proportions in Danube populations and none in Serbia populations (Jelic et al. 2015; Cortan et al. 2016).

5.4. Genetic Diversity Pattern for Populations

Although all loci in the studied populations were polymorphic, observed and effective allele number and allelic richness were lower than similar studies carried out previously with the common markers (Jelic et al. 2015; Cortan et al. 2016; Çiftçi et al. 2017). This can be explained by the presence of some alleles with low frequencies in

the studied loci and larger total sample size and wider sampling area of the previous studies than current study. For the present study, all populations had a similar number of effective and observed alleles. Allelic richness did not vary significantly among populations. Genetic diversity is assessed generally by private alleles and the number of different alleles. The highest mean allelic richness with the highest genetic diversity (He=0.60) and private allele number was observed in MID-KRK population, whereas the lowest was found in the UP-KAY population. Due to the direction of the river from UP-KAY to MID-KRK, the MID-KRK population possibly accumulates greater diversity by receiving seeds and branches from upstream and midstream populations of the river. Although UP-KAY and MID-KRK populations have close altitude values, sampling areas of MID-KRK population has a relatively enlarged bed and various ecosystems that vary in the field depending on topographic structure and elevation (Büyükdemirci 2012) protecting genetic resources of the species when compared to location of UP-KAY population. These diverse habitats may have served as refuge areas to protect genetic resources of the species in MID-KRK population.

The determined level of genetic diversity of the European black poplar from the major river systems in Turkey were different from the previous studies (Van der Schoot et al. 2000; Smulders et al. 2001; Imbert and Lefevre 2003; Smulders et al. 2008; Rathmacher et al. 2010). When considering size of study area and number of studied loci, the results of the current study represent the reliable genetic diversity characterization of European black poplar populations in Turkey. Majority of studies SSR loci being selected from different chromosomes not only allowed reliable detection of genetic diversity, but also increasing statistical and discriminative powers of estimates. Observed heterozygosity values for the studied populations from the Kızılırmak and Göksu river systems were found to be significantly high compared to the results of previous studies from different European river systems using many common SSR loci, whereas expected heterozygosity values were found to be lower (Jelic et al. 2015; Cortan et al. 2016). Although, the higher observed heterozygosity

values were also reported by Pospikova and Bartakova (2004) and DeWoody et al. (2015), expected heterozygosity values of these studies were also higher than those in the present study. When estimation of expected and observed heterozygosities were carried out with only common loci used in present and previous studies (Jelic et al. 2015 (WPMS03, WPMS05, WPMS09, WPMS12, WPMS16, WPMS18, PMGC14), Cortan et al. 2016 (WPMS09, WPMS14, WPMS16, WPMS18, WPMS20, PMGC14, PMGC2163)), higher observed heterozygosity (0.91, 0.80) and lower expected heterozygosity values (0.62, 0.58) were obtained for the current study. Lower expected heterozygosity value indicates highly reduced level of genetic of diversity in the studied European black poplar populations. Higher observed heterozygosity value is an indicator of the more heterozygote individuals than homozygote in current populations.

All studied populations indicated highly significant departures from HWE due to higher observed heterozygosity value. The low Garza-Williamson index values point out that populations from both river systems may have experienced recent bottleneck event which may resulted in excess of heterozygosity, severe past reductions in population sizes and retaining small number of alleles in the populations of two river systems. When looking the frequency of alleles in each locus, it was found that several alleles for particular loci have been seemed commonly with higher frequency than other present alleles. Despite of reduced allelic diversity, occurrence of the high level of observed heterozygosity in the studied populations could be explained by the loss of allelic richness faster than loss of allelic size range and loss of heterozygosity caused by recent bottleneck (Garza and Williamson 2001). It is understood from this result; European black poplar populations in Turkey have been affected severely from bottleneck and dramatically lost its genetic diversity. DeWoody et al. (2015) also found excess of heterozygosity in their Western Europe populations due to recent bottleneck. The other explanation for higher heterozygosity values found in two river systems could be natural and human mediated selections of trees with heterozygote

superiorities. Heterozygote trees with high growth performance and resistant stem forms have been propagated and distributed over the years through local people. An example of this situation was demonstrated in the previous study which phenotypically selected one tree which was heterozygote in the most of the studied loci was duplicated and distributed whole Turkey in a varying number (Çiftçi et al. 2017).

5.5. Genetic Differentiation of Populations

The genetic differentiation among Göksu and Kızılırmak river systems across the 20 SSR loci was found to be low (F_{ST} = 0.06). This is also revealed by the results of AMOVA (F_{CT}=0.08). The greatest amount of total genetic variation was due to among trees within population of the river systems (89.4%). About 8.76 % of total genetic variation was attributed to between two river systems, while a low level of genetic variation (1.78%) was among populations within the river systems. Upstream, middle and downstream populations of Kızılırmak river, which are sampled from geographically different locations show no significant differentiation from each other (F_{ST} = 0.03), suggesting that the existence of high level of gene flow throughout the dynamic river system. When compared to other forest trees, several studies dealing with *Populus* and *Salix* species belonging to the family Salicaceae have reported low genetic differentiation of populations between and among river systems (Gebhardt et al. 2002, F_{ST} =0.053 for *P. nigra*; Hall et al. 2007, F_{ST} = 0.015 for *P. tremula*; Cole 2005 , F_{ST} = 0.045 for *P. tremuloides,* Trybush et al. 2012, F_{ST} = 0.05 for *Salix viminalis*; Perdereau et al. 2014, G_{ST} = 0.07 for *Salix caprea*) as a result of extensive gene flow promoted by wind and river flow (Van Splunder et al. 1995). High level of gene flow among European black poplar populations throughout the river basins was recorded in different river basin of Europe due to wind-pollinating, outcrossing and clonal reproductive nature of the species (Pospiskova and Bartokova 2004, Smulders et al. 2008, Jelic et al. 2015).

Principal Coordinate Analysis performed with pairwise F_{ST} values clearly discriminated Göksu population from Kızılırmak populations. Distinction of Göksu from Kızılırmak populations could be explained by higher number of private alleles (1.05), differences in allele frequencies of studied loci and absence of common alleles (Number of Common alleles<0.50) in GKS population. As parallel to the PCoA result, the DAPC, MSN and genetic distance based Neighbour- Joining analyses provided additional support that trees sampled from the Göksu population were clearly separated from Kızılırmak populations. When looking the phenetic relationship of populations from two rivers, it is clear that Göksu and Kızılırmak populations were clustered into two groups. Four populations collected from Kızılırmak are close to each other as a result of vegetative material circulation and high level of gene flow among these locations. The results of Mantel test indicated that differentiation between population pairs is not the result of isolation by distance since low level of correlation between genetic and geographic distances was estimated (P value= 0.043). The result of barrier detection analysis indicated that there is one possible barrier between Göksu and Kızılırmak river populations causing possibly differentiation of populations. This barrier acting as a geographic barrier in shaping the spatial genetic structure of European black poplar between two river systems is Taurus mountain range which possibly hinders extreme gene flow between two river systems.

The STRUCTURE program applying a model-based clustering algorithm placed 104 European black poplar trees into two inferred clusters with distinctive allele frequencies and generally high estimated membership probability. All populations from Göksu and Kızılırmak rivers, assigned in to the first inferred cluster, recruited trees from two different genetic groups. The collections from GKS and Kızılırmak rivers were not separated from each other. There is tendency in STRUCTURE results that K, cluster number, is usually reported as 2. The optimal number of cluster should be determined by careful evaluation of STRUCTURE results and biology of studied species (Jane et al. 2017). When considering the second largest value of ΔK, five

populations were assigned to four clusters. Also results of the repeated different STRUCTURE runs gave the inferred cluster numbers as K=4. When comparing to STRUCTURE analysis, DAPC analysis define clusters more accurately because it is free of assumptions about HWE and linkage diseqilibrium. In addition, DAPC considers contribution of individual alleles to population structure to make visual assessment of population differentiation. With K=4 inferred cluster number, the Göksu river appeared to be a distinct subpopulation with membership up to 0.84 (The proportion of estimated membership to third inferred genetic group) in agreement with the results displayed by DAPC, MSN and genetic distance based neighbor joining analyses. Attempting to identify biologically meaningful differences, inferred cluster number was considered as K= 4 with the highest probability maintaining a small variance. Many individuals from Kızılırmak river were assigned into second genetic cluster. Based on the ancestry values of all of the individuals, it was found that some of individuals from upstream, middle and downstream populations were placed to first and fourth genetic clusters with high inferred membership values. Eventhough, DAPC analysis identified six genetic group for five European black poplar populations, it is clear that Göksu population has a different genetic structure from Kızılırmak river populations. When considering genetic clustering result of the DAPC analysis, it is found that several members of Göksu and MID-KRK populations were placed into two additional genetic clusters compared to STRUCTURE result. Presence of these two cluster within Göksu and MID-KRK populations could be explained by individuals with private alleles at several loci in these two populations.

When considering Kızılırmak river, genetic structure analysis provided that upstream, middle and downstream populations of the river were assigned to three genetic groups without a distinct differentiation among them (F_{ST} = 0.03) with higher inferred membership values. It was observed that many trees from four populations of the Kızılırmak river were originated from a major gene pool. Besides, trees assigned to the second and third clusters were possibly coming from different natural stands.

MSN analysis also detected close relationships among several individuals of each studied populations in Kızılırmak river with low genetic distance among them. The existence of high level of gene flow throughout the river cause to low level of differentiation among Kızılırmak populations. Similarly, high level of gene flow in different river basin of Europe was recorded (Pospiskova and Bartokova 2004; Smulders et al. 2008; Jelic et al. 2015).

In terms of genetic admixture, DOWN-COR population seemed to be more homogenous than midstream and upstream populations. High level of admixture in MID-KRK population could be explained by extensive human assisted clonal material circulation in watersheds of the river located mostly unforested high plateau. Additionally, the river accumulation of seeds and branches from UP-KAY and MID-KIR populations could cause to admixture in MID-KRK population. Conversely, downstream population seemed to be less affected by human as a result of its forested sampling area, Blacksea region, where there is less need for the poplar wood. Riverbanks of Samsun region including DOWN-COR population are not easily accessible to local people to influence the genetic structure of populations. In addition, constructed three dams between MID-KRK and DOWN-COR populations may have contributed to less admixture.

Easy dispersal of seeds via wind and water, vegetative reproducing ability of species and intensive human mediated dispersal of vegetative materials throughout the Anatolia for many years have shaped current genetic structure of populations in not only two river systems, but also throughout the natural range of species. European black poplar trees from natural stands have been cultivated traditionally by farmers in Anatolia for a millennium and scattered throughout the country (Tunçtaner 1998) with historical and recent internal migration events (Berkman et al. 2008). The results of current study indicate that extensive human-assisted migration and selection activities

and generative reproduction in its natural ecosystems have been changed the genetic structure of local gene pools of European black poplar.

CHAPTER 6

CONCLUSION

We characterize the actual genetic diversity and structure of natural populations of European black poplar from highly fragmented Kızılırmak and Göksu river systems in Turkey by screening 104 trees with 20 polymorphic microsatellite loci. Genetic integrity of the studied trees was determined by using informative and diagnostic alleles of *P. nigra* and *P. deltoides* trees at six loci.

The results of the study indicated that the natural genetic resources of the species are highly degraded due to anthropogenic effect and biological process (gene pollution from hybrid poplar and a recent bottleneck). When compared to similar studies, the genetic variation of the studied populations is found to be low level. All populations have experienced a recent reduction in population size, having excess of heterozygosity. Vegetative reproducing ability of species, easy dispersal of its seeds and intensive human mediated dispersal of vegetative materials appeared to have major role in determining current genetic structure of populations in not only two river systems, but also throughout the natural range of species. Past bottleneck event have reduced adaptive potential of population and result in collapse of the species gene pool in a short time by increasing inbreeding level and loss of genetic variation. It is urgent to declare *Populus nigra* habitats as national protected areas to conserve and manage Turkey's valuable black poplar heritage for future generations

European black poplar is an important tree species in terms of social, economic and ecological interest in Turkey. Although, high amount of poplar plantations meets the needs of the economy, conservation and restoration of natural genetic resources of the species have not been given high priority. Although, a countrywide conservation program including *in situ* and *ex situ* studies has been initiated, there are no restrictions or regulations on plantations and harvest of European black poplar in its natural range because of exemption of the species from the national forestry regulations. Urbanization, disruption and fragmentation of the species distribution area make it difficult to *in situ* conservation of the genetic resources of European black poplar along Kızılırmak and Göksu rivers.

In order to make efficient conservation and sustainable management of existing natural populations, effective *in situ* and *ex situ* conservation programs should be started in Turkey. To increase genetic diversity of European black poplar populations in the Göksu and Kızılırmak river systems, populations could be diversified by redistributing genotypes from different natural stands. The genetic data generated in this study provide a starting point for conserving valuable natural resources of European black poplar to prevent further genetic degradation and to improve efficiency of breeding programs concerning this species.

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APPENDICES

A. CTAB DNA EXTRACTION PROTOCOL

1. 2xCTAB extraction buffer (2% (w/v) CTAB, 5M NaCI, 0,5M EDTA, 1M Tris-HCI, pH 8.0) was heated at 65°C in a water bath for an hour.
2. 0.5g leaf tissue was weighed and placed in a 2 ml Eppendorf tube for each leaf sample.
3. 1500µL of the heated CTAB solution was added into 2ml Eppendorf tubes with leaf tissue. 75µL β-mercaptoethanol and 5µL Proteinase K were added to each tube and incubated in water bath at 65°C for 1 hour.
4. The tubes were centrifuged at 4°C for 20 minutes at 13000 rpm. 800 µL aqueous phase was collected to a new 2 ml Eppendorf tube.
5. 0.8V Chloroform/Octanol (24:1) was added on the aquous phase. The tubes were inverted gently a few times and centrifuged at 4°C for 15 minutes at 13000rpm.
6. 500µL supernatant was taken in a new 1.5mL Eppendorf tube and 500 µL ice cold Isopropanol was added. Tubes were incubated at -80°C for 1 hour.
7. Samples were centrifuged at 13000rpm for 25 minutes at 4 °C. The supernatant was removed and the pellet was washed with cold 70% EtOH, twice.
8. Air dryed pellets were resuspended in 75µL TE buffer o/n.

B. BUFFERS CHEMICALS AND EQUIPMENTS

Buffers and solutions for DNA isolation

2X CTAB: 2 gr CTAB (Cetyl Trimethyl Ammonium Bromide), (SIGMA)

4 ml (pH: 8) 0.5 M EDTA, (FLUKA)

10 ml (pH: 8) Tris HCL, (SIGMA)

28 ml NaCl is completed with 100 mL distilled water

Phenol, (AMRESCO)**:** Pure phenol

Chloroform isoamil alcohol, (FLUKA): (24/1)

Ethanol: 70% in distilled water

β mercapto ethanol, (SIGMA): 17,5 ml β mercapto ethanol is completed with 250 ml with distilled water

TE buffer: 10mm Tris HCL (pH: 7) 10mm ethylene diamine tetra acetic acid disodium salt (EDTA)

Isopropanol, (FLUKA): Pure Isopropanol, ice cold

Buffers and solutions for PCR

Sterile water

Taq DNA Polymerase (SIGMA Red *Taq*): 1U/µl

10X PCR buffer including MgCl2 (SIGMA)

dNTPs (SIGMA): 10mM

DNA: 10ng/ µl

Primer Pairs: 10µM

Agarose Gel Electrophoresis Buffers and Gel System

10X TBE Buffer: 108 gr Trizma Base, (SIGMA), 55 gr Boric Acid, (SIGMA)

Running Buffers: X TBE prepared in distilled water

Ethyidium Bromide, (SIGMA):4 mg/ ml

Agarose, (SIGMA): 3 % Agorose Gel

40 ml EDTA, (FLUKA) (0.5 M, pH: 8) completed with 1000 ml with distilled water

Low molecular weight DNA Ladder (SIGMA)

Equipments

Autoclave: Yamato

Centrifuge: Nüve- NF048

Electrophoresis System: Thermo Scientific

Thermocyclers: Eppendorf- Mastercycler

Deepfreezer: UĞUR- Freezer

Magnetic Stirrer: Labor Brand – Hotplate L-81

Refrigerator: Siemens

UV Transilluminator: Vilbor Lourmant

Vortex: Nüve- NM110

Water Bath: Memmert

Oven: Dedeoğlu

Micropipettes: Gılson

PHmeter: Hanna Inst.

C. ASSAY PROCEDURE WAS DONE BY THE BM LABOSİS

1. Registration of PCR product (by Customer)

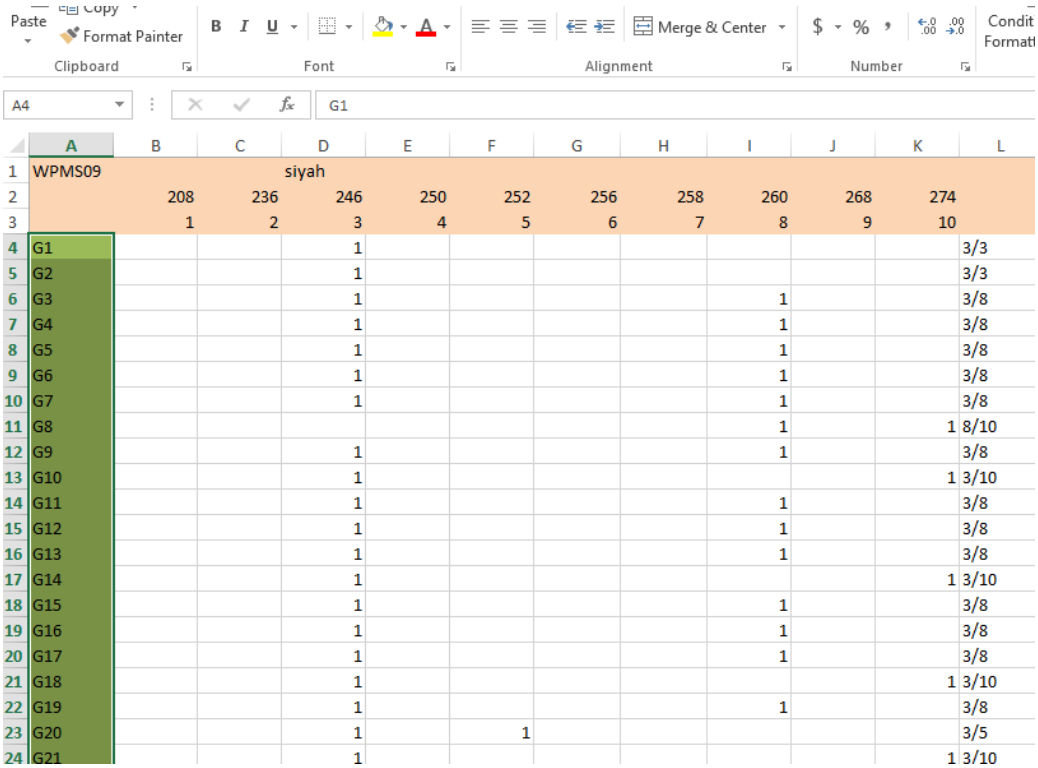
2. PCR product + Hi-Di formamide + size standard * size standard type: 400HD

3. Denaturation

4. 3730xl running by using Dye set: DS-30 set for internal standard size marker 400HD, DS-33 set for internal standard size marker 400HD

5. Genemapper v.5 analysis

D. A PART OF EXCEL MATRIX SHOWING GENOTYPES ALLELE SIZES



	A	B	C	D	E	F	G	H	I	J	K	L
1	WPMS09			siyah								
2		208	236	246	250	252	256	258	260	268	274	
3		1	2	3	4	5	6	7	8	9	10	
4	G1			1								3/3
5	G2			1								3/3
6	G3			1					1			3/8
7	G4			1					1			3/8
8	G5			1					1			3/8
9	G6			1					1			3/8
10	G7			1					1			3/8
11	G8								1		1	8/10
12	G9			1					1			3/8
13	G10			1							1	3/10
14	G11			1					1			3/8
15	G12			1					1			3/8
16	G13			1					1			3/8
17	G14			1							1	3/10
18	G15			1					1			3/8
19	G16			1					1			3/8
20	G17			1					1			3/8
21	G18			1							1	3/10
22	G19			1					1			3/8
23	G20			1		1						3/5
24	G21			1							1	3/10

E. A SUMMARY OF THE STATISTICS USED IN THESIS

The statistics used in the MICRO-CHECKER

Brookfield (1996) Null Allele Estimator 1

Assuming there are no null allele homozygotes or ignoring all non-amplified samples as degraded DNA, human error, etc., the Brookfield (1996) estimate of the null allele frequency is given by:

r = (He - Ho) / (1 + He)

The Confidence Interval

Bonferroni (Dunn-Sidak) adjusted 95% confidence interval is used. The values for a randomised locus are placed in an ordered list. If, for example, the 95% Confidence Interval is selected, the top 2.5% of the values and the bottom 2.5% of the values are removed from the list. The highest and lowest values in the remaining list provide the range of the Confidence Interval.

The Bonferroni (Dunn-Sidák) adjusted Confidence Interval is based on the formula:

$$a' = 1 - (1 - a)^{1/k}$$

where a is 0.05 (the complement of 0.95 or 95%) and k is the number of comparisons (Sokal and Rohlf, 1995).

The statistics used in GenAlEx 6.5

Number of different alleles (Na)

Determined by direct count. GenAlEx also provides the arithmetic mean across loci.

Effective number of alleles (Ne)

Ne represents an estimate of the number of equally frequent alleles in an ideal population. Ne enables meaningful comparisons of allelic diversity across loci with diverse allele frequency distributions. The formula is as follows;

$$Ne = \frac{1}{1-He}$$

Ne via *Frequency* is calculated by locus from HE for each population.

Number of private alleles

Equivalent to the number of alleles unique to a single population in the data set.

Shannon's Information Index (I)

Calculated on a single-locus basis, where ln = the natural logarithm and pi is the frequency of the i^{th} allele. Equivalent to the Shannon-Weaver Index of ecology. Unlike He, not bounded by 1 and may therefore be a better measure of allelic and genetic diversity, though largely overlooked in genetic studies. The formula is as follows;

$$I = \sum pi \mathrm{Ln}\, pi$$

pi is the allele frequency of the i^{th} allele at the locus in question for the specified population.

Expected heterozygosity (*He*)

He is the Expected Heterozygosity or Genetic Diversity within a population. Calculated per locus as 1 minus the sum of the squared allele frequencies, pi^2. The formula is as follows;

$$He = 1 - \sum pi^2$$

Expected Heterozygosity averaged across populations (Mean He)

The average *He* or genetic diversity per population, also called *Hs* and used in the calculation of *F*- statistics. Where *Hes* is the expected heterozygosity in the *s*-th population; *k* is the number of populations. The formula is as follows;

$$Hs = \overline{He} = \frac{\sum H_{Es}}{k}$$

Observed Heterozygosity (Ho)

Observed heterozygosity for a single locus within a population, where the number of heterozygotes is determined by direct count, *N* = sample size. The formula is as follows;

$$Ho = \frac{Number\ of\ heterozygotes}{N}$$

Observed heterozygosity, averaged across populations (Mean Ho)

The average observed heterozygosity of a collection of populations, used in the calculation of F-statistics. Here, Hos is the observed heterozygosity in the s-th population; k is the number of populations. The formula is as follows;

$$\overline{\boldsymbol{H}}_{\boldsymbol{o}} = \frac{\sum \boldsymbol{H_{Os}}}{\boldsymbol{k}}$$

Fixation Index (F)

Calculated on a per locus basis. GenAlEx also provides the arithmetic mean across loci. Values close to zero are expected under random mating, while substantial positive values indicate inbreeding or undetected null alleles. Negative values indicate excess of heterozygosity, due to negative assortative mating, or heterotic selection. The formula is as follows;

$$F = \frac{H_e - H_o}{H_e}$$

Probability of Identity (PI)

The Probability of Identity PI provides an estimate of the average probability that two unrelated individuals, drawn from the same randomly mating population, will by chance have the same multilocus genotype. Also called Population Match Probability. PI is an indication of the statistical power of a specific set of marker loci.

PI is the frequency of the *i*th allele at a locus. For multiple loci calculated as the product of individual locus *PI*'s. *PI* represents the average probability of a match for any genotype, rather than for a specific genotype, as in Genotype Probability (Taberlet and Luikart 1999; Waits et al. 2001). The formula is as follows;

$$PI = 2\left(\sum p_i^2\right)^2 - \sum p_i^4$$

F statistics

F statistics show the statistically expected level of heterozygosity in a population. It measures the degree of a reduction in heterozygosity relative to Hardy-Weinberg expectations (Allendorf and Luikart, 2007).

Individual Heterozygosity (Hı)

H-indiv = the proportion of loci that are heterozygous across an individual, where nH is the number of heterozygous loci, and nL is the number of loci. When compared across individuals H-indiv can offer important clues about the amount and distribution of inbreeding in populations.

$$H_I = \frac{nH}{nL}$$

Average within Population heterozygosity (Hs)

Identical to the mean He, being the average of the within population expected heterozygosity across populations.

$H_e = 1 - \sum p_i^2$; $H_S = \overline{H_E} = \frac{\sum H_{Es}}{k}$

Total expected heterozygosity (Ht)

Ht is the expected heterozygosity if all populations were pooled (no subdivision). Calculated as 1 minus the sum of the average allele frequencies over populations.

$$H_T = 1 - \sum_{i=1}^{h} \overline{p}_i^2$$

There are three indices used in F statistics (Wright, 1951);

F_{IS}, (Inbreeding coefficient) the measure of reduction of heterozygosity of an individual due to nonrandom mating within subpopulations. The formula is;

$$F_{IS} = 1 - \left(\frac{H_I}{H_S}\right)$$

F_{IT}, the measure of reduction of heterozygosity of an individual in relation to the total population and the formula is;

$$F_{IT} = 1 - \left(\frac{H_I}{H_T}\right)$$

F_{ST}, (Fixation index within subpopulations, relative to the total) provides a measure of the genetic differentiation among populations. That is, the proportion of the total genetic divergence that separates the populations. F_{ST} is typically greater than or equal to zero (but can be slightly negative). If all subpopulations are in Hardy-Weinberg equilibrium with the same allele frequencies, $F_{ST} \approx 0$. (Note that the s used for subpopulations in the notation for Fst). The formula is as follows;

$$F_{ST} = 1 - \left(\frac{H_S}{H_T}\right)$$

The genetic structure of populations can be estimated by the formula

$$(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$$

where H_I represents the average observed heterozygosity in subpopulations, H_S represents the average expected heterozygosity estimated from each subpopulation and H_T represents the total gene diversity or expected heterozygosity in the total population as estimated from the pooled allele frequencies (Allendorf and Luikart, 2007).

Number of Migrants (Nm)

Where F_{ST} represents the degree of population genetic differentiation.

$$Nm = \frac{\left[\left(\frac{1}{F_{ST}}\right) - 1\right]}{4}$$

Nei's Genetic Distance

Nei's genetic distance D, where I is Nei's Genetic Identity.

$$D = -\ln(I)$$

Nei's Genetic Identity

p_{ix} and p_{iy} are the frequencies of the i^{th} allele in populations x and y. For multiple loci, J_{xy}, J_x and J_y are calculated by summing over all loci and alleles and dividing by the number of loci. These average values are then used to calculate I**.** (Nei, 1972;1978)

$$I = \frac{J_{xy}}{\sqrt{(J_x J_y)}} \; , J_{xy} = \sum_{i=1}^{k} P_{ix} P_{iy} \; , J_x = \sum_{i=1}^{k} P_{ix}^2 \quad , \; J_y = \sum_{i=1}^{k} P_{iy}^2$$

Standard Error

SE is the standard error of the mean and is widely reported when the arithmetic mean and other summary statistics are reported. Where s is the standard deviation and n is the sample size.

$$SE = \frac{s}{\sqrt{n}}$$

Polymorphic Information Content (PIC)

Polymorphic information content (PIC) is a measure of informativeness related to expected heterozygosity and likewise is calculated from allele frequencies (Botstein *et al.* 1980; Hearne *et al.* 1992). It is commonly used in linkage mapping.

CERVUS calculates an average PIC across all loci, the arithmetic average of the PIC values at each locus. PIC value is calculated for each primer not for each allele as follows:

Therefore PIC is calculated by the formula:

PIC= 1-∑(pi2)

Pi will be calculated for each allele. pi is the frequency of the i^{th} allele

pi=no. of alleles/no. of genotype

PIC Values range between 0 and 1. Primers whose pic value is zero or less than zero (i.e negative value) should not be used for analysis.

Percentage of Polymorphic Loci (%P)

P = mean proportion of loci polymorphic in population

$$P = \sum \frac{P_i}{N}$$

P_i = proportion of loci polymorphic in a population and N = number of populations.

Garza-Williamsion Index

Garza-Williamson index (G-W)

Following Garza and Wlliamson (2001), the G-W statistic is given as

$$G - W = \frac{k}{R + 1}$$

where k is the number of alleles at a given loci in a population sample, and R is the allelic range. Originally, the denominator was defined as just R in Garza and Wlliamson (2001), but this could lead to a division by zero if a sample is monomorphic. This adjustment was introduced in Excoffier *et al*. (2005).

This statistic was shown to be sensitive to population bottleneck, because the number of alleles is usually more reduced than the range by a recent reduction in population size, such that the distribution of allele length will show "vacant" positions. Therefore the G-W statistic is supposed to be very small in population having been through a bottleneck and close to one in stationary populations.

Statistics used to select K

To evaluate the K, *ad hoc* quantity (ΔK) was calculated. When ΔK shows the top peak it means the true value of K is obtained (Evanno *et al.,* 2005).

Ln P(D) shows the log likelihood for each K in STRUCTURE output obtained by first computing the log likelihood of the data at each step of the Markov Chain Monte Carlo(MCMC). The LnP(K) gives the mean likelihood over 10 runs for each K, the average of 10 values of Ln P(D). Steps for evaluating ΔK were as below;

The mean difference between successive likelihood values of K was plotted;

$$Ln'(K)=LnP(K)-LnP(K-1)$$

First the difference between values L′(K) were calculated and then, absolute value was taken;

$$|\ Ln''(K)=Ln'(K+1)-Ln'(K)\ |$$

Estimated ΔK as the mean of the absolute values of Ln″(K) was averaged over 10 runs and divided by the standard deviation of LnP(K);

$$\Delta K= (m|Ln''(K)|)/(s|LnP(K)|)$$

F. FILE FORMATS OF SOFTWARES

ARLEQUIN FILE FORMAT-ARP FILE

arlequinkavakzlgks.arp - Notepad

File Edit Format View Help

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Results from GENEPOP

Wed Jul 26 21:23:50 AWST 2017

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        LocusSeparator=WHITESPACE
        GameticPhase=0
        RecessiveData=0
        MissingData="?"

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        [[Samples]]
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          SampleSize=25
          SampleData= {
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          269  274  282  218  260  244  174  243  210  159  231  234  216  228  204  200  351  138  138  150
2     1   269  248  278  218  246  248  164  210  204  144  225  220  192  224  190  192  357  136  136  150
          281  274  278  218  274  248  174  243  210  159  225  234  198  224  194  212  363  160  138  160
3     1   263  248    ?  218  246  248  166  231  204  144  222  234  198  224  190  200  351  136  136  148
          269  248    ?  218  260  248  174  243  210  159  231  234  216  228  194  208  357  162  138  150
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FSTAT FILE FORMAT-DAT FILE

fstatkavakkzlgks.dat - Not Defteri

Dosya Düzen Biçim Görünüm Yardım

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WPMS20
WPMS04
WPMS05
WPMS07
WPMS09
WPMS10
WPMS12
WPMS14
WPMS15
WPMS16
WPMS18
WPMS03
PMGC14
PMGC21
PMGC27
PMGC28
PMGC93
PE5
PE13
PE14
1       263269 248274 278282 218218 246260 210244 164174 231243 204210 144159 222231 234234 198216 224228 190204
1       269281 248274 278278 218218 246274 248248 164174 210243 204210 144159 225225 220234 192198 224224 190194
1       263269 248248 000000 218218 246260 248248 166174 231243 204210 144159 222231 234234 198216 224228 190194
1       269281 274274 262278 218230 246274 248248 164174 210243 204210 144150 225225 220234 192198 224242 190194
1       269281 274274 262278 218218 246274 248248 000000 213243 204210 144150 225225 220234 192198 242242 190194
1       269281 274274 278278 218218 246274 248248 164174 210243 204210 144159 225231 220234 192198 224242 190194
1       269281 274274 278278 218218 246274 248248 164174 234252 204210 135144 225231 220226 189207 224242 202216
1       263263 274274 284288 218218 236252 248268 164164 210243 195195 144150 222228 220226 189207 242242 190194
1       269281 274274 278278 218218 246274 248248 164174 210243 204210 144150 225225 220234 192198 224242 190194
1       263269 274274 278278 218218 246260 248248 164174 231243 204210 144150 225231 220226 189207 224242 190194
1       269281 274274 278278 218230 246246 248248 000000 210243 204210 144150 222228 220234 192198 224242 190194
1       269281 274274 278278 218218 246260 248248 164174 231243 204210 144150 225225 234234 198216 000000 190194
1       269281 274274 278278 218218 246274 248248 166174 231243 204210 144150 225225 220234 198198 224242 190204
1       269281 248274 278282 218230 246260 248248 166174 210243 204210 144150 225225 234234 198216 224242 190204
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GDA FILE FORMAT-TXT FILE

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     format tokens missing=? datapoint=standard;
               locusallelelabels

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               2 'WPMS04'   [/ 247 249 254 256 260 264 274 286],
               3 'WPMS05'   [/ 224 226 228 232 236 246 262 268 274 278 280 282 284 286 288 290],
               4 'WPMS07'   [/ 214 218 220 224 226 230 232 234 236 240 246 250 254 258 262],
               5 'WPMS09'   [/ 208 236 246 250 252 256 258 260 268 274],
               6 'WPMS10'   [/ 210 220 236 238 244 246 248 254 268],
               7 'WPMS12'   [/ 156 160 164 166 174 178],
               8 'WPMS14'   [/ 210 213 216 225 231 234 237 243 246 249 252 260],
               9 'WPMS15'   [/ 183 195 204 210 213 216 219],
               10 'WPMS16'  [/ 130 136 141 144 150 153 156 159],
               11 'WMPS18'  [/ 220 225 228 231 234 237 246 249 252 261],
               12 'WPMS03'  [/216 220 224 226 228 234 236],
               13 'PMGC14'  [/ 189 192 195 198 201 204 207 210 213 216 219 225],
               14 'PMGC2163' [/ 186 192 198 206 210 216 220 222 224 228 234 240 242 246 248 252 256 260 266 268],
               15 'PMGC27'  [/ 190 194 198 200 202 204 208 210 216],
               16 'PMGC28'  [/ 180 184 190 192 198 200 202 208 210 212 216],
               17 'PMGC93'  [/ 348 351 354 357 363],
               18 'Pe13'  [/ 126 128 130 134 136 138 140 142 158],
               19 'Pe5'  [/ 130 134 136 138 152 158 160 162 168 174],
               20 'Pe14'  [/ 140 144 148 150 160];

matrix
GOKSU:                                                    |
G1       263/269 249/274 278/282 218/218 246/260 210/244 164/174 231/243 204/210 144/159 220/231 234/234 198/216 224/228 190/204
G2       269/281 249/274 278/278 218/218 246/274 248/248 164/174 210/243 204/210 144/159 225/225 220/234 192/198 224/224 190/194
G3       263/269 249/249  -1/-1  218/218 246/260 248/248 166/174 231/243 204/210 144/159 220/231 234/234 198/216 224/228 190/194
G4       269/281 274/274 262/278 218/230 246/274 248/248 164/174 210/243 204/210 144/150 225/225 220/234 192/198 224/242 190/194
G5       269/281 274/274 262/278 218/218 246/274 248/248  -1/-1  213/243 204/210 144/150 225/225 220/234 192/198 242/242 190/194
G6       269/281 274/274 278/278 218/218 246/274 248/248 164/174 210/243 204/210 144/159 225/231 220/234 192/198 224/242 190/194
G7       269/281 274/274 278/278 218/218 246/274 248/248 164/174 234/252 204/210 136/144 225/231 220/226 189/207 224/242 202/216
G8       263/263 274/274 284/288 218/218 236/252 248/268 164/164 210/243 195/195 144/150 220/228 220/226 189/207 242/242 190/194
G9       269/281 274/274 278/278 218/218 246/274 248/248 164/174 210/243 204/210 144/150 225/225 220/234 192/198 224/242 190/194
```

GENALEX FORMAT- EXCEL FILE

20	109	5	25	20	22	28	14						
Example			Goksu	Avanos	Beynam	Kalecik	Bafra						
	Pop	WPMS20	WPMS20	WPMS04	WPMS04E	WPMS05A	WPMS05B	WPMS07A	WPMS07B	WPMS09A	WPMS09B	WPMS10A	WPMS10B
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2	G2	269	281	248	274	278	278	218	218	246	274	248	248
3	G3	263	269	248	248	0	0	218	218	246	260	248	248
4	G4	269	281	274	274	262	278	218	230	246	274	248	248
5	G5	269	281	274	274	262	278	218	218	246	274	248	248
6	G6	269	281	274	274	278	278	218	218	246	274	248	248
7	G7	269	281	274	274	278	278	218	218	246	274	248	248
8	G8	263	263	274	274	284	288	218	218	236	252	248	268
9	G9	269	281	274	274	278	278	218	218	246	274	248	248
10	G10	263	269	274	274	278	278	218	218	246	260	248	248
11	G11	269	281	274	274	278	278	218	230	246	246	248	248
12	G12	269	281	274	274	278	278	218	218	246	260	248	248
13	G13	269	281	274	274	278	278	218	218	246	274	248	248
14	G14	269	281	248	274	278	282	218	230	246	260	248	248
15	G15	263	269	248	274	278	282	218	218	246	260	244	248
16	G16	263	269	248	274	278	280	218	218	246	260	248	248
17	G17	263	263	260	260	278	288	218	218	208	208	248	248
18	G18	269	281	274	274	278	278	218	218	246	274	248	248
19	G19	269	281	274	274	278	278	218	218	246	274	248	248
20	G20	269	281	248	274	278	278	218	218	0	0	248	248
21	G21	269	281	274	274	278	278	218	218	246	274	248	248
22	G22	269	281	274	274	278	278	218	218	246	274	248	248
23	G23	269	281	274	274	278	278	218	218	268	268	0	0
24	G24	269	281	0	0	0	0	218	218	246	274	236	246
25	G25	269	269	260	260	284	288	218	218	236	252	220	236
26	ka1	269	281	274	274	274	274	230	230	246	274	248	248
27	ka2	269	281	274	274	278	278	230	230	246	246	248	248

GENCLONE 2.0 FILE FORMAT-TXT FILE

geneclone2017..txt - Not Defteri

Dosya Düzen Biçim Görünüm Yardım

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1	999	999	269281	274274	228278	226230	246246	248248	164174	210243	204210	144150
2	999	999	263269	274274	278284	230232	246246	248248	164174	210243	204210	150150
3	999	999	251269	246246	278284	226262	246260	248248	164174	210243	204210	144150
4	999	999	263269	246274	278284	230262	246260	248248	164174	231243	204210	144150
5	999	999	263269	246274	278284	230262	246260	248248	164174	231243	204210	144150
6	999	999	251251	246274	278284	230230	246260	248248	166174	231243	204210	144150
7	999	999	263269	246274	278284	230262	246260	248248	166174	231243	204210	144150
8	999	999	251269	246246	278284	226262	260274	248248	166174	210243	204210	144150
9	999	999	263269	246246	278284	230262	246260	248248	166174	231243	204210	144150
10	999	999	263281	274274	278284	230230	246274	248248	164174	231243	204210	144144
11	999	999	263269	246274	278284	230262	246260	248248	166174	231243	204210	144150
12	999	999	263281	246246	278284	230262	246260	248248	166174	243243	204210	144144
13	999	999	263269	246274	278284	230262	246260	248248	166174	231243	204210	144150
14	999	999	269281	248274	278278	226230	246274	248248	166174	210243	204210	144150
15	999	999	263269	246274	278284	230262	246260	248248	166174	231243	204210	144150
16	999	999	263269	246274	278284	230262	246260	248248	164174	231243	204210	144150
17	999	999	263269	246274	278284	230262	246260	248248	166174	231243	204210	144150
18	999	999	269281	246248	278278	226230	246274	248248	164174	210243	204210	144150
19	999	999	263269	246248	278284	226262	246260	248248	166174	210231	204210	144150
20	999	999	263269	246248	278278	230240	246252	248248	164174	231231	210216	144150
21	999	999	269281	264274	278284	226230	246274	236248	160174	210246	204210	129144
22	999	999	269281	246274	278280	226230	246256	238248	164174	210231	204210	144150
23	999	999	269281	246274	278284	226230	246274	248248	164174	210243	204210	144150
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26	999	999	269281	274274	278278	226230	246274	248248	164174	210243	204210	144150
27	999	999	263281	274274	278278	226230	246274	248248	164174	210231	204210	144150
28	999	999	269281	246274	278278	226230	246274	248248	164174	210243	204210	144150
29	999	999	269281	246274	278278	226230	246274	248248	164174	210243	204210	144150
30	999	999	269281	274274	278278	226230	246274	238248	164174	210243	204210	144150

GENEPOP FILE FORMAT-TXT FILE

GENEPOP20PRIMERkzlgks.txt - Not Defteri

Dosya Düzen Biçim Görünüm Yardım

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WPMS04
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WPMS07
WPMS09
WPMS10
WPMS12
WPMS14
WPMS15
WPMS16
WPMS18
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PMGC21
PMGC27
PMGC28
PMGC93
PE5
PE13
PE14
Pop
1_      ,       263269 248274 278282 218218 246260 210244 164174 231243 204210 144159 222231 234234 198216
1_      ,       269281 248274 278278 218218 246274 248248 164174 210243 204210 144159 225225 220234 192198
1_      ,       263269 248248 000000 218218 246260 248248 166174 231243 204210 144159 222231 234234 198216
1_      ,       269281 274274 262278 218230 246274 248248 164174 210243 204210 144150 225225 220234 192198
1_      ,       269281 274274 262278 218218 246274 248248 000000 213243 204210 144150 225225 220234 192198
1_      ,       269281 274274 278278 218218 246274 248248 164174 210243 204210 144159 225231 220234 192198
1_      ,       269281 274274 278278 218218 246274 248248 164174 234252 204210 135144 225231 220226 189207
1_      ,       263263 274274 284288 218218 236252 248268 164164 210243 195195 144150 222228 220226 189207
1_      ,       269281 274274 278278 218218 246274 248248 164174 210243 204210 144150 225225 220234 192198
1_      ,       263269 274274 278278 218218 246260 248248 164174 231243 204210 144150 225231 220226 189207
1_      ,       269281 274274 278278 218230 246246 248248 000000 210243 204210 144150 222228 220234 192198
1_      ,       269281 274274 278278 218218 246260 248248 164174 231243 204210 144150 225225 234234 198216
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MICROCHECKER FILE FORMAT-TXT FILE

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MPMS04
MPMS05
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MPMS09
MPMS10
MPMS12
MPMS14
MPMS15
MPMS16
MPMS18
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PMGC21
PMGC27
PMGC28
PMGC93
PE5
PE13
PE14
Pop
1_      ,       269281 274274 228278 226230 246246 248248 164174 210243 204210 144150 225231 220234
1_      ,       263269 274274 278284 230232 246246 248248 164174 210243 204210 150150 225234 220234
1_      ,       251269 246246 278284 226262 246260 248248 164174 210243 204210 144150 231234 234234
1_      ,       263269 246274 278284 230262 246260 248248 164174 231243 204210 144150 231234 234234
1_      ,       263269 246274 278284 230262 246260 248248 164174 231243 204210 144150 231234 216234
1_      ,       251251 246274 278284 230230 246260 248248 166174 231243 204210 144150 225231 224236
1_      ,       263269 246274 278284 230262 246260 248248 166174 231243 204210 144150 231234 234236
1_      ,       251269 246246 278284 226262 260274 248248 166174 210243 204210 144150 231231 220234
1_      ,       263269 246246 278284 230262 246260 248248 166174 231243 204210 144150 231234 234234
1_      ,       263281 274274 278284 230230 246274 248248 164174 231243 204210 144144 225234 234234
1_      ,       263269 246274 278284 230262 246260 248248 166174 231243 204210 144150 231234 234234
1_      ,       263281 246246 278284 230262 246260 248248 166174 243243 204210 144144 231234 220234
1_      ,       263269 246274 278284 230262 246260 248248 166174 231243 204210 144150 231234 234234
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STRUCTURE FILE FORMAT-TXT FILE

structure kzlgks - Kopya.txt - Not Defteri

Dosya Düzen Biçim Görünüm Yardım

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86	1	269	274	278	230	246	248	164	210	204	144	225	220
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87	1	269	274	278	230	246	248	164	210	204	144	225	220
87	1	281	274	278	230	274	248	174	243	210	150	231	234
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90	1	281	274	278	230	274	248	174	243	210	150	231	234
91	1	269	274	278	226	246	248	164	210	204	144	225	220
91	1	281	274	278	230	274	248	174	243	210	150	231	234
92	1	269	274	278	226	246	248	164	210	204	144	225	220
92	1	281	274	278	230	274	248	174	243	210	150	231	234
93	1	263	246	278	230	246	248	166	231	204	144	231	234
93	1	269	274	284	262	260	248	174	243	210	150	234	234
94	1	263	246	278	230	246	248	166	231	204	144	231	234
94	1	269	274	284	262	260	248	174	231	210	150	234	234
95	1	263	246	278	230	246	248	166	231	204	144	231	234
95	1	269	274	284	262	260	248	174	261	210	150	234	234
96	1	263	246	278	226	246	248	166	231	204	144	231	234
96	1	269	274	284	262	260	248	174	243	210	150	234	234
99	1	269	274	278	220	246	248	164	210	204	144	225	220
99	1	281	274	278	230	274	248	174	243	210	150	231	234
102	1	263	246	278	230	246	248	166	231	204	144	231	234
102	1	269	274	284	262	246	248	174	243	210	150	234	234
103	1	263	246	278	230	246	236	166	231	204	144	231	234
103	1	269	274	284	262	260	248	174	243	210	150	234	234
104	1	269	264	278	226	246	248	166	210	210	150	228	228
104	1	281	264	280	236	246	248	174	243	219	150	231	234
105	1	263	246	278	230	246	248	166	231	204	144	231	234
105	1	269	274	284	262	260	248	174	243	210	150	234	234
106	1	269	274	278	226	246	248	164	210	204	144	225	220
106	1	281	274	278	230	274	248	174	243	210	150	231	234

NEWHYBRIDS FILE FORMAT

kzlg.dat - Not Defteri

Dosya Düzen Biçim Görünüm Yardım

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NumIndivs 119
NumLoci 6
Digits 3
Format Lumped
1       246260  144159  220231  199217  224228  190204
2       246274  144159  225225  193199  224224  190194
3       246260  144159  220231  199217  224228  190194
4       246274  144150  225225  193199  224242  190194
5       246274  144150  225225  193199  242242  190194
6       246274  144159  225231  193199  224242  190194
7       246274  135144  225231  190208  224242  202216
8       236252  144150  220228  190208  242242  190194
9       246274  144150  225225  193199  224242  190194
10      246260  144150  225231  190208  224242  190194
11      246246  144150  220228  193199  224242  190194
12      246260  144150  225225  199217  0       190194
13      246274  144150  225225  199199  224242  190204
14      246260  144150  225225  199217  224242  190204
15      246260  144150  225225  193199  224242  190204
16      246260  135141  220231  217217  224228  202216
17      208208  144150  225225  199199  224242  190194
18      246274  144150  225225  193199  224242  190194
19      246274  144150  225225  193199  224228  0
20      0       144150  225231  193199  224242  190194
21      246274  144150  225225  193199  224242  190194
22      246274  144150  225225  193199  224242  0
23      268268  144150  225225  193199  224242  190194
24      246274  144150  225225  199199  224242  190194
25      236252  144150  220231  190208  186242  202216
26      246274  144150  225231  196202  224240  190194
27      246246  144150  225231  196202  224240  190194
28      246274  144150  225231  196202  224240  190194
29      246274  144150  225231  196202  224240  190194
30      246274  144150  225231  196202  224240  190194
31      246274  144150  225231  196202  224240  190194
32      246274  144150  225231  196202  224240  190194
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PUBLICATIONS

Ciftci A, Karatay H, Karaosmanoğlu F, Karahan A, Kaya Z, (2017) Genetic diversity of *Populus nigra* population assessed by microsatellite DNA markers. Tree Genetic and Genomes 13: 69 DOI 10.1007/s11295-017-1154-8

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