GENETIC DIVERSITY PATTERN IN FEMALE AND MALE TREES OF *POPULUS EUPHRATICA* POPULATIONS SAMPLED FROM GÖKSU RIVER BASIN

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submitted by **NEHİR NEBİOĞLU** in partial fulfillment of the requirements for the degree of **Master of Science** i**n Biology, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Ayşe Gül Gözen
Head of the Department, **Biological Sciences** _____

Prof. Dr. Zeki Kaya
Supervisor, **Biological Sciences, METU** _____

Examining Committee Members:

Prof. Dr. Sertaç Önde
Biological Sciences, METU _____

Prof. Dr. Zeki Kaya
Biological Sciences, METU _____

Prof. Dr. İrfan Kandemir
Biology, Ankara University _____

Date: 15.02.2021

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name :

Signature :

ABSTRACT

GENETIC DIVERSITY PATTERN IN FEMALE AND MALE TREES OF *POPULUS EUPHRATICA* POPULATIONS SAMPLED FROM GÖKSU RIVER BASIN

Nebioğlu, Nehir
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Supervisor: Prof. Dr. Zeki Kaya

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Populus euphratica, one of the four *Populus* species naturally distributed in Southern and Southeastern Turkey, can survive under extreme conditions like high temperatures, low moisture, salty and calciferous soils. *P. euphratica* carries great significance for both the sustainability of a healthy river ecosystem and the supply of renewable energy resources. Meanwhile, due to the continuous increase in the human population, water resources decrease, and habitat destruction becomes unavoidable for this species. Recent studies confirm the reduction in the range of species and loss in its genetic resources. Thus, studying the magnitude and structure of the genetic diversity between genders held on great importance to generate information for the use of gene resources and effective conservation.

In this study, the genetic diversity and genetic structure of female and male individuals in *P. euphratica* populations from the Göksu river ecosystem were studied with seven microsatellite markers (SSR: simple sequence repeats). The microsatellite markers were selected according to several sex-determination studies in *Populus* species. The existence of sex chromosomes in *Populus* species was proposed, yet the location of the sex-determining region on the sex chromosome of *P. euphratica* has not been determined. Differentiation of sex at early stages of growth is exceptionally critical to developing the breeding strategies because the

genetic diversity programs cannot be employed until the species reached the reproductive stage (10 to 12 years). Thus, generated data on gender related diversity and gender determination in this study have great importance for augmentation in breeding programs, genetic improvements, and conservation studies of *P. euphratica.*

Among *P. euphratica* populations from the Göksu basin, private alleles were mostly found in male individuals. Most of the detected private alleles were found in the BPCA90 locus, which had male- and female-specific private alleles. Also, BPCA90 was also the most informative and effective locus in our study according to genetic diversity parameters.

Genetic diversity analysis revealed no significant variation between female and male tree populations detected with the studied loci. Moreover, population structure analyses asserted that the gene regions have used for gender determination in several *Populus* species showed high similarity in female and male trees of *P. euphratica* populations in our study. There were two homogeneous gene pools observed in both genders with equal membership values. Different genders might be expressed by the same genotype of *P. euphratica* trees in our study, depending on the environmental factors. To continue breeding in adverse environmental conditions, these deviations from strict dioecism have been observed in several *Populus* species.

The results of this study indicated considerably narrowed genetic diversity. According to effective population size analysis and calculated GW indexes, natural populations of *P. euphratica* species in the Göksu river appeared to have experienced severe past reductions in population sizes and are in danger of breakdown.

Keywords: *Populus euphratica,* sex determination, microsatellite markers, genetic diversity, genetic structure

ÖZ

GÖKSU NEHRİ HAVZASINDAN ÖRNEKLENEN FIRAT KAVAĞI POPÜLASYONLARINDAKİ ERKEK VE DİŞİ AĞAÇLARIN GENETİK ÇEŞİTLİLİK YAPISI

Nebioğlu, Nehir
Yüksek Lisans, Biyoloji
Tez Yöneticisi: Prof. Dr. Zeki Kaya

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Güney ve Güneydoğu Türkiye'de doğal olarak dağılmış dört kavak türünden biri olan *Populus euphratica* (Fırat kavağı), yüksek sıcaklık, düşük nem, tuzlu ve kireçli topraklar gibi ekstrem koşullarda dahi varlığını sürdürebilmektedir. Fırat kavağı, hem sağlıklı bir nehir ekosisteminin sürdürülebilirliği hem de yenilenebilir enerji kaynaklarının temini açısından büyük önem taşır. Bununla birlikte, insan nüfusundaki devamlı artış sebebiyle su kaynakları azalmakta ve Fırat kavağı için habitat tahribatı kaçınılmaz hale gelmektedir. Son çalışmalar, tür içi çeşitliliğin azaldığını ve genetik kaynaklardaki kaybı doğrulamaktadır. Dolayısıyla, gen kaynaklarının doğru kullanımı ile tür için etkin koruma sağlayabilmek adına cinsiyetler arası genetik çeşitliliğin ve genetik yapılanmanın incelenmesi büyük önem taşımaktadır.

Bu Yüksek Lisans Tezinde, Göksu nehri ekosisteminden örneklenen Fırat kavağı popülasyonlarında yer alan dişi ve erkek bireylerin genetik çeşitliliği ve genetik yapısı yedi mikrosatelit belirteci (SSR: basit tekrar dizileri) ile incelenmiştir. Çalışmada kullanılan mikrosatelit belirteçleri, daha önce kavak türlerinde yapılan cinsiyet belirleme çalışmalarına göre seçilmiştir. Kavaklarda cinsiyet kromozomlarının varlığı öne sürülmüş, ancak Fırat kavağı için cinsiyet kromozomundaki cinsiyet belirleyici bölgenin yeri belirlenememiştir. Bu çalışmada

diğer Kavak türlerinde cinsiyete özgü olduğu tespit edilen mikrosatelit belirteçleriyle Fırat kavağı için cinsiyetin moleküler düzeyde tanımlaması ve popülasyonlardaki genetik çeşitliliğin ortaya konulması amaçlanmıştır. Cinsiyetin büyümenin erken aşamalarında belirlenmesi üreme stratejilerinin geliştirilmesinde oldukça önemlidir zira genetik çeşitlilik programları, tür üreme aşamasına gelene kadar (10 ila 12 yıl) kullanılamaz. Dolayısıyla, çalışmadan elde edilen veriler tür için yapılacak ıslah çalışmalarında ve eşeye bağlı genetik çeşitliliğin araştırılmasında büyük önem taşır. Göksu havzasından örneklenen Fırat kavağı popülasyonlarında özel aleller çoğunlukla erkek bireylerde tespit edilmiştir. BPCA90 lokusu tespit edilen özel alellerin çoğuna sahip olmakla birlikte, erkek ve dişiye özgü özel aleller de bulundurmaktadır. Ayrıca BPCA90, genetik çeşitlilik parametrelerine göre çalışmamızdaki en bilgilendirici ve etkili lokustur.

Yapılan genetik çeşitlilik analizleriyle incelenen lokuslarla dişi ve erkek ağaç popülasyonları arasında önemli bir varyasyon görülmemiştir. Popülasyon yapı analizleri, çeşitli kavak türlerinde cinsiyet tayini için kullanılan gen bölgelerinin Fırat kavağı popülasyonlarının dişi ve erkek ağaçlarında yüksek benzerlik gösterdiğini ortaya koymuştur. Her iki cinsiyette de eşit ihtimalle dağılan iki homojen gen havuzu gözlenmiştir. Çevresel faktörlere bağlı olarak üremeyi devam ettirmek için Fırat kavağı ağaçlarının aynı genotip ile farklı cinsiyetlerde çoğalması söz konusu olabilir. Olumsuz koşullarda oluşabilen bu sapmalar birçok kavak türünde gözlemlenmiştir. Bu çalışmanın sonuçları, Göksu nehrindeki Fırat kavağına popülasyonların genetik çeşitliliğinin tehlikede olabileceğini göstermektedir. Etkili popülasyon büyüklüğü analizi ve hesaplanan GW indekslerine göre, geçmişte popülasyon boyutlarında ciddi düşüşler yaşanmış olabilir ve günümüzde hayatta kalan popülasyonlar da bozulma tehlikesiyle karşı karşıya kalmaktadır.

Anahtar Kelimeler: Fırat kavağı, cinsiyet belirlenmesi, mikrosatelit belirteçleri, genetik çeşitlilik, genetik yapılanma

To mom and dad

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%P	Percentage of Polymorphic Loci
AMOVA	Analysis of Molecular Variance
Ar	Allelic Richness
bp	Base pairs
CLUMPP	Cluster Matching and Permutation Program
CTAB	Cetyl Trimethyl Ammonium Bromide
EDTA	Ethylenediaminetetraaceticacid disodium salt
F	Inbreeding Coefficients
F_{CT}	Difference Among Groups For The Total Population
F_{IS}	Inbreeding Coefficient Within Individuals
F_{IT}	Inbreeding Coefficient Within Total Population
F_{SC}	Differences Among Population Within Groups
F_{ST}	Inbreeding Coefficient Within Subpopulations
GDA	Genetic Data Analysis
G-W Index (M)	Modified Garza-Williamson Index
uHe	Unbiased Expected Heterozygosity
Ho	Observed Heterozygosity
HWE	Hardy–Weinberg Equilibrium
I	Shannon's Information Index Markov Chain Monte
MCMC	Carlo
$MgCl_2$	Magnesium Chloride
Na	Number of Alleles
Ne	Number of Effective Alleles
Nm	Number of Migrants
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PI	Probability of Identity
PIC	Polymorphism Information Content
R_{ST}	Inbreeding Coefficient Within Subpopulations (based
SSR	on size) Simple Sequence Repeats
Ta	Annealing temperature
TE	Tris EDTA
TUBIVES	Türkiye Bitkileri Veri Servisi
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean

CHAPTER 1

INTRODUCTION

1.1 *Populus euphratica* Oliv.

Populus (Poplar) is a genus of deciduous trees belonging to the Salicaceae family (Bremer et al., 2003). One of the thirty-five known species of *Populus*, *Populus euphratica*, is an ecological pioneer species for the riparian forest of arid regions (Ferreira et al., 2006). *P. euphratica*, also known as the Euphrates poplar, salt poplar, or desert poplar, has a self-supporting growth form (Jun Wang et al., 2013), meaning it can hold its static weight while withstanding the dynamic forces (Rowe & Speck, 2005). The species can grow in saline and arid environments and naturally distributed in desert forest ecosystems in China and Middle Eastern countries (Zhang et al., 2019) and grow in shelterbelts along riversides (Du et al., 2013). One of the most important characteristics of *P. euphratica* is the ability to survive under extreme conditions like high temperatures, drought, and salt stress (Chen & Polle, 2010; Gu et al., 1999; Ma et al., 1997; Tschaplinski et al., 1998). As a result of its ability, the species is accepted as a model to study the mechanisms responsible for the survival of woody plants under heat stress (Ferreira et al., 2006).

1.1.1 Biology

Populus euphratica is a unique poplar species that survive under unfavorable conditions such as extreme cold and hot temperatures, alkaline soils, and salt stress. *P. euphratica* has polymorphic leaves on the same tree or even in the same branch (Ma et al., 2012). The polymorphic leaf forms of the species are narrowly lanceolate, linear-lanceolate, and willow-like (Al-joboury, 2017). The polymorphic leaves of *P. euphratica* from Göksu samples were in Figure 1.1.

Figure 1.1 The polymorphic leaf forms of *Populus euphratica*.

Stems of the trees may be straight, but they also grow twisted or bent. Different growth forms of *P. euphratica* were illustrated in Figure 1.2 (Miao et al., 2020). Over the entire distribution spectrum, the maximum tree size extensively varies. According to the literature, depending on the distribution of location, Euphrates poplar trees can be 6m (Spain), 10m (Turkey and India), and 15m heights (China and Pakistan) (Ma et al., 1997). Also, the height of 20–30m can be possible under optimal growing conditions (Weisgerber & Han, 2001).

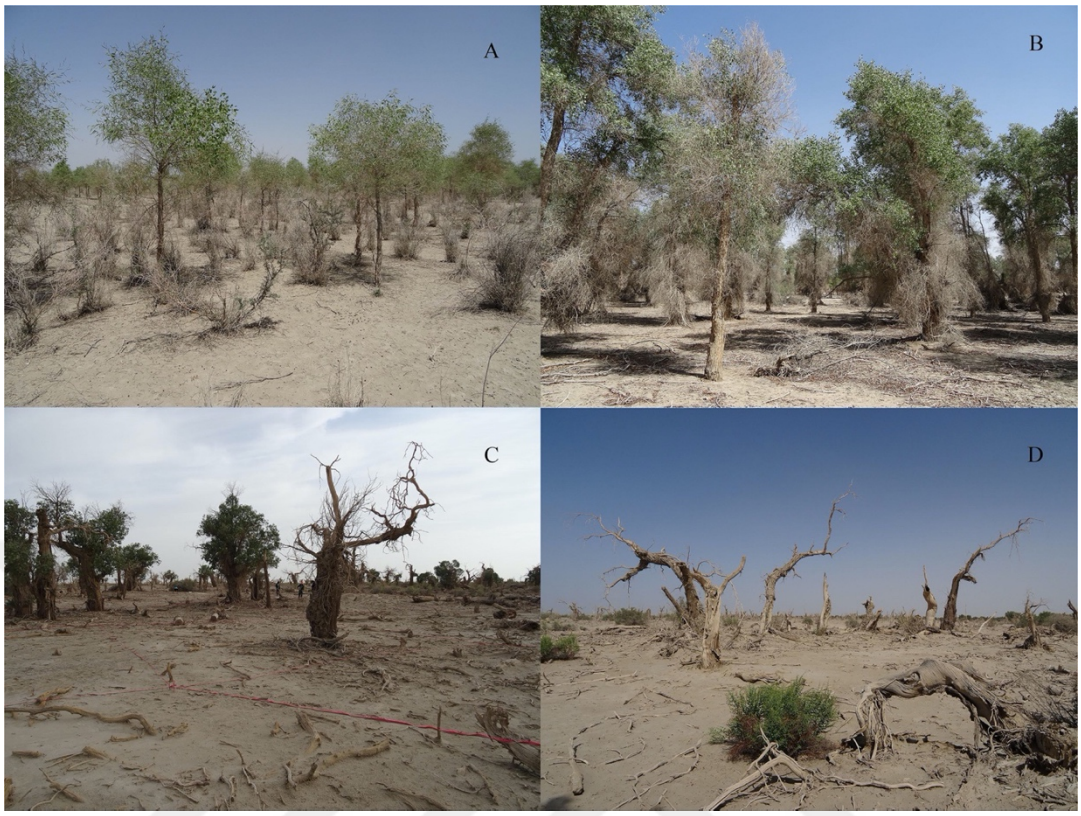

Figure 1.2 Photos from *Populus euphratica* forest from the different growth and death stages: A. Juvenile forest plot, B. Mature forest plot, C. Dying forest plot, D. Dead forest plot (Miao et al., 2020)

Populus euphratica is a wind-pollinated species with female and male flowers on separate individuals (Figure 1.3). Female and male flowers of *P. euphratica* are catkins, a type of compact inflorescence. In natural populations, trees achieve reproductive maturity at 10–15 years under optimal conditions (Wiehle et al., 2009). The flowering typically occurs in the springtime, continues 1-2 weeks, and follows the leaves' appearance (Botstein et al., 1980). Wind pollination extends over one to two months, and during pollination, the dispersal distance of pollens can reach incredibly variable (Eckenwalder, 1996). Fruits are capsules of tiny seeds generated in large amounts with cotton-like appendages (Soleimani et al., 2014). The seeds with cotton-like appendages are dispersed effectively via wind at great distances.

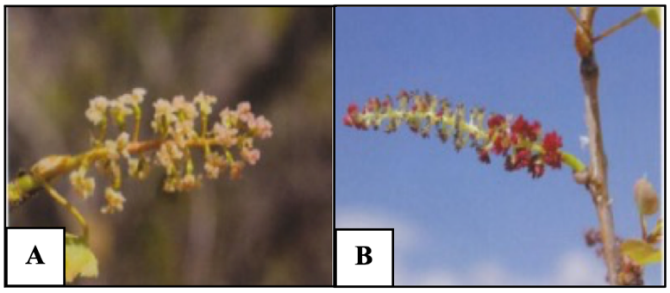

Figure 1.3 *Populus euphratica* flowers. A. Female catkins, B. Male catkins (Mamıkoğlu, 2007)

Members of the genus *Populus* are dioecious, which present separate genders on individual trees generally (Hughes et al., 2000; McLetchie & Tuskan, 1994; Nagaraj, 1952; Slavov et al., 2010). Although all *Populus* species, including *P. euphratica* have the capacity of sexual reproduction, they also can undergo vegetative propagation naturally with broken branches or via root suckers (Kramp et al., 2018). In this way, it enables genotypes to spread over great distances (Legionnet et al., 1997). It can be an effective strategy to preserve the desired characteristics under unfavorable environmental conditions (Peterson & Jones, 1997).

1.1.2 Distribution and Importance

Olivier (1801) described the Euphrates poplar as called after the Euphrates River due to the existence of large populations in there (Weisgerber & Han, 2001). According to Agroforestry Database 4.0 (Orwa et al., 2009), the total distribution of *P. euphratica* ranges from Morocco in the west over North Africa, West, and South Asia to Central Asia as far as Mongolia (Figure 1.4). In Asia, *P. euphratica* appears to be only distributed in patches on the west side (Peper et al., 2010), while in the eastern part of its distribution range, it forms widespread woodlands called Tugai forests (Ma et al., 1997). An isolated population assumed to have an anthropogenic origin was identified in Spain (Fay et al., 1999). The species have disjunct

distribution patterns with isolated populations (Fay et al., 1999; Weisgerber & Han, 2001). Today, the most extensive contiguous forests are in Kazakhstan and China (Ma et al., 1997). Even though the trees arise in the vertical direction up to 2000 m above sea level, it has been stated to reach 3000–4000 m in Pakistan and Tibet (Weisgerber & Han, 2001).

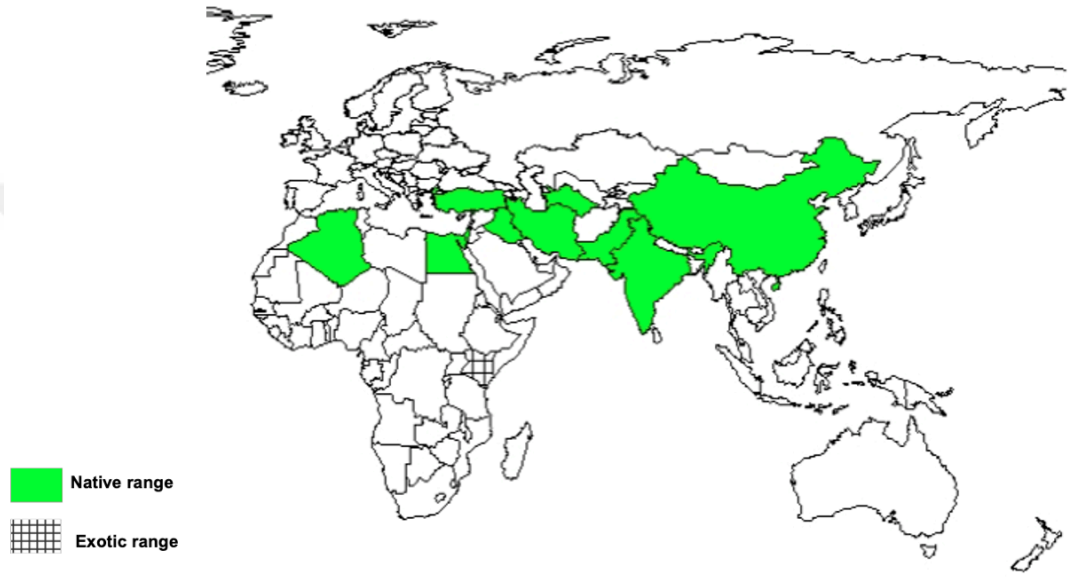

Figure 1.4 Documented distribution of *Populus euphratica*. Retrieved from Agroforestry Database 4.0 (Orwa et al., 2009). Native range including Algeria, China, Egypt, India, Iran, Iraq, Israel, Libyan Arab Jamahiriya, Pakistan, Syrian Arab Republic, Turkey, and Turkmenistan; exotic range includes Kenya.

In Turkey, the natural stands of the species occur in South and Southeastern Anatolia narrows between the Dicle River and its tributaries as the Eastern border and Bozyazı River in Anamur as the Western border (Velioğlu & Akgül, 2016). In the Euphrates and the Göksu river basins, the densest stands of the species exist (Figure 1.5).

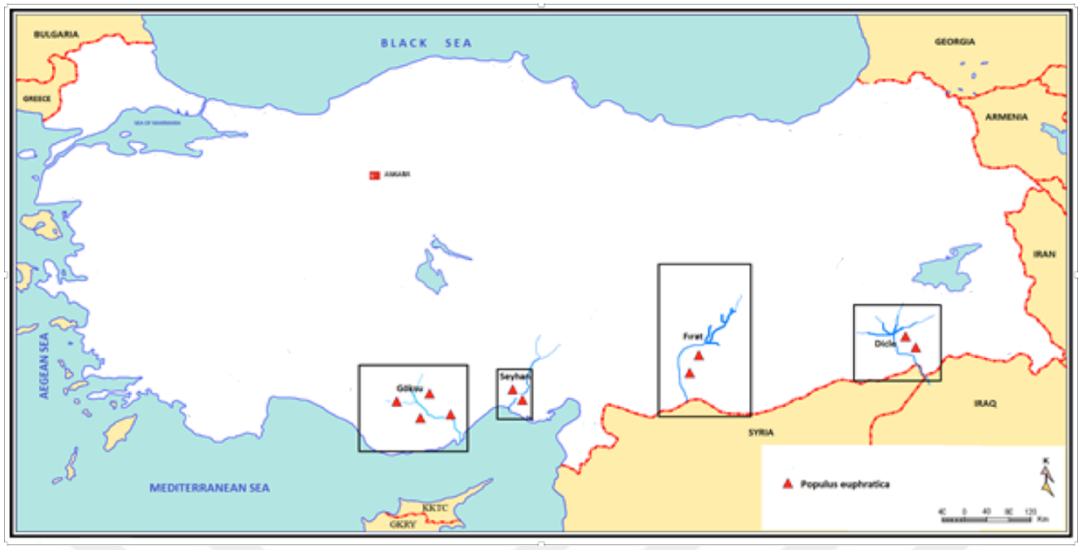

Figure 1.5 Distribution of *Populus euphratica* in Turkey. Four main populations are represented in frames. Retrieved from Country Progress Report of the National Poplar Commission (Velioğlu and Akgül, 2016)

In northwestern China's river corridors, *P. euphratica* plays critical ecological functions as the keystone species for all regions' biodiversity (Wiehle et al., 2009). It has considerable economic value for the local human population as the primary source of wood and a hardy crop plant; however, the species suffers from intense pressure from humans and their livestock (Ma et al., 1997; Reich et al., 1996). Since the antique ages, common *Populus* species (*Populus euphratica, Populus tremula*, *Populus nigra,* and *Populus alba*) have been of great importance for wood and timber products for local people. *P. euphratica* is also planted for windbreaks and soil stabilization to ensure the ecosystem's utility. The species play an essential role in maintaining healthy river ecosystems where are habitats of several essential animal species (Wu et al., 2010).

In Turkey, natural populations of *P. euphratica* are marginally located in the South and Southeastern Anatolia regions. The Euphrates, The Tigris, and the Göksu river basins, where the densest stands of this species exist, possess highly fragmented habitats (Browicz and Yaltırık, 1982). Due to the dam constructions in rivers, *P.*

euphratica is restricted to a minor section of these river basins. Moreover, due to the agricultural activities and urbanization, suitable habitats of *P. euphratica* in Göksu and Euphrates river basins have become narrow. The first population genetic study of *P. euphratica* in the Göksu river was conducted by Kansu & Kaya (2020) reported that the marginal populations of this species were at an extreme hazard of extinction. To provide the sustainability of these marginal *P. euphratica* populations, *in situ* conservation of this species should be started immediately.

1.1.3 Genetics

Harrison (1924) stated that the basic chromosome number in *Populus* was 19 by studying seven poplar species (Blackburn & Harrison, 1924). Thenceforward, it has been clear that *Populus* species, including *Populus euphratica*, exist in the diploid form (2n = 38) (Smith 1943), but also triploid or tetraploid genets have been observed uncommonly, especially in interspecific crosses (Bradshaw et al., 2000; Bradshaw & Stettler, 1993; Einspahr et al., 1963). Although hybridization is common in poplar species, natural hybridization between *P. euphratica* and other *Populus* species was not observed. Still, successful hybrids were obtained with *P. deltoides*, *P. nigra,* and *P. simonii* (Su et al., 1996), and *P. alba* (Mofidabadi et al., 1998). Similar hybridization studies between *P. euphratica* and other poplar species were performed in Turkey, but viable pollens or seeds were not achieved to produce hybrids (Özel et al., 2010).

Genetic studies performed with *Populus* species detected the haploid genome size of *Populus* is 550 million base pairs (bp), relatively small nuclear genome (2C = 1.2 pg) (Bradshaw & Stettler, 1993; Dhillon, 1988). The small *Populus* genome, a convenient feature for standard molecular genetics techniques like gene cloning, Southern blotting, etc., contributes to the usage of the species as a model organism. Moreover, *Populus's* physical/genetic distance ratio is almost the same as

Arabidopsis (200kb/cM), advantageous for map-based (positional) cloning of genes (Stirling et al., 2001).

1.1.3.1 Genetic studies of *Populus euphratica*

To date, a limited number of studies had investigated the level of genetic diversity and population structure in natural populations of *Populus euphratica*. Previous studies that evaluated genetic variation in *P. euphratica* populations were based on molecular markers such as allozymes and amplified fragment length polymorphism (AFLP) markers (Cervera et al., 2005; Fay et al., 1999; Jianming et al., 2006) and random amplified polymorphic DNA (RAPD) markers (Saito et al., 2002). Additionally, nuclear and chloroplast DNA (cpDNA) markers have been used to study the phylogeographic patterns of *P. euphratica* (Zeng et al., 2018). However, simple sequence repeat (SSR) markers have recently gained wide use (Du et al., 2013; Kansu & Kaya, 2020; Wang et al., 2011; Wu et al., 2008; Xu et al., 2013). SSR markers, also named microsatellites or short tandem repeats (STRs), are DNA segments that include a basic repeat unit having less than seven base pairs (Labbé et al., 2011). Unlike the other common molecular markers, SSR markers are codominant; thus, they can provide the determination of the heterozygotes from homozygotes (Ben-Ari & Lavi, 2012). Microsatellite markers have exceptional characteristics such as extensive genome coverage, locus specificity, codominant inheritance, hypervariability, relative abundance, and reproducibility (Parida et al., 2009; Powell et al., 1996). They can be used in both low and high throughput genotyping approaches (Vieira et al., 2016). These features of microsatellites make them an attractive option for genome mapping, parentage analysis, forensics, population & conservation genetics (Kalia et al., 2011). Recently, with the application of SSR markers, genetic studies of tree species have accelerated (Xia et al., 2017). Microsatellite markers have been preferred to estimate genetic diversity in several studies performed with different *Populus* species (Ciftci et al., 2017; Ciftci

et al., 2020; Ciftci & Kaya, 2019; Kansu & Kaya, 2020; Rathmacher et al., 2010; Sun et al., 2020; Zhang et al., 2019).

1.1.4 Sex Determination Studies of *Populus*

Gender determination is a fundamental developmental and evolutionary forced process (Tuskan et al., 2012). In sex determination research of plants, biochemical studies provide limited information comparing to gender-specific DNA sequences, which offer a more efficient and practical method (Qacif et al., 2007). There have been various efforts to understand the genetic basis of sex determination of plants with molecular tools (Hormaza et al., 1994; Restivo et al., 1995). Theoretically, successive mutations in two linked genes with complementary dominance on the ancestral autosomes cause the evolution of sex chromosomes (B. Charlesworth & Charlesworth, 1978; D. Charlesworth, 2002). While the existence of separate sexes on different individuals in *Populus* species is known via genetically driven, the details about gender-determining systems still are unclear (Tuskan et al., 2012; Yang et al., 2020). By a pair of heteromorphic sex chromosomes in male heterogamety form (XY system), sex determination can be accomplished in *Populus* (Ming et al., 2011; Xue et al., 2020). Tuskan et al. (2012) suggested that during the evolutionary process, selective pressure on the genome structure and gene composition of the peritelomeric region of the chromosome XIX has caused to arising of a distinctive genome structure that includes a cluster of genes leading to gender determination in *Populus* species. The challenging part is the location of a gender determination locus with alternate positions on chromosome XIX for different *Populus* species (Pakull et al., 2011; Paolucci et al., 2010; Yin et al., 2008) (Figure 1.6). After all, further studies on chromosome XIX of *Populus* species will give more accurate conceptions about the control of sex determination.

Microsatellite markers will give a new impulse to determine sex by constructing linkage maps using polymerase chain reaction (Jia et al., 2015; Song et al., 2012; Yang et al., 2020). The gender determination studies with *Populus trichocarpa* and *Populus tremula* x *Populus tremuloides* hybrid detected 12 microsatellite loci close to gender determination locus on the XIX chromosome (Pakull et al., 2011; Paolucci et al., 2010; Tuskan et al., 2004; Yin et al., 2008).

Recent studies revealed an XY system of sex determination on chromosome XIX of *P. euphratica* (Yang et al., 2020). By performing a genome-wide association study (GWAS), 310 single nucleotide polymorphisms (SNPs) were identified as sex associated. Furthermore, in this study, nearly all genotypes of these sex-associated loci were homozygous in females, but most of the genotypes were heterozygous in males (Yang et al., 2020). Also, the phylogenetic analysis of these genes indicated that the X and Y alleles started to diverge after speciation could be evidence of relatively recent development of sex determination regions of *P. euphratica.* This is a common feature of the sex chromosomes of the Salicaceae species studied up to now (Geraldes et al., 2015; Pucholt et al., 2017; Zhou et al., 2018, 2020).

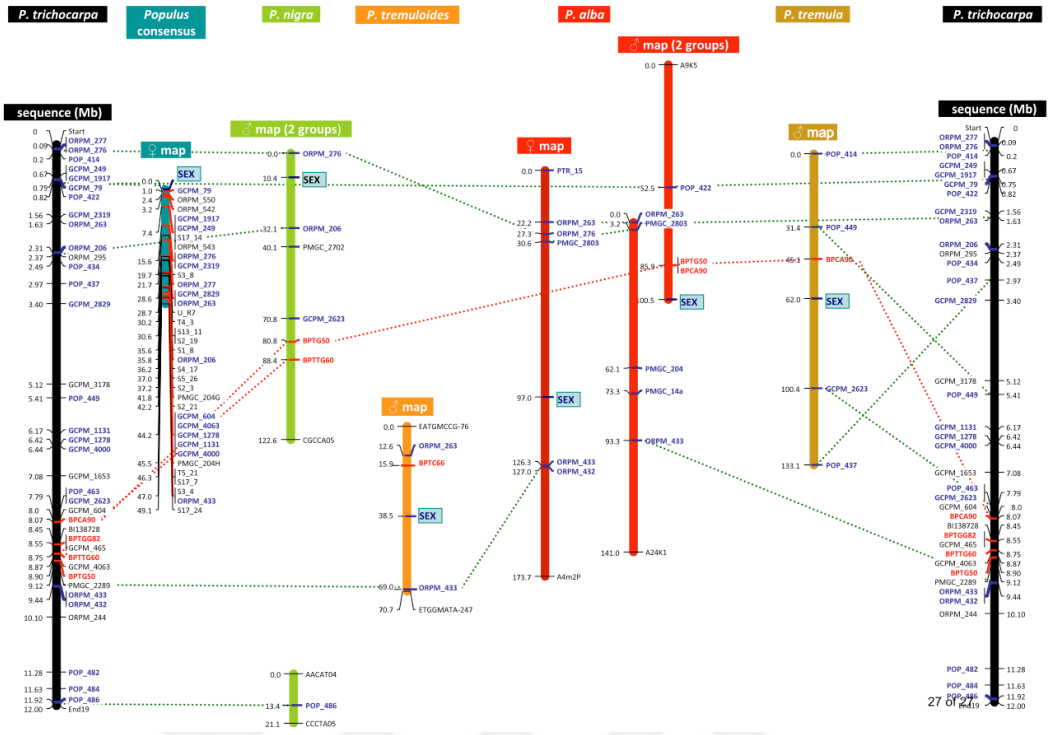

Figure 1.6 Comparative mapping of sex locus position on chromosome XIX from different *Populus* species. Blue: The consensus map of *P. trichocarpa* and *P. deltoids* (Yin et al., 2008); Green: The map of *P. nigra*; Orange: The map of *P. tremuloides*; Red: The maps of *P. alba* (Paolucci et al., 2010); Mustard: The map of *P. tremula* (Pakull et al., 2009, 2011). The broken lines indicate the connection of common microsatellite markers. The red microsatellite markers were introduced as sex-linked (Pakull et al., 2011).

CHAPTER 2

OBJECTIVES OF THE STUDY

Populus euphratica is a considerably essential species for the maintenance of healthy river ecosystems due to its survival ability under stress conditions, like high tolerance to salinity, temperature, and atmospheric drought. It is one of the poplar species naturally distributed in Turkey and holds remarkable significance for renewable energy resources. Consequently, understanding of potential genetic diversity in female and male tree populations of *Populus euphratica* has great importance in terms of species survival and adaptation as well as effective use and conservation, management, and use of the species' gene resources. In the Göksu River basin, the genetic resources of the species are under a significant threat due to anthropogenic pressures, such as agricultural activities, recently built dams and levees.

The main objective of the study was to investigate genetic diversity and genetic differentiation of female and male tree populations of *Populus euphratica* sampled from the Göksu River, Turkey, through available microsatellite data. The specific goals of this study include:

- to investigate genetic diversity and structure among female and male populations of *Populus euphratica* in the Göksu River basin with microsatellite markers,
- to detect potential genetic structure to analyze contributions from genders,
- to detect the gender of a tree using sex-linked markers if it is possible,
- to acquire valuable information for starting well-organized conservation programs and using genetic resources in the Göksu River basin effectively for future breeding programs.

Furthermore, in addition to inspecting genetic variation between genders, the geographical distribution of populations was considered in the study to compare the genetic diversity of female and male tree populations of *Populus euphratica.*

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Material and Field Work

For sampling female and male flowers on different individuals, the field studies were held on in April 2017. There were three sampling locations in Goksu River set to characterize upstream tributaries (Gökcay and Ermenek), middle (Mut), and downstream (Silifke) of the river. Corresponding locations were given in Figure 3.1. According to morphological observations of *Populus* flowers, 96 leaf samples, which consisted of 46 female and 50 male tree samples, were selected randomly (Table 3.1). To prevent clonal sampling, samples were collected from individuals at least approximately 200 m apart. For DNA extraction, leaf samples from each tree were stored up silica gel containing zipper bags.

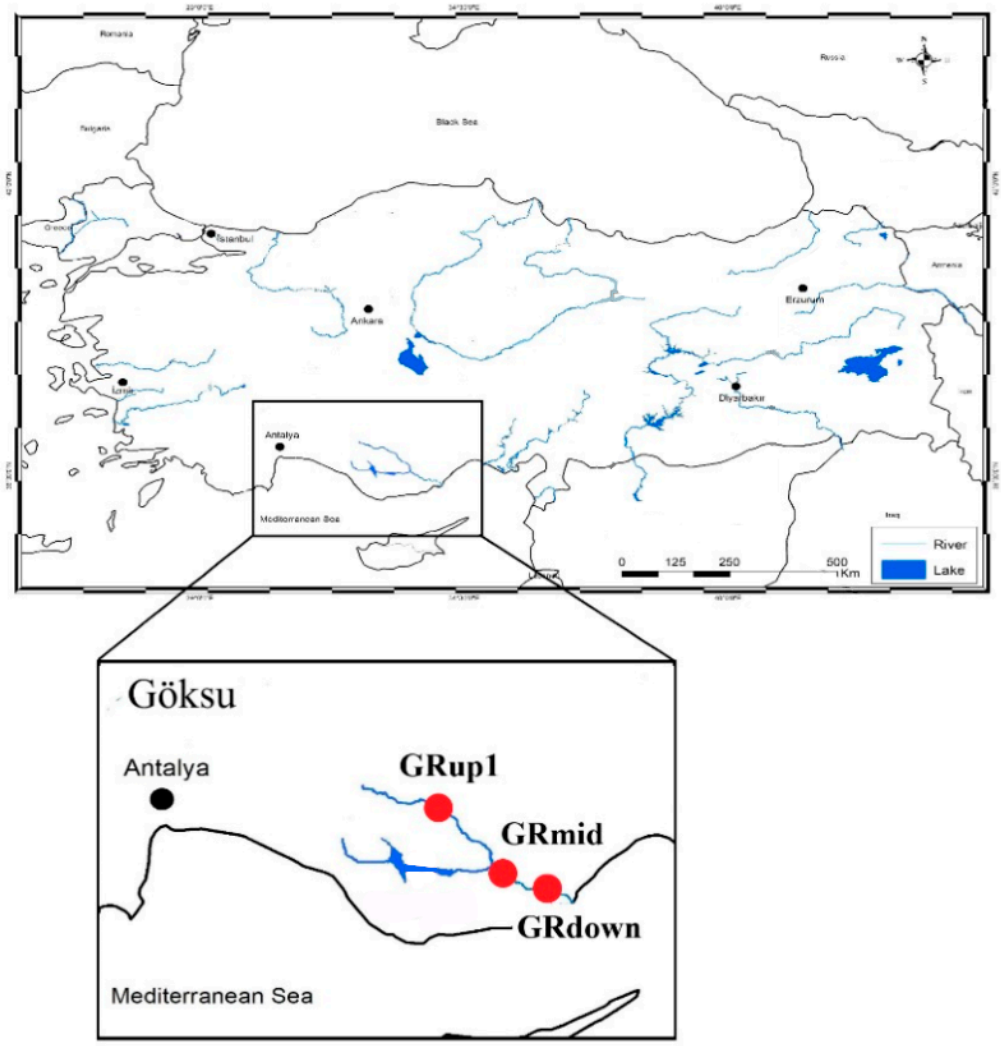

Figure 3.1 Sampling locations and corresponding populations in Göksu river in Turkey river map (Kansu & Kaya, 2020) (GRup: Göksu River upstream; GRmid: Göksu River middle; GRdown: Göksu River downstream tributaries)

Table 3.1 Sample sizes with respect to locations and gender

River	Population ID	Altitude Range	Female	Male	Total
Göksu	GUP (Gökcay&Ermenek)	441-490m	15	18	33
	GMID (Mut)	294-367m	16	15	31
	GDOWN (Silifke)	98-169m	15	17	32

3.2 Molecular Studies

3.2.1 DNA Extraction

Each leaf sample was ground with mortar and grinder with liquid nitrogen (-196°C). After this step, approximately 0.05g acquired powder was used for DNA extraction. Doyle (1991) CTAB extraction method was performed with some alterations (Appendix B). Using NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific), the concentration and purity of obtained DNA samples were measured by calculating the 260:280 OD ratios. Each measurement was repeated three times, and each value was recorded.

3.2.2 PCR Amplification of Microsatellite Loci

For the investigation of sex-linked genetic variations, ten microsatellite markers were selected. These markers have a potential utility of sex determination based on adjacency of peritelomeric region of chromosome XIX in *Populus* genus, according to genetic mapping results reported in previous studies (Pakull et al., 2011; Tuskan et al., 2004). Those loci were used to check the amplification success of the corresponding markers. The amplification could not be achieved with "GCPM" - prefix SSR markers, and they were discarded from further experiments. The repeat motifs, forward and reverse sequence of primers, and expected product sizes for each microsatellite marker were given in Table 3.2. In PCR reactions, 5x HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia), which included 15mM $MgCl_2$, was used with diluted 20 ng of isolated DNA samples. The components of the amplification mixtures and the Polymerase Chain Reaction (PCR) conditions performed for each of the mentioned loci were given in Table 3.3 and Table 3.4, respectively. The amplification products were confirmed by running 3% agarose gel. The gels were observed under UV light by using a gel imaging system (Vilbor Lourmat, France) (Figure 3.3).

Table 3.2 Characteristics of microsatellite loci

Locus ID	Repeat Motif	Left Primer (5’-3’)	Product Size	Reference
GCPM-1917	AT	AAGGCTTATAGAATGTGGCA	158	Oak Ridge National Laboratory
GCPM-249	AT		172	
GCPM-79	AT	TTACTTCGACAAGCTTTCCT	230	
ORPM-206	GCT	CCGTGGCCATTGACTCTTTA	196	Tuskan *et al*.,2004
ORPM-263	AT	AGCACATCTTTCGAGCATGA	243	
ORPM-276	TA	GCAGGAGAAAACACCAGGAA	205	
ORPM-433	TA	CCATCAGTTTCGAGGAGATTC	203	
BPTTG60	TTG	CAAGAACTCAGACATGATCAGATC	-	Pakul *et al*.,2011
BPTGG82	TGG	CTTGAAGAGCGAAAACTCAGCAG	-	
BPCA90	CA	CCTAGCCTTCATTCTCATTCAGC	-	

Table 3.3 PCR conditions and cycling parameters ("ORPM-" Prefix Loci)

Loci	Ingredients	**Volume(µL)**	PCR Cycling Parameters			
ORPM-206	dH_2O	16	Initial Denaturation		3 min.	95°C
	Master Mix	4	28 Cycle	Denaturation	30 sec.	95°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	4		Extension	50 sec.	72°C
	Total	25	Final Extension		20 min.	72°C
ORPM-263	dH_2O	14	Initial Denaturation		3 min.	95°C
	Master Mix	5	28 Cycle	Denaturation	30 sec.	95°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sex.	72°C
	Total	25	Final Extension		20 min.	72°C
ORPM-276	dH_2O	15.4	Initial Denaturation		3 min.	95°C
	Master Mix	3	28 Cycle	Denaturation	30 sec.	95°C
	Primers (10µM)	0.8 + 0.8		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sec.	72°C
	Total	25	Final Extension		20 min.	72°C
ORPM-433	dH_2O	14	Initial Denaturation		3 min.	95°C
	Master Mix	5	28 Cycle	Denaturation	30 sec.	95°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sec.	72°C
	Total	25	Final Extension		20 min.	72°C

Table 3.4 PCR conditions and cycling parameters ("BP-" Prefix Loci)

Loci	Ingredients	Volume(µL)	PCR Cycling Parameters			
BPTTG60	dH_2O	15	Initial Denaturation		3 min.	94°C
	Master Mix	4	30 Cycle	Denaturation	30 sec.	94°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sec.	72°C
	Total	25	Final Extension		20 min.	72°C
BPTGG82	dH_2O	15	Initial Denaturation		3 min.	94°C
	Master Mix	4	30 Cycle	Denaturation	30 sec.	94°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sex.	72°C
	Total	25	Final Extension		20 min.	72°C
BPCA90	dH_2O	15	Initial Denaturation		3 min.	94°C
	Master Mix	4	30 Cycle	Denaturation	30 sec.	94°C
	Primers (10µM)	0.5 + 0.5		Annealing	40 sec.	Ta
	DNA (10ng/µL)	5		Extension	50 sec.	72°C
	Total	25	Final Extension		20 min.	72°C

Table 3.5 Annealing temperatures of the markers for the microsatellite loci and the fluorescent dyes used

Locus ID	Ta (°C)	Fluorescent Dye
ORPM-206	60	ROX
ORPM-263	52	JOE
ORPM-276	54	6-FAM
ORPM-433	59	TAMRA
BPTTG60	56	TAMRA
BPTGG82	56	HEX
BPCA90	57	6-FAM

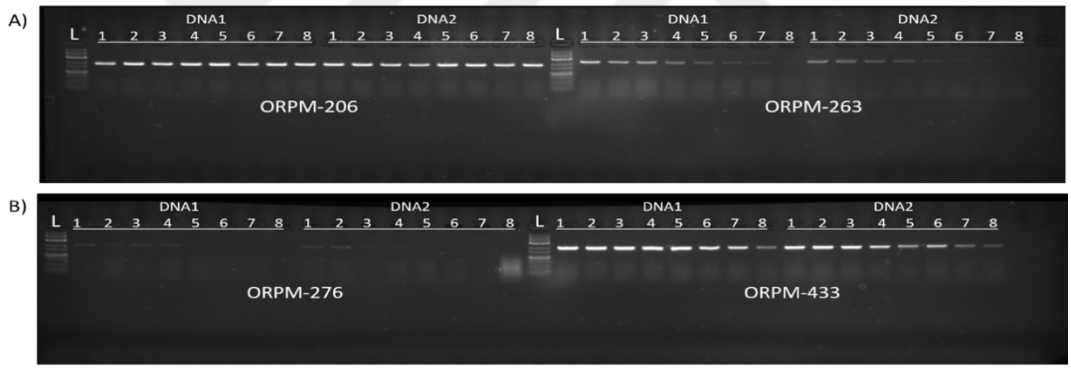

Figure 3.2 Images of PCR products belong to "ORPM" -prefix SSR loci in %3 agarose gel. A. ORPM-206 and ORPM-263 B. ORPM-276 and ORPM-433 (L represents the reference band of DNA. The various temperatures implied by numbers were used to check with two different DNA samples, DNA1 and DNA2, for amplification success.)

3.2.3 DNA Fragment Analysis for Microsatellite Genotyping

The fragmental analysis of amplified products with labelled primers was performed in BM Laboratory Systems Facilities, Ankara. The Applied Biosystems 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) was used with an internal standard size marker, The GeneScan ROX labelled 400HD, for the assay protocol for fragment analysis. Then, by comparing fragments with the standard curve, the relative size of each fragment for each locus was examined. An electrophenogram example was provided in Figure 3.3. The Peak Scanner 2.0 software (Biosystems, 2006) was used to determine allele sizes for each individual per locus to acquire microsatellite genotypes. In Peak Scanner™ v2.0 (Biosystems, 2006) software, as an analysis method, "Sizing Default NPP (NPP: No Primer Peaks)" and "size standard GS400" were adjusted.

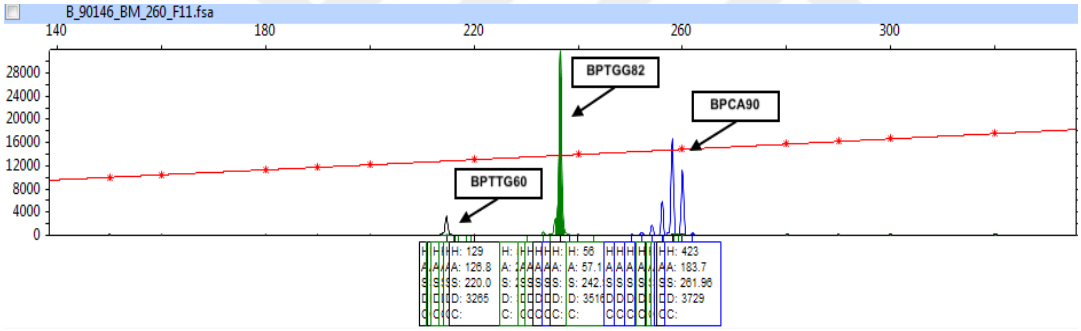

Figure 3.3 A microsatellite Electrophenogram example of three different microsatellite loci used in the study (y-axis: Relative Fluorescence Units, x-axis: Size of the fragment). Each primer labelled with a different fluorescent dye gave fragment peaks of different allele base pairs.

3.3 Population Genetic Analyses

DNA analyses by microsatellite markers have been optimized to examine the level of genetic diversity in female and male *Populus* tree populations. Highly

polymorphic microsatellite markers were used to identify the species' multiallelic nature, codominant inheritance, and clonal identity.

3.3.1 Detection of Clones

Salicaceae is a predominantly dioecious plant family that has the characteristics of clonal reproduction. The genus *Populus* has the ability to reproduce asexually, often by sprouting from the root collar of fallen trees or from broken branches that were inserted into the soil. However, clonal production could result in highly biased explicit sex ratios with unreliable genetic diversity and structure analysis. (Alliende & Harper, 1989; Balloux et al., 2003; Douhovnikoff et al., 2005; Kemperman & Barnes, 1976). Although in the study, samples were collected from individuals at least approximately 200 m apart from each other to prevent clonal sampling, the existence of clones must be checked by microsatellite markers as a universal and reliable method for clone identification. In order to identify any clonal sampling among genotypes in the female and male trees, the collected data from the fragment analysis of 7 microsatellite loci were evaluated with GenClone 2.0 software (Arnaud-Haond & Belkhir, 2007).

3.3.2 Presence of Null Allele

A null allele is an allele at a microsatellite locus that continuously fails to amplify to detected levels via the polymerase chain reaction (PCR) in definition (Dakin & Avise, 2004). Reduced primer annealing is one of the potential sources of null alleles caused by point mutations or indels in the nucleotide sequence, either in one or both flanking primers. Mostly, key mutations were thought to be exceptionally destructive to PCR amplification at the 3' end of the priming site, where extension starts (Kwok, 1990). Null alleles can also be formed from differential amplification size-variant alleles (Wattier et al., 1998). Short-length alleles often amplify more effectively than larger ones due to the competitiveness of PCR, indicating that only the shorter of two alleles from a heterozygous individual can be identified. Another source of null

alleles comes from the low DNA template quality cause by PCR failure (Garcia de Leon et al., 1998). On the other hand, there might be false evidence for null alleles, which include sex linkage. Since heterogametic sex of diploid organisms carries only one allele at every locus contained on a sex chromosome, overlook sex linkage at a locus could lead to a misconstruction of associated locus-specific 'heterozygote deficit' as revealing of null alleles (Dakin & Avise, 2004).

The existence of null alleles could lead to biased estimation of genetic parameters and cause overestimating homozygosity (Becquet et al., 2009; Coimbra et al., 2009; Weetman et al., 2005) and inbreeding levels of populations (Peterson & Jones, 1997; Xu et al., 2001). There were different estimating methods (Brookfield, 1996; Chakraborty et al., 1992; Dempster et al., 1977; Kalinowski et al., 2006; Marshall et al., 1998; Van Oosterhout et al., 2004; Weir & Cockerham, 1984) for the estimation of the null allele to prevent the deviations from Hardy-Weinberg equilibrium. Based on those various methods, various software can be used for the estimation of null allele frequency (r), including Cervus v3.0.7 (Marshall et al., 1998), MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004), ML-NullFreq (Kalinowski et al., 2006), and Genepop v4.2 (Rousset, 2008).

In this study, null allele frequencies were estimated to prevent the deviations from Hardy-Weinberg equilibrium by two different methods: The Maximum-Likelihood (ML) estimator based on the EM algorithm of (Dempster et al., 1977) implemented in Genepop 4.2 (Raymond & Rousset, 1995) and the ML estimator based on observed and expected heterozygosities defined by Chakraborty et al. (1992) with the modification of Brookfield (1996) as implemented in MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004).

3.3.3 Microsatellite Polymorphism and Parameters of Genetic Diversity

Genetic diversity parameters were calculated after completing the testing of clones in samples and the null allele presence. According to Greenbaum et al. (2014), variation in sample sizes could lead to bias in estimating genetic diversity parameters. Thus, to eliminate the sample size bias, first, allelic richness (Ar) was

estimated by FSTAT v2.9.3.2 (Goudet, 1995). Based on the smallest sample size (in this study, it corresponds to females, N=46), the standardized number of alleles was acquired. Thereafter, the number of alleles (Na), mean effective number of alleles (Ne), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), fixation index (F), Shannon's Information Index (I), and private alleles with their frequencies were calculated by GenAlEx v6.503 (Peakall & Smouse, 2012) for each locus and population.

By using Cervus v3.0.7 (Kalinowski et al., 2007), polymorphism information content (PIC) was estimated for each locus. Furthermore, the proportion of polymorphic loci (%P) and the probability of identity (PI) were determined for each gender with GenAlEx v6.503 (Peakall & Smouse, 2012). The Genepop v4.2 software (Raymond & Rousset, 1995) was used to assess F statistics, including F_{IS}, F_{ST}, and F_{IT} for each locus among genders, and perform precise assessments to test the Hardy-Weinberg deviations. A Markov chain (MC) algorithm with default parameters (Dememorization number:1000, Number of batches:100, Number of iterations per batch:1000) was used to estimate the exact p-value of this test with no bias (Guo & Thompson, 1992). Estimation of gene flow was acquired from F_{ST} (N_m= 0.25 (1-F_{ST})/F_{ST}).

For prediction and estimation of effective population size (N_e), Neestimator v2 (Do et al., 2014) was used. For the estimations of contemporary effective population size, both the linkage disequilibrium and heterozygote-excess methods were implemented by NeEstimator v2 (Do et al., 2014).

The Garza-Williamson Index (Garza & Williamson, 2001) was estimated with the Arlequin v3.5.1.2 software for all loci and populations to check for any experienced population decline in effective population size (Excoffier & Lischer, 2010).

The analysis of molecular variance (AMOVA) for all loci was performed by Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) to calculate the partition of the variation among locations, among genders within locations and within trees. Two different analyses were present for AMOVA in Arlequin v3.5.1.2 (Excoffier & Lischer, 2010); the number of different alleles (F_{ST}) based, and the sum of squared

size difference (R_{ST}) based. In the study, both methods were applied for the estimation of molecular variance.

3.3.4 Genetic Structure of Populations

Pairwise F_{ST} values examined genetic differentiation between genders. By using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010), the pairwise F_{ST} matrix and their corresponding N_m values were acquired.

In order to analyze the expression of the genetic differences over genders, a principal coordinates analysis (PCoA) based upon the covariance matrix with data standardization was produced with the pairwise F_{ST} values by GenAlEx v6.503 (Peakall & Smouse, 2012).

STRUCTURE v2.3.4 (Pritchard et al., 2000) software was used to characterize gender related genetic structure considering the geographical distribution of populations. A Bayesian iterative algorithm was performed to specify individuals into clusters as applied in STRUCTURE v2.3.4 (Pritchard et al., 2000). It was presumed the Admixture model 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=6. It was performed for both genders and the geographical distribution of populations with ten replications for each K.

With the run results of STRUCTURE v2.3.4 (Pritchard et al., 2000), web-enabling software Structure Harvester (Earl & vonHoldt, 2012) was used to evaluate the "true K" (most likely value of K) for the revealing the number of genetic groups existing in the data as implemented in Evanno method (Evanno et al., 2005).

By using the CLUMPP (Jakobsson & Rosenberg, 2007) software, multiple runs for the “true K” value were evaluated to identify the best alignment to the replicate results of the cluster analysis findings. Finally, for visualization, the Pophelper software (Francis, 2017) was used for the data generated from the admixture analysis.

CHAPTER 4

RESULTS

4.1 Quality Control of the Data

4.1.1 Detection of Clones and Identification of Possible Null Alleles

In the field studies, leaf samples were obtained at least 200 m apart from each other to avoid clonal sampling. The data was checked with GenClone v2.0 software (Arnaud-Haond & Belkhir, 2007) detected no clonal sampling.

The MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004) was used to detect possible null allele existence and their frequencies for each locus in each population. The results obtained from these analyses were given in Table 4.1 and 4.2, for female and male populations, respectively. The results indicated that there was no locus showing evidence of a null allele. Additional null allele detection analyses were performed with the Genepop v4.2 (Arnaud-Haond & Belkhir, 2007) and the results were given in Appendix C.

Table 4.1 Estimation of Null Allele frequency for each locus for female tree populations (MICRO-CHECKER v2.2.3) (Brookfield 1996; Chakraborty *et al.* 1992)

Population / Locus	Female GUP		Female GMID		Female GDOWN	
	Chakraborty	**Brookfield**	**Chakraborty**	**Brookfield**	**Chakraborty**	**Brookfield**
ORPM-206	-0.0480	-0.0197	-0.0535	-0.0207	-0.0345	-0.0079
ORPM-263	-0.1640	-0.1354	-0.2549	-0.2549	-0.2056	-0.1881
ORPM-276	-0.1321	-0.0802	-0.1429	-0.0909	-0.0909	-0.0435
ORPM-433	-0.0714	-0.0289	0	0	0	0
BPTTG60	0	0	0	0	0	0
BPTGG82	-0.2785	-0.2664	-0.2615	-0.2509	-0.3043	-0.2908
BPCA90	-0.1232	-0.1081	-0.1730	-0.1730	-0.1420	-0.1306

Table 4.2 Estimation of Null Allele frequency for each locus for male tree populations (MICRO-CHECKER v2.2.3) (Brookfield 1996; Chakraborty *et al.* 1992)

Population / Locus	Male GUP		Male GMID		Male GDOWN	
	Chakraborty	**Brookfield**	**Chakraborty**	**Brookfield**	**Chakraborty**	**Brookfield**
ORPM-206	-0.0815	-0.0441	-0.0256	-0.0059	-0.0226	-0.0047
ORPM-263	-0.1648	-0.1536	-0.2139	-0.2050	-0.2014	-0.1865
ORPM-276	0.1787	0.0585	-0.0714	-0.0289	-0.1525	-0.1009
ORPM-433	-0.0141	-0.0015	0	0	-0.0149	-0.0016
BPTTG60	0	0	0	0	0	0
BPTGG82	-0.3077	-0.2956	-0.2520	-0.2299	-0.3061	-0.2934
BPCA90	-0.1514	-0.1514	-0.1475	-0.1417	-0.1429	-0.1376

4.2 Genetic Diversity Measures

4.2.1 Microsatellite Polymorphism and Genetic Diversity Parameters

Table 4.3 illustrates the detected alleles in each locus for all female and male tree populations. According to detected alleles of seven SSR loci, BPTTG60 was monomorphic for female and male populations. The remaining six loci were polymorphic, and BPCA90 was the most variable locus with eight alleles, while ORPM-276 and ORPM-433 had the lowest variation, having only two alleles. Private alleles were genuine for ORPM-206 and BPCA90 within the seven SSR loci. The allele size of seven SSR loci ranged from 169 bp to 272 bp (Table 4.3).

Table 4.3 Observed alleles in each locus (Private alleles were shown in bold).

Locus	Alleles detected (bp)
ORPM_206	**179**, 182,188,191,200
ORPM_263	236, 238, 240
ORPM_276	169, 197
ORPM_433	186, 204
BPTTG60	214
BPTGG82	233, 236, 239
BPCA90	244, 250, **252**, 256, 258, **264**, **268**, 272

Genetic diversity parameters of seven microsatellite loci were provided in Table 4.4. The number of alleles per locus (Na) varied between 6.5±0.5 for BPCA90 and 1±0 for BPTTG60 (monomorphic). The number of effective alleles per locus (Ne) ranged from 1±0 to 3.30±0.25 for BPTTG60 and BPCA90, respectively. Shannon's information index (I) denoted the unifying measure of diversity and had a mean value of 0.66±0.12 for all loci. The average weight of I indicated the efficiency of SSR loci to reveal the variation present. The mean value of observed heterozygosity (Ho) was 0.49±0.11, while the mean value of unbiased expected heterozygosity (uHe) was

0.35±0.07 for all studied loci. All polymorphic loci had higher Ho than uHe, meaning that excess of heterozygotes; thus, having a negative fixation index (F). Allelic richness (Ar) values of seven SSR loci varied between 1 (for BPTTG60) to 6.435 (for BPCA90). The lowest probability of identity (PI) value was detected as 0.141 in BPCA90, and the highest PI value was 0.884 for ORPM-422 locus in between all studied polymorphic loci. By obtaining Polymorphism Information Content (PIC) value, which was introduced by Botstein et al. (1980) as a measure of polymorphism, the usefulness of the marker for linkage analysis can be measured. In the study, the PIC values varied between 0 (for BPTTG60) and 0.650 (for BPCA90).

F statistics of seven SSR loci for all female and male populations were provided in Table 4.5. F-Statistics present all polymorphic loci in the study had negative F_{IS} values for all female and male tree populations, which indicated an excess of heterozygotes (outbreeding). F_{ST} values varied between 0.003 for BPTGG82 and 0.072 for ORPM-433 with a mean of 0.027±0.002 over all polymorphic loci and all populations (Table 4.5).

Table 4.4 Genetic diversity parameters of the seven microsatellite loci

Locus	N	Na	Ne	I	Ho	uHe	F	Ar	PI	PIC
ORPM-206	48±2	4.5±0.5	1.25±0	0.47±0.01	0.22±0	0.20±0	-0.08±0	4.371	0.643	0.195
ORPM-263	48±2	3±0	2.50±0.17	0.99±0.04	0.89±0.02	0.60±0.03	-0.48±0.04	3.000	0.234	0.532
ORPM-276	48±2	2±0	1.47±0.5	0.50±0.03	0.38±0.06	0.32±0.02	-0.18±0.10	2.000	0.517	0.267
ORPM-433	48±2	2±0	1.07±0.03	0.14±0.04	0.63±0.02	0.06±0.02	-0.03±0.01	1.981	0.884	0.059
BPTTG60	47±1	1±0	1±0	0.00	0.00	0.00	-	1.000	1	0
BPTGG82	47±1	3±0	2.07±0.04	0.77±0.04	0.93±0.01	0.52±0.01	-0.79±0.02	2.934	0.348	0.403
BPCA90	47±1	6.5±0.5	3.30±0.25	1.38±0.08	0.93±0.03	0.70±0.02	-0.33±0	6.435	0.141	0.650
Grand Mean	47.58± 0.48	3.14± 0.48	1.81± 0.22	0.66± 0.12	0.49± 0.11	0.35±0.07	-0.32±0.07	-		0.301

N: Sample size, Na: mean allele number, Ne: effective number of alleles, I:Shannon's Information Index, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, F: inbreeding coefficient, Ar:allelic richness, PI: Probability of Identity, PIC: polymorphism information content

Table 4.5 F-Statistics and Nm estimates for each locus

LOCUS	F_{IS}	F_{IT}	F_{ST}	N_M
ORPM-206	-0.111	-0.079	0.029	8.408
ORPM-263	-0.507	-0.472	0.023	10.421
ORPM-276	-0.210	-0.188	0.018	13.703
ORPM-433	-0.113	-0.033	0.072	3.201
BPTTG60	Monomorphic			
BPTGG82	-0.797	-0.792	0.003	80.163
BPCA90	-0.346	-0.327	0.014	17.358
MEAN	-0.348±0.108	-0.315±0.108	0.027±0.002	22.209±10.429

4.2.2 Genetic Diversity Among Genders

In female and male tree populations, detected private alleles for each locus were shown with respective frequencies in Table 4.6. Allelic patterns across populations were generated with the mean values of Na (number of different alleles), Na with Freq≥5%, Ne (number of effective alleles), I (Shannon's Information Index), No. LComm Alleles ≤ 50% and He: expected heterozygosity (Table 4.7). The allelic patterns between populations were illustrated in Figure 4.1. The genetic diversity parameters of all studied female and male tree populations were summarized in Table 4.8.

Private alleles were detected only in two populations: male trees from the upstream site of Göksu and female trees from the middle section of the Göksu river (Table 4.6). The mean values of private alleles were observed in male trees from the upstream of the Göksu river and female trees from the middle section and ranged from 0.43±0.30 and 0.14±0.14, respectively. The mean number of alleles per locus (Na) varied from 2.14±0.40 for female trees of Göksu downstream population to 3.00±0.76 for male trees of Göksu upstream population with a mean value of

2.60±0.21. The effective number of alleles was comparable for gender structured populations with a mean value of 1.79±0.12 (Table 4.7).

As shown in the genetic diversity parameters table (Table 4.8), the mean value of Shannon's information index (I) was 0.58±0.07 for female and male populations. The average weight of %P, which indicated the percentages of polymorphic loci among populations was 78.57%±3.19% and relatively higher in male tree populations (80.95% for males and 76.19% for females). The observed heterozygosity (Ho) values were higher than unbiased expected heterozygosity (uHe) values for all female and male tree populations. The mean values of Ho and uHe were 0.49±0.11 and 0.35±0.07, respectively. As a result of the excess of heterozygosity, fixation index (F) was found to be negative for all populations with the mean of -0.36±0.05. The overall differentiation between populations was represented by F_{ST} value, which was detected as 0.17±0.13 for the study.

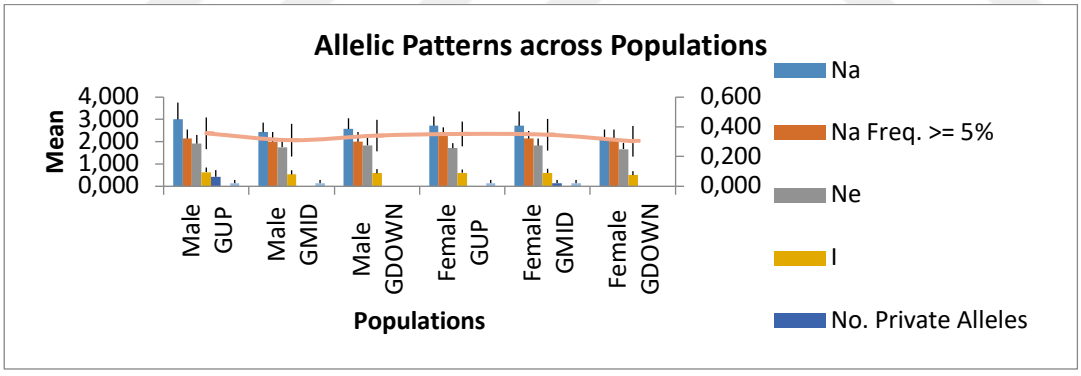

Figure 4.1 Allelic patterns across populations. Na: No. of Different Alleles, Na (Freq≥5%): No. of Different Alleles with a Frequency≥ 5%, Ne: No. of Effective Alleles, I: Shannon's Information Index, No. Private Alleles: Mean No. of Alleles Unique to a Single Population

Table 4.6 Private alleles and respective frequencies by locus in studied gender populations

Population	Locus	Allele	Frequency
Male GUP	ORPM-206	179	0.028
Male GUP	BPCA90	264	0.029
Male GUP	BPCA90	268	0.029
Female GMID	BPCA90	252	0.029

Estimation of effective population size (N_e) was performed to have better understanding about the history of the populations regarding inbreeding and genetic drift. The effective population size provides the prospects for the adaptive potential under environmental change as a function of vulnerability to genetic drift. As given in the estimation of effective population size table (Table 4.9), infinity results in confidence intervals of the LD method indicated little power to make any inferences about effective population size. From the estimation with heterozygote excess method results, N_e/N_c ratios (effective population size over current population size) were calculated. In theory, N_e/N_c ratio in the wild should be ≥ 0.1 (Frankham, 2008) to protect the adaptive potential of populations. This condition only meets in GUP (Gökcay&Ermenek) population when 0.01 were used as the lowest allele frequency. The rest of the estimations from all populations, N_e/N_c ratios were below the critical value which could be an indication of experienced genetic drift.

Table 4.7 Mean allelic patterns across populations

Population	Na	Na Freq. >= 5%	Ne	I	Mean No. Private Alleles	No.LCom Alleles (≤50%)	Ho	uHe
Male GUP	3.00±0.76	2.14±0.40	1.92±0.39	0.64±0.20	0.43±0.30	0.14±0.14	0.49±0.17	0.37±0.11
Female GUP	2.71±0.42	2.29±0.36	1.71±0.22	0.61±0.14	0.00	0.14±0.14	0.48±0.13	0.36±0.09
Male GMID	2.43±0.43	2.00±0.44	1.76±0.33	0.53±0.19	0.00	0.14±0.14	0.45±0.17	0.32±0.11
Female GMID	2.71±0.64	2.14±0.34	1.83±0.32	0.60±0.19	0.14±0.14	0.14±0.14	0.52±0.17	0.36±0.11
Male GDOWN	2.57±0.48	2.00±0.44	1.84±0.36	0.59±0.19	0.00	0.00	0.51±0.17	0.35±0.11
Female GDOWN	2.14±0.40	2.14±0.40	1.67±0.28	0.50±0.18	0.00	0.00	0.45±0.16	0.32±0.11

Na: No. of Different Alleles, Na (Freq≥5%): No. of Different Alleles with a Frequency≥ 5%, Ne: No. of Effective Alleles, I: Shannon's Information Index, Mean No. Private Alleles: Mean number of Alleles Unique to a Single Population, No. LCom Alleles (≤50%) : No. of Locally Common Alleles (Freq.≥5%) Found in 50% or Fewer Populations, Ho:observed heterozygosity, uHe:unbiased expected heterozygosity

Table 4.8 Genetic diversity parameters of *Populus euphratica* populations

Population	N	Na	Ne	I	%P	G-W index (M)	Ho	uHe	F	F_{ST}
Male GUP	17.57±0.2	3.00±0.76	1.92±0.39	0.64±0.20	85,71%	0.28±0.22	0.49±0.17	0.37±0.11	-0.26±0.15	0.17±0.13
Female GUP	16.00±0	2.71±0.42	1.71±0.22	0.61±0.14	85,71%	0.28±0.20	0.48±0.13	0.36±0.09	-0.32±0.08	
Male GMID	15.00±0	2.43±0.43	1.76±0.33	0.53±0.19	71,43%	0.33±0.20	0.45±0.17	0.32±0.11	-0.35±0.10	
Female GMID	17.00±0	2.71±0.64	1.83±0.32	0.60±0.19	71,43%	0.32±0.20	0.52±0.17	0.36±0.11	-0.45±0.10	
Male GDOWN	14.57±0.2	2.57±0.48	1.84±0.36	0.59±0.19	85,71%	0.27±0.22	0.51±0.17	0.35±0.11	-0.39±0.13	
Female GDOWN	15.00±0	2.14±0.40	1.67±0.28	0.50±0.18	71,43%	0.32±0.23	0.45±0.16	0.32±0.11	-0.40±0.12	
Mean	15.86± 0.18	2.60± 0.21	1.79± 0.12	0.58± 0.07	78,57% ±3,19%	0.30±0.22	0.49± 0.11	0.35±0.07	-0.36±0.05	

N: Sample size, Na: mean allele number, Ne: effective number of alleles, I: Shannon's Information Index, %P: Percentage of Polymorphic loci, G-W: Garza- Williamson, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, F: the fixation index over loci FST: the proportion of the diversity in the sample that' s due to allele frequency differences among populations

Table 4.9 Estimations of Effective Population Size

Region	Method	Lowest Allele Freq. Used	N_e	95% CI for N_e
GUP (Gökcay&Ermenek) (N=33)	Heterozygote Excess Method	0.050	2.6	1.6 – 12.1
		0.020	2.8	1.7 – 13.4
		0.010	3.3	1.9 – 18.4
	LD Method	0.050	156.5	14.1 – Infinite
		0.020	Infinite	19.7 – Infinite
		0.010	89.6	17.3 – Infinite
GMID (Mut) (N=31)	Heterozygote Excess Method	0.050	1.9	1.3 – 5.0
		0.020	2.1	1.3 – 5.6
		0.010	2.2	1.4 – 6.3
	LD Method	0.050	Infinite	184.5 – Infinite
		0.020	774.5	13.3 – Infinite
		0.010	Infinite	18.1 – Infinite
GDOWN (Silifke) (N=32)	Heterozygote Excess Method	0.050	1.8	1.2 – 5.0
		0.020	2.2	1.4 – 7.3
		0.010	2.4	1.5 – 8.6
	LD Method	0.050	Infinite	14.5 – Infinite
		0.020	Infinite	23.5 – Infinite
		0.010	13.6	3.2 – 89.7

In order to analyze the possible past bottlenecks arisen in the populations, Garza-Williamson index, M values were calculated for populations and shown in Table 4.8 (Garza & Williamson, 2001). M value, which is the mean ratio of the number of alleles to the range in allele size calculated from a population sample of microsatellite loci is useful to identify the reductions in population sizes in the past. Moreover, Garza-Williamson indexes at different loci were given for each population (Figure 4.2) and for male and female tree populations combined from all locations (Figure 4.3). For all populations, M values varied between 0.27±0.22 and 0.33±0.20 which were below the critical value (0.68), indicating that the bottleneck occurred in the populations.

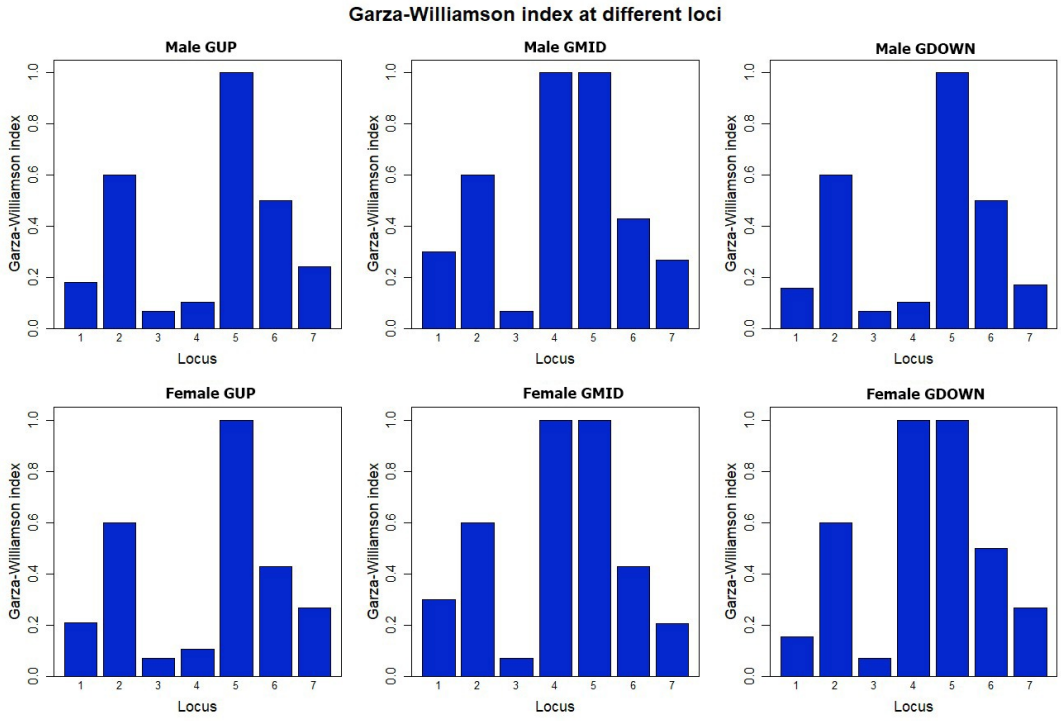

Figure 4.2 The Garza-Williamson index at polymorphic loci in each population. Numbers 1, 2, 3, 4, 5, 6 and 7 indicate loci ORPM-206, ORPM-263, ORPM-276, ORPM-433, BPTTG60, BPTGG82 and BPCA90, respectively.

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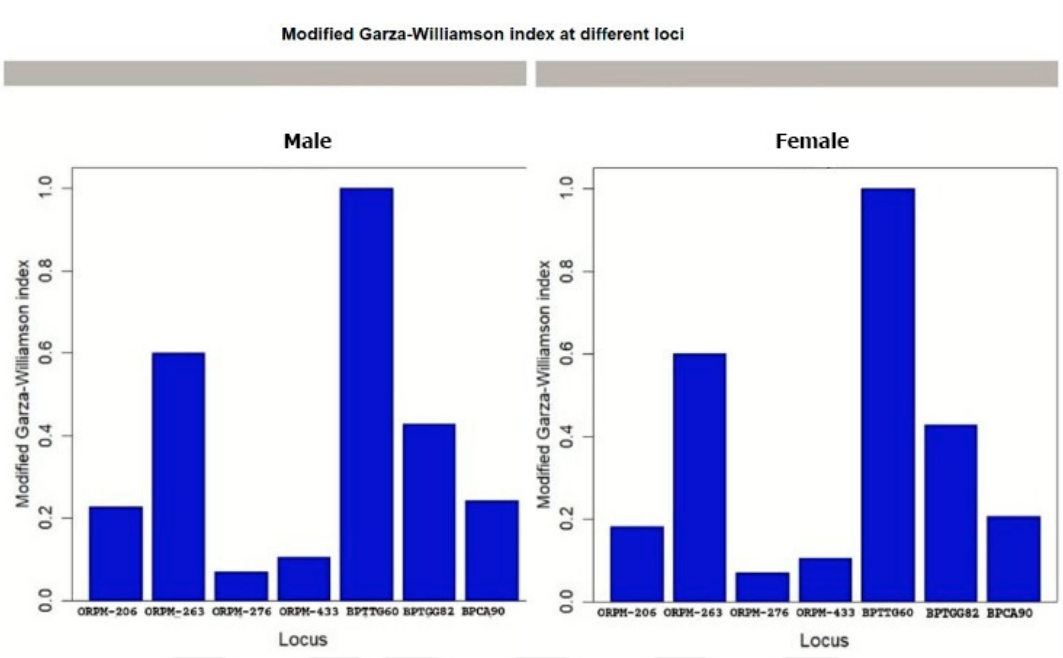

Figure 4.3 The Garza-Williamson index at polymorphic loci for male and female tree populations combined from all locations.

4.2.2.1 Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was performed by using Arlequin v3.5.2.2 (Excoffier & Lischer, 2010). F_{ST} based and R_{ST} based models were used to perform this analysis (Table 4.10). In both models, the total genetic variance was explained significantly by the differences among trees within genders.

Table 4.10 Analysis of molecular variance (AMOVA) for female and male *Populus euphratica* populations by using seven microsatellite loci between genders/regions

	Source of variation	Sum of squares	Variance components	Percentage of variation
Based on F_{ST}	**Among locations**	2.017	0.00607	0.52%
	Among genders within locations	1.876	-0.01772	0
	Among trees within genders	221.310	1.18984	99.48%
	Total	225.203	1.17819	100.00%
Based on R_{ST}	**Among locations**	153.336	-1.83624	0
	Among genders within locations	581.814	0.76720	0.46%
	Among trees within genders	31526.554	169.49760	99.54%
	Total	32261.703	168.42856	100.00%

4.3 Population Genetic Structure

4.3.1 Principal Coordinate Analysis with Pairwise F_{ST} matrix

Calculated Pairwise F_{ST} values were given in Table 4.11 and illustrated in Figure 4.3. As given in Table 4.11, the highest p value of F_{ST} found between male and female populations from middle region of Göksu. Except the F_{ST} values between genders in middle region of Göksu, detected F_{ST} values were quite low (Figure 4.4).

Table 4.11 Pairwise F_{ST} matrix (p-values) of populations

	Male GUP	**Female GUP**	**Male GMID**	**Female GMID**	**Male GDOWN**	**Female GDOWN**
Male GUP	-					
Female GUP	0.25±0.04	-				
Male GMID	0.25±0.04	0.07±0.02	-			
Female GMID	0.27±0.03	0.13±0.04	0.81±0.02	-		
Male GDOWN	0.16±0.04	0.28±0.04	0.11±0.02	0.35±0.06	-	
Female GDOWN	0.27±0.04	0.43±0.06	0.77±0.04	0.49±0.04	0.47±0.03	-

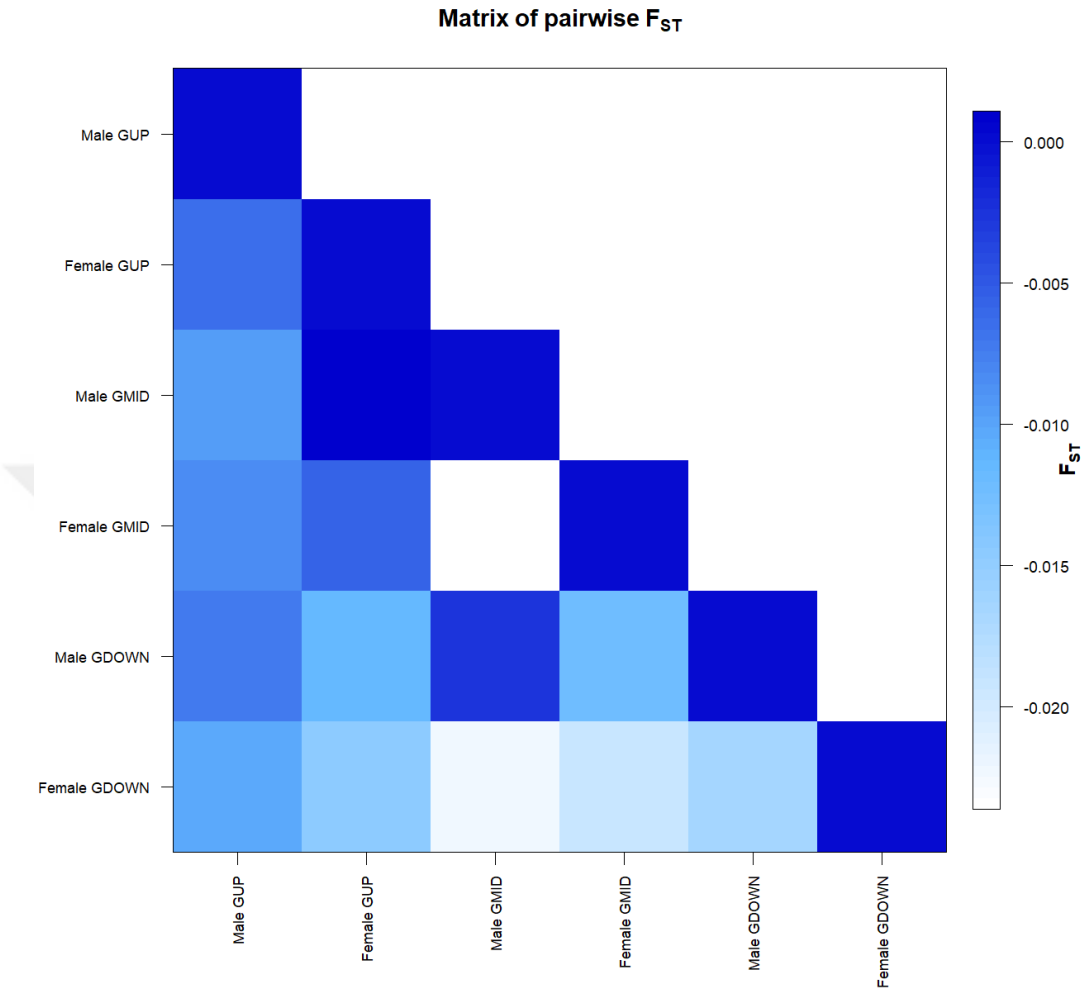

Figure 4.4 Matrix of pairwise F_{ST} among populations.

Principal Coordinates Analysis (PcoA) was used to detect the distribution structure of genetic variation across geographical location and sample background (McVean, 2009). PcoA was accomplished with GenAlEx 6.503 (Peakall & Smouse, 2012) based on population pairwise F_{ST} values and shown in Figure 4.4. The first principal component explained 42.76% of the total variance, and the second principal component accounted for 21.91% of the total variation among studied populations (Figure 4.5).

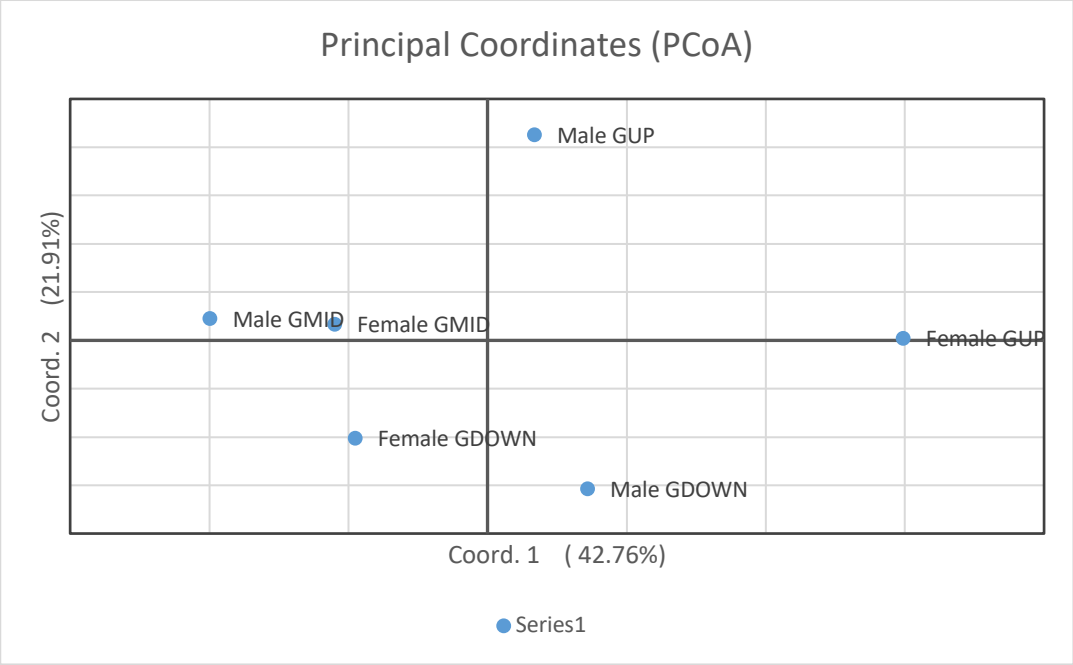

Figure 4.5 Principal component analysis (PCoA) based on gendered structure population pairwise F_{ST} values

4.3.2 Clustering

To identify the patterns of genetic structures in the study, alterations in the distribution of genetic variants were analyzed with a Bayesian iterative algorithm by STRUCTURE v2.3.4 (Pritchard et al., 2000). The patterns of genetic structures were evaluated without giving prior information about individuals. This means, all the female and male trees from different localities were introduced as a whole population. By using the web-based program Structure Harvester, which was implemented the Evanno Method (Evanno et al., 2005), true K with the highest ΔK and lowest standard deviation was detected as two (Table 4.12 and Figure 4.6). The clustering patterns of populations were given for the first two highest values of K (K=2 and K=5) in Figure 4.7.

Table 4.12 Evanno table illustrates ΔK values for clustering analysis of populations (Evanno et al., 2005).

K	Reps	Mean LnP(K)	StDev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-825.8400	0.2633	NA	NA	NA
2*	10	-827.1800	0.7569	-1.340000	1.570000	2.074266
3	10	-826.9500	0.6241	0.230000	NA	NA
4	10	-826.5300	0.5813	0.230000	0.270000	0.464490
5	10	-826.0300	0.4715	0.500000	0.710000	1.505761
6	10	-826.2400	0.3658	-0.210000	0.380000	1.038943
7	10	-826.0700	0.5397	0.170000	0.500000	0.926526
8	10	-826.4000	0.4853	-0.330000	0.700000	1.442286
9	10	-826.5300	0.5813	0.230000	0.270000	0.464490
10	10	-826.0300	0.4715	0.500000	0.710000	1.505761

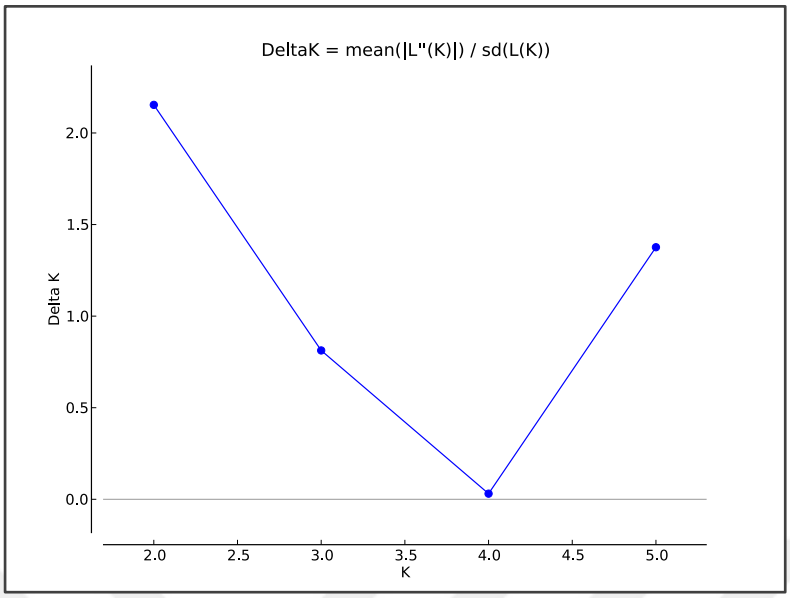

Figure 4.6 The estimated deltaK value by delta-K method of Evanno et al. (2005) for female and male tree populations of Göksu river.

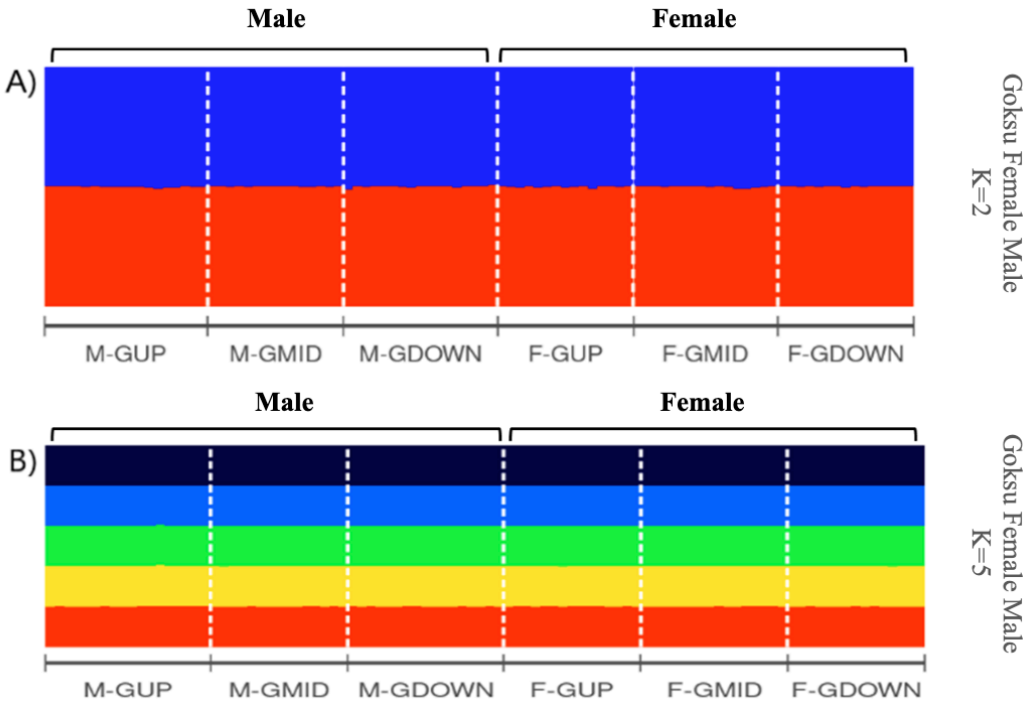

Figure 4.7 STRUCTURE (Pritchard et al., 2000) analyses of female and male *Populus euphratica* trees in the Göksu river A. K=2 and B. K=5. Each color represents a different cluster.

CHAPTER 5

DISCUSSION

In this thesis, the genetic diversity and genetic structure of female and male individuals in *Populus euphratica* populations from the Göksu river have been screened for the first time with selected microsatellite markers. The main objective of this study was the identification of microsatellite markers that could be used to differentiate male and female *Populus euphratica* trees. The microsatellite markers used in this study were designed initially for other genetic linkage maps belonging to several sex-related studies in *Populus*. In addition to other *Populus* species, those microsatellite markers were assumed to get the potential genetic and genomic features in the peritelomeric region of chromosome XIX, which was stated as developing characteristics of a sex chromosome, for *Populus euphratica* as well (Pakull et al., 2009, 2011; Paolucci et al., 2010; Yin et al., 2008).

Flowering in *Populus euphratica* starts at the age of 10 to 12 years for both sexes (Wiehle et al., 2009). Therefore, early differentiation of young seedlings into males and females can provide augmentation in breeding and advancement research programs for genetic improvements of *Populus euphratica.* The results obtained from this study have unique characteristics in comparison with previous gender-determination studies of *Populus*. Furthermore, interpretations about the genetic diversity potential within the male and female individuals and information about the genetic structure of *P. euphratica* populations in the Göksu river were analyzed and presented. Therefore, acquired results can offer essential information for gender determination and conservation studies in the future.

5.1 Genetic Diversity of The Microsatellite Loci

The microsatellite markers with the prefix ORPM (Oak Ridge *Populus* microsatellite) were designed firstly for *Populus trichocarpa* (Tuskan et al., 2004).

The rest of the markers, the ones with prefix BP, mapped in the cross *P. tremula* x *P. tremuloides* had amplified with primers based on the *P. trichocarpa* genomic sequence (Pakull et al., 2011). These markers were on a list of nucleotide repeats on the linkage group on chromosome XIX of *P. trichocarpa* (Yin et al., 2008). While selecting these markers, we considered that they could serve as the primary bridge connecting the peritelomeric region of chromosome XIX in *P. trichocarpa* to the gender determination region of *Populus euphratica.*

Among the studied seven microsatellite loci, BPTTG60 was found as monomorphic for all female and male tree populations. According to a previous study in a hybrid aspen population (*Populus tremula x Populus tremuloides),* paternal alleles of the BPTTG60 marker were inherited from the father 100% sex-linked (Pakull et al., 2011). Recent studies also revealed that in the monoecious (bisexual) *Populus tremula,* BPTTG60 was detected as homozygous (Fladung et al., 2019). Even though the BPTTG60 locus was monomorphic, it was used in further genetic diversity analyses to evaluate similarity among female and male individuals of *Populus euphratica.*

Among the polymorphic markers, polymorphism information content (PIC) was ranged between 0.059 and 0.650 with an average of 0.351. According to Botstein et al. (1980), locus could be accepted as highly informative if PIC>0.5, reasonably informative if 0.5>PIC>0.25, and slightly informative if PIC < 0.25. For each locus, the PIC value was calculated relatively with polymorphism content. Within the polymorphic markers, those with the prefix ORPM, ORPM-263 was highly informative (0.532), and ORPM-276 was reasonably informative (0.267). However, ORPM-206 and ORPM-433 loci were slightly informative (0.195 and 0.059, respectively), suggesting less discriminatory power of these markers under study. On the other hand, BPCA90 was detected as highly informative with the highest PIC value (0.650), and BPTGG82 was reasonably informative (0.403).

Probability of identity (PI) is one of the most used theoretical estimators to approach the relied statistical for individual identification and measure the genetic diversity in populations (Dokupilová et al., 2014; Mollet et al., 2015; Waits et al., 2001). PI value

demonstrates the average probability of the identical genotype in two independent individuals (Peakall & Smouse, 2012), associated with a low quantity of identified alleles if higher than 0.546 (Hvarleva et al., 2007). In this study, we found that ORPM-263, BPTGG82, and BPCA90 loci had sufficiently low PI values (0.234, 0.348, and 0.141, respectively), and ORPM-276 had relatively high, but an acceptable PI value (0.517). However, ORPM-206 and ORPM-433 loci had high PI values (0.643 and 0.884, respectively), and these markers were also stated as slightly informative due to their low PIC values. Moreover, these loci also had low Shannon's information index (I) values, which is used for the estimation markers' efficiency to characterize species diversity in a community (Niklaus et al., 2001; Poole & Catling, 1974). The rest of the polymorphic loci, ORPM-263, ORPM-276, BPTGG82, and BPCA90, had relatively high I value. Taking account for all, it can be stated that ORPM-206 and ORPM-433 are not as sufficiently informative as the others for analyzing the genetic diversity when they are used separately for *P. euphratica* populations.

For estimation of genetic diversity, allelic richness (Ar) and heterozygosity are two mostly used measures, yet heterozygosity is implemented more regularly (Greenbaum et al., 2014). However, Ar measures are critical for the characteristic of a population's genetic diversity concerning the long-term evolutionary potential of the population (Allendorf, 1986; Petit et al., 1998). Among the polymorphic loci in our study, the highest allelic richness (6.435) was found in the BPCA90 locus, which has also the highest heterozygosity and PIC values. Moreover, among six polymorphic loci, three of the four detected private alleles were found in locus BPCA90. Therefore, it can be stated that for certain, BPCA90 was the most informative and effective locus having high polymorphism and heterozygosity values in our study. Despite its low PIC and high PI values of ORPM-206, the second-highest Ar value (4.371) was detected in this locus. There have been similar Ar values detected in ORPM-206 locus from studies with *P. tremula* species (4.753) from the Tisza river hybrid zone, and *P. alba* species (5.020) from the Ticino river hybrid zone (Caseys et al., 2015). Among the studied loci, ORPM-206 was one the

most examined locus with several poplar species which also carried potential for *P. euphratica* due to its high allelic richness for this study.

Observed and expected heterozygosity values of ORPM-206 and ORPM-276 loci detected in our study were slightly lower than those found in Eastern Alps and Sweden populations of *P. tremula* (MacAya-Sanz et al., 2011). Also, for ORPM-276 locus, higher heterozygosity values were stated in Danube and Tisza populations of *P. alba* (MacAya-Sanz et al., 2011) and *P. tremula* populations in Sweden (Hall et al., 2007). When considering that sample sizes, distinct geographical areas, and different strains applied to the aforementioned studies, the variation in heterozygosity values among European *Populus* species and the Göksu river populations of *P. euphratica* could be comprehensible. Still, all the polymorphic markers in this study had higher observed heterozygosity (Ho) values than unbiased expected heterozygosity (uHe) values. ORPM-263, BPTGG82, and BPCA90 were showing significant deviations from Hardy-Weinberg equilibrium. The deviation from the Hardy-Weinberg equilibrium might result from anthropogenic pressures, such as habitat fragmentation, caused to failure for the trees to find a potential mate for reproduction (Kansu & Kaya, 2020). Besides non-random mating, the advantages of heterozygote individuals in populations and vegetative reproduction of the trees could be possible reasons for the deviation from Hardy-Weinberg equilibrium (Balloux et al., 2003; Balloux & Lehmann, 2003; Chen, 2010).

Heterozygote excess was observed from the negative F_{IS} values for all six polymorphic loci. In the study with *P. alba,* ORPM-206 had negative F_{IS} values for both males and females, but ORPM-276 had negative F_{IS} value only for female individuals (MacAya-Sanz et al., 2011). Similarly, ORPM-276 locus had positive F_{IS} for *P. tremula* populations indicating an excess of homozygotes (Hall et al., 2007). Estimation of $F_{ST,}$ which indicates interspecific differentiation, yielded nearly the same values among the studied loci and has a mean of 0.027±0.002 for all. Estimated F_{ST} value is in the range 0–0.05 indicates slight genetic differentiation, and a value between 0.05 and 0.15 indicates moderate differentiation (Charlesworth, 1999; Wright, 1978). According to this, moderate differentiation was indicated only

by ORPM-433 locus. The rest of the polymorphic loci (ORPM-206, ORPM-263, ORPM-276, BPTGG82, and BPCA90) contributed slightly to genetic differentiation of gender structured populations. The low level of migration could affect the F_{ST} estimation due to the sensitivity to the mutation rate of microsatellite markers (Balloux & Lugon-Moulin, 2002). Nonetheless, a recent study in the Göksu river populations of *P. euphratica* revealed similar estimations of $F_{ST,}$ which was explained by the absence of geographical barriers (Kansu & Kaya, 2020).

5.2 Genetic Diversity and Population Structure Among Genders of *Populus euphratica*

Understanding the population dynamics of the *Populus euphratica,* sex determination, magnitude, and structure of genetic diversity of female and male tree populations were investigated in the current study.

Genetic diversity parameters acquired in female and male individuals of *Populus euphratica* from the Göksu River were compared with previous studies of *P. euphratica.* In the study from Israel, the value of mean effective number of alleles was detected as 1.9, which is quite similar to our results (1.79±0.12) (Rottenberg et al., 2000). In this study, observed heterozygosity values were considerably higher than those detected in *P. euphratica* populations in Israel (Rottenberg et al., 2000) and Turkey (Kansu & Kaya, 2020). The reason behind this might be the recent gene flow event between populations separated by physical barriers before (Charlesworth, 1999). Our results revealed a relatively high level of heterozygosity values, regardless of gender and geographic area. In the literature, this phenomenon can be explained by isolate breaking, which is the opposite of the Wahlund effect (Wahlund, 2010). Recent secondary contact with divergent populations could cause heterozygote excess (Ho > He), and in the absence of new immigration, it would continue for several generations (Cornuet & Luikart, 1996). Besides, there would be no significant increase in allelic diversity regardless of heterozygote excess. According to Greenspoon & M'Gonigle (2014), negative assortative mating or

disassortative mating, which is the non-random mating with phenotypically different individuals, leads to an excess of heterozygotes in populations and results in the deviation from Hardy–Weinberg proportions. Furthermore, excess of heterozygotes would be distributed roughly comparable in the subpopulations (Hedrick et al., 2016). For our study, along with disassortative mating, heterozygote advantage could bring about heterozygote excess without a significant allelic variation. In a given population, heterozygosity advantage occurs when the stronger condition that all heterozygotes have higher fitness than the fitness of both homozygotes (Penn et al., 2002). This phenomenon more likely happened in old and structured populations with small subpopulations (Nielsen, 2005) like *P. euphratica* populations from the Göksu river, which were defined as a large metapopulation that includes clusters of subpopulations distributed in a patchy environment (Kansu & Kaya, 2020).

When genders in *P. euphratica* populations from same locations were examined separately, observed/expected heterozygosity values of female and male trees revealed no significant differences between them. Moreover, in compliance with the detected allelic richness of female and male individuals, as well as the effective number of alleles that they had, there was no significant variation amongst gender. On the other hand, analyses demonstrated the distribution inequality of private alleles on female and male individuals. Private alleles were detected only in two populations: male trees from the upstream of the Göksu river and female trees from the middle section. A greater proportion of these private alleles was present in male individuals. In male trees, allele 179 for ORPM-206 and, alleles 264 and 268 for BPCA90 loci were detected. While female trees had only allele 252 for BPCA90 locus was identified. Private or rare alleles in populations play an essential role in successful adaptation when environmental changes occur (Jaramillo-Ramirez et al., 2010). For the long-term preservation of genetic diversity, phylogenetic history, and the species' evolutionary potential, carrying private alleles could be incommensurably critical (Hampe & Petit, 2005). Also, the occurrence of private alleles in the studied loci can contribute to the determination of sex. Although the number of private alleles among *P. euphratica* populations from the Göksu basin

was detected as quite low, these loci with detected private alleles had a potential for sex determination studies, especially BPCA90 locus. The potential utility of BPCA90 in sex determination can be supported by a recent study which was carried out self-pollination experiments on the bisexual *Populus tremula* (Fladung et al., 2019). According to this study, BPCA90 locus had specific alleles segregate with sex chromosomes; males (YY), females (XX) and supermales (XY) revealed different bands (Fladung et al., 2019).

Overall, to get a better understanding the alleles could be determined as specific to male or female trees based on their frequencies in the total population by screening large number of trees from several geographical regions with BPCA90 locus.

While the inbreeding coefficient or fixation index (F) had negative values for all female and male subpopulations due to the heterozygotes excess, a lower value of inbreeding coefficient was detected in females. Also, male trees of *P. euphratica* had a relatively higher percentage of polymorphic loci (%P), and most of the private alleles were discovered in male trees. Female trees in our study presented relatively low genetic diversity compared to male trees. This situation could result from a population bottleneck (Cornuet & Luikart, 1996; Piry et al., 1999). Moreover, estimation of effective population size indicated a genetic drift. When the size of a population was severely reduced, the bottleneck event could have arisen as an extreme example of genetic drift. To test if the studied gender structured populations experienced a past bottleneck, the analysis was performed by Arlequin v3.5.2 (Excoffier & Lischer, 2010). As an output of this analysis, M values were calculated and compared to the critical value. The results suggested that female and male tree populations of *P. euphratica* from the Göksu River faced some bottleneck events in the past. According to Garza and Williamson (2001), allelic diversity is reduced faster than heterozygosity during a bottleneck. Since rare alleles have little effect on heterozygosity, a rapid loss in rare alleles cause a transient excess in heterozygosity. This means heterozygosity excess occurs relative to expected in a population of constant size is formed with the same number of alleles. The experienced bottleneck was responsible for the reduction of population effective size and the excess of

heterozygotes. Due to the bottleneck events, the loss of genetic variation could increase, which causes to decrease in the adaptive potential of populations and may result in the extinction of populations.

In this study, compared with the literature relatively high differentiation among female and male populations of *P. euphratica* was detected. Acquired low F_{ST} value is a characteristic of Salicaceae family members (Ciftci & Kaya, 2019; Čortan et al., 2016; Degirmenci et al., 2019; Sitzia et al., 2018; Zeng et al., 2018). In the recent study with Euphrate poplar populations in the Göksu river basin, the low variation was detected (Kansu & Kaya 2020). Thus, the same trees were relatively differentiated for these loci in our study. Still, this relative differentiation might be related to the potential sex-determining role of these loci in *P. euphratica*.

According to the AMOVA results, it was clear that no differentiation was detected between female and male tree population of *P. euphratica* from the Göksu River. This was also supported by the results from the Principal Coordinate Analysis (PCoA). This situation could be explained by wind-pollination, outcrossing, and clonal reproduction of the species throughout the river basin. Almost nonexistence of elevation difference between female and male tree populations of *P. euphratica* could be the consequence of extensive gene flow due to the absence of physical barriers. This argument was also supported by the studies of Wang (2011) and Eusemann et al. (2013). In metapopulations, events like recolonization and local extinctions frequently strike (Harrison & Taylor, 1997). Moreover, human impact on the Göksu river basin contributes the habitat fragmentations, which could contribute to recolonization and gene flow between subpopulations.

Interpretation of population structure analysis was performed by Bayesian algorithm and Evanno Method (Evanno et al., 2005), and as the most likely value for K, $\Delta K=2$ was identified. Regardless of gender and location, all trees of *P. euphratica* in the study had an equal possibility to be related to two genetically different groups. This could indicate there is no particular differentiation between genders and geographic locations for the loci we studied. The absence of differentiation might result from extensive gene flow between populations (Wei et al., 2012). In the field studies, all

sampled *P. euphratica* populations in the Göksu river had an almost equal number of female and male individuals. When this is considered with experienced bottleneck event, marginal populations could arise, and gene exchange occurs only within these individuals without external gene flow to generate the mentioned population structure (Mandak et al., 2005). Population structure analysis revealed that two homogeneous gene pools were observed in both genders with equal membership values. Thus, according to those loci, both genders had the same gene combinations. But for a determination of sex, the expression profiles might change. Moreover, depending on the environmental factors, the same genotype could produce individuals of different sexes to continue breeding in adverse environmental conditions. Deviations from strict dioecism have been observed in different *Populus* genus (Çiftçi et al., 2020; D. Einspahr & Joranson, 1960; Fladung et al., 2019; Lester, 1963; Novotná & Štochlová, 2013; Rottenberg et al., 2000; Sabatti et al., 2020; Song et al., 2012; Stettler, 1971). Monoecious trees and trees with unusual catkin morphology and hermaphrodite flowers were observed in small and fragmented or peripheral populations (Huff & Wu, 1992; Humeau et al., 1999).

Overall, effective population size analysis and GW indexes in our study strongly indicated some bottleneck events which caused to loss in diversity and might end with the extinction of the species. Among the polymorphic loci in our study, BPCA90 was the most informative and effective locus having male and female specific alleles. For *P. euphratica*, this locus had the highest probability to close-range sex determining regions on chromosome XIX. By studying with higher number of individuals from different locations this potential can be understood well.

CHAPTER 6

CONCLUSION

Populus euphratica, one of the fundamental species for maintaining river ecosystems stability, is distributed naturally in South and Southeastern regions of Turkey. The species provides suitable habitats for several other plants and animals by conserving the water resources and providing food resources. *P. euphratica* has great potential for future breeding programs, mainly to develop resilient genotype plantations for drought and salt stress in dry and saline environments. To accelerate the future breeding programs, sex-determination at the early stages of growth is very critical. Therefore, we focused on distinguishing the reliable microsatellite markers for sex in *P. euphratica* and investigating molecular sex differentiation.

Results demonstrated that using species-specific markers impact the estimates of genetic diversity. BPCA90 locus, which has shown the highest He, Ar, and PIC values, was subsequently the most informative and useful marker in our study. Moreover, three of the four detected private alleles existed in this locus might indicate BPCA90 as a promising candidate marker for *P. euphratica*. Thus, we suggested using of BPCA90 marker to determine the sex of *P. euphratica* as a tool for future studies.

Population structure analysis revealed that two homogeneous gene pools were observed in both genders with equal membership values. Although the same gene combinations were found in both genders, for sex-determination the expression profiles might be different. Moreover, to continue breeding in adverse environmental conditions, the same genotype could produce individuals of different genders. There have been observations of deviations from strict dioecism in several *Populus* species and this situation might have arisen in *P. euphratica* populations from Göksu river.

Effective population size analysis and GW indexes strongly showed that the populations experienced some bottleneck events. Additionally, *P. euphratica* populations from the Göksu river maintained a low genetic diversity when compared to other poplar species. The loss in diversity might end with the extinction of the species. Hence, it is critical to start *in situ* conservation of *P. euphratica* populations from the Göksu river. The conservation program must include strict regulations of agricultural activities and have to create public awareness policy.

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APPENDICES

Appendix A

POPULATION DETAILS

Sample Code	Sample ID	Latitude	Longitude	Altitude	Explanation	Population
1	GKS1-1	36 39 35 N	33 22 08 E	442 ft	Male	Upstream (GUP)
3	GKS1-2	36 39 37 N	33 22 18 E	452 ft	Male	Upstream (GUP)
5	GKS1-3	36 39 32 N	33 22 12 E	442 ft	Female	Upstream (GUP)
7	GKS1-4	36 39 21 N	33 22 08 E	442 ft	Male	Upstream (GUP)
9	GKS1-5	36 39 27 N	33 21 53 E	441 ft	Male	Upstream (GUP)
11	GKS1-6	36 39 27 N	33 21 54 E	441 ft	Female	Upstream (GUP)
15	GKS1-8	36 39 28 N	33 21 58 E	442 ft	Female	Upstream (GUP)
17	GKS1-9	36 39 33 N	33 21 46 E	459 ft	Male	Upstream (GUP)
19	GKS1-10	36 39 33 N	33 21 48 E	450 ft	Female	Upstream (GUP)
21	GKS1-11	36 39 33 N	33 21 55 E	446 ft	Male	Upstream (GUP)
23	GKS1-12	36 39 33 N	33 21 51 E	442 ft	Female	Upstream (GUP)
25	GKS1-13	36 40 32 N	33 21 52 E	470 ft	Male	Upstream (GUP)
27	GKS1-14	36 40 31 N	33 21 54 E	448 ft	Male	Upstream (GUP)
28	GKS1-15	36 40 31 N	33 21 54 E	448 ft	Female	Upstream (GUP)
30	GKS1-16	36 40 27 N	33 21 58 E	453 ft	Male	Upstream (GUP)
32	GKS1-17	36 40 26 N	33 21 59 E	464 ft	Female	Upstream (GUP)
34	GKS1-18	36 40 21 N	33 22 10 E	451 ft	Female	Upstream (GUP)
36	GKS1-19	36 40 20 N	33 22 13 E	449 ft	Male	Upstream (GUP)
37	GKS1-20	36 40 20 N	33 22 13 E	449 ft	Female	Upstream (GUP)
38	GKS1-21	36 40 19 N	33 22 13 E	451 ft	Male	Upstream (GUP)
40	GKS1-22	36 40 19 N	33 22 15 E	451 ft	Female	Upstream (GUP)
42	GKS1-23	36 40 19 N	33 22 15 E	451 ft	Male	Upstream (GUP)
44	GKS1-24	36 41 00 N	33 22 48 E	490 ft	Male	Upstream (GUP)
45	GKS1-25	36 41 00 N	33 22 48 E	490 ft	Female	Upstream (GUP)
47	GKS1-26	36 41 46 N	33 21 39 E	475 ft	Male	Upstream (GUP)
49	GKS1-27	36 41 43 N	33 21 41 E	472 ft	Male	Upstream (GUP)
51	GKS1-28	36 41 43 N	33 21 41 E	472 ft	Female	Upstream (GUP)

52	GKS1-29	36 41 46 N	33 21 39 E	476 ft	Female	Upstream (GUP)
54	GKS1-30	36 38 27 N	33 21 01 E	449 ft	Female	Upstream (GUP)
55	GKS1-31	36 39 19 N	33 22 21 E	448 ft	Male	Upstream (GUP)
56	GKS1-32	36 39 19 N	33 21 56 E	442 ft	Male	Upstream (GUP)
58	GKS1-33	36 39 19 N	33 21 56 E		Female	Upstream (GUP)
59	GKS2-1	36 32 21 N	33 26 54 E	336 ft	Male	Midstream (GMID)
60	GKS2-2	36 32 20 N	33 26 52 E	336 ft	Female	Midstream (GMID)
62	GKS2-3	36 32 19 N	33 26 51 E	342 ft	Female	Midstream (GMID)
63	GKS2-5	36 32 19 N	33 26 51 E	335 ft	Male	Midstream (GMID)
64	GKS2-6	33 32 18 N	33 26 49 E	346 ft	Female	Midstream (GMID)
66	GKS2-7	36 32 18 N	33 26 49 E	349 ft	Male	Midstream (GMID)
67	GKS2-8	36 32 17 N	33 26 48 E	350 ft	Female	Midstream (GMID)
69	GKS2-9	36 32 16 N	33 26 47 E	367 ft	Male	Midstream (GMID)
70	GKS2-10	36 32 12 N	33 26 42 E	326 ft	Female	Midstream (GMID)
72	GKS2-11	36 32 12 N	33 26 42 E	326 ft	Male	Midstream (GMID)
73	GKS2-12	36 32 11 N	33 26 40 E	323 ft	Female	Midstream (GMID)
75	GKS2-13	36 31 51 N	36 31 51 E	322 ft	Male	Midstream (GMID)
77	GKS2-14	36 31 51 N	33 28 09 E	314 ft	Female	Midstream (GMID)
78	GKS2-15	36 31 50 N	33 28 10 E	310 ft	Male	Midstream (GMID)
80	GKS2-16	36 31 49 N	33 28 13 E	308 ft	Female	Midstream (GMID)
81	GKS2-17	36 31 49 N	33 28 13 E	308 ft	Male	Midstream (GMID)
83	GKS2-18	36 31 49 N	33 28 14 E	313 ft	Female	Midstream (GMID)
85	GKS2-19	36 31 46 N	33 28 22 E	314 ft	Female	Midstream (GMID)
87	GKS2-20	36 31 47 N	33 28 31 E	313 ft	Female	Midstream (GMID)
89	GKS2-21	36 31 45 N	33 28 32 E	309 ft	Male	Midstream (GMID)
91	GKS2-22	36 30 36 N	33 29 51 E	301 ft	Male	Midstream (GMID)
93	GKS2-23	36 30 36 N	33 29 52 E	299 ft	Female	Midstream (GMID)
95	GKS2-24	36 30 37 N	33 29 55 E	301 ft	Male	Midstream (GMID)
97	GKS2-25	36 30 38 N	33 29 57 E	299 ft	Male	Midstream (GMID)
99	GKS2-26	36 30 38 N	33 29 57 E	298 ft	Female	Midstream (GMID)
100	GKS2-27	36 30 39 N	33 29 59 E	295 ft	Male	Midstream (GMID)
102	GKS2-28	36 30 30 N	33 30 02 E	294 ft	Female	Midstream (GMID)
104	GKS2-29	36 30 40 N	33 35 00 E	300 ft	Male	Midstream (GMID)
106	GKS2-30	36 30 40 N	33 30 06 E	298 ft	Female	Midstream (GMID)
108	GKS2-31				Male	Midstream (GMID)
109	GKS2-32				Female	Midstream (GMID)
110	GKS3-1	36 26 02 N	33 45 34 E	146 ft	Male	Downstream (GDOWN)

112	GKS3-2	36 26 02 N	33 45 34 E	161 ft	Female	Downstream (GDOWN)
114	GKS3-3	36 26 03 N	33 45 45 E	143 ft	Female	Downstream (GDOWN)
116	GKS3-4	36 26 03 N	33 45 41 E	169 ft	Male	Downstream (GDOWN)
117	GKS3-5	36 28 08 N	33 46 13 E	137 ft	Male	Downstream (GDOWN)
118	GKS3-6	36 24 54 N	33 47 32 E	141 ft	Female	Downstream (GDOWN)
119	GKS3-7	36 24 51 N	33 47 37 E	123 ft	Male	Downstream (GDOWN)
121	GKS3-8	36 24 51 N	33 47 37 E		Female	Downstream (GDOWN)
123	GKS3-8	36 24 51 N	33 47 36 E	126 ft	Male	Downstream (GDOWN)
124	GKS3-9	36 24 49 N	33 47 42 E	119 ft	Male	Downstream (GDOWN)
126	GKS3-10	36 24 43 N	33 47 53 E	121 ft	Male	Downstream (GDOWN)
128	GKS3-11	36 24 27 N	33 48 12 E	113 ft	Male	Downstream (GDOWN)
130	GKS3-12	36 24 17 N	33 48 14 E	137 ft	Male	Downstream (GDOWN)
132	GKS3-13	36 24 17 N	33 48 14 E	137 ft	Female	Downstream (GDOWN)
134	GKS3-14	36 24 16 N	33 48 15 E	120 ft	Male	Downstream (GDOWN)
135	GKS3-15	36 24 13 N	33 48 18 E	104 ft	Female	Downstream (GDOWN)
137	GKS3-16	36 24 12 N	33 48 18 E	113 ft	Male	Downstream (GDOWN)
138	GKS3-17	36 24 10 N	33 48 17 E	129 ft	Female	Downstream (GDOWN)
140	GKS3-18	36 24 09 N	33 48 29 E	131 ft	Female	Downstream (GDOWN)
141	GKS3-19	36 24 09 N	33 48 32 E	119 ft	Male	Downstream (GDOWN)
143	GKS3-20	36 24 12 N	33 48 33 E	99 ft	Male	Downstream (GDOWN)
144	GKS3-21	36 24 12 N	33 48 33 E	99 ft	Female	Downstream (GDOWN)
146	GKS3-22	36 24 12 N	33 48 35 E	98 ft	Male	Downstream (GDOWN)
148	GKS3-23	36 24 12 N	33 48 35 E	98 ft	Female	Downstream (GDOWN)
150	GKS3-24	36 24 12 N	33 48 36 E	102 ft	Male	Downstream (GDOWN)
152	GKS3-25				Female	Downstream (GDOWN)
154	GKS3-26	36 24 12 N	33 48 38 E	103 ft	Female	Downstream (GDOWN)
156	GKS3-27	36 24 13 N	33 48 39 E	103 ft	Female	Downstream (GDOWN)
158	GKS3-28	36 24 13 N	33 48 39 E	103 ft	Male	Downstream (GDOWN)
160	GKS3-29	36 24 13 N	33 48 41 E	104 ft	Female	Downstream (GDOWN)
162	GKS3-30	36 24 13 N	33 48 43 E	100 ft	Male	Downstream (GDOWN)
164	GKS3-31	36 24 13 N	33 48 43 E	101 ft	Female	Downstream (GDOWN)

Appendix B

CTAB DNA EXTRACTION PROTOCOL

1. 2xCTAB extraction buffer was prepared with 2% (w/v) CTAB + 5M NaCI + 0.5M EDTA + 1M Tris-HCI at pH 8.0.
2. 2% (w/v) polyvinylpyrollidine (PVP-40) was added to the newly prepared 2xCTAB extraction buffer, and the mixture was heated at 65 °C in a water bath for approximately an hour.
3. Approximately 0.1g leaf tissue was placed in a mortar together with 2000µL preheated CTAB solution. Leaf tissue was ground until a homogeneous liquid was acquired.
4. Obtained ~1800µL of the liquid was moved into a 2ml Eppendorf tube. For each tube, 100µL β- mercaptoethanol and 5µL Proteinase K were added.
5. The tubes were incubated in the water bath at 65°C for an hour.
6. The tubes were centrifuged at 4°C for 20 minutes at 15000 rpm. From each tube, ~800µL of the aqueous phase was collected and moved to a new 2 ml Eppendorf tube.
7. For each tube, 0.8V Chloroform/Isoamylalcohol (24:1) was added and inverted a few times gently.
8. The tubes were centrifuged at 4°C for 15 minutes at 14000rpm. From each tube, ~500µL supernatant was taken in a new 1.5mL Eppendorf tube.
9. For each tube, ice-cold Isopropanol was added with 1:1 ratio. Tubes were gently flip-flopped a few times and then incubated at -80°C for an hour.
10. The tubes were centrifuged at 4 °C for 15 minutes at 14000rpm. The supernatant was eliminated, and the pellet was washed with cold 70% EtOH two times.
11. The pellet was air-dried and resuspended in 60µL TE buffer o/n.

Appendix C

NULL ALLELE DETECTION

Genepop v4.2 (Arnaud-Haond & Belkhir, 2007)

(Locus by population) table of estimated null allele frequencies

Locus	Male	Female
ORPM-206	0.0000	0.0000
ORPM-263	0.1052	0.0000
ORPM-276	0.8124	0.7518
ORPM-433	0.9798	0.9555
BPTTG60	No info	No info
BPTGG82	0.0000	0.0000
BPCA90	0.0000	0.0000

Confidence intervals for null allele frequencies

Locus	Population	Frequency estimate	0.0250 bound	0.9750 bound
ORPM-206	Male	0.0000	*(No info for Cl)*	
	Female	0.0000	*(No info for Cl)*	
ORPM-263	Male	0.1052	0.0163	0.2577
	Female	0.0000	*(No info for Cl)*	
ORPM-276	Male	0.8124	0.7203	0.8846
	Female	0.7518	0.6463	0.8393
ORPM-433	Male	0.9798	0.9292	0.9977
	Female	0.9555	0.8929	0.9868
BPTTG60	Male	No info		
	Female	No info		
BPTGG82	Male	0.0000	*(No info for Cl)*	
	Female	0.0000	*(No info for Cl)*	
BPCA90	Male	0.0000	*(No info for Cl)*	
	Female	0.0000	*(No info for Cl)*	

Appendix D

POPULATION GENETICS STATISTICS

Number of Different alleles (Na)

It can be detected by direct count.

Effective number of alleles (Ne)

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

Ne: In an ideal population, the estimation of the number of equally frequent alleles.
He: Expected heterozygosity

Number of private (rare) alleles

The number of alleles that are unique to a single population in dataset

Shannon's Information Index (I)

$$\mathrm{I} = \sum p_i \ln p_i$$

I: Calculated on a single-locus basis which is the equivalence to the Shannon-Weaver Index of ecology
p_i: The allele frequency of the i^{th} allele at a stated locus for the specified population

Observed Heterozygosity (Ho)

$$\mathrm{Ho} = \frac{\text{Number of Heterozygotes}}{\mathrm{N}}$$

N: Population size

Expected Heterozygosity (He)

$$\mathrm{He} = 1 - \sum {P_i}^2$$

p_i: The allele frequency of the i^{th} allele at a stated locus for the specified population

Fixation Index (F)

$$F = \frac{He - Ho}{He}$$

F: Fixation index or inbreeding coefficient
He: Expected heterozygosity
Ho: Observed heterozygosity

Wright's F-Statistics (F_{IS}, F_{ST} and F_{IT})

$$F_{IS} = \frac{\overline{He} - \overline{Ho}}{\overline{He}}$$

F_{IS}: The inbreeding coefficient which represents total variation in individuals relative to the variation in their subpopulation
$\overline{He}$: The mean expected heterozygosity averaged across subpopulations
$\overline{Ho}$: The mean observed heterozygosity averaged across subpopulations

$$F_{ST} = \frac{H_T - \overline{He}}{H_T}$$

F_{ST}: The measure which represents the variation in the subpopulations relative to the total population
H_T: The expected heterozygosity
$\overline{He}$: The mean expected heterozygosity averaged across subpopulations

$$F_{IT} = \frac{H_T - \overline{Ho}}{H_T}$$

F_{IT} : The overall fixation index which represents the variation in individuals relative to the variation in the total set of subpopulations
H_T : The expected heterozygosity
$\overline{Ho}$: The mean observed heterozygosity averaged across subpopulations
The following equation establishes a connection among the F-statistics:

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

Number of Migrants (Nm)

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

Probability of Identity (PI)

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

p_i: The allele frequency of the i[th] allele at a stated locus for the specified population

Polymorphic Information Content (PIC)

$$\mathrm{PIC} = 1 - \sum ({p_i}^2)$$

p_i: The allele frequency of the i[th] allele at a stated locus for the specified population

Percentage of Polymorphic Loci (%P)

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

P_i: The proportion of polymorphic loci in a population
N: The number of populations

Garza-Williamson Index (G-W)

$$\mathrm{GW} = \frac{\mathrm{k}}{\mathrm{R}+1}$$

k: The number of alleles at a stated locus
R: The allelic range

Appendix E

FILE FORMATS OF SOFTWARES

Arlequin File Format (.arp)

```
[Profile]
  Title = "Male and Female Populus euphratica from Goksu"
  NbSamples = 6
  DataType = MICROSAT
  GenotypicData = 1
  LocusSeparator = WHITESPACE
  MissingData = "?"
  GameticPhase = 0
  RecessiveData = 0


[Data]
  [[Samples]]
    SampleName = "m_GKS1"
    SampleSize = 18
    SampleData = {
         1         1       188 236 197 204 214 233 256
                           188 238 197 204 214 236 258
         3         1       188 236 197 204 214 236 256
                           188 238 197 204 214 236 258
         7         1       188 238 197 204 214 233 250
                           188 238 197 204 214 236 256
         9         1       188 238 197 204 214 233 256
                           188 240 197 204 214 236 258
         13        1       188 236 197 204 214 233 244
                           188 238 197 204 214 236 256
         17        1       188 236 197 204 214 233 256
                           188 238 197 204 214 236 258
         21        1       188 238 197 204 214 233 256
                           200 240 197 204 214 236 258
         25        1       188 238 197 204 214 233 256
                           191 240 197 204 214 236 258
         27        1       188 240 169 204 214 233 250
                           191 240 197 204 214 236 256
         30        1       188 238 197 204 214 233 244
                           188 240 197 204 214 236 250
         36        1       188 236 169 204 214 233 244
                           188 238 197 204 214 236 256
         38        1       188 236 197 204 214 233 244
                           191 238 197 204 214 236 250
         42        1       179 238 169 204 214 233 256
                           188 240 197 204 214 236 268
         44        1       188 236 197 186 214 233 256
                           188 238 197 204 214 236 264
         47        1       188 236 197 204 214 233 258
                           191 238 197 204 214 236 272
         49        1       188 236 197 204 214 233 256
                           188 238 197 204 214 236 258
         55        1       188 236 169 204 ? ? ?
                           188 240 169 204 ? ? ?
         56        1       188 238 197 204 214 233 250
                           191 240 197 204 214 236 256
         }
  [[Samples]]
```

Cervus File Format (.csv)

Sexdata_Cervus

Population	ID	O_206A	O_206B	O_263A	O_263B	O_276A	O_276B	O_433A	O_433B	B_60A	B_60B	B_82A	B_82B	B_90A	B_90B
Pop1	1	188	188	236	238	197	197	204	204	214	214	233	236	256	258
Pop1	3	188	188	236	238	197	197	204	204	214	214	236	236	256	258
Pop1	7	188	188	238	238	197	197	204	204	214	214	233	236	250	256
Pop1	9	188	188	238	240	197	197	204	204	214	214	233	236	256	258
Pop1	13	188	188	236	238	197	197	204	204	214	214	233	236	244	256
Pop1	17	188	188	236	238	197	197	204	204	214	214	233	236	256	258
Pop1	21	188	200	238	240	197	197	204	204	214	214	233	236	256	258
Pop1	25	188	191	238	240	197	197	204	204	214	214	233	236	256	258
Pop1	27	188	191	240	240	169	197	204	204	214	214	233	236	250	256
Pop1	30	188	188	238	240	197	197	204	204	214	214	233	236	244	250
Pop1	36	188	188	236	238	169	197	204	204	214	214	233	236	244	256
Pop1	38	188	191	236	238	197	197	204	204	214	214	233	236	244	250
Pop1	42	179	188	238	240	169	197	204	204	214	214	233	236	256	268
Pop1	44	188	188	236	238	197	197	186	204	214	214	233	236	256	264
Pop1	47	188	191	236	238	197	197	204	204	214	214	233	236	258	272
Pop1	49	188	188	236	238	197	197	204	204	214	214	233	236	256	258
Pop1	55	188	188	236	240	169	169	204	204	0	0	0	0	0	0
Pop1	56	188	191	238	240	197	197	204	204	214	214	233	236	250	256
Pop1	59	188	188	238	240	169	197	204	204	214	214	233	236	250	256
Pop1	63	188	188	238	240	197	197	204	204	214	214	236	236	250	256
Pop1	66	188	188	240	240	169	197	204	204	214	214	233	236	244	250
Pop1	69	188	188	236	238	197	197	204	204	214	214	233	236	256	258
Pop1	72	188	188	238	240	197	197	204	204	214	214	236	239	250	250
Pop1	75	188	188	238	240	197	197	204	204	214	214	236	236	256	258
Pop1	78	188	188	238	240	197	197	204	204	214	214	233	236	256	258

FSTAT File Format (.dat)

```
2 7 272 3
O_206
O_263
O_276
O_433
B_60
B_82
B_90
1   188188 236238 197197 204204 214214 233236 256258
1   188188 236238 197197 204204 214214 236236 256258
1   188188 238238 197197 204204 214214 233236 250256
1   188188 238240 197197 204204 214214 233236 256258
1   188188 236238 197197 204204 214214 233236 244256
1   188188 236238 197197 204204 214214 233236 256258
1   188200 238240 197197 204204 214214 233236 256258
1   188191 238240 197197 204204 214214 233236 256258
1   188191 240240 169197 204204 214214 233236 250256
1   188188 238240 197197 204204 214214 233236 244250
1   188188 236238 169197 204204 214214 233236 244256
1   188191 236238 197197 204204 214214 233236 244250
1   179188 238240 169197 204204 214214 233236 256268
1   188188 236238 197197 186204 214214 233236 256264
1   188191 236238 197197 204204 214214 233236 258272
1   188188 236238 197197 204204 214214 233236 256258
1   188188 236240 169169 204204      0      0      0
1   188191 238240 197197 204204 214214 233236 250256
1   188188 238240 169197 204204 214214 233236 250256
1   188188 238240 197197 204204 214214 236236 250256
1   188188 240240 169197 204204 214214 233236 244250
```

GenAlEx File Format (.xlsx)

7	96	6	18	15	15	16	17	15	1	96	0	0			
sex data			M_GKS1	M_GKS2	M_GKS3	F_GKS1	F_GKS2	F_GKS3							
Sample #	Pop	ORPM_206	ORPM_206	ORPM_263	ORPM_263	ORPM_276	ORPM_276	ORPM_433	ORPM_433	BPTTG60	BPTTG60	BPTGG82	BPTGG82	BPCA90	BPCA90
1	1	188	188	236	238	197	197	204	204	214	214	233	236	256	258
3	1	188	188	236	238	197	197	204	204	214	214	236	236	256	258
7	1	188	188	238	238	197	197	204	204	214	214	233	236	250	256
9	1	188	188	238	240	197	197	204	204	214	214	233	236	256	258
13	1	188	188	236	238	197	197	204	204	214	214	233	236	244	256
17	1	188	188	236	238	197	197	204	204	214	214	233	236	256	258
21	1	188	200	238	240	197	197	204	204	214	214	233	236	256	258
25	1	188	191	238	240	197	197	204	204	214	214	233	236	256	258
27	1	188	191	240	240	169	197	204	204	214	214	233	236	250	256
30	1	188	188	238	240	197	197	204	204	214	214	233	236	244	250
36	1	188	188	236	238	169	197	204	204	214	214	233	236	244	256
38	1	188	191	236	238	197	197	204	204	214	214	233	236	244	250
42	1	179	188	238	240	169	197	204	204	214	214	233	236	256	268
44	1	188	188	236	238	197	197	186	204	214	214	233	236	256	264
47	1	188	191	236	238	197	197	204	204	214	214	233	236	258	272
49	1	188	188	236	238	197	197	204	204	214	214	233	236	256	258
55	1	188	188	236	240	169	169	204	204	0	0	0	0	0	0
56	1	188	191	238	240	197	197	204	204	214	214	233	236	250	256
59	2	188	188	238	240	169	197	204	204	214	214	233	236	250	256
63	2	188	188	238	240	197	197	204	204	214	214	236	236	250	256
66	2	188	188	240	240	169	197	204	204	214	214	233	236	244	250
69	2	188	188	236	238	197	197	204	204	214	214	233	236	256	258
72	2	188	188	238	240	197	197	204	204	214	214	236	239	250	250
75	2	188	188	238	240	197	197	204	204	214	214	236	236	256	258
78	2	188	188	238	240	197	197	204	204	214	214	233	236	256	258
81	2	188	188	236	238	197	197	204	204	214	214	233	236	256	258
89	2	188	188	238	240	197	197	204	204	214	214	233	236	256	258
91	2	188	188	238	240	197	197	204	204	214	214	233	236	256	258
95	2	182	188	236	238	197	197	204	204	214	214	233	236	256	258
97	2	188	188	238	240	197	197	204	204	214	214	233	236	244	258
100	2	188	188	238	240	169	197	204	204	214	214	233	236	250	256
104	2	188	191	238	240	197	197	204	204	214	214	233	236	250	256
108	2	188	188	236	238	169	197	204	204	214	214	233	236	250	256
110	3	182	188	238	240	169	197	186	204	214	214	233	236	250	272
116	3	188	188	236	238	169	197	204	204	214	214	233	236	250	256
117	3	188	188	238	240	197	197	204	204	214	214	233	236	258	272
119	3	188	188	236	238	197	197	204	204	214	214	233	236	250	256
123	3	188	188	236	238	169	197	204	204	214	214	233	236	250	256
124	3	188	188	238	238	169	197	204	204	214	214	233	236	250	256

GenClone File Format (.txt)

96	999	999 7	2	O_206	O_263	O_276	O_433	B_60	B_82	B_90
1	999	999	188188	236238	197197	204204	215215	237237	258260	
3	999	999	188188	236238	197197	204204	215215	237237	258260	
7	999	999	188188	238238	197197	204204	215215	237237	250258	
9	999	999	188188	238240	197197	204204	215215	237237	258260	
13	999	999	188188	236238	197197	204204	215215	237237	244258	
17	999	999	188188	236238	197197	204204	215215	237237	258260	
21	999	999	188200	238240	197197	204204	215215	237237	258260	
25	999	999	188191	238240	197197	204204	215215	237237	258260	
27	999	999	188191	240240	169197	204204	215215	237237	250258	
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Genepop File Format (.gen)

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Title line: "Male and Female Samples of Populus euphatica from Göksu Rive
O_206
O_263
O_276
O_433
B_60
B_82
B_90
Pop
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7       ,       188188  238238  197197  204204  215215  237237  250258
9       ,       188188  238240  197197  204204  215215  237237  258260
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17      ,       188188  236238  197197  204204  215215  237237  258260
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25      ,       188191  238240  197197  204204  215215  237237  258260
27      ,       188191  240240  169197  204204  215215  237237  250258
30      ,       188188  238240  197197  204204  215215  237237  244250
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38      ,       188191  236238  197197  204204  215215  237237  244250
42      ,       179188  238240  169197  204204  215215  237237  258268
44      ,       188188  236238  197197  186204  215215  237237  258264
47      ,       188191  236238  197197  204204  215215  237237  260272
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69      ,       188188  236238  197197  204204  215215  237237  258260
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81      ,       188188  236238  197197  204204  215215  237237  258260
89      ,       188188  238240  197197  204204  215215  237237  258260
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97      ,       188188  238240  197197  204204  215215  237237  244260
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Microchecker File Format (.txt)

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Title line: "Male and Female Samples of Populus euphatica from Göksu Rive
O_206
O_263
O_276
O_433
B_60
B_82
B_90
Pop
1       ,       188188  236238  197197  204204  215215  237237  258260
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7       ,       188188  238238  197197  204204  215215  237237  250258
9       ,       188188  238240  197197  204204  215215  237237  258260
13      ,       188188  236238  197197  204204  215215  237237  244258
17      ,       188188  236238  197197  204204  215215  237237  258260
21      ,       188200  238240  197197  204204  215215  237237  258260
25      ,       188191  238240  197197  204204  215215  237237  258260
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30      ,       188188  238240  197197  204204  215215  237237  244250
36      ,       188188  236238  169197  204204  215215  237237  244258
38      ,       188191  236238  197197  204204  215215  237237  244250
42      ,       179188  238240  169197  204204  215215  237237  258268
44      ,       188188  236238  197197  186204  215215  237237  258264
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55      ,       188188  236240  169169  204204  000000  000000  000000
56      ,       188191  238240  197197  204204  215215  237237  250258
59      ,       188188  238240  169197  204204  215215  237237  250258
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66      ,       188188  240240  169197  204204  215215  237237  244250
69      ,       188188  236238  197197  204204  215215  237237  258260
72      ,       188188  238240  197197  204204  215215  237237  250250
75      ,       188188  238240  197197  204204  215215  237237  258260
78      ,       188188  238240  197197  204204  215215  237237  258260
81      ,       188188  236238  197197  204204  215215  237237  258260
89      ,       188188  238240  197197  204204  215215  237237  258260
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Structure File Format (.txt)

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3	1	188	238	197	204	214	236	258
7	1	188	238	197	204	214	233	250
7	1	188	238	197	204	214	236	256
9	1	188	238	197	204	214	233	256
9	1	188	240	197	204	214	236	258
13	1	188	236	197	204	214	233	244
13	1	188	238	197	204	214	236	256
17	1	188	236	197	204	214	233	256
17	1	188	238	197	204	214	236	258
21	1	188	238	197	204	214	233	256
21	1	200	240	197	204	214	236	258
25	1	188	238	197	204	214	233	256
25	1	191	240	197	204	214	236	258
27	1	188	240	169	204	214	233	250
27	1	191	240	197	204	214	236	256
30	1	188	238	197	204	214	233	244
30	1	188	240	197	204	214	236	250
36	1	188	236	169	204	214	233	244
36	1	188	238	197	204	214	236	256
38	1	188	236	197	204	214	233	244
38	1	191	238	197	204	214	236	250
42	1	179	238	169	204	214	233	256
42	1	188	240	197	204	214	236	268
44	1	188	236	197	186	214	233	256
44	1	188	238	197	204	214	236	264
47	1	188	236	197	204	214	233	258