GENETIC DIVERSITY DIFFERENCES BETWEEN PARENTAL AND PROGENY POPULATION OF *POPULUS EUPHRATICA* POPULATIONS IN A FRAGMENTED RIVER ECOSYSTEM

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ABSTRACT

GENETIC DIVERSITY DIFFERENCES BETWEEN PARENTAL AND PROGENY POPULATION OF *POPULUS EUPHRATICA* POPULATIONS IN A FRAGMENTED RIVER ECOSYSTEM

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Euphrates poplar, also known as desert poplar in the literature, grows along riverbanks in arid and semi-arid regions and is well-known for its high tolerance to broad temperature changes, drought, and soils with high salt content. The presence of healthy Euphrates poplar forests in riparian ecosystems is of high importance due to their direct effect on local biodiversity as being pioneer species within their habitats. In addition to its positive impact on biodiversity, the species' healthy and unfragmented populations provide valuable ecosystem services such as watershed protection, riverbank stabilization, erosion prevention, and windbreak formation. However, current populations show a decreasing trend across the globe due to various anthropogenic factors, including the transformation of rivers' hydrological characteristics due to improper water management, groundwater pollution, and excessive logging. Therefore, determining the species' genetic potential with an age-based perspective can create the backbone of future conservation measures by revealing information regarding the extent of genetic diversity transfer between mature and young stands, as well as the generative regeneration capacity of natural populations.

In this study, the genetic diversity of mature and young stands found along three distinct locations (upstream, midstream, and downstream) of the Göksu River were compared via genotypic data created by 15 microsatellite markers. Low to moderate allelic diversity and heterozygosity values were obtained in all age structures, pointing out the gene pool shrinkage associated with sudden reductions in population size. Nevertheless, low clonality and highly similar genetic diversity values of mature and young stands revealed the relative success of genetic diversity transfer in all populations. The genetic structure of age groups also exhibited the lack of age-based genetic differentiation due to high-degree gene flow between the mature and young stands found in the same locality.

As being the most distant population in terms of genetic structure, the downstream location (GDOWN) has the highest genetic diversity values together with the highest number of private alleles found in its young population. Therefore, in order to avoid further loss of genetic diversity, the downstream population can be subject to both *in-situ* and *ex-situ* conservation measures.

Keywords: SSR, *Populus euphratica*, age, genetic diversity, genetic differentiation, clonality

ÖZ

PARÇALI BİR NEHİR EKOSİSTEMİNDEKİ *POPULUS EUPHRATICA* POPULASYONLARININ EBEVEYN VE SOY POPULASYONLARI ARASINDAKİ GENETİK ÇEŞİTLİLİK FARKLILIKLARI

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Literatürde çöl kavağı olarak da bilinen Fırat kavağı, kurak ve yarı kurak bölgelerde nehir kıyıları boyunca yetişir ve geniş sıcaklık değişimlerine, kuraklığa ve yüksek oranda tuz içeriğine sahip topraklara oldukça tolerans göstermesi ile bilinmektedir. Fırat kavağı bulunduğu habitatlarda öncü tür olarak yerel biyoçeşitliliğe doğrudan etkisi nedeniyle, sağlıklı nehir ekosistemlerinin oluşumunda büyük önem taşımaktadır. Biyoçeşitlilik üzerinde olumlu etkisine ek olarak, türün sağlıklı ve parçalanmamış popülasyonları, su havzası koruması, nehir kıyısı stabilizasyonu, erozyon önleme ve rüzgar siperi oluşturma gibi değerli ekosistem hizmetleri sağlamaktadır. Fakat, mevcut popülasyonlar, yanlış su yönetimi, yeraltı suyu kirliliği ve fazla ağaç kesimi sebebi ile nehirlerin hidrolojik özelliklerinin dönüşümüne yol açan çeşitli insan kaynaklı faktörler nedeniyle dünya genelinde azalan bir eğilim göstermektedir. Dolayısıyla, türün genetik potansiyelini yaşa dayalı bir bakış açısıyla belirlemek, yetişkin ve genç meşreceler arasındaki genetik çeşitlilik aktarımının kapsamının yanı sıra doğal popülasyonların jeneratif yenilenme kapasitesine ilişkin bilgileri ortaya çıkararak gelecekteki koruma önlemlerinin omurgasını oluşturabilir.

Bu çalışmada, Göksu Nehri'nin üç farklı lokasyonunda (yukarı, orta ve aşağı nehir bölümleri) bulunan yetişkin ve genç meşcerelerin genetik çeşitliliği, 15 mikrosatelit belirteci ile oluşturulmuş genotip verileri vasıtasıyla karşılaştırılmıştır. Tüm yaş gruplarında popülasyon büyüklüğündeki ani azalmalarla ilişkili gen havuzu daralmasını işaret eden, düşük ile orta dereceli alelik çeşitlilik ve heterozigotluk değerleri elde edilmiştir. Bununla birlikte, yetişkin ve genç meşcerelerin düşük klonalite ve oldukça benzer genetik çeşitlilik değerleri, tüm popülasyonlardaki yaş grupları arasında genetik çeşitlilik aktarımının nispi başarısını ortaya koymuştur. Yaş gruplarının genetik yapısı, aynı bölgede bulunan yetişkin ve genç meşcereler arasındaki yüksek dereceli gen akışı nedeniyle yaşa dayalı bir genetik farklılaşma olmadığını da göstermiştir.

Genetik yapı açısından en farklı popülasyon olan aşağı havza lokasyonu, genç popülasyonunda bulunan en yüksek sayıda özel alel ile birlikte en yüksek genetik çeşitlilik değerlerine sahiptir. Bu nedenle, genetik çeşitliliğin daha fazla kaybını önlemek için, aşağı havza popülasyonu hem *in-situ* hem de *ex-situ* koruma önlemlerine tabi tutulabilir.

Anahtar Kelimeler: SSR, *Populus euphratica*, yaş, genetik çeşitlilik, genetik farklılaşma, klonalite

To Bahtiyar

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LIST OF ABBREVIATIONS

%P	Percentage of Polymorphic Loci
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
Ar	Allelic Richness
BMBF	German Federal Ministry of Education and Research
bp	Base pairs
C	Clonality
CLUMPP	Cluster Matching and Permutation Program
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraaceticacid disodium salt
ESRI	Environmental Systems Research Institute
EST-SSR	Expressed Sequence Tag-Simple Sequence Repeats
EWDP	Ecological Water Diversion Program
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
FAO	Food and Agriculture Organization
GDA	Genetic Data Analysis
GDOWN	Downstream Population
GDOWNM	Mature Population of the Downstream Location
GDOWNY	Young Population of the Downstream Location
GMID	Midstream Population
GMIDM	Mature Population of the Midstream Location
GMIDY	Young Population of the Midstream Location
GUP	Upstream Population

GUPM	Mature Population of the Upstream Location
GUPY	Young Population of the Upstream Location
G-W Index (M)	Modified Garza-Williamson Index
uHe	Unbiased Expected Heterozygosity
Ho	Observed Heterozygosity
HWE	Hardy–Weinberg Equilibrium
I	Shannon's Information Index
IUCN	The International Union for Conservation of Nature
LC	Least Concern
MCMC	Markov Chain Monte Carlo
$MgCl_2$	Magnesium Chloride
MLG	Multilocus Genotype
Na	Number of Alleles
Ne	Number of Effective Alleles
Nm	Number of Migrants
NGS	Next Generation Sequencing
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PI	Probability of Identity
PIC	Polymorphism Information Content
QTL	Quantitative Trait Loci
RAPD	Random Amplification of Polymorphic DNA
SSR	Simple Sequence Repeats
Ta	Annealing Temperature
TE	Tris EDTA
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean

CHAPTER 1

INTRODUCTION

Distributed throughout temperate, boreal, and tundra regions of the northern hemisphere, the Salicaceae family consists of deciduous and dioecious individuals of trees and shrubs, which are traditionally allocated into *Populus* and *Salix* genera having approximately 480 species altogether (Liu et al., 2016). Recent advances in molecular phylogeny made it possible to increase the resolution of taxonomic relationships at the family level. Using the sequence data derived from plastid DNA, Chase et al. (2002) demonstrated the presence of a common ancestor shared between some tribes of Flacourtieae and Salicaceae and suggested a revision for the classification of these taxa. Accordingly, The Angiosperm Phylogeny Group (2003) acknowledged the family Salicaceae *sensu lato*, comprising tribes *Saliceae (Populus, Xylosma, and Salix), Abatiae, Bembiciea, Flacourtiae, Homalieae, Prockieae, Samydeae,* and *Scolopieae*. However, traditional taxonomic classification is still predominant, primarily because of the limited sampling problem of molecular phylogenetic studies.

A study on Salicaceae evolution using fossil records provided the discovery of Pseudosalix, which existed from early Eocene to early Oligocene with an estimated time of 30-52 million years ago (Boucher et al., 2003). In addition to this effort, genome organization studies of the *Populus* genus revealed the syntenic blocks shared between the *Populus* and *Salix* genome. According to Tuskan et al. (2006) and Hou et al. (2019), this large-scale genome-wide similarity was originated from the salicoid duplication event within and between genomes of the two genera. This event led to the divergence of *Salix* and *Populus* genera from a common paleotetraploid ancestor approximately 58-65 million years ago.

1.1 *Populus* Genus

Widely regarded as a model organism, the *Populus* genus is well adapted to either riparian habitats or drylands. Some *Populus* species have evolved to persist in both floodplains and dry regions (Farmer, 1996; Jansson & Douglas, 2007). All members of the genus have a chromosome number of 2n=38. According to Eckenwalder (1996), there are 30 to 40 *Populus* species worldwide, and they are partitioned into six sections, which are Abaso, Aigeiros (Cottonwoods), Leucoides (Swamp Poplars), Populus (Aspens), Tacamahaca (Balsam Poplars), and Turanga. Nevertheless, the exact number of species within the genus is controversial due to the extensive genus-wide hybridization phenomenon (Cronk, 2005; Eckenwalder, 1984). Although interspecific hybridization within sections leading to the formation of hybrid swarms in nature is prevalent, successful hybridization can also occur between the species within different sections of the *Populus* genus, previously thought of as incompatible (Floate, 2004; Liesebach et al., 2011). *Populus* vegetations undertake critical functions, especially in riparian ecosystems. They ensure healthy river hydrodynamics and enhance the overall biodiversity of their habitat. Species of the *Populus* genus are also intertwined with socioeconomic activities due to their distinctive biological features such as rapid growth, extensive vegetative reproduction, and ease of hybridization. In Turkey, they are significant sources of industrial wood products and are mainly utilized for construction and furniture (Ministry of Forest and Water Affairs, 2012).

Members of the genus are deciduous and generally show seasonal variation in their leaf morphology, termed heterophylly. Early leaves, also named preformed leaves, arise with spring flush and become neoformed leaves (late leaves) in time as shoots lengthen during the post-flowering period (Eckenwalder, 1996).

Populus trees are dioecious, wind pollinating, and can reproduce sexually and asexually (Braatne et al., 1996; Cronk, 2005). Under suitable conditions, maturation time in reproductive ability is 10-15 years for individuals within natural populations (Stanton & Villar, 1996). *Populus* flowers are clustered in pendant catkins, and the

1-2 weeks of blooming period occurs in early spring preceded by leaf emergence (Braatne et al., 1996; Eckenwalder, 1996; Stanton & Villar, 1996). On the other hand, pollution lasts for 1-2 months, and pollen grains can disperse variable distances depending on environmental factors (Slavov et al., 2009; Tabbener & Cottrell, 2003). After successful fertilization, fruit capsules dehisce within 4-6 weeks, and numerous seeds with cotton-like appendages thrive afterward (Braatne et al., 1996). Also, all poplars can propagate via root suckers owing to their clonal expansion ability, and the extent of clonal growth is species-specific. This trait enables replicating robust genotypes within a suitable environment and provides long-term persistence for natural populations (Dickmann, 2001).

Flora of Turkey and Aegean Islands listed four native *Populus* species in Turkey distributed mainly along the major rivers of Anatolia: *Populus euphratica* Oliv. (Euphrates poplar), *Populus alba* (White poplar), *Populus tremula* (Aspen poplar), and *Populus nigra* (Black Poplar), respectively (Browicz & Yaltırık, 1982).

1.2 *Populus euphratica* Olivier

1.2.1 Bio-ecological Characteristics and Distribution

A medium to large-sized deciduous tree having a bushy crown with broad leaves, *Populus euphratica* Oliv. is a significant component of naturally occurring riparian vegetations as being a pioneer species within its habitat (Miao et al., 2020). Being a member of section Turanga, which harbors the most drought-tolerant species of the genus, the species is diploid and has a chromosome number of n=19. Individual trees can grow up to 8-15 m in height within natural stands, and their growth heavily depends on environmental parameters such as soil type and water availability (Orwa et al., 2009; Qisen et al., 2010).

P. euphratica trees have the ability to change their leaf morphology, a phenomenon known as heterophylly. Its heteromorphic leaves can be categorized into young,

developing, and mature leaves alternating from the lower to upper crown of the tree, each demonstrating a different anatomical and morphological character related to their habitat adaptability. The leaf shapes corresponding to the mentioned development stages are lanceolate, ovate, and broad-ovate, respectively. Another noticeable transformation occurs at leaf margins, which increases its dentation during the maturation process. Besides, wax crystals and trichomes increase in number together with specialized cells such as mucilage cells and crystal idioblasts, making the mature leaf more stress-resistant and photosynthetically more active (Liu et al., 2015; Mamıkoğlu, 2007).

The species is wind-pollinated and exhibits dioecy meaning that female and male organs (flowers and catkins) are found in distinct individuals. Generally lasting longer for female trees, the flowering phenological period starts in mid-March and continues until mid-April (Li et al., 2019). Successful pollination followed by fruit production creates small capsules bearing numerous seeds. Its small, light seeds are enveloped in silky hairs, facilitating their dispersal by the wind (Cao et al., 2012). Germination and seedling establishment require moist soil with low salt content and occur better on freshly deposited riverbanks (Thevs et al., 2008a). Therefore, seed rain season should coincide with flooding events. In addition, groundwater contact is vital for seedling survival, making *P. euphratica* an obligate phreatophyte.

P. euphratica trees can grow vertical deep tap root systems rapidly after their establishment in order to achieve contact with the capillary fringe of the water table. They can also create root suckers stretching horizontally from the parent tree to produce clones as an alternative reproductive strategy to generative reproduction. Root suckering begins 10-15 years after the successful tree establishment, and spacer roots can grow up to 40 meters away from the parent tree (Wiehle et al., 2009).

The species is characterized by its high tolerance to abiotic stress, as well as wide temperature differences, and individual trees can grow in hyper-arid areas with high levels of soil salinity and alkalinity (Chen et al., 2011; Chen et al., 2006). This tolerance is derived from the development of several acclimation mechanisms that

were positively selected under drought and salt stress (Chen et al., 2001). Despite having these types of abilities, extreme conditions can decrease the growth performance of trees by reducing the net photosynthetic rate (Ma et al., 1997). Also, high salt and low moisture content in soil can prevent seed germination and lead to a shift in populations' reproduction mode, which may have a negative effect on genetic diversity (Zhang et al., 2019). Hukin et al. (2005) also elicited the species' vulnerability to cavitation induced by a severe drought, disturbing the hydraulic processes of trees.

Figure 1.1 illustrates the distribution of the species' native and exotic populations around the globe. The species is found across Africa and Asia continents and its natural populations are distributed through North Africa to the Middle East, Middle Asia, and China (Barstow, 2018). There are also two isolated exotic Euphrates poplar populations situated in Spain and Kenya (Fay et al., 1999; Thomas et al., 2016). Besides its widespread occurrence, the species is most prevalent in the Tugai forests of the Tarim River Basin, which harbors nearly 54% of Euphrates poplar vegetation of the world (Lang et al., 2016).

Four major natural Euphrates poplar populations are found in Turkey (Figure 1.2), and they are distributed over the Mediterranean and the Southeastern Anatolia region, in the south of the 37th parallel north: Göksu population, Seyhan population, Euphrates population, and Tigris-Botan population, respectively (Karatay, 2015). However, there was no Euphrates poplar vegetation observed along the Seyhan River during our field studies, hence this population may have gone extinct.

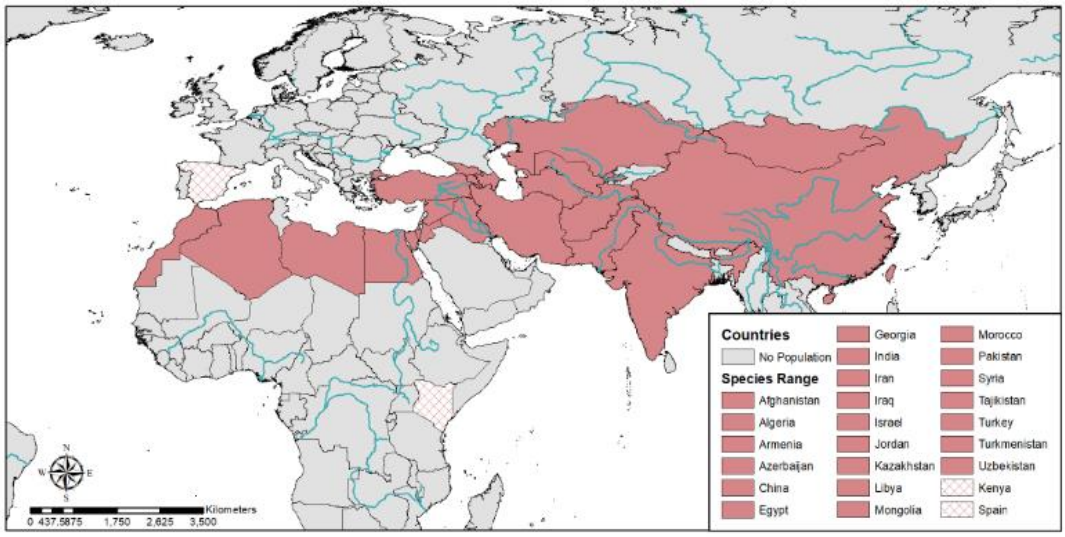

Figure 1.1: Distribution of native and exotic *P. euphratica* populations across the globe (Barstow, 2018). The map was manually created with Arcmap 10.7 (ESRI, 2019)

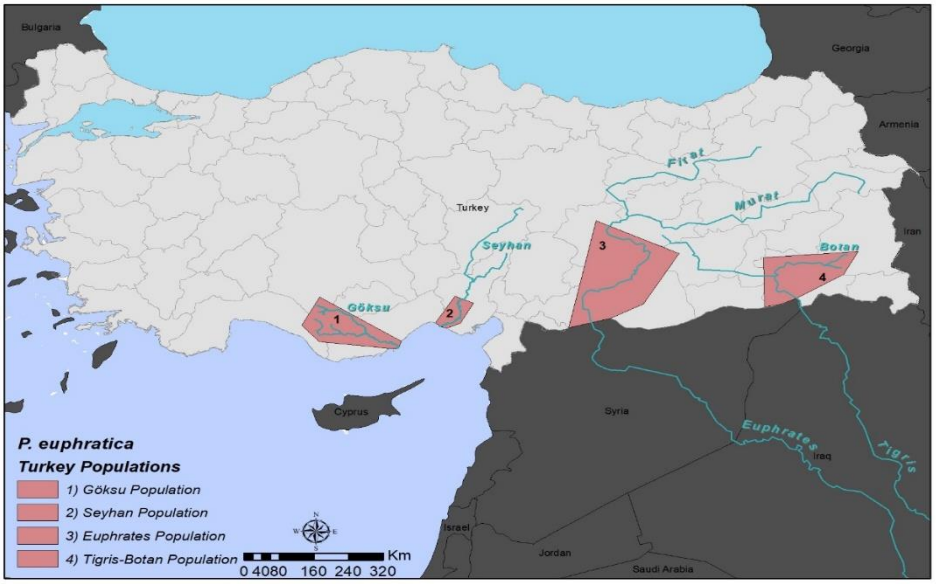

Figure 1.2: Distribution of *P. euphratica* populations in Turkey (Karatay, 2015). The map was manually created with Arcmap 10.7 (ESRI, 2019).

1.2.2 Significance, Threats, and Conservation Measures

Along with being a significant component of riparian habitats, *P. euphratica* forests augment biodiversity by providing habitat for a wide variety of ecologically important plant and animal species (Aishan et al., 2013). Euphrates poplar forests also render several ecosystem services of great importance, such as watershed protection, riverbank stabilization, windbreak formation, and erosion prevention (Mamat et al., 2018). Additionally, deep *P. euphratica* roots are capable of distributing water to upper soil layers. This process is known as hydraulic redistribution, which increases the water content of topsoil, thereby providing a water source for shallow-rooted plants to make the plant community under the forest canopy more stable (Yang et al., 2014). Apart from ecosystem services, they provide wood materials, and its biomass is utilized for fuel, construction, wood production, and livestock grazing (Gries et al., 2005).

P. euphratica is subjected to several transcriptome and metabolite profiling studies to identify the genes involved in abiotic stress (Brosché et al., 2005; Gu et al., 2004; Qiu et al., 2011; Tao et al., 2013). There are also studies shedding light on specific stress-related genes and metabolic pathways which evade the effects of salt stress (Chen et al., 2009; Ottow et al., 2005; Sun et al., 2010)

The species is assigned as the least concern (LC) by the IUCN Red List. However, the current populations are in a decreasing trend over the globe due to habitat destruction caused by construction works, excessive human use, and agricultural activities (Barstow, 2018; Gries et al., 2005). Moreover, dam construction and increased use of irrigation water alter the hydrological regime of rivers, thereby decreasing the frequency and magnitude of flooding events, which is indispensable for seed germination and seedling establishment. Water scarcity also leads to a decline in groundwater levels due to inadequate recharging. (Chen et al., 2008; Lang et al., 2016; Thevs et al., 2008b). Thus, these factors deteriorate the regeneration and growth capacity of degraded Euphrates poplar populations and obstruct their self-recovery via generative or vegetative reproduction (Keyimu et al., 2018)

Both *in-situ* and *ex-situ* conservation actions were initiated to mitigate the effects causing degradation of *P. Euphratica* forests, and they are currently in progress. In 2000, the Chinese government started a big-scale Tugai forest regeneration program for the lower reaches of the Tarim River and invested nearly 1.8 billion US dollars to implement the Ecological Water Diversion Program (EWDP) with accompanying research projects (Aishan et al., 2015). German Federal Ministry of Education and Research (BMBF) also funded extensive joint research projects to reveal relationships between the population dynamics of Euphrates poplar stands and hydrogeochemical characteristics of Tarim River and evaluate the effectiveness of the water diversion project (Halik et al., 2019). Moreover, UNESCO (2010) nominated the *P. euphratica* vegetation found in the Tarim River Basin for World Heritage List in line with FAO's suggestion. Besides *in-situ* conservation measures, seven *ex-situ* conservation programs include Euphrates poplar within their collections (Barstow, 2018).

1.3 Properties of Microsatellite Markers

Microsatellites, also known as simple sequence repeats (SSRs), are the tandem repeats of 1 to 6 bp sequence units (motifs) interspersed through the genome (Tautz & Renz, 1984). Their average occurrence rate in the plant genome is one SSR per 50 kbp DNA sequence. (Maughan et al., 1995). SSRs are categorized based on motif homogeneity within their composition. An SSR is termed as "perfect" if it is composed of repeats of only one motif; however, multiple different motif bearing SSRs are classified as "imperfect" or "compound" (Mason, 2015).

SSRs can be found in prokaryotes and eukaryotes. Their occurrence is not only confined in nuclear DNA of eukaryotes; mitochondrial DNA and chloroplast DNA also have SSRs distributed within their sequences (Vieira et al., 2016). They are present within both coding and non-coding regions of the nuclear genome, whereas their occurrence frequency is higher in non-coding regions than coding regions. (Li et al., 2004). SSRs arise primarily through polymerase strand-slippage errors during

the DNA replication process and mutate over the same mechanism with a high rate of 10^{-2}-10^{-3} per generation. This process results in addition or separation of a repeat motif and gives rise to repeat (length) polymorphism (Ashley & Dow, 1994)

The hypervariable nature of SSRs makes them very practical in terms of building up marker libraries. Since their discovery in 1984, they have been widely utilized in the scope of various molecular studies owing to their additional distinctive characteristics such as allelic codominance, transferability among closely related species, reproducibility, and extensive genome coverage (Miah et al., 2013). Especially, SSR markers are highly useful in genetic diversity assessment, estimation of genetic distance and gene flow rates, and genetic structure assignment within natural plant populations (De-Lucas et al., 2008; Fischer et al., 2017; Gadissa et al., 2018; Moges et al., 2016; Smulders et al., 2008). Also, they became widespread in molecular taxonomy studies to reveal the evolutionary relationships among the plant species (Gammon et al., 2007; Kaur et al., 2014). Apart from their application in natural plant populations, their usage is also common among crops for mapping QTLs, determining linkage groups, estimation of kinship degree, and marker-assisted selection (Kong et al., 2012; Miah et al., 2013).

1.4 Population Genetics Studies on *P. euphratica*

Population genetics studies measuring the genetic variability of *P. euphratica* natural populations started in the early 2000s. The first study attempted to assess genetic polymorphism levels of natural populations found along the Jordan River, using isozyme markers (Rottenberg et al., 2000). Then, Saito et al. (2002) investigated the genetic diversity of populations in northwestern China with RAPD markers. Two years later, Bruelheide et al. (2004) concentrated on clone presence within the populations situated along the Qira River using AFLP markers. The first comprehensive study aiming to develop species-specific SSR markers was performed by Wu et al. (2008). Following year, SSR markers previously developed for black cottonwood and aspen were screened via multiplex PCR to weigh their

informative value in *P. euphratica* (Eusemann et al., 2009). In 2010, a clonality study was carried out with AFLP markers for *P. euphratica* populations at the southern rim of the Taklamakan Desert (Vonlanthen et al., 2010). Subsequently, genetic diversity and differentiation patterns among ten populations found in northwest China were analyzed with species-specific SSR markers (Wang et al., 2011a). Wang et al. (2011b) conducted a more comprehensive study in the same year to reveal genetic differentiation and delimitation between *P. euphratica* and *P. Pruinosa* species using SSR markers in combination with cpDNA and ITS sequences. Later on, Xu et al. (2013) analyzed the magnitude of genetic variation of populations found in Western China and Inner Mongolia using SSR markers. Petzold et al. (2013) investigated sex ratios of *P. euphratica* stands in Xinjiang, China at different levels with SSR markers. In the same year, EST-SSR markers were developed for the species (Du et al., 2013). In 2015, Wang et al. (2015) enriched the species-specific SSR marker library of *P. euphratica* by isolating SSRs from RAPD products. Then, Kramp et al. (2018) carried out a clonality study for the populations situated along different reaches of the Tarim River. In the same year, Zeng et al. (2018) conducted a comprehensive genetic diversity study on *P.euphratica* populations found in northern and southern Xinxiang regions. The first attempt of determining the magnitude of genetic diversity possessed by *P. euphratica* populations found in Turkey was realized in 2020 by sampling the populations from the Göksu and the Euphrates River basin (Kansu & Kaya, 2020). The most recent study aiming to evaluate the demographic history and genetic variation of a Northwest China population was conducted with NGS data (Jia et al., 2020).

CHAPTER 2

JUSTIFICATION AND OBJECTIVES

The sustainability of healthy and unfragmented Euphrates poplar forests in riparian ecosystems are of utmost importance in terms of biodiversity maintenance and continuity of valuable ecosystem services that they provide. As the current population trend is decreasing across the globe due to anthropogenic factors such as dam construction and agricultural activities leading to severe habitat loss, both *in-situ* and *ex-situ* conservation strategies should be designed immediately to mitigate the effects that may cause further loss of the species' natural populations. As an essential part of these conservation actions, determining the degree of genetic diversity possessed by *P. euphratica* populations gives critical clues regarding the current population structure and generative regeneration capacity of extant populations. However, population genetics studies on Euphrates poplar are limited in number in the literature, and there is no study linking populations' age structure and genetic diversity. To this end, an age-based perspective was utilized while taking the species' reproductive characteristics and biogeographical features of the Göksu River basin into consideration to determine:

- The degree of clonality and genetic diversity in young and mature stands
- The extent of genetic diversity transfer between the mature and young stands of three distinct locations situated along the Göksu River basin
- Age-based genetic differentiation and structure patterns

CHAPTER 3

MATERIAL AND METHODS

3.1 Sampling Locations and Strategy

As the first step of the study, a field trip was performed on April 20, 2019, within the blooming season. Young leaf samples were collected from both young and mature individual trees for subsequent molecular analysis from three different locations: upstream, midstream, and downstream of the Göksu River, respectively. Geographical coordinates and altitude information were recorded for each mature individual, and populations were visualized using ArcMap 10.7 (ESRI, 2019) software, utilizing satellite images (Landsat-7, 2019) as a base map (Figure 3.1). Sampled trees are partitioned into three populations according to their locality information. Each population was then further structured as mature and young subpopulations, composing six subpopulations in total (Table 3.1). A stratified sampling strategy was used for mature and young individuals due to the species' clonal growth habit via root suckers. Using GPS data, mature individuals that are approximately 200 m away were selected in order to represent a single genet, and they were within almost the same age group. Sampling of young individuals, on the other hand, was realized outside of a circumferential area having 40 m diameter by marking mature individuals as the center to avoid sampling ramets of the same clone. Samples were placed in zipper bags containing silica gels to prevent decay for further DNA extraction. Pictures that were taken during the field trip were given in Figure 3.2.

Table 3.1: Number of mature and young individuals sampled from three populations situated along the Göksu River Basin

Population	Altitude Range	Mature Population Identifier	Number of Mature Individuals	Young Population Identifier	Number of Young Individuals	Total
GUP	134-149 m	GUPM	32	GUPY	34	67
GMID	89-106 m	GMIDM	31	GMIDY	43	74
GDOWN	30-51 m	GDOWNM	32	GDOWNY	42	74
Total	-		95		119	214

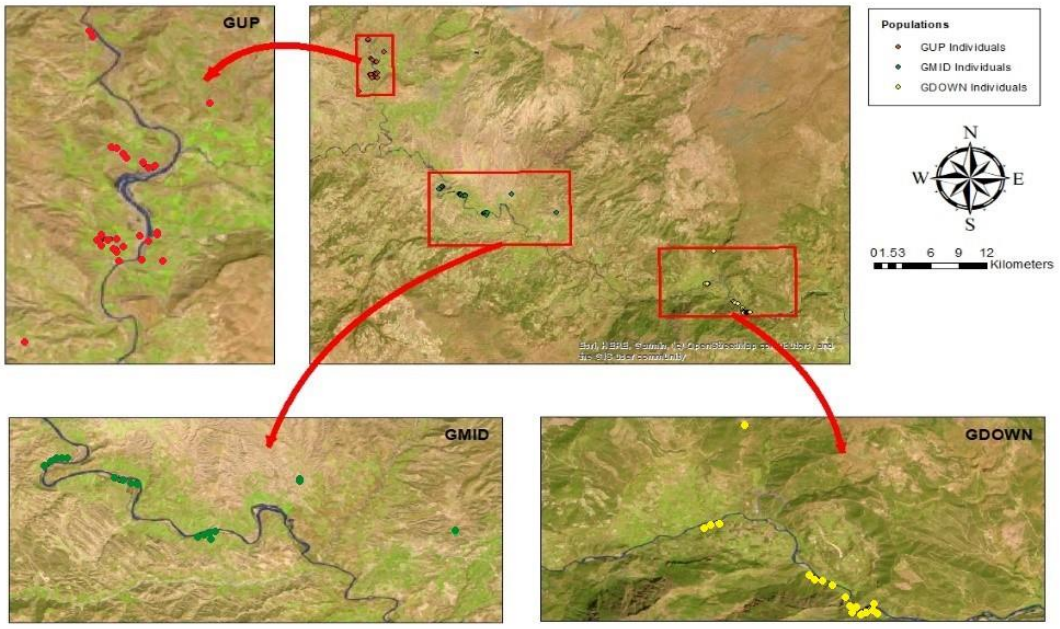

Figure 3.1: Sampling locations of upstream, midstream, and downstream populations located along the Göksu River

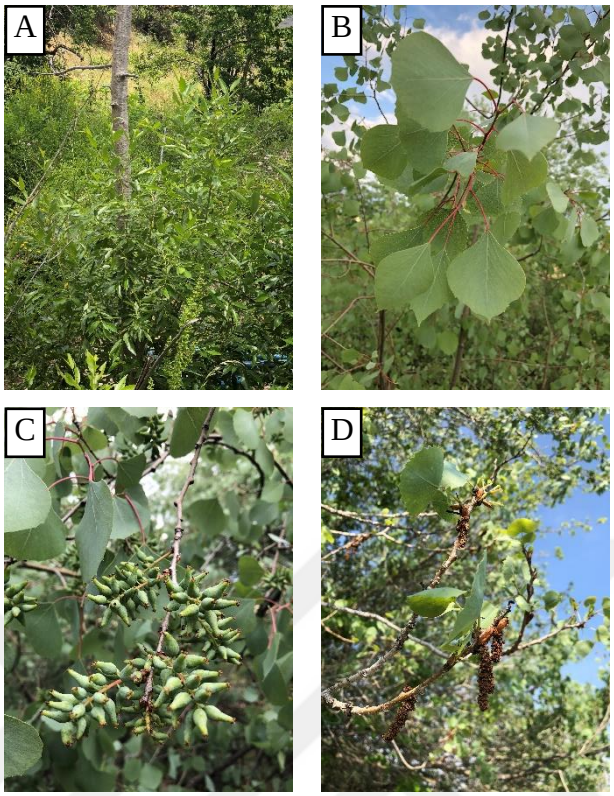

Figure 3.2: Photos that were taken during the field trip A: A young *P.euphratica* individual B: Mature leaves of *P. euphratica* C: *P. euphratica* fruits D: Catkins of a male *P. euphratica* individual

3.2 Molecular Studies

3.2.1 DNA Extraction

Collected leaf materials were grounded using pestle and mortar with the aid of liquid nitrogen until it reaches at a fine dust form. The leaf samples were stored in a deep freezer at − 80 °C until DNA isolation. DNA extraction was performed as per the modified CTAB extraction protocol (Doyle, 1991), and the quality of total DNA samples was checked via NanoDrop Spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The DNA extraction process was iterated for samples with low purity, and absorbance ratios were examined again until they fall within the optimal range (A260/A280=1.7-2.0). The template DNA concentration was diluted to 20 ng/µl before it was used.

3.2.2 Amplification of Microsatellite Loci

Fifteen microsatellite loci were chosen from previous SSR enrichment studies of the genus *Populus*. Species-specific markers having “Pe” and “Popeu” prefix were used together with SSRs developed for *P. nigra* (WPMS prefix) and *P. thiocarpa* (PMGC prefix) owing to cross-species transferability of SSR markers. Each SSR locus has a different repeat motif pattern and expected product size, so different primers were designed for each marker according to their sequence information (Table 3.2). Using 5x HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) in all reactions, PCR conditions were optimized for each SSR locus (Table 3.3, Table 3.4). After the optimization step, forward primers were labeled with a fluorescent dye before the amplification process in order to genotype the SSR loci successfully (Table 3.5). All SSR loci were then amplified for each individual utilizing a thermocycler, and reaction products were run via 3% agarose gel electrophoresis to verify their presence.

Table 3.2: Repeat motif, forward and reverse primer, and expected product size of each locus

Loci	Repeat Motif	Forward Primer (5'-3')	Reverse Primer (3'-5')	Product Size	Reference
WPMS5	GT	TTCTTTTTCAACTGCCTAACTT	TGATCCAATAACAGACAGAACA	280	Smulders et al. 2001; van der Schoot et al. 2000
WPMS14	CGT	CAGCCGCAGCCACTGAGAAATC	GCCTGCTGAGAAGACTGCCTTGAC	245	
WPMS18	GTG	CTTCACATAGGACATAGCAGCATC	CACCAGAGTCATCACCAGTTATTG	245	
PMGC14	CTT	TTCAGAATGTGCATGATGG	GTGATGATCTCACCGTTTG	210	The International *Populus* Genome Consortium
PMGC2163	GA	CAATCGAAGGTAAGGTTAGTG	CGTTGGACATAGATCACACG	220	
PMGC2889	GA	CCCAAGATCCGATTTTTGGG	CACAATGTACAAATCGCTGTC	207	
PMGC93	CTT	ATCATGCGTTCGGCTACAGC	CTCAAACTCCAACTGTTATAAC	350	
Pe2	$(CT)_9(CA)_{11}$	CGCTATCATCCTCATCTCTGATCTC	CCATGCTGGGATGAAATATGTAAAC	128–147	Wu et al. 2008
Pe5	TC	ACCCACCCAATGTGCAGCCCTGCAA	CTCGCCCTCTATATATCTCTATGAA	164–206	
Pe6	TG	GAACATGGGTCCATATCAAGTAG	CACCCCACACACCCACAC	175–233	
Pe8	$(TG)_{12}(GA)_{11}$	AACACGGAAGCAAGAAAAAATGAAG	CACACATTCCACCCTCCACACCACT	146–197	
Pe13	$(CT)_6(GT)_5$	TTCAACTTGACTAGTTGTAACTCTC	CACTTTCCCAGCTATCCCTTTCTAA	121–137	
Pe14	CT	TCGAAATGGGAGATCTGTAGAGGTG	CACCACAACAGCGTACAGAAATGAA	137–178	
Pe15	AG	TGTTTAAGAAGAGATCAAAAGGGGA	TTTCTCAGGTATCCAAGCGATGCTG	124–161	
Popeu13	AT	TCTGGAGCACAATTGAATTTTTG	TCTTTGAAATTCATGCTACTTCA	270–330	Wang et al. 2015

Table 3.3: Optimized PCR conditions for studied SSR Loci

Content	**WPMS14**	**PMGC93**	**PMGC14**	**PMGC2163**	**PMGC2889**	**Pe13**	**WPMS5**	**Pe6**
dH20	14 µl	14 µl	14 µl	14 µl	14 µl	12 µl	14 µl	12 µl
5x HOT FIREPol® Blend Master Mix	4 µl	4 µl	5 µl	5 µl	5 µl	5 µl	4 µl	5.5 µl
Primers (10 µM)	0.5 µl + 0.5 µl	0.5 µl + 0.5 µl	0.5 µl + 0.5 µl	0.5 µl + 0.5 µl	0.5 µl + 0.5 µl	0.5 µl + 0.5 µl	1 µl + 1 µl	0.75 µl +0.75 µl
DNA (20 ng/µl)	6 µl	6 µl	5 µl	5 µl	5 µl	7 µl	5 µl	6 µl
Total	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl
	Pe8	**Pe5**	**Pe2**	**Pe14**	**Pe15**	**Popeu13**	**WPMS18**	
dH20	12 µl	12 µl	11 µl	12 µl	12 µl	10.4 µl	13.5 µl	
5x HOT FIREPol® Blend Master Mix	4.5 µl	4.5 µl	6 µl	5.5 µl	5 µl	5 µl	5 µl	
Primers (10 µM)	0.75 µl +0.75 µl	0.75 µl +0.75 µl	0.5 µl + 0.5 µl	0.75µl +0.75 µl	1 µl + 1 µl	0.8 µl + 0.8 µl	0.75 µl +0.75 µl	
DNA (20 ng/µL)	7 µl	7 µl	7 µl	6 µl	6 µl	8 µl	5 µl	
Total	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	25 µl	

Table 3.4: PCR Program used for amplification of SSR loci

Phase	**Temperature (°C)**	**Time**
Initial Denaturation	94	3’
Denaturation	94	30’’
Annealing	Ta	30’’
Extension	72	40’’
Final Extension	72	20’

Table 3.5: Annealing temperatures, fluorophore dyes used, and number of PCR cycles for each SSR locus

Locus	**Ta(°C)**	**Fluorophore**	**Number of Cycles**
WPMS5	55	6-FAM (Blue)	25
WPMS14	60	HEX (Green)	25
WPMS18	55	TAMRA (Black)	25
PMGC14	55	6-FAM (Blue)	25
PMGC2163	55	HEX (Green)	25
PMGC2889	55	HEX (Green)	25
PMGC93	55	TAMRA (Black)	25
Pe2	58	100-VIC (Green)	28
Pe5	58	NED (Black)	25
Pe6	59	VIC (Green)	25
Pe8	60	6-FAM (Blue)	25
Pe13	57	PET (Red)	25
Pe14	58	6-FAM (Blue)	25
Pe15	54	NED (Black)	25
Popeu13	54	300-VIC (Green)	28

3.2.3 DNA Fragment Analysis

Following the amplification of SSR loci, DNA fragment analysis was performed by getting service from BM Laboratory Systems Facilities, Ankara. The analysis procedure was performed with the Applied Biosystems 3730 XL DNA Analyzer (Applied Biosystems Inc., Foster City, CA), utilizing the GeneScan ROX labeled 400 HD internal standard size marker. This assay uses the capillary electrophoresis method, which separates previously labeled DNA fragments according to their base-pair size while utilizing a size standard marker to estimate the exact base pair-size of the fragments. The output of this assay was given in the form of an electropherogram (Figure 3.3), which visualizes the allele calls as peaks, each having a different color. Each allele call was assigned using the PeakScanner 2.0 software (Applied Biosystems Inc., Foster City, CA), and allele sizes were recorded for each locus to build a microsatellite genotype profile of the entire sample thereafter.

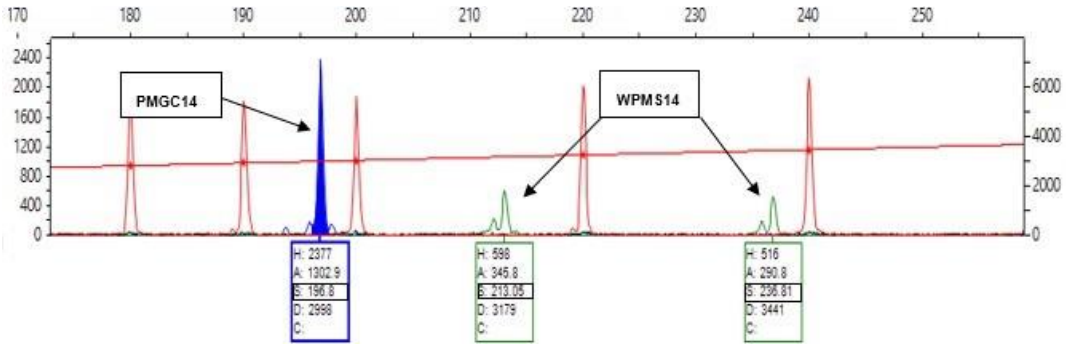

Figure 3.3: Example of electropherograms for two SSR loci

3.3 Population Genetics Analyses

3.3.1 Quality Check of Markers and Detection of Alleles

Raw genotype data checked via MICRO-CHECKER 2.2.4 (Van Oosterhout et al., 2004) to detect genotyping errors sourced from DNA degradation, low DNA concentrations, and primer-binding site mutations. Stutter peaks, large allele dropouts were eliminated, and typographic errors were corrected before commencing population genetics analyses. Moreover, alleles were determined for each locus along with their numbers and allelic size ranges by scanning genotype data with MICRO-CHECKER 2.2.4 (Van Oosterhout et al., 2004).

3.3.2 Detection of Clones

Clonal expansion habit is widespread among *Populus* species owing to their root sucker generation ability (Cristóbal et al., 2014). These opportunistic suckers emerge from root buds after induced by an external disturbance leading to the formation of a clone that is identical to its parent tree in terms of its genetic background (Wiehle et al., 2009). In certain aspects, clonal growth can be an advantageous strategy by securing the colonization process of unoccupied areas where seedling recruitment is unlikely and by enhancing the species' competitive power and survival rate in marginal habitats (Jeník, 1994). However, clonal expansion is also a well-known phenomenon in terms of its negative effect on the genetic diversity of natural plant populations. This adverse effect may lead to a reduction in effective population sizes with an accompanying decrease in overall fitness and adaptability in the long term, thus making the species more vulnerable to sudden changes in the environment (Barsoum et al., 2004).

The number of clones was calculated by GenClone 2.0 (Arnaud-Haond & Belkhir, 2007) software, which scans genotype data acquired from DNA fragment analysis to specify identical individuals with respect to their genotype at all 15 loci. Moreover,

by using the equation *Clonality (C)=1-(Number of Unique Genotypes-1)/(Number of Sampled Individuals-1)* as formulated by Dorken & Eckert (2001), clonality was calculated for each population.

3.3.3 Null Allele Presence

Even though microsatellite markers are widely used in population genetic studies due to their several favorable specifications, their utilization is prone to null allele presence (Chapuis & Estoup, 2007). Null alleles appear as a result of (i) mutations that occurred within the primer annealing regions of SSR loci in which high mutation rate is observed, (ii) amplification process favoring the emergence of short alleles due to insufficient DNA template quality, and (iii) DNA polymerase slippage events encountered during amplification via PCR (Dabrowski et al., 2015; Gagneux et al., 1997).

An ideal genotyping process includes determining the exact length of previously amplified SSR loci. However, the presence of null alleles rather than true ones within genotype data appears as homozygote excess, leading to deviations in genetic diversity parameters (Pemberton et al., 1995). Population structure assignment would also be error-prone due to the biased increase of genetic differentiation among populations (Carlsson, 2008; Paetkau & Strobeck, 1995). There are several null allele frequency estimation methods available in the literature embedded within various software packages. These methods check whether a population is in Hardy-Weinberg equilibrium or not and relates the deviations towards heterozygote deficit with null allele presence (Dabrowski et al., 2014).

Dabrowski et al. (2015) reported that the final performance of the estimators is enhanced when they are used together. Thus, the presence of null alleles was monitored with MICRO-CHECKER 2.2.4 (Van Oosterhout et al., 2004), which implements the null allele frequency estimators of Brookfield (1996) and Chakraborty et al. (1992) in combination.

3.3.4 Genetic Diversity Parameters

Basic genetic diversity statistics such as mean number of alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), observed heterozygosity (Ho) and unbiased expected heterozygosity (uHe), Fixation Index, and Private allele frequencies were calculated with GenAlEx 6.503 (Peakall & Smouse, 2012) to assess genetic polymorphism attributed for each locus and age group. Initially developed in Information theory, Shannon's diversity index (I) is a unifying measure of genetic diversity, and it allows to find out the variation inherent to loci sensitively (Konopiński, 2020). The two most common genetic diversity indicators in the scope of population genetics studies are observed heterozygosity (Ho) and expected heterozygosity (He). Together, they create the framework of Wright's F-statistics, allowing to make deductions regarding inbreeding and genetic drift operating within natural populations. Furthermore, as a standardized value of the number of alleles, allelic richness (Ar) was assigned by FSTAT version 2.9.4 for each locus (Goudet, 1995). FSTAT software circumvents the sample size bias using Hurlbert's rarefaction index (Hurlbert, 1971), as suggested by El Mousadik & Petit (1996). As Ar is not a frequency-based measure such as heterozygosity, it is more sensitive to allelic loss caused by demographic shrinkage events (Leberg, 2002). Therefore, it is a more relevant measure than heterozygosity within the context of long-term evolutionary potential, especially the selection capacity and eventual fitness of the natural populations (Greenbaum et al., 2014).

In order to effectuate a complete insight regarding polymorphism levels of each locus, Cervus 3.0.7 (Kalinowski et al., 2007) was utilized to measure polymorphism information content (PIC). Moreover, Genalex 6.503 (Peakall & Smouse, 2012) was used for the estimation of the quantities, such as the percentage of polymorphic loci (%P) and the probability of identity (PI). Probability of identity (PI) estimator represents the probability of that two randomly selected individuals from a population have the same multilocus genotype (Eichmann et al., 2005). Polymorphism Information Content (PIC), on the other hand, is an estimator of a

genetic marker's ancestral source and is used to evaluate its functionality (Elston, 2005).

Fixation indices (Fis, Fst, Fit) and deviations from Hardy-Weinberg Equilibrium per locus were computed by Genepop 4.2 (Rousset, 2008) to examine the effects of inbreeding and genetic drift. Genepop 4.2 uses heterozygote excess and deficit information together with a Markov chain algorithm, reducing the bias over p-value calculation. Besides, the magnitude of gene flow was (Nm) measured for each locus by GenAlEx 6.503 (Peakall & Smouse, 2012).

Possible bottleneck events in population history, which might have caused a sudden reduction in population size was checked with Arlequin version 3.5.2 (Excoffier & Lischer, 2010), which calculates Garza-Williamson Index (Garza & Williamson, 2001) per locus.

3.3.5 Genetic Structure of Age Groups

Genetic differentiation patterns between the age groups situated in three different localities along the Göksu River basin were analyzed with Arlequin 3.5.2 (Excoffier & Lischer, 2010), which creates a pairwise Fst matrix with corresponding Nm values to reveal genetic distance among populations. Subsequently, principal coordinates analysis (PCoA) was performed with GenAlEx 6.5.0.3 (Peakall & Smouse, 2012) to visualize the differentiation patterns in the form of a graph for convenient observation of genetic distances.

Phenetic relationships between the age groups were evaluated by GDA software (Lewis & Zaykin, 2001), which constructs a cladogram as an output. This software executes an algorithm that employs the Unweighted Pair-Group Method with Arithmetic Averaging (UPGMA) hierarchical clustering method.

The extent of genetic clustering within natural populations relies on several genetic and demographic determinants such as the magnitude of genetic drift and gene flow, temporal differences in reproductive episodes, and adult densities shaping the stand

structure (Chung et al., 2003). Among these, gene flow is featured due to its considerable effect on genetic backgrounds at the individual level. The genetic structure of mature and young tree stands was analyzed with STRUCTURE version 2.3.4 (Pritchard et al., 2000), which assigns each individual into clusters with or without the prior information to which population the individual belongs. STRUCTURE executes an iterative Bayesian algorithm and provides four alternative modeling decisions according to different assumptions regarding individuals' ancestry while calculating the posterior probabilities of cluster memberships. The admixture model option was chosen with the run parameters of 10 replications for each possible cluster number (K) ranging from 1 to 10, and 250,000 MCMC (Markov Chain Monte Carlo) iterations along with 50,000 burn-in length. A downstream web-based tool, STRUCTURE HARVESTER (Earl & vonHoldt, 2012), which implements Evanno Method (Evanno et al., 2005), was utilized afterward to find out the actual cluster number that best matches the data output generated by STRUCTURE. Then, outputs of all ten runs for the true K were collated by CLUMPP version 1.1.2 (Jakobsson & Rosenberg, 2007) to figure out the optimal alignment of the replicates. The output from CLUMPP that contains individuals' membership coefficients was used to visualize the genetic groups via Pophelper (Francis, 2017).

3.3.6 Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was performed with two different grouping structure based on location (Upstream, Midstream and, Downstream) and age (Young and Mature) to estimate genetic variability among groups (Va), among populations within groups (Vb), and within populations (Vc). The primary rationale behind two different grouping strategies is to uncover whether location or age difference affects the genetic variation more. Implemented within Arlequin 3.5.2 (Excoffier & Lischer, 2010), Fst based AMOVA was carried out for both grouping strategies. Fst based AMOVA design utilizes the infinite allele model grounded upon the number of different alleles.

CHAPTER 4

RESULTS

4.1 Detection of Alleles

To evaluate the genetic diversity levels per locus, detected alleles were assigned for 15 loci along with their numbers and size range at first (Table 4.1). The number of alleles ranged between 2 and 11, and the loci having these scores are Pe13 and Popeu13, respectively. The locus having the highest allelic size range was Pe5, with a 26 base pair difference between its shortest and longest alleles.

Table 4.1: Observed alleles and allelic size range of SSR each locus

Locus	Number of Alleles	Alleles Detected	Size Range
WPMS5	3	287, 289, 291	4 bp
WPMS14	6	210, 213, 216, 219, 231, 237	27 bp
WPMS18	3	225, 228, 231	6 bp
PMGC14	3	188, 197, 200	12 bp
PMGC2163	4	195, 201, 203, 205	10 bp
PMGC2889	7	181, 183, 185, 187, 189, 191, 193	12 bp
PMGC93	5	348, 351, 354, 357, 369	21 bp
Pe2	7	97, 99, 101, 103, 105, 107, 119	21 bp
Pe5	8	156, 160, 162, 168, 170, 172, 174, 182	26 bp
Pe6	3	153, 155, 161	8 bp
Pe8	5	142, 146, 152, 154, 158	16 bp
Pe13	2	126, 130	4 bp
Pe14	6	140, 144, 146, 148, 150, 152	12 bp
Pe15	9	128, 130, 134, 138, 140, 142, 144, 148, 152	24 bp
Popeu13	11	278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298	20 bp

4.2 Detection of Duplicate Genotypes

Within the three study locations (GUP, GMID, GDOWN), a total of 214 individuals were chosen for genetic analysis with a stratified sampling strategy. The main purpose of the stratified sampling strategy was to avoid clonal sampling. However, there were 28 clones detected among 214 multilocus genotypes (MLGs) allocated within 12 clone groups (Table 4.2). All clones were young individuals that are in close proximity to their parent trees.

Clonality was calculated for each population immediately after the number of unique genotypes had been obtained (Table 4.3). Downstream population (GDOWN) showed the highest clonality among the populations (C=0.096), whereas the upstream population (GUP) has the lowest score (C=0.046). In addition, the midstream population (GMID) had the highest number of unique MLGs, which is 68.

Sixteen young clone genotypes were excluded from the study while keeping one genotype per clone group. Thus, all further analyses were performed with unique 198 MLGs.

Table 4.2: Clone groups (Young Individuals were highlighted)

Population	Clone groups	Clones excluded from further analysis	Number of Excluded Genotypes
GUP	7, 8 31a, 31b, 31c	8 31b, 31c	3
GMID	73,74a,74b,74c 97,98 103b,103c 104,107a	74a,74b,74c 98 103c 107a	6
GDOWN	124,125 126,127a 129a,129b 138, 139a,139b 141,142 164,165	125 127a 129b 139a,139b 142 165	7
Total			16

Table 4.3: Clonality levels of each population

	GUP	GMID	GDOWN	Total
Genotypes Sampled	66	74	74	214
Unique Genotypes	63	68	67	198
Duplicate Genotypes (Clones)	5	10	13	28
Clonality (C)	0.046	0.082	0.096	0.07

4.3 Null Allele Presence

Determination of null allele presence within studied loci was carried out using the Brookfield (1996) and Chakraborty et al. (1992) frequency estimators (Table 4.5). These estimators detected several loci possessing null alleles; especially Popeu13 locus has null alleles in more than one population (Table 4.4). Fortunately, any amplified loci showed null allele presence in all populations, thereby exerting no meaningful effect on further statistical analyses. Thus, the further evaluation continued with all available locus data, assuming no null alleles were present within loci.

Table 4.4: Null allele possessing loci

Population	**Null Allele Possessing Loci**
GUPM	PMGC2163, PMGC2889, Popeu13
GUPY	WPMS14
GMIDM	Popeu13
GMIDY	Pe5
GDOWNM	-
GDOWNY	Pe13, Popeu13

Table 4.5: Null allele frequencies calculated by two different estimators

Population	GUPM		GUPY		GMIDM		GMIDY		GDOWNM		GDOWNY	
Method / Locus	Chakraborty	Brookfield	Chakraborty	Brookfield	Chakraborty	Brookfield	Chakraborty	Brookfield	Chakraborty	Brookfield	Chakraborty	Brookfield
WPMS5	0.0496	0.0375	0.0798	0.0591	-0.0396	-0.0327	-0.04	-0.0333	0.0869	0.0597	0.0071	0.0056
WPMS14	0.1284	0.0877	0.2094*	0.1142*	0.1251	0.0853	0.0574	0.0437	0.0485	0.0373	0.0619	0.0483
WPMS18	0.1148	0.0765	0.1498	0.0904	-0.0293	-0.0221	-0.04	-0.0318	0.0333	0.0234	0.0937	0.0584
PMGC14	-0.0076	-0.0004	-0.0084	-0.0005		0		0		0	-0.0072	-0.0004
PMGC2163	0.2961*	0.1201*	0.0781	0.0282	0.1276	0.0511	0.1386	0.0631	0.0702	0.0321	0.06	0.0219
PMGC2889	0.1239*	0.0953*	0.0981	0.0769	0.0791	0.0631	-0.0057	-0.0048	-0.0454	-0.0396	-0.0433	-0.0371
PMGC93	0.1357	0.0894	0.0324	0.0241	0.1517	0.0976	0.0964	0.0627	-0.0483	-0.0387	0.1075	0.0697
Pe2	-0.0858	-0.0779	-0.0634	-0.0557	0.0069	0.0056	-0.0412	-0.0364	-0.0336	-0.0287	-0.1023	-0.0931
Pe5	0.0145	0.0064	-0.1098	-0.0631	0.0392	0.0219	0.1509*	0.1003*	0.0534	0.0287	-0.0759	-0.0552
Pe6	-0.0905	-0.0771	-0.0729	-0.0575	-0.1372	-0.1177	-0.1374	-0.1182	0.0376	0.0283	-0.0796	-0.0603
Pe8	0.1538	0.0378	0.1189	0.0371	0.1548	0.0632	0.0438	0.0176	-0.0018	-0.001	-0.0765	-0.0526
Pe13	0.0815	0.0502	0.0327	0.0205	-0.0206	-0.0139	0.0812	0.0494	0.0794	0.0485	0.3148*	0.1582*
Pe14	-0.1109	-0.1009	-0.1013	-0.0891	-0.0533	-0.0474	-0.0634	-0.0541	-0.042	-0.0375	-0.126	-0.1218
Pe15	-0.0323	-0.0287	0.0658	0.0512	-0.0377	-0.0327	-0.0251	-0.0223	-0.0446	-0.0398	-0.1168	-0.1128
Popeu13	0.1969*	0.1241*	0.0116	0.0085	0.1973*	0.1232*	0.0526	0.0344	0.0585	0.0442	0.2578*	0.1715*

*: Null allele possessing loci

4.4 Amount of Genetic Variation Among Loci

None of the loci were monomorphic, but the least variable one is PMGC 14 with respect to its mean number of alleles (Na). Na values ranged between 1.5 and 6.3 within loci, having a grand mean of 3.9±0.16 per locus (Table 4.6). As for the effective number of alleles (Ne), all loci had lower values due to low-frequency alleles within loci. Popeu13 has the highest allelic richness (Ar) value of 6.47, and the lowest value (1.4) belonged to PMGC14. PMGC28, Pe2, Pe14, and Pe15 have lower Probability of Identity (PI) values ranged from 0.1 to 0.15, inferring their higher power of discriminating MLGs compared with other loci. As another indicator of polymorphism levels among loci, Polymorphic Information Content (PIC) values ranged from 0.01 to 0.71, which belongs to PMGC14 and Pe15, respectively. Shannon's Information Index (I) ranged from 0.04 at PMGC14 to 1.44 at Pe15 with a grand mean value of 0.97±0.04, demonstrating the moderate level of variability inherent to loci.

The observed heterozygosity (Ho) values ranged from 0.02 at PMGC14 to 0.82 at Pe14, having a grand mean of 0.52±0.03. The unbiased expected heterozygosity differed between 0.02 and 0.75 at PMGC14 and Pe14, respectively. The higher value of mean expected heterozygosity (0.55±0.02) could be attributed to the heterozygote deficiency of the ten loci, also having positive fixation indices (F). Besides, five loci showed significant deviations from HWE.

Ten loci with positive inbreeding coefficients (Fis) observed among fifteen loci further support the overall heterozygosity deficit (Table 4.7). Also, one negative and fourteen positive Fst values of loci ranged from -0.01 to 0.1, with a mean of 0.02. Corresponding Nm values ranged from 2.34 at Popeu13 to 26.21 at PMGC14, having a mean of 11.85±1.87.

Table 4.6: Genetic diversity parameters of studied loci

Locus	N	Na	Ne	Ar	PI	PIC	I	Ho	uHe	F	HW
WPMS5	32.7±0.95	3±0	2.9±0.08	3.000	0.19	0.591	1.08±0.01	0.62±0.03	0.66±0.01	0.04±0.04	*
WPMS14	31.7±0.95	5±0.365	2.81±0.2	5.144	0.17	0.607	1.19±0.07	0.52±0.05	0.64±0.03	0.19±0.04	**
WPMS18	32.3±1.17	3±0	2.3±0.08	2.996	0.27	0.483	0.9±0.03	0.52±0.04	0.57±0.01	0.09±0.06	NS
PMGC14	33±1.06	1.5±0.22	1.02±0.01	1.405	0.97	0.015	0.04±0.03	0.02±0.01	0.02±0.01	-0.02±0	ND
PMGC2163	31.7±0.71	2.6±0.21	1.44±0.04	2.547	0.52	0.264	0.51±0.03	0.23±0.01	0.31±0.02	0.22±0.05	*
PMGC2889	31.7±0.61	5.5±0.22	3.78±0.16	5.423	0.10	0.709	1.42±0.03	0.69±0.03	0.74±0.01	0.06±0.06	NS
PMGC93	31.7±0.88	4.2±0.31	2.45±0.07	4.382	0.20	0.564	1.1±0.02	0.51±0.04	0.6±0.01	0.14±0.06	NS
Pe2	32±0.82	4.7±0.33	3.39±0.1	4.669	0.13	0.675	1.3±0.02	0.78±0.03	0.71±0.01	-0.12±0.04	NS
Pe5	31.5±1.12	4.7±0.76	1.82±0.18	5.468	0.32	0.429	0.84±0.12	0.41±0.04	0.43±0.05	0.01±0.08	NS
Pe6	32.8±0.94	3±0	2.48±0.1	3.000	0.23	0.532	0.98±0.02	0.7±0.03	0.6±0.01	-0.18±0.06	NS
Pe8	33±1.06	3.17±0.3	1.5±0.1	3.396	0.48	0.301	0.56±0.07	0.29±0.07	0.31±0.05	0.11±0.07	NS
Pe13	32.5±1.09	2±0	1.97±0.01	2.000	0.38	0.375	0.68±0	0.41±0.03	0.50±0	0.16±0.07	NS
Pe14	33 ±1.06	4.8±0.31	3.38±0.18	5.137	0.13	0.662	1.33±0.05	0.82±0.02	0.71±0.02	-0.18±0.03	*
Pe15	31.8±0.83	5.5±0.22	3.83±0.12	5.789	0.10	0.714	1.44±0.03	0.79±0.04	0.75±0.01	-0.07±0.05	NS
Popeu13	31.8±1.1	6.3±0.56	2.68±0.21	6.473	0.15	0.635	1.24±0.06	0.47±0.03	0.63±0.03	0.22±0.06	***
Grand Mean	32.2±0.24	3.9±0.1	2.51±0.09				0.97±0.04	0.52±0.03	0.55±0.02	0.05±0.02	

N: Sample size, Na: Mean allele number, Ne: Effective number of alleles, Ar: Allelic Richness, PI: Probability of Identity, PIC: Polymorphism information content, I: Shannon's Information Index, Ho: Observed heterozygosity, uHe: Unbiased expected heterozygosity, F: Inbreeding coefficient, HW: Hardy-Weingberg deviation (ND: non-deviating, NS: non-significant, ***:p<0.001, **:p<0.01, *:p<0.05)

Table 4.7: F-statistics and Nm values across the studied loci

Locus	F_{is}	F_{it}	F_{st}	N_m
WPMS5	0.05*	0.06	0.01	11.64
WPMS14	0.19***	0.21	0.03	6.07
WPMS18	0.10	0.10	0.00	18.68
PMGC14	-0.0002	0.0038	0.004	26.21
PMGC2163	0.25	0.24	-0.01	26.01
PMGC2889	0.07*	0.09	0.01	9.40
PMGC93	0.15**	0.17	0.02	8.43
Pe2	-0.10	-0.08	0.02	8.74
Pe5	0.05*	0.08	0.03	5.64
Pe6	-0.17	-0.14	0.02	7.97
Pe8	0.07***	0.12	0.05	4.44
Pe13	0.18	0.18	0.00	14.5
Pe14	-0.17	-0.16	0.01	16.29
Pe15	-0.06*	-0.05	0.01	11.43
Popeu13	0.24***	0.32	0.10	2.34
Mean	0.05	0.07	0.02	11.85 ±1.87

Fis: Inbreeding Coefficient Within Individuals, Fit: Inbreeding Coefficient Within Total Population, Fst: Inbreeding Coefficient Within Subpopulations, Nm: Number of Migrants (***: $p<0.001$, **: $p<0.01$, *: $p<0.05$)

4.5 Amount of Genetic Variation Among Age Groups

Allelic diversity parameters across the age structure of populations showed low to moderate polymorphism levels (Table 4.8, Figure 4.1). The mean number of alleles varied from 3.66 at young stands of the upstream population (GUPY) to 4.2 at young stands of the midstream population (GMIDY), having a mean of 3.94±0.16. All age populations had a lower effective number of alleles, with a mean of 2.52±0.09 when overall loci were considered. Shannon's information index (I) values varying from 0.9 to 1.01 also supported the moderate level of allelic diversity inherent to the age

structures of populations. The grand mean of polymorphic loci percentage was 96.67%±1.49% among the age structures, while the individuals of GUPM, GUPY, and GDOWNY were polymorphic within all loci.

Descriptive statistics regarding genetic variation among the age structures of populations demonstrated a moderate level of diversity intrinsic to young and mature stands (Table 4.9). As observed heterozygosity (Ho) differed between 0.48 and 0.56, unbiased expected heterozygosity (uHe) varied from 0.52 to 0.56 among all age populations, and their average values were 0.52±0.02 and 0.55±0.02. All age structures exhibited heterozygote deficits with positive F values. Low Fst value (0.03±0.006) among all age structures indicated a low level of genetic differentiation.

Populations with the highest number of private alleles were midstream (GMID) and downstream (GDOWN) populations (Table 4.10). Both populations had five private alleles shared between their young and mature stands. Besides, no private alleles were detected in the upstream population's mature stands (GUPM), while young stands of the upstream population (GUPY) had only one. Detected within the young stands of the downstream population (GDOWNY), the private allele “152” of locus Pe15 had the highest frequency with a value of 0.076.

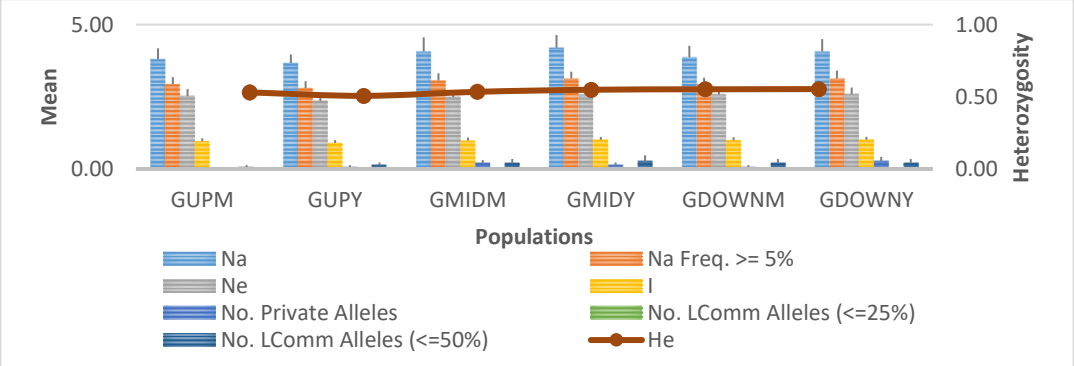

Figure 4.1: Graph showing the allelic diversity patterns across age groups (Na: No. of Different Alleles, Na (Freq≥5%): No. of Different Alleles with a Frequency≥ 5%, Ne: No. of Effective Alleles, I: Shannon's Information Index, No. LComm Alleles (≤50 %) No. of Locally Common Alleles (Freq.≥5%) Found in 50% or Fewer Populations He: expected heterozygosity)

Table 4.8: Mean allelic diversity patterns across age groups

Population	Na	Na Freq. >= 5%	Ne	I	No. Private Alleles	No. LComm Alleles (<=50%)	Ho	uHe
GUPM	3.8±0.38	2.93±0.25	2.52±0.25	0.95±0.11	0	0.07±0.07	0.49±0.07	0.54±0.06
GUPY	3.66±0.3	2.8±0.24	2.35±0.23	0.9±0.1	0.07±0.07	0.13±0.09	0.48±0.06	0.52±0.06
GMIDM	4.07±0.49	3.07±0.25	2.48±0.23	0.98±0.1	0.2±0.11	0.2±0.14	0.51±0.06	0.54±0.05
GMIDY	4.2±0.44	3.13±0.24	2.56±0.23	1.01±0.1	0.13±0.09	0.27±0.21	0.54±0.06	0.56±0.05
GDOWNM	3.86±0.4	2.93±0.23	2.58±0.23	0.99±0.11	0.07±0.07	0.20±0.14	0.54±0.06	0.56±0.05
GDOWNY	4.06±0.43	3.13±0.27	2.59±0.23	1.01±0.1	0.27±0.15	0.20±0.14	0.56±0.07	0.56±0.05
Mean	3.94±0.16		2.52±0.09	0.98±0.04			0.52±0.02	0.55±0.02

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. LComm Alleles (≤50%): No. of Locally Common Alleles (Freq.≥5%) Found in 50% or Fewer Populations Ho: observed heterozygosity, uHe: unbiased expected heterozygosity

Table 4.9: Mean genetic diversity parameters across age groups of populations

Population	N	Na	Ne	I	%P	G-W Index (M)	Ho	uHe	F	Fst
GUPM	32.33±0.19	3.8±0.38	2.52±0.25	0.95±0.11	100.00%	0.352±0.127	0.49±0.07	0.54±0.06	0.1±0.05	0.03±0.006
GUPY	29.07±0.21	3.66±0.3	2.35±0.23	0.9±0.1	100.00%	0.369±0.137	0.48±0.06	0.52±0.06	0.05±0.05	
GMIDM	30.73±0.15	4.07±0.49	2.48±0.23	0.98±0.1	93.33%	0.353±0.130	0.51±0.06	0.54±0.05	0.05±0.05	
GMIDY	35.53±0.29	4.2±0.44	2.56±0.23	1.01±0.1	93.33%	0.364±0.107	0.54±0.06	0.56±0.05	0.03±0.04	
GDOWNM	31.73±0.12	3.86±0.4	2.58±0.23	0.99±0.11	93.33%	0.364±0.123	0.54±0.06	0.56±0.05	0.03±0.03	
GDOWNY	33.8±0.34	4.06±0.43	2.59±0.23	1.01±0.1	100.00%	0.350±0.145	0.56±0.07	0.56±0.05	0.01±0.06	
Mean	32.2±0.24	3.94±0.16	2.52±0.09	0.98±0.04	96.67%	0.359±0.128	0.52±0.02	0.55±0.02	0.05±0.02	

N: Sample size, Na: Mean allele number, Ne: Effective number of alleles, I: Shannon's Information Index, %P: Percentage of polymorphic loci, G-W (M): Garza Williamson Index, Ho: Observed heterozygosity, uHe: Unbiased expected heterozygosity, F: Fixation index, Fst: Overall genetic distance among age groups

Table 4.10: Detected private alleles across age groups

Pop	Locus	Allele	Freq
GUPY	Pe2	105	0.017
GMIDM	Pe5	182	0.017
GMIDM	Pe8	152	0.032
GMIDM	Pe15	134	0.016
GMIDY	PMGC28	181	0.015
GMIDY	Pe8	146	0.014
GDOWNM	Pe2	97	0.016
GDOWNY	PMGC14	188	0.014
GDOWNY	Pe15	152	0.076
GDOWNY	Popeu13	294	0.015
GDOWNY	Popeu13	298	0.015

During population bottleneck events, the allelic size range is lost slower than the number of alleles (Garza & Williamson, 2001). To determine sudden reductions in population sizes within population history, the Garza-Williamson index (M), which utilizes the ratio of the mean number of alleles to allelic size range, was computed and illustrated with a bar graph for each age structure of populations (Table 4.9, Figure 4.2). With a mean of 0.359±0.128, M values varied between 0.350 and 0.369 among age structures of populations, which are well below the threshold value (0.68).

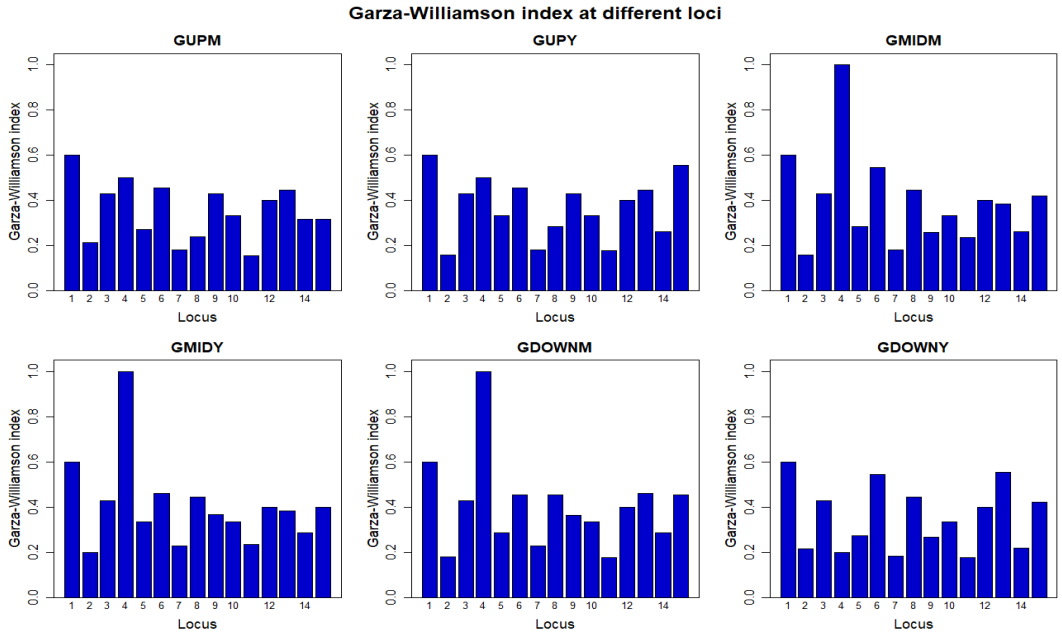

Figure 4.2: Garza-Williamson Index across loci for each age group of populations

4.6 Genetic Structure of Age Groups

4.6.1 Molecular Differentiation

Pairwise Fst values were computed together with their corresponding Nm values and visualized in the form of a heatmap (Table 4.11, Figure 4.3). All young populations of studied locations (GUPY, GMIDY, GDOWNY) showed their lowest pairwise Fst values with mature populations (GUPM, GMIDM, and GDOWNM) belonging to the same location. Surprisingly, GMIDM had its lowest pairwise Fst value with GUPM, rather than GMIDY. The lowest Fst value was observed between GUPM and GUPY, indicating almost no differentiation between mature and young populations of the upstream location. The highest Fst, on the other hand, was observed between young populations of upstream and downstream locations, which is the most distant population pair.

Table 4.11: Pairwise Fst matrix of age groups of the studied populations

	GUPM	**GUPY**	**GMIDM**	**GMIDY**	**GDOWNM**	**GDOWNY**
GUPM	-					
GUPY	0.006 (44.03)	-				
GMIDM	0.007 (34.18)	0.010 (24.82)	-			
GMIDY	0.013 (18.36)	0.019 (12.66)	0.009 (26.46)	-		
GDOWNM	0.020 (12.57)	0.027 (9.18)	0.022 (10.89)	0.023 (10.42)	-	
GDOWNY	0.026 (9.38)	0.032 (7.50)	0.024 (10.19)	0.025 (9.88)	0.009 (28.30)	-

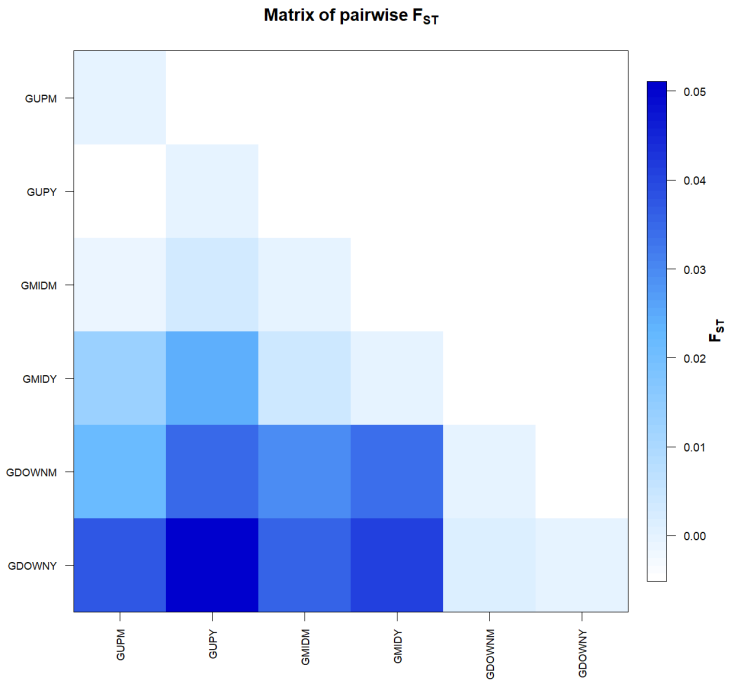

Figure 4.3: Pairwise Fst heatmap of age groups

Principal Coordinates Analysis (PCoA) was carried out to scale the genetic distances between the age structures of populations in two dimensions (Figure 4.4) using Pairwise Fst values. The first principal component separated age structures in the downstream population (GDOWNM and GDOWNY) from age structures in upstream and midstream populations with a 56.07% variance. Besides, Age structures of the midstream population (GMIDM and GMIDY) were separated from age structures of upstream and downstream populations by the second principal component, which accounts for 22.67% of total variance.

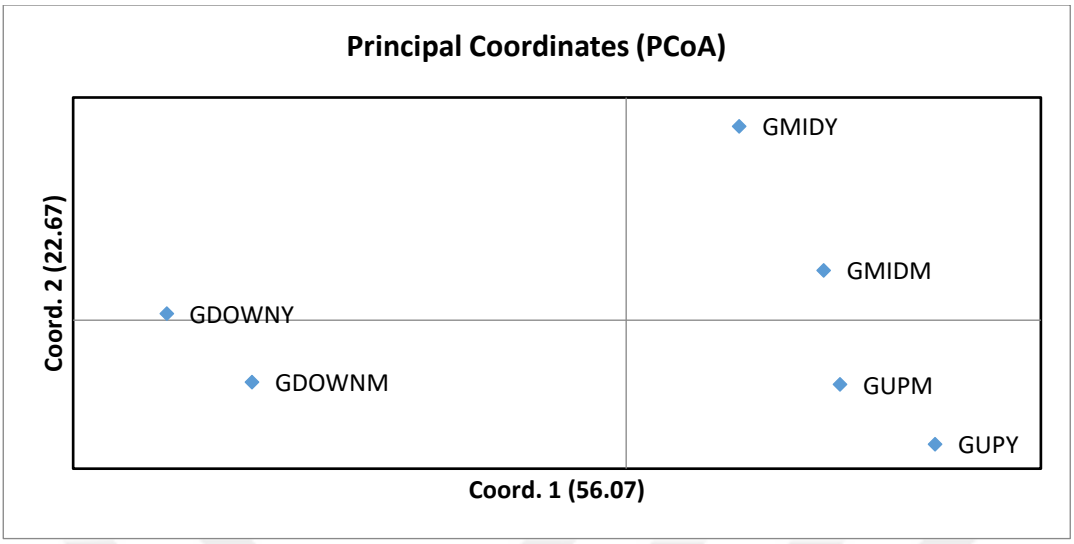

Figure 4.4: Scaled genetic distances among age structures of studied populations

4.6.2 Phenetic Relationships Among Age Groups

A cladogram was constructed using the UPGMA method to unveil phenetic relationships among age structures of the studied populations(Figure 4.5). Two young populations that formed a distinct clade with mature age structures of the same population were GUPY and GDOWNY. The GMIDM became the outgroup of clade GUPM-GUPY cluster, and GMIDY formed a second outgroup near this cluster

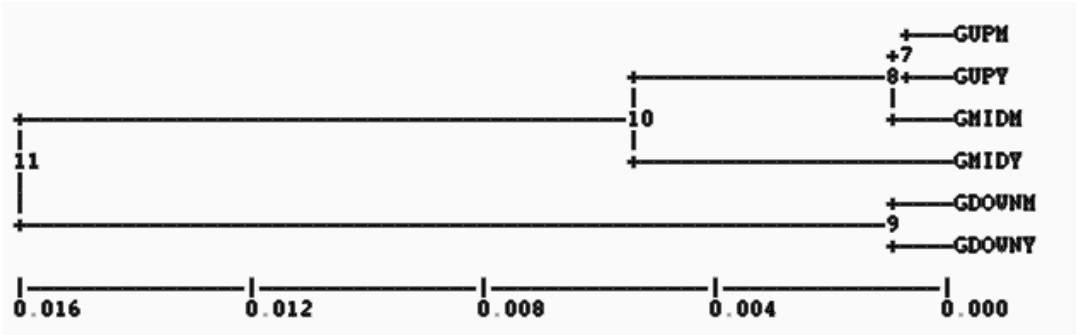

Figure 4.5: UPGMA Tree

4.6.3 Genetic Clusters

Ancestry groups shaping the genetic structure of individuals were determined by computing their membership coefficients to genetic clusters with and without prior information about their localities. Both analysis results were used for further discussion because different results were obtained from each type of analysis. With prior information, population structure analysis specified two genetic clusters; however, three genetic clusters were determined by analysis without prior location information.

The ad hoc statistic ΔK computed by the Evanno method (Evanno et al., 2005) specified three major genetic clusters without prior information regarding the location for the individuals of six age grouped populations (Table 4.12, Figure 4.6). Genetic cluster assignments elicited a low differentiation between the age and location groups and a high admixture level in most age structures (Figure 4.7). Mature and young age structures from the upstream and midstream population composed of individuals which are the members of Cluster 1 (represented as dark brown bars) and Cluster 3 (represented as light green bars). However, individuals from mature and young age structures of the downstream population primarily belonged to Cluster 2 (represented as dark green bars). A high admixture level was observed for the mature and young age structures of the upstream and midstream population. Age structures of the downstream population, on the other hand, hosts a portion of individuals having a more homogenous genetic substructure, admixed less than the age structure members situated along the upper reaches of the Göksu River.

Table 4.12: Delta K values estimating three ancestral groups without prior location information

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-6042.29000	0.056765	—	—	—
2	10	-5946.77000	9.850217	95.520000	25.32000	2.57050
3*	10	-5825.93000	8.471790	120.84000	81.52000	9.62252
4	10	-5786.61000	54.729627	39.320000	1.220000	0.02229
5	10	-5748.51000	14.081860	38.100000	4.550000	0.32311
6	10	-5714.96000	8.327625	33.550000	59.34000	7.12568
7	10	-5740.75000	62.299086	-25.790000	29.73000	0.47721
8	10	-5736.81000	110.258176	3.940000	31.86000	0.28895
9	10	-5701.01000	44.445608	35.800000	25.19000	0.56676
10	10	-5690.40000	42.027425	10.610000	—	—

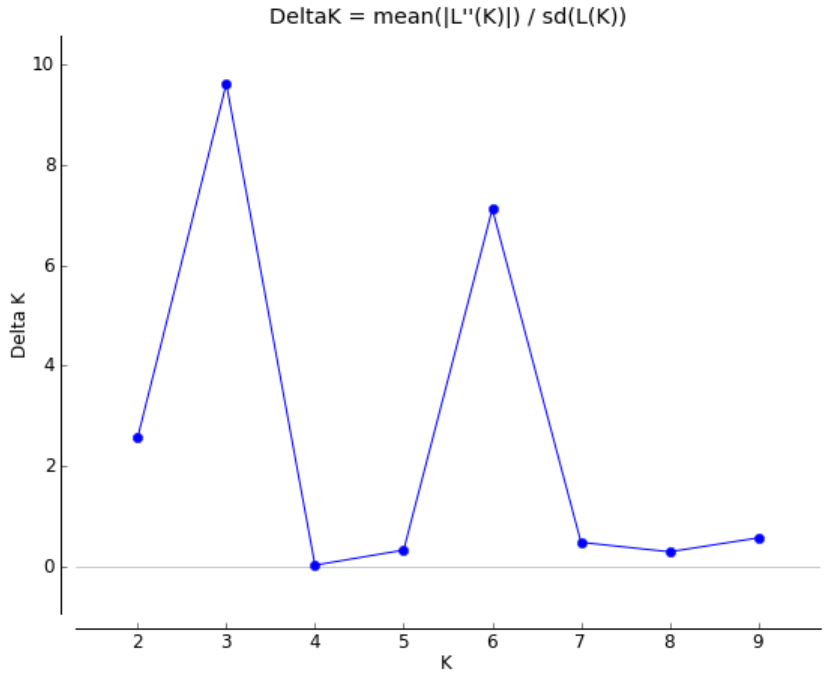

Figure 4.6: Line graph of Delta K values estimating three ancestral groups without prior location information

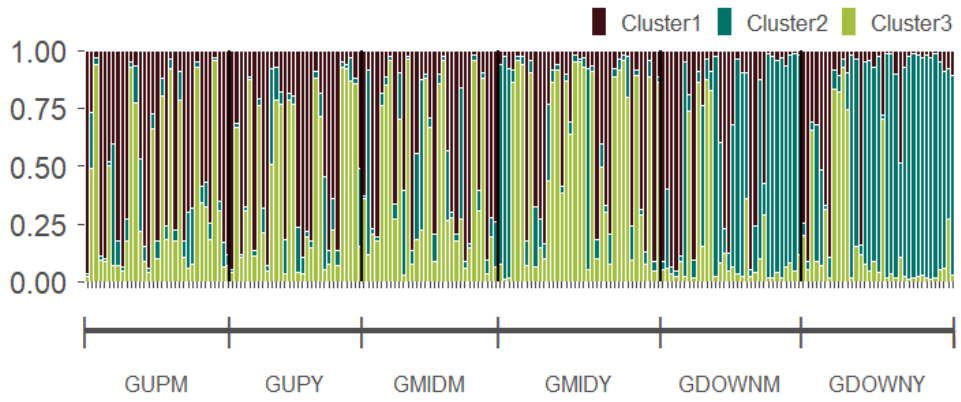

Figure 4.7: Genetic structure of age groups for K=3 without prior location information. Each color stands for a different ancestry group.

As for the analysis with prior location information, the highest ΔK was observed for two genetic clusters (Table 4.13, Figure 4.8). Again, genetic differentiation levels were low among the age structures of upstream and midstream populations (Figure 4.9), and their members mostly belonged to cluster 2 (represented as red bars). On the other hand, admixture levels were low for the age structures of upstream and midstream populations for this time, which has a contrasting pattern compared with the genetic structure analysis without prior location information. Another contrasting pattern was observed for admixture levels in the age structures of the downstream population whose individuals assigned to both cluster 2 and cluster 1 (represented as blue bars), thus admixed more than the individuals of upstream and downstream populations.

Table 4.13: Delta K values estimating two ancestral groups with prior location information

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-6042.43000	0.449815	—	—	—
2*	10	-5917.67000	9.366851	124.760000	100.970000	10.779504
3	10	-5893.88000	57.004928	23.790000	53.020000	0.930095
4	10	-5817.07000	32.457666	76.810000	1.220000	0.037587
5	10	-5739.04000	30.936394	78.030000	78.570000	2.539727
6	10	-5739.58000	50.033984	-0.540000	35.170000	0.702922
7	10	-5775.29000	131.387937	-35.710000	66.300000	0.504613
8	10	-5744.70000	82.603914	30.590000	7.030000	0.085105
9	10	-5707.08000	130.491505	37.620000	68.790000	0.527161
10	10	-5738.25000	118.655845	-31.170000	—	—

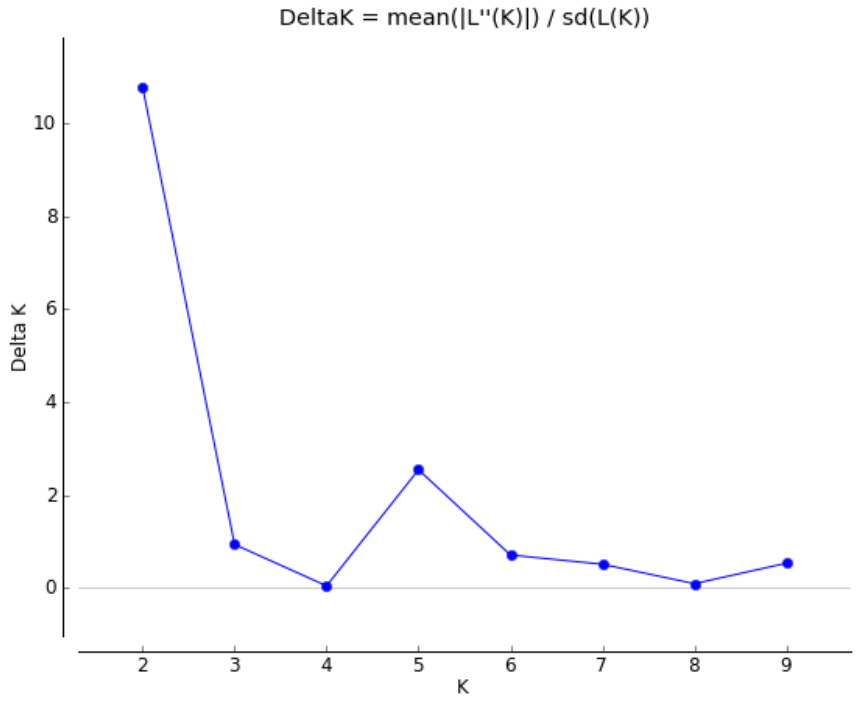

Figure 4.8: Line graph of Delta K values estimating two ancestral groups with prior location information

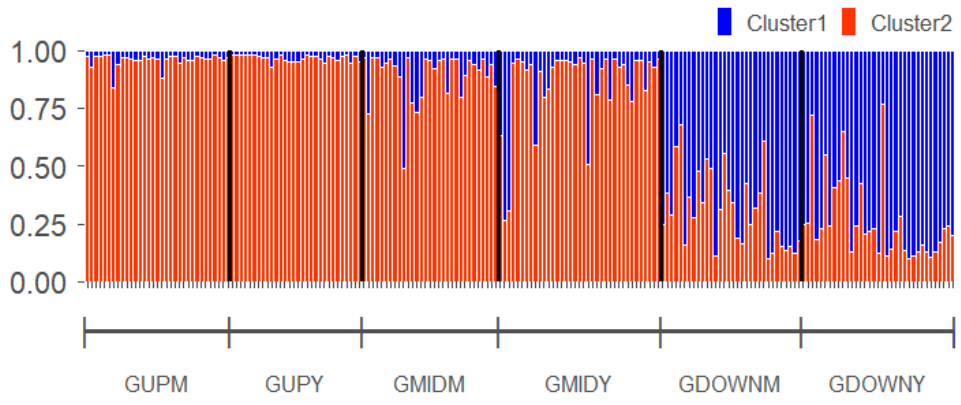

Figure 4.9: Genetic structure of age groups for K=2 with prior location information. Each color stands for a different ancestry group.

4.7 Analysis of Molecular Variance (AMOVA)

Fst-based AMOVA was carried out with two different grouping structure based on location and age (Table 4.14). Grouping structure based on location partitioned populations into three separate groups, each having mature and young structures of three different locations. On the other hand, grouping structure grounded on age partitioned populations into two separate groups, each having only mature and only young populations.

Fst-based AMOVA with location groups revealed that the greater percentage of variation (97.19%) is sourced from differences among individuals within populations. 2.71% of the variation, on the other hand, was attributed to differences among three different locations. The lowest source of variation was the differences among populations within groups; only 0.086% of variation came from here. Differences among population within groups represent the differences between age groups belonging to same population, and the variation sourced from these

differences is non-significant (Fsc=0.00089, p=0.474) according to results of the Fst-based AMOVA with location groups.

Similarly, Fst-based AMOVA with age groups determined the highest source of variation as differences among individuals within populations (98.09%). However, an opposite situation was observed for the other two variance components compared with the results of the first grouping strategy. The variation coming from differences among populations within groups (2.85%) was higher than the variation sourced from differences among groups (-0.95%). Here, differences among groups corresponds to differences between age groups, and the variation coming from differences between age groups is non-significant (F_{CT}=-0.00951, p=0.998).

Table 4.14: Fst-based Analysis of Molecular Variance (AMOVA) for two different grouping strategy

Grouping Structure	Groups			Source of variation	Sum of Squares	Variance components (Va, Vb, Vc)	Percentage of Variation	Fixation Indices (Fct, Fsc, Fst)	P value
Location (UP, MID, DOWN)	GUPM GUPY	GMIDM GMIDY	DOWNM GDOWNY	Among Groups	38.000	0.11468	2.71723	0.02717	0
				Among Populations within groups	13.004	0.00365	0.08648	0.00089	0.47410
				Within Populations	1557.191	4.10200	97.19628	0.02804	0
				Total	1608.194	4.22032	-	-	
Age (Young, Mature)	GUPM GMIDM GDOWNM		GUPY GMIDY GDOWNY	Among Groups	4.124	-0.03977	-0.95105	-0.00951	0.99804
				Among Populations within groups	46.880	0.11930	2.85308	0.02826	0
				Within Populations	1557.191	4.10200	98.09797	0.01902	0
				Total	1608.194	4.18153	-	-	

CHAPTER 5

DISCUSSION

Clonality, genetic diversity, and genetic structure of *P. euphratica* populations located along three different reaches of Göksu River were assessed with an age-based perspective to investigate the extent of genetic diversity transfer and genetic differentiation between the mature and young stands of natural populations. This study is distinctive in terms of being the first effort with its age-based approach among the population genetics studies of Euphrates Poplar.

5.1 Clonality

As for natural Euphrates poplar populations, the high prevalence of unique MLGs rather than clones is an indicator of successful seed germination and seedling establishment within the floodplain. These events strongly depend upon breeding areas' exposure to active runoff during the seed dispersal season to provide optimal conditions for seedling recruitment. Successive flooding events generated by active runoff deposit fresh alluvial soil with adequate moisture and low salt content to the riverbanks and recharge groundwater table to ensure seedling survival (Thevs et al., 2008a).

Our findings demonstrated that generative reproduction is the dominant reproduction mode within all populations, which have substantially lower clonality compared with the results of other studies found in the literature (Eusemann et al., 2013; Kramp et al., 2018; Vonlanthen et al., 2010; Zeng et al., 2018). Moreover, most of the clone groups are composed of a parent tree (genet), and young clones (ramets) sampled close to the parent tree. These results represent the relative success of the stratified sampling strategy deliberately designed to avoid clonal sampling. Besides, most of

the sampled individuals are close to the active riverbed and located on river-parallel germination rows where the most feasible germination conditions are present. Göksu River hydrodynamics seems to have provided suitable conditions favoring generative reproduction along the germination rows during the early succession stage of Euphrates Poplar populations.

Considerable variations were observed in clonality among the three populations. The downstream population (GDOWN) has more than twice duplicated trees comparing to the upstream population (GUP). This result might be a consequence of spatiotemporal heterogeneity among different localities of the Göksu River Basin, having varying levels of flooding intensity and resource availability. However, a lower number of young individuals was sampled per mature individual within each locality from the upstream population (see Appendix A) compared to the other two populations; hence sampling method appears to be more decisive for this case.

Göksu River basin has a moderate level of salinity within its soil and groundwater content (Güner et al., 2018), which can meet the low salinity requirement of sexual reproduction. Nevertheless, we do not have the information on whether the current flow trend of the Göksu River can satisfy the timing requirement of flooding events, which must coincide with the peak of seed release occurring between mid-July and mid-August. Göksu River's annual highest flow velocity occurs in April (241.14 m^3/s). It decreases rapidly immediately after its peak, reaching a flow velocity between 40 m^3/s and 50 m^3/s in July and August primarily because of the presence of water storage facilities and irrigation channels withdrawing water from the river course (Palta et al., 2019). Moreover, monthly average precipitation values recorded between 1929 and 2016 by Silifke Meteorology Station shows that the lowest precipitation occurs during July and August within the Göksu River Basin (Yıldırım et al., 2018). A combination of these factors appears to create an arid environment during the seed dispersal season, which may hamper seed germination and seedling establishment. However, Ling et al. (2015) demonstrated that the most appropriate flooding pattern for generative regeneration of *P. euphratica* stands was two to three times per year with a duration of 15-20 days and an intensity of 25-30 m^3/s.

Therefore, a flow velocity between 40 m^3/s and 50 m^3/s might be more than sufficient for maintaining the hydrological function of the river to form a suitable environment for seedling recruitment. However, this argument needs further investigation due to lack of information about the frequency and duration of the Göksu River's flooding events and riverbed topography.

5.2 Genetic Diversity Levels of the Loci

None of the loci are monomorphic among the studied 15 loci, but PMGC14 is almost monomorphic. Nearly all individuals are homozygous and possess two identical copies of SSRs having 197 bp length at PMCG14 locus, while other alleles have very low frequencies. This result is also evident in the effective number of alleles (Ne) value of 1.02 at PMGC14, which is practically fixed for allele "197". This situation might be a consequence of a reduction in population size, causing a loss of alleles followed by genetic drift. Another possible reason is the directional or disruptive selection, which operates in favor of allele "197" within this locus, yet this argument requires verification via neutrality tests.

PMGC14, PMGC2163, and Pe8 have PI values higher than 0.4 along with low PIC values, implying the low informative value of these loci for the Göksu River Euphrates Poplar populations in terms of measuring their genetic diversity levels. Other loci demonstrate low to moderate PI values in combination with moderate to high PIC values, thereby possessing an adequate discrimination capacity with relatively high informative power while assessing the genetic diversity of the populations studied.

The lowest I values were observed at PMGC14, PMGC2163, and Pe8 loci, that is congruent with the deduction made with PI and PIC values, further supporting the finding of their insufficient informativeness. Moreover, a moderate mean value of I (0.97 ± 0.04) indicates the markers' overall efficacy in terms of revealing the genetic variation inherent to *P. euphratica* populations.

Four loci (WPMS18, PMGC2163, PMGC14, and Pe13) showed an Ar value lower than three with low He and PIC values. The remaining eleven loci have an Ar value of three or greater than three, especially Popeu13, Pe15, Pe5, WPMS14, and Pe14 has an Ar value of greater than five. These five loci also showed superiority with respect to other diversity parameters except for the Pe5, which has a low effective number of alleles due to its non-uniform allele frequency distribution. Thus, Popeu13, Pe15, WPMS14, and Pe14 can be accounted as highly informative for the Göksu River Euphrates poplar populations if all diversity parameters are considered.

Speaking of the locus-wise mean heterozygosity values obtained by SSR genotyping of *P.euphratica* populations in the literature (Wang et al., 2011a; Wang et al., 2011b; Xu et al., 2013), it seems that the species' populations found along Tarim River and its tributaries have considerably higher mean heterozygosity values than of our findings. However, a previous study realized for the Göksu River Euphrates Poplar populations (Kansu & Kaya, 2020) revealed a slightly lower locus-wise mean heterozygosity value than this study most probably due to the presence of additional loci showing less polymorphism within their locus set. Apart from *P. euphratica* genetic diversity studies, low heterozygosity values were also reported for other poplar species (Du et al., 2012; Lexer et al., 2005; Namroud et al., 2005; Shen et al., 2014). Ten loci showed heterozygote deficit with corresponding positive fixation indices (F), and 4 of them deviated significantly from Hardy-Weinberg Equilibrium. This type of locus-wise heterozygosity pattern must be derived from the frequent occurrence of inbreeding, which emerges through non-random mating among the individuals related by shared ancestry. Additionally, the effects of inbreeding can amplify consecutively through generations if propagule mobility is restrained by several human-mediated practices. In our case, these practices include dam construction and agricultural practices leading to habitat destruction. Another possible explanation lying behind this locus-wise heterozygosity pattern is the directional or disruptive selection operating on some loci, causing fixation of a specific allele over generations. However, this explanation needs to be verified by neutrality tests that determine the outlier loci to become valid.

5.3 Genetic Diversity among Age Structures

Levels of allelic diversity averaged across loci (Na and Ne) were very similar among age structures and not congruent with higher mean allelic diversity values obtained in previous genetic diversity studies of Euphrates poplar performed with SSR markers (Wang et al., 2011a). Observed and expected heterozygosity values were also very close among age structures; no significant difference was observed between the mature and young populations in terms of heterozygosity values. Besides, studies with a similar scope with respect to the species in question assessed higher population-wise heterozygosity values (Eusemann et al., 2009; Wang et al., 2011a). Inconsistency among similar studies is acceptable to a certain degree, considering the difference in sampling strategies as well as the distinct nature of demographic and evolutionary dynamics operating among different study sites. Nevertheless, low population-wise genetic diversity measures among age structures obtained in this study appear to be an outcome of a putative gene pool shrinkage event realized within population history. These events are termed population bottlenecks, co-occurring with the drastic genome-wide loss of allelic variants previously present in the original population. The prospective detrimental effect of these events is the increased vulnerability of less frequent alleles to genetic drift. Less frequent alleles can easily be swept from the gene pool if they cannot be transferred to subsequent generations (Parisod et al., 2005). This scenario is strongly supported by Garza-Williamson index (M) values, which are well below the critical value of 0.68 for all age structures. The pioneer genetic diversity study on the Göksu River's Euphrates poplar populations (Kansu & Kaya, 2020) also revealed a highly similar diversity pattern while pointing out the demographic dynamics as the primary source of reduced genetic diversity.

One of the most important findings of this study is the success of genetic diversity transfer from mature stands to young stands, which is apparent in similar population-wise genetic diversity values between the age structures belonging to the same population. In fact, young populations of midstream (GMIDY) and downstream

(GDOWNY) locations showed slightly higher allelic diversity and heterozygosity values than of mature populations of the same locations, but these differences are not statistically significant. These results represent the populations' moderate capacity for successful seedling recruitment within their habitats. Thus, genetic material seems to be replenished over generations, even if it nourishes from a limited gene pool.

Private alleles are population-specific alleles that may function in increasing the evolutionary fitness of a population, and their abundance guides conservation strategies by assisting the determination of conservation targets (Szpiech & Rosenberg, 2011). The number of private alleles is the highest in the young population of the downstream location (GDOWNY), which also has the highest genetic diversity values. Hence, GDOWNY can be subject to future conservation studies in Turkey if genetic sources of the Göksu River population are desired to be protected.

5.4 Molecular Differentiation

Genetic differentiation patterns revealed a poor subdivision among age groups within three distinct locations, as it was portrayed by low pairwise Fst (0.006-0.009) values concomitant with the high number of migrants (Nm). Low genetic differentiation is common among the native populations of *Salicaceae* family members due to their reproductive characteristics (Ciftci & Kaya, 2019; Čortan et al., 2016; Degirmenci et al., 2019; Du et al., 2012). All young populations exhibited their lowest pairwise value with mature populations belonging to the same location, indicating the close ancestral relationship between the mature and young stands. Young individuals originate more commonly from the reproductive events performed by mature individuals of the same locality rather than gene flow from different localities. Besides, a high number of migrants between mature and young stands is most likely a consequence of the temporal homogeneity of reproductive episodes. It seems that a limited portion of mature individuals participates in pollen

generation and pollination during a narrow reproductive period, thus generating new but genetically not distant young individuals. AMOVA results also strengthen the finding of low differentiation between mature and young populations. Age difference accounts for only %0.086 (Fsc=0.0089, p=0.474) of variation among age structures within location groups if age structures are grouped according to location. Even more striking results were obtained when age structures were grouped based on age, such that there is no attributable variation (-0.95%) to the age difference (Fct=-00951, p=0.998). Close relatedness between mature and young stands is also evident in Principal Coordinate Analysis (PCoA); the scaled distances between each location's age structure pairs are low compared to those among three separate localities. Moreover, as illustrated in the UPGMA tree, young population of the upstream location (GUPY) and young population of the downstream (GDOWNY) location formed a distinct clade with their mature population pair belonging to the same location.

Pairwise Fst values among three separate locations exhibit low genetic differentiation among localities, but it is still higher than age-based differentiation. Especially, mature population of the midstream location (GMIDM) had its lowest pairwise Fst value (0.007) with mature population of the upstream location (GUPM). This obvious resemblance between upstream (GUP) and midstream populations (GMID) in terms of genetic background must be the consequence of a geographical barrier absence that allows propagule dispersal between these localities. This type of location-based differentiation pattern is also valid for Euphrates poplar populations found along the Tarim River (Eusemann et al., 2013; Wang et al., 2011a; Wang et al., 2011b). Downstream population (GDOWN), on the other hand, is the genetically most distant population among the three populations with its relatively higher pairwise Fst values varying between 0.020 and 0.032. PCoA also clearly discriminated GDOWN from GUP and GMID with first principal component representing 56.07% of the total variance. Therefore, despite not so much, GDOWN is more isolated than populations found along the upper reaches of the Göksu River in terms of reproduction.

5.5 Clustering Patterns

Population structure analysis supports the findings gained through molecular differentiation analyses of six age structured populations. There is a weak age-based clustering pattern, as the individuals of age structure pairs are usually derived from the same ancestry groups. This pattern, governed by a variety of factors, reveals the close ancestral relationship between the mature and young stands belonging to the same locality. First, young individuals are mostly the progeny of mature individuals belonging to the same locality, and the participants of reproductive episodes are limited in number. Second, there is no considerable temporal variation inherent to reproductive episodes. These episodes represent a single and narrow period of opportunity in terms of reproduction. Third, a prominent spatial substructure does not exist among different localities, especially the individuals of upstream (GUP) and midstream (GMID) populations have nearly the same ancestral backgrounds due to the homogenizing effect of high-degree gene flow between these localities. Moreover, age structure pairs belonging to the same locations are admixed at the same level even if admixture levels observed for two ancestry groups (K=2) and three ancestry groups (K=3) are contrasting at spatial scale. This type of age-based admixture pattern supports the presence of aforomentioned factors, which are in action for each locality studied.

Results obtained from both population structure analyses (K=2 and K=3) distinguish the GDOWN from GUP and GMID at spatial scale in terms of ancestry groups in which the individuals were assigned. In other words, most of the individuals of GDOWN are members of different genetic clusters irrespective of the number of ancestral groups assessed with (K=2) or without (K=3) prior location information. GDOWN appears to be isolated partially from the populations found along the upper reaches of the Göksu River, most probably due to geographical and artificial barriers that prevent pollen and seed dispersal between these localities. As a result, the location-based population structure of Göksu populations fits well with the infinite island model of gene flow proposed by Slatkin (1985), meaning that there are no

barriers to gene flow, thereby no marked population structure. Göksu River's Euphrates poplar populations exhibit the characteristics of a large and single metapopulation.

The discrepancy on admixture levels between the analysis without prior location information (K=3) and the analysis with prior location information (K=2) must be the consequence of a relatively weak structure signal due to low location-based genetic differentiation. As STRUCTURE v.2.3.4 (Pritchard et al., 2000) software make use of the population locations to aid clustering, it decreased the number of ancestry groups from 3 to 2 and assigned the individuals of GUP and GMID to a single cluster with no admixture due to exceedingly low pairwise Fst values observed between these locations. In the meantime, prior location information assisted the Bayesian algorithm to create a more distinctive population structure for GDOWN. However, a portion of individuals of GDOWN having a more homogenous ancestral background without location information became more admixed and assigned to two ancestral groups when the number of ancestral groups decreased by one with location information. In other words, the Bayesian algorithm with prior location information increased the discrimination power at the population level while lowering it at the individual level due to the conditional link between prior location information and posterior cluster membership coefficients. All in all, two ancestry groups (K=2) represent the age and location-based genetic structure better by revealing a more pronounced genetic structure pattern than of three ancestry groups (K=3) but have a disadvantage with regard to unveiling the admixture pattern at the individual level.

CONCLUSION

Understanding the extent of genetic diversity transfer and differentiation between the mature and young stands of *P. euphratica* populations allows us to make valuable deductions regarding the species' overall evolutionary potential. The comprehensive portrait of this potential is full of information, which is the projection of the species' demographic dynamics and reproductive status. Accordingly, future conservation attempts can set up their foundations using the data provided by this study.

Findings associated with the clonality levels within three distinct locations pointed out the infrequent presence of clone individuals due to the sampling strategy used. Also, most of the sampling locations were very close to the active riverbed, where the most suitable germination conditions are present. Even though the simultaneous occurrence of these conditions is difficult, spatiotemporal dynamics of the Göksu River Basin can somehow assemble a complex chain of environmental factors to create a favorable environment for the generative reproduction of *P. euphratica* individuals.

Locus-wise descriptive statistics revealed the sufficient informativeness of the studied loci. Especially Popeu13, Pe15, WPMS14, and Pe14 loci could be utilized for future genetic diversity studies on Euphrates poplar populations found along the Göksu River due to their high polymorphism levels. Only the PMGC14 locus is nearly monomorphic among the 15 loci, and it is nearly fixed for one specific allele. Besides, all allelic diversity values showed low to moderate variation. Reduced polymorphism levels within loci were associated with past demographic dynamics, including at least one bottleneck event, which led to a loss of alleles. Heterozygote deficit was observed within ten loci, and four of them deviated significantly from Hardy-Weinberg Equilibrium due to the effect of inbreeding.

Levels of genetic diversity averaged across loci are low among age structures. There is no significant difference between mature and young individuals belonging to the

same locality in terms of descriptive statistics evaluating the genetic diversity inherent to age structure pairs. This result represents the achievement of genetic diversity transfer between the parent and progeny via generative reproduction even if the total genetic variation is limited. The young population of the downstream location (GDOWN) has the highest number of private alleles together with the highest genetic diversity parameter values, demonstrating its superior overall fitness and adaptability to changing environmental conditions in the long-term.

There is no prominent genetic differentiation and structure pattern observed between mature and young individuals for each location studied. The origin of young individuals is mostly the reproductive events performed by mature individuals belonging to the same locality rather than gene flow from different localities. Low differentiation between age groups also indicates the temporal homogeneity of reproductive periods and limited participation of mature individuals. Likewise, there is no evident location-based differentiation and structure pattern between locations except that of GDOWN, which is partially isolated from the populations found along the upper reaches of the Göksu River. The upper reaches of the Göksu River do not seem to have any geographic or artificial barrier that may prevent gene flow.

In short, this study revealed valuable information regarding the extent of genetic diversity transfer and genetic differentiation between mature and young *P. euphratica* stands. However, there is still much unknown about the sophisticated nature of the Göksu River hydrodynamics and its relation to stand structure and development patterns of *P. euphratica* populations. Therefore, future population ecology studies should incorporate a meta-analysis approach while considering the increasing intensity of environmental and human-mediated impacts that deteriorate *P. euphratica* populations' regeneration capacity.

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APPENDICES

A. Sampled *P. euphratica* Individuals

UPSTREAM POPULATION (GUP)					
Sample Code	**Sample ID**	**Latitude**	**Longitude**	**Altitude**	**Sex/Age**
1	GKS1-1	36º 39' 35" N	33º 22' 08" E	442 ft	Male
2	GKS1-1				Young
3	GKS1-2	36º 39' 37" N	33º 22' 18" E	452 ft	Male
4	GKS1-2				Young
5	GKS1-3	36º 39' 32" N	33º 22' 12" E	442 ft	Female
6	GKS1-3				Young
7	GKS1-4	36º 39' 21" N	33º 22' 08" E	442 ft	Male
8	GKS1-4				Young
9	GKS1-5	36º 39' 27" N	33º 21' 53" E	441 ft	Male
10-a	GKS1-5				Young
10-b	GKS1-5				Young
11	GKS1-6	36º 39' 27" N	33º 21' 54" E	441 ft	Female
12	GKS1-6				Young
13	GKS1-7	36º 39' 28" N	33º 21' 58" E	442 ft	Male
14	GKS1-7				Young
15	GKS1-8	36º 39' 28" N	33º 21' 58" E	442 ft	Female
16	GKS1-8				Young
17	GKS1-9	36º 39' 33" N	33º 21' 46" E	459 ft	Male
18	GKS1-9				Young
19	GKS1-10	36º 39' 33" N	33º 21' 48" E	450 ft	Female
20	GKS1-10				Young
21	GKS1-11	36º 39' 33" N	33º 21' 55" E	446 ft	Male
22	GKS1-11				Young
23	GKS1-12	36º 39' 33" N	33º 21' 51" E	442 ft	Female
24	GKS1-12				Young
25	GKS1-13	36º 40' 32" N	33º 21' 52" E	470 ft	Male
26	GKS1-13				Young

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
27	GKS1-14	36º 40' 31" N	33º 21' 54" E	448 ft	Male
28	GKS1-15	36º 40' 31" N	33º 21' 54" E	448 ft	Female
29	GKS1-15				Young
30	GKS1-16	36º 40' 27" N	33º 21' 58" E	453 ft	Male
31-a	GKS1-16				Young
31-b	GKS1-16				Young
31-c	GKS1-16				Young
31-d	GKS1-16				Young
32	GKS1-17	36º 40' 26" N	33º 21' 59" E	464 ft	Female
33-a	GKS1-17				Young
33-b	GKS1-17				Young
34	GKS1-18	36º 40' 21" N	33º 22' 10" E	451 ft	Female
35	GKS1-18				Young
36	GKS1-19	36º 40' 20" N	33º 22' 13" E	449 ft	Male
37	GKS1-20	36º 40' 20" N	33º 22' 13" E	449 ft	Female
38	GKS1-21	36º 40' 19" N	33º 22' 13" E	451 ft	Male
39	GKS1-21				Young
40	GKS1-22	36º 40' 19" N	33º 22' 15" E	451 ft	Female
41	GKS1-22				Young
42	GKS1-23	36º 40' 19" N	33º 22' 15" E	451 ft	Male
43	GKS1-23				Young
44	GKS1-24	36º 41' 00" N	33º 22' 48" E	490 ft	Male
45	GKS1-25	36º 41' 00" N	33º 22' 48" E	490 ft	Female
46-a	GKS1-24&25				Young
46-b	GKS1-24&25				Young
47	GKS1-26	36º 41' 46" N	33º 21' 39" E	475 ft	Male
48	GKS1-26				Young
49	GKS1-27	36º 41' 43" N	33º 21' 41" E	472 ft	Male
50	GKS1-27				Young
51	GKS1-28	36º 41' 43" N	33º 21' 41" E	472 ft	Female
52	GKS1-29	36º 41' 46" N	33º 21' 39" E	476 ft	Female
53-a	GKS1-29				Young
53-b	GKS1-29				Young

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
53-c	GKS1-29				Young
53-d	GKS1-29				Young
54	GKS1-30	36º 38' 27" N	33º 21' 01" E	449 ft	Female
55	GKS1-31	36º 39' 19" N	33º 22' 21" E	448 ft	Male
56	GKS1-32	36º 39' 19" N	33º 21' 56" E	442 ft	Male
57	GKS1-32				Young
58	GKS1-33	36º 39' 19" N	33º 21' 56" E		Female
MIDSTREAM POPULATION (GMID)					
59	GKS2-1	36º 32' 21" N	33º 26' 54" E	336 ft	Male
60	GKS2-2	36º 32' 20" N	33º 26' 52" E	336 ft	Female
61	GKS2-2				Young
62	GKS2-3	36º 32' 19" N	33º 26' 51" E	342 ft	Female
63	GKS2-5	36º 32' 19" N	33º 26' 51" E	335 ft	Male
64	GKS2-6	33º 32' 18" N	33º 26' 49" E	346 ft	Female
65-a	GKS2-6				Young
65-b	GKS2-6				Young
66	GKS2-7	36º 32' 18" N	33º 26' 49" E	349 ft	Male
67	GKS2-8	36º 32' 17" N	33º 26' 48" E	350 ft	Female
68-a	GKS2-8				Young
68-b	GKS2-8				Young
68-c	GKS2-8				Young
69	GKS2-9	36º 32' 16" N	33º 26' 47" E	367 ft	Male
70	GKS2-10	36º 32' 12" N	33º 26' 42" E	326 ft	Female
71-a	GKS2-10				Young
71-b	GKS2-10				Young
72	GKS2-11	36º 32' 12" N	33º 26' 42" E	326 ft	Male
73	GKS2-12	36º 32' 11" N	33º 26' 40" E	323 ft	Female
74-a	GKS2-12				Young
74-b	GKS2-12				Young
74-c	GKS2-12				Young
75	GKS2-13	36º 31' 51" N	36º 31' 51" E	322 ft	Male
76-a	GKS2-13				Young
76-b	GKS2-13				Young

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
76-c	GKS2-13				Young
77	GKS2-14	36º 31' 51" N	33º 28' 09" E	314 ft	Female
78	GKS2-15	36º 31' 50" N	33º 28' 10" E	310 ft	Male
79	GKS2-15				Young
80	GKS2-16	36º 31' 49" N	33º 28' 13" E	308 ft	Female
81	GKS2-17	36º 31' 49" N	33º 28' 13" E	308 ft	Male
82-a	GKS2-16&17				Young
82-b	GKS2-16&17				Young
83	GKS2-18	36º 31' 49" N	33º 28' 14" E	313 ft	Female
84-a	GKS2-18				Young
84-b	GKS2-18				Young
85	GKS2-19	36º 31' 46" N	33º 28' 22" E	314 ft	Female
86	GKS2-19				Young
87	GKS2-20	36º 31' 47" N	33º 28' 31" E	313 ft	Female
88-a	GKS2-20				Young
88-b	GKS2-20				Young
88-c	GKS2-20				Young
89	GKS2-21	36º 31' 45" N	33º 28' 32" E	309 ft	Male
90	GKS2-21				Young
91	GKS2-22	36º 30' 36" N	33º 29' 51" E	301 ft	Male
92-a	GKS2-22				Young
92-b	GKS2-22				Young
93	GKS2-23	36º 30' 36" N	33º 29' 52" E	299 ft	Female
94-a	GKS2-23				Young
94-b	GKS2-23				Young
95	GKS2-24	36º 30' 37" N	33º 29' 55" E	301 ft	Male
96-a	GKS2-24				Young
96-b	GKS2-24				Young
97	GKS2-25	36º 30' 38" N	33º 29' 57" E	299 ft	Male
98	GKS2-25				Young
99	GKS2-26	36º 30' 38" N	33º 29' 57" E	298 ft	Female
100	GKS2-27	36º 30' 39" N	33º 29' 59" E	295 ft	Male
101-a	GKS2-27				Young

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
101-b	GKS2-27				Young
101-c	GKS2-27				Young
102	GKS2-28	36º 30' 30" N	33º 30' 02" E	294 ft	Female
103a	GKS2-28				Young
103b	GKS2-28				Young
103c	GKS2-28				Young
104	GKS2-29	36º 30' 40" N	33º 35' 00" E	300 ft	Male
105-a	GKS2-29				Young
105-b	GKS2-29				Young
105-c	GKS2-29				Young
106	GKS2-30	36º 30' 40" N	33º 30' 06" E	298 ft	Female
107-a	GKS2-30				Young
107-b	GKS2-30				Young
107-c	GKS2-30				Young
108	GKS2-31				Male
109	GKS2-32				Female
DOWNSTREAM POPULATION (GDOWN)					
110	GKS3-1	36º 26' 02" N	33º 45' 34" E	146 ft	Male
111-a	GKS3-1				Young
111-b	GKS3-1				Young
111-c	GKS3-1				Young
112	GKS3-2	36º 26' 02" N	33º 45' 34" E	161 ft	Female
113	GKS3-2				Young
114	GKS3-3	36º 26' 03" N	33º 45' 45" E	143 ft	Female
115-a	GKS3-3				Young
115-b	GKS3-3				Young
116	GKS3-4	36º 26' 03" N	33º 45' 41" E	169 ft	Male
117	GKS3-5	36º 28' 08" N	33º 46' 13" E	137 ft	Male
118	GKS3-6	36º 24' 54" N	33º 47' 32" E	141 ft	Female
119	GKS3-7	36º 24' 51" N	33º 47' 37" E	123 ft	Male
120-a	GKS3-7				Young
120-b	GKS3-7				Young
121	GKS3-8	36º 24' 51" N	33º 47' 37" E		Female

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
122	GKS3-8				Young
123	GKS3-8	36º 24' 51" N	33º 47' 36" E	126 ft	Male
124	GKS3-9	36º 24' 49" N	33º 47' 42" E	119 ft	Male
125	GKS3-9				Young
126	GKS3-10	36º 24' 43" N	33º 47' 53" E	121 ft	Male
127-a	GKS3-10				Young
127-b	GKS3-10				Young
128	GKS3-11	36º 24' 27" N	33º 48' 12" E	113 ft	Male
129-a	GKS3-11				Young
129-b	GKS3-11				Young
130	GKS3-12	36º 24' 17" N	33º 48' 14" E	137 ft	Male
131	GKS3-12				Young
132	GKS3-13	36º 24' 17" N	33º 48' 14" E	137 ft	Female
133	GKS3-14				Young
134	GKS3-14	36º 24' 16" N	33º 48' 15" E	120 ft	Male
135	GKS3-15	36º 24' 13" N	33º 48' 18" E	104 ft	Female
136-a	GKS3-15				Young
136-b	GKS3-15				Young
137	GKS3-16	36º 24' 12" N	33º 48' 18" E	113 ft	Male
138	GKS3-17	36º 24' 10" N	33º 48' 17" E	129 ft	Female
139-a	GKS3-17				Young
139-b	GKS3-17				Young
139-c	GKS3-17				Young
140	GKS3-18	36º 24' 09" N	33º 48' 29" E	131 ft	Female
141	GKS3-19	36º 24' 09" N	33º 48' 32" E	119 ft	Male
142	GKS3-19				Young
143	GKS3-20	36º 24' 12" N	33º 48' 33" E	99 ft	Male
144	GKS3-21	36º 24' 12" N	33º 48' 33" E	99 ft	Female
145	GKS3-21				Young
146	GKS3-22	36º 24' 12" N	33º 48' 35" E	98 ft	Male
147	GKS3-22				Young
148	GKS3-23	36º 24' 12" N	33º 48' 35" E	98 ft	Female
149	GKS3-23				Young

Sample Code	Sample ID	Latitude	Longitude	Altitude	Sex/Age
150	GKS3-24	36º 24' 12" N	33º 48' 36" E	102 ft	Male
151-a	GKS3-24				Young
151-b	GKS3-24				Young
152	GKS3-25				Female
153-a	GKS3-25				Young
153-b	GKS3-25				Young
154	GKS3-26	36º 24' 12" N	33º 48' 38" E	103 ft	Female
155-a	GKS3-26				Young
155-b	GKS3-26				Young
156	GKS3-27	36º 24' 13" N	33º 48' 39" E	103 ft	Female
157-a	GKS3-27				Young
157-b	GKS3-27				Young
157-c	GKS3-27				Young
157-d	GKS3-27				Young
158	GKS3-28	36º 24' 13" N	33º 48' 39" E	103 ft	Male
159	GKS3-28				Young
160	GKS3-29	36º 24' 13" N	33º 48' 41" E	104 ft	Female
161-a	GKS3-29				Young
161-b	GKS3-29				Young
162	GKS3-30	36º 24' 13" N	33º 48' 43" E	100 ft	Male
163-a	GKS3-30				Young
163-b	GKS3-30				Young
163-c	GKS3-30				Young
164	GKS3-31				Female
165	GKS3-31				Young

B. Modified CTAB DNA Extraction Protocol

1. 2% (w/v) polyvinylpyrollidine (PVP-40) mixed with 2xCTAB extraction buffer, and the final solution was heated at 65° in a preheated water bath for half an hour
2. 0.1 g grounded leaf tissue added in a mortar along with 2000 µL preheated CTAB solution and grounded with a pestle until a homogenous green mixture is obtained.
3. 1800 µL of the mixture was transferred into a 2 ml Eppendorf tube, and 100 uL β-mercaptoethanol and 5 µL Proteinase K were added into tubes containing mixtures. Then, tubes were incubated in a 65° water bath for 1 hour with occasional gentle swirling.
4. The tubes were centrifuged at 15000 rpm for 20 minutes at 4°C, and 800 µL aqueous phase was transferred to a new 2 ml Eppendorf tube thereafter.
5. 640 µL Chloroform/Isoamyl alcohol (24:1) was added to the previously collected aqueous phase and inverted gently two or three times. Tubes were centrifuged at 14000 rpm for 15 minutes at 4°C.
6. 500µL supernatant was collected and transferred into a new 1.5mL Eppendorf tube, 500 µL ice-cold Isopropanol was added, and tubes were inverted gently two or three times. Then, the tubes were incubated at -80°C for 1 hour.
7. Tubes were centrifuged at 14000 rpm for 15 minutes at 4°C. The supernatant was poured off while paying attention not to drop the pellet. Then, the pellet was washed twice with 70% cold EtOH.
8. The pellet was dried with air in a laminar flow hood and dissolved in 60 µL TE buffer.

C. Reagents and Equipments

Chemicals and Buffers Used in DNA Extraction

2X CTAB Buffer (500ml): 10 gr CTAB (Cetyl Trimetyl Ammonium Bromide) - SIGMA

50 ml Tris HCL (1M, pH:8) – SIGMA

40 ml EDTA (0.5M, pH: 7.8) – FLUKA

41 g NaCl

Distilled water up to 500 mL

Tris HCl 1M pH:8 (250ml): 30.35 gr Tris Base – SIGMA

Concentrated 1M HCI to adjust pH

Distilled water up to 250 ml

PVP-40

Isopropanol: Pure isopropanol – FLUKA

Chloroform – FLUKA

Isoamyl alcohol – FLUKA

β-mercaptoethanol – SIGMA

Proteinase K

TE Buffer: 1 mL Tris HCl (1M, pH:7) – SIGMA

200 µL EDTA (0.5M, pH 8.0) – FLUKA

Distilled water up to 100 mL

Chemicals and Buffers Used in Agarose Gel Electrophoresis

10X TBE Buffer (1000 mL): 108 gr Trizma Base – SIGMA

55 gr Boric Acid – SIGMA

40 ml EDTA (0.5 M, pH:8) – FLKA

Distilled water up to 1000 mL

Running Buffer: 1X TBE Buffer

3% Agarose Gel (300 mL): 9 gr Agarose – SIGMA

1X TBE buffer up to 300 mL

Ethidium Bromide (4 mg/mL) – SIGMA

Low Molecular Weight DNA Ladder – SIGMA

Equipments

Autoclave – Yamato

Centrifuge – Nüve NF048

Deepfreezer – UĞUR Freezer

Electrophoresis System – Thermo Scientific

Thermocycler – Eppendorf Mastercycler

Magnetic Stirrer – Labor Brand Hotplate L-81

Oven – Dedeoğlu

pH meter – Hanna Instruments

Refrigerator – Siemens

Spectrophotometer – NanoDrop2000

UV Transilluminator – Vilbor Lourmant

Vortex – Nüve NM110

Water Bath – Memmert

Micropipettes - Gilson

D. Descriptive Population Genetics Statistics

Number of Different alleles – Na

Specified by direct count.

Effective number of alleles – Ne

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

Where He is expected heterozygosity.

Number of private alleles

The number of alleles that are unique to a single population.

Shannon's Information Index (I)

$$I = \sum p_i \ln p_i$$

Where p_i is the allele frequency of the i^{th} allele for a specified locus within a certain population.

Observed Heterozygosity (Ho)

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

Where N is the population size.

Expected Heterozygosity (He)

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

Where p_i is the allele frequency of the i^{th} allele for a specified locus within a certain population.

Fixation Index (F)

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

Fixation index (F) is also known as the inbreeding coefficient. He is expected heterozygosity and Ho is observed heterozygosity.

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

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

F_{IS} is the inbreeding coefficient and represents mean reduction in heterozygosity of a subpopulation due to non-random mating. $\overline{He}$ is the mean He within a random mating subpopulation. Ho is the mean Ho within a subpopulation.

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

F_{ST} is the fixation index, it represents population subdivision and mean reduction in heterozygosity of subpopulations due to genetic drift. H_T is the expected heterozygosity in a random mating total (pooled) population. $\overline{He}$ is the mean He within a random mating subpopulation.

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

F_{IT} is the overall fixation index, it combines the effects of inbreeding and genetic drift altogether to represent decrease in heterozygosity. H_T is the expected heterozygosity in a random mating total (pooled) population. Ho is the mean Ho within a subpopulation.

Following equation establishes a connection among the F-statistics:

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

Number of Migrants (Nm)

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

Where Fst is the fixation index, representing population subdivision.

Probability of Identity (PI)

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

Where p_i is the allele frequency of the i^{th} allele for a specified locus within a certain population.

Polymorphic Information Content (PIC)

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

Where p_i is the allele frequency of the i^{th} allele for a specified locus within a certain population.

Percentage of Polymorphic Loci (%P)

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

Where P_i is the proportion of polymorphic loci in a population and N is the number of populations.

Garza-Williamson Index (G-W)

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

Where k is the number of alleles at a given locus and R is the allelic range.

E. Input File Formats of Software

Arlequin Input File Format (.arp file)

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    GameticPhase=0
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2     1   287  219  225  197  201  185  348  099  168  153  142  126  152  130  280
          289  231  231  197  201  189  351  103  170  161  142  130  152  144  280
3     1   287  219  225  197  201  189  369  099  170  153  154  126  152  130  280
          291  237  231  197  201  189  369  099  170  161  154  130  152  148  280
4     1   289  213  225  197  195  189  348  099  170  153  154  130  144  142  278
          289  213  231  197  201  191  351  101  170  155  154  130  152  148  280
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6     1   287  213  225  197  201  183  348  103  170  155  154  126  144  130  282
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8     1   289  213  225  197  195  185  351  099  174  155  154  130  144  142  282
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9     1   289  213    ?  197  201  183  348  101  170  153  154  126  150  142  280
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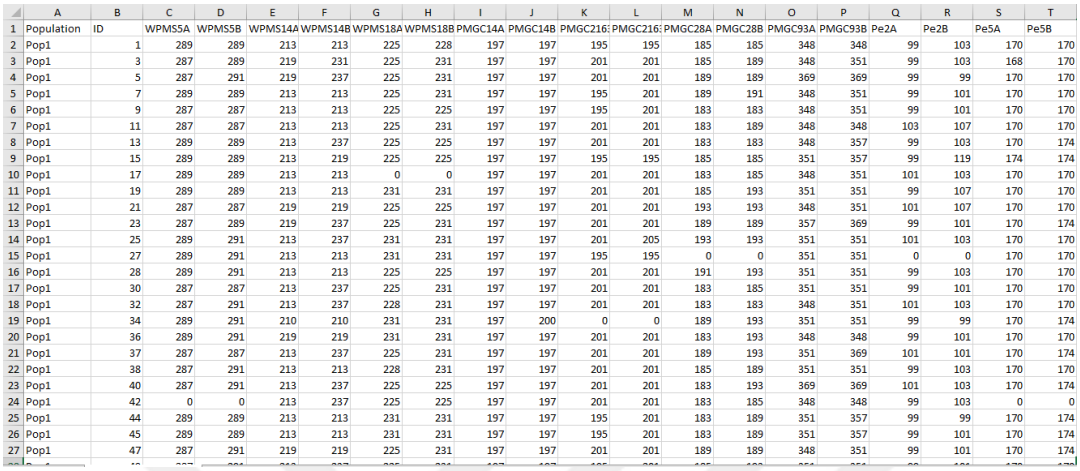
Cervus Input File Format (.csv file)



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
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3	Pop1	3	287	289	219	231	225	231	197	197	201	201	185	189	348	351	99	103	168	170
4	Pop1	5	287	291	219	237	225	231	197	197	201	201	189	189	369	369	99	99	170	170
5	Pop1	7	289	289	213	213	225	231	197	197	195	201	189	191	348	351	99	101	170	170
6	Pop1	9	287	287	213	213	225	225	197	197	195	201	183	183	348	351	99	101	170	170
7	Pop1	11	287	287	213	213	225	231	197	197	201	201	183	189	348	348	103	107	170	170
8	Pop1	13	289	289	213	237	225	225	197	197	201	201	183	183	348	357	99	103	170	174
9	Pop1	15	289	289	213	219	225	225	197	197	195	195	185	185	351	357	99	119	174	174
10	Pop1	17	289	289	213	213	0	0	197	197	201	201	183	185	348	351	101	103	170	170
11	Pop1	19	289	289	213	213	231	231	197	197	201	201	185	193	351	351	99	107	170	170
12	Pop1	21	287	287	219	219	225	225	197	197	201	201	193	193	348	351	101	107	170	170
13	Pop1	23	287	289	219	237	225	231	197	197	201	201	189	189	357	369	99	101	170	174
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15	Pop1	27	289	291	213	213	231	231	197	197	195	195	0	0	351	351	0	0	170	170
16	Pop1	28	289	291	213	213	225	225	197	197	201	201	191	193	351	351	99	103	170	170
17	Pop1	30	287	287	213	237	225	231	197	197	201	201	183	185	351	351	99	101	170	170
18	Pop1	32	287	291	213	237	228	231	197	197	201	201	183	183	348	351	101	103	170	170
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FSTAT Input File Format (.dat file)

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PMGC28
PMGC93
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Pe5
Pe6
Pe8
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Pe15
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GDA Input File Format (.nex file)

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_13_    289/289 213/237 225/225 197/197 201/201 183/183 348/357 099/103 170/174 161/161 154/154 126/130 144/152 142/144 284/284
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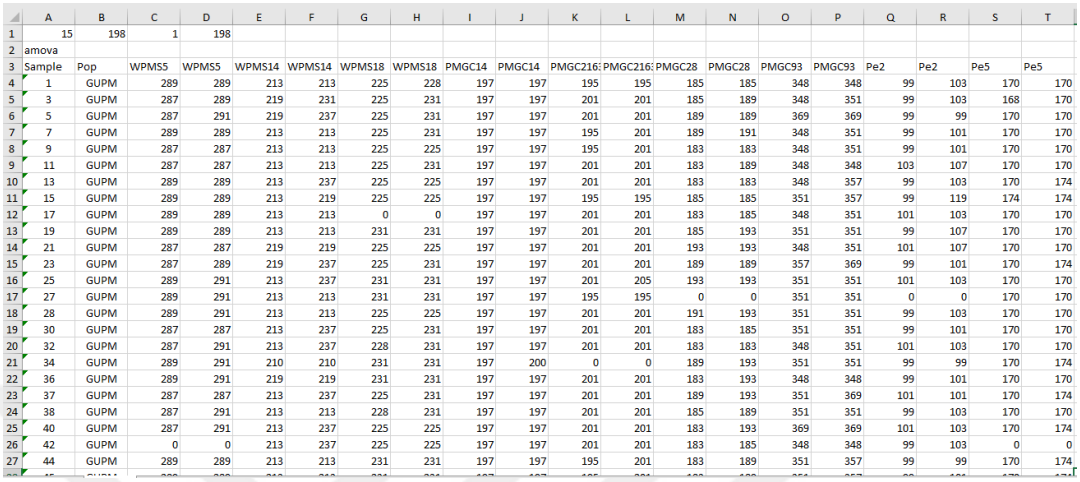
GenAlEx Input File Format (.xlsx file)



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7	7	GUPM	289	289	213	213	225	231	197	197	195	201	189	191	348	351	99	101	170	170
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23	37	GUPM	287	287	213	237	225	231	197	197	201	201	189	193	351	369	101	101	170	174
24	38	GUPM	287	291	213	213	228	231	197	197	201	201	185	189	351	351	99	103	170	170
25	40	GUPM	287	291	213	237	225	225	197	197	201	201	183	193	369	369	101	103	170	174
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GenClone Input File Format (.txt file)

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Genepop Input File Format (.gen file)

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27  ,   289291 213213 231231 197197 195195 000000 351351 000000 170170 155161 154154 126126 144152 000000 280280
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WPMS5
WPMS14
WPMS18
PMGC14
PMGC2163
PMGC28
PMGC93
Pe2
Pe5
Pe6
Pe8
Pe13
Pe14
Pe15
Popeul3
Pop
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Structure Input File Format (.txt file)

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