

Genetic diversity of marginal populations of *Populus euphratica* Oliv. from highly fragmented river ecosystems

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Abstract

Populus euphratica Oliv. (Euphrates poplar) is one of the naturally distributed poplar species and limited to south and southwestern Turkey. The species possesses great importance for both renewable energy resources and persistence of a healthy river ecosystem. Due to increased habitat destructions and fragmentation by human activities, the distribution area of this species has become narrower. Hence, searching for potential genetic diversity present in species' genetic resources is of great importance in terms of its resilience to changing environment as well as breeding and use. To explore genetic structure and diversity of Euphrates poplar, natural populations in the Göksu and Euphrates river ecosystems were studied with 21 microsatellite DNA loci. Results demonstrated reduced level of genetic diversity ($H_o:0.44$, $uHe:0.45$) and low differentiation among two river populations ($F_{ST}=0.07$), suggesting a common origin. It appears that severe past reductions in population sizes have resulted in loss of genetic variation in the species. Native populations of this species in two rivers seemed to be marginal with continued gene pool shrinkage. Therefore, they are in great danger of collapsing, mainly because of continued habitat loss and fragmentation. Genetic data generated with the current study provide important information which could be useful for future restoration and conservation studies of the species.

Keywords: : *Populus euphratica*, *Microsatellite loci*, *genetic structure*, *genetic diversity*, *habitat fragmentation*

Introduction

Populus euphratica Oliv. (Euphrates poplar) is a dioecious, broadleaved, bushy tree which belongs to the section *Turanga* of genus *Populus* in Salicaceae family. This poplar has the ability to regenerate by root suckers besides seed/seedlings and propagating via broken branches is rare, if occurs. Clonal growth by root suckering up to 40 m begins when the plants reach at an age of 11–15 years (Wiehle et al., 2009). Euphrates poplar is one of the essential components of riparian ecosystems in arid areas due to its tolerance to drought, high temperature, high salt concentration and dust storms. Furthermore, it is well known for its phreatophytic habit of growth in deserts (Gries et al., 2005).

The natural distribution range of Euphrates poplar extends from northern Africa over the Middle East to Central Asia, northern India and China. The largest stands of Euphrates poplar are found in Kazakhstan and China (Thevs et al., 2008; Wang et al., 1996). There is also one isolated population with anthropogenic origin in Spain (Fay et al., 1999). This extraordinary species has latitudinal and altitudinal ranges from 48° North in Kazakhstan to 15° North in Yemen, and from 390 m below sea level in the Dead Sea depression to 4500 m in Kashmir (Browicz 1977).

Euphrates poplar, together with other poplar species, is important for wood and timber production for rural people since antique ages. It is also used as feeds for animals, wind-breaks and stabilization of soil in its natural range as an ecosystem service and indicator species for sustainable usage of river ecosystems. Besides, Euphrates poplar maintains the ecological balance of river basins which provide natural habitats for

many economically and ecologically important species. Since natural reproduction of the species is problematic due to groundwater access requirement for survival of seedlings, its role in desert and river ecosystems needs to be studied thoroughly.

Although Euphrates poplar was assessed as Least Concern in IUCN Red List, the number of populations and individuals of this species has dropped considerably in recent years to the point where it should be now considered to be endangered all over the world (Bruehlheide et al., 2004). The number of Euphrates poplar populations is declining by clearance, overharvesting and modification of hydrological regimes for energy production (Beardmore et al., 2014; Cao et al., 2012). In addition, The Forest Tree Genetic Resources Panel of the Food and Agriculture Organization of the United Nations (1995) declared that Euphrates poplar was one of the threatened boreal species.

Euphrates poplar is one of the four native species of poplars in Turkey and possesses great importance for both renewable energy resources and persistence of a healthy river ecosystem. The populations of the species in Turkey are located at the margins of the natural range distribution of the species. Although a wider natural distribution of the species in Turkey is reported in the literature (Browicz and Yaltirik 1982; Velioğlu and Akgül 2016), in our field studies, we found that most of the populations were deteriorated and some are extinct. Indeed, the extant habitats of the species are either fragmented or disappeared due to constructed dams and levees which diminish flooding and lowering groundwater tables. Thus, survival and establishment of Euphrates poplar seedlings in highly fragmented river ecosystems are significantly lowered. While suitable habitats are available in south and southeastern Turkey, natural populations are marginally located and restricted to most ecologically suitable habitats only, which are Göksu river basin in south and Euphrates and Tigris rivers in southeastern Turkey. The densest stands of this species are spatially disjunct in the Euphrates and the Göksu river basins which possess highly fragmented habitats (Browicz and Yaltirik 1982; Mamikoğlu 2007). Moreover, agricultural activities such as clearance of riverbanks to extend the land for cultivation have been further reduced suitable habitats for the species in Göksu and Euphrates river basins. Though Euphrates river is approximately 1230 km long in Turkey, Euphrates poplar is restricted to a smaller section due to presence of five dams along the river. Especially habitats in the northern tributaries of the river basin were lost. The extant Euphrates poplar remains as patches of stands in southern part of Euphrates river across 70 km range between two dams. The peripheral populations inhabit near margins of natural range that are often patchily distributed, subjected to greater isolation, more limited resources and habitat variability (Brown 1984; Brussard 1984; Eckert et al., 2008). To be able to assess future sustainability of the marginal (peripheral) Euphrates poplar populations, it is important to know how much genetic diversity still remains after habitat fragmentation and loss. The effects of environmental changes are likely to be stronger and more rapid in marginal populations. Thus, the assessment of the genetic variation in these

populations is of great importance for conservation of the genetic resources of the species and critical to adaptive management of these populations under future climate change scenarios which may offer new suitable habitats for recolonization of species further upstream of the rivers of south and southwestern Turkey.

Molecular and population genetics studies of Euphrates poplar are very recent and limited to a few studies (Bruehlheide et al., 2004; Eusemann 2010; Eusemann et al., 2009, 2013; Petzold et al., 2013; Rottenberg et al., 2000; Saito et al., 2002; Wang et al., 2011a; Wang et al., 2011b; Xu et al., 2013; Zeng et al., 2018). Recently, new species specific microsatellite loci based markers (SSR) were developed and tested in genetic variation studies (Wang et al., 2015; Wu et al., 2008). However, there is no study investigating genetic diversity and structure of marginal Euphrates poplar populations in highly fragmented river ecosystems. Here with this study, magnitude and structuring of genetic diversity in marginal/peripheral populations were extensively studied in two highly fragmented river systems and consequences of habitat fragmentation on genetic diversity were reported.

Materials and Methods

Plant Materials

For DNA extraction and further molecular analysis, young leaf samples were collected from *P. euphratica* trees in late Spring to early Summer. There were five sampling sites in two main rivers, Göksu and Euphrates, respectively (Table 1, Figure 1). Göksu river populations were determined to represent upstream tributaries Gökçay (GRup1) and Ermenek (GRup2), middle (GRmid) and downstream (GRdown) of the river. Due to fragmentation and loss of habitats in the river ecosystems, there is only one population available for sampling in the Euphrates river which is located near the Turkey-Syria border (EUPH). In field, leaf samples were collected from individual trees (genotypes) at least approximately 200 m apart in order to prevent sampling of ramets from cohorts. The number of trees in each population varied according to the size of populations and conditions of habitats in river ecosystems.

DNA Extraction

For the DNA extraction, leaf samples were first ground with mortar and pestle using liquid nitrogen (-196°C). From the powder obtained, ~0.1g was used for DNA extraction. Total DNA extraction was performed according to Doyle (1991) CTAB extraction protocol with minor modifications. Visual confirmation of total DNA was done with agarose gel electrophoresis and concentration and purity of the obtained DNA samples were measured using NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific).

PCR Amplification of Microsatellite Loci

For the population genetics study, first a panel of thirty-two microsatellite loci (SSR loci) were used to check the amplification success of the corresponding primers. Among those,

twenty-two of the loci were successfully amplified. The repeat motif, primers and their reference, and expected product size for each locus are given in Supplementary Table 1S. The microsatellite loci used in this study are all from *Populus* origin, but only markers encoded with “Pe” prefix are species-specific markers. WPMS and PMGC are cross-species markers which were developed in *Populus nigra* L. and *Populus trichocarpa* Torr & Gray., respectively.

In order to perform microsatellite genotyping for the individuals, forward primer of each pair SSR locus was modified to incorporate a fluorescent dye prior to amplification (Supplementary materials, Table 2S). The Polymerase chain reaction conditions for the loci are given in Table 3S of the Supplementary materials. In PCR reactions 5x HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia), which included 15mM MgCl₂, was used. The presence of products was verified by running 3 % agarose gel electrophoresis. Capillary electrophoresis was done in BM Laboratory Systems Facilities, Ankara. Assay procedure for fragment analysis was done with the Applied Biosystems 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using The GeneScan ROX labelled 400HD as standard size marker. Base callings were manually checked in Peak Scanner 2.0 software (Applied Biosystems) and allele sizes were recorded for each individual per locus to obtain SSR genotypes.

Table 1
Details of the populations studied in two rivers

River	Population ID	Altitude Range	Number of Individuals
Göksu	GRup1	250-288m	46
	GRup2	331-359m	23
	GRmid	86-112m	39
	GRdown	30-92m	32
Euphrates	EUPH	33-351m	20

Data Analysis

Detection of Duplicated Sampling of Clones

The data obtained from the fragment analysis of 22 microsatellite loci were analyzed with GenClone 2.0 software (Arnaud-Haond and Belkhir 2007) in order to discriminate distinct multilocus genotypes (MLGs) and to determine any duplicated sampling among genotypes of populations from both Göksu and Euphrates rivers.

Null allele presence

For the null allele presence, Brookfield's (1996) estimator method implemented with Genepop 4.2 software (Raymond and Rousset 1995) was first used to detect null allele frequencies for each locus in each population. But Dabrowski et al. (2014) suggested that the null allele detection may be improved further by combining results of several methods. Thus, subsequent analyses were done with MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) to check scoring errors and allele dropouts in addition to presence of null alleles.

SSR Loci Polymorphism and Genetic Diversity Parameters

Genetic diversity parameters were assessed by calculating number of alleles (Na) and mean effective number of alleles (Ne), observed (Ho) and unbiased expected (uHe) heterozygosity using GenAlEx 6.503 (Peakall and Smouse 2012). In addition to number of alleles and mean effective number of alleles, allelic richness (Ar) was computed by FSTAT version 2.9.3.2 (Goudet 1995). Polymorphism information content (PIC) of each locus was calculated by Cervus version 3.0.7 (Kalinowski et al., 2007). In addition, proportion of polymorphic loci (% P), the probability of identity (PI), private alleles and their frequencies for each population were estimated with GenAlEx 6.503 (Peakall and Smouse 2012). Genepop 4.2 (Raymond and Rousset 1995; Rousset 2008) was used to assess F statistics for each

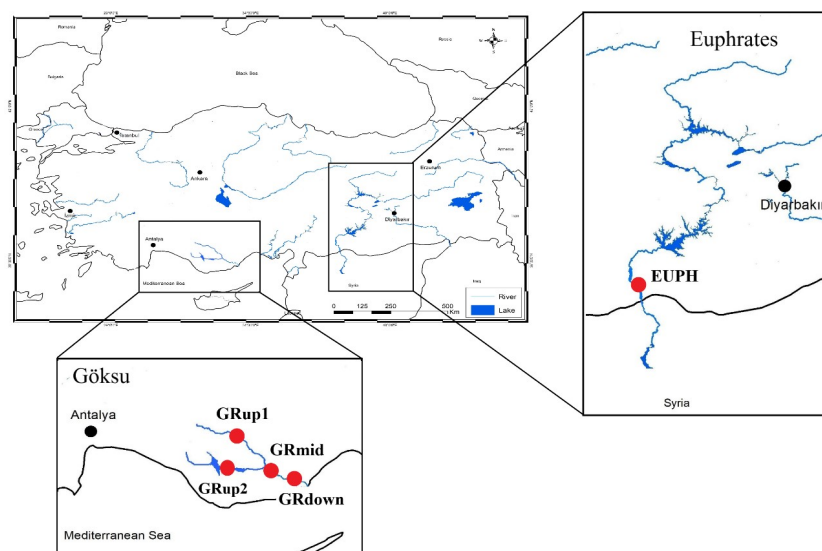


Figure 1
Sampling sites of *Populus euphratica* populations on Turkey map

locus among populations and to implement exact tests for testing the Hardy-Weinberg deviations. A Markov chain (MC) algorithm with default parameters was used to estimate without bias the exact p-value of this test (Dememorization number:1000, Number of batches:100, Number of iterations per batch:1000; Guo and Thompson 1992). Gene flow was estimated from F_{ST} obtained from GenAlEx 6.503 ($Nm = 0.25 (1-F_{ST})/F_{ST}$; Peakall and Smouse 2012). To test for any experienced population reductions in effective size of populations, Garza-Williamson Index (Garza and Williamson 2001) was calculated for all loci and populations with the software Arlequin version 3.5.1.2 (Excoffier and Lischer 2010). To partition total variation into between rivers, among populations within river and within populations, the analysis of molecular variance (AMOVA) was carried out for all loci as implemented in Arlequin version 3.5.1.2 (Excoffier and Lischer 2010). Mantel test was employed with the package adegenet (Jombart 2008) to identify any correlation between geographic and genetic (F_{ST}) distances.

Population Genetic Structure

To demonstrate the genetic differences over multilocus microsatellite genotypes, the pairwise F_{ST} values were used to generate a principal coordinate analysis (PCoA) based on the covariance matrix with data standardization as implemented in GenAlEx 6.503 (Peakall and Smouse 2012). To identify the genetic structure of populations, a Bayesian iterative algorithm was used to assign individuals to clusters as implemented in STRUCTURE v2.3.4 (Pritchard et al., 2000). Admixture model was assumed and run settings were as follows: a burn-in of 50,000 and 250,000 Markov chain Monte Carlo iterations, possible cluster numbers (K) tested from K=1 to K=10 over ten simulation runs. Correlation in allele frequencies was allowed while the remaining parameters were set to the default values. The Structure Harvester (Earl and vonHoldt 2012), a web-based tool, was used to assess the most likely value of K (true K or genetic groups) from STRUCTURE run results by implementing Evanno method (Evanno et al., 2005). In addition, the distribution of the log-likelihoods was examined for the value with the highest probability and lowest variance. Multiple runs for the true K value were analyzed with the CLUMPP software (Jakobsson and Rosenberg 2007) to identify the best alignment to the replicate results of the cluster analysis. The graphical display of population structures was realized with the Pophelper software (Francis 2016).

Since model based approaches like STRUCTURE determine clusters by sorting individuals into assigned populations which are in Hardy-Weinberg equilibrium, Discriminant Analysis of Principal Components [DAPC (Jombart et al., 2010)] was also carried out to see the population structure as implemented with adegenet software (Jombart 2008).

Gene Flow and Effective Population Size

The historic gene flow and Effective population sizes (N_e) were investigated with Migrate-n software (Beerli and Felsenstein 1999; Beerli 2006; Beerli and Palczewski 2010) implemented in CIPRES Science Gateway V 3.3 (Miller et al., 2010). by calculating the parameters θ and M. The parameter θ is the

mutation-scaled population size [equals to $4 \times N_e \times \mu$ (N population size and μ is the mutation rate for the microsatellite data per locus and generation)] and M is the mutation-scaled immigration rate from one population to other [equals to immigration rate divided by the mutation rate per locus and generation]. Coalescent-theory based Bayesian inference method and the Brownian motion model were chosen, starting θ values estimated from F_{ST} with static heating scheme with 4 chains (starting temperatures of 1.0, 1.5, 3.0, and 100000), a constant mutation rate was assumed. Estimation parameters were as follows: 1 long chain, 50 000 recorded steps, an increment of 100 and 10 000 discarded trees as initial burn-in. We adopted mutation rate of 10^{-3} per gamete per generation in poplars, as suggested by Lexer et al. (2005) and Wang et al. (2011b).

Demographic History

To elucidate population history of *P. euphratica* populations from the two rivers, six alternative scenarios were tested using the Approximate Bayesian Computations (ABC) procedure (Beaumont et al., 2002) implemented in DIYABC v2.1.0 (Cornuet et al., 2014). Detailed information on parameter settings are given in Supplementary materials (Table 4S). Direct and logistic regression approaches were applied to estimate the posterior probability of the best supported scenario. We adopted 20 years to represent generation time as suggested by Zeng et al. (2018).

Results

SSR Loci Diversity

The data searched for any possible duplicated clones in samples by comparing multilocus genotypes (MLGs) and indicated that there was no clonal duplication. Among the studied loci, there was one locus (Pe17) with slightly high null allele frequencies in all studied populations. This null allele possessing locus Pe17, which may have had large allelic dropouts, was discarded from further analysis (Table 5S and 6S in Supplementary materials).

Genetic diversity parameters of the studied microsatellite loci were provided in Table 2. Among the analyzed twenty-one loci, two of them were monomorphic for the studied populations. The rest of them were polymorphic having a mean of 3.50 alleles per locus. The least variable ones were WPMS7 and Pe13 loci with 2 alleles. The most variable one was Pe2 locus, displaying ten alleles. Probability of identity (PI) is demonstrating that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits et al. 2001). In the present study, WPMS10 and WPMS15 loci showed high PI values, but the rest of the loci had sufficiently low PI values. Eleven loci were highly informative ($PIC > 0.5$). Allelic richness (A_r) ranged from 1.41 in PMGC14 to 7.36 in Popeu13 locus. The mean observed and unbiased expected levels of heterozygosity were 0.44 and 0.45, respectively (Table 2).

Table 2
Genetic diversity parameters of the 21 microsatellite loci

Locus	N	Na	Ne	Ar	PI	PIC	Ho	uHe	F _{IS}	F _{ST} (Nm)
WPMS5	32	2.8±0.20	2.27±0.25	3.00	0.308	0.530	0.43±0.03	0.54±0.06	0.23	0.08(2.46)
WPMS7	32	1.8±0.20	1.54±0.16	2.00	0.564	0.297	0.15±0.15	0.32±0.09	0.71	0.07(2.14)
WPMS10	32	1.8±0.37	1.06±0.04	1.80	0.901	0.049	0.05±0.04	0.06±0.04	0.22	0.03(5.31)
WPMS12	29.2	3±0.32	1.43±0.08	2.90	0.536	0.259	0.21±0.06	0.30±0.04	0.28	0.0012.02
WPMS14	32	4.2±0.20	2.49±0.22	4.12	0.238	0.601	0.65±0.08	0.59±0.05	-0.07	0.11(1.65)
WPMS15	32	2.4±0.24	1.28±0.18	2.55	0.743	0.172	0.23±0.13	0.18±0.09	-0.24	0.28(0.68)
WPMS18	32	3±0	2.33±0.15	2.99	0.276	0.514	0.53±0.10	0.57±0.02	0.03	0.02(7.65)
WPMS20	31.8					Monomorphic				
PMGC14	32	1.4±0.24	1.04±0.03	1.41	0.937	0.025	0.02±0.01	0.04±0.02	0.48	0.03(5.97)
PMGC2163	32	2.4±0.24	1.74±0.04	2.56	0.414	0.354	0.55±0.06	0.43±0.01	-0.29	0.01(8.63)
PMGC2889	31.8	5.6±0.24	3.71±0.25	5.40	0.115	0.728	0.73±0.05	0.74±0.02	0.00	0.04(4.10)
PMGC93	32	4.2±0.37	2.79±0.21	4.43	0.200	0.614	0.63±0.06	0.64±0.03	0.07	0.02(7.70)
Pe2	31.6	6±0.63	4.02±0.56	6.54	0.117	0.720	0.79±0.03	0.75±0.03	-0.05	0.04(4.33)
Pe5	32	4.8±0.20	2.40±0.26	4.81	0.240	0.545	0.71±0.07	0.58±0.04	-0.24	0.04(5.45)
Pe6	32	3±0	2.64±0.15	3.00	0.221	0.572	0.65±0.09	0.63±0.03	-0.11	0.03(6.18)
Pe7	32					Monomorphic				
Pe8	31.8	3.8±0.20	1.73±0.19	3.84	0.416	0.409	0.43±0.05	0.40±0.06	-0.10	0.19(1.01)
Pe13	31.8	2±0	1.87±0.07	2.00	0.400	0.368	0.46±0.06	0.47±0.02	0.07	0.04(6.11)
Pe14	32	5±0.55	3.24±0.27	5.37	0.151	0.685	0.68±0.06	0.69±0.03	0.00	0.06(3.06)
Pe15	30.8	6.4±0.40	3.88±0.43	6.43	0.117	0.740	0.75±0.02	0.74±0.03	-0.01	0.05(3.31)
Popeu13	30.8	7.8±0.49	3.93±0.68	7.36	0.117	0.701	0.48±0.10	0.73±0.05	0.34	0.03(4.35)
Mean	31.69	3.50±0.19	2.26±0.11	-	-	-	0.44±0.03	0.45±0.03	0.03	0.06(4.39)

N: Sample size, Na: mean allele number, Ne: effective number of alleles, Ar: allelic richness, PI: Probability of Identity, PIC: polymorphism information content, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, F_{IS}: inbreeding coefficient, F_{ST}: genetic differentiation, Nm: number of migrants

Genetic Diversity and Structure of Populations

The genetic diversity parameters for each studied population were presented in Table 3. The mean number of alleles per locus (Na) ranged between 3 (GRup2) and 3.81 (GRmid) with a mean value of 3.50. Effective number of alleles varied between 2.10 (GRup2) and 2.53 (EUPH) with a mean of 2.26. There were no private alleles for GRdown population as expected due to river flow direction. On the other hand, the highest number of private alleles were detected in EUPH population. Average percentage of polymorphic loci among populations was 84.76 %, with highest value observed in EUPH population. Observed heterozygosity (Ho) was lower than unbiased expected heterozygosity for all populations except EUPH (Ho:0.50, uHe:0.49) in which there was slight excess of heterozygosity. Differentiation among populations was low (F_{ST}=0.075). Additionally, comparative results on genetic diversity and population differentiation from *P. euphratica* and other arid region poplars were provided in Table 4. Considering any possible link between genetic and geographic distance, Mantel test indicated there is no correlation between geographic and genetic distances of the studied populations (p=0.056). Calculated Garza and Williamson M values ranged between 0.363 (EUPH) and 0.394 (GRup1) (Table 3), which are much smaller than the critical value 0.68, indicating past reductions in the sizes of all populations in the study.

A hierarchical analysis of molecular variance (AMOVA) of the populations in two rivers showed that only 11.73 % of the variation was account for the differences between rivers. 1.87

% of the variation was attributed to differences among populations within rivers and 86.40% of total variation was within populations (Table 5).

Overall differences among populations were further explored with PCoA analysis based on pairwise F_{ST}. The results pointed out that the first principal component clearly discriminated the populations of two rivers and explained 64.57 % of the total variance while the second principal component explained 19.40 % of the variation among populations within the rivers (Figure 2).

Genetic structure patterns of populations were assessed with prior information about the localities of individuals using STRUCTURE v2.3.4 software (Figure 3c). The delta-K method of Evanno et al. (2005) indicated the presence of three genetic groups (K values) (Figure 3a). In order to identify the mostly likely biologically meaningful value of K, we also considered the K of the highest probability maintaining a small variance, which is K = 5 (the second highest peak in delta-K plot) (Figure 3b). There are conflicting ideas about the cluster analysis with STRUCTURE, especially on estimating the number of genetic groups (K value) because the Bayesian iterative algorithm that the program uses makes assumptions while assigning genotypes to clusters. Thus, the population differentiation was further investigated with a DAPC analysis, implemented in R (Figure 4), which identified the number of clusters (K) to be 5 in a multivariate analysis.

Table 3
Genetic diversity parameters of *Populus euphratica* populations

Population	Na	Ne	% P	Number of Private alleles	G-W index (M)	Ho	uHe	F _{IS}	F _{ST}
GRup1	3.52±0.42	2.21±0.21	85.71%	2	0.394± 0.133	0.41±0.07	0.44±0.06	0.087±0.08***	
GRup2	3±0.33	2.10±0.22	80.95%	1	0.383± 0.165	0.40±0.07	0.42±0.06	0.021±0.08***	
GRmid	3.81±0.50	2.29±0.23	85.71%	3	0.387± 0.126	0.43±0.07	0.46±0.06	0.049±0.07***	
GRdown	3.38±0.42	2.15±0.22	80.95%	-	0.375± 0.126	0.43±0.07	0.43±0.06	0.006±0.07***	0.031
EUPH	3.76±0.46	2.53±0.34	90.48%	9	0.363± 0.135	0.50±0.07	0.49±0.06	-0.037±0.08***	
Mean	3.50±0.19	2.26±0.11	84.76%±1.78%	-	-	0.44±0.03	0.45±0.03	0.025±0.03	0.075a

Na: mean allele number, Ne: effective number of alleles, % P: Percentage of Polymorphic loci, G-W: Garza-Williamson, Ho: observed heterozygosity, uHe: unbiased expected heterozygosity, F_{IS}: inbreeding coefficient, F_{ST}: genetic differentiation, HWE deviations ***: p<0,001, **: p<0,01, *: p<0,05, a: F_{ST} over all populations

Table 4
Comparison of genetic diversity and genetic differentiation for representative arid region *Populus* species (Studies with microsatellite markers in literature)

Publication	Species	Loci number	Sample size	Diversity and Differentiation
This study	<i>P. euphratica</i>	21 loci	160 indiv.	Ho:0.44, He: 0.45 FST: 0.075
Eusemann et al.,2009a	<i>P. euphratica</i>	8 loci	145 indiv.	Ho:0.498, He:0.596
		No commonb	158 indiv.	Ho:0.40, He:0.55
			133 indiv.	Ho:0.460, He:0.56
Eusemann et al.,2013	<i>P. euphratica</i>	7 loci	1701 indiv.	Dest: 0.014
		No commonb		GST: 0.005
Wang et al.,2011a	<i>P. euphratica</i>	8 loci	170 indiv.	Ho:0.932, He:0.839
		6 in common		FST:0.093
Wang et al.,2011b	<i>P. euphratica</i>	8 loci	200 indiv.	Ho:0.935, He:0.746
		5 in common		GST:0.625
	<i>P. pruinosa</i>	8 loci 5 in common	200 indiv.	Ho:0.64, He:0.65 GST:0.492
Wiehle et al.,2016	<i>P. laurifolia</i>	8 loci 2 in common	600 indiv.	Ho:0.534, He:0.528 FST:0.137
	<i>P. euphratica</i>	17 loci 4 in common	552 indiv.	Ho:0.538, He:0.558 FST:0.062
Zeng et al.,2018	<i>P. pruinosa</i>	17 loci 4 in common	102 indiv.	Ho:0.484, He:0.560 FST:0.009

a: No F_{ST} result is given, b: No common loci with current study

Gene flow and Demographic History of Populations

For the gene flow and effective population size, θ ($4N_e\mu$, where N_e = effective population size and μ =mutation rate) and M ($4Nm = \theta \times M$) were used in calculation. M parameter gives information on how much more migration is relative to mutation to bring new variants into a population. The results were presented in Table 6.

In DIYABC analysis, comparison of the posterior probabilities (obtained from both direct approach and logistic

regression, Supplementary Materials Figure 2S) of the six scenarios indicated that Scenario 2 was the best explanatory scenario among all (Figure 5, see all scenarios in Supplementary Materials, Figure 1S). According to Scenario 2, *P. euphratica* populations had an initial effective population size of 7410 (NA). The Euphrates and Göksu river populations diverged approximately 16720 years ago and all populations had experienced both past and recent bottleneck events (Table 7).

Table 5
Hierarchical partitioning of genetic variation using AMOVA [twenty-one microsatellite loci, two groups/regions (The Goksu river and the Euphrates river)]

Source of variation	Sum of squares	Variance components (Va, Vb, Vc)	Percentage of variation
Among rivers	53.227	0.63541	11.73
Among populations within rivers	34.741	0.10132	1.87
Within Populations	1458.036	4.67866	86.40
Total	1546.004	5.41539	
Fixation indices			
F_{ST}		0.136***	
F_{SC}		0.021***	
F_{CT}		0.117***	

(***p<0,001, **p<0,01, *p<0,05)

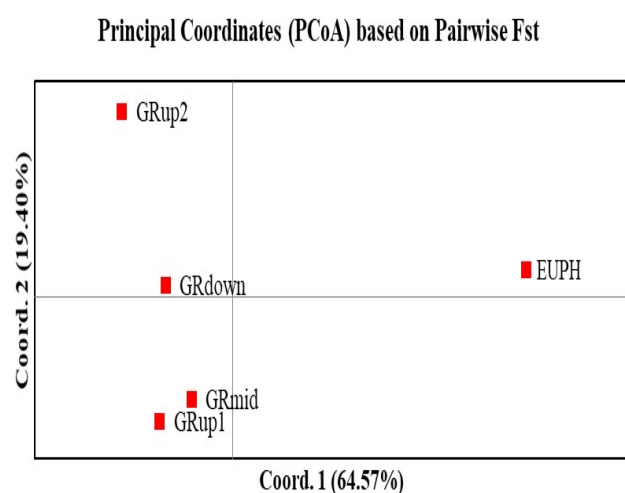


Figure 2
Principal coordinate analysis based on population pairwise F_{ST} values

Discussion

The results obtained in this study are unique and precious in that it is the first population genetics study conducted with marginally located, disjunct and fragmented Euphrates poplar populations. Analysis of microsatellite data revealed the magnitude and structure of genetic diversity which the species possess will provide fundamental information for future use, conservation and breeding of the species.

Among the analyzed 21 microsatellite loci, only WPMS 20 and Pe7 were monomorphic for the studied populations though these loci were reported to be polymorphic in literature with high number of alleles. The reason of monomorphism could stem from reduced effective sizes of the studied populations due to fragmentation. Moreover, based on the geographical region that the sampling was done, there could be primer binding site mutations in flanking sequences of the repeat regions which can prevent amplification. The WPMS10, WPMS15 and PMGC14 loci had high PI values together with low PIC meaning that they are not sufficiently informative for analyzing the genetic diversity if they are used one by one. The resolution of rest of the loci was high with sufficiently low PI values. This was also in accordance with their PIC values indicating acceptable discrimination power. Some authors argued that allelic richness may indicate populations' long-term potential more effectively than heterozygosity does (Allendorf 1986; Petit et al., 1998). Among the polymorphic loci, WPMS14, PMGC2889, PMGC93, Pe2, Pe5, Pe14, Pe15 and Popeu13 had high A_r values (>3.0). In fact, species-specific microsatellites loci had higher A_r values than cross-species microsatellite loci. Consequently, those aforementioned loci were the most informative that they could be considered as choice of markers in future Euphrates poplar population genetic studies.

The average and effective number of alleles per locus, observed and expected heterozygosity were lower as compared to similar previous studies (Eusemann et al., 2009; Wang et al., 2011a; Wang et al., 2011b; Xu et al., 2013). Similar low levels of genetic diversity were also reported in other poplar species (Du et al., 2012; Lexer et al., 2005; Namroud et al., 2005). Only one recent study (Zeng et al., 2018) dealing large number of Euphrates poplar populations found low level of genetic diversity, being similar to current study ($H_o = 0.538$ and $H_e = 0.558$). In all studied populations, except for EUPH heterozygote deficiencies were found. Consequently, the results indicate that the effects of inbreeding and subsequent genetic drift on genetic diversity are evident in the surviving remnants of Euphrates poplar following habitat destructions.

Low genetic diversity and increased inbreeding coefficients (F_{IS} values) of the studied populations point out gene pool shrinkage of Euphrates poplar populations. Considering low number of alleles observed for the species, Euphrates poplar populations may have experienced bottleneck(s) in the past and retained small number of alleles. This has been supported by low Garza-Williamson index values observed in all populations. Accordingly, these results were also in agreement with our DIYABC analyses, which indicated that the best explanatory scenario includes both past and present bottlenecks. Under

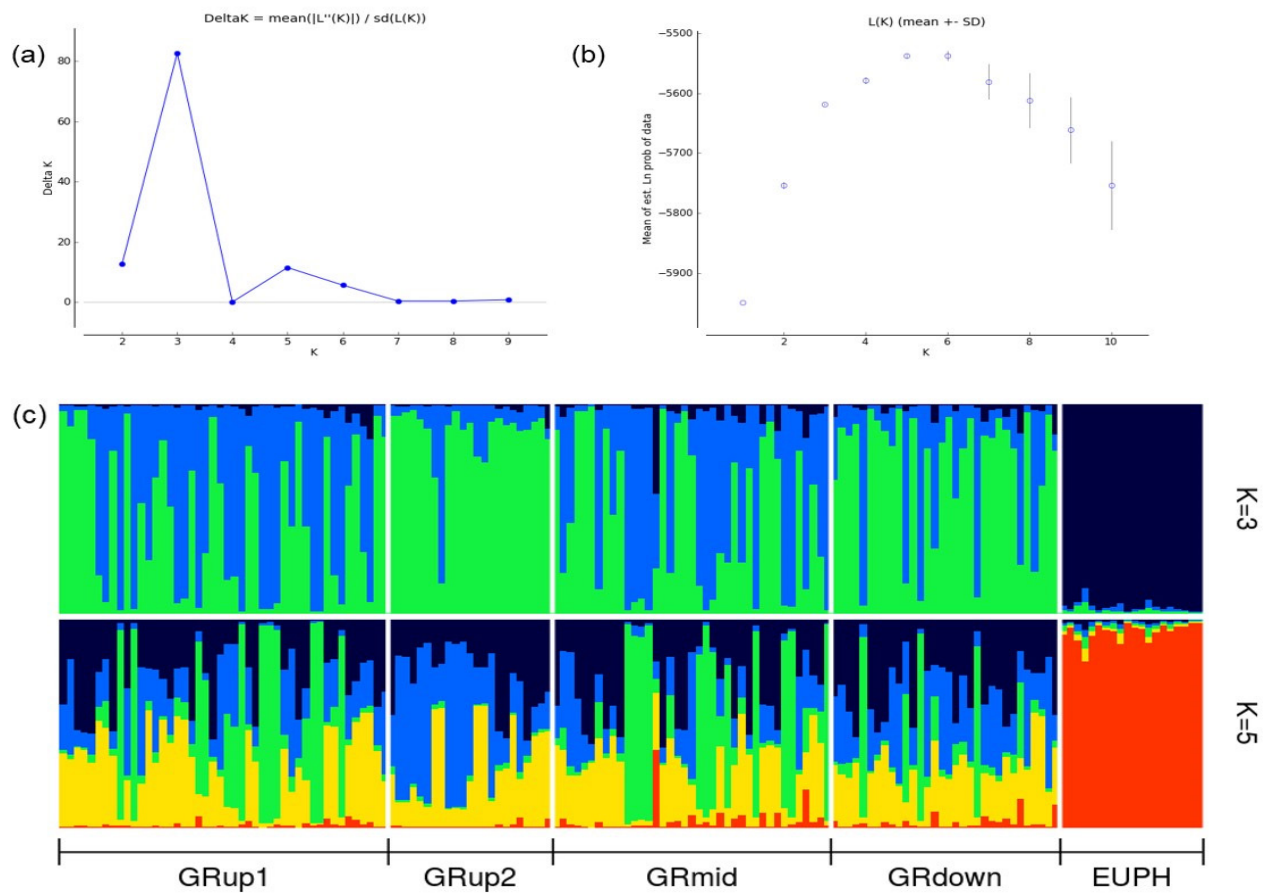


Figure 3
STRUCTURE results for 160 *Populus euphratica* genotypes from the Göksu and the Euphrates rivers. a) Delta K and b) Mean L(K) statistics. c) Histogram of individual assignments when K = 3 and K = 5. Each vertical bar represents one individual

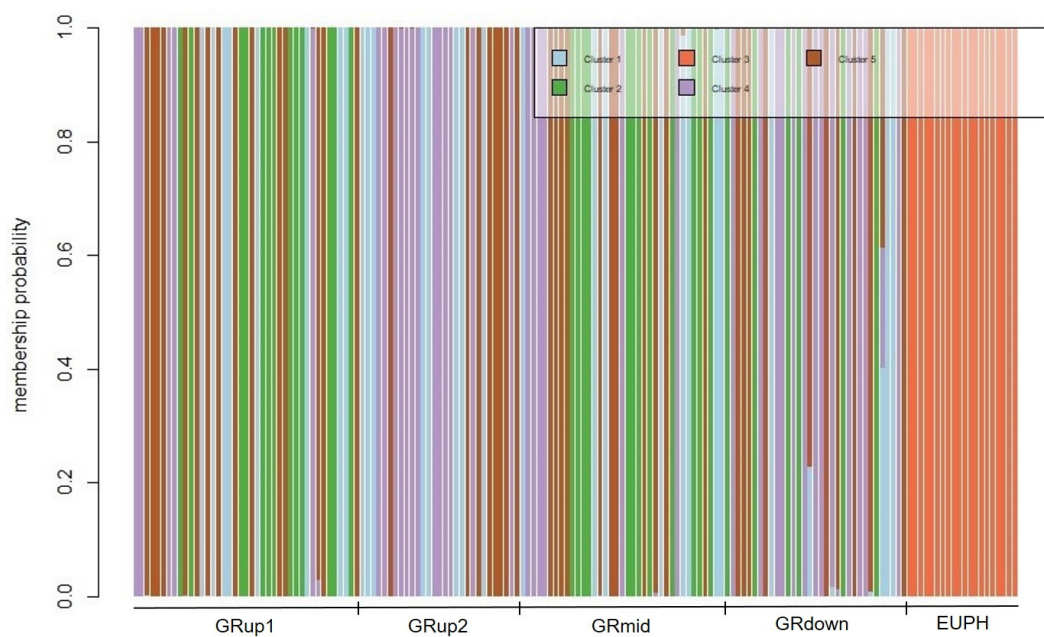


Figure 4
DAPC plot of *Populus euphratica* populations for K=5

Table 6

Historic gene flow and the effective population sizes of the five populations from the Goksu the Euphrates rivers

	M (m/ μ)						
	GRup1	GRup2	GRmid	GRdown	EUPH	θ	Ne
GRup1	-	5.37	89.43	17.56	53.37	1.03	258.33
GRup2	37.57	-	23.9	28.3	55.9	1.17	291.67
GRmid	51.03	32.9	-	86.23	16.03	21.97	5491.67
GRdown	24.63	20.43	26.5	-	21.23	1.50	375
EUPH	73.23	70.1	48.43	36.57	-	20.57	5141.67

M: mutation-scaled immigration rate; m: immigration rate; μ : mutation rate; Ne: effective population size; θ : mutation-scaled population size

*Receiving populations presented in rows

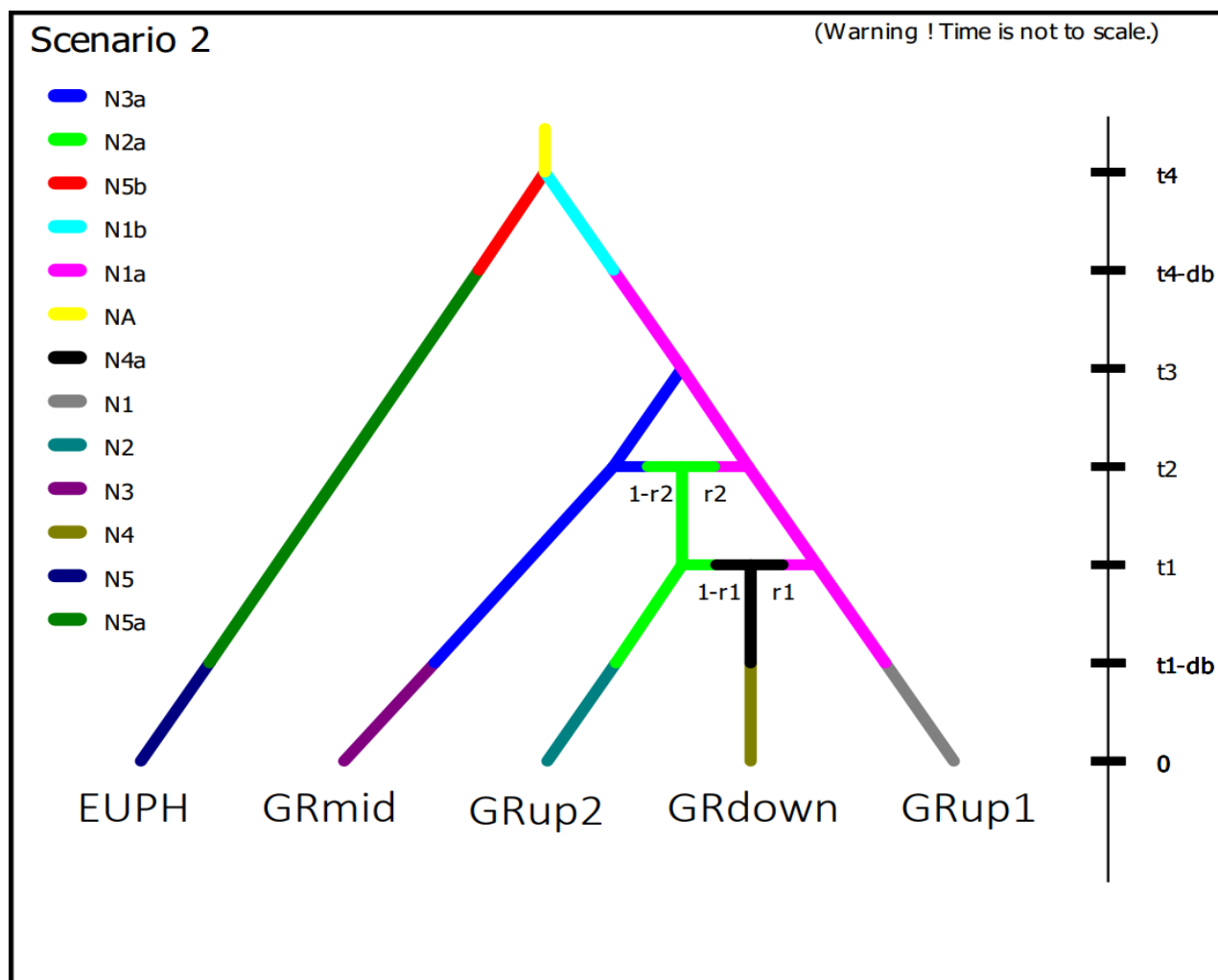


Figure 5

Best supported scenario for the demographic history of populations

Table 7

Estimates of posterior distributions of parameters obtained from the DIYABC analysis for the best scenario (Scenario 2) for the demographic history of *Populus euphratica*

Parameter	Mean	Median	Mode	5%	95%
N1	963	823	608	105	2340
N2	877	706	342	97.8	2270
N3	1470	1450	1200	252	2730
N4	1280	1190	803	181	2660
N5	1430	1390	1090	243	2720
t1	1622	1350	776	398	3600
db	4920	4820	2620	740	9380
N1a	3010	2970	2560	1530	4640
N3a	3840	3890	3870	2600	4870
N5a	4140	4210	4230	3100	4910
N2a	2210	1980	1480	752	4420
N4a	3000	2980	2670	1230	4770
r1	0.459	0.451	0.432	0.118	0.839
t2	2920	2020	1502	704	8000
r2	0.483	0.478	0.460	0.0862	0.9
t3	5840	4380	3380	1806	13740
t4	16720	11400	8600	3900	48600
N1b	3530	3570	3450	1080	5760
N5b	4060	4230	5550	1670	5830
NA	7410	8030	9180	2850	9630
$\hat{\mu}_{mic_1}$	1.27×10^{-4}	1.2×10^{-4}	1.0×10^{-4}	1.01×10^{-4}	1.74×10^{-4}
pmic_1	0.206	0.206	0.206	0.130	0.279

Time parameter (t and db) (unit of time: years) was scaled by an assumed generation time of 20 years.

$\hat{\mu}_{mic_1}$: estimated microsatellite mutation rate; pmic_1: marker parameter of the geometric distribution of the length in number of repeats of mutation events

the circumstances, it is expected that the rare alleles are lost by drift more often than common alleles. Bottlenecks can further increase rate of inbreeding and loss of genetic variation, which in turn can reduce adaptive potential of populations so that populations may face high probability of extinction in the future.

Coalescent-based demographic analyses (DIYABC) of nSSR data also indicated that the two river populations had diverged 16720 years ago in the late stages of The Last Glacial Period (LGP), after the Last Glacial maximum (LGM). During the LGM only high mountainous areas (above 2000m) were glaciated, while the inner part of Anatolia was undercover of desertic steppe (Atalay 1996). These unglaciated regions could have increased the distribution range of the drought adapted species. In turn, this would have provided opportunity for survival of Euphrates poplar in inner Anatolia during the LGM. Actually there were records of a few remnant Euphrates poplar trees in upper tributaries of Euphrates river, near Keban Dam in Eastern Anatolia (Toplu 1999). Thus, during Holocene, populations

might have diverged and migrated towards The Göksu and The Euphrates valleys.

These aforementioned results point out that studied populations have small effective sample size, spatial isolation (fragmentations) and peripheral distribution, so that they may be considered as "marginal populations" in both river systems (Brown 1984; Brussard 1984; Eckert et al., 2008). Eventually, they are expected to have reduced genetic diversity within populations, that is the prevailing situation for all studied populations (Dixon et al., 2013; Vakkari et al., 2009). Although there are debates on whether marginal populations worth to be conserved (Lesica and Allendorf 1995), they could have the evolutionary potential to withstand the burden of geographical distribution shifts as a result of climate change. Despite inbreeding and genetic drift, this is the case for the EUPH population in our study that it has the potential with high private allele number and homogeneous gene pool. Once, there were populations residing northern part of the Euphrates river, but these gene pools are now mostly lost. Thus, the marginal

populations of Euphrates poplar have the potential to shift habitats and colonize northern tributaries of the studied rivers in future in response to climate change.

In the current study, lack of suitable habitats due to water shortage and low groundwater table in watershed of the two rivers could be the main factors for possible past bottlenecks. This situation forced Euphrates poplar trees reproduce clonally and resulted in a situation that few genotypes breed constantly, eventually leading to reduction in genetic diversity. If access of Euphrates poplar seed and seedlings to groundwater are available, this will promote reproduction by seed and recolonization of disturbed habitats (Cao et al., 2012; Westermann et al., 2008). Especially, in EUPH population, recolonization by seeds in disturbed habitats will be more effective due to high number of private alleles since private and rare alleles are crucial to adapt future environmental changes.

In general, genetic differentiations among populations are low in both poplar (Ciftci et al., 2017; Cortan et al., 2016, Zeng et al., 2018) and willow species (Degirmenci et al., 2019, Sitzia et al., 2018). This situation is valid for Euphrates poplar indeed (Wang et al., (2011a); Zeng et al., 2018). Our results showed low differentiation among Göksu river populations ($F_{ST} = 0.031$) as expected since there is no obvious geographical barriers to prevent gene flow. When EUPH river population is considered together with Göksu river populations, genetic differentiation increased slightly ($F_{ST} = 0.075$). The results from Principal Coordinate Analysis based on pairwise F_{ST} values showed that the first principal component, clearly discriminated EUPH population and explained 64.57 % of the variance. These results showed clearly that EUPH population is slightly differentiated from the Göksu river populations. In fact, the studied populations from two rivers have separated from each other by a long geographical distance. On the contrary to expectation due to long distance geographic isolation, the EUPH population did not show substantial genetic differentiation from Göksu river populations, suggesting a common origin. The historical migration rates ($M=m/\mu$) between the populations were asymmetrical and strikingly high. It could be possible that the two river populations were of greater in size in the past and also connected by other extinct populations that might have acted as bridges for the migrants. Actually, in literature there were records of populations in the Seyhan River in Adana province located between the rivers of the current study (Toplu 1994). Although there is Southeastern Taurus mountain range between the two river systems, there was high historical gene flow between GRmid and EUPH populations as depicted in Migrate analysis. The Taurus mountains were formed during the Alpine orogenic period, which occurred at the end of the Oligocene (Atalay et al., 2008). However, the populations seemed to be diverged during Holocene. Thus, the gene flow was continuous despite of the presence of those mountain ranges.

The results from admixture analysis were in accordance with the Principal coordinate analysis for the five populations under consideration. Population clustering showed the presence of two genetically separated groups (for both $K=3$ and $K=5$). It appears that EUPH population has a homogeneous

gene pool. The bar graph plot of DAPC for $K=5$ showed substantial similarity to the corresponding STRUCTURE plot for $K=5$. Thus, the results from the DAPC further supported the homogeneous gene pool of EUPH population and the presence of high admixture in Göksu river. Among the Göksu river populations, individuals from GRup1 and GRmid populations displayed the highest admixture whereas GRup2 is differentiated with low admixture. The river dynamics in the GRup1 and GRmid population sites provide a large area suitable for colonization of the species, that is, the river bed is extending with meanders in the river which are suitable for germination and seedling establishment during flooding. Thus, highest intensity of admixture was observed in those populations. It can be argued that there are no barriers to gene flow in the Göksu river basin and Göksu populations form one large metapopulation, which includes groups of populations/subpopulations in a patchy environment. This is also supported by the studies of Eusemann et al. (2013) and Wang et al. (2011a). The local populations/subpopulations persist by patchy colonization, extinction and recolonization. These repeated founding events may decrease genetic diversity. Furthermore, the effects of the founding events could be robust if the founder population is coming from a single deme.

Conclusion

Great majority of studied SSR markers, especially species specific ones, were highly informative for analyzing the genetic diversity in Euphrates poplar.

Studied Euphrates poplar populations in two rivers maintained a low genetic diversity as compared to previous studies. Euphrates poplar populations seemed to have experienced past bottleneck events which resulted in a gene pool shrinkage and created "marginal" populations of the species. Further, continued habitat deteriorations and fragmentations are likely to contribute greatly to extinction of remnant populations of the species. On the contrary to expectation due to long distance geographic isolation, the EUPH population did not show substantial genetic differentiation from the Göksu river populations, suggesting a common origin.

The results of present study provide important genetic data which could be used to restore and conserve genetic resources of highly degraded extant population of the species.

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