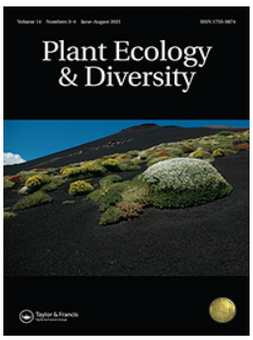

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Genetic diversity and population structure of *Salix alba* across river systems in Turkey and their importance in conservation management

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ABSTRACT

Background: *Salix alba* is a pioneer species of river ecosystems throughout Turkey. Its genetic diversity and population structure across these ecosystems is currently unknown.
Aims: We investigated genetic diversity in Turkish *S. alba* to assess factors likely to shape the genetic structure of the species and to assist with conservation recommendations.
Methods: Six hundred and forty-six individuals from 10 major river systems in Turkey were genotyped using 15 microsatellite markers. Between one and five sub-populations were sampled from each river system with 23 sub-populations sampled in total.
Results: Populations contained moderately high levels of genetic diversity. Five genetic groups were detected by Bayesian clustering, with samples from particular river systems mainly assigned to particular genetic groups. This revealed a geographic structure, also detected by principal coordinate analysis, showing that particular river system populations in different parts of Turkey were genetically similar to each other but different from those in other parts of the country.
Conclusion: Genetic isolation caused by geographic distance (in part) and natural barriers among river systems appear to have shaped the genetic structure of populations. The results have important implications for the conservation of genetic resources within *S. alba* and restoration of degraded Turkish populations of the species.

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Conservation; genetic diversity; genetic isolation; population structure; river ecosystems

Introduction

Anatolia, the Asian part of Turkey, is located at the intersection of the Caucasus, Irano-Anatolian, and Mediterranean biodiversity hotspots (Gür 2016). The Anatolian peninsula has a high central plateau, a narrow coastal plain, and several high mountain ranges (the western Anatolian mountains, the Taurus mountains, the northern Anatolian mountains, and the Anatolian diagonal) (Çiner 2004; Akçar et al. 2017). The region is also characterised by strong climatic contrasts, consisting of a Mediterranean climate in coastal areas reaching 800 m elevation and inland along the major river valleys, and more continental regimes beyond the coastal mountains (Thompson 2020).

Anatolia never experienced Quaternary glaciation and instead acted as a continental refuge and source area for colonisation of northern areas of Eurasia by animals and plants during post-glacial periods (Ansell et al. 2011; Korkmaz et al. 2014; Gür 2017). The escape of Anatolia from the last glaciation, its geographical location between temperate and subtropical regions, and the presence of diverse phytogeographic regions have contributed to the remarkable levels of plant diversity and endemism now found within this region (Kaya and Raynal 2001; Şekercioğlu et al. 2011). This high plant diversity is exemplified by the presence of more than 300 tree species (Thompson 2020). One of these species, *Salix alba* L. (White willow), is an important pioneer tree species of floodplain ecosystems. Along with *Populus nigra* L. (Black poplar), *S. alba* occurs as an early successional riparian tree species often in small groups or as individuals widely distributed in river basins across a wide range of different climatic and ecological zones in Turkey and worldwide (Avcı 1999; Barsoum 2002; Terzioğlu et al. 2014). The species is broadleaved, deciduous and dioecious, and in addition to sexual reproduction, reproduces clonally forming small colonies through rhizome development and broken branches that root (Kuzovkina et al. 2008). White willow is effective in the phytoremediation of river banks and in ecosystem rehabilitation and is used as a short-rotation plantation species for biomass production (Mleczek et al. 2010; Malik et al. 2020). It also

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provides habitat for different forms of native wildlife, including birds, amphibians and insects, and is considered as an indicator species of healthy riparian ecosystems (Rotach 2004; Cao and Berent 2021).

Due to anthropogenic effects, Turkey faces a significant challenge with regard to conserving biodiversity, especially in river and floodplain ecosystems. Many natural riparian ecosystems in the country have either disappeared or are highly fragmented because of unplanned urbanisation, construction of dams, and hydroelectric power stations (Barsoum 2002; Şekercioğlu et al. 2011; Energy Atlas 2019). Consequently, it is essential to determine the genetic structure of Turkish natural *S. alba* populations for the continuity of healthy riparian ecosystems and the restoration of degraded ones. Population genetic studies of this species are expected to reveal patterns of genetic diversity and provide valuable information about the evolutionary and ecological potential of the species (Hughes et al. 2008; Hague and Routman 2016).

Studies of genetic diversity, divergence and gene flow among populations have been conducted on several *Salix* species (e.g., *S. hukaoana, S. viminalis, S. daphnoides, S. caprea, S. psammophila, S. myrsinifolia, S. alba*) using various genetic markers (Kikuchi et al. 2011; Trybush et al. 2012; Sochor et al. 2013; Berlin et al. 2014; Perdereau et al. 2014; Mirski et al. 2017; Değirmenci et al. 2019; Hao et al. 2019). However, to date, there has been no comprehensive study of population-level genetic variation in *S. alba* sampled from diverse riparian ecosystems. Since mode of reproduction type, dispersal, other natural processes and human impacts shape the distribution range and spatial genetic structure of plant species (Barrett and Husband 1990; Hamrick and Godt 1996), a study describing quantitatively the genetic structure of *S. alba* populations would be valuable for future conservation and restoration work of species with fragmented riparian ecosystems.

In this study, we assessed the genetic diversity and population structure of *S. alba* across Turkish riparian ecosystems, and considered which factors may have shaped the genetic structure of this species in these ecosystems. We hypothesised that differences in geographical distance, climate, topography and degree of habitat disturbance and fragmentation within and between river systems will have significantly impacted the magnitude and pattern of genetic diversity in *S. alba* in this region. To test this hypothesis, we surveyed genetic diversity in *S. alba* within and between 10 river systems located in seven climatically and geographically distinct parts of Turkey. Our results provide pointers to how historical and contemporary factors may have shaped the genetic structure of the species in Turkey, and yield information relevant to how genetic resources in this species may be maintained and managed in the future for conservation and restoration purposes.

Material and methods

Sampling area and plant material

For a range-wide characterisation of genetic diversity of *S. alba* in Turkey, leaf samples were collected from 23 locations across 10 Turkish river systems (Figure 1). For analysis, all samples from a particular river system were considered to represent the population for that river system, while all samples taken from a particular location within a river system were considered to constitute a sub-population. Within each river system, one to five sub-populations were sampled and when possible, these were selected to represent the upper, middle, and lower sections of the rivers. A minimum of 20 individuals were sampled from each sub-population per river system, with at least 200 m distance maintained between individuals, so as to prevent sampling ramets of the same genet more than once. Differences between river systems in climate, geographical barriers, topography, degree of fragmentation and length of the rivers, were recorded (Table 1).

DNA extraction and amplification of microsatellite markers

Genomic DNA was extracted with a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987) from freshly collected leaves of 646 individuals representing 23 sub-populations from 10 river systems. Fifteen well-amplified and polymorphic microsatellite loci were used to assess the genetic diversity of the sampled populations (Table S1).

Extracted DNAs were amplified in 20 µl total volume containing different concentrations of 5x HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.25 µM each primer pair, 20 ng template DNA. Two PCR protocols were used for

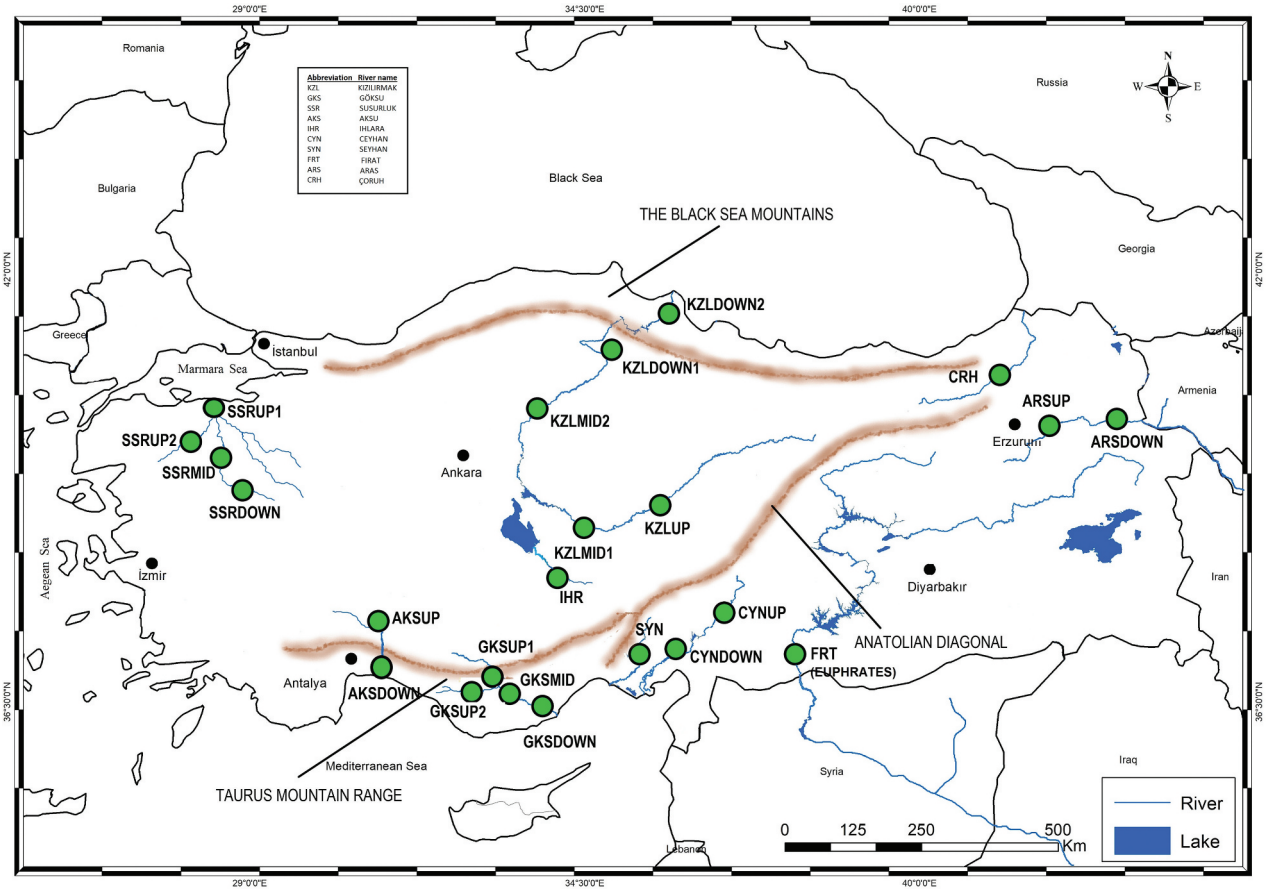

Figure 1. Locations of 23 *Salix alba* sub-populations (green dots) from ten river systems in Turkey.

amplification of template DNAs with selected microsatellite loci (Table S2). The SSR fragment analysis was carried out by BM Labosis Company (Çankaya, Ankara) using an Applied Biosystems 3730 XL DNA Analyser (Applied Biosystems, Foster City, CA, USA) and an internal standard size marker (The GeneScan 400HD ROX dye). To check fragment sizes and allele calls, electropherograms were visualised with Peak Scanner Software 2.0 (Applied Biosystems Inc. Foster City, CA).

Genetic diversity

To estimate null allele frequencies at each microsatellite locus, method proposed by Brookfield (1996) was implemented to obtain maximum likelihood estimates of gene frequency using MICRO-CHECKER Software (Van Oosterhout et al. 2004). Some loci exhibited null alleles in certain river system populations (Table S3a). However, preliminary analyses of the data detected no notable differences between diversity estimates with or without these loci present. Therefore, all further analyses included all loci without consideration of presence of null alleles (Table S3b). The occurrence of replicated multi-locus genotypes among samples (likely due to clonal reproduction) was checked using the GenClone 2.0 software (Arnaud-Haond and Belkhir 2007). Replicates of multi-locus genotypes were excluded from further analyses.

Linkage disequilibrium (LD) between loci was estimated with the R *poppr* package (Kamvar et al. 2014) with indices of association ($\bar{r}_d$) (Agapow and Burt 2001) determined for pairs of loci across river system populations and as a multi-locus measure of LD across samples within each river system population (Figure S1(a,b)). Diversity measures across loci, including mean number of observed alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and F statistics, were calculated using GenAlEx (Peakall and Smouse 2012), while estimates of allelic richness (Ar) and polymorphic information content (PIC) were determined, and tests of Hardy-Weinberg Equilibrium (HWE) conducted using FSTAT (Petit et al. 1998), CERVUS (Marshall et al. 1998; Kalinowski et al. 2007) and GENEPOP (Rousset 2008), respectively. FSTAT standardises Ar to the smallest sample size by incorporating a rarefaction option (Petit et al. 1998), thus removing potential bias caused by sample size variation.

Table 1. Site information on the 23 *Salix alba* sub-populations sampled across ten river systems in Turkey.

River system	Population code	Province/County	Elevation (m)	River condition (Akbulut et al. 2009)	Climate type of region (Sensoy et al. 2012)	Mean annual temperature (°C)*	Mean annual precipitation (mm)*	River length (km)	Number of dams/HPP
Kızılırmak	KZLUP	Kayseri	789–1113	Intermediate	Semi dry-less humid, Semi dry	9.4	443	1355	15
	KZLMID1	Kırşehir	640–816	Fragmented		11.3	417		
	KZLMID2	Kırıkkale	730–1269			11.5	418		
	KZLDOWN1	Çorum	358–424			10.6	484		
	KZLDOWN2	Bafra	0-2			12.6	638		
Çoruh	CRH	Artvin	604–2099	Fragmented, dam and HPP construction	Very humid	6.3	775	438	6
Göksu	GKSUP1	Mut	246–284			11.4	451	260	7
	GKSUP2	Ermenek	333–342	Good	Semi dry- less humid	14.2	578		
	GKSMID	Mut	91–104			11.4	451		
	GKSDOWN	Silifke	27–58			14.2	578		
Ceyhan	CYNUP	Osmaniye	19–60	Intermediate	Semi dry- less humid	15.5	709	509	10
	CYNDOWN	Ceyhan	0–30			16.9	781		
Seyhan	SYN	Seyhan	0–13	Fragmented	Semi dry- less humid	15.5	781	560	8
Aksu	AKSUP	Aksu	39–329	Fragmented, dam and HPP construction	Humid	14.2	667	162	1
	AKSDOWN		2–17						
Susurluk	SSRUP1	Balıkesir	46–86	Good	Humid	13.2	695	321	2
	SSRUP2	Balıkesir	38–128			13.2	695		
	SSRMID	Bursa	9–55			14.4	737		
	SSRDOWN	Balıkesir	30–72			13.2	695		
Ihlara	IHR	Nevşehir	1102	Good	Semi dry	11.2	399	14	
Aras	ARSUP	Erzurum	1508–2012	Intermediate	Semi humid	5.6	584	1072	14
	ARSDOWN	Iğdır	850–870			8.2	398		
Fırat (Euphrates)	FRT	Urfa	337–358	Fragmented	Semi dry- less humid	18.0	462	2800	5

*Mean annual temperature and precipitation values were obtained from *https://climateknowledgeportal.worldbank.org/country/turkey/climate-data-historical*. Seasonality of temperature / precipitation for seven regions of Turkey are given as supplementary information (see Table S7a).

Genetic structure

The number of genetic clusters (groups) across all samples was determined using STRUCTURE V.2.3.4 (Pritchard et al. 2000; Falush et al. 2007) without prior population information. Ten runs were made with a burn-in length of 50,000 and an MCMC of 250,000 using the admixture model. The possible number of groups (K) was tested from 1 to 10, taking into consideration that the total number of river system populations was 10. The most likely number of genetic groups was determined using the ΔK method (Evanno et al. 2005) in the web-based STRUCTURE HARVESTER software (Earl and vonHoldt 2012). For graphical representation of groups, output data from CLUMPP (Jakobsson and Rosenberg 2007) was used as input data in POPHELPER (Francis 2017). Principal coordinate analysis (PCoA) was also conducted on the dataset using GenAlEx to determine population groupings, while partitioning of variation among river system populations and sub-populations within river systems was carried out by analysis of molecular variance (AMOVA) in Arlequin 3.1 (Excoffier and Lischer 2010).

To detect correlations between geographic and genetic distances, a Mantel test was conducted on matrices of pairwise geographic distance and pairwise F_{ST} estimates between river system populations using the *ade4* package (Thioulouse et al. 1997). This was followed by an analysis to determine if barriers to gene flow exist among the populations, using their point coordinates and pairwise genetic distances and applying the Monmonier algorithm in the *adegenet* R package (Jombart 2008). Finally, to test for the occurrence of past genetic bottlenecks (>100 generations), the Garza-Williamson index (M value, Garza and Williamson 2001) was estimated using Arlequin 3.1

Results

Genetic diversity

Of the 646 *S. alba* samples examined, 644 represented different multi-locus genotypes. Among these genotypes, three possessed four alleles and were considered tetraploid. These together with the two samples that replicated the genotype of another sample were excluded from further analysis.

Table 2. Genetic diversity estimates at 15 nuclear microsatellite loci calculated across all samples of 23 *Salix alba* sub-populations across ten river systems in Turkey.

Locus	Number of alleles	Ar	PIC	H_o	H_e	F_{IS}	F_{IT}	F_{ST}	HWE
Sare03	20	12.56	0.92	0.69	0.83	0.16	0.25	0.10	***
Sare04	25	13.13	0.93	0.72	0.84	0.14	0.22	0.10	***
Sare08	27	12.20	0.91	0.67	0.82	0.18	0.26	0.10	***
SB24	17	8.35	0.85	0.54	0.71	0.25	0.37	0.16	***
SB194	5	3.17	0.54	0.55	0.52	-0.05	-0.02	0.03	***
SB196	6	2.26	0.11	0.12	0.11	-0.08	-0.00	0.07	***
SB233	29	11.18	0.79	0.70	0.73	0.05	0.13	0.09	***
SB243	5	3.94	0.66	0.65	0.61	-0.07	0.01	0.07	***
SB265	5	3.40	0.34	0.27	0.33	0.19	0.27	0.10	***
SB493	12	4.39	0.63	0.53	0.50	-0.06	0.16	0.21	***
W293	12	6.73	0.63	0.77	0.59	-0.30	-0.20	0.08	***
W784	6	3.01	0.56	0.99	0.55	-0.80	-0.76	0.02	***
gSIMCT024	8	5.62	0.76	0.66	0.72	0.08	0.15	0.08	***
PMGC2709	22	10.86	0.84	0.64	0.71	0.11	0.23	0.14	***
PMGC2889	21	8.72	0.83	0.74	0.76	0.02	0.11	0.09	***
Mean	14.6	7.30		0.62	0.62	-0.01	0.08	0.10	***

Ar: Allelic richness, PIC: Polymorphism information content, H_o: Observed heterozygosity, H_e: Expected heterozygosity, F statistics: F_{IS}: Inbreeding coefficient within sub-populations, F_{IT}: Inbreeding coefficient for whole population and F_{ST}: fixation index, HWE= Hardy-Weinberg Equilibrium, ***, P<0.001.

Table 3. Descriptive statistics of genetic variability for ten Turkish river system populations of *Salix alba*.

Pop	N	N_a	N_e	P	GWindex (M)	H_o	H_e	F
Göksu	111.13 ± 0.26	10.26 ± 1.64	4.31 ± 0.68	100	0.27 ± 0.10	0.70 ± 0.07	0.66 ± 0.06	−0.07 ± 0.07
Kizilirmak	144.67 ± 0.16	11.47 ± 1.80	4.73 ± 0.75	100	0.30 ± 0.11	0.71 ± 0.06	0.68 ± 0.06	−0.06 ± 0.07
Ceyhan	72.80 ± 0.79	6.07 ± 0.97	2.93 ± 0.43	93.33	0.17 ± 0.08	0.48 ± 0.06	0.56 ± 0.06	0.08 ± 0.10
Seyhan	28.33 ± 0.23	4.93 ± 0.67	2.92 ± 0.44	100	0.14 ± 0.06	0.49 ± 0.07	0.56 ± 0.06	0.08 ± 0.11
Aksu	55.80 ± 1.07	7.27 ± 1.16	3.37 ± 0.51	100	0.19 ± 0.07	0.53 ± 0.06	0.61 ± 0.06	0.10 ± 0.09
Ihlara	28.47 ± 0.75	5.13 ± 0.74	3.50 ± 0.49	100	0.14 ± 0.07	0.63 ± 0.06	0.62 ± 0.06	−0.03 ± 0.11
Aras	54.20 ± 0.55	7.53 ± 1.05	3.61 ± 0.57	100	0.22 ± 0.10	0.53 ± 0.06	0.62 ± 0.06	0.09 ± 0.09
Firat (Euphrates)	23.60 ± 0.72	5.67 ± 0.79	2.77 ± 0.38	93.33	0.15 ± 0.10	0.45 ± 0.07	0.54 ± 0.07	0.11 ± 0.11
Susurluk	80.73 ± 1.88	9.07 ± 1.37	4.26 ± 0.77	100	0.26 ± 0.12	0.65 ± 0.05	0.68 ± 0.05	0.01 ± 0.07
Coruh	24.27 ± 1.10	7.60 ± 0.73	4.09 ± 0.53	100	0.23 ± 0.10	0.59 ± 0.05	0.72 ± 0.03	0.12 ± 0.09
Mean	62.40 ± 3.18	7.44 ± 0.40	3.65 ± 0.18	98.67	0.21 ± 0.09	0.58 ± 0.02	0.63 ± 0.02	0.04 ± 0.03

N, Number of individuals; N_a, Number of different alleles; N_e, Number of effective alleles; P, Percentage of polymorphic loci; G-W index (M), Garza-Williamson index; H_o, Observed heterozygosity; H_e, Expected heterozygosity; F, Inbreeding coefficient within sub-populations; Values are means ± standard errors.

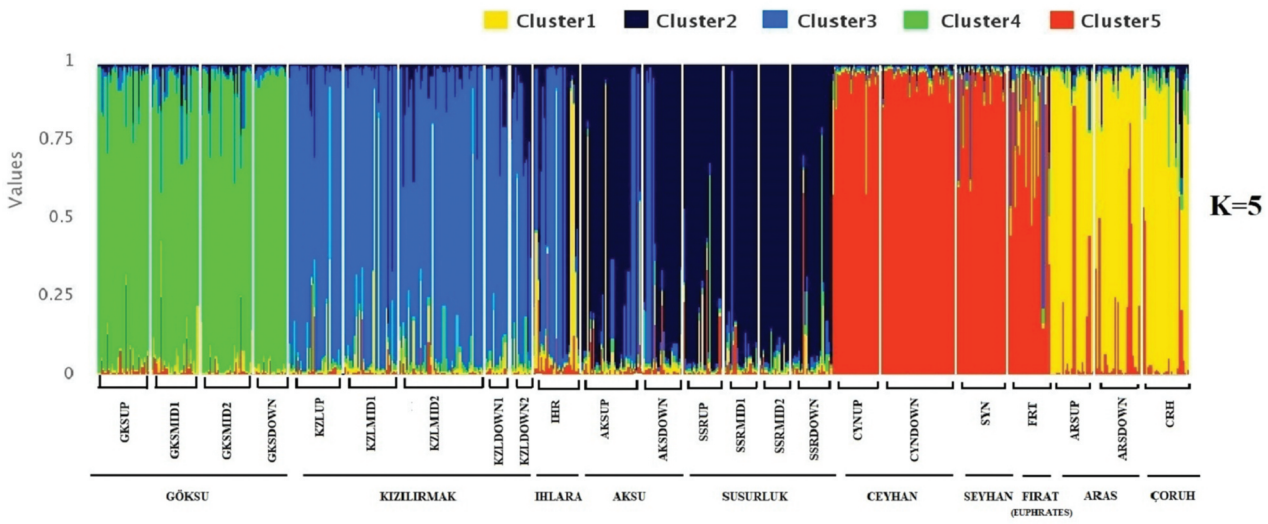

Figure 2. Assignment of 641 *S. alba* individuals to five genetic groups/clusters (represented by different colours) detected by STRUCTURE. White vertical lines separate assumed sub-populations.

Estimates of LD in terms of the index of association ($\dot{r}_d$) for pairs of loci across all samples, for all loci across all samples, and for all loci across samples within river systems, were one and fell outside the expected distribution range for permutations ($P < 0.001$) for no LD (Figure S1(a,b)). Because the majority of loci (SB194, SB243, SB24, SB80, W784, PMGC2709, PMGC2889, PMGC2163, and WPMS18) are known to be located on different chromosomes (Hanley et al. 2002; Gaudet et al. 2008), LD between them cannot be attributed to linkage.

The lowest number of alleles detected per locus across populations was five for the SB194, SB243, and SB265 loci, while the highest was 29 for the SB233 locus (Table 2). Eleven loci were highly informative (PIC>0.5) and mean observed and expected heterozygosity values per locus were of the same magnitude (0.62). HWE tests revealed significant departures from HWE ($P < 0.001$) at each locus (Table 2).

The average number of observed alleles per locus (Na) ranged from 4.93 in the SYN (Seyhan) population to 11.47 in the KZL (Kızılırmak) population with a mean of 7.44 across all populations (Table 3). The number of private alleles per population did not appear to be associated with sample size or river length. Thus, four private alleles were detected in the KZL population along the longest river (Kızılırmak River) which contained the highest number of sampled trees, whereas five private alleles were recorded in the Çoruh River (CRH) population containing only 27 sampled trees (Table S4). Expected heterozygosities (H_e) of populations varied from 0.54 in FRT to 0.72 in CRH with a mean of 0.63. Moderate levels of genetic differentiation were found among populations ($F_{ST} = 0.10$, $P < 0.001$).

Genetic structure

Analysis of the genetic structure of *S. alba* across all samples using STRUCTURE indicated that the most likely number of genetic groups/clusters (K) within the dataset was 2 or 5 (ΔK values are shown in Supplementary Figure S3). Because PCoA also indicated the presence of five groups in the dataset (Figure 3(a)), it was concluded that $K = 5$ best represents the number of genetic clusters present. Bar charts indicating the assignment of samples to genetic clusters when $K = 5$ and $K = 2$ are shown in Figure 2 and Figure S4, respectively. With $K = 5$, a geographic structure is evident with samples of populations from river systems located in northeastern Turkey (Aras and Çoruh populations) mainly assigned to Cluster 1 (Figure 2), those from populations in the Mediterranean and Aegean region Turkey(Aksu and Susurluk rivers) mainly assigned to Cluster 2, those from populations in the Kızılırmak and Ihlara river systems of central and northern Turkey mainly assigned to Cluster 3, those from the Göksu river populations in southern Turkey mainly assigned to Cluster 4, and those from three other river systems in southern Turkey (Ceyhan, Seyhan and Euphrates) mainly assigned to Cluster 5. Ancestry values (proportion of membership to different clusters) fluctuated across samples, but only the mean ancestry values for the Ihlara population indicated assignment to more

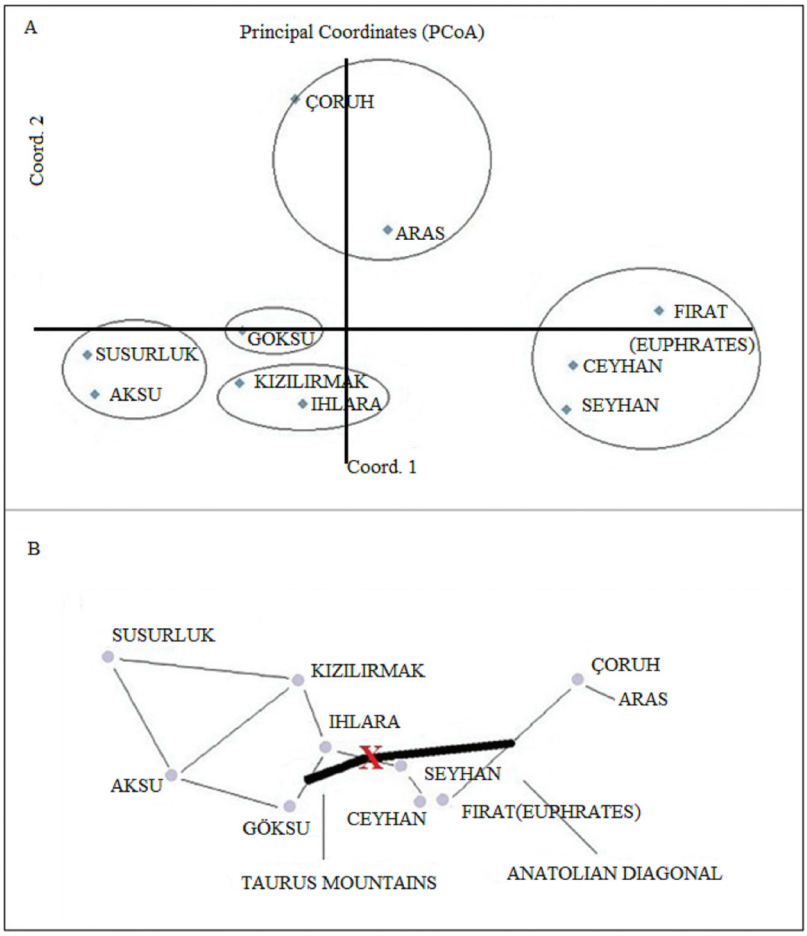

Figure 3. (a) Plot of first and second principal coordinates from a principal coordinate analysis (PCoA) based on pairwise F_{ST} values between ten river system populations of *S. alba*. (b) Placement of genetic barriers according to a barrier detection analysis conducted on ten river system populations of *S. alba*. The lines connecting populations in Figure 3(a) refer to distance between populations based on map coordinates of populations. The thick black lines indicate two physical barriers to gene flow ($P < 0.05$) between river system populations. The 'X' indicates the border between the two geographical barriers. The geographical barrier to the left of the X is caused by the Taurus mountains and the one to the right by the Anatolian diagonal.

Table 4. Analysis of molecular variance (AMOVA) as weighted averages over 15 microsatellite loci for ten *Salix alba* populations.

Source of variation	Sum of squares	Variance components	Percent of total variation	Fixation index
Among genetic groups	337.81	0.27	5.06	$F_{ST} = 0.09$
Among populations within groups	288.53	0.21	3.89	
Among individuals within population	6046.89	4.80	91.04	
Total	6673.24	5.28	100	
Among river system populations	485.31	0.35	6.58	$F_{ST} = 0.09$
Among sub-populations within river systems	141.03	0.11	2.14	
Within river systems	6046.89	4.80	91.28	
Total	6673.24	5.26	100	

F_{ST}, Variance among the coefficients of individuals relative to the total variance.

than one group (Table S5). Principal coordinate analysis revealed a similar genetic structure of populations to that revealed by STRUCTURE (Figure 3(a)).

AMOVA showed that variation among the five different genetic groups was significant and contributed 3.89% to total variation, while differences among populations within clusters were also significant and contributed 5.06% to total variation (Table 4). AMOVA of molecular variance among the ten river system populations showed that differences among these populations were significant and contributed 6.58% to total variation, while differences among sub-populations within river systems were also significant and contributed 2.14% to total variation (Table 4).

Although a Mantel test revealed an absence of correlation between genetic and geographic distance ($r = 0.16$, $P = 0.15$) across the entire distribution of *S. alba* populations surveyed, barrier analysis suggested the existence of two possible geographic barriers between populations from different river systems. One barrier was apparent between river system populations in north-eastern Turkey (Cluster 1) and

southern Turkey (Cluster 5), while another barrier was indicated to exist between populations in the Mediterranean and Aegean regions (Cluster 2) and the Göksu river system population in the south (Cluster 4, Figure 3(b)).

Garza-Williamson index values (M) for all studied populations were found to be lower than the critical value of 0.68, indicating a recent bottleneck event (Table 3).

Discussion

Our survey of microsatellite variation among samples of *S. alba* from 10 river system populations in Turkey, detected the presence of five different genetic groups (clusters). Individuals from geographically close river populations tended to cluster in the same genetic group and geographic barriers were indicated to be a partial cause of this. Our study further indicated that *S. alba* in Turkey experienced a recent genetic bottleneck event, however moderately high levels of genetic diversity were detected both within and between river system populations.

Genetic diversity in *S. alba*

Although clonal reproduction is common in the Salicaceae family, all but two of the 646 samples surveyed differed in genotype. This shows that our sampling method was successful in preventing the collection of multiple samples from the same clone, and that clones are not large and extensively distributed within and among sub-populations. The latter finding has also been reported for other willow species, such as *S. huakoana* (Kikuchi et al. 2011) and *S. arctica* (Steltzer et al. 2008). Despite this, estimates of $\hat{r}_d$ indicated that linkage disequilibrium (LD) exists between all pairs of loci tested, even though the majority of the 15 loci are located on different chromosomes. The occurrence of LD between loci is likely to be a legacy of clonal reproduction, although other causes, such as population differentiation and isolation by distance, as well as natural selection, might contribute to it.

In general, the 10 river system populations contained similar and moderately high levels of genetic diversity as reflected by estimates of N_a, N_e, H_o and H_e, regardless of low sample sizes for some river systems (Seyhan, Ihlara, Fırat, and Çoruh). However, genetic diversity was not evenly distributed among sub-populations within river systems, with a deficiency of heterozygotes noted in all but four of them (Table 3). This possibly reflects the partial isolation of sub-populations within river systems due to fragmentation of habitat and topographic structure, especially along longer river systems. In the three river system populations (Göksu, Kızılırmak and Ihlara) where there was a slight excess of heterozygotes ($H_o > H_e$), this might have been caused by increased disturbance resulting from higher density of human populations which, in turn, could lead to increased gene flow, non-random mating, phenotypic selection (heterozygote advantage) and frequent dispersion of trees among sub-populations (Delmotte et al. 2002; Galeuchet et al. 2005).

Genetic structure

STRUCTURE analysis showed that five different genetic groups were present among *S. alba* individuals sampled across the 10 river systems. Although a Mantel test indicated no correlation between genetic and geographic distance across the entire distribution of *S. alba* in Turkey, genetic differentiation among river system populations was moderately high (F_{ST} = 0.10). Moreover, it was evident from the STRUCTURE analysis that individuals within geographically close river populations tended to be assigned to the same genetic group. Thus, samples of populations from river systems located in north-eastern Turkey were mainly assigned to one group (Figure 2), those from river systems in the Mediterranean and Aegean region were mainly assigned to another group, those from central and northern Turkey were mainly assigned to a third group, those from the Göksu river system in southern Turkey were mainly assigned to a fourth group, and those from three other river systems in southern Turkey (Ceyhan, Seyhan and Euphrates) were mainly assigned to a fifth group. A similar geographic structure was revealed by principal coordinate analysis. It seems, therefore, that river system populations which cluster together genetically are isolated (or were historically isolated) from those that form clusters with other populations.

In the absence of significant isolation by distance, barriers to gene flow between river system populations are most likely to have arisen from geographical and/or ecological barriers (e.g., climatic differences) or fragmentation of the species distribution in the past. Climatic differences exist between river systems (Table 1); however, it is not known if selection might have produced

locally adapted forms in response, which are isolated from each other as a result. The occurrence of geographical barriers between some riparian ecosystem populations, however, was detected by a barrier analysis. This showed the presence of two possible geographic barriers: The Taurus mountain range separating populations of the Kızılırmak from those of Göksu, Ceyhan and Seyhan river systems; and the Anatolian diagonal mountain belt separating populations of Kızılırmak, Göksu, Aksu, Ihlara and Susurluk (located on the west side of the diagonal) from those of the Ceyhan, Seyhan, Fırat and Aras river systems (on the east side of the diagonal). The Anatolian diagonal has an important role in the biodiversity of Anatolia, dividing the Irano-Turanian phytogeographical region into eastern and mid-western sections, with consequent effects on shaping current species compositions and distributions (Davis 1971; Ekim and Güner 1986). Most of the 1200 endemic plant species within species-rich families (Asteraceae, Lamiaceae, Boraginaceae) are distributed either just west or east of the Anatolian diagonal (Noroozi et al. 2019).

The placement of the Göksu population into a separate genetic group is interesting as Göksu populations of *Populus nigra* (Çiftçi and Kaya 2019) and *P. euphratica* (Kansu and Kaya 2020) have also been shown to be genetically distinct from other populations in these species, with geographic isolation by the Taurus mountain range and Anatolian diagonal held responsible, respectively. Although in the current study only two geographic barriers were detected by barrier analysis, it is possible that the Black Sea mountains may act as a further barrier causing the downstream subpopulation (KZLDOWN2) of the Kızılırmak river to be genetically distinct from other subpopulations in this river system (Table S6, Figure S). Further analysis is required of additional geographical and ecological barriers which might have been important in establishing the occurrence of the five genetic groups in *S. alba*. Moreover, the possibility should be investigated that the distribution of the species in Turkey underwent repeated episodes of fragmentation during Pleistocene glaciations with populations isolated in different refugia diverging genetically from each other. Although Anatolia was not glaciated during these periods, it is possible that the distribution of *S. alba* became fragmented in response to lowered temperatures as suggested to have occurred in other species in unglaciated parts of the Mediterranean (Comes and Abbott 1998; Peredo et al. 2009; Thompson 2020), thus providing the conditions for divergence.

Conclusions

The existing moderately high level of genetic diversity that we have detected in *S. alba* suggests that the species might be resilient to habitat fragmentation that has occurred to date and able to withstand/adapt to climate change. Nonetheless, there is a need to conserve as much genetic diversity as possible for the restoration of populations of the species in riparian ecosystems and for the maintenance of diversity across the Turkish distribution of the species. With regard to *in situ* conservation, populations with high genetic diversity and unique alleles across the five genetically different groups should be prioritised. It will also be important to set up *Ex situ* conservation programmes to capture genetic resources from the five distinct genetic groups of the species for future breeding and restoration before genetic resources of the species are further diminished or threatened by anthropogenic action.

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