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Norway maple (*Acer platanoides*) and pedunculate oak (*Quercus robur*) demonstrate different patterns of genetic variation within and among populations on the eastern border of distribution ranges

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Abstract: Norway maple (*Acer platanoides* L.) is a key species of broadleaved forests whose population genetics is poorly studied using modern genetic tools. We used ISSR analysis to explore genetic diversity and differentiation among 10 Russian populations on the eastern margin of the species range of distribution, and to compare the revealed patterns with the results of our population genetic studies of pedunculate oak (*Quercus robur* L.). In the first set comparatively high heterozygosity and allelic diversity were found (expected heterozygosity H_E = 0.160 ± 0.033, number of alleles n_a = 1.440 ± 0.080, effective number of alleles n_e = 1.271 ± 0.062) in comparison with strongly fragmented and geographically isolated small maple stands of the second set (H_E = 0.083 ± 0.011, n_a = 1.281 ± 0.031, n_e = 1.136 ± 0.019). A relatively high genetic differentiation among populations was detected (the proportion of the inter-population component of total genetic variation, G_{ST} = 0.558 ± 0.038). In the Cis-Urals, local groups of populations that are confined to the northern, middle and southern parts of the Urals were identified. On the contrary, the current significant fragmentation of the pedunculate oak distribution area in the same study area did not lead to any noticeable genetic differentiation among the majority of populations, the values of the population genetic diversity were very similar in different parts of the Southern Urals.

Keywords: Norway maple; pedunculate oak; genetic diversity; ISSR markers

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In the European part of Russia, deciduous tree species *Quercus robur* L. and *Acer platanoides* L. are main components of stands in the subzone of mixed forests and in the two vast zones of broadleaved forests and forest-steppe (Hytteborn et al. 2005). The maple, as a rule, occurs with a much lower frequency in stand composition in comparison with the oak (Smirnova et al. 2017). The exception is the eastern border of the distribution range of both species. Extensive stands with dominance of *A. platanoides* are still present on the western macroslope of the Southern Urals in Bashkortostan (Gorchakovsky 1972), a region of Russia on the border of Europe and Asia. At the same time, intensive declines and strong fragmentation of the broadleaved forests occurred in the last centuries in neighbouring plains of the Bashkir Cis-Urals, a part of the Republic with the area of about 90 000 km^2. As a result, stands with the maple occupy mainly relatively small patches of rugged landscapes where the relief does not allow using lands for agricultural purposes. In comparison with *Q. robur*, *A. platanoides* is a more sensitive species to the forest decline due to the relative narrowness of ecological niches. Some ecological traits of the maple (such as limited seed dispersal and entomophily) and comparatively low density in the stand composition (Caudullo, de Rigo 2016) lead to a stronger reproductive isolation of habitats.

Consequences of the fragmentation of the Russian broadleaved forests over large areas, which led to the loss of biodiversity and degraded the functions of the local ecosystems, are well studied (Smirnova et al. 2017). Much less is known about the genetic consequences of fragmentation of stands of this vegetation type into geographically isolated patches with small population sizes. The occurrence of small-sized isolated populations significantly decreases possibilities of the gene flow among populations in the mixing of their gene pools, induces the genetic drift, and increases mating among relatives (Bertolasi et al. 2015). All these processes pose threats to the adaptability and adaptation of trees (Kremer, Hipp 2020) because of the influence on population genetic diversity (Cortés et al. 2020). When the fragmentation has been persisting over several successive generations, it is predicted that a reduced number of parents, random drift and inbreeding may result in the accumulation of deleterious alleles, decreased fecundity of individuals, increasing mortality rate of seeds and plants, and reducing rate of their growth (Aguilar et al. 2008).

The existence of two types of stands with *A. platanoides* in Bashkortostan, large areas with dominance of the species and territories with highly isolated habitats give an opportunity to investigate the impact of the habitat fragmentation on the genetic diversity and differentiation among populations. Recently, 70.9% of all the Russian maple forests were present in Bashkortostan (Bukshtinov 1982), which occupy less than 1% of the country's territory. According to the database "Forests of Russia" of the Federal Agency "ROSLESINFORG" (http://178.176.30.40:8282/#/), their greatest part (89.2%) is concentrated in a relatively narrow meridional band on the western macroslope of the Southern Urals Mountains (51°40'–55°10' N). The Bashkir Cis-Urals occupies almost 2/3 of the territory of the Republic. However, the area of maple forests there is almost 8 times smaller (17.385 ha). A strongly limited genetic flow which causes a reduction in the effective population size and subsequent loss in allelic diversity (Porth, El-Kassaby 2014) seems unavoidable under this situation.

When compared with other European broadleaved forest tree species, *A. platanoides* has been little studied using modern genetic markers (Panchuk et al. 2019). At the same time, genetic diversity and population structure of *Q. robur* is well investigated in Western and Central Europe (Petit et al. 2002) and, in the last years, in the Russian part of the distribution range, including the Southern Urals. (Bushbom et al. 2011; Degen et al. 2019, 2021a, 2021b, 2021c; Blanc-Jolivet et al. 2020; Semerikova et al. 2021). Having got results on genetic diversity of *A. platanoides,* it seems important to compare genetic diversity of these two associate representatives of broadleaved forests from theoretical and practical points of view. They have common history of development in the Southern Urals and now both together are fragmented in a part of this region due to human impacts in the last few centuries.

The objectives of this study were to (*i*) analyse the genetic diversity and differentiation of *A. platanoides* populations in the Southern Urals using ISSRs (Inter Simple Sequence Repeats) as genetic markers; and (*ii*) discuss these data in the light of our results on population genetic diversity of *Q. robur*, another associate species of broadleaved forests of this region.

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MATERIAL AND METHODS

Study area and sampling. The study area is located on the western mountainous macroslope of Southern Urals and Cis-Urals lowlands. Ten stands (Table 1) were selected in the territory, which represent the eastern margin of the *A. platanoides* distribution range. They are located mainly in the territory of the Republic of Bashkortostan, a Russian region with an area of 143 600 km^2, which stretches in the north-south direction to 550 km (Figure 1). The trial plots are represented by stands of 8 forest enterprises of the region and a neighbouring part of the Perm Region. Most of the stands studied represent districts of relatively low forest cover areas (trial plots BRS, BUR, UFA, TMZ, ALS, SLV and KMR) with different proportions of the maple forests. Forest enterprises with relatively large forest cover also have both comparatively high (ARC) and low (KUD and ASK) participation of *A. platanoides* stands in broadleaved forests. Samples for laboratory analysis were collected on each trial plot from 31–32 trees of reproductive age, which are located at a distance of at least 50 m from each other.

Laboratory procedures. DNA was isolated from 100 mg of leaves using a method (Rogers, Bendich 1985) with the addition polyvinylpolypyrrolidone. The concentration and quality of DNA were measured on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and the concentration was increased to 10 ng·μL^{-1}. We chose ISSRs (Inter Simple Sequence Repeats) to detect the polymorphism of populations (Zietkiewicz et al. 1994). The most informative five ISSR primers X10, M3, M27, ISSR-10 and CR-215 were selected when testing 22 primers available. We used 25 μL of the reaction mixture for the polymerase chain reaction, which includes 2 units of Tag polymerase, 2.5 μL of standard 10× buffer, 25 pM of a primer,

Table 1. Information on forests of the study area and trial plots of *Acer platanoides*

Forest enterprises	Forest cover (%)	Area of forests (ha)			Trial plots	Coordinates (latitude, longitude)	Sample size
		S1	S2	S3			
Kueda	45.6	227 701	33 823	4 (0.01)	1. KUD	56.554042, 55.709419	31
Askino	48.2	199 469	44 553	140 (0.31)	2. ASK	55.939444, 56.611059	31
Birsk	28.4	148 351	66 151	737 (1.11)	3. BRS	55.590846, 55.600944	32
					4. BUR	55.990671, 55.174831	32
Ufa	16.7	163 531	91 465	2 519 (2.75)	5. UFA	54.771547, 55.731663	32
Arkhangelsk	55.4	231 328	124 238	13 242 (10.66)	6. ARC	54.421835, 56.795232	32
Tuimazy	18.3	201 563	82 293	4 582 (5.57)	7. TMZ	54.529813, 53.590570	32
Alshey	13.1	83 304	29 413	1 190 (4.05)	8. ALS	53.983732, 54.796708	32
Sterlitamak	14.4	187 900	104 408	10 885 (10.43)	9. SLV	53.245329, 55.979297	32
					10. KMR	52.781621, 55.836891	32

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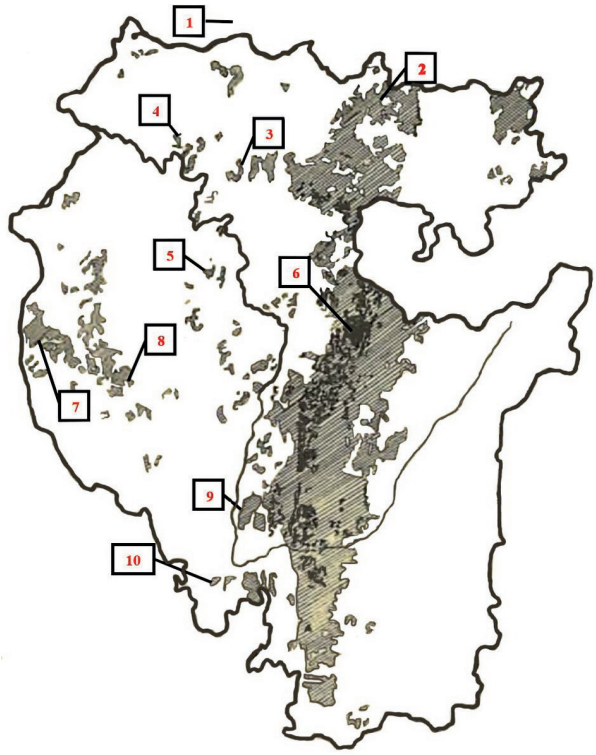

Figure 1. Location of stands with dominance of *Acer platanoides* (dark spots), broadleaved forests with the participation of the species (dashed zones) and the location of trial plots on the map of the Republic of Bashkortostan (see Table 1)

2.5 mM Mg^{2+}, 0.25 mM dNTP, 5 µL of genomic DNA. Amplification was performed in the GeneAmp PCR System 9700 amplifier (Applied Biosystems, USA) according to the following program: predenaturation 94 °C, 2 min; the first five cycles 94 °C, 20 s; annealing temperature, 10 s; 72 °C, 10 s; in the next thirty-five cycles 94 °C, 5 s; annealing temperature, 5 s; 72 °C, 5 s. The last cycle of elongation lasted 2 min at 72 °C. Depending on the G/C composition of the primers, the annealing temperature varied from 52 °C to 64 °C. The amplification products were separated using electrophoresis in 1.7% agarose gel in 1× TBE buffer. We stained the gels with ethidium bromide and photographed them in transmitted ultraviolet light in the Gel-Doc XR system (Bio-Rad, USA). The length of the DNA fragments was determined using molecular weight markers (100 bp + 1.5 Kb + 3 Kb DNA Ladder) (SibEnzim-M, Russia) and the Quantity One program in the Gel-Doc XR gel documentation system (Bio-Rad, USA). The analysis of DNA polymorphism was carried out using 318 trees (Table 1).

Data analysis. Computer analysis of the obtained data was carried out using the POPGENE1.31 program (Yeh et al. 1996) and GenAlEx6 macros (Peakall, Smouse 2006) with calculations of the proportion of polymorphic loci P_{95}, the number of alleles per locus n_a, the effective number of alleles per locus n_e, the expected heterozygosity H_E. The genetic structure of populations was described using the expected proportion of heterozygotes in the total population H_T, the expected proportion of heterozygotes in each individual population H_S, and the population differentiation measure G_{ST}. The STRUCTURE 2.3.4 program was used to identify the genotype clustering (Falush et al. 2003). Visualization of the results, their mathematical confirmation, and determination of the most probable number of genetic groups were performed using the STRUCTURE Harvester program (Earl, von Holdt 2012). We calculated a matrix of genetic distances (Nei 1972) based on a matrix of binary traits and unweighted pair group method (UPGMA) using computer programs Treecon 1.3 b (Van de Peer, De Wachter 1994) and POPGENE 1.31. (Yeh et al. 1996). The genetic distance between populations (D) was determined by the formulas (Nei, Li 1979). In addition, the principal component analysis (PCA), implemented in the GenAlEx6 program (Peakall, Smouse 2006), was used.

RESULTS AND DISCUSSION

Genetic diversity. The ISSR primers used amplified 117 fragments, 95 of which were polymorphic. Each primer (Table 2) initiated the synthesis of 23.4 ± 2.2 DNA fragments, including 19.0 ± 1.9 polymorphic ones (81.1 ± 2.1% of all the fragments). It is noteworthy that the proportions of polymor-

Table 2. ISSR primers used to study genetic diversity in *Acer platanoides* populations; proportions of polymorphic loci are given in parentheses

Primers	Sequence (5'→ 3')	Size range (bp)	No. of bands	
			all	polymorphic
X10	$(AGC)_6C$	180–1 750	24	21 (87.5%)
M3	$(AC)_8CT$	150–2 150	30	23 (76.7%)
M27	$(AG)_8C$	190–1 020	26	22 (84.6%)
ISSR-10	$(ATG)_7C$	120–1 040	19	15 (78.9%)
CR-215	$(CA)_6GT$	140–650	18	14 (77.8%)

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Table 3. Number and frequency of polymorphic amplified DNA fragments identified in *Acer platanoides* populations by used ISSR primers; proportions of polymorphic fragments are given in parentheses

Trial plots	ISSR primers					In total
	X10	M3	M27	ISSR-10	CR-215	
1. KUD	8 (0.533)	10 (0.526)	5 (0.625)	5 (0.417)	9 (0.818)	37 (0.569)
2. ASK	6 (0.500)	6 (0.500)	10 (0.667)	10 (0.769)	5 (0.556)	37 (0.607)
3. BRS	4 (0.444)	11 (0.611)	5 (0.417)	7 (0.583)	7 (0.583)	34 (0.540)
4. BUR	6 (0.462)	10 (0.500)	9 (0.600)	4 (0.400)	3 (0.273)	32 (0.464)
5. UFA	4 (0.333)	4 (0.364)	5 (0.294)	3 (0.250)	5 (0.365)	21 (0.323)
6. ARC	13 (0.722)	21 (0.913)	19 (0.905)	17 (0.944)	6 (0.545)	76 (0.835)
7. TMZ	6 (0.427)	1 (0.100)	6 (0.462)	5 (0.385)	4 (0.364)	22 (0.361)
8. ALS	3 (0.250)	16 (0.727)	12 (0.632)	10 (0.769)	7 (0.636)	48 (0.623)
9. SLV	3 (0.250)	17 (0.773)	8 (0.500)	8 (0.571)	6 (0.545)	42 (0.560)
10. KMR	4 (0.333)	15 (0.833)	11 (0.733)	7 (0.700)	8 (0.615)	45 (0.662)

phic bands are very similar (coefficient of variation is 5.8%). As a rule, values of the parameter in individual populations are much lower (Table 3). Only the Arkhangelsk stand is close in the proportion of polymorphic bands to the average level of this indicator for all the samples. Four out of five primers used allowed showing the largest polymorphism of this population (except for CR-215). This result was obtained due to the higher occurrence of relatively rare variants in the sample. We did not reveal any ISSR fragments with a frequency of less than 5% in 8 of the 10 stands studied.

Such 3 fragments were found in trees of the trial plot KUD with a frequency of 8%. But 16 of the 76 polymorphic bands are classified as rare ones in the Arkhangelsk population (21.1%). More pronounced patterns of population differences are found when calculating two parameters of the genetic diversity (Table 4). We confirmed that the Arkhangelsk stand represents the genetically most diverse population. The values of the parameters used were higher 1.73–4.92 (H_E), 1.19–1.39 (n_a) and 1.15–1.34 (n_e) times in trees of the trial plot ARC in comparison with other samples. This population represents the part of the region where the largest areas of forests with dominance of *A. platanoides* are concentrated (Table 1, Figure 1). Other three stands (ASK, SLV and KMR), which are also situated on the western macroslope of the Southern Urals with a significant participation of the species, are characterized by comparatively higher expected heterozygosity and number of alleles. The most part of populations (five out of six, except ALS) of the lowland Bashkir Cis-Urals with geographically isolated and mainly small maple stands has a relatively low genetic diversity.

Population structure. In general, the analysis of the population genetic structure confirmed the regularities in the spatial distribution of expected heterozygosity and number of alleles in the *A. platanoides* stands. The primers used demonstrated that the values of H_S are 2.0–2.9 times lower than those of H_T (Table 5). Accordingly, a relatively high genetic differentiation between populations was detected. Depending on a primer, the variation among populations reached relatively high values

Table 4. Genetic diversity in *Acer platanoides* populations; standard deviations are given in parentheses

Trial plots	H_E	n_a	n_e	R
1. KUD	0.099 (0.016)	1.308 (0.464)	1.164 (0.300)	3
2. ASK	0.122 (0.017)	1.316 (0.467)	1.191 (0.327)	0
3. BRS	0.077 (0.014)	1.299 (0.460)	1.127 (0.270)	0
4. BUR	0.069 (0.012)	1.274 (0.448)	1.105 (0.225)	0
5. UFA	0.073 (0.014)	1.205 (0.406)	1.127 (0.283)	0
6. ARC	0.256 (0.019)	1.675 (0.470)	1.450 (0.393)	16
7. TMZ	0.052 (0.011)	1.197 (0.399)	1.081 (0.208)	0
8. ALS	0.128 (0.017)	1.402 (0.492)	1.212 (0.323)	0
9. SLV	0.113 (0.015)	1.385 (0.489)	1.181 (0.289)	1
10. KMR	0.148 (0.019)	1.385 (0.489)	1.261 (0.376)	0
On average	0.113 (0.58)	1.401 (0.136)	1.213 (0.106)	0

H_E – expected heterozygosity; n_a – absolute number of alleles; n_e – effective number of alleles; R – number of private bands

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Table 5. Parameters of the genetic structure and differentiation of *Acer platanoides* populations; standard deviations are given in parentheses

ISSR primers	H_T	H_S	G_{ST}
X10	0.234 (0.026)	0.080 (0.009)	0.659
M3	0.239 (0.033)	0.117 (0.006)	0.511
M27	0.316 (0.026)	0.120 (0.005)	0.619
ISSR-10	0.272 (0.033)	0.129 (0.008)	0.447
CR-215	0.274 (0.037)	0.122 (0.013)	0.555
All samples	0.266 (0.030)	0.113 (0.008)	0.576

H_T – expected proportion of heterozygous genotypes in the total population; H_S – expected proportion of heterozygous genotypes in each individual population; G_{ST} – population differentiation measure (Nei 1975)

of 44.7–65.9% (on average, G_{ST} = 0.558 ± 0.038). The constructed dendrogram (Figure 2) shows that some populations make an unequal contribution to the population differentiation. They are represented by the genetically most diverse population ARC from the western macroslope of the Southern Urals, where the maple often dominates the composition of broadleaved forests on large areas. The stand KUD from the northern margin of the distribution area of *A. platanoides*, where the species occurs only sporadically, also has a peculiar genetic structure. A pair of genetically similar populations UFA and TMZ from the lowland Bashkir Cis-Urals, which are characterized by the least genetic diversity, formed their own cluster. The average genetic distance among ten studied populations was D = 0.247 ± 0.012 with changes from 0.103 to 0.431. The sequential removal of the samples ARC, KUD, and UFA/TMZ from the calculations resulted in a significant decrease of the parameter (D = 0.216 ± 0.010, D = 0.197 ± 0.010 and D = 0.169 ± 0.010, respectively).

These data are compatible with the results of another analysis. The STRUCTURE program allowed identifying several genetic groups. When the genotypes were analysed using a model with K = 5 (Figure 3), the most part of clusters (Figure 4) corresponded to populations of the northern Bashkortostan (trial plots BRS, BUR and, to a lesser extent, ASK), middle (TMZ and UFA) and southern (KMR, SLV and ALS) parts of the Republic. Two populations with contrasting characteristics of maple forests (trial areas ARC in the forest enterprise with the largest areas of maple forests and KUD on the margin of the *A. platanoides* distribution range) differ from each other and from the other six stands studied. These patterns are visible to a greater extent when using the principal component analysis on the basis of genetic distances D between populations (Figure 5).

In the present study, ISSRs as genetic markers were used to study genetic diversity of *A. platanoides* in two groups of populations. Relatively large stands with dominance of the maple on the meridional western macroslope of the Southern Urals have comparatively higher genetic diversity.

Simultaneously, the mountainous populations (ARC, ASK, the pair SLV and KMR) are comparatively different in gene pools. When using nuclear microsatellite loci, we observed this pattern in *Q. robur* populations of the Southern Urals western macroslope (Degen et al. 2019). Maple popu-

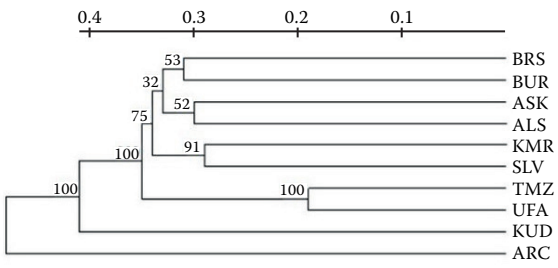

Figure 2. Clustering of *Acer platanoides* populations; the scale demonstrates genetic distances D (Nei 1979), bootstrap values are shown in the dendrogram in percent

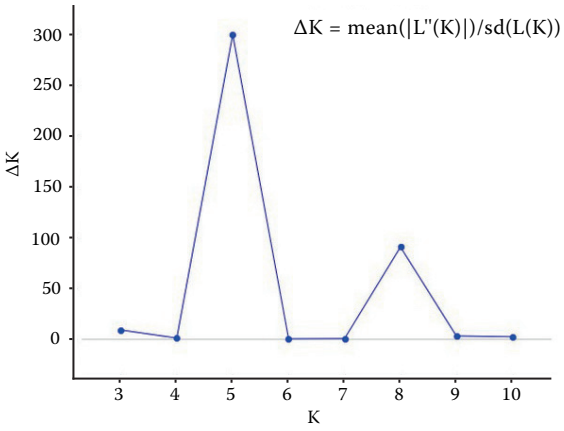

Figure 3. ΔK values for the tested genetic groups
K – tested genetic groups (2–10 in STRUCTURE Harvester program); L''(K) – model choice criterion; sd – standard deviation of L(K)

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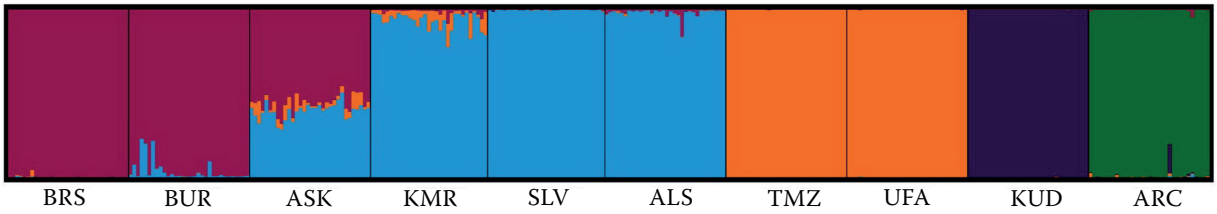

Figure 4. Estimated population structure of *Acer platanoides* populations; vertical bars represent individuals of trial plots, which are represented by colours proportional to the average membership to clusters

lations in the Cis-Urals were found to have lower genetic diversity, as measured by expected heterozygosity and number of alleles. On average, the genetic diversity of 5 out of the 6 populations of the downlands is lower by 53.8% (expected heterozygosity), 12.7% (number of alleles) and 11.8% (effective number of alleles) in comparison with that in the forest mountainous zone. The differences in the group averages exceeded a 95% level of statistical significance for H_E and n_a ($P = 0.025$ and $P = 0.045$, respectively).

The current great fragmentation of the distribution range of maple in the Southern Urals did not lead to synchronous visible genetic divergence in most population sets. At any rate, we revealed a greater genetic similarity of neighbouring populations within northern (trial plots BRS and BUR, partially ASK), central (TMZ and Ufa) and southern (KMR, SLV and ALS) parts of Bashkortostan (Figures 4 and 5). The exceptions to this pattern are stands KUD and ARC with minimal and maximal portions of stands with dominance of *A. platanoides*, respectively, in forest enterprises Kueda and Archangelsk (Table 1). When analysing frequencies of ISSR bands, we revealed their differences from other populations. A part of the bands in KUD (3 of 64 revealed, or 4.7%) are private common ones (Table 4). The frequencies of other bands also significantly distinguish the trees of the trial plot from other samples. The stand ARC is characterized by the uniqueness of ISSR marker number and frequencies. Their comparatively large part (16 of 91 or 17.6%) are private ones. The third set of samples, which has a single private band (1 of 75 or 1.3%), is represented by another mountainous population (SLV). All the studied trees of the remaining 7 stands possessed only common DNA fragments with frequencies more 5%.

Some possible explanations can be given for the interpretation of results on the lower genetic diversity of most Cis-Urals populations of *A. platanoides*. Maple stands KUD, BUR, BRS, TMZ and UFA are

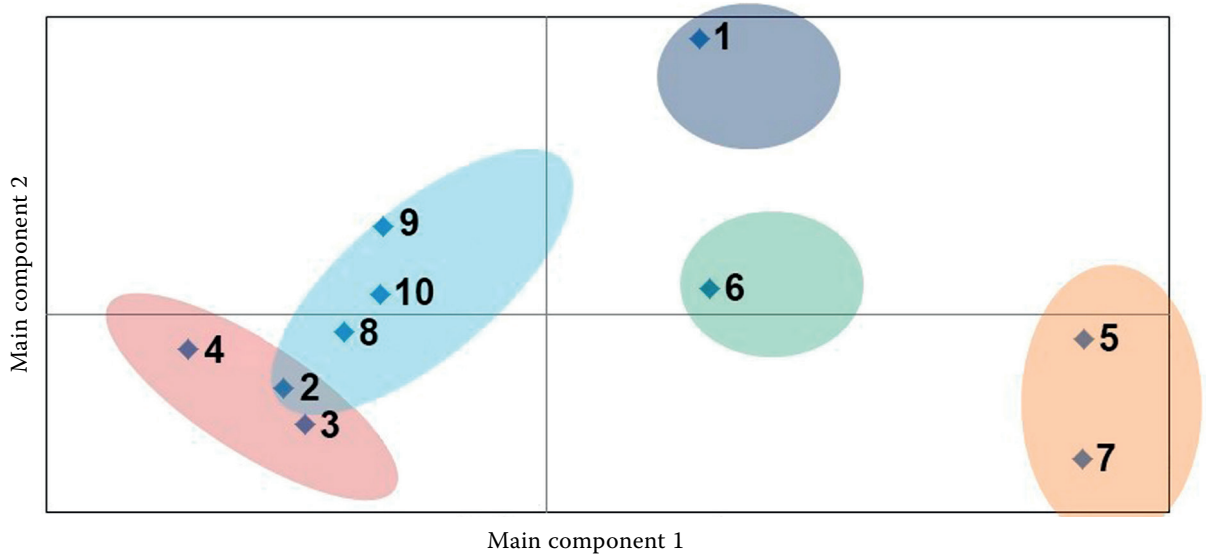

Figure 5. Principal component analysis of *Acer platanoides* populations from the Southern Urals
1 – KUD; 2 – ASK; 3 - BRS; 4 – BUR; 5 – UFA; 6 – ARC; 7 – TMZ; 8 – ALS; 9 – SLV; 10 – KMR

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situated in a lowland part of Bashkortostan, which is very poor in relict and endemic plant species. It is argued (Popov 1980) that broadleaved forests have colonized this territory after the last glacial event. Earlier, broadleaved forests (*A. platanoides*, *Q. robur* and other forest tree species of this type of vegetation) were situated only in local glacial shelters of the Southern Urals. Probably, the stands ARC, SLV and KMR are examples of populations with greater historical age and one more confirmation of the relatively high genetic diversity of refugia (Hewitt 2000). Having survived here in small favourable spots, broadleaved tree species of the Southern Urals Mountains may be sources for their expanding towards the lowlands of the Bashkirian Cis-Urals after climate warming. There are evidences that colonization of new habitats leads to a partial loss of alleles due to an effect of multiple foundation events (Petit et al. 2001). This seems to be the most likely explanation for the relatively low heterozygosity and number of alleles in trial plots BRS, BUR, TMZ and UFA. The population ALS has a relatively high level of genetic diversity, which is not typical of *A. platanoides* of Cis-Urals stands. It is located on the Belebey hills. When compared with the eastern macroslope of the Southern Urals, the composition of relict and endemic plant species on this geomorphological formation is almost the same (Popov 1980). This indirectly indicates greater age of local vegetation, including *A. platanoides*, on these hills. In addition, pollen analysis showed (Neustadt 1957) the existence of a glacial shelter of broadleaved forests to the south of this place on a vast upland, Obshchij Syrt. In general, the process of colonization leads to a decrease of allelic richness, but colonization from different sources leads to higher heterozygosities, because of the admixture of different alleles (Petit et al. 2003). Thus, the mixing of *A. platanoides* population gene pools from Obshchij Syrt and the eastern macroslope of the Southern Urals may determine a comparatively high genetic diversity revealed in trees of the trial plot ALS, which is untypical of other Cis-Urals stands TMZ, UFA, BUR, BRS and KUD.

The occurrence of small and geographically isolated populations, such as *A. platanoides* Cis-Urals ones, may result in the genetic drift (Bertolasi et al. 2015). But the patterns in the diversity and differentiation of the maple populations we have identified do not allow us to consider this possibility as an already performing process. The existence of sets of populations with similar gene pools (trial plots BRS and BUR, TMZ and UFA) and the absence of private and rare ISSR markers in Cis-Urals maple stands contradict the features that should be observed under the action of gene drift (Wright 1984). This is not a result of the successful external gene flow into geographically isolated stand fragments, which would mix their gene pools and rescue small populations from this microevolution force (Bushbom et al. 2011), as *A. platanoides* is an entomophilic species. Deforestation of the vast forest-steppe and forest zones of the European part of Russia has been occurring at an increasingly rapid pace in the last millennium in connection with the agricultural development of the territory (Neustadt 1957). But this process began intensively in the Southern Urals relatively not so long ago, since agriculture was not the main field of activity of the local nomadic Bashkir people. A few centuries ago, forests occupied almost 72% of the territory of Bashkortostan (Popov 1980). Now this cover has dropped to almost 40%. Given the relatively large forest cover of the mountainous part of the Republic (81.2–92.0%), the scale and speed of deforestation in other parts of the region seem impressive. At the same time, this relatively short period of deforestation means that the fragmented populations of long-lived *A. platanoides* in Bashkir Cis-Urals have been isolated for only few generations. Perhaps this is not enough for the effect of gene drift to become noticeable in the formation of population gene pools because of the "lag time" phenomenon (Smulders et al. 2009). This may be one of the reasons for the lack of a direct relationship between the genetic diversity of populations and the level of forest cover and the proportion of maple stands in broadleaved forests. Symptoms of gene drift appear only in trees of the stand KUD, a population on the species margin of the distribution range with extremely low density and number of individuals.

Considering the above-mentioned arguments, we assume that the fragmentation of the *A. platanoides* distribution range into small-sized and geographically isolated habitats could not yet become a significant reason for the relatively low heterozygosity and number of alleles in trees of the Bashkir Cis-Urals stands. This conclusion may have a significant impact on the development of a strategy of the conservation and use of maple genetic resources on the region scale and therefore its additional verification would be desirable. Different approaches can be used to do this. We decided to compare the

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data acquired with our results on genetic diversity of Cis-Urals populations of *Q. robur*, another obligatory key tree species of Russian broadleaved forests (Gorchakovsky 1972). This approach of a comparative parallel genetic analysis of species of the same region was successfully demonstrated using allozyme loci in a study of *A. platanoides* and *Betula pendula* populations of Northern Europe (Rusanen et al. 2003). Using double digest restriction site associated DNA sequencing (ddRAD) and Miseq, we lately developed a large set of new geographically highly informative loci of nuclear and plastid DNA of the oak (Degen et al. 2021b). A genetic inventory of 97 populations from the eastern part of the species distribution range using 412 SNP (Single-Nucleotide Polymorphism) loci allowed us to select 9 populations of the Cis-Urals from this larger set to compare the *Q. robur* genetic diversity with the data on *A. platanoides* of this study. The trial plots of the oak and maple were situated in close, and sometimes in the same locations (KUD, BRS, BUR, TMZ, UFA). In comparison with the maple, which demonstrated the existence of a local spatial genetic structure in the southern, middle, and northern parts of the Bashkir Urals, the *Q. robur* gene pool was much more homogeneous throughout all this territory. A genetically efficient pollen flow over long distances may be reason for maintaining similarities of the oak populations. Using microsatellite loci and reconstruction of possible parental pairs of alleles in embryos of a geographically isolated oak stand, we demonstrated earlier that genetic efficiency of pollen flow erases differences between populations on tens of kilometres (Bushbom et al. 2011). Obviously, this scale is much larger than the flight distances of the insect pollinators of the entomophilic maple. Scattered populations of insect-pollinated *A. platanoides* were also less heterozygous and much more differentiated in allozyme loci than wind-pollinated *B. pendula* with light wind-dispersed seeds and a more continuous distribution (Rusanen et al. 2003). However, we revealed that genetic differences in the Bashkir Cis-Urals oak populations increase on a geographic scale by the type of isolation by distance (Degen et al. 2021a). The correlation coefficient between genetic and geographic distances was statistically significant ($r = 0.498$, $P < 0.05$). The heterozygosity and number of alleles were very similar in the stands and varied within the slight limits H_E = 0.172–0.206 (on average H_E = 0.197 ± 0.003) and n_e = 1.289–1.349 (1.334 ± 0.006), respectively. The similarity of the genetic diversity of isolated small-sized populations and the pattern of "isolation by distance" are contradictions to expectations under genetic drift. Thus, the results on *Q. robur* confirm our previous conclusion about no significant effect of considerable habitat fragmentation of the Bashkir Cis-Urals broadleaved forests on *A. platanoides* regional gene pools.

There are evidences that nemoral broadleaved tree species migrated in the north-east direction during the past warm climate periods and even reached all together Western Siberia (Velichko et al. 1997). It is predicted that the current climatic change, which significantly affects the species geographical distribution (Sun et al. 2020), may cause new colonization of more northern and eastern territories. When implementing this scenario, our results demonstrate that *Q. robur* from the vast majority of stands in Bashkortostan and *A. platanoides* from broadleaved forests of the Southern Urals western macroslope will form new populations with relatively high genetic diversity. And vice versa, Cis-Urals geographically isolated stands of the maple will have a lower potential in this field and it may decrease in future. It seems that this problem will be much worse for the maple forests outside the studied area. Using the "Forests of Russia" database (http://178.176.30.40:8282/#/), we calculated areas of forests with dominance of *A. platanoides* in 35 regions of the European part of Russia. The average area of stands with dominance of the species per region (9 020 ha) does not reflect the real patterns of geographical distribution. Except Bashkortostan with its rich maple resources, four regions of the Volga region and the Urals (Tatarstan, Samara, Orenburg and Saratov), which are also located in the eastern part of the distribution range of the species, have 33.8% of the Russian maple forests areas. Only 13.3% of the *A. platanoides* stands are presented in 12 regions, and 18 ones have about 2.0%. Therefore, we assume that the decrease in genetic diversity in the maple forests of the Bashkir Cis-Urals we revealed can be expressed much more in the Russian regions with fewer resources of *A. platanoides*. A large part of forest fragments, which were isolated from each other throughout the last millennium due to agricultural development of the forest-steppe zone (Neustadt 1957), can be too small to reproduce gene pools at a sufficient level to sustain the viability of populations. When collecting seeds in such stands, various negative

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changes in gene pools are possible (Degen et al. 2021c). This argues the need for an extended study of this possible threat, both for the maple and other associate broadleaved forest tree species.

CONCLUSION

In this study, ISSR markers were used to explore genetic diversity and differentiation among *A. platanoides* populations on the eastern margin of the species range of distribution. We revealed relatively large differences in heterozygosity and number of alleles between two groups of stands which are situated on the western macroslope of the Southern Urals and Bashkir Cis-Urals plains. In the second group, representing strongly fragmented geographically isolated small maple stands, lower heterozygosity and allelic diversity of most populations were found. Local groups of populations that are confined to the northern, middle and southern parts of the Urals were identified. The patterns of the revealed population spatial structures are probably caused by the multiplicity of refugia in the Southern Urals and neighbouring geomorphological formations and the complexity of the migration routes of *A. platanoides* from glacial shelters. Data on SNPs allowed us to find out in *Q. robur* another associate species of broadleaved forests, relatively similar levels of population genetic diversity in the same area and an absence of the local spatial structure of genetic pools. All these patterns indicate that the fragmentation of the broadleaved forests of this vast Bashkir Cis-Urals into geographically isolated habitats has not yet led to noticeable genetic consequences.

The presented research results may be useful for developing strategies for the conservation and use of *A. platanoides* genetic resources in the Southern Urals. We can already offer some solutions to this problem. The genetic uniqueness of the central mountainous population and its relatively high genetic diversity may be used for long-term gene pool conservation purposes and reforestation at local forest enterprises. Distinctly different ecological conditions of the western macroslope of the Southern Urals and Bashkir Cis-Urals require a careful approach to the use of seed material from mountain populations for reforestation in the plain part of the studied area. The enrichment of the relatively low genetic diversity of Cis-Urals populations of the species can be achieved using an admixture of gene pools of local populations.

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