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Population genetic diversity in *Quercus robur* and *Ulmus laevis* in Southern Urals (Russia): a comparatively study of adults and progeny in localities with contrast forest cover

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Abstract

Extensive forest areas decreased and fragmented during anthropogenic development of the zone of broad-leaved forest tree species in Russia. The pedunculate oak (*Quercus robur* L.) and the European white elm (*Ulmus laevis* Pall.), important key components of these ecosystems, suffered last few centuries of extreme climate events, attacks of insects and diseases. Using ISSR genetic markers, we compared expected heterozygosity and allelic diversity of these two species in natural and artificial stands, planted and naturally regenerated progeny. Weak differences in the genetic diversity in *Q. robur* and *U. laevis* were revealed in areas with different forest cover and participation of the species in a stand composition. Overall, we found that the genetic diversity of parent natural and artificial stands is well reproducing in natural offspring and planted saplings. But the tree species studied express both higher and lower heterozygosity in planted trees in comparison to natural stands.

Key words: broad-leaved tree species; forest decline; genetic diversity; ISSR markers

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1. Introduction

In the European part of Russia, broad-leaved forests cover a vast territory from the western borders of the country to the Southern Urals. As a rule, they form communities with polydominant tree layer of species with wide ecological traits (Baisheva et al. 2019). The biggest geographical type of these forests mainly includes mixed stands of the pedunculate oak (*Quercus robur* L.), small-leaved linden (*Tilia cordata* Mill.), Norway maple (*Acer platanoides* L.) and elms (*Ulmus laevis* Pall. and *U. glabra* Huds.). Numerous and extensive paleo- and geobotanical investigations (Neyshtadt 1957; Popadyuk et al. 1995) have demonstrated that irreversible shifts have occurred in both the natural boundaries and species composition caused by past long-term climatic changes and anthropogenic disturbance of the territory. As a result, the former relatively unbroken zone crumbled into fragments of dif-

ferent sizes surrounded by agrarian and industrial lands. Birches (*Betula pendula* Roth.), trembling aspen (*Populus tremula* L.) and Scots pine (*Pinus sylvestris* L.), as pioneer species, get into the communities of broad-leaved tree species and gradually displace them. *Q. robur* and *U. laevis* are two the most affected participants of these processes (Popadyuk et al. 1995). The long-time decline of the pedunculate oak was reinforced in the XXth century due to extremely low winter temperatures, wide spread of pathogens and multiple mass insect attacks (Oleksyn & Przybyl 2007). In comparison to other species, *U. laevis* especially suffers of transformation in land uses due to the narrowness of ecological niches (Collin 2003; Čurn et al. 2014), comparatively low density of occurrence in a stand and diseases (Venturas et al. 2015). It is expected that climate change will amplify such disturbances (Dyderski et al. 2018; Augustynczik et al. 2019). The patterns mentioned pose a serious threat to the survival of broad-

leaved forests in a large part of the area of their distribution. Ultimately, all these processes create a threat to population genetic diversity (Cortés et al. 2020), which is a base for the adaptability and adaptation of tree populations (Prunier et al. 2016; Kremer & Hipp 2020). Various forest management and silvicultural operations (seed collection, artificial and natural regeneration, thinning, etc.) affect genetic composition of stands (Degen et al. 2021). Possible genetic consequences of these works are well studied in Western and Central Europe (Bradshaw 2004). They can cause a restricted gene flow, decreasing effective population size, loss of rare alleles, appearance of founder effects, etc. (Ratnam et al. 2014; Aravanopoulos 2018). European and Russian broad-leaved tree species have the same ecological traits but another space distribution, composition, demography and disturbance of stands. Unfortunately, influence of these features and impact of domestic forestry practices on genetic diversity of local populations have been little investigated. This prevents the implementation of conservation measures, especially in fragmented forests (Aguilar et al. 2008).

The aim of this pilot study was to provide results on levels of genetic diversity in populations of *Q. robur* and *U. laevis* eastern species' range of distribution in the Southern Urals. Of particular interest, were three questions: (a) do we have evidences of differences in population genetic diversity in localities with contrast forest cover? (b) how does the genetic diversity of parental populations is reproducing in progeny? (c) to what extent are natural stands and plantations different in genetic diversity? To answer these questions, we used ISSRs, relatively reproducible and repeatable dominant markers that have been successfully used in plant genetics (Vijayan 2005). We tested the hypothesis that small isolated stands in areas with relatively low forest cover have less genetic diversity and greater genetic differentiation among populations, and this affects the genetic diversity of offspring during artificial and natural seed regeneration.

2. Materials and methods

2.1. Study area and sampling

The study area is located on the Southern Urals, which are the biggest part of the Ural Mountains and the natural boundary between Europe and Asia. Long meridional ridges of this geomorphological formation are a natural barrier to the moist and warm Atlantic air masses. Therefore, the climate of the Trans-Urals is more continental, hotter in summer, colder and drier in winter. The natural vegetation of the Southern Urals is characterized by unique species richness due to the climate-regulating role of mountains, the absence of Pleistocene glaciation and the survival of ancient plants, contacting of the vegetation of mountain taiga, deciduous broad-leaved and boreal forests, clear latitude and altitude zonality, diverse ecological conditions (Baisheva et al. 2019). At the same time, the Southern Urals is a well-defined physical and geographical boundary of the distribution areas of many European nemoral plant species, such as *Q. robur, A. platanoides, U. laevis, U. glabra* and *T. cordata* (Gorchakovsky 1988). Bashkortostan is a Russian region (Fig. 1) that occupies most of the Southern Urals. Total area of the Republic is about 14.3 million hectares, of which forest cover accounts for almost 40% (5.7 million ha). The region has lost about half of its forest cover in last few centuries. Today, forest cover in some districts decreased by 8–10% (Popov 1984). The broad-leaved forest-forming tree species occupy 36.2% of the forest cover. A part of stands is located in the lowland Cis-Urals, which have been greatly fragmented during agricultural development in last few centuries. Relatively undisturbed forests dominated by the broad-leaved tree species survived on a relatively narrow meridional band of the foothills and low mountains of the western macrosclope of the Southern Urals.

According to the information of the database "Forests of Russia" (https://maps.roslesinforg.ru/#/), we determined for each of forestry enterprises of the Republic of Bashkortostan the forest cover values and proportions of *Q. robur* and *U. laevis* in the forest area. After that,

Table 1. Information on the trial plots.

FE	Forest cover [%]	Forest area [ha]	Areas of the forests with predominance		Trial plots	
			Q. robur	*Ulmus* spp.	Abbr.	Coordinates [latitude, longitude]
AR	50.6	211 270	5 982 (2.8)	10 027 (4.7)	O–ATn	54.4220, 56.8004
					O–ATa	54.5333, 56.8035
					O–ASn	54.5333, 56.8035
					E–ATn	54.4671, 57.1068
					E–ASn	54.4671, 57.1068
TM	22.4	207 538	16 625 (8.0)	522 (0.3)	O–TTn	54.5801, 54.0960
					O–TSn	54.5801, 54.0960
					O–TSa	54.5813, 54.0941
					E–TTa	54.6211, 53.7529
					E–TSn	54.6213, 3.7530
KD	45.6	227 701	194 (0.1)	65 (<0.1)	O–KTn	56.5509, 55.6933
					O–KSn	56.5514, 55.7145
Ufa	16.5	150 941	12 170 (8.1)	3 278 (2.2)	E–UTa	54.7660, 55.7563

FE – forest enterprises. The abbreviation of the names of trial areas includes: O – oak, E – elm, A – Arkhangelsk, T – Tuymazy, K – Kueda, T – trees, S – saplings, letters n and a indicate natural and artificial origins, respectively. Proportions of a species in the forest area of a forest enterprise are shown in the brackets (%).

Fig. 1. Location of the Republic of Bashkortostan and the study areas.

forestry enterprises with contrasting values of these parameters were selected. An additional criterion used was the presence of both natural stands and plantations of each of these species in the same locality.

Thirteen trial plots were established in natural and man-made stands of *Q. robur* and *U. laevis* in three localities of the Republic of Bashkortostan (Arkhangelsk, Tuymazy and Ufa districts) and a neighboring part of Perm region in the district of Kueda (Fig. 1), differing in forest cover. These species have comparitively small areas (Table 1) but they form extensive mixed stands dominated by linden and maple. *T. cordata* is the most distributed tree species in the forest areas of forest enterprises (FE) in Ufa and Arkhangelsk (48.7 and 45.0%, respectively). *A. platanoides* is one of the most distributed species only in Arkhangelsk FE (6.3%). In Kueda and Tuymazy FE, stands with domination of *Q. robur* and *U. laevis* occupy 27.3% and 14.8% of the forest areas, respectively.

One set of samples is selected on the territory of Arkhangelsk FE (Table 1). The broad-leaved forests of this forest enterprise are still exist without being affected by noticeable human influence. For the study *Q. robur*, we selected a natural stand (trial plot O–ATn), a neighboring plantation of reproductive trees (O–ATa) and natural saplings (O–ASn) under canopy of this artificial stand. Two trial plots of *U. laevis* were also established in Arkhangelsk FE, which represent a natural stand (E–ATn) and its natural progeny (E–ASn).

A set of trial plots presents forests of both species in Tuymazy FE on the Bugulma-Belebey Upland, which is separated from the mountains by a wide valley of the Belaya river. Over past several centuries, the broad-leaved forests of this area have been exchanged by agricultural territories (mainly arable lands). Now, stands occupy mainly small sites of rugged landscapes where the relief precludes to use them for agrarian uses. Trees of *Q. robur* remain relatively common up to now in local mixed forests but trees of *U. laevis* are comparatively rare in a stand composition. A set of oak trial plot includes a natural stand (O–TTn), saplings of natural regeneration under its canopy (O–TSn) and planted saplings in the neighboring habitat (O–TSa). A field-protective forest belt of *U. laevis* trees and a neighboring area with an abundant natural offspring of this plantation was found in Tuymazy FE. They were used to establish trial plots E–TTa and E–TSn, respectively.

Saplings of the only plantation of *U. laevis* in a forest lands we found in the Republic of Bashkortostan are presented by the trial plot E–UTa. The plantation is in the territory of a local forest enterprise in Ufa, the capital of Bashkortostan. This area has comparatively low forest cover. The elm is presented mostly in the wide valley of the Belaya river, protected areas and stands near settlements as scattered groups of trees.

A set of two trial plots was established in the zone of southern taiga (Perm region, Kueda FE), where areas of *Q. robur* have decreased gradually over time in last few millenniums due to climatic fluctuations. The last northern outposts of the species are situated here, they are presented as few geographically isolated small stands and groups of trees. *U. laevis* is absent in this area. The

samples of *Q. robur* in the trial plot O–KTn include only 8 reproductive trees and 23 not mature young trees and saplings (30–700 cm in height) under canopy of a mixed stand. In an abandoned pasture at the edge of this forest, we found a comparatively abundant natural regeneration of the oak with heights of saplings from 10 cm to 8 m. The trial plot O–KTn was established in this site.

2.2. DNA isolation and ISSR analysis

In general, leaf samples of trees and saplings were collected from 32 individuals in all the trial plots, excluding O-KTn with sample size of 31 plants. For the ISSR genetic analysis (Zietkiewicz et al. 1994), fresh leaves of trees and saplings were dried using silica gel. Genomic DNA was isolated using a standard method (Rogers & Bendich 1985). Quantitative analysis and spectral characterization of the DNA isolated (10 ng·µl⁻¹) were done on a Spectrophotometer™ NanoDrop 2000 ("Thermo scientific", USA). For the PCR amplification, we used MJ MiniCycler ("Bio-rad", USA). As a negative control, 5 µl of deionized water was added to the reaction mixture. Products of amplification were separated using electrophoresis in 1.7% agarose gels in 1xTBE buffer, stained with ethidium bromide and photographed under UV light (Gel Doc XR, BioRad, USA). To determine sizes of DNA fragments, molecular weight markers ("LLC-Sibenzym-M", Russia) were used. Five primers with the best clearly identifiable and repeatable amplification products for both the species were selected for the genetic analysis during a pre-screen among 20 (*Q. robur*) and 19 (*U. laevis*) available ISSR primers. All the analyses were performed in triplicate. Samples with well repeated patterns were used for our further work.

The mean number of alleles per locus (n_a), mean effective number of alleles (n_e), mean expected heterozygosity (H_E) and their respective standard errors were determined. Gene parameters were calculated using POPGENE 1.31 and specialized macros GenAlEx 6.3.

3. Results

Five primers used for *Q. robur* produced a total of 108 clearly identifiable bands of which 83 (76.9%) were polymorphic (Table 2). Each of them reveals relatively similar portions of polymorphic bands (76.6±3.8%, changes are within 70.0–90.5%). The average expected heterozygosity was H_E=0.211±0.009 and the average allelic diversities were n_a=1.76±0.027 and n_e=1.34±0.015, respectively. The greatest genetic diversity was recorded in the plantation O–ATa (Table 3). Under the canopy of this stand, the expected heterozygosity and number of alleles slightly decreases in natural progeny (O–ASn). Natural population (O–ATn) have comparatively lower levels values of H_E, n_a and n_e. In contrast to the Arkhangelsk population, genetic diversity of the natural oak grove

in Tuymazy FE (O–TTn) exceeded heterozygosity and number of alleles in natural progeny (O–TSn) under canopy of this stand. The lowest level of genetic diversity was revealed in planted saplings (O–TSa). Surprisingly, the level of genetic diversity in natural saplings (O–KSn) in abandoned agricultural land was relatively high, although they are offspring of a geographically isolated small stand O–KTn with only 8 reproductive trees of *Q. robur*. In an abandoned pasture 76 polymorphic variants were found in the individuals, 8 of which are absent in trees of the parental population.

Table 2. Information on the ISSR genetic markers of *Quercus robur*.

Primers	Repeat motif (5'→ 3')	Size range, bp	No of fragments	
			In total	Polymorphic*
M1	(AC)₈CG	200–1800	20	14 (0.700)
M3	(AC)₈CT	90–1000	21	19 (0.905)
M27	(GA)₈C	100–1000	20	15 (0.750)
X11	(CA)₆G	120–1800	27	21 (0.778)
X1	(AGC)₆G	120–2200	20	14 (0.700)

* Proportions of polymorphic bands are shown in the brackets.

Table 3. Expected heterozygosity and allelic diversity in adult trees, naturally regenerated and planted saplings in *Quercus robur*.

Samples	H_E	n_a	n_e	Comments
O–ATn	0.202±0.016	1.75+0.43	1.3±0.30	Natural stand
O–ATa	0.249±0.015	1.86±0.35	1.40±0.31	Artificial stand
O–ASn	0.231±0.017	1.82±0.38	1.37±0.33	Natural progeny of O–ATa
O–TTn	0.221±0.017	1.78±0.41	1.36±0.34	Natural stand
O–TSn	0.191±0.015	1.77±0.42	1.30±0.30	Natural progeny of O–TTn
O–TSa	0.170+0.016	1.63±0.48	1.27+0.31	Planted saplings
O–KTn	0.208±0.017	1.66±0.47	1.33±0.32	Trees and saplings of a small stand
O–KSn	0.215±0.017	1.77±0.43	1.35±0.35	Natural progeny of O–KTn

The proportion of polymorphic loci in all the samples of *Ulmus laevis* (Table 4), depending on the ISSR primer analyzed, varied from 45.5 to 86.6% (on average, 67.1±6.5). Excluding M1, other primers revealed relatively similar levels of their portions (45.5 vs. 66.7–86.6%). The average expected heterozygosity was H_E=0.200±0.010 and the average allelic diversities were n_a=1.65±0.011 and n_e=1.34±0.019. However, both artificial and natural stands E–TTa and E–UTa from the areas with less forest cover demonstrate lower values of H_E (Table 5) in comparison to the population in Arkhangelsk FE with relatively rich resources of the species (Table 1). Like the oak plantation in this forest enterprise (trial plot O–ATa), natural progeny of the elm (E–ASn) possess much higher expected heterozygosity than the parental trees (E–ATn). When comparing trees of the plantation E–TTa in Tuymazy FE with saplings of natural regeneration (E–TSn) under canopy of this man–made stand, this pattern is not observed. The values of H_E, n_a and n_e in these samples are almost identical. The genetic diversity in another plantation (E–UTa) in Ufa is similar with expected heterozygosity and number of alleles in samples of Tuymazy FE.

Table 4. Information on the ISSR genetic markers of *Ulmus laevis*.

Primers	Repeat motif (5'→ 3')	Size range, bp	No of fragments	
			In total	Polymorphic*
M1	$(AC)_8CG$	200–820	11	5 (45.5)
ISSR–4	$(TG)_8GC$	230–2800	21	14 (66.7)
ISSR–5	$(AG)_8CA$	240–970	15	13 (86.6)
ISSR–7	$(CTC)_6C$	320–790	10	7 (70.0)
ISSR–9	$(ACG)_7G$	230–970	18	12 (66.7)

* Portions of polymorphic bands are shown in the brackets.

Table 5. Expected heterozygosity and allelic diversity in adult trees, naturally regenerated and planted saplings in *Ulmus laevis*.

Samples	H_E	n_a	n_e	
E–ATh	0.201±0.023	1.65±0.48	1.35±0.38	Natural stand
E–ASn	0.239±0.023	1.69±0.46	1.41±0.38	Natural progeny of E–ATh
E–TTa	0.189±0.022	1.65±0.48	1.32±0.36	Artificial stand
E–TSn	0.183±0.022	1.63±0.49	1.30±0.35	Natural progeny of E–TSn
E–UTa	0.187±0.023	1.63±0.45	1.32±0.39	Planted saplings

4. Discussion

There are numerous causes of deforestation and they form long causation chains that eventually lead to the forest cover decline (Contreras-Hermosilla 2000). Reducing the size of stands of forest tree species populations and increasing distances between them, the fragmentation of habitats can have an impact on the gene flow between populations, induce genetic drift in populations, and increase mating among relatives (Bertolasi et al. 2015). All the processes can lead to the loss of genetic diversity within populations. In general, more fragmented populations have comparatively greater genetic differentiation between populations (Vranckx et al. 2012; Poudel et al., 2014). When planning the study, we also expected comparatively lower genetic diversity in small isolated stands in areas with relatively low forest cover (FE in Tuymazy and Ufa) or low participation (Kueda FE) of *Q. robur* and/or *U. laevis* in a stand composition. Our expectations were confirmed only as a tendency for the elm. The heterozygosity of the Arkhangelsk population with relatively large resources of the species was 6.0–8.9% higher than other samples compared (the differences are not statistically significant). Alleles diversity in both species has very similar values in all the samples. The extensive Arkhangelsk population of *Q. robur* with a large participation of the oak is even slightly less heterozygous than the natural stand in Tuymazy FE with relatively low forest cover and the isolated and extremely small population in the southern taiga out range of distribution of the species (Kueda FE). This can be caused by the effectiveness of gene flow on far distances. Two nearest oak stands are located at 18 and 31 km from the trial plot O–KTn. Pollen flow from these sources may counteract a loss of genetic variation in the isolated and small population O–KTn with few reproductive trees. It has been shown that the immigration of pollen and seeds from outside counteracts the decrease in genetic diversity in the next generation, even in stands with a small number of mother

trees (Kramer et al. 2008; Bacles & Jump 2011). Thus, efficient gene flow may be one of the factors leading to relatively high genetic diversity and relatively weak differentiation between populations that we have identified for two wind-pollinated species, *Q. robur* and *U. laevis*. We demonstrated earlier the realism of this assumption using 9 microsatellite loci and reconstruction of possible parent alleles in trees of two geographically isolated Trans-Ural small stands of *Q. robur*. It was revealed that at least 35% of pollination was carried out by "alien" pollen from other oak groves, which are situated on distances of tens km from this population (Bushbom et al. 2011).

However, the efficiency of the genetic flow alone cannot be the only explanation for the similar levels of genetic variation of the populations studied in localities with contrasting fores cover, especially for *U. laevis*. We do not exclude the effectiveness of step pollen flow between fragmented broad-leaved forests with this wind-pollinated tree species from localities with greater forest cover. However, good empirical data on gene flow are necessary to estimate genetic consequences of forest fragmentation of forests (Lowe et al. 2005; Kremer & Hipp 2020). When using microsatellite loci, it was indicated that seeds of the *U. laevis* mother trees disperse not far (on average 37 m, maximal distance reaches 1,165 km), although they are winged and adapted for wind-dispersal (Nielsen & Kjær 2010). That's probably why this species possesses comparatively low levels of genetic diversity in European populations. This pattern could be also due to a historic bottleneck of the species in Western Europe (Nielsen & Kjær 2010; Čurn et al. 2014). For this reason, there should be another reason for the comparable levels of expected heterozygosity and allelic diversity of the populations of *U. laevis* of the Southern Urals in areas with contrasting forest cover. Fossil pollen studies (Neyshtadt 1957) argue an existence of Pleictocene and Holocene refugia of on the western macroslope of the Sothern Urals and the Bugulma-Belebey Upland. This circumstance and absence of such bottleneck could be a next reason of a comparatively high genetic diversity of these two species in the area studied. It is possible that the decrease in the effective population sizes of both species studied could have occurred here only in historically recent times. On the forestry inventory information in Bashkortostan, the forest cover decreased in the region mainly in last two centuries (Baisheva et al. 2019). It is possible that the decrease in the effective population sizes of both species could have occurred here only in this historically short time and too few generations have been changed to reveal a serious genetic erosion. Forest cover in the Republic has decreased from 72 to 40% only in last two centuries of intensive development of agriculture (Popov 1984). The longevity of the oak and elm trees may retard population extinction and preserve former genetic diversity.

Thus, a genetically efficient pollen flow and a relatively short period of forest decline in the Southern

Urals may be the main reasons for the comparatively high genetic diversity and relatively weak differentiation of populations of *Q. robur* and *U. laevis* in areas with contrasting forest cover. A similar interpretation is made when studying *Taxus cuspidata* populations, fragmented during a large-scale deforestation in China (Su et al. 2018). Chloroplast and mitochondrial DNA haplotypes showed high degrees of genetic diversity of 26 natural and transplanted populations, extensive gene flow over the entire distribution range and historical stability of effective population size. Ancestor haplotypes were widespread among the populations studied. The short time of fragmentation, long-distance pollination, seeds transfer and potential adaptation to past climatic oscillations were selected as explanation of this pattern. No considerable influence of serious reduction in the populations size due to decreasing the forest cover on the microsatellite loci genetic diversity in natural juvenile cohort of Norway spruce from Poland was revealed in other study (Chomic-Zegar et al. 2015).

Our results on the gene pools of *Q. robur* and *U. laevis* also show that the possibility of restoring broadleaved forests of the Urals by establishing plantations and assisting natural regeneration has not yet been lost in localities with low forest cover. At the same time, it is necessary to take into account the results of numerous studies which have demonstrated that silvicultural practices of stand establishment impact the tree genetic composition (Bradshaw 2004). The decrease in forest cover during such works leads to the inbreeding, gene flow, mating system, selection, demographics and the population gene pools (Fageria & Rajora 2014). It was shown by computer simulations that collecting seeds in large stands from many relatively distant seed trees makes it possible to sufficiently reflect the populations genetic structure. And vice versa, a limited number of parental trees causes changes in genetic diversity in next generation (Blanc-Jolivet & Degen 2014). We confirmed these predictions by our empirical results, but they are not systematic on the patterns revealed. A local population of *Q. robur* in Arkhangelsk FE is less heterozygous (by 23.3%) and has lower number of alleles (6.3%) than a local artificial stand. According to the information from the forest enterprise, seeds were collected from many local stands and trees to establish the plantation. There are also other evidences that the origin of stands (natural or artificial) and their genetic diversity are not strongly related (Wehenkel et al. 2009). At the same time, a pair of artificial and natural stands of the species in Tuymazy FE showed an opposite result. This plantation has lower values of heterozygosity (by 13.6%) in comparison to a local natural population and all the stands in forest enterprises in Arhangelsk and Kueda. There is no an information about origin of seeds used to establish this man-made plantation. We can only assume that the effect of small size of seed trees is observed here. The offspring of both man-made stands of *U. laevis* and *Q. robur* is similar in the genetic diversity with natural populations or exceed it. Such patterns were revealed when comparing genetic diversity in natural and man-made forests of other forest tree species (Gauli et al. 2009). When studying Norway spruce in Poland, similar genetic diversity in progeny of stands in localities with of both relatively large and low forest cover (Chomic-Zegar et al. 2015). If the seed collection has been done using few seed trees, usually this creates a genetic bottleneck, decrease of initial genetic diversity of a parent stand in next generations (Blanc-Jolivet & Degen 2014). Even during natural regeneration, the establishing of strong family structures can be seen (Jolivet et al. 2012; Vakkari et al. 2020). Using the SNPs of 366 polymorphic loci mentioned, we revealed 6 full-sibs and 12 pairs of half-sibs in an artificial small park stand of *Q. robur* with comparatively high genetic diversity (Ae = 1.39, H_O = 0.278, H_E = 0.226) (Degen et al. 2021). We concluded that the seeds were collected from a very limited number of trees. An isolated natural stand with few parental trees of the oak with comparatively high heterozygosity and number of alleles (Ae = 1.40, H_O = 0.255, H_E = 0.238) includes 12 pairs of half-sibs. This argues that family genetic clustering can be found even in natural stands with relatively high genetic diversity. Conversely, collecting seeds from many trees and plantings can provide high genotypic diversity in plantations. Using this set of loci, we have confirmed (Degen et al. 2021) that planted trees of the trial plot O–ATa have higher levels of genetic diversity in comparison with the natural pure stand O–ATn (Ae = 1.44, H_O = 0.266, H_E = 0.255 and Ae = 1.42, H_O = 0.263, H_E = 0.245, respectively). So, both types of markers, ISSRs and SNPs, showed the same patterns on these samples.

In general, we concluded that that present forest decline and fragmentation has not yet caused a serious erosion of *Q. robur* and *U. laevis* populations gene pool in the Southern Urals in localities with different forest cover.

Acknowledgements

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