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Phenetic analysis of *Populus nigra*, *P. laurifolia* and *P.* × *jrtyschensis* in natural hybridization zone

A.V. Klimov[1] ✉, B.V. Proshkin[2]

[1] Novokuznetsk Branch of Kemerovo State University, Novokuznetsk, Russia
[2] Novosibirsk State Agrarian University, Novosibirsk, Russia

The wide spread of hybridization in the genus *Populus*, including spontaneous hybridization, caused by cultivars, requires studying the variability and inheritance of morphological traits by hybrids for initial tracking of these processes. The analysis of endogenous, intra- and inter-population variability was performed on 533 individual poplar trees in seven populations of *P. nigra*, seven populations of *P. laurifolia* and four populations of *P.* × *jrtyschensis* in the Tom river basin. On each specimen, 15 leaves from short mid-crown branches were collected to determine the shape of the leaf blade, the shape of its tip and base, as well as the branch morphotype. Some biometric indicators were proposed for geometric assessment of the leaf blade shapes of poplar species. The analysis showed that of all the traits examined only the leaf blade shape did not meet the criterion for "phene", since it is usually represented by several forms in the crown of one and the same tree. In all the species studied, the level of their intra-population diversity was found to be much higher than the inter-population one. According to the increase of intra-population variability of qualitative traits, the taxa could be ranked as *P. nigra* < *P. laurifolia* < *P.* × *jrtyschensis*. The share of inter-population diversity differed among the species studied, accounting for 21.5% in *P. laurifolia*, 13.8% in *P. nigra* and 8.0% in *P.* × *jrtyschensis*. The *P. laurifolia* populations showed the greatest inter-population differentiation, most likely because of a disjunct distribution due to narrow specialization to the montane river environment. The lower differentiation in *P. nigra* is probably due to the facts that this species dominates the poplar stands of the Tom River basin; its populations are not fragmented and are linked by vast gene flow. In *P. nigra*, an increase in the diversity of qualitative characteristics and phenotypes was observed in populations confined to hybridization centers. Natural selection is most likely the factor governing the inter-population differentiation in *P.* × *jrtyschensis*, leading to the predominance of F_1 hybrids in populations and hence to a sharp decrease in inter-population variability.

Key words: *Populus*; hybrids; qualitative traits; morphological markers; morphometry; phenotypic diversity.

Фенетический анализ *Populus nigra*, *P. laurifolia* и *P.* × *jrtyschensis* в зоне гибридизации

А.В. Климов[1] ✉, Б.В. Прошкин[2]

[1] Новокузнецкий институт (филиал) Кемеровского государственного университета, Новокузнецк, Россия
[2] Новосибирский государственный аграрный университет, Новосибирск, Россия

Широкое распространение гибридизации в роде *Populus*, в том числе спонтанной, вызванной использованием культиваров, требует изучения изменчивости и наследования морфологических признаков гибридами для первичного отслеживания этих процессов. Анализ эндогенной, внутри- и межпопуляционной изменчивости выполнен на 533 особях в семи популяциях *P. nigra*, семи – *P. laurifolia* и четырех – *P.* × *jrtyschensis* бассейна реки Томи. На каждой особи на 15 листьях с укороченных побегов в средней части кроны исследовались: форма листовой пластинки, форма ее верхушки, основания и морфотип побегов. Предложен вариант использования биометрических показателей для геометрической оценки формы листовой пластинки видов тополя. Проведенный анализ показывает, что из исследованных признаков критерию фена не соответствует только форма пластинки, поскольку обычно представлена несколькими типами в кроне одного дерева. У всех изученных видов уровень их внутрипопуляционного разнообразия значительно выше межпопуляционного. По нарастанию внутрипопуляционной изменчивости по качественным признакам таксоны располагаются в следующем порядке *P. nigra* < *P. laurifolia* < *P.* × *jrtyschensis*. Доля межпопуляционного разнообразия отличается у исследованных видов: *P. laurifolia* – 21.5 %, *P. nigra* – 13.8 %, *P.* × *jrtyschensis* – 8.0 %. Популяции *P. laurifolia* отличаются наибольшей межпопуляционной дифференцировкой, что, вероятно, связано с дизъюнктивным распространением вида в силу узкой специализации к условиям горных рек. Меньшая дифференциации у *P. nigra* обусловлена тем, что этот вид доминирует в топольниках бассейна р. Томи, его популяции не фрагментированы и связаны между собой обширным потоком генов. У *P. nigra* наблюдается увеличение разнообразия качественных признаков и фенотипов в популяциях, приуроченных к очагам гибридизации. Фактором, определяющим межпопуляционную диффе-

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✉ e-mail: populus0709@mail.ru

ренцировку у *P.* × *jrtyschensis*, является действие отбора, приводящего к преобладанию в популяциях гибридов F_1, что ведет к резкому снижению межпопуляционной изменчивости.

Ключевые слова: *Populus*; гибриды; качественные признаки; морфологические маркеры; морфометрия; фенотипическое разнообразие.

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The hybridization process, which is quite common among the *Populus* species, increases morphological diversity and often seriously impedes taxon attribution. The latter is especially important as the use of adventitious species and cultivars for parking and afforestation resulted in spreading of spontaneous hybridization (Meirmans et al., 2010; Thompson et al., 2010; Talbot et al., 2012; Vanden Broeck et al., 2012; Roe et al., 2014; Hu et al., 2017). The threat of genetic contamination of natural populations of aboriginal species via uncontrolled interbreeding with subsequent introgression urges for extensive studies of variability and inheritance of morphological traits, caused by poplars' hybridization. Molecular markers are the best tools to identify hybrids; however, the primary screening of these processes in populations should be based on phenetic data, which will also facilitate and focus phenetic studies.

In Siberian areas, where *P. laurifolia* (*Tacamahaca* Mill. section) and *P. nigra* (*Aigeiros* Lunell section) grow together, their natural hybridization resulted in the emergence of the hybrid taxon named *P.* × *jrtyschensis* Ch. Y. Yang. Previously, we studied some quantitative and qualitative traits of these species, as well as their asymmetrical hybridization in the Tom River basin (Klimov, Proshkin, 2016, 2017; Proshkin, Klimov, 2017a).

The aim of the present study was to analyze the diversity of qualitative traits of *P. nigra, P. laurifolia* and *P.* × *jrtyschensis* in the area of their natural hybridization according to the "phene" criteria.

Materials and methods

The variability of morphological traits was analyzed in poplar populations along the up- and mid-stream Tom River (Table 1). The Tom River is the right tributary of the Ob River, with its mouth at the 68 m a.s.l. located at 56°50′00″N and 84°29′20″E. The Tom River begins in the south of the Kuznetsk Alatau Mountains in the area where they join the Abakan Ridge, its source being at 903 a.s.l. at 53°39′05″N; 89°45′50″E. The river basin is located at the bound of the West Siberian Lowland and the Altai-Sayan Mountains. The valley of the up-stream Tom River is deeply cut with a V-shaped profile and the width exceeding 1 km. The valley slopes are high and steep. The average altitude of the water-collecting area is 900 m, with altitude amplitudes exceeding 600 m. In the environs of the Mezhdurechensk town after merging with the Usa River the Tom River valley widens to 3–4 km. In mid-stream areas below the Novokuznetsk city, the Tom River becomes a typical plain river, albeit with sometimes increased inclines and stream flow speed. Such large tributaries as the Belsu River (83 km long), the Usa River (179 km long), the Verkhnyaya, Srednyaya and Nizhnyaya Ters Rivers (95, 114 and 110 km, respectively), the Taidon River (110 km) and others flow into the Tom River from the west slope of the Kuznetsk Alatau. All of them are typical montane rivers with narrow valleys, rapids and a high flow speed (Zhukov, 2012).

In the Tom River basin the black poplar *P. nigra*, called "osokor" in Russian, prefers growing mostly in the plain or, to a lesser degree, in low mountain areas, while the laurel poplar *P. laurifolia* prefers highland areas. In the area of the joint growth, their natural hybridization results in the emergence of the Irtysh hybrid called *P.* × *jrtyschensis*. Hybridization in the basin studied does not have a wide spread character, occurring only at certain sites, which can be regarded as hybridization centers. The Irtysh poplar populations studied in the Tom River basin are of both natural and anthropogenic origin (Klimov, Proshkin, 2017).

As morphological markers to examine the intra-species structure with, we chose the traits important for identification of the poplar species studied. For each individual tree, we determined the branch morphotype. For 15 leaves sampled from the middle part of the short branches in the mid-crown of the mature trees, we determined the shape of the leaf blade, its base and tip (Proshkin, Klimov, 2017b).

For each leaf, the following characteristics were measured: L, leaf blade length (mm); D, maximal leaf blade width (mm); P, petiole length (mm); A, the distance between the widest part of a leaf blade and its base (mm). Measurements of the major morphometric traits were carried out with the help of Axio Vision 4.8.2 software. Since different studies may drastically differ in terminology employed to describe leaf characteristics, we described and determined leaf blade shape according to the patterns proposed by Fedorov et al. (1956), using the biometric characteristics obtained.

Analysis of herbaria samples as well as literature sources (Bolshakov, 1992; Koropachinsky, Vstovskaya, 2012) showed that leaves of the poplar species studied are often described as egg-shaped triangular. Because this shape is absent from Fedorov's patterns, we chose R1/2 as a point to distinguish it. To elaborate, the egg-shaped form geometrically represents an ovoid, i.e. a closed box curve with one axis of symmetry and two supporting circumferences differing in diameter (Talalay, 2011), with R1 being the radius of the bigger circumference. The R1/2 point can be easily determined on the

Table 1. The poplar populations studied

Population name	Geographical coordinates	The number of trees/leaves		
		P. nigra	*P. laurifolia*	*P.* × *jrtyschensis*
		Up-stream Tom River populations		
Belsu	53°41′35″ N, 88°22′44» E	–	30/450	–
Studeny Ples	53°39′49″ N, 88°20′01″ E	–	30/450	–
Vorony	53°66′10″ N, 88°29′52″ E	–	30/450	–
Chistenky	53°66′19″ N, 88°28′56″ E	30/450	–	–
Maizas	53°37′24″ N, 88°12′48″ E	30/450	30/450	39/585
Kiizak	53°72′27″ N, 87°94′58″ E	30/450	–	–
Shveinik	53°48′34″ N, 87°28′42″ E	30/450	–	–
Karlyk	53°49′21″ N, 87°28′03″ E	–	–	41/615
		Mid-stream Tom River populations		
Novokuznetsk	53°49′04″ N, 87°07′23″ E	30/450	–	23/345
Kazankovo	53°99′08″ N, 87°29′44″ E	30/450	–	–
Slavino	54°02′05″ N, 87°22′55″ E	30/450	–	–
Erunakovo	54°09′32″ N, 87°47′45″ E	–	30/450	–
Verkhnyaya Ters	54°13′00″ N, 87°39′48″ E	–	30/450	10/150
Srednyaya Maganakova	54°19′33″ N, 87°58′57″ E	–	30/450	–
Total		210/3150	210/3150	113/1695

Table 2. General characteristic of branch morphotypes

Morphotype	Description
	P. nigra
1Pn	Young branches are cylindrical along the entire length, the short branches are leptoblasts, branches and leaves have no hairs
2Pn	The same as above; the petiole at the base of the leaf blade has long scarce outstanding hairs
	P. laurifolia
1Pl	Young branches are ribbed along the entire length, the short branches are leptoblasts and discoblasts; branches have no hairs; the petiole has short scarce hairs, while the leaf blade has none
2Pl	The same as above; the petiole at the base of the leaf blade has long scarce hairs
3Pl	The same as above; branches and leaves are densely covered with short hairs
	P. × *jrtyschensis*
1P×j	Young branches are cylindrical along the entire length, the short branches are leptoblasts and discoblasts; branches and leaves have no hairs
2P×j	Young branches are ribbed along the 1/2–1/3 of their length and then cylindrical, the short branches are leptoblasts and discoblasts, branches have no or scarce hairs, the leaves along the veins are scarcely haired
3P×j	Young branches are ribbed along the entire length, the short branches are leptoblasts and discoblasts, the branches and leaves along the veins are densely haired

leaf blade by the A/L ratio (Pravdin, 1964). However, since morphology studies usually consider living variable objects rather than strict geometric forms, we analyzed leaf shapes using Fedorov's patterns (Fedorov et al., 1956), the following ranges: < 0.25, triangular; 0.25–0.35, egg-shaped triangular; 0.35–0.45, egg-shaped; 0.45–0.65, elliptic; and 0.65 >, reversed egg-shaped forms.

The branch morphotypes were determined by examining the hairiness of leaves and branches (Table 2). The degree of trichomes' development was studied using an MBS-10 stereomicroscope. Phenotype attribution of an individual tree was carried out based on the combination of morphological qualitative traits.

We used one-way ANOVA to check the differences between poplar species and their hybrids in qualitative traits, adopting the threshold as $p < 0.001$ and using principal components analysis. The intra- and inter-population variability was assessed using the indices proposed by Zhivotovsky (1991), Putenikhin et al. (2004), and Boronnikova et al. (2009). The diversity of qualitative traits meeting the criteria of the "phene" was analyzed as recommended by Vidyakin (2004).

Statistical analyses were performed with the help of Excel and SPSS 23.0 software.

Results

The one-way ANOVA revealed statistically significant differences among the studied species in their qualitative traits; the result was also confirmed by PCA. PC1 accounted for 57.6 % of the total data variance, while PC2, PC3 and PC4 accounted for 22.0 %, 17.0 % and 3.4 % of it, respectively. PC1 is closely correlated with the shape of the leaf blade tip ($r = 0.94$, $p << 0.00001$) and branch morphotype ($r = 0.73$, $p << 0.00001$), while PC2 was correlated with the leaf blade shape ($r = 0.85$, $p << 0.00001$), and PC3 was correlated with the shape of the leaf blade base ($r = 0.57$, $p << 0.00001$). The taxa studied have four types of leaf blade shapes, namely

Table 3. Leaf blade and branch morphotype variability in poplar populations as assessed by Zhivotovsky's index ($\mu \pm s_{\mu}$)

Populations	Trait leaf blade shape	Trait leaf blade base shape	Trait leaf blade tip shape	Branch morphotype
		P. nigra		
Chistenky	1.347 ± 0.044	1.000 ± 0.000	1.000 ± 0.000	1.359 ± 0.170
Maizas	1.713 ± 0.033	1.000 ± 0.000	1.000 ± 0.000	1.988 ± 0.028
Kiizak	1.708 ± 0.033	1.000 ± 0.000	1.000 ± 0.000	1.597 ± 0.146
Shveinik	1.844 ± 0.025	1.000 ± 0.000	1.000 ± 0.000	1.359 ± 0.170
Novokuznetsk	1.776 ± 0.029	1.000 ± 0.000	1.000 ± 0.000	1.960 ± 0.051
Kazankovo	1.390 ± 0.043	1.000 ± 0.000	1.000 ± 0.000	1.359 ± 0.170
Slavino	1.617 ± 0.037	1.000 ± 0.000	1.000 ± 0.000	1.495 ± 0.158
		P. laurifolia		
Belsu	2.374 ± 0.057	1.000 ± 0.000	1.000 ± 0.000	2.496 ± 0.204
Studeny Ples	2.289 ± 0.060	1.000 ± 0.000	1.000 ± 0.000	2.483 ± 0.206
Vorony	2.528 ± 0.051	1.000 ± 0.000	1.000 ± 0.000	2.768 ± 0.146
Maizas	2.772 ± 0.037	1.000 ± 0.000	1.000 ± 0.000	2.735 ± 0.155
Erunakovo	2.377 ± 0.057	1.000 ± 0.000	1.000 ± 0.000	2.732 ± 0.156
Verkhnyaya Ters	2.368 ± 0.057	1.960 ± 0.013	1.000 ± 0.000	2.647 ± 0.176
Srednyaya Maganakova	2.310 ± 0.059	1.798 ± 0.028	1.000 ± 0.000	2.765 ± 0.147
		P. × *jrtyschensis*		
Maizas	1.990 ± 0.005	1.435 ± 0.037	1.594 ± 0.033	2.579 ± 0.166
Karlyk	2.062 ± 0.056	1.890 ± 0.058	1.425 ± 0.036	2.380 ± 0.189
Novokuznetsk	2.152 ± 0.074	2.262 ± 0.071	1.990 ± 0.007	2.390 ± 0.251
Verkhnyaya Ters	1.943 ± 0.117	1.597 ± 0.065	1.597 ± 0.065	2.893 ± 0.175

Note: μ, Zhivotovsky's index of intra-population diversity; s_{μ}, error of Zhivotovsky's index.

Table 4. Intra- and inter-population phenotypic diversity (Shannon's index)

Trait	H_{es}	H_n	F_n	F_{ip}
		P. nigra		
LBS	0.544	0.513	0.943	0.056
LBBS	0.000	0.000	0.000	0.000
LBTS	0.000	0.000	0.000	0.000
Mt	0.671	0.535	0.796	0.203
Mean	0.304	0.262	0.862	0.138
		P. laurifolia		
LBS	1.223	1.114	0.910	0.090
LBTS	0.836	0.404	0.483	0.517
LBTS	0.000	0.000	0.000	0.000
Mt	1.467	1.253	0.854	0.146
Mean	0.881	0.692	0.785	0.215
		P. × jrtyschensis		
LBS	0.962	0.864	0.898	0.102
LBBS	0.549	0.539	0.981	0.019
LBTS	0.821	0.671	0.817	0.183
Mt	1.185	1.170	0.987	0.013
Mean	0.879	0.811	0.920	0.080

Notes: LBS, leaf blade shape; LBBS, leaf blade base shape; LBTS, leaf blade tip shape; Mt, branch morphotype; H_{es}, the diversity index for the entire data set; H_n, the mean value of the diversity index for populations' data sets; F_n, the diversity index of the intra-population data sets; F_{ip}, the index of the inter-population diversity.

Table 5. Intra-population diversity of poplar qualitative traits and phenotypes

Population	μ_1	$S_{\mu 1}$	μ_2	$S_{\mu 2}$
		P. nigra		
Chistenky	18.721	1.162	1.763	0.211
Maizas	22.230	1.368	2.238	0.303
Kiizak	20.891	1.288	2.588	0.370
Shveinik	20.462	1.263	2.039	0.265
Novokuznetsk	22.520	1.375	3.471	0.535
Kazankovo	18.880	1.171	1.763	0.211
Slavino	20.201	1.248	1.923	0.243
		P. laurifolia		
Belsu	26.152	3.977	4.490	0.276
Studeny Ples	25.897	3.930	4.862	0.429
Vorony	27.604	4.247	3.383	0.146
Maizas	28.291	4.374	5.326	0.345
Erunakovo	30.415	4.659	5.745	0.220
Verkhnyaya Ters	30.980	4.764	6.786	0.220
Srednyaya Maganakova	30.514	4.677	5.683	0.245
		P. × jrtyschensis		
Maizas	31.505	1.001	11.614	0.842
Kiizak	30.780	1.101	7.447	0.645
Novokuznetsk	35.141	1.568	6.563	0.410
Verkhnyaya Ters	31.618	2.134	4.721	0.131

Notes: μ_1, Zhivotovsky's index of intra-population diversity for qualitative traits; $S_{\mu 1}$, error of μ_1; μ_2, Zhivotovsky's index of intra-population diversity for phenotypes; $S_{\mu 2}$, error of μ_2

triangular, egg-shaped triangular, egg-shaped and elliptical. At the endogenous level, different shapes can be seen at once, but one shape usually predominates.

The *P. nigra* trees were found to have triangular and egg-shaped triangular leaves, the latter predominating (78–96 %) in all populations. It should be noted that 70 % of the black poplar trees with the 2Pn morphotype and hairiness were found to have leaf blades of the irregular rhomboidal shape. Such trees comprised from 7 to 20 % of the data set. Their A/L ratio ranged from 0.35–0.47, i.e. being typical of egg-shaped leaves, which are not common for *P. nigra*. Thus the emergence of the 2Pn morphotype is most likely due to introgression (Klimov, Proshkin, 2017), and the observed frequencies of leaf blade shapes can be regarded as a morphological marker.

The *P. laurifolia* trees were also found to have three forms of leaves: egg-shaped triangular, egg-shaped and elliptical. In most of the poplar stands studied, the egg-shaped leaves were the most frequent (50–70 %). The *P. × jrtyschensis* trees were found to have all the leaf forms common for the parental species. The hybrid species populations were found to have egg-shaped triangular leaves prevailing (55–82 %), with triangular and elliptical leaves showing very low frequencies.

At the individual level, the lowest variability of the leaf blade shape was observed in *P. nigra*, while the highest variability was displayed by *P. laurifolia* trees. The *P. × jrtyschensis* populations were characterized by variability indices ranging between those of the parental species (Tables 3, 4).

The short mid-crown branches on one and the same tree always display only one leaf blade tip shape and one leaf blade base shape. The *P. nigra* leaves in all the populations studied had long pointed tips and wedge-shaped bases.

The *P. laurifolia* trees had pointed leaf blade tips of all the leaves. The variability of the leaf blade base of the laurel poplar trees within the study area is due to the presence of tree forms that differ not only in the trait being examined, but also in their bark colour (Proshkin, Klimov, 2017c). The most widely spread grey-bark form has a rounded wedge-shaped base of the leaf blades, while the white-bark form, common for mid-stream populations, has a heart-shaped leaf blade base.

The *P.* × *jrtyschensis* trees commonly had leaves with long pointed tips and rounded wedge-shaped bases, but few had rounded and sinuate bases. The greatest variability of the traits was observed in the Novokuznetsk population (see Table 3).

The branch morphotype did not vary at the endogenous level. Most of the *P. nigra* populations were found to have morphotype 1Pn dominating, with exceptions of the Maizas and Novokuznetsk data sets collected in the stands mixed with *P. laurifolia* and *P.* × *jrtyschensis*, where morphotype 2Pn contributed 56.7 % and 36.7 %, respectively.

P. laurifolia also showed a tendency to change the relative abundance of morphotypes in hybridization centers. For instance, the Maizas and Verkhnyaya Ters populations displayed a sharp increase in the relative abundance of morphotype 1Pl (up to 60 and 66.7 %, respectively). Most poplar stands beyond the hybridization centers were dominated by trees with branches and leaves scarcely covered by long hairs.

At all the study sites for *P.* × *jrtyschensis*, morphotype 2P×j was predominating. The lowest variability of the traits was observed in *P. nigra,* while *P. laurifolia* and *P.* × *jrtyschensis* displayed similar variability (see Table 3). The species studied revealed a higher level of intra-population diversity in qualitative traits than that of inter-population diversity: its share was 21.5 % in *P. laurifolia,* 13.8 % in *P. nigra* and 8.0 % in *P.* × *jrtyschensis* (see Table 4).

Assessing the inter-population variability by Zhivotovsky's index showed that qualitative traits and phenotype number were more polymorphic in *P. laurifolia* than *P. nigra*. The *P.* × *jrtyschensis* populations were the most diverse (Table 5).

The Maizas and Novokuznetsk *P. nigra* populations in hybridization centers displayed an increased diversity of qualitative traits and phenotypes due to an increased share of the forms with hairy leaves (see Table 5). The mid-stream *P. laurifolia* populations displayed increased polymorphism due, as we noted previously, to the presence of white-bark forms.

Discussion

Analysis of the morphological traits studied for meeting the "phene" criterion showed that the shapes of the leaf blade tip and base, as well as the branch morphotype can be regarded as phenes, since they display no endogenous variability. Although leaf blade shape as a trait does not meet all criteria of phenes, and, in particular, alternativity, when the set is rather large, the leaf blade shape becomes an important indicator of specific variability of qualitative traits of *Populus* species. Overall, the tip and base of leaf blades of *P.* × *jrtyschensis* represent a combination of parental taxa traits. Hybrids always inherit short branches, i.e. discoblasts, common for species in the section *Tacamahaca*. This trait is most likely controlled by nuclear genes (Proshkin, Klimov, 2017d).

A high share of intra-population variability was reported by some researchers for *P. nigra* (Šiler et al., 2014; Čortan et al., 2015; Jiang et al., 2015) and *Populus* species in general (Joshi et al., 2011). Gene flow over large distances leads to decrease in the inter-population variability of wind-pollinated plants (Zitte et al., 2007). The floodplain poplar species have a considerable number of seeds transported by water flow (Hidalgo et al., 2010). According to the increase in intra-population variability, the poplar species studied can be ranked as *P. nigra* < *P. laurifolia* < *P.* × *jrtyschensis*.

A relatively small share of inter-population differentiation in *P. nigra* is very likely related to its dominance in the Tom River basin poplar stands and to the fact that its populations are not fragmented and hence are connected by vast gene flow. The *P. nigra* populations in hybridization centers have an increased diversity of qualitative traits and phenotypes.

By contrast, as was shown by our research of *P. laurifolia* carried out in the areas of the Tom, Biya and Katun River basins, this poplar species in the northwestern part of the Altai-Sayan Mountains has a disjunct distribution, resulting in an increased inter-population variability. The species preference for well-aerated gravel-boulder alluvium results in a narrower ecological amplitude of *P. laurifolia*, growing mostly in multi-stream areas of montane rivers. In our view, the growth of laurel poplar in plain areas is hampered by low oxygen in dense sandy-silt sediments there.

The laurel poplar is spread both in the up- and mid-stream areas of the Tom River. It is quite numerous up-stream, forming well-developed stands. In mid-stream areas with small alluvium, *P. laurifolia* is very scarce, growing mostly as sole clones. Its numbers increase sharply in the floodplains of the major montane tributaries of the Verkhnyaya and Srednyaya Ters Rivers. The mid-stream group of *P. laurifolia* populations is relatively separated from the up-stream populations, as not only are they rather well apart (*ca.* 50 km), but also the river flow in these areas is different. The up-stream Tom River flows from east to west, while the mid-stream of it flows from south to north. Taking into consideration the prevailing directions of air flow, such river flow prevents pollen transportation, leaving only one way for gene flow to link the populations, namely seed transportation via water flow, resulting in one-way gene flow. However, fragmented *P. laurifolia* populations grow mostly in the floodplains of the right montane tributaries of the Tom River, and the efficiency of such gene flow should be studied in detail.

The observed diversity of *P. laurifolia* forms, in particular, the occurrence of the white-bark form in the populations near montane tributaries of the mid-stream Tom River alongside the widely spread grey-bark form results in increased polymorphism both in qualitative traits and phenotypes. Different studies showed that spatial separation of *Populus spp.* populations is governed by historical processes, mainly by events in the Pleistocene and Holocene (Keller et al., 2010; Macaya-Sanz et al., 2012; Dewoody et al., 2015; Meirmans et al., 2017; Fan et al., 2018). Therefore, the variability of *P. laurifolia* population structure in the Tom River basin is the subject for a further extended research.

Theoretically, the highest rate of inter-population differences should be displayed by *P.* × *jrtyschensis*, because (1) this is the least common species; (2) its stands are fragmented; and (3) its populations are 20–100 km apart, which seriously impedes gene flow among them. However, the phenotypic structure of *P.* × *jrtyschensis* populations is determined by extremely strong natural selection, resulting in the prevalence of F_1 hybrids, and rejection of next-generation hybrids and backcrosses early in ontogenesis before reproductive maturity (Jiang et al., 2016; Proshkin, Klimov, 2017a). All these lead to drastic decrease in inter-population variability. We believe that significant differences in phenotypic diversity in the *P. × jrtyschensis* populations studied are determined by population origin. They emerge at sites with disturbed vegetation cover, where selection factors and their pressure are site-specific. The Maizas and Karlyk populations grow in floodplains, but the former emerged due to the degradation of the vegetation cover by erosion and accumulation caused by streambed activity, while the latter emerged due to anthropogenic disturbance. The Verkhnyaya Ters population is under a strong influence of factors associated with streambed dynamics. The Verkhnyaya Ters River is a typical montane river with gravel-boulder alluvium prevailing. Thus the environment there is optimal for *P. laurifolia*, but unfavourable for *P. × jrtyschensis* and *P. nigra*, which results in a low population of their hybrids. The Novokuznetsk population is of anthropogenic origin; it is located on the upper fluvial terrace, where the spectrum of adverse factors might be broader, as compared to the natural populations where the main abiotic selection factors are determined by the riverbed dynamics.

Conflict of interest

The authors declare no conflict of interest.

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