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GENETIC EVIDENCE OF NATURAL HYBRIDIZATION BETWEEN SIBERIAN STONE PINE, *PINUS SIBIRICA* DU TOUR, AND DWARF SIBERIAN PINE, *P. PUMILA* (PALL.) REGEL

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ABSTRACT

Sympatric populations of two Eurasian bird-dispersed pine species *Pinus sibirica* and *P. pumila*, and their putative natural hybrid were analyzed using 28 allozyme loci controlling 14 enzyme systems. The *Adh–1, Fe–2*, and *Lap–3* loci in the hybrid had genotypes that are typical for *P. sibirica*, but did not occur or are unlikely in *P. pumila*. The *Gdh, Got–2, Got–3, Pgm–1* and *Pgm–2* loci had carried alleles and genotypes unknown for *P. sibirica*, but were common in *P. pumila*. The *Skdh–2* locus was heterozygous for alleles, one of which was specific to *P. sibirica*, the other to *P. pumila*. Some embryos from seeds of the hybrid were likely resulted from selfing, while others from backcrosses with parental species. This is the first genetic evidence of natural hybridization and possible gene exchange between Siberian stone pine and dwarf Siberian pine.

Key words: Siberian stone pine, *Pinus sibirica*, dwarf Siberian pine, *P. pumila*, isozyme loci, natural hybridization

INTRODUCTION

Siberian stone pine (*Pinus sibirica* Du Tour) is a forest tree widely distributed in Siberia and in the northeast of the Ural Mountains. Its range extends eastward to the Transbaikalia (the Baikal Lake region) and southern Sakha-Yakutia, where it comes into contact with the closely related dwarf Siberian pine (also known as Japanese stone pine), *P. pumila* (Pall.) Regel (Fig. 1). These two species share several common evolutionary traits, such as large, wingless seeds, that are retained within mature "indehiscent" cones, which promote bird-mediated seed dispersion (LANNER 1996). Though some early botanical descriptions of *P. pumila* (Flora Rossica 1784, cited in BOBROV 1978) treated it as an ecological or geographical variety of *P. sibirica*, the characteristic traits of this pine were later interpreted as distinct from *P. sibirica* (REGEL 1859, cited in BOBROV 1978), and since that time the species status of dwarf Siberian pine has been widely accepted. Both pines are traditionally included in the subsection Cembrae, section Strobus, subgenus Strobus (CRITCHFIELD & LITTLE 1966), although this classification seems to be disputable in view of the recent data on genetic differentiation among pines of the section Strobus obtained using isozyme loci (BELOKON *et al.* 1998).

Unlike *P. sibirica*, which is typically a large tree up to 35 m tall, *P. pumila* exists mainly as a shrub-like or crawling form, only rarely displaying a tree-like phenotype (Fig. 2). Some botanists studying stone pines (*e.g.*, SUKACHEV 1929; GALAZII 1954) have believed that the tree-like form of *P. pumila* is mainly (though not exclusively) attributed to the zone of sympatry between Siberian stone pine and dwarf Siberian pine. Based on this fact, these authors assumed that upright forms of *P. pumila* are, in fact, inter-specific hybrids. This assumption could explain not only unusual living form, but also some other traits intermediate between these pine species. However, to the best of our knowledge, there are no genetic data or study proving the hybrid origin of these suspected trees. The objective of this paper is to present genetic data confirming the hybrid origin (*P. pumila* x *P. sibirica*) of a tree growing in the zone of contact of these two species using electrophoretic analysis of seed isozymes.

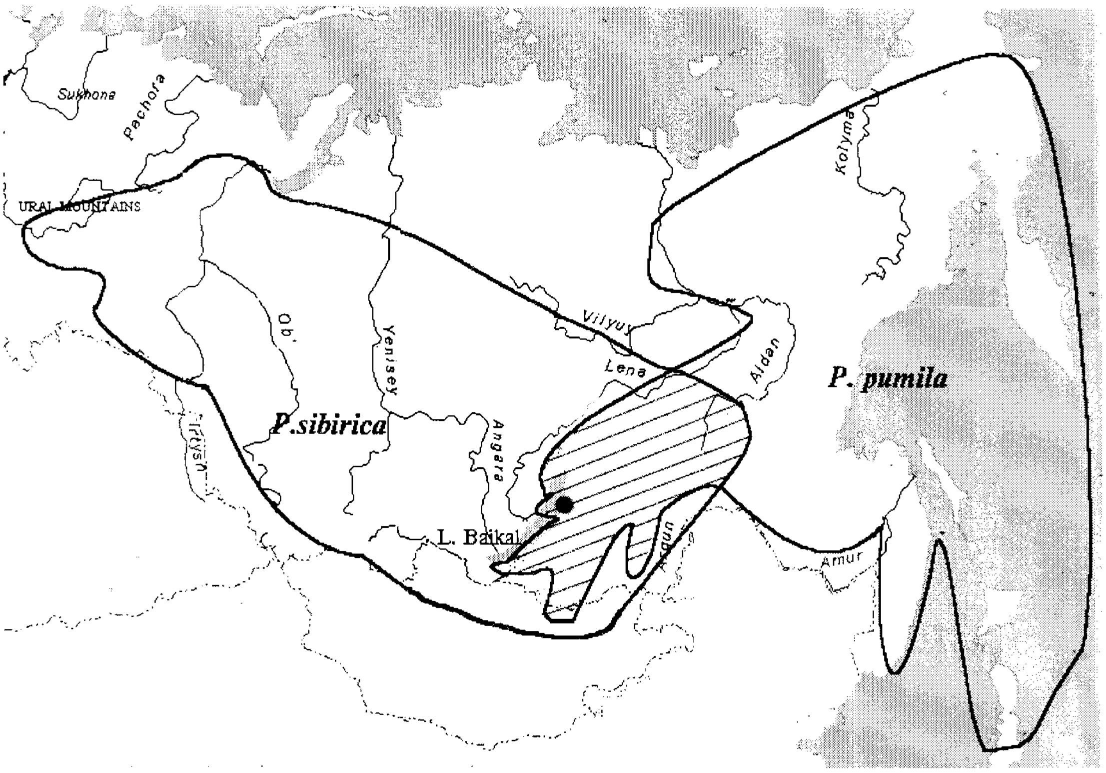

Figure 1. Natural ranges and sympatry zone of the two stone pine species. The black dot indicates the location where the samples were collected.

MATERIALS AND METHODS

Plant material

Open-pollinated seeds were collected from one suspected hybrid pine tree (referred to further as the "hybrid") grown on the territory of Barguzin Biosphere State Reserve (Buryat Republic, Russian Federation) in 1991 in the mixed stand among typical *P. sibirica* and *P. pumila*. The seeds were processed and stored at –20 °C in the Institute of Dendrology (Kornik, Poland) and part of them were handed over to the Institute of General Genetics (Moscow, Russia) by L. E. Mejnartowicz in 1992. The sampled hybrid had morphological traits intermediate between *P. sibirica* and *P. pumila* being a small tree about 4 m tall with broad open crown, and dark brown bark (Fig. 2B). Cones of the hybrid (Fig. 3B) were smaller than those of *P. sibirica* (Fig. 3A) but larger and thicker and not as much elongated as *P. pumila* cones (Fig. 3C). Size of the seeds from the hybrid was also intermediate between typical *P. sibirica* and *P. pumila* seeds, as shown in Fig. 3.

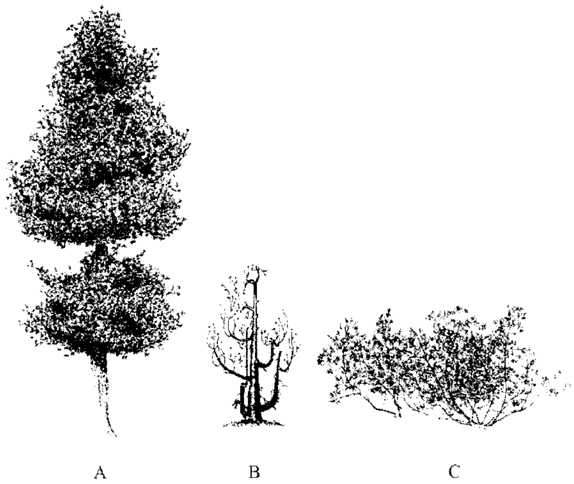

Figure 2. Typical *Pinus sibirica* (**A**), tree-like, supposedly hybrid, form of *P. pumila* (**B**) tree from the Barguzin Mountains, Malaya Cheremshana River drainage, and typical *P. pumila* (**C**) (**A** and **C** are taken from FARJON 1984, and **B** from MOLOZHNIKOV 1975).

Control seed samples were collected from 50 and 80 typical *P. sibirica* and *P. pumila* pines, correspondingly, growing in the same region where the hybrid was collected. In addition, extensive population genetic

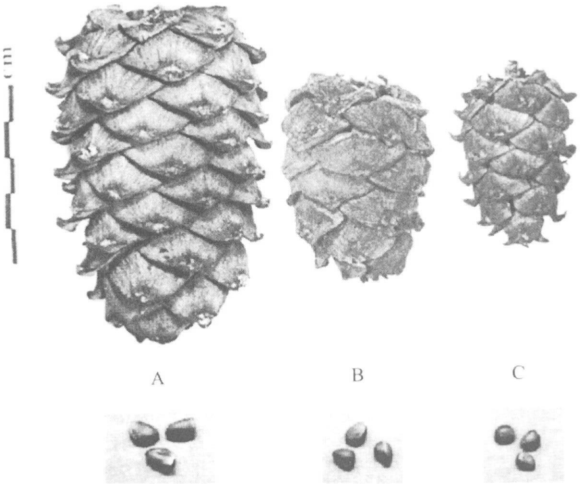

Figure 3. The cone and seed morphology of typical *P. sibirica* (A), hybrid (B), and typical *P. pumila* (C) trees. Photo of cones by L. Mejnartowicz.

data obtained previously for several hundreds of trees from the numerous allopatric populations of these two species and published elsewhere (*e.g.*, KRUTOVSKII *et al.* 1988, 1989, 1990, 1994, 1995; KRUTOVSKII & POLITOV 1992; POLITOV 1989; POLITOV *et al.* 1992; POLITOV & KRUTOVSKII 1994) were also summarized and used to compare the isozyme allele composition of the hybrid with that of pure *P. sibirica* and *P. pumila*. All available 20 haploid megagametophytes and 8 diploid embryos (other 12 seeds contained dead or no embryo) of the hybrid were analyzed electrophoretically.

Isozyme analysis

Haploid megagametophyte and diploid embryo tissues of intact pine seeds were extracted and ground separately in 0.05 M tris-HCl buffer, pH 7.7, containing 3% polyvinylpyrrolidone. Homogenates were separated electrophoretically in 13 % starch gels using three buffer systems: (A) tris-citrate pH 7.4 (KONNERT & MAURER 1995), (B) tris-EDTA-borate pH 8.6 (Markert & Faulhaber 1965) and (C) tris-citrate pH 8.5/LiOH-borate pH 8.1 (Ridgeway *et al.* 1970). Thin, horizontal gel slices were stained for enzyme activity of alcohol dehydrogenase (ADH, 1.1.1.1), fluorescent esterase (FE, 3.1.1.56), formate dehydrogenase (FDH, 1.2.1.2), fumarase (FUM, 4.2.1.2), glutamate dehydrogenase (GDH, 1.4.1.2-4), glutamate oxaloacetate transaminase (GOT, 2.6.1.1), leucine aminopeptidase (LAP, 3.4.11.1), malate dehydrogenase (MDH, 1.1.1.37), phosphoglucose isomerase (PGI, 5.3.1.9), phosphogluconate dehydrogenase (PGD, 1.1.1.44), phosphoglucomutase (PGM, 5.4.2.2), shikimate dehydrogenase (SKDH, 1.1.1.25), sorbitol dehydrogenase (SDH, 1.1.1.14), and superoxide dismutase (SOD, 1.15.1.1). SKDH, FUM, PGD and MDH were separated in buffer system A; ADH, FE, GDH, PGI, PGM and SOD in B; FDH, GOT, LAP and SDH in C. Earlier we described the genetic control of most of these enzymes in *P. sibirica* (KRUTOVSKII *et al.* 1987). FDH, FUM and SDH, which were not described in our earlier publications, are each coded by a single locus, PGD and SOD are coded by three loci each. Enzymes in *P. pumila* migrate to the same positions as those in *P. sibirica*, and thus, are assumed to be controlled by orthologous loci. They were numbered according to the relative electrophoretic mobility of the corresponding activity zones (the most anodal was numbered 1, etc.). The alleles were designated according to the relative mobility of the corresponding allozymes, with the most frequent alleles in *P. sibirica* taken as 100. The allelic variation in the corresponding loci was compared among the putative hybrid, and typical *P. pumila* and *P. sibirica* trees to make a conclusion about a possibility of an inter-specific hybrid origin of the studied specimen.

RESULTS

The hybrid produced sound seeds of intermediate size (Fig. 3), although percentage of empty seeds was about 25 %. Most filled seeds contained embryos that appeared normal, although only for 8 of them we were able to obtain allozyme genotypes for many loci, and others had either no embryo or insufficient activity in the embryo tissue. In total, 28 loci controlling 14 enzyme systems were investigated. Four loci, *Fe–3, Lap–1, Mdh–1* and *Skdh* were monomorphic and fixed for the same alleles in both species. *Mdh–2, Mdh–3, Mdh–4* and *Pgi–2* were polymorphic in control samples, and the same alleles common in both species were also found in the putative hybrid. Species-specific alleles existed at low and medium frequencies at some polymorphic loci (*Fdh, Fum, Got–1, Pgd–1, Pgd–2, Sod–2* and *Skdh–1*), but they were not found among seeds from the hybrid. It was difficult to determine a correspondence of alleles between species for some loci (*e.g., Adh–2, Lap–2*, and *Pgi–1*), because of a weak difference in electrophoretic mobility or other methodological problems. We excluded all the above mentioned loci from further consideration, since they did not provide information allowing us to confirm or disprove hybridization. The remaining 10 loci, listed in Table 1, served as the basis for our conclusions. In Table 1 we present allele frequencies for these loci averaged for all allopatric populations of *P. sibirica* and *P. pumila* studied in previous range-wide population

Table 1. Allele frequencies for 10 polymorphic loci averaged for all allopatric populations of *P. sibirica* and *P. pumila* studied in previous wide-scale population studies, for typical *P. sibirica* and *P. pumila* trees from neighboring stands surrounding the hybrid, and for the multilocus genotype of the hybrid.

Locus/allele	*P. sibirica*		Hybrid	*P. pumila*	
	allopatric	neighbor		neighbor	allopatric
Adh-1^{109}	0.0013	0.0140	0	0.0000	0.0000
Adh-1100	0.7586	0.7430	0.5	0.0000	0.0051
Adh-1/91	0.2401	0.2430	0.5	0.9820	0.9814
Adh-1/88	0.0000	00000	0	0.0180	0.0135
Fe-2/127	0.2715	0.0590	0.5	0.0000	0.0000
Fe-2/120	0.0000	0.0000	0	0.1000	0.1622
Fe-2/100	0.7260	0.9410	0.5	0.9000	0.7489
Fe-2/87	0.0025	0.0000	0	0.0000	0.0889
Gdh/111	0.0000	0.0000	0.5	0.6250	0.5394
Gdh/100	0.9975	1.0000	0.5	0.3750	0.4606
Got-2/112	0.0000	0.0000	0.5	0.2500	0.0867
Got-2/100	1.0000	1.0000	0.5	0.7500	0.9133
Got-3/134	0.0000	0.0000	0	0.0000	0.0056
Got-3/100	1.0000	1.0000	0.5	0.4120	0.3152
Got-3/53	0.0000	0.0000	0	0.0000	0.0562
Got-3/34	0.0000	0.0000	0.5	0.5880	0.6230
Lap-3/100	0.8611	0.7350	1	0.0500	0.0610
Lap-3/96	0.0000	0.0000	0	0.9000	0.8199
Lap-3/92	0.0250	0.0590	0	0.0500	0.1134
Lap-3/87	0.1139	0.2060	0	0.0000	0.0057
Pgm-1/104	0.0039	0.0000	0	0.0000	0.0139
Pgm-1/100	0.9489	1.0000	0.5	0.7500	0.5793
Pgm-1/96	0.0472	0.0000	0	0.1250	0.2480
Pgm-1/94	0.0000	0.0000	0.5	0.1250	0.1588
Pgm-2/111	0.0000	0.0000	0.5	0.1500	0.0756
Pgm-2/100	1.0000	1.0000	0.5	0.8500	0.9230
Pgm-2/97	0.0000	0.0000	0	0.0000	0.0014
Skdh-2/123	0.0000	0.0000	0	0.0500	0.0494
Skdh-2/110	0.0000	0.0000	0.5	0.9500	0.9490
Skdh-2/100	1.0000	1.0000	0.5	0.0000	0.0000
Skdh-2/95	0.0000	0.0000	0	0.0000	0.0016
Sod-1/100	1.0000	1.0000	1	0.8750	0.9166
Sod-1/68	0.0000	0.0000	0	0.1250	0.0834

Note: Shaded **raw cel** show the unambiguous or the most likely origin of the allele in the hybrid.

studies (KRUTOVSKII *et al.* 1988, 1989, 1990, 1994, 1995; KRUTOVSKII & POLITOV 1992; POLITOV 1989; POLITOV *et al.* 1992; POLITOV & KRUTOVSKII 1994; unpublished data), and for typical *P. sibirica* and *P. pumila* trees from neighboring stands surrounding the hybrid, and compare those to the multilocus genotype of the hybrid inferred from segregation analysis of haploid megagametophytes.

Adh–1 was polymorphic in *P. sibirica* with 3 alleles: 109, 100 and 91. The hybrid had a heterozygote genotype 100/91, typical for *P. sibirica* or very likely expected for hybrid, because allele 91 was almost fixed in *P. pumila*, and allele 100 was extremely rare and found only once in a heterozygous tree in the population of the southern Sakha-Yakutia located far away from the hybrid.

Fe–2 was polymorphic in both species with alleles 127 and 100 found in *P. sibirica*, and 120 and 100 in *P.*

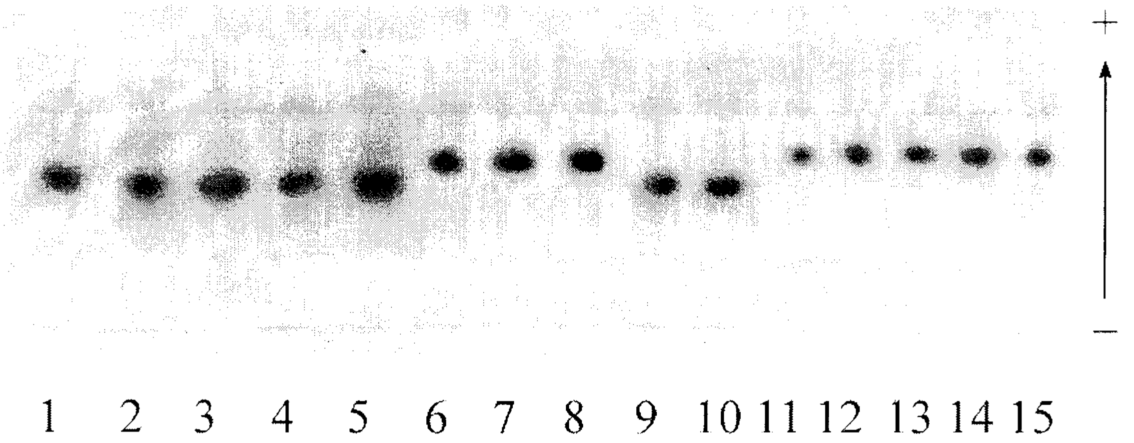

Figure 4. The isozyme electrophoretic pattern of *Skdh*–2 alleles in megagametophytes of pure *P. sibirica* (*1–6*), hybrid (*7–11*), and pure *P. pumila* (*12–16*) trees.

pumila. The hybrid was heterozygous 127/100, which would be likely for hybrid, but impossible for pure *P. pumila*.

Gdh was almost monomorphic in *P. sibirica*, while two alleles, 100 and 111, were detected in *P. pumila*. Allele 100 was common in *P. sibirica*, but allele 111 was not found in this species. The hybrid was heterozygous 111/100, and therefore resembled *P. pumila* for this locus, or obtained at least one allele from *P. pumila*.

Got–2 was invariant in *P. sibirica*. The allele fixed in this species was also the most common in *P. pumila*, with an additional allele, 112, reaching frequency 0.25 in stands surrounding the hybrid, which was heterozygous 112/100. Thus, as for the *Gdh* locus, the hybrid received at least one allele from *P. pumila*.

Got–3 had only allele, 100, in *P. sibirica*, while four alleles were found in *P. pumila*, with 100 and 34 being the most frequent. The hybrid was heterozygous 100/34, a genotype not observed in *P. sibirica*, but frequent in *P. pumila*, and likely for hybrid.

Lap–3 was polymorphic in both species, but alleles 100 and 87 were frequent in *P. sibirica*, while 96 and 92 were typical for *P. pumila*. The hybrid genotype 100/100 occurs commonly in *P. sibirica*, but is rare in pure *P. pumila*.

Pgm–1 and *Pgm–2*, both have genotypes in the hybrid that could result from either within *P. pumila* or inter–specific crosses, but not within *P. sibirica*.

Skdh–2 is the only locus that shares no common alleles in the two species and therefore it has the highest diagnostic value. *P. pumila* lacks allele 100, which is the only allele in *P. sibirica*. One could not expect to find heterozygote 100/110 in pure *P. sibirica* or *P. pumila*, and therefore, inter-specific hybridization is the only plausible explanation for its occurrence in the studied tree (Fig. 4).

Sod–1 has common allele 100 in *P. pumila* (frequency 0.87–0.92), which is fixed in *P. sibirica*. Hybrid tree was homozygous for allele 100, but one embryo of this tree was heterozygote 100/68.

Thus, some of the above-mentioned loci demonstrate genotypes typical for *P. sibirica* and impossible or very unlikely for *P. pumila* (*Adh–1, Fe–2,* and *Lap–3*), while others show a combination of alleles impossible for *P. sibirica* (*Gdh, Got–2, Got–3, Pgm–1,* and *Pgm–2*), and, finally, the *Skdh–2* locus gives a straightforward evidence that the studied specimen did result from an inter-specific cross between Siberian stone pine and dwarf Siberian pine. However, we cannot conclude whether it represents the first generation of inter-specific cross (F_1), since the same genotype could occur in the next generation as a result of either self-pollination of F_1 hybrid, cross between different hybrids, or backcross to *P. pumila*.

Considering the evolutionary significance of such inter-specific hybridization, it was also of interest to investigate the paternal contribution or effective pollen pool that was responsible for developed embryos of this tree. For this purpose all eight viable embryos available from the hybrid tree seeds were analyzed along with their megagametophytes. By assessing both the haploid megagametophyte and diploid embryo of a seed, we can distinguish between maternal and paternal zygotic contributions, and therefore, infer the source (*i.e.*, one of parental species or hybrid) of the pollen that fertilized each particular ovule. Unfortunately, because of an insufficient number of embryo-containing seeds, precise quantification was impossible. However, analysis of the paternal haplotypes in embryos of 8

Table 2. Multilocus haplotypes of 8 pollen (P1-P8) fertilized the hybrid, and their inferred origin.

Locus	P1	P2	P3	P4	P5	P6	P7	P8
Adh-1	91	91	100s	91	100s	–	100s	–
Fe-2	120p	127s	120p	127s	127s	127s	100	120p
Got-3	34p	100	100	100	100	100	34p	100
Lap-3	100	100	100	100	100	100	100	96p
Pgm-1	96p	100	100	100	100	94p	94p	100
Skdh-2	110p	100s	100s	100s	100s	100s	100s	110p
Sod-1	–	–	–	100	100	100	100	68p
Origin	*pumila*	*sibirica* or hybrid	hybrid	*sibirica* or hybrid	*sibirica* or hybrid	hybrid	hybrid	*pumila*

Note. Letters after allele names show the most likely origin of species–specific paternal allele (p – from *Pinus pumila*, s – from *P. sibirica*). Conclusions on the assumed origin of the pollen is given in the last raw.

available seeds from the hybrid revealed multiple source of pollen. At least 3 (~38%) embryos contained a combination of paternal alleles characteristic of two different species, and therefore resulted either from self-pollination or from crosses with other unknown hybrids (P3, P6, and P7, Table 2). Two other embryos could not be result of selfing and very likely originated from fertilization by *P. pumila* pollen (P1 and P8, Table 2), since they contained alleles specific for this species and lacking in the studied hybrid. Parental haplotypes of the rest three embryos might have originated from *P. sibirica* pollen (P2, P4, and P5, Table 2). However, in these cases selfing also cannot be excluded since *P. sibirica* in general possesses fewer species-specific alleles.

DISCUSSION

There are many instances of natural and artificial interspecific hybridization in most Pinaceae taxa, including cases confirmed by isozyme (*e.g.*, WANG *et al.* 1990; HÄCKER & BERGMANN 1991), cpDNA (WAGNER *et al.* 1987; STINE *et al.* 1989; WATANO *et al.* 1995, 1996), and mtDNA markers (WATANO *et al.* 1996). Authors of numerous papers on hybridization in white pines of the section Strobus (*e g.*, BLADA 1994; MIROV 1967; TITOV 1977; WATANO *et al.* 1995, 1996) as well as CRITCHFIELD (1986) who summarized available to date information on hybridization in this section did not mention any case of *P. sibirica* x *P. pumila* hybridization. We are also unaware of any well documented reports of recognized hybrids between these species, although some researchers (*e.g.*, SUKACHEV 1929; GALAZII 1954; POZDNIAKOV 1952) described forms of a stone pine intermediate between *P. pumila* and *P. sibirica* within the zone of their sympatry and believed them to be hybrids. It is unknown, however, how this form corresponds to the tree-like *P. pumila* found also on the Kamchatka Peninsula, far away from the Baikal Lake Region (MOLOZHNIKOV 1975).

BOBROV (1961, 1972, 1978) studied the problem of introgressive hybridization and regarded the Transbaikalia as a complex zone of introgression for many plant taxa, both conifers and angiosperms. However, in spite of an occurrence of broad zone of sympatry between *P. pumila* and *P. sibirica*, he emphasized that they occupy different elevations in the mountain taiga and subalpine ecosystems, and, therefore, do not hybridize. It is obvious that large-scale introgressive hybridization between them does not take place, otherwise one would have observed and described a variety of intermediate forms with different extents of similarity to parental species over a large territory of their sympatry. However, a possibility of occasional hybridization exists in places of the species contact. *P. pumila* in the Baikal Lake region occupies mainly the upper mountain belt forming consistent dense populations there, and contacting *P. sibirica* at the lower elevations, at the lower limit of *P. pumila*. These mixed stands are at the upper elevation limit for *P. sibirica* and represent tree line. Another area of sympatry is attributed to the zone directly on the coast of the Baikal Lake, where *P. pumila* also often grows together with *P. sibirica* (MOLOZHNIKOV 1975). The periods of pollination for these species overlap, so one cannot expect strong phenological isolation.

As far as we are aware, the genetic evidence presented here is the first direct confirmation of hybridization between the two species. Based on our preliminary data, we cannot conclude what generation the studied tree represents, F_1, F_2, or a backcross hybrid, or how well the offspring will survive and compete. Although the hybrid produced sound seeds with normal looking and potentially viable embryos, percentage of empty seeds (about 25 %) was relatively high compared to regular 15–20% (authors' data) in pure *P. pumila* stands. However, the limited number of available hybrid seeds did not allow us to make more vigorous estimations and conclusions.

Hypothetically, this particular gene exchange between *P. sibirica* and *P. pumila* may play a significant adaptive role. The zone of sympatry in the Baikal Region and Southern Yakutia is not optimal for both species and is intrinsically occupied by marginal populations. These species are adapted to different environmental optima (BOBROV 1978), and their survival outside the optimal zone may be promoted by genes from related species coming from another side of the sympatry zone with different environmental gradients. It can be equally hypothesized that the sympatry zone plays only a transitional role and the hybrids are not adapted and occur only due to recurrent, sporadic hybridization. However, occurrence of putative backcrosses among open-pollinated progeny of the hybrid tree (Table 2) demonstrates the potential for gene exchange and subsequent gene introgression. The frequency and distribution of hybrid-looking trees in the sympatry zone of Siberian stone pine and dwarf Siberian pine trees and their possible role in the species adaptation and evolution are still largely unknown and set up the objectives of our future studies. Mitochondrial and chloroplast DNA markers, representing maternal and paternal inheritance in conifers, respectively, can be applied to study direction of gene flow and phenomenon of hybridization more comprehensively.

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REFERENCES

BELOKON', M. M., POLITOV, D. V., BELOKON', YU. S., KRUTOVSKII, K. V., MALUCHENKO O. P. & ALTUKHOV, YU. P. 1998: Genetic differentiation in white pines of the section Strobus: Isozyme analysis data. *Doklady Biological Sciences* 358: 81–84 (translated from the *Proceedings of Russian Academy of Sciences: Doklady Akademii Nauk* [In Russian] **358**(5): 699–702).

BLADA, I. 1994: Interspecific hybridization of Swiss stone pine (*Pinus cembra* L.). *Silvae Genetica* **43**: 15–20.

BOBROV, E. G., 1961: Introgressivnaia gibridizatsia vo flore Baikal'skoi Sibiri (Introgressive hybridization in the flora of the Baikal region in Siberia). *Botanicheskii Zhurnal* (Botanical Journal) **46**: 313–327 [In Russian].

BOBROV, E. G. 1972: Introgressivnaia gibridizatsia, formoobrazovanie i smeny rastitel'nogo pokrova. [Introgressive hybridization, speciation and succession in plants]. *Botanicheskii Zhurnal* (Botanical Journal) **57**: 865–879 [In Russian].

BOBROV, E. G. 1978: Lesoobrazujushie khvoinye SSSR [Forest-forming conifers of the USSR]. Nauka, Leningrad, 189 pp. [IN RUSSIAN]

CRITCHFIELD, W. B. 1986: Hybridization and classification of the white pines (*Pinus* section *Strobus*). *Taxon* **35**: 647–656.

CRITCHFIELD, W. B. & LITTLE, E. L. 1966: Geographic distribution of the pines of the world. U.S. Dep. Agric., Washington, D. C. 98 pp.

FARJON, A. 1984: Pines: Drawings and descriptions of the genus *Pinus*. Leiden: E. J. Brill. 204 p.

Flora Rossica, 1784: 1(1): 5 tab.2 fig. f–h.

GALAZII, G. I. 1954: Tree-line in the mountains of Eastern Siberia and its dynamics. Proceedings of Komarov Botanical Institute, USSR Acad. Sci., V. 9, "Geobotanika" [In Russian].

HÄCKER, M. & BERGMANN, F. 1991: The proportion of hybrids in seed from a seed orchard composed of two larch species (*L. europaea* and *L. leptolepis*). *Ann. Sci. For.* **48**: 631–640.

KONNERT, M. & MAURER, W. 1995: Isozymic investigation on Norway spruce [*Picea abies* (L) Karst.] and European silver fir (*Abies alba* Mill.): Practical guide to separation methods and zymogram evaluation. German Federal-State Working Group "Conservation of Forest Gene Resources". 79 pp.

KRUTOVSKII, K. V., POLITOV, D. V. & ALTUKHOV, YU. P. 1987: Genetic variability in Siberian stone pine, *Pinus sibirica* Du Tour. I. Genetic control of isozyme systems. *Genetika* (Moscow) **23**(12): 2216–2228 (translated in English as *Soviet Genetics* (1988) **23**: 1568–1576).

KRUTOVSKII, K. V., POLITOV, D. V. & ALTUKHOV, YU. P. 1988: Genetic variability in Siberian stone pine, *Pinus sibirica* Du Tour. II. Level of allozyme variability in a natural population of Western Sayan. *Genetika* (Moscow) **24**(1): 118–124 (translated in English as *Soviet Genetics* (1988) **24**: 81–86).

KRUTOVSKII, K. V., POLITOV, D. V., ALTUKHOV, YU. P., MILUTIN L. I., KUZNETSOVA, G. V., IROSHNIKOV, A. I., VOROBJEV, V. N. & VOROBJEVA, N. A. 1989: Genetic variability in Siberian stone pine, *Pinus sibirica* Du Tour. IV. Genetic diversity and differentiation between populations. *Genetika* (Moscow) **25**(11): 2009–2032 (translated in English as *Soviet Genetics* (1990) **25**: 1343–1362).

KRUTOVSKII, K. V., POLITOV, D. V. & ALTUKHOV, YU. P. 1990: Interspecific genetic differentiation of Eurasian stone pines for isoenzyme loci. *Genetika* (Moscow) **26**(4): 694–707 (translated in English as *Soviet Genetics* (1990) **26**:440 -450).

KRUTOVSKII, K. V. & POLITOV, D. V. 1992: Study of intra- and interspecific genetic differentiation between Eurasian stone pines using isozyme loci and multidimensional analysis. In: Molecular Mechanisms of Genetic Processes. Proceedings of All Union Meeting, pp. 87-96. Moscow, Nauka Publishers [In Russian].

KRUTOVSKII, K. V., POLITOV, D. V. & ALTUKHOV, YU. P. 1994: Study of genetic differentiation and phylogeny of stone pine species using isozyme loci. In: Proceedings – International Workshop on Subalpine Stone Pines and Their Environment: the Status of Our Knowledge (W. C. Schmidt and F.-K. Holtmeier, Comps.), St. Moritz, Switzerland, Sept. 5-12, 1992. USDA For. Serv. Intermountain Res. Stat. Gen. Tech. Rep. INT-GTR-309, pp. 19-30.

KRUTOVSKII, K. V., POLITOV, D. V. & ALTUKHOV, YU. P. 1995: Isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pine species (subsection Cembrae, section Strobi, subgenus Strobus). In: Population Genetics and Genetic Conservation of Forest Trees (Baradat, Ph., Adams, W. T. & Müller-Starck, G., Eds.). Proceedings of the International Symposium, Carcans Maubuisson, France, 24-28 August 1992. SPB Academic Publishing, Amsterdam, The Netherlands, pp. 279-304.

LANNER, R. M. 1996: Made for Each Other: A Symbiosis of Birds and Pines. Oxford University Press, Inc., New York, Oxford, 160 pp.

MARKERT, C. L. & FAULHABER, I. 1965: Lactate dehydrogenase isozyme patterns in fish. *J. Exp. Zool.* **159**(2): 319-332.

MIROV, N. T. 1967: The Genus Pinus. The Ronald Press Company. New York. 577 pp.

MOLOZHNIKOV, V. N. 1975: Kedrovii stlanik gornikh landshaftov Severnogo Pribaikailia. [Dwarf Siberian pine in mountainous landscape of the North Baikal region]. Nauka, Leningrad, 203 pp. [In Russian].

POLITOV, D. V. 1989: Allozyme polymorphism, genetic differentiation and mating system of Siberian stone pine *Pinus sibirica* Du Tour. Ph. D. Thesis. Moscow, 1989, 170 pp. (in Russian).

POLITOV, D. V., KRUTOVSKII, K. V. & ALTUKHOV, YU. P. 1992: Isozyme loci characteristics of gene banks of populations of cedar pines. *Genetika* (Moscow) **27**(1): 93-114 (translated in English as *Soviet Genetics* (1992) **27**: 76-95).

POLITOV, D. V. & KRUTOVSKII, K. V. 1994: Allozyme polymorphism, heterozygosity, and mating system of stone pines (Pinus, subsection Cembrae). In: Proceedings – International Workshop on Subalpine Stone Pines and Their Environment: the Status of Our Knowledge (W. C. Schmidt & F.-K. Holtmeier, Comps.), St. Moritz, Switzerland, Sept. 512, 1992. USDA For. Serv. Intermountain Res. Stat. Gen. Tech. Rep. INT-GTR-309, pp. 36-42.

POZDNIAKOV, L. K. 1952: Drevovidnaia forma kedrovogo stlanika. [Tree-like form of dwarf Siberian pine]. *Botanicheskii Zhurnal* (*Botanical Journal*) **37**(5):688-691 [In Russian].

REGEL, Index Sem. Horti Petropol.1858: 23: 1859.

RIDGWAY, G. L., SHERBURNE, S. W. & LEWIS, R. D. 1970: Polymorphism in the serum esterase of Atlantic herring. *Trans. Am. Fish Soc.* **99**: 147-151.

STINE, M., SEARS, B. B. & KEATHLEY, D. E. 1989: Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. *Theor. Appl. Genet.* **78**: 768-774.

SUKACHEV, V. N. 1929: Predvaritel'nii otchet o Baikal'skoi expeditcii AN v 1926 g. [Preliminary report on the Baikal Expedition of the Academy of Sciences in 1929). *Otchety o deiatel'nosti AN SSSR* (Reports on the USSR Academy of Sciences Activity), Leningrad **2**: 10-18 [In Russian].

TITOV, E. V. 1977: Opit skreschivanija kedra sibirskogo s drugimi sosnami v uslovijakh severo-vostochnogo Altaia. [Experimental crosses of Siberian stone pine with other pines in the Northeast Altai]. *Lesovedenie* (*Forestry*) 4: 81-87 [In Russian].

WAGNER, D. B., FURNIER, G. R., SAGHAI-MAROOF, M. A., WILLIAMS, S. M., DANCIK, B. P. & ALLARD, R. W. 1987: Chloroplast DNA polymorphism in lodgepole and jack pines and their hybrids. *Proc. Natl. Acad. Sci. USA* **84:** 2097 -2100.

WANG, X.-R., SZMIDT, A.E., LEWANDOVSKI, A.& WANG, Z.-R. 1990: Evolutionary analysis of *Pinus densata* Masters, a putative Tertiary hybrid. *Theor. Appl. Genet.* **80**: 635-640.

WATANO, Y., IMAZU, M. & SHIMIZU, T. 1995: Chloroplast DNA typing by PCR-SSCP in the *Pinus pumila – P. parviflora* var. *pentaphylla* complex (Pinaceae). *Journal of Plant Research* **108**: 493-499.

WATANO, Y., IMAZU, M. & SHIMIZU, T. 1996: Spatial distribution of cpDNA and mtDNA haplotypes in a hybrid zone between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae). *Journal of Plant Research* **109**: 403-408.