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Artificial crosses and hybridization frequency in five-needle pines

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Abstract: The capability of conifers for interspecific hybridization is well known. Five-needle pines from the section *Quinquefoliae* of the subgenus *Strobus* play an important role in ecosystems and have great economic significance. Interspecific hybridization that occurred in nature or under controlled pollination suggests that reproductive isolation is relative, being expressed to different degrees. Controlled pollination allows us to gather knowledge about reproductive compatibility and provides valuable material for breeding work. Artificial crosses of five-needle pines began with a purely practical purpose to increase blister rust resistance and it was subsequently suggested that species crossability should be taken into account in their classification. We carried out a number of controlled pollinations using *Pinus sibirica*, *P. koraiensis* and natural *P. sibirica* × *P. pumila* hybrid clones as maternal trees along with pollen of 11 species and four hybrids of the five-needle pines. For the first time, seeds were obtained from the interspecific crosses *P. koraiensis* × *P. armandii*, *P. koraiensis* × *P. monticola*, *P. koraiensis* × *P. sibirica*, *P. sibirica* × *P. ayacahuite*, *P. sibirica* × *P. armandii*, and *P. sibirica* × *P. wallichiana*, as well as from some combinations with hybrids. Based on the results and available information on the crossability of the species, we concluded that complete reproductive isolation does not exist among five-needle pines, and confirmed that interspecific gene flow is common in this section.

Keywords: soft pines, controlled pollination, reproductive isolation, compatibility

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Introduction

It has long been known that natural interspecific hybridization is widespread especially among plants. Many species of conifers have a high capacity for hybridization. For example; within the *Larix* genus, well-known hybrids between *L. sibirica* and *L. gmelinii* exist – described as *L. × czekanowskii* (Abaimov & Koropachinskiy, 1984; Semerikov & Lascoux, 2003) – and hybrids between *L. kaempferi* and *L. decidua* have been documented, referred to as *L. × marschlinii*

(Sander & Läänelaid, 2007). Both native and introduced *Larix* species are easily cross-pollinated in plantations (Avrov, 1982; Meirmans et al., 2014). Hybridization is also an inherent process of the *Picea* genus; a well-known example is the introgression of the *Picea abies* and *P. obovata*, which has resulted in a vast hybrid zone which reaches widths of up to 1000 km (Tsuda et al., 2016). Hybridization of the *Abies* species does not only lead to hybrid species, but has also resulted in the emergence of the hybrid section, *Balsamea* (Xiang et al., 2015). Although the Pinaceae

family exhibits large amounts of interspecies gene flow, it is not one of the 25 plant families that are most prone to hybridization (Whitney et al., 2010).

The most numerous genus of the Pinaceae family – *Pinus* – includes about 110 species of the subgenera *Pinus* or *Strobos*. All five-needle pines with five leaves per fascicle were classified into section *Strobos* of the subgenus *Strobos*. Section *Strobos* has previously been divided into two subsections, *Strobi* (white pines) and *Cembrae* (stone pines); based primarily on the female cone structure, seed morphology and mode of distribution (Critchfield & Little, 1966). Species with dehiscent cones and wind-dispersed small winged seeds were classed as *Strobi*, while species with indehiscent cones and bird-dispersed large wingless seeds were considered as *Cembrae*, although there were no clear boundaries between the subsections. As a consequence, additional group (*Flexiles*) was created including *P. flexilis* and *P. armandii*, which have large seeds with rudimentary wings and are dispersed by birds rather than wind (Shaw, 1914). The current classification is based on molecular markers and classifies all five-needle pines into a single group, namely subsection *Strobos* of the section *Quinquefoliae* of the subgenus *Strobos* (Gernandt et al., 2005; Syring et al., 2007).

The first artificial pollination experiments involving five-needle pines were carried out with the aim of increasing resistance to blister rust caused by *Cronartium ribicola* J.C. Fisch. Almost all American species of five-needle pines are susceptible to this pathogen, which can decimate forest stands (McDonald & Hoff, 2001). Efforts to transfer the genes associated with resistance resulted in a number of artificial crosses among five-needle pines, mostly between American and Asian white pine species (Callaham, 1962; Kriebel, 1983; Critchfield & Kinloch, 1986). Indeed,

interspecies hybrids between American species *P. strobus* and Asian species (*P. parviflora* and *P. koraiensis*) are distinguished by significantly higher resistance to blister rust (Lu et al., 2005).

Three species of the five-needle pines; the Siberian stone pine (*Pinus sibirica* Du Tour), Siberian dwarf pine (*P. pumila* (Pall.) Regel) and Korean pine (*P. koraiensis* Sieb. & Zucc.) previously part of the subsection *Cembrae*; are widespread in Russia. There have been few efforts to hybridize Russian five-needle pines with other related pines. Artificial crosses of Siberian stone pine were only carried out in the 1960s and 1970s (Dokuchaeva, 1967; Titov, 1977). In the thirty years since the hybridization of five-needle pines was summarized by W.B. Critchfield (1986), significant new data has accumulated and a new summary is needed. Although molecular methods have come to the forefront for classification, species crossability is still of great theoretical and practical interest. The aim of the current study was to determine the crossability of species from the subsection *Strobos* of the section *Quinquefoliae*, based on controlled pollination experiments and review of the available literature.

Material and Methods

The experiment was carried out in 2008 and 2012 at the “Kedr” field station, managed by the Institute of Monitoring of Climatic and Ecological Systems and situated 30 km south of Tomsk (Tomskaya oblast, Russia, 56°13N 84°51E, 78 m a.s.l.). Reciprocal crosses of *P. sibirica* and *P. koraiensis* were performed in 2008. In 2012, *P. sibirica*, *P. koraiensis* and the natural hybrids *P. sibirica* × *P. pumila* were pollinated using the pollen of 11 different pine species and four interspecies hybrids from section *Quinquefoliae* (Table

Table 1. Number of pollinated and collected cones in controlled crosses

Species origin	Pollen	<i>P. koraiensis</i>		<i>P. sibirica</i>		<i>P. sibirica</i> × <i>P. pumila</i>	
		pollinated	collected	pollinated	collected	pollinated	collected
North America	<i>P. ayacahuite</i>	26	6	10	10	–	–
	<i>P. monticola</i>	28	21	15	14	–	–
	<i>P. strobus</i>	17	8	29	28	–	–
	<i>P. ×schwerinii</i>	9	0	4	4	–	–
Europe	<i>P. cembra</i>	7	4	32	32	–	–
	<i>P. peuce</i>	4	4	10	10	–	–
Asia	<i>P. armandii</i>	37	13	27	20	–	–
	<i>P. koraiensis</i> *	–	–	18	13	–	–
	<i>P. parviflora</i>	28	9	29	27	70	51
	<i>P. pumila</i>	2	2	13	3	–	–
	<i>P. sibirica</i> *	5	3	–	–	–	–
	<i>P. wallichiana</i>	37	16	19	17	–	–
	<i>P. pumila</i> × <i>parviflora</i>	–	–	9	9	–	–
	<i>P. sibirica</i> × <i>koraiensis</i>	40	23	3	2	–	–
	<i>P. sibirica</i> × <i>pumila</i>	3	1	15	9	35	27

“–” – controlled pollination was not conducted; * – experiment 2008.

1). Mother plants of the species and the hybrids were cloned by grafting. They were of varying ages in the reproductive phase. All pollen was collected at the "Kedr" field station and from Czech arboretums (Kostelec and Sofronka) in the year of the experiment, with the exception of *P. koraiensis* pollen. Pollen release in *P. koraiensis* occurs much later than the flowering time of *P. sibirica*. *P. koraiensis* pollen was collected a year prior to pollination and was stored with CaCl_2 in a refrigerator. Pollen was collected from at least three trees of each species and hybrids, with the exception of *P. × schwerinii* (*P. strobus* × *P. wallichiana*) and *P. pumila* × *P. parviflora*, with one paternal tree of each. Before the controlled pollination experiment, pollen viability was examined by germination test in 5% sugar solution and was not lower than 70%. In total, 15 *P. koraiensis* clones, 11 *P. sibirica* clones and seven *P. sibirica* × *P. pumila* clones were used as mother plants. One ramet per clone was used for species and 2–3 ramets were used for the *P. sibirica* × *P. pumila* hybrids. The number of pollinated macrostrobili was 243 in *P. koraiensis*, 233 in *P. sibirica* and 105 in the hybrids. Female cones of the same clones obtained from open pollination were used as controls. The number of cones collected for open pollination experiments was 25 in *P. koraiensis*, 36 in *P. sibirica* and 10 in *P. sibirica* × *P. pumila* hybrids.

For controlled pollination, the female cones were isolated in Kraft paper bags 1–2 days before pollen release. After isolation we observed female cone development every day and carried out pollination twice in the receptive phase. We used a syringe to add pollen to the isolation bag through a little cut that was carefully sealed after pollen application. Isolation bags were removed after closure of macrostrobili scales. All seeds in the mature cone were categorized as either full-grown (normal size) or aborted (rudimentary, much smaller). The quality of full-grown seeds was checked using X-ray analysis (Shcherbakova, 1965).

Results

Conelet survival varied greatly in the different crosses, with all conelets from the *P. koraiensis* × *P. × schwerinii* cross being dropped. However, on average, conelet drop was less than 7% per pollination year. In the year after pollination, cone drop was high in some crosses. For example in the *P. koraiensis* × *P. ayacahuite* cross, more than 75% conelets has dropped.

Cones from controlled crosses, in which *P. sibirica* and *P. sibirica* × *P. pumila* hybrids were the mother trees, produced approximately the same number of seeds as cones from open pollination did. In some cases, crosses of *P. sibirica* with *P. wallichiana*, *P. koraiensis* or *P. pumila* × *P. parviflora* pollen produced notably

fewer seeds (Table 2). On the contrary, seed number was consistently reduced in cones of *P. koraiensis*, except when pollinated with *P. monticola*, *P. cembra* or *P. sibirica* pollen. There were no seeds in some *P. koraiensis* cones; cones without seeds occurred in crosses with *P. peuce*, *P. wallichiana*, and *P. ayacahuite*.

We did not obtain filled seeds from *P. koraiensis* crossed with *P. ayacahuite*, *P. strobus*, *P. peuce*, *P. cembra*, *P. pumila*, *P. wallichiana* and with *P. sibirica* × *P. pumila* hybrids. The highest number of filled seeds was obtained in back-crosses *P. koraiensis* × (*P. sibirica* × *P. koraiensis*). Among crosses attempted, interspecies crosses *P. koraiensis* × *P. sibirica* and *P. koraiensis* × *P. armandii* were the most successful whereas crosses *P. koraiensis* × *P. parviflora* and *P. koraiensis* × *P. monticola* resulted in one sound seed only.

P. sibirica did not produce filled seeds in crosses with the species: *P. monticola*, *P. strobus*, *P. peuce*, *P. parviflora*, *P. × schwerinii*. The best compatibility with more than 63% of filled seeds was recorded in the *P. sibirica* × *P. cembra* cross. *P. sibirica* × *P. koraiensis* and *P. sibirica* × *P. wallichiana* were less successful crosses. Several tens of sound seeds were obtained in back-crosses *P. sibirica* × (*P. sibirica* × *P. pumila*) and *P. sibirica* × (*P. sibirica* × *P. koraiensis*). In crosses of *P. sibirica* with *P. parviflora* × *P. pumila* hybrid a few filled seeds were obtained. Crosses of *P. sibirica* with *P. armandii*, *P. ayacahuite*, and *P. pumila* resulted in one sound seed only.

Controlled pollination of the natural hybrids *P. sibirica* × *P. pumila* was successful in both cases. The same hybrid clone (inventory number 28) was used in controlled self-pollination and open pollination. The portion of filled seeds was similar in both cases (Chi-square 3.36, $p=0.0668$). Seven clones of the hybrids were pollinated by *P. parviflora* pollen with only four filled seeds obtained.

Almost all filled seeds had a well-developed embryo, less than 5% of the seeds had a small poorly developed embryo, and an empty cavity was observed extremely rarely. In the (*P. sibirica* × *P. pumila*) × *P. parviflora* cross, only one of the four filled seeds obtained had a well-developed embryo.

Discussion

Controlled pollination in conifers, including five-needle pines, is a rather labor-intensive experiment and is rarely performed. Controlled crosses of *P. sibirica* with *P. koraiensis* were conducted in the early 1960s by M.I. Dokuchaeva (1967). Using the pollen mix of *P. sibirica* and *P. koraiensis* author obtained seed progeny from *P. sibirica* × *P. koraiensis* crosses that were grown in Ivanteevka arboretum (Moskovskaya oblast). Hybrid nature of some plants of the progeny was proved by isozyme analysis (Politov, 2007).

Table 2. Seed quality in different controlled crosses

Pollen	Seeds per cone			Analyzed full-grown seeds			
	Total	Aborted seeds, %	Full-grown seeds, N	Total	Filled seeds, N/%	Seeds with CM ¹ , N/%	Empty seeds, N/%
<i>P. koraiensis</i>							
Open pollination	63.9	6.8	59.5	752	568/75.5	7/0.9	177/23.5
<i>P. ayacahuite</i>	27.0	7.4	4.2	25	0/0	0/0	25/100
<i>P. monticola</i>	50.8	2.6	49.5	1039	1/0.1	0/0.0	1038/99.9
<i>P. strobilus</i>	22.6	4.4	21.6	168	0/0	0/0	168/100
<i>P. cembra</i>	81.3	0.6	80.8	352	0/0	0/0	352/100
<i>P. peuce</i>	0.25	0.0	1.0	1	0/0	0/0	1/100
<i>P. armandii</i>	11.7	7.9	10.8	137	14/10.2	1/0.7	122/89.1
<i>P. parviflora</i>	8.8	2.5	8.6	74	1/1.4	0/0.0	73/98.6
<i>P. pumila</i>	20.0	15.0	17.0	34	0/0	0/0	34/100
<i>P. sibirica</i> *	78.0	1.3	77.0	230	26/11.3	1/0.4	203/88.3
<i>P. wallichiana</i>	6.6	3.8	6.4	102	0/0	0/0	102/100
<i>P. sibirica</i> × <i>koraiensis</i>	32.6	40.3	19.5	415	244/58.8	5/1.2	166/40.0
<i>P. sibirica</i> × <i>pumila</i>	29.0	3.4	28.0	28	0/0	0/0	28/100
<i>P. sibirica</i>							
Open pollination	58.7	2.3	57.3	1551	1105/71.2	63/4.1	383/24.7
<i>P. ayacahuite</i>	61.3	7.6	56.6	488	1/0.2	0/0.0	487/99.8
<i>P. monticola</i>	40.9	4.7	38.9	491	0/0	0/0	491/100
<i>P. strobilus</i>	58.6	4.0	56.3	1466	0/0	1/0.07	1465/99.93
<i>P. ×schwerinii</i>	67.5	5.2	64.0	203	0/0	0/0	203/100
<i>P. cembra</i>	60.4	7.0	56.2	1580	1004/63.5	44/2.8	532/33.7
<i>P. peuce</i>	51.2	3.7	49.3	439	0/0	0/0	439/100
<i>P. armandii</i>	58.8	3.9	56.5	1018	1/0.1	0/0.0	1017/99.9
<i>P. koraiensis</i> *	35.6	2.6	34.6	385	16/4.2	10/2.6	359/93.2
<i>P. parviflora</i>	50.6	4.1	48.5	810	0/0	0/0	810/0
<i>P. pumila</i>	74.3	5.4	70.3	191	1/0.5	0/0.0	190/99.5
<i>P. wallichiana</i>	22.1	1.6	21.7	353	45/12.7	0/0.0	308/87.3
<i>P. pumila</i> × <i>parviflora</i>	35.7	6.2	33.4	270	3/1.1	3/1.1	264/97.8
<i>P. sibirica</i> × <i>koraiensis</i>	57.5	5.2	54.5	98	33/33.7	7/7.1	58/59.2
<i>P. sibirica</i> × <i>pumila</i>	54.1	7.4	50.1	381	158/41.5	16/4.2	207/54.3
<i>P. sibirica</i> × <i>P. pumila</i>							
Open pollination	25.4	36.6	16.1	248	37/14.9	24/9.7	187/75.4
<i>P. parviflora</i>	18.1	28.2	13.0	522	4/0.8	20/3.8	498/95.4
<i>P. sibirica</i> × <i>pumila</i>	23.0	27.8	16.6	295	62/21.0	22/7.5	211/71.5

* – experiment 2008, CM¹ – Collapsed Megagametophyte.

The same hybrid plants were grafted in 2006 at the “Kedr” field station serving as the pollen donors in our experiment. E.V. Titov (1988) has also reported that cross of *P. sibirica* × *P. koraiensis* was successful producing sound seeds. The species *P. cembra* is the most closely related to *P. sibirica* and it was successfully crossed with North American species *P. monticola* and with Asian species *P. wallichiana* (Blada, 1994).

We obtained sound seeds from the following interspecific crosses: *P. koraiensis* × *P. armandii*, *P. koraiensis* × *P. monticola*, *P. koraiensis* × *P. sibirica*, *P. koraiensis* × (*P. koraiensis* × *P. sibirica*), *P. sibirica* × *P. ayacahuite*, *P. sibirica* × *P. armandii*, *P. sibirica* × *P. wallichiana*, *P. sibirica* × (*P. pumila* × *P. parviflora*), *P. sibirica* × (*P. sibirica* × *P. koraiensis*). The seeds obtained were sown and the progenies are grown at the “Kedr” field station (Tomskaya oblast).

A previous study has shown that the *P. sibirica* × *P. pumila* hybrids can be successfully crossed with the parental species serving either as a mother plant or as a pollen donor (Vasilyeva & Goroshkevich, 2013). The hybrids are characterized by a high proportion of rudimentary seeds. When the parental species (*P. sibirica* or *P. pumila*) were used as the mother plants, no more than 10% of seeds were rudimentary but when the hybrids were used as the mother plants, the proportion of rudimentary seeds was 25% or more (Vasilyeva & Goroshkevich, 2013). The results of the current experiment indicate that hybrids always have a plenty of rudimentary seeds what is in contrast with pure parental species which do not exhibit this phenomenon. This confirms that rudimentary seed production is determined by the mother plant and does not depend on pollen origin.

Our knowledge about the development of reproductive structures in five-needle pines under interspecific crosses is scarce. In the interspecific crosses *P. peuce* × *P. cembra*, *P. peuce* × *P. koraiensis*, seed development is normal until the proembryo stage, at which point it stops (Hagman & Mikkola, 1963). A similar pattern of development was observed in *P. strobus* pollinated by *P. flexilis*, *P. cembra* and *P. koraiensis* pollen (Kriebel, 1972); the author concluded that in white pines inviability of the embryo is a key factor underlying the incompatibility barrier. However, this statement is not valid for all five-needle pines. Seed development ceases before fertilization although the corrosion cavity is formed in the megagametophyte in *P. monticola* pollinated with *P. lambertiana* pollen (Fernando et al., 2005). Cytoembryological research on the ovules and developing seeds of *P. sibirica* pollinated by *P. armandii*, *P. parviflora*, *P. strobus*, *P. wallichiana*, *P. monticola* and *P. pumila* × *P. parviflora* pollen showed that archegonium development was normal but fertilization was absent in spite of corrosion cavity formation (Tretyakova & Lukina, 2017).

Interestingly, *P. sibirica* and *P. parviflora* did not intercross but in the crosses *P. sibirica* × (*P. pumila* × *P. parviflora*) and (*P. sibirica* × *P. pumila*) × *P. parviflora* we have obtained a few sound seeds. Hence, the hybrids serve as a genetic bridge for species that are not able to intercross directly. It is possible that more attempts and the involvement of more trees in controlled pollination could lead to obtaining hybrid seed progeny. Moreover, the selection of mother trees is needed especially for species with weak crossability (Kriebel, 1972) because maternal control is a powerful factor controlling embryo abortion (Korbecka et al., 2002).

Artificial crosses of different five-needle pines were described in a few scientific publications and summarized by W.B. Critchfield (1986). Natural hybridization was reported between *P. flexilis* and *P. strobiformis* (Steinhoff & Andresen, 1971; Menon et al., 2018), *P. flexilis* and *P. monticola* (Mirov, 1967; Hoff, 1968). The species *P. chiapensis* naturally hybridized with *P. ayacahuite* (Perry, 1991) and *P. ayacahuite* with *P. strobiformis* (Frankis, 2009). The species *P. pumila* is actively involved in natural hybridization forming hybrid zones with *P. parviflora* (Watano et al., 1996; Ito et al., 2008) and *P. sibirica* (Goroshkevich, 1999; Polotov et al., 1999; Goroshkevich et al., 2008). A study of the chloroplast DNA in both *P. lambertiana* and *P. albicaulis* has shown that hybridization occurred between these species in the past (Liston et al., 2007). The Asian species *P. armandii*, *P. kwantungensis*, *P. morrisonicola*, *P. fenzeliana*, *P. bhutanica*, *P. dabeshanensis* and *P. wangii* are closely related (Liu et al., 2014; Li et al., 2015) and have probably crossed successfully but there is no information about this in the literature. It is presumed that introgression has occurred between

P. armandii and *P. kwantungensis* as well as between *P. armandii* and *P. bhutanica* (Liu et al., 2014). South Asian five-needle pines are similar in such degree that sometime they are not considered to be separate species. For example, *P. kwantungensis* is regarded as a subspecies of *P. wangii*, and *P. bhutanica* as a subspecies of *P. wallichiana* (Businský, 2004). The species *P. dalatensis* is an endemic species from Vietnam with a very narrow geographic distribution which is closely related to subtropical eastern Asian species (Nghia, 2008; Hao et al., 2015). Our results together with literature data on five-needle pine hybridization suggest that there are weak reproductive barriers among species (Fig. 1).

As illustrated in the figure, there are 37 reproductive combinations that have arisen as a result of natural hybridization and successful artificial crosses

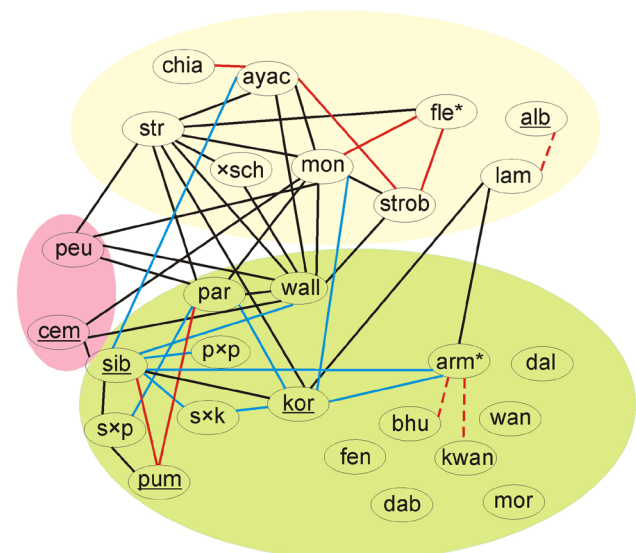


Fig. 1. Crossability of five-needle pines from the subsection *Strobus* of section *Quinquefoliae* subgenus *Strobus*. American species are shown in the yellow field, European – in the pink field, and Asian in the green field. Species symbols: peu – *P. peuce*, str – *P. strobus*, chia – *P. chiapensis*, mon – *P. monticola*, ayac – *P. ayacahuite*, strob – *P. strobiformis*, lam – *P. lambertiana*, fle – *P. flexilis*, alb – *P. albicaulis*, sib – *P. sibirica*, cem – *P. cembra*, pum – *P. pumila*, kor – *P. koraiensis*, par – *P. parviflora*, arm – *P. armandii*, wall – *P. wallichiana*, kwan – *P. kwantungensis*, mor – *P. morrisonicola*, fen – *P. fenzeliana*, bhu – *P. bhutanica*, dab – *P. dabeshanensis*, wan – *P. wangii*, dal – *P. dalatensis*, xsch – *P. × schwerinii*, s×p – *P. sibirica* × *P. pumila* hybrids, s×k – *P. sibirica* × *P. koraiensis* hybrids, p×p – *P. pumila* × *P. parviflora* hybrids. Underline – species that were previously in subsection *Cembrae*, * – species that were previously in *Flexiles* group. Red line shows natural hybridization, red dashed line ancient hybridization, blue line artificial crosses first carried out in the given experiment, black line artificial crosses earlier known (Little & Righter, 1965; Dokuchaeva, 1967; Titov, 1977; Critchfield, 1986; Blada, 1994; Lu et al., 2005; Vasilyeva & Goroshkevich, 2013)

between 23 species of the section *Quinquifoliae*, without taking into account crossability with hybrids. It turns out that on average, each species ($37/23 = 1.6$) is able to cross with at least one other species and as a maximum with eight species, as in the case of *P. wallichiana*. We believe that this is a conservative estimate, since many interspecific crosses have not yet been carried out or have not been confirmed or have not yet been completed successfully. Our knowledge about the wide hybridization capability among the five-needle pines indicates the importance of genetic exchange in their evolution, its potential as a method of speciation and as a source of genetic enrichment of species' gene pools. According to recent studies, some species (*P. pumila*, *P. koraiensis* and *P. parviflora*) provide evidence of historical hybridization, which is currently being found as an incongruence of phylogenetic trees based on mitochondrial, chloroplast and nuclear DNA markers (Tsutsui et al., 2009; Wang & Wang, 2014; Hao et al., 2015).

Reproductive isolation of biological species is considered to be a key criterion of species delimitation (Mayr, 1942; 1970). It has previously been believed that crosses between true species and another species do not result in fertile and promising hybrids and only in sterile and maladapted ones. This is an extreme view that has been challenged since the evolutionary importance of interspecific hybridization has become commonly accepted (Rieseberg & Carney, 1998; Mallet, 2005; Soltis & Soltis, 2009 and other). Simultaneously with the enrichment of our knowledge about hybridization frequency and its real impact on species evolution, the term "reproductive isolation" is being revised in the context of biological species concept. As written by E. Mayr (1996), isolating mechanisms do not always prevent the occasional interbreeding of non-conspecific individuals, but they nevertheless prevent the complete fusion of such species populations. Such understanding of isolating mechanisms is consistent with current ideas about reproductive barriers that are considered as semipermeable to gene flow and at the same time maintain species boundaries (Wu, 2001; Rieseberg et al., 2003; Hausdorf, 2011; De La Torre et al., 2014). The five-needle pines as a group of species isolated primarily by geographic and ecological factors and secondly by slowly developing incompatibility are a convincing illustration of the current concept of species boundaries.

Conclusion

This study showed the potential crossability of the Asian species *P. sibirica*, *P. koraiensis* and *P. sibirica* × *P. pumila* hybrids with related five-needle pines of different geographic origins. Artificial and natural interspecific hybrids can serve as a mediator between

species with pronounced incompatibility, as shown in the pair of *P. sibirica* and *P. parviflora*. A summary of the results and literature review suggests that species from section *Quinquifoliae* of the subgenus *Strobis* possess weak reproductive barriers and high interbreeding potential that, under climatic change or anthropogenic factors, may lead to formation of the new hybrid zones.

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