Ann. For. Res. 51: 19-30, 2008

ANNALS OF FOREST RESEARCH
www.e-afr.org

Population genetic structure and mating system in the hybrid zone between *Pinus sibirica* Du Tour and *P. pumila* (Pall.) Regel at the Eastern Baikal Lake shore

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Petrova E.A., Goroshkevich S.N., Belokon M.M. , Belokon Y.S., Politov D.V. 2008. Population genetic structure and mating system in the hybrid zone between *Pinus sibirica* Du Tour and *P. pumila* (Pall.) Regel at the Eastern Baikal Lake shore. Ann. For. Res. 51: 19-30.

Abstract. Genetic structure of sympatric *Pinus sibirica* Du Tour and *P. pumila* (Pall.) Regel populations and putative interspecific hybrids between them was analyzed in the Baikal Lake region (Barguzin Biosphere Natural Reserve, Davsha River basin) by means of 31 allozyme loci controlling 18 enzyme systems. Several alleles at loci *Adh-1, Fest-2, Lap-3, Pgi-1, Sod-3* and *Skdh-1* were diagnostic for *P. sibirica*, while alleles typical for P. pumila were detected at loci Gdh, Got-3, Lap-3, *Mdh-2, Mdh-4, Pepca, Pgi-1, Pgd-2, Pgd-3, Pgm-1* and *Pgm-2*. All hybrids were heterozygous for the diagnostic *Skdh-2* locus. Classification into hybrids and parental species using PCA analysis of multilocus allozyme genotypes had good correspondence with diagnoses made by morphological and anatomical analyses. Approximately 27% of embryos in *P. pumila* seeds had *P. sibirica* paternal contribution, and 8% of haplotypes in effective pollen pool combined alleles typical for *P. pumila* and *P. sibirica*, and therefore were classified as pollinated by the hybrids. About 83% of embryos in seeds from the hybrids most likely originated from fertilization by *P. sibirica* pollen, 14% from *P. pumila* and 3% from hybrid trees. This result favours the view that hybrids make both male and female contributions to the reproductive output of the population and confirm the presence of backcrosses and F_2 hybrids.

Keywords: natural hybridization, *Pinus sibirica*, *Pinus pumila*, allozymes, mating system, genetic structure, Baikal Lake.

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Introduction

Interspecific hybridization is common in higher plants. It is a frequent and important component of plant evolution and speciation (Arnold 1997, Barton & Hewitt 1985). It has been argued that hybrids occur more frequently at geographical and ecological margins of parental species when their ranges overlap (Grant 1981, Stebbins 1950). Usually hybrids show lower fitness in parental environments (Barton 1979, Barton & Hewitt 1985), but possibly increased fitness in novel or intermediate environments (Moore 1977). Reproductive success can be considered as evidence of the evolutionary potential of hybrids. Thus, the study of genotypic structure and mating patterns, especially the contribution of hybrids to the formation of next generation's gene pool, is of primary importance for geneticists dealing with a hybrid zone.

The Lake Baikal drainage is located in Siberia at the boundaries of three floristic provinces and has traditionally been viewed as a region of mass natural hybridization (Bobrov 1961). This is the zone where the ranges of two related *Pinus* species of subsection Cembrae overlap: Siberian stone pine (*Pinus sibirica* Du Tour) and Siberian dwarf pine (*Pinus pumila* (Pall.) Regel). The possibility of natural hybridization between these species was first noticed in the first half of the twentieth century (Sukachev 1929). Only a few presumably hybrid individuals intermediate in morphology were found (Galazii 1954, Molozhnikov1975, Pozdniakov 1952). The initial discovery of natural hybrids between the two stone pine species in two sites at the Baikal Lake coast was reported by D. Politov (1998) and confirmed by allozyme analysis. Genetic evidence for natural hybridization between *P. sibirica* and *P. pumila* was obtained using electrophoretic analysis of seed isozymes (Politov et al. 1999). In that study, only eight seeds from one putative hybrid were analyzed. Occurrence of putative backcrosses among open-pollinated progeny of the tree was assumed (Politov et al. 1999). S. Goroshkevich (1999, 2004) was the first to describe mass hybridization of these species in nature. The study was based on reproductive and growth traits (growth form, cone morphology and needle structure) and confirmed the intermediate position of the putative hybrids relative to parental species (Goroshkecich 1999, 2004).

In this paper, we present for the first time the results of genetic analysis of a substantial number of natural hybrids and representatives of parental species, along with embryos in seeds collected from these trees. This approach allows to recognize the hybridization events at an early ontogenetic stage and to describe potential gene introgression.

Materials and methods

The study site is located in the eastern part of Lake Baikal region on the territory of Barguzin Biosphere Natural Reserve (Buryat Republic, Russia). *Pinus sibirica-Larix* forests dominated by *P. sibirica* are distributed at the site. Generative *P. pumila* individuals are scarce; for the most part there are few old-growth thickets up to 50 m in diameter occurring in the narrow (50-100 m wide) Lake Baikal riparian zone. Open-pollinated seeds were collected in September, 2005 from typical *P. sibirica* and *P. pumila* trees and from putative hybrids in the mixed stand in the lakeside zone in Davsha Bay. Identification of species and hybrids *in situ* were conducted using two main characters: tree habit and coloration of maturating cones. Plants with green cones and crooked or prostrate stems were assigned to *P. pumila*, and plants with violet cones and upright stems were assigned to *P. sibirica*. Plants with violet cones but not having upright (crooked, crawling or inclined) stem were classified as hybrids. In total, the ratio of (*P. sibirica*):(*P. pumila*):(hybrid) numbers was approximately 300:10:1. According to the analysis of cone scars on shoot bark, in this stand *P. sibirica* produces abundant cone yields regularly. In contrast, *P. pumila* has a lot of aborted cones from year to year and only several cones per tree reach maturation.

Haploid megagametophyte and diploid embryo tissues of intact pine seeds were extracted and ground separately in distilled water. Homogenates were separated electrophoretically in 13% starch gels using three buffer systems: A - morpholine citrate, pH 7.8 (Clayton & Tretiak 1972), B - Tris-EDTA borate, pH 8.6 (Markert & Faulhaber 1965), and C - Tris-citrate/LiOH borate (Ridgeway et al. 1970). Thin, horizontal gel slices were stained for enzyme activity of aconitase (ACO, 4.2.1.3), alcohol dehydro-genase (ADH, 1.1.1.1), formate dehydro-genase (FDH, 1.2.1.2), fluorescent esterase (FE, 3.1.1.1), fumarase (FUM, 4.2.1.2), glutamate dehydrogenase (GDH, 1.4.1.2-4), glutamate oxaloacetate transarninase (GOT, 2.6.1.1), (IDH, 1.1.1.42), leucine aminopeptidase (LAP, 3.4.11.l), malate dehydrogenase (MDH, 1.1.1.37), menadione reductase (MNR, 1.6.99.2), phospho-enolpyruvate carboxylase (PEPCA, 4.1.1.31), phosphoglucose isomerase (PGI, 5.3.1.9), 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44), phosphoglucomutase (PGM, 5.4.2.2), shikimate dehydrogenase (SKDH, 1.l.1.25), sorbitol dehydrogenase (SDH, 1.1.1.14), and superoxide dismutase (SOD, 1.15.1.1). SKDH, FUM, 6-PGD, MDH, PEPCA, MNR, IDH and ACO were separated in buffer system A; ADH, FE, GDH, PGI, PGM and SOD in B; FDH, GOT, LAP and SDH in C. Multiple activity zones and the corresponding gene loci were designated according to the abbreviated names of enzymes and numbered in the order of decreasing electrophoretic mobility from the anode to the cathode. Genetic control of these enzymes was described earlier for *P. sibirica, P. pumila* and other pines of the section Strobus (Belokon et al. 2005, Krutovskii et al. 1987, Politov et al. 1999, 2006).

Computations were performed based on matrices of multilocus genotypes of open-pollinated embryos and for adult (maternal) plants. The maternal tree genotypes were inferred from segregation of allelic variants in haploid seed endosperms (megagametophytes); at least eight endosperms were analyzed for each tree. Matrices of genotypes of maternal trees and embryos were used to calculate the mean number of polymorphic loci, mean number of alleles per locus, observed (*HO*) and expected (*HE*) heterozygosity, and intrapopulation inbreeding coefficient $F_{IS} = (HE\text{-}HO)/HE$. Distributions of multilocus genotypes of trees and embryos were tested for deviations from Hardy-Weinberg equilibrium using the program GenAlEx V.6 (Peakall & Smouse 2006). Multidimensional analysis of genotypes was performed by Principal Components Analysis with Statistica software (StatSoft 1998).

Results

Genetic structure of adult tree samples

Multilocus genotypes were determined for 24 individuals of *P. pumila*, 21 hybrids and 20 *P. sibirica* trees. In total, 31 loci controlling 18 enzyme systems were analyzed. Allele frequencies for parental species and hybrid samples are presented (table 1). Only *Mdh*-1 and *Sdh* loci were completely monomorphic in all three samples. Loci *Fdh, Gdh, Got-3, Idh, 6-Pgd-2, 6-Pgd-3, Pgi-1, Pgm-2, Skdh-2* and *Sod-2* were fixed in *P. sibirica* tree sample. Some of these loci are monomorphic across the species' range (*Got-3, 6-Pgd-2, Pgi-1, Pgm-2, 6-Pgd-3*), while other loci (e.g., *Fdh, Skdh-2*) are variable in other parts of the *P. sibirica* distribution (Krutovskii et al. 1989, Petrova et al. 2006, Politov 1989). In *P. pumila* the number of polymorphic loci was 80%; only loci *Adh-1, Got-1, Pgi-1* and *Sod-3* were monomorphic. There were several alleles at loci *Adh-1, Fest-2, Lap-3, Pgi-1, Sod-3* and *Skdh-1* found only in *P. sibirica* sample, while alleles typical for *P. pumila* were detected in loci *Gdh, Got-3, Idh, Lap-3, Mdh-2, Mdh-4, Mnr-1, Pepca, Pgi-1, Pgd-2, Pgd-3, Pgm-1, Pgm-2, Sod-2* and *Skdh-2*. For most of the loci the hybrids had a combination of alleles typical for both *P. pumila* and *P. sibirica*, confirming their hybrid origins. For instance, all hybrids were heterozygous at *Skdh-2* locus, combining alleles typical for *P. pumila* and *P. sibirica.* This locus was previously recommended as a diagnostic genetic marker to recognize *P. pumila* × *P. sibirica* hybrids (Politov et al. 1999).

Most of the genotype frequency distributions observed in the samples of pure species and hybrids corresponded to the Hardy-Weinberg proportions. A significant deviation towards the heterozygote deficiency was detected at two loci (*Idh* and *6-Pgd-3*) in *P. pumila.* Among the hybrids and in the *P. sibirica* samples, a significant excess of heterozygotes at three loci (*Adh-1, Lap-2* and *Skdh-2*) and at one locus (*Skdh-1*) was observed, respectively. Genetic diversity values calculated for 31 loci and for 24 loci analyzed in embryos are given in table 1. The values of genetic diversity

Table 1 Allelic frequencies and parameters of genetic variability in pure species and hybrids estimated in samples of adult trees and embryos

Loci	Allele	*Pinus pumila*		Hybrids		*Pinus sibirica*	
		Adults	Embryos	Adults	Embryos	Adults	Embryos
Aco	*1*	0.250	0.370	0.143	0.092	0.025	0.014
	2	0.750	0.630	0.857	0.908	0.975	0.986
Adh-1	*1*	0.000	**0.098+**	0.310	0.426	0.550	0.581
	2	1.000	0.902	0.690	0.574	0.450	0.419
Adh-2	*1*	0.938	–	0.786	–	0.850	–
	2	0.000	–	0.071	–	0.100	–
	null	0.063	–	0.143	–	0.050	–
Fdh	*1*	0.917	0.906	0.905	0.974	1.000	0.996
	2	0.021	0.010	0.071	0.019	0.000	0.004
	3	0.063	0.083	0.024	0.007	0.000	0.000
Fest-2	*1*	0.000	**0.008+**	0.119	0.126	0.125	0.131
	2	0.292	0.337	0.071	0.061	0.000	0.000
	3	0.708	0.655	0.786	0.798	0.850	0.858
	4	0.000	0.000	0.024	0.015	0.025	0.011
Fum	*1*	0.396	0.401	0.286	0.305	0.275	0.282
	2	0.604	0.599	0.714	0.695	0.725	0.718
Gdh	*1*	0.583	0.469<	0.190	0.089	0.000	0.000
	2	0.417	0.531	0.810	0.911	1.000	1.000
Got-1	*1*	0.000	0.000	0.000	0.000	0.050	0.026
	2	1.000	1.000	1.000	1.000	0.950	0.974
Got-2	*1*	0.000	0.004	0.048	0.030	0.000	0.000
	2	0.958	0.989	0.929	0.970	0.975	0.996
	3	0.000	0.000	0.024	0.000	0.000	0.000
	null	0.042	0.007	0.000	0.000	0.025	0.004
Got-3	*1*	0.083	0.053<	0.000	0.000	0.000	0.000
	2	0.500	0.598	0.762	0.894	1.000	1.000
	3	0.417	0.350	0.238	0.106	0.000	0.000
Idh	*1*	0.917	0.965	0.976	0.992	1.000	1.000
	2	0.083	0.035	0.024	0.008	0.000	0.000
Lap-2	*1*	0.000	–†	0.024	–	0.000	–
	2	0.667	–	0.810	–	0.975	–
	3	0.167	–	0.071	–	0.000	–
	null	0.167	–	0.095	–	0.025	–
Lap-3	*1*	0.021	0.195>	0.476	0.605	0.875	0.865
	2	0.958	0.787<	0.452	0.316	0.000	0.000
	3	0.021	0.007<	0.024	0.015	0.025	0.011
	4	0.000	**0.011+**	0.048	0.064	0.100	0.124
Mdh-2	*1*	0.313	0.461>	0.571	0.748	0.975	0.967
	2	0.313	0.207<	0.262	0.172	0.025	0.022
	3	0.375	0.332<	0.167	0.080	0.000	0.011
Mdh-3	*1*	0.125	–	0.190	–	0.300	–
	2	0.875	–	0.810	–	0.700	–
Mdh-4	*1*	0.083	–	0.024	–	0.000	–
	2	0.167	–	0.405	–	0.575	–
	3	0.500	–	0.500	–	0.375	–
	4	0.250	–	0.071	–	0.000	–

Table 1 (continuation)

Loci	Allele	*Pinus pumila*		Hybrids		*Pinus sibirica*	
		Adults	Embryos	Adults	Embryos	Adults	Embryos
	5	0.000	–	0.000	–	0.050	–
Mnr-1	*1*	0.021	0.017	0.000	0.000	0.000	0.000
	2	0.479	0.621>	0.690	0.775	0.975	0.975
	3	0.479	0.338<	0.310	0.225	0.025	0.025
	4	0.021	0.025	0.000	0.000	0.000	0.000
Pepca	*1*	0.521	0.591>	0.762	0.896	1.000	1.000
	2	0.479	0.409<	0.238	0.104	0.000	0.000
6-Pgd-2	*1*	0.021	0.004<	0.000	0.000	0.000	0.000
	2	0.813	0.839>	0.929	0.940	1.000	1.000
	3	0.167	0.157<	0.071	0.060	0.000	0.000
6-Pgd-3	*1*	0.042	0.009<	0.000	0.000	0.000	0.000
	2	0.917	0.969>	0.976	0.996	1.000	1.000
	3	0.042	0.022	0.024	0.004	0.000	0.000
Pgi-1	*1*	0.000	–	0.571	–	1.000	–
	2	1.000	–	0.429	–	0.000	–
Pgi-2	*1*	0.083	0.068	0.143	0.146	0.025	0.054
	2	0.917	0.932	0.857	0.854	0.975	0.946
Pgm-1	*1*	0.625	0.727>	0.762	0.870	0.975	0.986
	2	0.313	0.231<	0.167	0.101	0.025	0.014
	3	0.063	0.042<	0.071	0.029	0.000	0.000
Pgm-2	*1*	0.125	0.157	0.119	0.072	0.000	0.000
	2	0.875	0.843	0.881	0.928	1.000	1.000
Skdh-1	*1*	0.000	0.000	0.024	0.024	0.025	0.033
	2	0.000	**0.018+**	0.167	0.214	0.400	0.308
	3	0.021	0.050	0.262	0.313	0.250	0.370
	4	0.979	0.932	0.548	0.448	0.225	0.243
	null	0.000	0.000	0.000	0.000	0.100	0.047
Skdh-2	*1*	0.125	0.119	0.048	0.046	0.000	0.000
	2	0.875	0.733<	0.452	0.256	0.000	0.000
	3	0.000	**0.148+**	0.500	0.697	1.000	1.000
Sod-2	*1*	0.854	0.882	0.952	0.952	1.000	1.000
	2	0.146	0.118	0.048	0.048	0.000	0.000
Sod-3	*1*	1.000	–	0.810	–	0.895	–
	2	0.000	–	0.190	–	0.105	–
Sod-4	*1*	0.417	–	0.190	–	0.050	–
	2	0.583	–	0.810	–	0.950	–
A	31 loci	2.19		2.47		1.81	
A	24 loci		2.44		2.29		1.76
P	31 loci	80.65		90.32		58.06	
P	24 loci		88.00		84.00		52.00
H_O	31 loci	0.267		0.373		0.136	
H_O	24 loci	0.261	0.215	0.365	0.225	0.113	0.086
H_E	31 loci	0.269		0.301		0.130	
H_E	24 loci	0.265	0.280	0.278	0.221	0.130	0.099
F_{IS}	31 loci ‡	0.031		-0.194		-0.032	
F_{IS}	24 loci ‡	0.037	0.192	-0.235	-0.016	-0.040	0.109

Table notes:
† not analyzed in embryos
+ allele typical for *P. sibirica* and absent in *P. pumila* adults
< alleles in *P. pumila* embryo sample with lower frequency than *P. pumila* adults, approaching a frequency typical for *P. sibirica*
> alleles in *P. pumila* embryo sample with higher frequency than *P. pumila* adults, approaching frequency typical for *P. sibirica*
‡ mean value for polymorphic loci only among all loci analyzed in adults and embryos.

criteria obtained for *P. pumila* and *P. sibirica* samples in this study are in agreement with those reported in literature (Goncharenko et al. 1993a, 1993b, Krutovskii et al. 1994, Politov 1989, Politov et al. 2006, Politov & Krutovskii 1994, Tani et al. 1996). As expected, the mean number of alleles per locus, percentage of polymorphic loci, and observed and expected heterozygosity were higher in the hybrid tree sample. FIS was slightly positive in *P. pumila*, slightly negative in *P. sibirica* and substantially negative in the hybrids (-0.194 for 31 loci). This result was expected since hybrids are not produced in freely mating populations but by crossing between individuals representing substantially different gene pools.

Hybrids from both parental species were discriminated by Principal Components - Analysis (PCA) of genotypic data (figure 1). All three classes were successfully discriminated by the first factor. Classification into these three groups corresponded well with diagnoses made by preliminary morphological analyses. The *P. pumila* genotypes are more scattered on the PCA plot (figure 1) than *P. sibirica* genotypes, possibly due to greater genetic diversity of the former species.

Genetic structure of embryo samples

Multilocus allozyme genotypes of 158 *P. pumila*, 164 hybrids and 138 *P. sibirica* seed embryos were determined. Allozyme genotypes of embryos were obtained for 24 out of the 31 loci used to genotype adult trees (except for *Adh-2, Lap-2, Mdh-3, Mdh-4, Pgi-1, Sod-3* and *Sod-4*). Allele frequencies and genetic variability for these 24 loci are given in Table 1. As in maternal plants, Sdh and *Mdh-1* loci were monomorphic in all three types of embryo samples. *Got-1* was invariable in *P.*

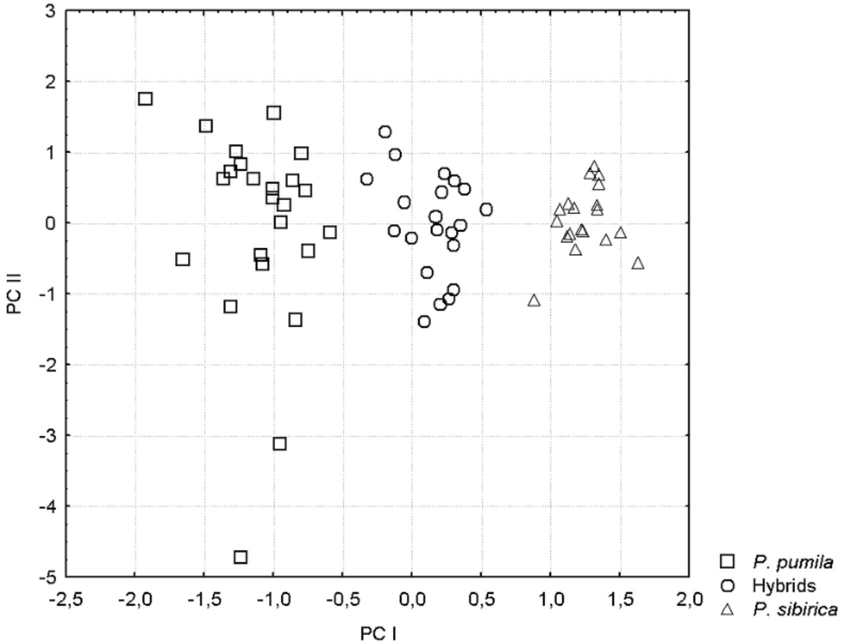

Figure 1 2D-plot of PCA factor 1 separation for *P. sibirica, P. pumila* and hybrid individuals.

pumila and in embryos from hybrid trees, while loci *Gdh, Got-3, Idh, Pepca, 6-Pgd-2, 6-Pgd-3, Pgi-1, Pgm-2, Skdh-2, Sod-2* were monomorphic in *P. sibirica* embryos. Significant differences between observed and expected (according to Hardy-Weinberg equilibrium) genotype distributions were found at *Aco, Fdh, Fest-2, Fum, Idh, Mdh-2, Mnr-1, Pepca, 6-Pgd-2, 6-Pgd-3, Pgm-2, Skdh-2,* and *Sod-2* in *P. pumila*, at *Pgm-1* in embryos from the hybrids, and at *Fest-2, Mnr-1, Pgm-1, Skdh-1* and *Fum* in *P. sibirica* embryos. All cases of deviations were related to heterozygote deficiencies (F>0). The mean inbreeding coefficient was positive in embryos of pure species (significantly different from zero in *P. sibirica*) and slightly negative in embryos from the hybrids.

Mating system analysis

A mixed mating system combining a certain proportion of outcrossing (cross-pollination) and selfing (self-pollination) is the main factor causing deficiency of heterozygous genotypes at early developmental stages in conifers. Commonly, *P. pumila* occurs in a shrub or prostrate form unusual for pines. Low ratio of the height of the plant to the area it occupies can promote self-pollination (Politov et al. 2006). Besides, the scattered distribution of the plants at the study site also increases the probability of self-pollination and explains positive F_{IS} values in *P. pumila* embryos. Observed heterozygosity was lower in embryos than in adult trees in both parental species and the hybrids. Age-related increase in heterozygosity is common in conifer species and this is commonly explained by elimination of inbred progenies and balancing selection in favour of heterozygotes (Belokon et al. 2005, Bush & Smouse 1992, Politov 1989, Politov et al. 2006, Politov & Krutovskii 1990). The inbreeding estimates for *P. pumila* and *P. sibirica* embryo samples agree with previous results (Krutovskii et al. 1995, Politov 1998, Poitov et al. 2006, Politov & Krutovskii 1994).

In embryo samples, several alleles absent in the corresponding sample of mature trees were found (table 1). In some cases it was related to the limited number of mature trees analyzed, where the alleles were typical for the parental species (at loci *Fdh, Mdh-2* in *P. sibirica*). The presence of specific alleles of one parental species among embryos of another species suggests that hybridization takes place and indicates the direction of gene flow. In *P. pumila* embryos we found alleles specific for *P. sibirica* at loci *Adh-1, Fest-2, Lap-3, Skdh-1,* and *Skdh-2*. These additional alleles in *P. pumila* embryos evidently resulted from interspecific crossing events, and increased the mean number of alleles, percentage of polymorphic loci and expected heterozygosity relative to those of *P. sibirica* and their hybrids.

Comparing multilocus pollen haplotypes and maternal tree genotypes identifies the source of the pollen that fertilized each ovule and yields quantitative estimates of the mating system. When the mating system in a hybrid zone is examined, one should consider species-specific alleles in pollen haplotypes to estimate all possible hybridization events. According to our data, approximately 27% of embryos of *P. pumila* seeds had *P. sibirica* paternal contribution, and 8% of haplotypes in the effective pollen pool combined alleles typical for *P. pumila* and *P. sibirica*, and therefore were classified as pollen from hybrids (figure 2a). In contrast, there were no *P. pumila*-specific alleles detected among pollen that fertilized *P. sibirica* ovules (figure 2b).

We compared genotypes of mature hybrid trees with genotypes of their open-pollinated embryos to reveal cases of cross pollination. Only 56% of embryos contained alleles absent in maternal trees, and therefore were directly classified as cases of cross-pollination. Among these, we tried to further classify them by the source of pollen. However, it was difficult to unambiguously distinguish pollen originating from hybrids and pure species because one cannot fully exclude the possibility that hybrids produce pollen with haplotypes of pure species at marker loci. Moreover, a portion of recombinant pollen types in hybrids might be eliminated by meiotic irregularities. As a result of this analysis of paternal contribution in evidently outcrossed embryos, we classified each pollen as (1) a sibirica/hybrid when its haplotype did not contain alleles typical for *P. pumila,* or (2) a pumila/hybrid when alleles typical for *P.*

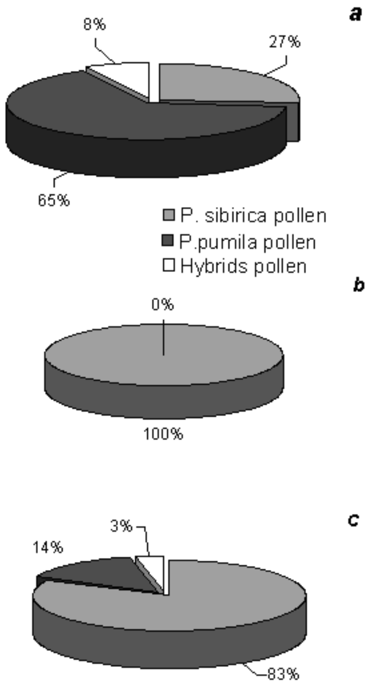

Figure 2 Composition of effective pollen pools in the hybrid zone when maternal parents are: *P. pumila* (a), *P. sibirica* (b) and hybrids (c).

sibirica were lacking, or (3) a hybrid when a pollen haplotype combined alleles specific for *P. sibirica* and *P. pumila.* This analysis showed that about 83% of the embryos originated from fertilization by *P. sibirica* (or hybrids), 14% from fertilization by *P. pumila* (or hybrids) and 3% from hybrid trees (figure 2c).

Discussion

The main indicators of the fitness of a discrete evolutionary unit at the aggregate (population to species) level are: the quantity of mature individuals, their reproductive output, and their contribution to the formation of the next generation (Grant 1981). Basing on these criteria, our results attest that *P. sibirica* × *P. pumila* hybrids are relatively successful in the evolutionary sense.

Using isozyme markers we confirmed the hybrid nature of a number of previously described putative *P. sibirica* × *P. pumila* hybrids that had intermediate growth habit, morphological and anatomical traits. The presence of alleles typical for both species in a number of individuals derived to be hybrids and intermediacy of allozyme frequencies in the 'hybrid' sample provide genetic evidence of mass hybridization between the 5-needle pine species in the Baikalian part of Eastern Siberia. Marginal parts of species' distribution ranges, ecotones, disturbed and artificial habitats facilitate spread of interspecific hybrids, where hybrid genotypes may be more fit than parental species (Abbott 1992, Moore 1977). The studied site is a distinct habitat marked by a unique combination of ecological factors. *P. pumila* and *P. sibirica* likely have no specific adaptations to this environment, so hybrids between them may exhibit adaptive success comparable with those of the parental species.

In addition, our data favors the view that hybrids make both male and female contributions to reproductive output of the mixed population and confirm the existence and survival of backcrosses and F_2 hybrids. These results demonstrate that at least the initial stages of introgressive hybridization take place in the studied mixed stand of the two stone pine species. Particularly interesting is the unidirectional pattern of introgression, characterized by extensive pollen flow from *P. sibirica* to *P. pumila* and hybrid plants and the absence of *P. pumila*-specific alleles in the embryos studied in *P. sibirica* seeds. A similar unidirectional introgression pattern was observed in the hybrid zone between *P. pumila* and another 5-needle pine, a representative of the subsection Strobi, *P. parviflora* var. *pentaphylla* (Watano et al. 1995, 1996). *Pinus* species exhibit paternal chloroplast inheritance and maternal mitochondrial inheritance (Neale & Sederoff 1989). The pattern of cytoplasmic introgression between the species was examined, revealing cpDNA introgression has occurred unidirectionally from *P. parviflora* var. *pentaphilla* to *P. pumila* (Watano et al. 1995, 1996). Therefore, in both zones of *Pinus pumila* sympatry with 5-needle pines (*P. sibirica* in the west, *P. parviflora* in the east), unidirectional patterns of pollen introgression occur and *P. pumila* appears to be a universal recipient of germplasm mediated by pollen flow. *P. pumila* has noticeably higher genetic diversity compared to other Cembrae pines (Politov & Krutovskii 2004), and this phenomenon can be partly accounted for by the enrichment of the species' gene pool by novel genes through interspecific introgression.

There are several possible explanations for unidirectional pollen flow between sympatric species. First, a one-way phenological isolation caused by differences in flowering regimes of the species. This mechanism would occur if dichogamy (an asynchronous development of male and female strobili) takes place in the studied *P. pumila* and *P. sibirica* sympatric populations due to environmental conditions of the site, and if the pollination time of *P. sibirica* contemporized with *P. pumila* ovule receptivity, earlier than mass *P. pumila* pollen maturation. This is quite possible because distinct dichogamy was observed in West Siberia where a series of neighboring *P. sibirica* stands with contrasting soil and climatic conditions, promoting integration of the stands into a single functional population (Avrov 1993).

Second, the unidirectional incompatibility between the two pine species may be due to gametic incompatibility or selection at earlier ontogenetic stages against the embryos having the combination of *P. pumila* paternal and *P. sibirica* maternal gametes. Such incomplete reproductive isolation has been widely observed in angiosperms (Harder et al. 1993), but information on incompatibility experiments in *Pinus* is quite limited (Kriebel 1972, 2004, Kormutak 1990, Wachowiak et al. 2005). Interspecific pollen competition is another force acting at the gametic stage that may contribute to the success of *P. sibirica* pollen in sipring *P. pumila* offspring. Third, the phenomenon of reduced male reproductive function under environmental stress, "environmental emasculation", can also account for the asymmetric pattern of introgression in the *P. pumila-P. sibirica* hybrid zone. These two types of unilateral isolation have been well studied in angiosperm species too (Chapman et al. 2005, Klips 1999, Williams et al. 1999). Multiple crossing experiments are needed to clarify the roles of these factors in pine hybridization, particularly with respect to the unidirectional pattern of introgression.

Fourth, the biased pollen contributions of the parental species are also likely responsible for the asymmetry of introgression. It is evident that the total amount of pollen produced by P. *sibirica* is far beyond the amount that scarce and partially depressed *P. pumila* plants can contribute to the total pollen pool.

It is likely that a combination of the above-mentioned reasons provide the most suitable explanation for the total pollen quantity and quality and the introgression pattern, at least at the study site. In this part of the Lake Baikal coast, *P. pumila* is scattered and occurs only in the narrow riparian zone; its sexual reproduction is depressed, and paternal contributions to the mixed population embryo pool are reduced. Unidirectional formation of *Pinus taeda-P. echinata* natural hybrids was reported (Edwaeds-Burke et al. 1997). All hybrid plants had the cpDNA of *P. echinata*, suggesting they were sired by pollen of this species.

Chloroplast, mitochondrial and nuclear DNA markers should be used to evaluate precisely the degrees of nuclear and cytoplasmic introgression, and give insight into the mating pattern of the *P. sibirica-P. pumila* hybrid zone. Studies of this phenomenon should be extended to other sites in the hybrid zone characterized by a dissimilar proportion of parental tree species and varied environmental conditions.

Acknowledgements

This work was supported by the Russian Foundation of Basic Research (Project no. 07-04-00593-a); the fundamental research programs of the Russian Academy of Sciences "Biological Resources of Russia: Fundamentals of Rational Use," "Origin and Evolution of the Biosphere" and "Gene Pool Dynamics" and the Program for Support of Scientific Schools (NSh - 8596.2006.04).

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Rezumat. Petrova E.A., Goroshkevich S.N., Belokon M.M., Belokon Y.S., Politov D.V., 2008. Structura genetică a populaţiilor şi sistemul de încrucişare în zona hibridă dintre *Pinus sibirica* Du Tour şi *P. pumila* (Pall.) Regel din litoralul estic al Lacului Baikal. Ann. For. Res. 51:19-30.

Structura genetică a populaţiilor simpatrice de *Pinus sibirica* Du Tour şi *P. pumila* (Pall.) Regel şi hibrizii interspecifici prezumtivi dintre ele din regiunea Lacului Baikal (Rezervaţia Naturală a Biosferei Barguzin, Bazinul Râului Davsha) a fost analizată cu ajutorul a 31 izoenzime, care controleaza 18 sisteme enzimatice.

Câteva alele ale locilor *Adh-1, Fest-2, Lap-3, Pgi-1, Sod-3* si *Skdh-1* au constituit diagnostic pentru *P. sibirica*, în timp ce alelele tipice pentru *P. pumila* au fost detectate la locii *Gdh, Got-3, Lap-3, Mdh-2, Mdh-4, Pepca, Pgi-1, Pgd-2, Pgd-3, Pgm-1* si *Pgm-2*. Potrivit cu diagnoza locusului *Skdh-2*, toţi hibrizii au fost heterozigoţi.

Clasificarea în hibrizi şi specii parentale folosind analiza PCA a genotipurilor multilocus izoenzimatice a avut o bună corespondenţă cu diagnozele efectuate prin analizele morfologice şi anatomice.

Aproximativ 27 % din embrionii de *P. pumila* au avut pe *P. sibirica*, ca părinte patern, 8% dintre haplotipuri din masa efectivă de polen a combinat alelele tipice pentru *P. pumila* şi *P. sibirica* şi în consecinţă au fost clasificate ca fiind polenizate de către hibrizi.

Nicio alelă specifică pentru *P. pumila* nu a fost detectată în polenul care a fertilizat ovulele de *P. sibirica*. Aproximativ 83% din embrionii seminţelor hibride îşi au originea în fertilizarea cu polen de *P. sibirica*, 14% cu polen de *P. pumila* şi 3% cu polen de la arborii hibrizi.

Acest rezultat susține punctul de vedere potrivit căruia hibrizii pot contribui la fenomenul de reproducere a populațiilor atât ca părinți masculi, cât şi femeli, iar aceasta confirmă prezența backcross-ului în generația hibridă F_2.

Cuvinte cheie: hibridare naturală, *P. sibirica*, *P. pumila*, izoenzime, sistem de încrucişare, structură genetică, Lacul Baikal.

(Tradus de I. Blada)