Phylogenetics, Genogeography and Hybridization of Five-Needle Pines in Russia and Neighboring Countries

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Abstract—Phylogenetic and population genetic studies of native five-needle pines growing in Russia and neighboring countries were reviewed. Four species, *Pinus cembra, P. sibirica, P. pumila* and *P. koraiensis*, together with North American species *P. albicaulis,* comprise the subsection *Cembrae* (stone pines) of the section *Strobus* (white pines). They share bird-dispersal related traits such as seed winglessness and cone indehiscence that differentiate them from most other white pines and related section *Parrya*. Phylogenetic analysis showed that *P. cembra, P. sibirica* and *P. albicaulis* represented a close group based on isozyme loci and other molecular genetic markers. *Pinus pumila* and *P. koraiensis* also clustered together in phylogenetic trees, but they were closer to *P. parviflora* and other East Asian pines of the subsection *Strobi* than to the *cembra-sibirica-albicaulis* group. Therefore, we hypothesized that seed winglessness and other bird-dispersal related traits could either arise independently in these lineages or could have been introduced via occasional hybridization. Natural hybridization between *P. sibirica* and *P. pumila* in their zone of sympatry in the Baikal region was confirmed by isozyme methods, but no significant introgression was revealed. The possibility of producing hybrids in artificial crosses between *P. sibirica* and *P. cembra*, and *P. sibirica* with *P. koraiensis* was also confirmed by isozyme analysis. Intrapopulation genetic variation, measured as expected heterozygosity of isozyme loci (H_e), was relatively high in *P. koraiensis* ($H_e = 0.130$) and *P. sibirica* (0.106) and similar to the average for other soft pines, but significantly higher in *P. pumila* (0.198) and lower in *P. cembra* (0.082). There was no obvious difference in the level of heterozygosity between bird-dispersed pines of the subsection *Cembrae* and wind-dispersed pines of the closely related subsection *Strobi*. Relatively low inbreeding was observed in embryos in all five-needle pines and was primarily caused by self-pollination. However, Hardy – Weinberg equilibrium or even a slight excess of heterozygosity was usually observed among mature trees, apparently as a result of selection against inbred progeny and in favor of heterozygotes. Two types of polyembryony, monozygotic and polyzygotic, were found in *P. sibirica*. Gene geography studies in *P. sibirica* and *P. pumila* populations based on multivariate analysis of isozyme data was also discussed.

Key words: *Cembrae,* genogeography, isozyme, phylogenetics, *Strobi*, stone pines, white pines

Introduction

Four species of five-needle pines, also known as haploxylon or soft pines (genus *Pinus* L., subgenus *Strobus* Lemm.), are native in Russia and neighboring countries (fig. 1). These four species - *P. cembra* L. (Swiss stone pine), *Pinus sibirica* Du Tour (Siberian stone pine), *P. pumila* (Pall.) Regel (Siberian dwarf pine) and *P. koraiensis* Sieb. et Zucc. (Korean pine) - are classified with section *Strobus* Loud. (white pines) and subsection *Cembrae* Loud. (stone pines). *Pinus sibirica* forms forests of great economical importance in Siberia and the Far East, while *P. pumila* occupies vast territory in East Siberia and along the Asian Pacific coast. *Pinus koraiensis* is distributed in the Russian Far East, including the Amur Region, Khabarovsk and Primorskii Territories, and the Island of Sakhalin. *Pinus cembra* is scattered in the East Carpathian Mountains in Ukraine.

Figure 1—Geographic distribution of five-needle pines in Russia and neighboring countries.

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The *Cembrae* species have large edible wingless seeds that are dispersed by birds and play a key role in Siberian taiga (*P. sibirica, P. koraiensis*) and subalpine (*P. cembra, P. pumila*) ecosystems. Although *P. parviflora* is a five-needle pine species that occurs in Russia, it is also endemic to Japanese islands and occurs in a few isolated populations on the Kuril Islands along the Russian Pacific coast.

Evolutionary relationships and intraspecific population genetic structure of Eurasian *Cembrae* pines have been studied since early 1900s, primarily by traditional morphological methods (compare Bobrov 1978; Iroshnikov 1974; Lanner 1990, 1996, 1998; Smolonogov 1994). However, the levels of intrapopulation, intraspecific and interspecific genetic variation have been quantitatively estimated only after development of reliable molecular genetic markers.

This paper presents an overview of genetic studies of the above-mentioned five-needle pines. We have concentrated on results obtained using molecular genetic markers, but also included karyological and morphological data when needed.

Phylogenetics

All four *Cembrae* pine species produce functionally indehiscent cones and large wingless seeds. Based on those traits, these species and the North American whitebark pine (*P. albicaulis* Engelm.) are traditionally included in the subsection *Cembrae* (stone pines) within section *Strobus* (white pines). This section together with the species of the section *Parrya* Mayr. comprise the group of soft pines (subgenus *Strobus*, or Haploxylon) of the genus *Pinus*. We used the most widely accepted classification by Critchfield and Little (1966) with minor modifications and additions (Price and others 1998). According to this classification section *Strobus* is subdivided into two subsections *Cembrae* and *Strobi* Loud. that both have representatives in Eurasia and North America (fig. 2).

This subdivision is based on the occurrence of large wingless seeds and indehiscent (not opening upon ripening) macrostrobili (cones) in *Cembrae* pines. It has been widely accepted that both these traits evolved as a result of the

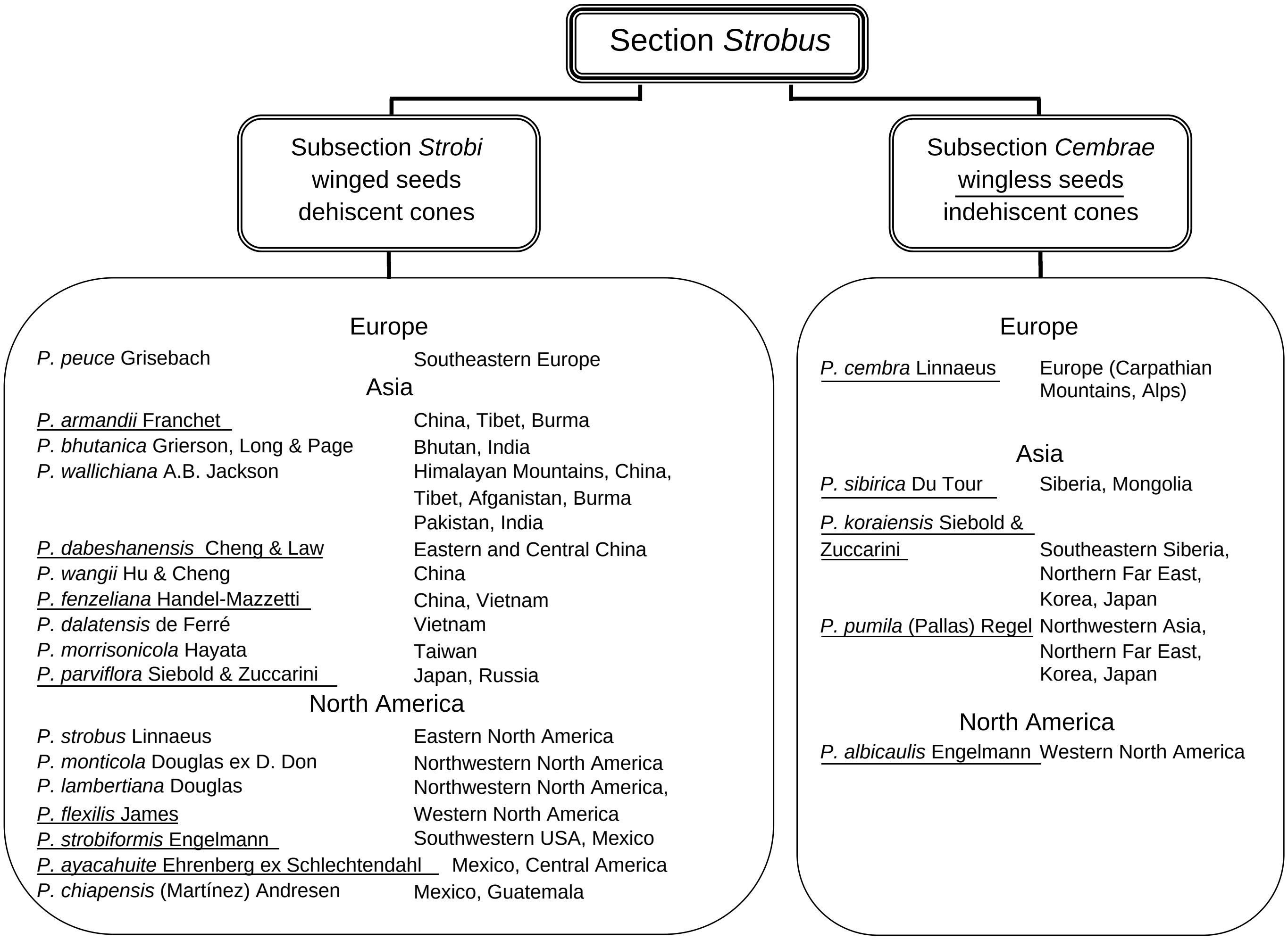

Figure 2—Taxonomic classification and generalized distribution of white pines (Critchfield and Little 1966; Price and others 1998). Underlined are species with wingless or almost wingless seeds (Lanner 1996, 1998).

adaptation to dispersal of their seeds by corvid birds (for example, Lanner 1996; Tomback and Linhart 1990), and particularly nutcrackers (*Nucifraga* spp.). The closely related subsection *Strobi* includes mainly typical wind-dispersed species with small winged seeds released from the cones upon ripening. However, seeds of some of *Strobi* species (underlined in fig. 2) are large and virtually maladapted to dispersal by wind. Moreover, traits that are normally characteristic of *Cembrae* pines (especially winglessness) sporadically occur also in *Strobi* pines as rare abnormalities or as within-population polymorphism. Reproductive barriers are incomplete between these two subsections, and there are numerous examples of artificial and natural interspecific hybridization both within and between subsections (Critchfield 1986; Blada 1994). The occurrence of intermediate forms and documented hybridization between species of different subsections make the hypothesis of monophyletic origin of *Cembrae* and *Strobi* subsections controversial. This problem stimulated repeated attempts to revise the taxonomy of the group and to rearrange the composition of the subsections. However, until recently, the problem was studied mainly using morphological comparisons (for example, Kupriyanova and Litvinceva 1974).

Muratova (1980) compared karyotypes of three Eurasian *Cembrae* pine species and found only slight differences in chromosome size and morphology, as well as in number and localization of nucleoli organizers. *Pinus cembra* and *P. sibirica* karyotypes were the most similar, although there were slight differences. The karyotype of *P. pumila* was closer to these species than to *P. koraiensis*, which partially contradicts molecular genetic data presented below.

The first molecular genetic evidence of great similarity between *P. cembra* and *P. sibirica* was provided with isozyme loci (Krutovsky and others 1990). This study also showed that *P. pumila* and *P. koraiensis* comprise the next closely related pair. These relationships among Eurasian *Cembrae* pines were confirmed later by other scientists (for example, Goncharenko and others 1991; Shurkhal and others 1992). *Pinus albicaulis* is the only North American *Cembrae* pine. This species together with all four Eurasian *Cembrae* pines were studied by Krutovsky and others (1994, 1995), which were the first studies where phylogenetic relationships were analyzed within the entire subsection *Cembrae* using molecular genetic markers. Two lineages were found again in *Cembrae* pines; *P. cembra* and *P. sibirica* formed a pair of the most closely related *Cembrae* species, while another lineage was represented by two Far Eastern species *P. pumila* and *P. koraiensis*. The position of *P. albicaulis* was not fully resolved at that time, but it was found to be closer to the *cembra–sibirica* group.

Krutovsky and others (1994, 1995) studied chloroplast DNA (cpDNA) restriction fragment length polymorphisms (RFLPs) in all five *Cembrae* pine species, as well as in four representatives of North American pines from subsection *Strobi* (*P. strobus* L., *P. lambertiana* Dougl., *P. monticola* Dougl., and *P. flexilis* James). Species of *Strobi* were close to *Cembrae* species, but they did not form a separate cluster in the dendrogram. A similar pattern was also observed in other studies based on cpDNA markers, although unfortunately neither of those studies included all representatives of *Cembrae* pines species (for example, Wang and Szmidt 1993; Wang and others 1999). For instance, Wang and Szmidt (1993) used cpDNA RFLP markers to study phylogenetic relationships in several Asian pine species including one *Cembrae* pine (*P. sibirica*) and five *Strobi* pines (*P. armandii* Franch., *P. griffithii* McClelland (syn. *P. wallichiana* A. B. Jacks.), *P. kwangtungensis* Chun & Tsiang, *P. parviflora* Sib. & Zucc., and *P. peuce* Griseb.). *Pinus sibirica* (*Cembrae*) clustered very closely with *P. parviflora* (*Strobi*) with 95 percent of the bootstrap support within the following topology in the maximum parsimony consensus tree: (*peuce* ((*parviflora*, *sibirica*) (*armandii* (*griffithii, kwangtungensis*)))). However, the absence of *Cembrae* species other than *P. sibirica* in the analysis made it impossible to draw any conclusions about phylogenetic relationships between *Cembrae* and *Strobi* pines. In a more recent study based on cpDNA markers (Wang and others 1999), the topology of the resulting consensus tree was different: (*peuce* (*strobus* (*monticola* (*parviflora* (*koraiensis*, *armandii*, *griffithii*, *cembra*, *kwangtungensis*, *pumila*))))). However, resolution within a group of six species belonging to both subsections *Strobi* (*P. armandii*, *P. griffithii* and *P. kwangtungensis*) and *Cembrae* (*P. cembra*, *P. koraiensis* and *P. pumila*) was insufficient to make any strong conclusions on the phylogeny of these species. The parsimony tree indicated that two North American (*P. strobus* and *P. monticola*) and one European (*P. peuce*) species of subsection *Strobi* appeared to be ancestral to all Asian white pine species including *P. cembra*. These results contradict our point of view that Asian pine species are ancestral to North American pines, which is supported by Belokon and others (1998) study based on more numerous nuclear markers and more representative set of white pine species. Liston and others (1999) used ribosomal internal transcribed spacer (ITS) DNA markers to study phylogeny of the genus *Pinus*. White pines of the section *Strobus* formed a separate cluster, but relationships within the section were unclear. The only clade well supported by bootstrap was comprised of *P. cembra* and *P. albicaulis*, which agreed with our earlier allozyme and cpDNA data (Krutovsky and others 1994, 1995). Belokon and others (1998) studied 13 white pine species using isozyme markers coupled with Principal Component Analysis (PCA) and also did not find a strong support for dividing section *Strobus* into the two traditional subsections *Cembrae* and *Strobi*. Instead, the study found even more evident differentiation between North American and Eurasian white pines. The updated results from the Belokon and others (1998) study with increased sample size and two additional species (*P. lambertiana* and *P. strobiformis*) are presented in figure 3 (Politov and others, unpublished). *Cembrae* pines were separated from *Strobi* pines along the first dimension on the two-dimensional (2D) plot obtained by multidimensional scaling of Nei's (1972) genetic distance matrix based on isozyme data, while the second dimension differentiated North American and Eurasian species. Among them, *P. peuce* of southern Europe was located (genetically) as the nearest species to North American *Strobi* pines (fig. 3). In general, Eurasian *Strobi* species were more differentiated, and *P. griffithii* (as *P. wallichiana* in the paper) was the nearest to the Asian *P. pumila* and North American *P. albicaulis* (*Cembrae* pines).

A representative of the related section *Parrya* (pinyons), *Pinus edulis* Engelm., was included as an outgroup in Belokon and others (1998) study. The dendrogram of white

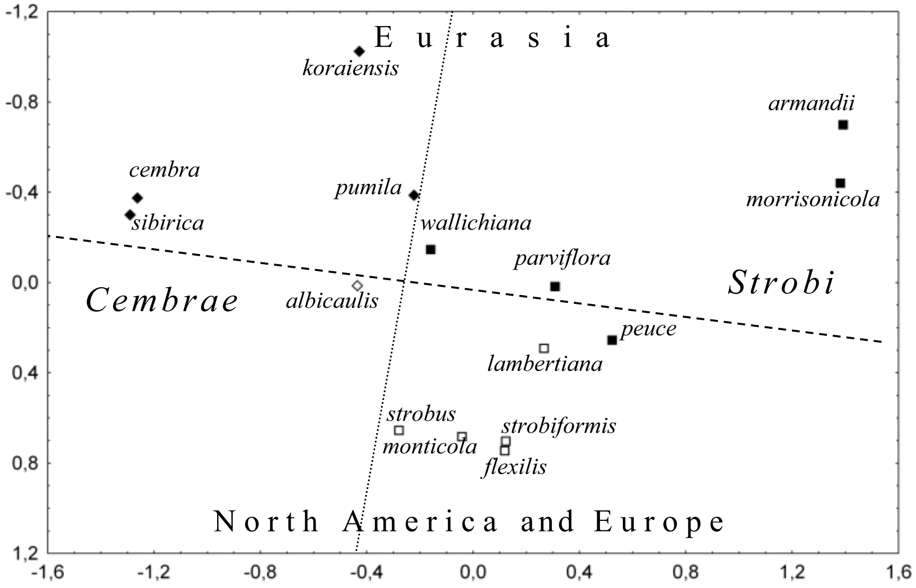

Figure 3—Two-dimensional (2D) plot of 13 white pine species (section *Strobus*) obtained by multidimensional scaling of Nei's (1972) genetic distance matrix based on isozyme loci. ◆ - Eurasian *Cembrae*; ◊ - North American *Cembrae*; ■, Eurasian *Strobi*; □ - North American *Strobi*.

pine species and *P. edulis* obtained in this study (fig. 1 in Belokon and others 1998) indicated that Asian *Strobi* pines are evolutionarily older than both American *Strobi* and all *Cembrae* pines. There is other evidence also supporting the hypothesis that subsection *Cembrae* is an evolutionarily younger taxonomic group. It is based on the relatively recent origin of nutcracker birds (*Nucifraga* spp.) that disperse seeds of *Cembrae* pines.

However, despite indicating evolutionary trends in section *Strobus,* none of the above mentioned phylogenetic studies was able to unambiguously confirm the validity of the subsection *Cembrae*. We believe that there can be several explanations for the controversial origin of *Cembrae* pines. Their wingless seeds and indehiscence could have been indeed inherited from a common ancestor (true monophyly), but sufficiently so long ago that relatedness is obscured by millions of years of evolution in diverse habitats. Allozyme variation is not necessarily neutral, at least in part, and may be shaped by selection of particular alleles or genotypes advantageous in particular environment reflecting local adaptation rather than phylogenetic relationships. The monophyly of subsection *Cembrae* assumed under this scenario does not necessarily mean monophyly of subsection *Strobi*. *Cembrae* pines could have arisen within wind-dispersed *Strobi*, but these two subsections are not necessarily two sister groups.

The second scenario assumes polyphyletic origin of *Cembrae* pines. Genetic divergence between *P. sibirica – P. cembra* (and perhaps *P. albicaulis*), from one side, and *P. pumila – P. koraiensis*, from another side, supports this hypothesis. It is relatively high and comparable to the level of divergence between species from different subsections, *Cembrae* and *Strobi*. This could mean an independent and multiple (polyphyletic) origins of wingless seeds and indehiscent cones in these two lineages. The wingless seeds occur also in several white pines of subsection *Strobi*. As to cone indehiscence, this trait is underlain by a simple mechanism; *Cembrae* cones lack specific tracheid fascicles (coarse fibers) in the scales. In other pine species, the fascicles contract, when a cone dries, causing scales to bend outward (Lanner 1990). The critical question is whether the absence of this tissue, a homologous trait, synapomorphic for all five *Cembrae* species. We think that this is not necessarily the case, since the lack of this tissue could arise independently in two evolutionary lineages *(P. sibirica - P. cembra - P. albicaulis* and *P. pumila - P. koraiensis)* as a result of nonhomologous mutations. Indehiscence then could be fixed via coevolution of pines and their dispersers, corvid birds (Tomback and Lindhart 1990), as this trait helps to hold the seeds in the cones making them more easily available for birds.

There is also a possibility of interspecific gene flow and multiple cases of gene exchange between different white pine species (Critchfield 1986). Interspecific hybridization and occurrence of natural interspecific hybrids were documented between *P. sibirica* and *P. pumila* within subsection *Cembrae* (Politov and others 1999), and between *P. pumila* and *P. parviflora* from different subsections *Cembrae* and *Strobi*, respectively (Watano and others 1995, 1996). Once indehiscence appeared, it could have crossed species borders as a result of sporadic hybridization events. Therefore, indehiscence could be a result of a "monophyletic" event (appearing once in the evolution). However, genes responsible for indehiscent cones could share the same ancestor gene or genes, while other genes in the genome may have a quite different evolutionary history. Past or present gene introgression may also be responsible for similarity of genetic markers in sympatric or nearly sympatric species, such as western North American white pines *P. monticola, P. flexilis* and *P. strobiformis* Engelm., or white pines from Taiwan, *P. morrisonicola* Hayata and *P. armandii* (which was represented by the Taiwanese variety in our material).

If introgressive hybridization indeed took place in the white pine evolutionary history, this could violate major principles of phylogenetic analysis, such as independence of compared operational taxonomic units (OTUs), and therefore, white pine evolution would be better described as reticulate evolution. Even a large number of molecular markers could be insufficient to resolve complicated reticulate patterns. Although molecular genetic markers have already provided valuable information for the understanding of genetic relationships in the section *Strobus*, their value for phylogeny is still not fully understood.

Genogeography

Pinus sibirica

Pinus sibirica was the first *Cembrae* pine in Russia that was studied by isozyme loci. Eleven isozyme systems coded by 19 loci were described more than a decade ago (Krutovsky and others 1987), and genetic differentiation among populations was estimated (Krutovsky and others 1989; Politov and others 1989; Politov and others 1992; Krutovsky and others 1994, 1995). The proportion of interpopulation variation in total species variation measured as F_{ST} value was relatively low (2.5 percent) in this species; nevertheless cluster analysis of 11 populations based on isozyme genetic distances showed good correspondence to their geographic origin. Populations from major regions that were substantially different in habitat types formed separate clusters: South Siberian Mountains, Western Siberian Plain, and Northern Siberia.

Using practically the same set of isozyme loci, Goncharenko and others (1993b) reported F_{ST} in *P. sibirica* to be 3.9 percent. Despite broader representation of samples (from northwestern Siberia to eastern Kazakhstan and to Lake Baikal Region in East Siberia), the concordance of UPGMA clustering to geographic origin was less pronounced in their study. We believe that this could have been caused by biased allele frequency estimations due to relatively low sample size, 12.9 trees on average per population as compared to 38.9 trees in Politov and others (1992) and Krutovsky and others (1994, 1995). Using seven isozyme loci analyzed in the needle tissue, 41 samples were studied by Podogas (1993), and the position of samples on the resulting PCA plot generally corresponded to the geographic location of populations while the F_{ST} value was also relatively low (3 percent).

Using 31 allozyme loci, Politov (1998) studied genetic differentiation among 15 populations in Lake Baikal region in East Siberia. A higher level of interpopulation diversity (F_{ST}=6.3 percent) was revealed in these populations, and genetic differentiation studied by PCA was in good concordance with geography (fig. 4). Comparative analysis of Baikalian populations together with earlier studied populations from West and Middle Siberia using 10 common loci showed that Baikalian populations were different from other major provinces (Politov 1998).

Pinus cembra

Allozyme variation in *P. cembra* populations was first studied by Szmidt (1982). The estimated population genetic differentiation (F_{ST} = 0.313) was unusually high compared to other pine species. In part, the high F_{ST} value could be explained by greater isolation among *P. cembra* populations. There are reasons, however, other than isolation that can explain that high F_{ST}. Based on the description of the geographic origin provided by the author, one of the samples appeared to be collected from a *P. sibirica* population in Chitinskaya Region in East Siberia. This area is sympatric for both *P. sibirica* and *P. pumila*. We compared genetic data obtained for East Siberian populations of *P. sibirica* and *P. pumila*, which revealed that this sample represented neither *P. cembra* nor *P. sibirica*. Several alleles in this sample are species specific for *P. pumila* and were never found in either *P. cembra* or *P. sibirica*. Correspondingly, we believe that this sample actually represented *P. pumila,* which is highly diverged from *P. sibirica* in isozyme loci. Subsequently, only a few single populations of *P. cembra* have been studied by isozymes (Krutovsky and others 1990, 1994, 1995; Goncharenko and others 1991; Politov and others 1992; Pirko 2001). However, recently Belokon, Belokon and Politov (in preparation) estimated genetic diversity in five *P. cembra* samples collected from the Alps (Switzerland and Austria) and the Carpathian Mountains populations in western Ukraine. The F_{ST} value was not exceptionally high (0.047). Therefore either stand isolation did not drastically affect population differentiation of this species or this isolation is possibly a recent event, and the stands were much more dense and widespread in the recent past. We conducted Correspondence Analysis (StatSoft 1998) on these five populations, and its results are shown in figure 5. Samples from the Alps and the Carpathian Mountains were well separated along the first dimension, while the second dimension differentiated northern and southern macroslopes of the eastern Carpathian Mountains. This differentiation showed that Alpine and Carpathian populations as well as populations from northern and southern macroslopes of the eastern Carpathian Mountains have different genetic constitutions, although the southern macroslopes were represented by only a single population in our study.

Gugerli and others (2001) studied 15 populations in *P. cembra,* one in *P. sibirica* and two in *P. pumila* using a few DNA markers of several different types, but no data on intraspecific variation were reported. The authors confirmed allozyme data that *P. pumila* was the most divergent species among these three species. However, they failed to differentiate *P. cembra* from *P. sibirica*, which clustered among *P. cembra* populations.

Pinus pumila

Genetic structure of *P. pumila* was first analyzed in studies on three populations from the north region of Kamchatka Peninsula (Krutovsky and others 1990; Politov and others 1992; Krutovsky and others 1994, 1995). F_{ST} value based on 22 allozyme loci was relatively low (2.1 percent), but dendrograms based on genetic distances corresponded to the geographic localization of the populations.

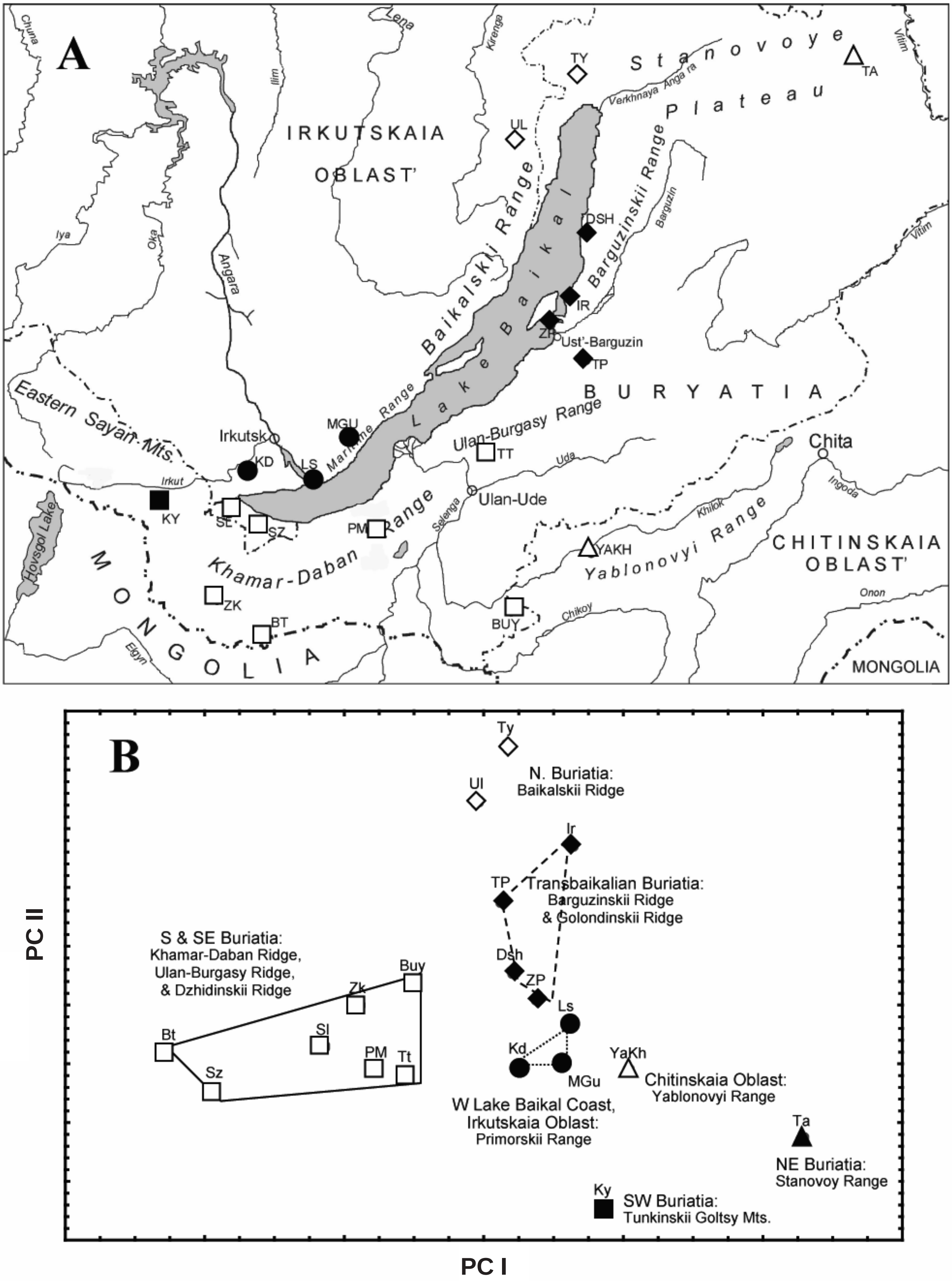

Figure 4—Sampling site locations (A) and results of Principal Component Analysis of 19 populations of *P. sibirica* based on allelic variation in 31 isozyme loci (B) studied in Baikal Lake region (Politov 1998).

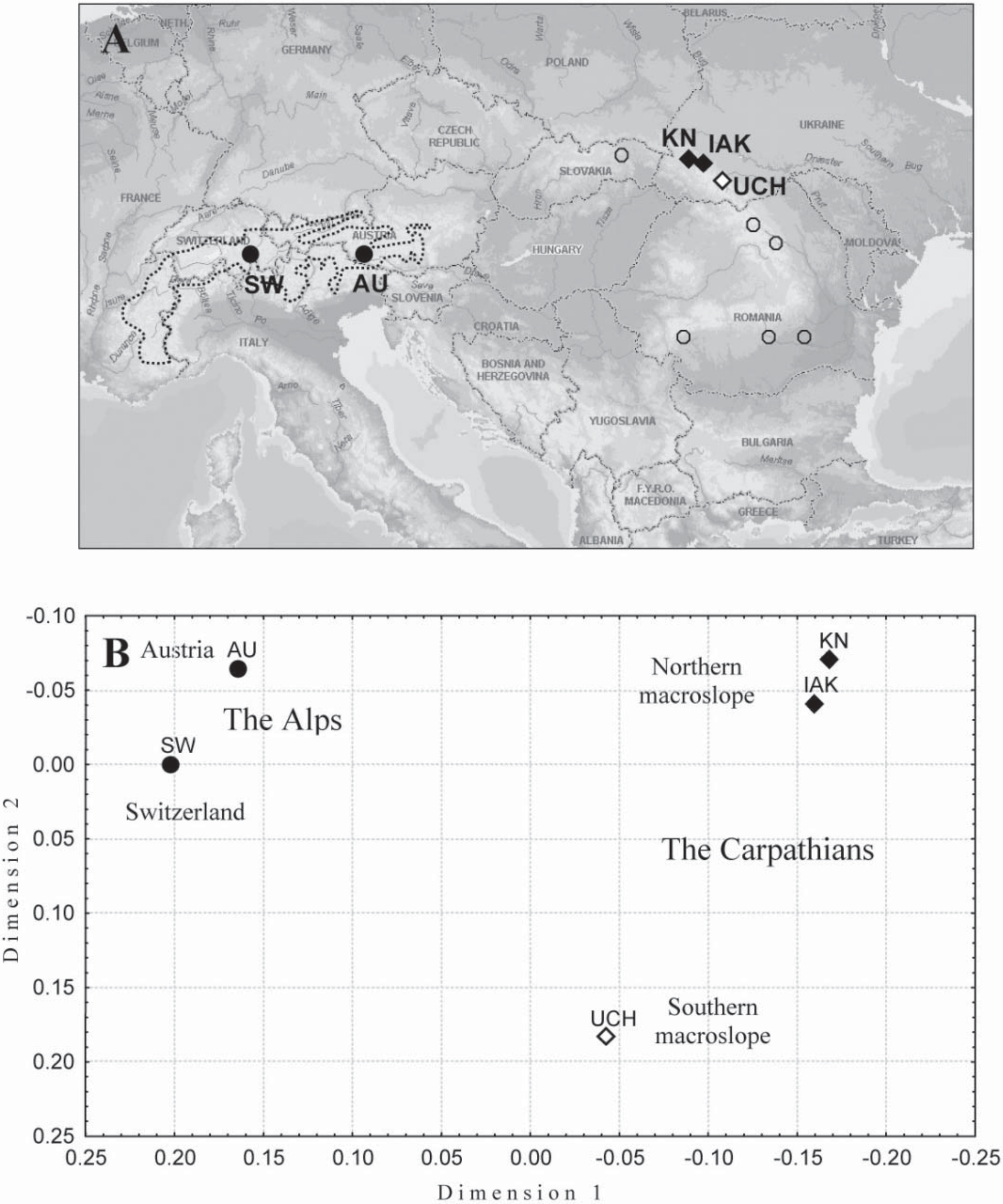

Figure 5—Sampling site locations (A) and results of Correspondence Analysis (B) of five populations of *P. cembra* based on allelic variation in 31 isozyme loci (Belokon, Belokon and Politov, unpublished). Eigenvalue 1 = 0.02466 (54.46 percent of inertia), eigenvalue 2 = 0.00891 (19,68 percent of inertia). Empty circles represent isolated populations or relatively small stands of *P. cembra* in the Carpathian Mountains that were not sampled.

Goncharenko and others (1993a) reported slightly higher differentiation (F_{ST}=4.3 percent) in five populations from two regions, the Chukotka Peninsula and Island of Sakhalin. However, the authors failed to find clear pattern in differentiation among populations. Similar to their abovementioned study of *P. sibirica*, this study has the same problem of the limited sample sizes (12.6 trees per population on average), which could bias estimation of allele frequencies in populations. Substantial genetic differentiation (F_{ST}=7.3 percent) was found in recent, more representative and wide ranging geographic studies of 22 populations of *P. pumila* sampled from the Lake Baikal region through the inland mountain ridges to the Pacific coast uncluding the Sakhalin Island (Maluchenko and others 1998; Politov 1998). The distribution of populations on a PCA plot based on 26 allozyme loci was in good correspondence to their geographic origin (fig. 6).

Pinus koraiensis

Early studies of three populations of P. koraiensis (Politov and others 1992; Krutovsky and others 1994, 1995) revealed a relatively low level of genetic differentiation (F_{ST}=4.0 percent). The F_{ST} value was even lower (1.5 percent) in a more representative study of 19 populations collected from most of *P. koraiensis*' range in Russia (Potenko and Velikov 1998), but the authors did not use any clustering technique to analyze genogeography. Belokon and Politov (2000) analyzed nine populations that included seven populations from three regions of Russian Far East (Primorski Territory, Amurskaya Oblast and Khabarovsk Territory) and two samples from northeastern China. However, populations did not cluster according to their geographic origin on the PCA plots. It appears that studies on genetic differentiation in *P. koraiensis* will require combined efforts of different scientists and more samples from the entire range (see also accompanying paper by V. V. Potenko).

Intra- and Interpopulation Genetic Variation in *Cembrae* Pines

The different number and type of isozyme loci or other genetic markers and the different size and origin of samples used in various studies make comparison among studies of levels of genetic variation between species and even within species problematic if not impossible. Estimates of genetic variation greatly varied in different studies of *Cembrae* pines. Expected heterozygosity (H_e) calculated from allele frequencies by assuming Hardy - Weinberg equilibrium in population and F_{ST} or G_{ST} parameters are the most common estimates of intrapopulation genetic variation and interpopulation genetic differentiation, respectively. We have summarized these estimates obtained in Russian populations of *Cembrae* pines (table 1). To make comparisons more accurate we presented only those H_e values that were based on the same set of loci. The F_{ST}/G_{ST} values based on the same or almost the same loci and obtained within a geographic range of the same or similar scale were also calculated and presented. Although these adjustments do not necessarily ensure correct comparisons, we believe that such comparisons are more objective than direct comparison of estimates based on unbalanced data. The H_e values increased in our study in the following order: *P. cembra* > *P. sibirica* > *P. koraiensis* > *P. pumila*. F_{ST} was also the greatest in *P. pumila* followed by *P. cembra*, *P. koraiensis*, and *P. sibirica* (fig. 7). The latter two species had similar F_{ST} values. We also calculated H_e values for 15 white pine species based on the

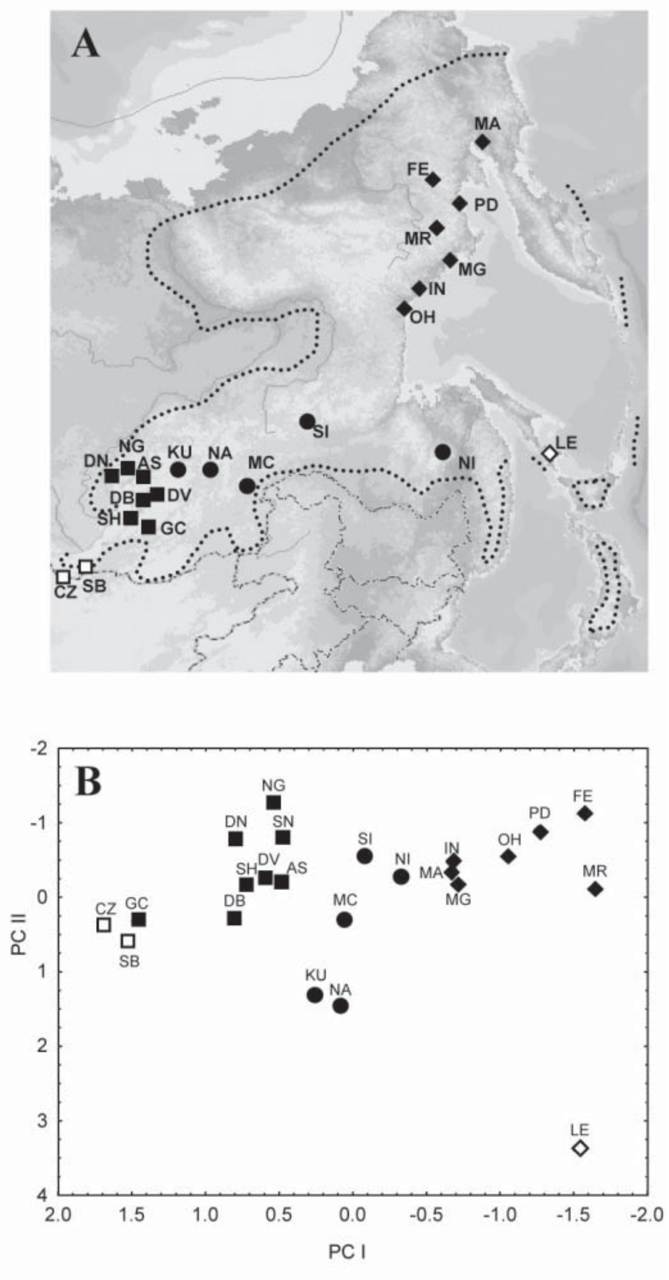

Figure 6—Sampling site locations (A) and results of Correspondence Analysis (B) of 23 populations of *P. pumila* based on allelic variation in 31 isozyme loci (Maluchenko and others 1998; Politov 1998). □ - Southeastern Baikal (Hamar-Daban Range), ■ - Northern Baikal, ● Inland Ridges, ◊ Sakhalin Island, ◆ - Northeastern coast of Sea of Okhotsk.

Table 1—Genetic diversity in Eurasian stone pine species (subsection *Cembrae*) estimated as expected heterozygosity (**H_e**) and interpopulation genetic differentiation (**F_{ST}** or **G_{ST}**) parameters using isozyme loci.

Species	Populations	Loci (mean)	F_{ST}/G_{ST}	F_{ST} [a]	H_e	Reference
P. cembra	5	30	0.047	0.065	0.093	Belokon, Belokon and Politov, unpublished
P. sibirica	32	19-31 (25)	0.025-0.063 [b]	0.035	0.124	Politov 1998
P. koraiensis	5	30	0.015-0.040	0.035	0.128	Belokon and Politov 2000
	19	26	-	-	0.182	Potenko and Velikov 1998
P. pumila	29	22-32 (30)	0.021-0.073	0.070	0.234	Politov 1998; Maluchenko and others 1998

[a] Calculated for populations within the same scale of geographic range and using the same set of loci for comparison.
[b] The range of estimates from different studies.

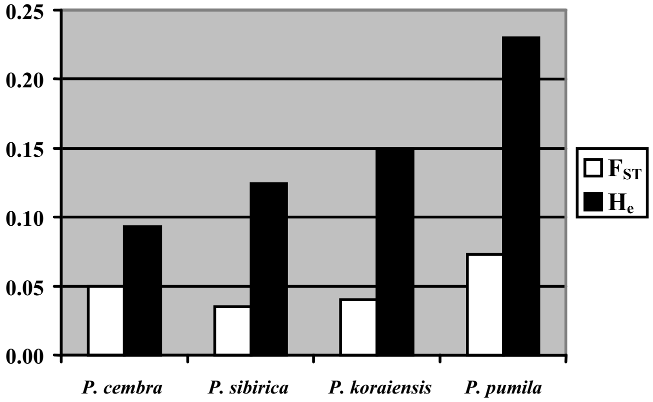

Figure 7—Comparison of intrapopulation (H_e) and interpopulation (F_{ST}) genetic diversity between four *Cembrae* pine species.

same set of 16 isozyme loci (table 2). There was considerable variation in H_e values among species, but differences were associated neither with subsections nor with subdivision of species into bird-dispersed versus wind-dispersed species (table 2). Birds are known as efficient dispersers that ensure substantial gene flow within *Cembrae* pine populations (Furnier and others 1987). It is commonly believed that nutcrackers may also be responsible for extensive migration among populations of pine species, with which they are associated (Bruederle and others 2001). However, although table 3 shows that F_{ST}/G_{ST} values were lower in bird-dispersed than in wind-dispersed pines, the variance of estimates was very high. Difference between mean values (0.057 ± 0.045 vs. 0.103 ± 0.082) for the two groups did not generally exceed the differences that often observed between different species within each group or between several independent studies conducted for the same species, but using different loci sets and/or different population range. Therefore, additional studies that are based on the same set of genetic markers and the same geographic and ecological range are needed to calculate F_{ST}/G_{ST} values that would allow a strong conclusion as to whether migration is higher in bird-dispersed pines.

In general, such factors as high gene flow (due to efficient mechanisms of seed and pollen dispersal) along with a high effective population size, a high stand density, and a predominantly outcrossing mating system should lead to a higher intrapopulation component of genetic variation and a lower differentiation among populations. This is a typical pattern of genetic structure observed in widely distributed coniferous species (Ledig 1998). However, the fact that F_{ST} values significantly vary among loci contradicts the common opinion that migration plays a major role in differentiation and possibly indicates a nonneutral nature of at least some of loci. Loci with low F_{ST} values can be subjected to balancing selection that equalize gene frequencies, while loci with higher than average values can be affected by diversifying selection, and loci with intermediate F_{ST} values can be neutral (Altukhov 1991). The introduction of new genetic markers that are more neutral than isozyme loci will provide an opportunity for testing this hypothesis and estimating the role of different factors in maintaining of genetic structure of *Cembrae* pines.

Heterozygosity Dynamics and Mating System

The combination of a haploid endosperm (megagametophyte) that represents a segregating maternal gamete with a diploid embryo in conifer seeds allows researchers to genotype two consecutive generations in seeds collected from individual trees. Genotypes of maternal trees can be inferred from segregation of isozyme alleles in megagametophytes. At the same time embryos of these seeds represent open-pollinated progenies (half- or full-siblings) that can be considered as next generation of individuals in a particular tree stand. Comparisons of observed genotype distributions in two generations with those that are expected assuming Hardy - Weinberg equilibrium revealed interesting and important trends of temporal changes in the level of heterozygosity in all Eurasian *Cembrae* pines. Embryos typically demonstrate slight to moderate deficiency of homozy-

Table 2—Genetic diversity in white pines (section *Strobus*) estimated as expected heterozygosity (**H_e**) parameter using the same 16 isozyme loci.

Species [a]	**H_e**
Subsection *Cembrae*	
P. cembra	0.082
P. sibirica	0.106
P. albicaulis	0.113
P. koraiensis	0.130
P. pumila	0.198
Mean	**0.126 ± 0.020**
Subsection *Strobi*	
P. armandii	0.080
P. wallichiana	0.091
P. strobus	0.093
P. parviflora	0.109
P. monticola	0.119
P. strobiformis	0.122
P. peuce	0.125
P. flexilis	0.128
P. morrisonicola	0.132
P. lambertiana	0.142
Mean	**0.114 ± 0.006**
Mean for bird-dispersed	**0.123 ± 0.012**
Mean for wind-dispersed	**0.112 ± 0.009**

[a] Underlined are species for which there is some evidence of bird-dispersal of seeds (Lanner 1996, 1998).

Table 3—Level of genetic differentiation between populations in bird- versus wind-dispersed white pines estimated as **F_{ST}** or **G_{ST}** parameters using isozyme loci.

Species	**Populations**	**Loci**	**Geographic scale**	**F_{ST} or G_{ST}**	**Reference**
			Bird-dispersed		
P. albicaulis	3	21	Local	0.004	Rogers and others 1999
	9	19	Local	0.025	Bruederle and others 1998
	14		Mediumwide	0.088	Yandell 1992
	30	17	Rangewide	0.034	Jorgensen and Hamrick 1997
P. sibirica	8	20	Mediumwide	0.039	Goncharenko and others 1993b
	11	17	Mediumwide	0.025	Krutovskii and others 1994, 1995
P. pumila	3	18	Local	0.021	Krutovskii and others 1994, 1995
	5	22	Mediumwide	0.043	Goncharenko and others 1993a
	18	19	Mediumwide	0.170	Tani and others 1996
	12	32	Rangewide	0.073	Maluchenko and others 1998
P. koraiensis	3	16	Mediumwide	0.040	Krutovskii and others 1995
	8	23	Mediumwide	0.059	Kim and others 1994
	10	26	Rangewide	0.063	Belokon and others unpublished
P. flexilis	8	10	Mediumwide	0.022	Schuster and others 1989
	30	24	Rangewide	0.101	Jorgensen and Hamrick 1997
	16	27	Rangewide	0.149	Hamrick and others 1994
	7	10	Rangewide	0.016	Latta and Mitton 1997
P. strobiformis	8	11	Rangewide	0.047	Ledig, pers. comm.
			Mean	**0.057 ± 0.045**	
			Wind-dispersed		
P. ayacahuite	2	23	Local	0.047	Ledig 1998
	14	23	Rangewide	0.222	Ledig 1998
P. monticola	28	12	Rangewide	0.148	Steinhoff and others 1983
P. strobus	27	12	Rangewide	0.080	Ryu and Eckert 1983
	10	18	Mediumwide	0.019	Beaulieu and Simon 1994
			Mean	**0.103 ± 0.082**	

gotes (Politov and others 1992; Politov and Krutovsky 1994; Krutovsky and others 1994, 1995; Politov 1998). Estimates of mating system parameters have shown this deficiency is caused by partial self-pollination that can occur with up to 15 percent in *P. sibirica* populations, 8 percent in *P. koraiensis,* and can be as high as 31 percent in *P. cembra.* The latter species exists in small isolated stands that can have a limited pollen flow distance, which often promotes self-pollination.

In contrast to embryos, heterozygote deficiency was not observed in mature trees (Politov and Krutovsky 1994; Krutovsky and others 1995). They typically demonstrate either Hardy – Weinberg equilibrium or slight heterozygote excess that likely indicates the selective elimination of progeny originated from self-pollination during early stages of life and probably, also the balancing selection in favor of heterozygotes in some loci (Politov and Krutovsky 1994; Krutovsky and others 1995). Assortative mating of parents with different genotypes and/or preferential fertilization of gametes with different haplotypes are mating system mechanisms that may potentially also contribute to the excess of heterozygosity, but it also would cause an excess of heterozygosity in both embryos and mature trees and would not explain the increase of heterozygosity with age. We are also unaware of any evidence or data that would prove that assortative mating of such kind occurs in pines or other conifers.

Interspecific Hybridization

Critchfield (1986) summarized data on interspecific crosses among white pines of the section *Strobus.* Successful crosses between pines within the subsection *Cembrae* such as *P. sibirica* x *P. cembra* were also mentioned in that review, although this successful hybridization is not surprising due to the phylogenetic proximity of the parental species. Titov (1988) obtained sound seeds from the cross between *P. sibirica* x *P. koraiensis*; however, these were never tested by genetic markers to confirm hybridity. Successful crosses between *Cembrae* and *Strobi* pines, such as *P. koraiensis* x *P. lambertiana* were also reported earlier (Bingham and others 1972).

Interspecific crosses of *P. sibirica* x *P. koraiensis* were conducted in Ivanteevka Arboretum (Moscow Region, Russia) in the late 1960s. Needles and buds from 27 putative "hybrid" mature trees were tested by 18 allozyme loci (Politov, Belokon and Belokon, unpublished). Only two trees were unambiguously identified as hybrids based on species specific alleles in loci *Gdh, Adh-1,* and *Lap-3*, while the other trees were apparently *P. sibirica.* It is worth noting that these hybrids were superior in growth and showed resistance to the pest *Pineus cembrae* Cholodkovsky 1888 (Adelgidae: Chermes).

Natural hybridization between *P. sibirica* and *P. pumila* in Lake Baikal region was confirmed genetically using isozyme analysis (Politov and others 1999). Both species were thoroughly studied in their allopatric areas and across a wide zone of their sympatric distribution. Putative natural hybrids were identified using 28 allozyme loci controlling 14 enzyme systems. The *Adh-1, Fe-2,* and *Lap-3* loci in the hybrids had genotypes that were typical for *P. sibirica*, but did not occur or were unlikely in *P. pumila*, while five other loci carried alleles and genotypes that are unknown in *P. sibirica*, but common in *P. pumila*. The *Skdh-2* locus was heterozygous for alleles, one of which was specific for *P. sibirica*, but another for *P. pumila*. Some embryos from the seeds of the hybrid were likely resulted from self-pollination while others from backcrosses with parental species. This was the first genetic evidence of natural hybridization and potential gene exchange between *P. sibirica* and *P. pumila*.

Hypothetically, 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. *P. sibirica* and *P. pumila* are adapted to different environments, and survival outside of their respective optimal environments may be promoted by genes from related species coming from another side of the sympatry zone with different environmental gradients. The frequency and distribution of hybrids in the sympatry zone of *P. sibirica* and *P. pumila* and their possible role in the species adaptation and evolution are still largely unknown. It is still questionable whether observed hybridization leads to extensive gene introgression, but if it is so the gene exchange could be a mechanism of increasing of total population fitness. Despite intercrossing among *Cembrae* species, we do not consider this fact alone as an evidence of their closer relationships to each other than to other white pines. Crossability and closeness of relationship can be correlated, but the lack of crossability is not convincing of a more distant relationship. Blada (1994) reported a number of successful artificial crosses between *P. cembra* and pines of subsection *Strobi.* Watano and others (1995, 1996) studied trees with morphological traits that were "intermediate" when compared to traits in "pure" *P. pumila* and *P. parviflora* using DNA markers, and proved the trees to be interspecific hybrids. High conservatism in the number and morphology of chromosomes probably facilitates hybridization of white pines as well as other *Pinaceae*, and interspecific gene exchange might be an important (although usually underestimated) factor of population genetic structure dynamics.

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