Maintenance of Genetic Structure in Progenies of Marginal Mountainous and Steppe Populations of Three Species of *Pinaceae* Lindl. Family in Ukraine

By I. I. KORSHIKOV[1)], N. N. PIRKO, E. A. MUDRIK and YA. V. PIRKO

The Donetsk Botanical Gardens, Nat. Acad. Scie. of Ukraine, Illich ave., 110, Donetsk, 83059, Ukraine

(Received 7th June 2004)

Abstract

Comparative investigations of the genetic variation have been conducted at 8 to 10 polymorphic allozyme loci in samples of embryos and maternal plants from marginal populations of *Abies alba* Mill., *Pinus pallasiana* D. Don and *Pinus sylvestris* var *cretacea* Kalenicz. ex Kom. in Ukraine. It was found that the allele frequencies of the loci analysed were maintained in progenies of maternal plants of these populations. However, the population genotype structure of embryos was shifted towards an excess of homozygous genotypes. The highly variable loci of the maternal plants make the greatest contribution to the homozygotation of progenies.

Key words: Pinaceae Lindl., marginal populations, embryos, allozyme variation, genetic structure.

Introduction

In the process of evolution, large populations of living organisms reach an adaptive optimum of genetic diversity, which is maintained during generations. In such populations local fluctuations of gene frequencies are possible in the following generations, however, in general they remain stable over time (ALTUKHOV et al., 1996; ALTUKHOV, 2003). At the periphery of the species range, the increasing impact of unfavourable factors affects genetic structure of populations and their progenies more significantly, because some factors or whole complexes of factors exert a selectively significant influence. Extreme environmental factors can have an effect on reproductive capacity of the population by inhibiting the reproductive potentials of individuals in the population, and therefore by changing their contribution to the next generation. Occasionally, it can affect demographic dynamics and the genetic structure of subsequent generations, especially in isolated marginal plant populations with a limited number of individuals (KORSHIKOV et al., 2002b; KORSHIKOV et al., 2002a).

The dynamics of gene pools in populations of conifers can be studied on the basis of allozyme variation beginning with the earliest stages of plant ontogenesis. Using the electrophoretic analysis of the isoenzyme composition of megagametophytes and embryos the genotype of coniferous maternal plants and their progenies can be assessed by allozyme loci. Comparative investigations of the genetic structure of mature plants and their progenies are especially interesting in marginal populations of species with irregular seed crops. *Abies alba* Mill. is such a species in Ukraine, as its good seed crops are restricted to once every 2 to 4 years on the eastern border of the range in the Ukrainian Carpathians. This kind of investigations is very important in small populations of open-pollinated species, because of the drift effect, which may affect the population. Among such populations in Ukraine an isolated population of *Pinus sylvestris* var. *cretacea* Kalenicz. ex Kom. This may influence the fitness of genotypes in future generations. Investigations of the genetic structure of mountainous populations in different seed years are also interesting. The genetic composition can be changed by differences in flowering phenology caused by differences in altitude.

The knowledge of temporal dynamics of genetic variation in marginal populations of these species, especially of the ones that are decreasing in number (*P. sylvestris* var. *cretacea*) are required to ensure sustainable maintenance of their genetic diversity in subsequent generations. It is known that in populations of many coniferous plants, heterozygote deficiencies are often observed at early ontogenetic stage and are reduced at later ontogenetic stages as a consequence of the elimination of inbred progenies.

It can be expected that in marginal populations of such species as *Pinus pallasiana* D. Don and *Abies alba* with their scattered distribution as well as in species with isolated populations (*Pinus sylvestris* var. *cretacea*) of limited size, homozygotic genotypes will be in excess among embryos due to increased proportion of self-pollination. Moreover, owing to the impact of suboptimal or limiting environmental factors, the harsh selection leads to changes of the frequencies of alleles at some loci in these populations. This will favour some alleles and thus contribute to differentiation of the next generation in a higher or lower proportion. Temporal dynamics of allele and genotype distribution in different generations of a population can be an indicator for selection affecting those alleles occurring during subsequent ontogenetic stages (ALTUKHOV et al., 2004).

The aim of this study was to conduct a comparative analysis of genetic variation in progenies (embryos) and in maternal plants of marginal and isolated populations of three species of *Pinaceae* Lindl. family in Ukraine with a view of documenting possible changes in the genetic structure at different ontogenetic stages.

[1)] E-mail: herb@herb.dn.ua

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

Materials and Methods

In the course of expeditions, seeds were collected from three indigenous coniferous species in preserved areas in Ukraine.

Abies alba. Four populations of *A. alba* from the Ukrainian Carpathians were included in this study: Samborskaya (SAM) – in Lvov region (Stary Sambor forestry), Breduletskaya (BRD) and Goverlyanskaya (GOV) in the territory of Carpathian National Natural Park and Delyatinskaya population (DEL, Delyatin state forestry, botanical reserve "Pravy") – in the Ivano-Frankovsk region. Samples of 29 to 44 trees were used in the analyses, the age of the trees were 100 to 180 years.

Pinus sylvestris var. *cretacea*. A single population was sampled in the National Park "Svyati Gory", situated at the right bank of Severskiy Donets River in Donetsk Region (PSC). Seeds were collected from 29 trees aged 40 to 80 years.

Pinus pallasiana. The population used for this investigation was located on the southern mountains of Crimea near Nikita settlement (NIK-400, situated at the altitude of 400 m above sea level). Seed collections from the same trees (21 trees aged 80 to 100 and more years) were carried out in two different crop years of 1996 (E-96) and 1997 (E-97).

Genetic markers used were isoenzymes of those enzyme systems that can successfully be separated under electrophoresis of their extracts from megagametophyte and embryo tissues. Only two enzyme systems were common for all three species studied: glutamate oxaloacetate transaminase (GOT, enzyme classification (E.C.) 2.6.1.1.) and acid phosphatase (ACP, E. C. 3.1.3.2.). For the study of *A. alba* we additionally used alcohol dehydrogenase (ADH, E. C. 1.1.1.1.) and esterase (EST, E. C. 3.1.1.1), and in the investigations of *Pinus pallasiana* and *P. sylvestris* var. *cretacea* – glutamate dehydrogenase (GDH, E. C. 3.4.11.1.), leucine aminopeptidase (LAP, E. C. 1.1.1.37.), malate dehydrogenase (MDH, E. C. 1.1.1.37.) and superoxide dismutase (SOD, E. C. 1.15.1.1.). The electrophoresis of enzymes of megagametophyte and embryo homogenates was conducted in vertical blocks of 7.5% polyacrylamide gel with separating gel pH 8.9, using tris-glycine electrode buffer, pH 8.3 (Davis, 1964; Korochkin et al., 1977). Genetic control of the above-stated enzymes in the populations studied was determined earlier in our research (Korshikov and Terlygia, 2000; Tunda and Korshikov, 1999; Korshikov, Morozova and Pirko, 2003). Allele and loci nomenclature followed Prakash and co-authors (Prakash et al., 1969). For determining the genotype of the maternal plant segregation among at least eight haploid megagametophytes was analysed. The genetic structure of populations of the progenies was revealed on the basis of the analysis of equal numbers of embryos from each maternal plant included into the investigations. From a random sample of seeds of each maternal plant of *P. pallasiana* and *P. sylvestris* var. *cretacea* eight seeds were analysed from each maternal plant, in the three populations of *A. alba* five embryos from each maternal plant, and in one population four embryos from each maternal plant were analysed. Following parallel electrophoresis of enzymes of megagametophytes and embryos, we identified 10 polymorphic loci in *A. alba*, 9 in *P. sylvestris* var. *cretacea* and 8 in *P. pallasiana*. Got-1 locus in embryos and maternal plants turned out to be monomorphic in the studied populations of the two latter species. Locus Sod-4 was also monomorphic in *P. pallasiana.*

Similarities and differences in the genetic structure of maternal plants and their progenies were defined on the basis of analysis of composition and frequencies of alleles and genotypes, their heterogeneity (standard χ^2-test), mean number of alleles (A) and genotypes (Pg) per locus, polymorphism (P_{99}), expected (H_E) and observed (H_O) heterozygosity, F-statistics (Wright, 1965) and Nei's G-statistics and Nei's genetic distance (D_N) (Nei, 1972). These parameters of populational genetic variation were calculated by means of computer program BIOSYS-1 (Swofford and Selander, 1981). The correspondence of the observed genotype distributions to the expected ones according to Hardy-Weinberg equilibrium was tested at each locus in the samples of maternal plants and embryos according to the standard χ^2-criterion. For the calculation of embryo genotype success we used a ratio of frequencies of genotypes (embryo/maternal plant).

Results

In aggregate samples of embryos (E) and maternal plants (Mp) of four populations of *A. alba*, 34 alleles of 10 polymorphic loci (*Table 1*), in *P. sylvestris* var. *cretacea* 29 alleles of 9 loci, and in *P. pallasiana* 25 alleles of 8 loci were found (*Table 2*). In most samples of maternal plants and embryos of the three species, the predominant allele (1.00) occurred with a frequency of more than 0.5 in all the loci studied.

Rare alleles

Attention should be paid to the distribution of rare alleles in *A. alba* populations. The Got-$2^{0.92}$ allele occurred only in maternal plants of Samborskaya population, and Est-$1^{1.06}$ allele was found only in the samples of embryos of Delyatinskaya population. Apart from these two very rare alleles, three other rare alleles (Got-$2^{0.92}$, Got-$3^{0.93}$ and Acp^{null}) also occurred in one of the populations of *A. alba* in the samples of maternal plants and embryos with the frequency of at least 0.025. Some more rare alleles were found particularly allele $Acp^{1.02}$ in *P. pallasiana* embryo samples, and in *P. sylvestris* var. *cretacea* such a rare allele was Mdh-$3^{0.94}$. Alleles, found only in embryos, are from trees as pollen donor that were not included in the samples.

Allele and genotype heterogeneity

In progenies of the populations of the species studied, allele diversity is reliably reproduced. This is confirmed by the comparative analysis of composition and frequencies of alleles of embryos and maternal plants (*Table 3*). Considerable allele heterogeneity between maternal plants and their progenies was found only in two populations of *A. alba*, and it related only to 1 to 2 of

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

Table 1. – Frequencies of alleles, expected (H_E) and observed (H_O) heterozygosity at 10 loci in samples of seed embryos and maternal plants of four populations of *Abies alba* Mill. in Ukrainian Carpathians.

Locus	Allele	Samborskaya		Breduletskaya		Goverlyanskaya		Delyatinskaya	
		Mp	E	Mp	E	Mp	E	Mp	E
Got-1	0.92	.000	.000	.011	.005	.000	.000	.000	.000
	1.00	.983	.987	.989	.986	1.00	.995	.987	.976
	1.08	.017	.013	.000	.009	.000	.005	.013	.024
	H_O	.034	.025	.023	.027	.000	.010	.026	.049
	H_E	.033	.026	.022	.028	.000	.010	.026	.047
Got-2	0.92	.017	.000	.000	.000	.000	.000	.000	.000
	0.97	.035	.000	.068	.045	.000	.000	.026	.011
	1.00	.948	1.00	.932	.955	1.00	1.00	.974	.989
	H_O	.103	.000	.136	.082	.000	.000	.051	.022
	H_E	.100	.000	.127	.086	.000	.000	.051	.022
Got-3	0.80	.000	.000	.045	.059	.013	.020	.013	.068
	0.93	.000	.000	.000	.000	.025	.023	.000	.000
	1.00	.759	.817	.898	.857	.824	.839	.872	.818
	1.27	.241	.183	.057	.084	.138	.118	.115	.114
	H_O	.483	.200	.205	.168	.275	.205	.256	.286
	H_E	.366	.299	.188	.255	.301	.281	.226	.313
Adh-1	1.00	.603	.721	.807	.834	.813	.822	.923	.943
	1.04	.397	.279	.193	.166	.187	.178	.077	.057
	H_O	.586	.425	.386	.314	.225	.235	.154	.114
	H_E	.479	.402	.312	.277	.304	.293	.142	.108
Adh-2	0.78	.293	.196	.273	.289	.250	.280	.320	.270
	0.92	.000	.000	.000	.000	.013	.008	.013	.011
	1.00	.569	.691	.670	.672	.637	.632	.629	.695
	1.04	.138	.113	.057	.039	.100	.080	.038	.024
	H_O	.448	.317	.386	.368	.550	.425	.538	.373
	H_E	.571	.471	.473	.463	.522	.516	.500	.443
Est-1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.995
	1.06	.000	.000	.000	.000	.000	.000	.000	.005
	H_O	.000	.000	.000	.000	.000	.000	.000	.011
	H_E	.000	.000	.000	.000	.000	.000	.000	.010
Est-2	0.97	.000	.000	.000	.000	.013	.025	.051	.032
	1.00	.831	.858	.739	.673	.737	.675	.744	.698
	1.06	.020	.017	.068	.075	.100	.130	.051	.084
	1.09	.149	.125	.193	.252	.150	.170	.154	.186
	H_O	.333	.100	.432	.350	.350	.270	.410	.341
	H_E	.287	.248	.412	.478	.424	.498	.418	.470
Est-4	0.75	.000	.004	.091	.105	.013	.023	.038	.057
	1.00	.750	.726	.716	.700	.697	.684	.770	.798
	1.17	.042	.029	.068	.038	.040	.025	.038	.024
	1.50	.208	.233	.125	.157	.250	.260	.141	.097
	2.25	.000	.008	.000	.000	.000	.008	.013	.024
	H_O	.417	.250	.432	.195	.375	.195	.410	.184
	H_E	.392	.418	.459	.473	.450	.463	.384	.349
Acp-1	0.96	.000	.000	.080	.021	.025	.005	.026	.019
	1.00	.970	.983	.875	.908	.800	.817	.910	.870
	1.04	.030	.017	.045	.071	.175	.178	.064	.111
	H_O	.069	.033	.250	.155	.300	.210	.128	.200
	H_E	.058	.033	.226	.170	.329	.301	.167	.230
Acp-3	Null	.000	.000	.000	.000	.013	.018	.000	.000
	0.80	.220	.400	.170	.234	.063	.115	.308	.351
	1.00	.530	.404	.580	.600	.587	.582	.410	.465
	1.24	.250	.196	.250	.166	.337	.285	.282	.184
	H_O	.586	.308	.568	.323	.525	.345	.564	.368
	H_E	.608	.638	.572	.558	.538	.567	.658	.627

MP – maternal plants; E – embryos.

Table 2. – Frequencies of alleles, expected (H_E) and observed (H_O) heterozygosity at 10 loci in samples of seed embryos and maternal plants of natural populations of *Pinus sylvestris* var. *cretacea* Kalenicz. ex Kom. in the Donetsk region and *Pinus pallasiana* D. Don in Mountainous Crimea.

Locus	Allele	*Pinus pallasiana*			*Pinus sylvestris* var. *cretacea*	
		NIK-400			PSC	
		Mp	E-96	E-97	Mp	E
Gdh	0.90	.047	.036	.033	.000	.000
	1.00	.929	.923	.940	.759	.796
	1.12	.024	.041	.027	.241	.204
	H_O	.095	.083	.071	.414	.254
	H_E	.134	.145	.115	.366	.325
Got-1	1.00	1.00	1.00	1.00	1.00	1.00
	H_O	.000	.000	.000	.000	.000
	H_E	.000	.000	.000	.000	.000
Got-2	1,00	.524	.497	.616	.603	.660
	1.12	.476	.503	.384	.397	.340
	H_O	.571	.530	.387	.517	.329
	H_E	.499	.500	.473	.479	.449
Got-3	0.60	.024	.015	.018	.000	.000
	1.00	.905	.929	.911	.707	.717
	1.15	.000	.000	.000	.086	.058
	1.50	.071	.056	.071	.207	.225
	H_O	.190	.131	.143	.414	.273
	H_E	.175	.134	.165	.450	.432
Sod-4	0.90	.000	.000	.000	.017	.021
	1.00	1.00	1.00	1.00	.983	.979
	Ho	.000	.000	.000	.034	.011
	He	.000	.000	.000	.033	.041
Acp	0.94	.286	.202	.220	.241	.296
	0.97	.024	.009	.012	.052	.036
	1.00	.690	.789	.747	.621	.592
	1.02	.000	.000	.021	.086	.076
	H_O	.333	.071	.083	.586	.337
	H_E	.442	.337	.393	.546	.555
Lap-1	0.95	.000	.000	.000	.069	.082
	0.97	.000	.000	.000	.035	.036
	1.00	.976	.991	.982	.879	.874
	1.05	.024	.009	.018	.017	.008
	H_O	.048	.006	.036	.172	.036
	H_E	.047	.018	.035	.221	.228
Lap-2	0.95	.000	.012	.003	.207	.205
	0.97	.000	.000	.000	.017	.013
	1.00	.929	.946	.961	.759	.764
	1.05	.071	.042	.036	.017	.018
	H_O	.143	.083	.054	.172	.018
	H_E	.132	.103	.075	.380	.374
Mdh-2	0.90	.000	.000	.000	.034	.028
	1.00	.976	.994	.991	.828	.814
	1.08	.024	.006	.009	.138	.158
	H_O	.048	.012	.018	.310	.316
	H_E	.047	.012	.018	.294	.312
Mdh-3	0.86	.000	.000	.000	.259	.220
	0.94	.000	.000	.000	.000	.007
	0.96	.024	.039	.012	.000	.000
	1.00	.690	.738	.720	.724	.756
	1.03	.000	.000	.000	.017	.013
	1.15	.286	.223	.268	.000	.004
	H_O	.333	.429	.298	.448	.385
	H_E	.442	.404	.410	.408	.380

analysed loci. Significant genotypic heterogeneity between maternal plants and embryos has been found in all the species studied. In *P. sylvestris* var. *cretacea* it was observed only at one locus analysed. In the Breduletskaya population of *A. alba* it was observed at three, and in Samborskaya population, at four loci. In Goverlyanskaya and Delyatinskaya populations of *A. alba*, no significant divergences were determined at any of the loci in allele and genotype diversity of maternal plants and embryos.

Allele and genotype heterogeneity of seed progenies of four *A. alba* seed populations is considerably higher than in maternal plants. Thus, comparing samples of maternal plants of four *A. alba* populations, significant allele heterogeneity was found at one, and genotypic heterogeneity at three loci. For the progenies of these populations, significant allele and genotype heterogeneity was determined for eight loci. Progenies for two consecutive years of the same trees of *P. pallasiana* mountainous populations were different in genetic structure: valid allele heterogeneity was revealed at three, and genotypic one occurred at two loci.

Parameters of genetic polymorphism

Mean values of the basic indices of genetic polymorphism of maternal plants and embryos of the species studied were different. Thus, the fraction of polymorphic loci in the samples of embryos was equal or slightly

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

Table 3. – Allele and genotypic heterogeneity of polymorphic loci in samples of seed embryos and maternal plants of natural populations of three species of *Pinaceae* Lindl. family, χ^2-test.

Locus	*Abies alba*								Locus	*Pinus pallasiana*				*Pinus sylvestris* var. *cretacea*
	Plants – Embryos				Heterogeneity by 4 populations					Plants – Embryos		Embryos		
	Samborskaya		Breduletskaya		Plants		Embryos			1996	1997	1996 – 1997		Plants – Embryos
	allele	genotype	allele	genotype	allele	genotype	allele	genotype		genotype	genotype	allele	genotype	genotype
Got-1	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	Gdh	n. s.	n. s.	n. s.	n. s.	n. s.
Got-2	12.61** (2)	12.61** (2)	n. s.	n. s.	n. s.	n. s.	33.53 *** (3)	32.90 *** (6)	Got-2	n. s.	n. s.	9.64 ** (1)	14.01 ** (2)	n. s.
Got-3	n. s.	11.12 ** (2)	n. s.	n. s.	n. s.	25.40 * (15)	61.98 *** (9)	67.50 *** (24)	Got-3	n. s.	n. s.	n. s.	n. s.	n. s.
Adh-1	n. s	n. s.	n. s.	n. s.	10.80* (3)	26.80** * (6)	55.52 *** (3)	66.05 *** (6)	Sod-4	n. s.	n. s.	–	–	n. s.
Adh-2	n. s	n. s.	n. s.	n. s.	n. s.	n. s.	39.37 *** (9)	49.62 *** (21)	Acp	14.55 ** (3)	20.21** (5)	7.84 * (3)	n. s.	n. s.
Est-2	n. s.	10.71* (4)	n. s.	n. s.	n. s.	n. s.	67.50 *** (9)	72.15 *** (27)	Lap-1	n. s.	n. s.	n. s.	n. s.	12.21 * (4)
Est-4	n. s.	n. s.	n. s.	19.10 ** (7)	n. s.	n. s.	90.87 *** (12)	84.38 *** (33)	Lap-2	n. s.	n. s.	n. s.	n. s.	n. s.
Acp-1	n. s.	n. s.	9.11* (2)	12.42 * (4)	n. s.	19.38 * (9)	57.75 *** (6)	52.90 *** (12)	Mdh-2	n. s.	n. s.	n. s.	n. s.	n. s.
Acp-3	6.54 * (2)	11.73 * (5)	n. s.	15.78 ** (5)	n. s.	n. s.	115.21*** (9)	90.10 *** (21)	Mdh-3	n. s.	n. s.	6.26 * (2)	15.91 ** (4)	n. s.

Note: the number of freedom degrees is given in brackets. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. – insignificant differences; the data concerning populations with the absent allele and genotypic heterogeneity, are not presented in the table.

lower than in the samples of maternal plants. The mean number of alleles per locus, on the contrary, was higher in embryos, with the exception of one Samborskaya population of *A. alba* (*Table 4*). In all the embryo samples analysed, the mean number of genotypes per locus was considerably higher than in maternal plants samples. This may be connected with the sizes of the samples as indicated above.

Maternal plant samples and corresponding samples of embryos of all three studied species did not differ considerably in the average level of expected heterozygosity (H_E). Mean values of observed heterozygosity (H_O) were significantly (Student criterion) lower in six out of seven embryo samples (exclusively, *P. pallasiana*, 1996 seed crops), than in samples of adult plants. For *A. alba* populations, mean values of H_O of embryos compared to maternal plants was 23.2 to 45.8% lower, for *P. sylvestris* var. *cretacea* 34.2% lower and for *P. pallasiana* was 23.3 to 38.1% lower. As slight heterozygote excess (*A. alba*) (1.1 to 5.9%) or their deficiency (1.2 to 9.4%) (in *A. alba*), 3.4% (*P. sylvestris* var. *cretacea*), 8.4% (*P. pallasiana*) were peculiar to maternal plants, significant lack of het-

Table 4. – Values of basic parameters of genetic polymorphism in samples of seed embryos and maternal plants in natural populations of three species of *Pinaceae* Lindl. family.

Populations	Plants (Mp), embryos (E)	Sample size	Polymorphic loci fraction, P_{99}	Mean number per locus of		Average heterozygosity		Wright's fixation index, F
				allele, A	genotypes, Pg	expected, H_E	observed, H_O	
Abies alba								
SAM	Mp	29	0.900	2.400	3.100	0.289±0.024	0.306±0.024	- 0.059
	E	120	0.800	2.400	3.600	0.254±0.011	0.166±0.010	0.346
BRD	Mp	44	0.900	2.600	3.400	0.279±0.019	0.282±0.020	- 0.011
	E	220	0.900	2.700	4.600	0.279±0.009	0.198±0.008	0.290
GOV	Mp	40	0.700	2.800	4.000	0.287±0.020	0.260±0.020	0.094
	E	200	0.700	3.000	5.500	0.293±0.009	0.190±0.008	0.352
DEL	Mp	39	0.900	2.900	3.800	0.257±0.019	0.254±0.020	0.012
	E	185	0.900	3.000	5.000	0.262±0.009	0.195±0.009	0.256
At an average	Mp	152	0.847	2.691	3.603	0.284±0.010	0.274±0.010	0.035
	E	725	0.828	2.810	4.785	0.280±0.005	0.190±0.004	0.321
Pinus sylvestris var. *cretacea*								
PSC	Mp	29	0.900	2.800	3.700	0.318±0.025	0.307±0.025	0.034
	E	267	0.900	3.000	4.000	0.309±0.009	0.202±0.008	0.346
Pinus pallasiana								
NIK-400	Mp	21	0.800	2.200	2.200	0.192±0.024	0.176±0.023	0.084
	E-96	168	0.600	2.300	3.100	0.165±0.008	0.135±0.007	0.182
	E-97	168	0.700	2.400	2.900	0.168±0.008	0.109±0.007	0.352

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

erozygotes was typical for embryo samples of all three species. In *A. alba* this deficiency was estimated as 25.6 to 34.6%, in *P. sylvestris* var. *cretacea* 34.6% and in *P. pallasiana* 18.2 to 35.2% (*Table 4*, Wright's fixation index).

It turned out by means of locus-by-locus analysis that observed heterozygosity in the maternal plant samples of *A. alba*, with some rare exception (five cases), was higher than in the embryo samples (*Table 1*). The most significant decrease in actual heterozygosity in samples of embryos compared to those of maternal plants was found in all populations of this species at loci Est-4 (40 to 55.1%), Acp-3 (34.3 to 47.4%), Est-2 (16.8 to 70%) and Adh-2 (4.7 to 30.7%). It is remarkable that they are the most variable loci in *A. alba*. Their mean observed heterozygosity in samples varied from 20.5 to 58.6%. Population mean of observed heterozygosity in maternal plants ranged for these loci from 39.1% (Est-2) up to 56.1% (Acp-3) and for embryos from 20.6% (Est-4) up to 37.1% (Adh-2). This level was significantly lower (Student criterion) in embryos than in maternal plants for all the loci. Consequently, the noticeable decrease in the mean level of actual heterozygosity in progenies of marginal populations of *A. alba* is especially correlated with a considerable reduction of frequencies of heterozygotes of the highly variable loci. Obviously, there seems to be a tendency for increased inbreeding during sexual reproduction of marginal populations of *A. alba*.

Tendencies in locus-by-locus variation of actual heterozygosity in maternal plants and their progenies of isolated population of *P. sylvestris* var. *cretacea* and mountainous populations of *P. pallasiana* are similar to those found in *A. alba*. That is the level of observed heterozygosity (H_O = 0.254 to 0.377) in 5 out of 6 highly variable loci in embryos of *P. sylvestris* var. *cretacea*, was determined to be lower than in plants (H_O = 0.414 to 0.586) (*Table 2*, values for Gdh and Acp, for example). The decrease in actual heterozygosity in embryos was found to be 34.1% (Got-3), 42.5% (Acp) and 14.1% (Mdh-3). Only one highly variable locus Mdh-2 had very similar values of observed heterozygosities in samples of maternal plants and embryos *P. sylvestris* var. *cretacea*. The largest decrease of heterozygotes numbers in embryos of this species was observed in two medium-polymorphic loci (Lap-2 and Lap-1), and one low-polymorphic locus (Sod-4), that was 89.5, 71.1, 77.6% respectively. From three highly variable (H_O = 0.333 to 0.571) loci of maternal plants of *P. pallasiana* the decrease of heterozygosity in embryo samples of the crops of two consecutive years was found to be stably at the Acp locus (75.1 to 78.7%) and not stably at the loci Got-2 (7.2 to 32.2%) and Mdh-3 (10.5%) in comparison with maternal plants. In embryo samples of *P. pallasiana*, the frequencies of heterozygotes of medium-polymorphic loci (Lap-2 and Got-3) were also considerably reduced.

Hardy-Weinberg equilibrium

It is evident that under significant heterozygote deficiencies in progenies of populations of the species studied, considerable deviations of the actual genotype distribution from the Hardy-Weinberg equilibrium should be expected in samples of embryos compared to maternal plants. In maternal plant samples of all three species, the observed genotype distribution significantly deviated from this equilibrium only at three loci (*Table 5*). The unbalanced genotype distribution in maternal plants of *A. alba* was found only in one population. Significant deviations in the actual distribution of genotypes from the expected Hardy-Weinberg equilibrium

Table 5. – Loci with significant deviation of the observed genotype distribution from the expected one according to Hardy-Weinberg rule in the samples of embryos and maternal plants from the populations of three species of *Pinaceae* Lindl. family in the territory of Ukraine.

Locus	*Abies alba*					Locus	*Pinus sylvestris* var. *cretacea*		*Pinus pallasiana*		
	SAM	BRD	GOV	DEL			PSC		NIK-400		
	E	E	E	Mp	E		Mp	E	Mp	E-96	E-97
Got-3	12.59 *** (0.96)	35.68*** (2.44)	20.29*** (2.28)	n. s.	7.62 * (2.55)	Got-2	n. s.	14.92 *** (0.99)	n. s.	n. s.	5.55 * (1.00)
Adh-1	n. s.	n. s.	7.37 ** (0.97)	n. s.	n. s.	Got-3	n. s.	50.72 *** (2.60)	n. s.	n. s.	n. s.
Adh-2	31.01*** (2.78)	10.03** (2.28)	7.81** (1.06)	n. s.	16.60 *** (1.29)	Gdh	n. s.	7.952 ** (0.98)	1.55* (0.22)	47.43*** (1.49)	79.48 *** (1.11)
Est-2	17.25*** (0.73)	33.07*** (2.76)	71.52*** (3.92)	8.69 * (2.41)	28.86 *** (4.25)	Sod-4	n. s.	12.96*** (0.25)	–	–	–
Est-4	1.293* (0.21)	197.44*** (4.81)	18.18*** (1.23)	n. s.	72.15 *** (3.59)	Acp	n. s.	103.88 *** (4.54)	n. s.	2.25* (0.35)	16.96 *** (1.25)
Acp-1	n. s.	n. s.	0,92 * (0.14)	n. s.	n. s.	Lap-1	2.230* (0.48)	50.71 *** (0.92)	n. s.	n. s.	n. s.
Acp-3	61.69*** (3.00)	72.75*** (2.96)	66.50*** (3.05)	n. s.	66.27 *** (2.98)	Lap-2	n. s.	62.18 *** (1.40)	n. s.	n. s.	n. s.
						Mdh-2	n. s.	12.62 ** (1.87)	n. s.	n. s.	n. s.
						Mdh-3	n. s.	n. s.	n. s.	11.91** (2.10)	3.06 ** (0.57)

Note: the number of freedom degrees is given in brackets. Differences are valid when *P < 0.05; **P < 0.01; ***P < 0.001; n.s. – differences are insignificant.; populations of maternal plants with the absence of valid differences are not presented in the table.

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

occurred in embryo samples. These deviations were observed in 7 out of 10 studied loci of *A. alba*, in 8 out of 9 loci of *P. sylvestris* var. *cretacea* and in 4 out of 8 loci of *P. pallasiana*. This imbalance was found in embryo samples of all four populations of *A. alba* at five loci (Got-3, Adh-2, Est-2, Est-4 and Acp-3). These five loci are characterized by the highest average heterozygosity both in plant and embryo samples (*Table 1*). In three out of four loci in embryo samples of *P. pallasiana* there was observed a considerable deviation from the expected equilibrium in actual distribution of genotypes, they were also characterized by the highest average values of heterozygosity (*Table 2*).

Thus, genetic structure differentiation of maternal plants and progenies of marginal populations of *A. alba* and *P. pallasiana* is especially pronounced at highly variable loci. In the small isolated population of *P. sylvestris* var. *cretacea*, 8 out of 9 loci studied in maternal plants that are characterized by different levels of variation, make a significant contribution to the variation of genotype structure of seed progenies.

Locus-by-locus analysis of genotype distributions with significant deviation from Hardy-Weinberg equilibrium in embryo samples of *A. alba* identified a number of peculiarities (*Table 6*). In 7 loci an excess (>) of homozygotes was found to be mainly connected with genotypes carrying less frequent alleles. Only 3 loci (Est-2, Est-4 and Acp-3) in some populations have been found to have a redundancy of homozygotes at the predominant allele (1.00). In 5 of the most variable loci (Got-3, Adh-2, Est-2, Est-4 and Acp-3) these cases are correlated with a significant shift in the distribution of the most frequent genotypes found at these loci. All the cases of significant deviations in heterozygotes distribution show deficiencies. A significant surplus of homozygotes in embryo samples of four A. alba populations occurred in 10 to 16 genotypes, and considerable insufficiency of heterozygotes in only 5 to 9 genotypes.

Consequently, genotypes with less frequent alleles make the main contribution to the increase of homozygosity level of progenies of marginal populations of *A. alba*. During later ontogenetic stages, young plants

Table 6. – Cases of significant deviation in genotype distribution from the expected Hardy-Weinberg equilibrium and embryo genotype success in embryos of four populations of *Abies alba* Mill. in Ukrainian Carpathians, χ^2-test.

Locus	Genotype	Sambor-skaya	Bredulet-skaya	Goverlyan-skaya	Delyatin-skaya	Embryo genotype success
		χ^2-test	χ^2-test	χ^2-test	χ^2-test	
Got-3	1.27/1.27	8.78 >	26.1 >	9.7 >	5.14 >	1.600 °
	1.27/1.00	3.9 <	6.9 <	–	–	0.603
	0.93/0.93	–	–	–	–	0.00 +
	0.80/0.80	–	7.8 >	–	–	0.00 +
Adh-1	1.04/1.04	–	–	5.06 >	–	0.722 °
Adh-2	1.00/1.04	–	–	6.2 <	–	0.615
	1.04/1.04	19.2 >	–	25.6 >	34.9 >	1.121 °
	1.00/0.92	–	–	6.2 <	–	0.512°
	1.00/0.78	–	5.47 <	–	–	0.814
	0.78/0.78	6.2 >	5.0 >	–	–	1.267
	0.92/0.92	–	–	–	36.4 >	0.000 +
Est-2	1.09/1.09	34.9 >	8.8 >	25.8 >	9.2 >	4.597 °
	1.09/1.00	9.7 <	4.7 <	9.5 <	4.1 <	0.520
	1.06/1.06	24.7 >	17.9 >	39.9 >	16.3 >	1.135 °
	1.00/1.00	–	–	4.8 >	–	1.045
	1.06/0.97	–	–	9.5 <	4.1 <	0.00 +
	0.97/0.97	–	–	6.1 >	–	0.192 °
	1.00/0.97	–	–	10.4 <	–	0.922 °
Est-4	1.50/1.00	6.8 <	7.0 <	29.9 <	4.7 <	0.494
	1.00/1.00	–	5.9 >	7.5 >	–	1.179
	1.17/1.17	37.3 >	41.8 >	28.1 >	6.3 >	0.200 °
	1.00/0.75	–	–	–	6.8 <	0.734 °
	0.75/0.75	–	140.7 >	33.9 >	68.8 >	1.787 °
	1.50/0.75	–	7.3 <	–	–	0.000 + *
	1.50/1.50	13.7 >	33.6 >	40.8 >	30.8 >	3.124 °
	2.25/2.25	-	–	–	76.4 >	0.000 +
Acp-1	1.04/1.04	–	–	11.8 >	–	1.269 °
	1.04/1.00	–	–	5.7 <	–	1.159
Acp-3	1.24/1.24	15.1 >	23.7 >	13.4 >	18.5 >	1.299
	1.24/1.00	–	9.0 <	8.9 >	14.8 <	0.411
	1.00/1.00	10.6 >	7.1 >	5.5 >	14.2 >	1.289
	1.00/0.80	13.4 <	11.6 <	8.9 >	11.5 <	0.982
	1.24/0.80	6.2 <	–	–	–	0.824
	0.80/0.80	14.9 >	21.0 >	26.4 >	7.7 >	3.026 °
	null/null	–	–	57.8 >	–	0.000 +

Note: + – absent in maternal plants; * – absent in maternal plants of Delyatinskaya population and in embryos of Goverlyanskaya population; ° – mean value of embryo genotype success calculated only for the populations with this genotype present in plants and embryos.

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

Table 7. – Cases of significant deviations in genotypes distribution from the expected Hardy-Weinberg equilibrium and embryo genotype success of *Pinus sylvestris* var. *cretacea* Kalenicz. ex Kom. and *Pinus pallasiana* D. Don., χ^2-test.

Locus	Allele	*Pinus sylvestris* var. *cretacea*	Embryo genotype success	Locus	Allele	*Pinus pallasiana*		Embryo genotype success
		E				E-96	E-97	
Acp	0.94/0.94	27.75 >	0.176	Gdh	0.90/0.90	36.17 >	–	0.000 + ••
	1.00/1.00	5.43 >	0.766		0.90/1.00	5.93 <	5.25 <	0.000 +
	1.02/1.02	54.90 >	0.761		1.00/1.12	–	6.63 <	4.666
	0.97/0.97	11.45 >	0.000 +		1.12/1.12	10.64 >	–	0.000 + ••
	0.94/0.97	15.49 >	1.400		1.12/0.90	38.61 >	201.38 >	1.327
	0.97/1.00	8.81 <	2.760					
Got-3	1.15/1.15	24.75 >	0.000 +	Mdh-3	0.96/0.96	12.13 >	–	0.000 + ••
	1.50/1.50	18.23 >	0.293		1.00/1.15	–	5.45 <	0.902
	1.00/1.50	9.42 <	1.181		1.15/1.15	–	8.19 >	2.531
Lap-2	0.95/0.95	94.85 >	0.746	Acp	0.94/0.94	12.09 >	23.68 >	0.000 +
	0.95/0.97	47.84 <	6.800		0.94/1.00	5.67 <	–	2.932
	1.00/1.00	6.95 >	0.094		0.97/0.97	–	40.01 >	0.000 + •
Sod-4	0.90/0.90	47.78 >	0.000 +					
	0.90/1.10	4.17 <	3.182					
Mdh-2	0.90/1.08	11.83 <	1.166					
Got-2	1.12/1.12	7.99 >	0.762					
	1.00/1.12	5.91 <	1.529					
Lap-1	0.95/0.95	123.50 >	0.000 +					
	0.97/0.97	27.00 >	0.972					
Gdh	1.12/1.12	4.76 >	0.459					

Note: + – absent in maternal plants; • – absent in embryos of 1996 crop; •• – absent in embryos of 1997 crop.

are exposed to selection, resulting in a less frequent representation of homozygotes in samples of adult plants of the populations studied.

The peculiarities in genotype distribution among embryos, similar to those described in *A. alba*, were also found in *P. sylvestris* var. *cretacea* and *P. pallasiana* (*Table 7*). The cases of deviation of the actual genotype distribution from the Hardy-Weinberg equilibrium in embryo samples of these species resulted from homozygote excess and heterozygote deficiency in most of the cases. Homozygote redundancy at the predominant allele (1.00) has been revealed only at two loci (Acp and Lap-2) in *P. sylvestris* var. *cretacea.*

Success of homozygous embryo genotypes of *A. alba*, *P. sylvestris* var. *cretacea* and *P. pallasiana* is evidently higher than that of heterozygous ones (*Table 6, 7*). Probably their higher frequency in populations is peculiar to parental gametes forming mainly homozygous and less heterozygous genotypes. This might be a consequence of redundant self-pollination of maternal plants. In the later stages of ontogenetic development, homozygotes are probably eliminated more frequently than heterozygotes, which leads to an equalized genotype distribution in compliance with Hardy-Weinberg rule of the adult stages of the populations studied.

To determine the contribution of homo- and heterozygous maternal plants to form redundant homozygotation of progenies, all the studied plant samples were divided into groups of homo- and heterozygous trees at each locus. In embryo samples of these groups the relation of the observed to the expected genotype distribution was determined according to the Hardy-Weinberg rule. The tendency for cases of significant bias in actual genotype distribution of embryos in the homozygous or heterozygous groups of maternal plants of all three species studied were the same as mentioned above. Thus, for instance, in groups of homozygous genotypes of maternal plants of four populations of *A. alba*, the total number of cases of significant bias in actual genotype distribution in embryos caused by heterozygous deficiency was 14 while 21 was caused by homozygous excess. In the groups of heterozygous genotypes of maternal plants of this species, the total number of such cases in embryo samples was slightly higher (41), the lack of heterozygote being peculiar to 10 of them and homozygotes surplus, to 31 of them. In embryo samples of homo- and heterozygous maternal plants of *P. sylvestris* var. *cretacea* and *P. pallasiana* no significant cases of deviation from the Hardy-Weinberg equilibrium by reason of heterozygous redundancy was found in the genotype distribution. Consequently, both homozygous and heterozygous genotypes of maternal plants in marginal populations of *A. alba*, *P. sylvestris* var. *cretacea* and *P. pallasiana* make a contribution by causing considerable heterozygotes deficiency in their seed progenies.

Inbreeding effects

Inbreeding in marginal populations of *A. alba* is also confirmed by calculations of inbreeding coefficients of an individual in relation to the population (F_{IS}) and inbreeding of an individual in relation to the species as a whole (F_{IT}). Positive values of these coefficients in embryo samples are one order higher than in maternal plants of the four compared populations of *A. alba* (*Table 8*). This points once again to the considerable shortage of heterozygotes in progenies of *A. alba* plants in the four marginal populations. The values of F_{ST} and G_{ST} coefficients characterizing subdivision of the studied maternal plants and embryo samples are not high and almost identical. Both in plants and embryos, the proportion of interpopulational variation is about 2%. The degree of genetic differentiation between populations of

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

Table 8. – Parameters of WRIGHT's F-statistics and NEI's G-statistic, NEI's genetic distance (D_N) in samples of embryos and maternal plants of natural populations of three species of *Pinaceae* Lindl. family.

Samplings of plants and embryos	Limits of variability of genetic indices				
	F_{IS}	F_{IT}	F_{ST}	G_{ST}	D_N
Abies alba					
Maternal plants of four populations	-0.004	0.017	0.021	0.021	0.007 – 0.020
Maternal plants and embryos of each population	0.113 – 0.211	0.115 – 0.211	0.001 – 0.006	0.000 – 0.007	0.001 – 0.008
Embryos of four populations	0.192	0.205	0.017	0.018	0.006 – 0.018
Pinus sylvestris var. *cretacea*					
Maternal plants and embryos	0.319	0.319	0.000	0.000	0.001
Pinus pallasiana					
Maternal plants and embryos of the crops of					
1996	0.180	0.181	0.001	0.000	0.002
1997	0.185	0.186	0.001	0.000	0.002
Embryos of the crops of two different years	0.200	0.202	0.003	0.001	0.002

Note: F_{IS} – inbreeding coefficient of an individual in relation to population; F_{IT} – inbreeding coefficient of an individual in relation to the species as a whole; F_{ST} and G_{ST} – inbreeding coefficients of a population in relation to the species as a whole.

A. alba is obvious in their progenies. This is evidenced by very similar values and by the amplitude of variability of Nei's genetic distance.

The level of individual heterozygosity of maternal plants does not influence creation of a heterozygous deficiency. Thus, the level of observed heterozygosity in the embryo samples from groups of trees heterozygous by 1 as well as 2, 3, 4 and 5 loci was essentially lower than that in the maternal plant samples (*Table 9*). In embryos of six groups of plants, the lack of heterozygotes varied within the limits of 22% (trees with 50% heterozygosity) to 55.7% (homozygous trees). In general, average actual heterozygosity in *A. alba* embryos was 32,1% lower than theoretically expected.

Discussion

Heterozygote deficiency found in embryos of the plants from the marginal and isolated populations of three species of *Pinaceae* family is not always found in other populations. For instance, in a population of the central part of the *Pinus sylvestris* L. species range in the Urals (Russia), in embryo samples of two different crop years according to the values of Wright's fixation index (F), heterozygote excess was observed (–0.137 and –0.174) and maternal plants were in a balanced state (F = –0.006). H_O values in plants and embryos were almost equal: 0.299 and 0.290 respectively (SHIGAPOV et al., 1996). Heterozygote surplus both in embryos (F = –0.066) and maternal plants (F = –0.074) has earlier been determined in *Picea glauca* (Moench) Voss. in Ontario, Canada (CHELIAK et al., 1985a). In a population of this species in Alberta (Canada) heterozygote deficiency was observed in adults (F = 0.039) and in embryos of two different seed crop years (F = 0.029–0.031) (KING et al., 1984). Mean values of F or F_{IS} and F_{IT} of many coniferous species in populations show that in most cases the frequency of heterozygote occurrence in embryos is lower than in adults. Shortage of heterozygotes in early ontogenetic stages (embryos) was probably caused by non-random mating between closely related individuals and by self-pollination contributing to the increase of the proportion of inbred progenies. Heterozygote surplus can be explained by pronounced family structure of populations (SHAW and ALLARD, 1982; YAZDANI et al., 1985; FINS and LIBBY, 1982; SHEA, 1987; KNOWLES et al., 1987; INNES and RINGIUS, 1990, LEWANDOWSKI et al., 1991; POLITOV et al., 1992).

In conifers with mixed mating system, self-pollination is one of the main causes of homozygote excess in the embryonal stage. This is confirmed by the assessments of outcrossing frequencies by the use of several loci. Multilocus index of cross-pollination (t_m) in coniferous populations varies from 0.70 to 0.99 (CHELIAK et al., 1985b; PERRY and DANCIK, 1986; MUONA and HARJU, 1989; INNES and RINGIUS, 1990; LEWANDOWSKI et al., 1991; EL-KASSABY et al., 1993). Self-pollination leads to

Table 9. – Distribution of embryo numbers and their average heterozygosity depending upon the number of heterozygous loci in maternal plants in aggregate sample of four *Abies alba* Mill. populations.

Number of heterozygous loci per maternal plants (observed heterozygosity)	Number of plants	Number of heterozygous loci per embryos (0-7)								Total number of embryos	Average heterozygosity of embryos		Wright's fixation index
		0	1	2	3	4	5	6	7		observed, H_O	expected, H_E	
		Number of embryos											
0 (0.000)	3	12	2	1	0	0	0	0	0	15	0.027±0.013	0.061±0.017	0.557
1 (0.100)	21	18	26	37	16	5	0	0	0	102	0.169±0.011	0.249±0.012	0.321
2 (0.200)	43	39	75	55	34	8	2	0	0	213	0.155±0.008	0.244±0.008	0.365
3 (0.300)	42	20	49	75	48	16	2	0	0	210	0.196±0.008	0.288±0.009	0.319
4 (0.400)	22	17	19	29	33	8	2	2	0	110	0.209±0.012	0.288±0.012	0.274
5 (0.500)	13	1	6	21	19	15	1	2	0	65	0.280±0.016	0.359±0.016	0.220
6 (0.600)	2	0	0	1	4	2	2	0	1	10	0.390±0.044	0.372±0.045	-0.048
Total	146	107	177	219	154	54	9	4	1	725	0.190±0.040	0.280±0.005	0.321

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

the reduction of genetic variability level in subsequent generations and to the decrease of effective sizes of populations (INGVARSSON, 2002). When the cross-pollination proportion is high, the genotype structure of progenies is usually correspond to Hardy-Weinberg expectations. For instance, in embryos of three different crop years of three populations of *Pinus contorta* var. *latifolia* in Alberta (Canada), when t_m = 0.926 to 0.983, there were observed no cases of significant deviation in actual genotype distribution from the expected under Hardy-Weinberg equilibrium (PERRY and DANCIK, 1986). In other species, the occurrence of unbalanced genotype distribution in embryos varied from 20 to 83.3%. As a rule, these cases are correlated with heterozygous deficiencies (SHEA, 1987; 1990; KNOWLES et al., 1987; INNES and RINGIUS, 1990; CHANGTRAGOON and FINKELDEY, 1995). In the two species studied in this survey the occurrence of an unbalanced genotype distribution was within the indicated bounds, 43.8% for *Pinus pallasiana* and 62.9% for *Abies alba*. It was extremely high for *P. sylvestris* var *cretacea*, 88.9%. The increased occurrence of deviations from the Hardy-Weinberg equilibrium in *P. sylvestris* var. *cretacea* embryos can be probably explained by the diffuse spatial distribution of plants in this small (60.5 ha) isolated population. Stands with low density are usually characterized by a reduced level of cross-pollination (RUDIN et al., 1977; FARRIS and MITTON, 1984; CHANGTRAGOON and FINKELDEY, 1995).

Heterozygote deficiencies in embryos are often reduced in the subsequent ontogenetic stages (seedlings, young plants) up to the complete absence of homozygotes. This is due to the elimination of inbred progenies (FARRIS and MITTON, 1984; YAZDANI et al., 1985; PLESSAS and STRAUSS, 1986; MUONA et el., 1987; POLITOV and KRUTOVSKII, 2004). However, in plants which have reached reproductive development stage, heterozygosity level can be changed in different age groups of one population (TIGERSTEDT et al., 1982, HAMRICK and LOVELESS, 1986).

Genotype structure of populations in the early ontogenetic stage (embryos) apparently depends upon the parameters and stability of mating system of plants. In marginal populations with low density and diffuse or mosaic pattern of distribution, the effectiveness of their outcrossing may be reduced due to discordance of flowering time, shifts of periods favourable for pollination or differences in fertility and pollen viability. This limits the free combination of male and female gametes in a population, which increases their divergence in the early ontogenetic stage. Probably these factors explain the fact that allele and genotype heterogeneity is considerably higher in embryos compared to maternal plants of four marginal populations of *A. alba*. Genetic heterogeneity can also be influenced by the sizes of plant and embryo samples studied. It is obvious that inbreeding and outcrossing balance in the progenies of mountainous populations can be affected by climatic factors determining a favourable period of flowering. This probably accounts for allele and genotype heterogeneity of embryos in different crop years in *P. pallasiana* population. In other coniferous species, genetic differences between population progenies of different years were not always found (KING et al., 1984; CHELIAK et al., 1985b).

Thus, in progenies of marginal and isolated populations of three studied species from *Pinaceae* family, allele diversity peculiar to maternal plants, is maintained. This was observed in populations of many coniferous species. The main quantitative contribution to the formation of homozygote excess in embryos of the species studied is due to highly variable loci in maternal plants. Heterozygote deficiency in embryos is eliminated by selection in the subsequent ontogenetic stages and in certain age groups it reaches an evolutionally determined genetic optimum specific for each species (ALTUKHOV, 2003). Since conditions for a species are usually less favourable at the periphery than in a central part of the range, it can be expected that dynamics and time to reach the adaptive optimum of heterozygosity in the course of ontogenetic process (ALTUKHOV, 2003) will be different in marginal populations. According to these results it is evident that in seed selection in isolated marginal populations, the genetic peculiarities of maternal plants must be taken into account in order to minimize the proportion of the inbred progeny. Genetic control on the embryonal stage is necessary for ascertaining this proportion. This will enable to decrease the level of plant elimination in the early ontogenetic stages and inbred depression in late ontogenesis.

Acknowledgments

The authors express sincere appreciation to the administration and stuff of “Carpathian Park” and National natural park “Svyati Gory” for the assistance during seed material collections.

References

ALTUKHOV, YU. P., (2003): Genetic processes in populations. Manual. 3ed., rev. and enl. PTC “Academkniga”, Moscow. 431 p.

ALTUKHOV, YU. P., et al., (2004): Dynamics of population gene pools under anthropogenic pressures. Nauka, Moscow. 619 p..

ALTUKHOV, YU. P., L. I. KOROCHKIN and YU. G. RYCHKOV (1996): Hereditary biochemical diversity in evolution and development. Genetika (Moscow) **32**: 1256–1275.

CHANGTRAGOON, S. and R. FINKELDEY (1995): Patterns of genetic variation and characterization of the mating system of *Pinus merkusii* in Thailand. Forest Genet. **2**: 87–97.

CHELIAK, W. M., J. A. PITEL and G. MURRAY (1985a): Population structure and mating system of white spruce. Can. J. For. Res. **15**: 301–308.

CHELIAK, W. M., B. P. DANCIK, K. MORGAN, F. C. H. YEH and C. SRTROBECK (1985b): Temporal variation of the mating system in a natural population of jack pine. Genetics **109**: 569–584.

DAVIS, B. J. (1964): Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 67–75.

EL-KASSABY, Y. A., M. D. MEAGHER and R. DAVIDSON (1993): Temporal variation in the outcrossing rate in a natural stand of western white pine. Silvae Genet. **42**: 131–135.

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics

FARRIS, M. A. and J. B. MITTON (1984): Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. Evolution **38**: 1151–1154.

FINS, L. and W. J. LIBBY (1982): Population variation in *Sequoiadendron*: seed and seedling studies, vegetative propagation, and isozyme variation. Silvae Genet. **31**: 102–110.

HAMRICK, J. L. and M. D. LOVELESS (1986): The genetic structure of tropical tree populations: Associations with reproductive biology. The evolutionary ecology of plants/Ed. by J. E. BOCK, Y. B. LINHART. Boulder etc.: Westview press: 129–146.

INGVARSSON, PAR K., (2002): A metapopulation perspective on genetic diversity and differentiation in partially self-fertilizing plants. Evolution (USA) **56**: 2368–2373.

INNES, D. J. and G. G. RINGIUS (1990): Mating system and genetic structure of two populations of white spruce (*Picea glauca*) in eastern Newfoundland. Can. J. Bot. **68**: 1661–1666.

KING, J. N., B. P. DANCIK and N. K. DHIR (1984): Genetic structure and mating system of white spruce (*Picea glauca*) in a seed production area. Can. J. For. Res. **14**: 639–643.

KNOWLES, P., G. R. FURNIER, M. A. ALEKSIUK and D. J. PERRY (1987): Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. **65**: 1087–1091.

KOROCHKIN, L. I., O. L. SEROV and A. I. PUDOVKIN et al. (1977): Genetics of isozymes. Nauka, Moskow. 278 p.

KORSHIKOV, I. I., N. N. MOROZOVA and YA. V. PIRKO (2003): Genetic control of Silver fir isozymes (*Abies alba* Mill.) of the Ukrainian Carpathian Mountains. Cytology and Genetics (Ukraine) **37**: 36–40.

KORSHIKOV, I. I., N. S. TERLYGA and S. A. BYCHKOV (2002a): Populyatsionno-geneticheskiye problemy dendrotechnogennoi introduktsii (na primere sosny krymskoi). "Lebed'", Donetsk. 325 p..

KORSHIKOV, I. I. and N. S. TERLYGA (2000): Genetic variability of *Pinus pallasiana* D. Don. in natural Crimean populations and artificial plantation in Krivbass. Cytology and Genetics (Ukraine). **34**: 21–29.

KORSHIKOV, I. I., T. I. VELIKORIDKO and L. A. BUTILSKAYA (2002b): Genetic structure and variation in *Pinus sylvestris* L. populations degrading due to pollution-induced injury. Silvae Genet. **51**: 45–49.

LEWANDOWSKI, A., J. BURCZYK and L. MEINARTOWICZ (1991): Genetic structure and the mating system in an old stand of Polish larch. Silvae Genet. **40**: 75–79.

MUONA, O., R. YAZDANI and D. RUDIN (1987): Genetic change between life stages in *Pinus sylvestris*: Allozyme variation in seeds and planted seedlings. Ibid. **36**: 39–42.

MUONA, O. and A. HARJU (1989): Effective population sizes, genetic variability and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genet. **38**: 221–228.

NEI, M., (1972): Genetic distance between populations. Amer. Natur. **106**: 283–292.

PERRY, D. J. and B. P. DANCIK (1986): Mating system dynamics of lodgepole pine in Alberta, Canada. Silvae Genet. **35**: 190–195.

PLESSAS, M. E. and S. H. STRAUSS (1986): Allozyme differentiation among populations, stands, and cohorts in Monterey pine. Canad. J. Forest Res. **16**: 1155–1164.

POLITOV, D. V., K. V. KRUTOVSKII and YU. P. ALTUKHOV (1992): Characterization of gene pools of cembrae pines of the set of isozyme loci. Genetika (Moscow) **28**: 93–114.

POLITOV, D. V. and K. V. KRUTOVSKII (2004): Phylogenetics, genogeography and hybridization of five-needle pines in Russia and neighbouring countries. USDA Forest Service Proceedings RMRS-P-032: 85–97.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY (1969): A molecular approach to the study of genetic variation in central, marginal and isolated populations of Drosophila pseudoobscura. Genetics **61**: 841–858.

RUDIN, D., G. ERIKSSON and M. RASMUSON (1977): Inbreeding in a seed tree stand of *Pinus sylvestris* L., in northern Sweden. A study by the aid of the isozyme technique. Department of Forest Genetics, Royal College of Forestry, Garpenberg, Sweden. Res. Note 25.

SHEA, K. L. (1987): Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. Evolution **41**: 124–136.

SHEA, K. L. (1990): Genetic variation between and within populations of Engelmann spruce and subalpine fir. Genome **33**: 1–8.

SHAW, D. V. and R. V. ALLARD (1982): Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. Silva Fenn. **16**: 115–121.

SHIGAPOV, Z. KH., A. SH. TIMER'YANOV, YU. A. YANBAEV and A. I. SHIGAPOVA (1996): Dynamics of genetic structure in generations on a seed plantation and in a natural population of Scotch pine *Pinus sylvestris* L. Genetika (Moscow) **32**: 1363–1370.

SWOFFORD, D. L. and R. B. SELANDER (1981): BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**: 281–283.

TIGERSTEDT, P. M. A., D. RUDIN, T. NIEMELA and J. TAMMISOLA (1982): Competition and neighbouring effect in a naturally regeneration populations of Scots pine. Silva Fenn. **16**: 122–129.

TUNDA, S. N. and I. I. KORSHIKOV (1999): Genotipicheskiye osobennosti plyusovykh dereviev *Pinus sylvestris* L. var. *cretaceae* Kalenicz. ex Kom. Introduktsiya i akklimatizatsiya rastenii. **32**: 176–197.

YAZDANI, R., O. MUONA, D. RUDIN and A. SZMIDT (1985): Genetic structure of *Pinus sylvestris* L. Seed-tree stand and naturally regenerated understory. Forest Sci. **31**: 430–436.

DOI:10.1515/sg-2007-0001
edited by Thünen Institute of Forest Genetics