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Allozyme variation in populations, full-sib families and selfed lines in *Betula pendula* Roth

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Abstract Changes in genetic variability in populations (stand origins), full-sib (FS) families and three generations of selfed lines of *Betula pendula* were observed based on 15 allozyme loci. Growth vigour, measured as stem volume, and its relationship with heterozygosity was studied to determine the effect of inbreeding. Pooled FS families showed a higher percentage of polymorphic loci (P) and allelic numbers per locus (A) than those of natural populations, but no difference in heterozygosity. There was no difference in allozyme variability between fast- and slow-growing family groups, and heterozygosity was not correlated with stem volume among FS families. Allozyme variability was significantly decreased in advancing generations of selfing, and the further the selfing generation, the lower the heterozygosity and the slower the growth. Observed heterozygosity after advancing generations of inbreeding was increasingly higher than expected, indicating overdominance effects or, alternatively, selection against deleterious homozygotes.

Key words *Betula pendula* · Allozyme variability · Stem volume · Population · Inbreeding

Introduction

Different levels of breeding materials in forest trees have been deployed in advancing order of refinement; seed production stands, plus trees, breeding populations, half- and full-sib families, and ultimately selfed lines and hybrid families between selfed lines as in *Betula pendula* Roth (Viherä-Aarnio 1991). With the goal of many breeding programmes being selection for higher yields, the genetic composition of the breeding materials is of great interest to the breeder. Significant differences in stem volume growth were noted among families and between the means of full- and half-sib families and stand origin controls in progeny tests in southern Finland (Raulo and Koski 1977; Hagqvist unpublished data). However, a basic knowledge of the genetic structure, for instance allozyme variation, changes in gene frequencies and genetic variability, in such breeding materials and the relationship between heterozygosity and growth vigour remain unexplored in this species.

Allozyme variability in species and within and among populations has been extensively studied in forest trees, especially in conifers (Hamrick and Godt 1990; Hamrick et al. 1992; Muona 1990). However, few studies have been made on the changes in genetic variability in advancing levels of breeding materials. Comparisons of allozyme variation among selected and randomly chosen populations of several conifer species have not shown substantial differences in genetic structure (Knowles 1985; Cheliak et al. 1988). However, a higher proportion of heterozygous genotypes after mass selection in the seedling stage in *Picea engelmannii* was found by Mitton and Jeffers (1989). Also, clear differences in the average degree of heterozygosity and the distribution of individual heterozygosity between random tree samples and selected orchard clones from the same *Picea abies* populations was reported by Bergmann and Ruetz (1991).

There have been several attempts to relate allozyme heterozygosity with quantitative characters in tree species (Mitton 1983; Mitton and Grant 1984; Bush and Smouse 1992). The results have given a complex picture. The correlation between allozyme heterozygosity and growth was found to be positive in *Populus tremuloides* clones (Mitton and Grant 1980) and *Pinus rigida* (Bush et al. 1987), but no relationship was observed in ponderosa pine (Knowles and Grant 1981) and *Pinus contorta* (Knowles and Mitton 1980). A relationship between heterozygosity and the stability of annual trunk growth in *Pinus attenuata* was noted by Strauss (1987).

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The objective of the study described here was to observe the allozyme variability in populations, full-sib families and three generations of selfed lines, and to relate changes in heterozygosity to growth vigour in fast- and slow-growing families and three generations of selfed lines in *B. pendula*.

Materials and methods

Plant materials

Samples were collected from three progeny tests in southern Finland: (1) test 816/1, planted in 1982, lat. 60°57′ N, long. 24°32′ E and alt. 85 m; (2) tests 602/1 and 950/1, planted in 1975 and 1983, lat. 60° 30′ N, long 24° 32′ E and alt. 100 m. A completely randomised block design with four to six blocks was used in all trials.

The materials used comprised three populations (natural stand origins), ten full-sib (FS) families and eight selfed lines from three generations of selfing (S_1, S_2 and S_3), all of southern Finnish origin. The three natural stand origins used as reference in this study were collected from two of the trials (816/1 and 950/1), where they severed as control entries (Table 2).

The ten FS families were chosen to represent five fast- and four slow-growing families, with one standard FS family being used as a control in progeny test series 950/1. Four fast- and three slow-growing families were chosen from trial 816/1. One fast- and one slow-growing family were chosen from another full- and half-sib progeny test (trial no. 602/1). The fast-growing family chosen from trial 602/1 is one of the highest yielding FS family selected in the silver birch breeding programme; its stem volume was 89% higher than that of the stand origin and 34% higher than that of all 20 selected FS families from southern Finland (unpublished data from Hagqvist 1991). Eight selfed lines were chosen from trial 950/1 (Table 2). The sample comprised three S_1, three S_2 and two S_3 lines.

Ten individuals from each FS family and each stand origin and eight to ten individuals from each selfed line were mechanically sampled. Inflated buds from each individual were separately collected and stored at −20 °C at the beginning of May 1994. Stem volume of the families and selfed lines were measured in the autumn of the same year.

Isozyme electrophoresis and detection

Since there was no procedure available for isozyme analysis in *B. pendula*, 31 isozyme systems were tested with seven extraction buffers and 19 electrode and gel buffer systems adopted from Soltis et al. (1983), Cheliak and Pitel (1984), Kephart (1990) and Pasteur et al. (1988). Inflated buds and young leaves as plant materials were also compared. Finally, the procedures were optimised as follows.

The frozen buds were homogenised in TRIS-HCl (pH 7.5) (Mattila et al. 1994) extraction buffer on ice. Three buffer systems were used to separate 10 isozyme systems (Table 1) in 12% starch gel electrophoresis. The 10 enzyme systems included diaphorase (DIA, EC 1.6.4.3), phosphoglucomutase (PGM, EC 2.7.5.1), shikimate dehydrogenase (SDH, EC 1.1.1.25), leucine-amino peptidase (LAP, EC 3.4.11.1), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), isocitrate dehygrogenase (IDH, EC 1.1.1.42), alcohol dehydgrogenase (ADH, EC 1.1.1.1), phosphoglucose isomerase (PG1, EC 5.3.1.9), esterase (EST, EC 3.1.1.1) and esterase (fluorescent)(FEST,EC 3.1.1.1).

Gels were kept cool by a RM6 LAUDA cooling system at 7°–9° C during the run. The applied current was about 55 m*A* per gel. The staining procedures were adopted from several sources (Cheliak and Pitel 1984; Pasteur, et al. 1988; Kephart 1990). Fifteen loci were scored: *6Pgd-1*, *6Pgd-2*, *Adh-1*, *Adh-2*, *Dia-2*, *Est-1*, *Est-2*, *Fest-3*, *Idh-1*, *Idh-2*, *Lap-1*, *Pgi-2*, *Pgm-2*, *Sdh*.

Data analysis

Four genetic parameters – percentage of polymorphic loci (P) (with 0.95 crition), mean number of alleles per loci (A), observed (H_o) and expected heterozygosity (H_e) – were calculated using the BIOSYS-1.7 computer programme (Swofford 1989). The H_e was an unbiased estimate because of the small samples (Nei 1978). The H_e values of the selfed lines were adjusted by subtracting the corresponding reduction in heterozygosity given by the inbreeding coefficient (F) (Hedrick 1985). At the population level, the parameters were calculated as a average over populations (Pop) to represent the population variability. For FS families, variability was calculated on the basis of both pooled FS families (P-FS), to represent the nature of the seed used in practical forestry, and on the averages over all FS families (*FS*), to indicate within-family variation. At the three levels of selfing (S_1, S_2 and S_3), the average variability over the selfed lines of each generation ($\bar{S}_1$, $\bar{S}_2$ and $\bar{S}_3$) was calculated.

The analysis of the relationship between heterozygosity and vigour among fast- and slow-growing FS families was only based on those families from trial 816/1, whereas the analysis of the same relationship among FS families and selfed lines from different generations of selfing was based on the materials from trial 950/1.

Results

Of the 15 loci examined, 3 loci (*Adh-1*, *Idh-2*, and *6Pgd-1*) were monomorphic in all of the individuals investigated. Most of the loci examined had three alleles per locus. There was one most frequent allele at each locus examined.

Table 1 Buffer systems for isozymes analysed

Buffer system	Usage	Composition	pH	Isozyme
TC 7.1	Electrode	0.135 *M* TRIS, 0.043 *M* citric acid	pH 7.1	DIA, PGM, SDH LAP
	Gel	1:14	pH 7.1	
TC 7.8	Electrode	0.135 *M* TRIS, 0.039 *M* citric acid	pH 7.8	6PGD, IDH, ADH
	Gel	1:14	pH 7.8	
Ashton 8.1	Electrode (AI)	0.5 *M* LiOH 0.277 *M* boric acid	pH 8.1	PG1, EST, FEST
	Gel	1 AI:1.14 AII AII: 0.256 *M* TRIS 0.048 *M* citric acid	pH 8.1	

Differences in variability between populations and FS families

The mean percentage of polymorphic loci (P) of the $\overline{Pop}$ was 55.5%, with a range from 53.3% to 60.0%. The P value of the P-FS was 64.3% (Table 2), which was about 10% higher than the $\overline{Pop}$ but not significantly different (Fig. 1). The mean number of alleles per locus (A) was 1.7 for the $\overline{Pop}$ and 2.5 for P-FS, with the difference being significant ($P < 0.05$, Fig. 2). The mean proportion of observed heterozygous individuals (H_o) of the $\overline{Pop}$ was 0.176, with a range from 0.148 to 0.200, and the mean expected heterozygosity (H_e) of the $\overline{Pop}$ was 0.170, with a range from 0.152 to 0.180. The H_o and H_e of the P-FS were 0.159 and 0.172, respectively, which did not significantly differ from that the $\overline{Pop}$ (Fig. 3).

The mean P value of $\overline{FS}$ was 44.6%, with a range from 33.3% to 60.0%; there was one exception of 13.3% for one fast-growing FS family (No. 40), which was 11% lower than that of the $\overline{Pop}$, but the difference was not significant (Fig. 1). The mean A value of $\overline{FS}$ was 1.5, with a range of 1.2–1.6, which was significantly lower than that of the $\overline{Pop}$ ($P < 0.05$, Fig. 2). The mean H_o and H_e of the $\overline{FS}$ were 0.166 and 0.144, with a range from 0.127 to 0.267 and from 0.113 to 0.215, respectively; there was one exception, family 40, with $H_o = 0.67$ and $H_e = 0.62$. The values of H_o and H_e of the $\overline{FS}$ were lower than those of the $\overline{Pop}$; however, the ranges of the variation in both H_o and H_e of the $\overline{FS}$ were wider than those of the $\overline{Pop}$ (Table 2 and Fig. 3).

Differences in variability between fast- and slow-growing FS families

Among the FS families, the mean P value of the slow-growing family group (48.3%) was slightly higher than that of the fast-growing family group (44.0%), but the difference was not significant (Table 2). This was due to the exceptionally low value of family 40 as mentioned above. Otherwise, the mean P values of the two groups were about the same. The situation was about the same for both H_o and H_e of the two groups. Thus, no difference was found between the two groups in the observed criteria of genetic variability.

Table 2 Materials chosen from three progeny tests for allozyme analysis and summary of allozyme variability of populations, FS families and selfed lines

Category	Group	Trial no.	Entry no.	Polymorphic loci (%)	Mean allelic no. per locus	Heterozygosity	
						Direct count H_o	Hdywbg exp. H_e
Population	Stand	816/1	2	60.0	1.7 (0.2)[a]	0.148 (0.043)	0.152 (0.041)
		950/1	1	53.3	1.7 (0.2)	0.181 (0.072)	0.180 (0.053)
		950/1	2	53.3	1.7 (0.2)	0.200 (0.058)	0.178 (0.051)
	$\overline{Pop}$		*Mean*	*55.5*	*1.7*	*0.176*	*0.170*
FS family	P-FS			64.3	2.5 (0.2)	0.159 (0.032)	0.172 (0.034)
	Fast	816/1	11	46.7	1.5 (0.1)	0.213 (0.062)	0.190 (0.055)
			18	60.0	1.6 (0.1)	0.187 (0.053)	0.168 (0.047)
			35	46.7	1.5 (0.1)	0.150 (0.067)	0.113 (0.040)
			40	13.3	1.2 (0.1)	0.167 (0.045)	0.062 (0.043)
		602/1	7	53.3	1.5 (0.1)	0.153 (0.054)	0.134 (0.044)
			Mean	*44.0*	*1.5*	*0.154*	*0.133*
	Slow	816/1	20	53.3	1.5 (0.1)	0.267 (0.081)	0.215 (0.058)
			31	53.3	1.5 (0.1)	0.200 (0.063)	0.184 (0.051)
			49	46.7	1.6 (0.2)	0.160 (0.065)	0.148 (0.051)
		602/1	9	40.0	1.4 (0.1)	0.127 (0.060)	0.120 (0.049)
			Mean	*48.3*	*1.5*	*0.189*	*0.167*
	Control	950/1	3	33.3	1.3 (0.1)	0.133 (0.059)	0.111 (0.048)
	$\overline{FS}$		*Weighted mean*	*44.6*	*1.5*	*0.166*	*0.144*
Generation of selfing	S_1	950/1	7	20.0	1.2 (0.1)	0.050 (0.027)	0.025
			8	40.0	1.4 (0.1)	0.103 (0.045)	0.053
			9	46.7	1.5 (0.1)	0.107 (0.047)	0.077
	$\overline{S_1}$		*Mean*	*35.3*	*1.4*	*0.087*	*0.052*
	S_2		39	26.7	1.3 (0.1)	0.060 (0.040)	0.020
			41	40.0	1.4 (0.1)	0.107 (0.041)	0.035
			48	26.7	1.3 (0.2)	0.030 (0.016)	0.025
	$\overline{S_2}$		*Mean*	*30.0*	*1.3*	*0.064*	*0.026*
	S_3		66	6.7	1.1 (0.1)	0.059 (0.059)	0.004
			67	6.7	1.1 (0.1)	0.027 (0.027)	0.003
	$\overline{S_3}$		*Mean*	*6.7*	*1.1*	*0.043*	*0.004*

[a] The numbers in brackets are standard errors

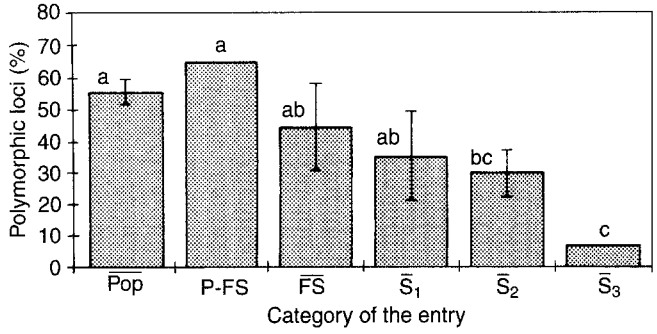


Fig. 1 Percentages of polymorphic loci (P) averaged over populations (Pop), for pooled FS families (*P-FS*), averaged over FS families (*FS*) and over the three generations of selfing (S_1, S_2 and S_3). *Error bars* indicate standard deviations from the means. Data with the *same letter* are not significantly different at the 0.05 level

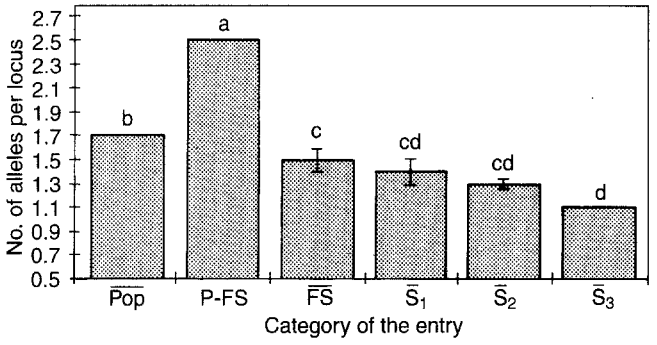


Fig. 2 Allelic number per locus (A) averaged over populations (Pop), for pooled FS families (*P-FS*), averaged over FS families (*FS*) and over the three generations of selfing (S_1, S_2 and S_3). *Error bars* indicate standard deviations from the means. Data with the *same letter* are not significantly different at the 0.05 level

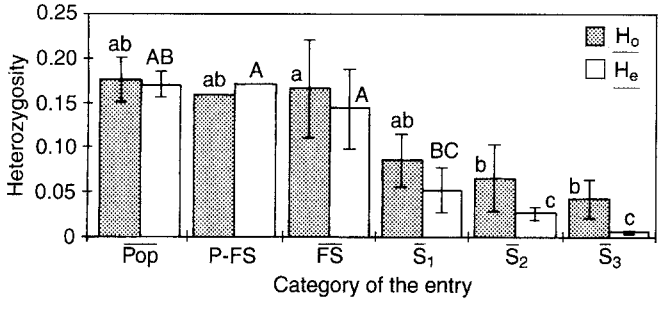


Fig. 3 Heterozygosity including H_o and H_e averaged over populations (*Pop*), for pooled *FS* families (*P-FS*), averaged over FS families (*FS*) and over three generations of selfing (S_1, S_2 and S_3). *Error bars* indicate standard deviations from the means. Data with the *same letter* are not significantly different at the 0.05 level

Differences in variability among the FS family and different generations of selfing

For the three generations of selfing, the mean P values of the S_1, S_2 and S_3 were 35.3%, 30.0% and 6.7% respectively, a decrease with advancing generations of selfing (Table 2 and Fig. 1). There was no significant difference in mean P value between S_1 and FS. However, the difference between the S_2 or S_3 and FS was significant ($P < 0.05$, Fig.1), and the overall trend was obvious.

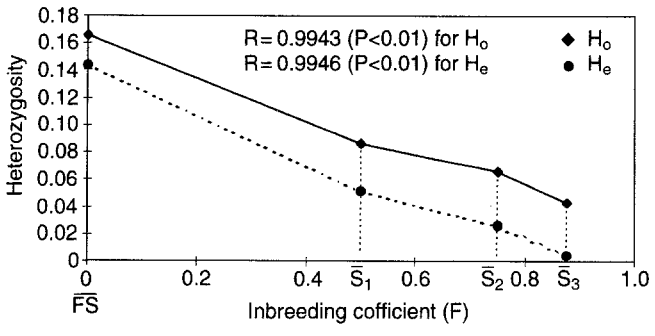
The mean allelic number per locus (A) was 1.4, 1.3 and 1.1 for the S_1, S_2 and S_3 respectively, or 93.3%, 86.7% and 73.3% of A of the FS. The number of alleles of the S_3 was significantly lower than that of the FS (Fig. 2); here again, the trend was obvious.

The mean H_o was 0.087, 0.064 and 0.043 for the S_1, S_2 and S_3, respectively, indicating a steady decrease with advancing generations of selfing, these were 52.4%, 38.6% and 25.9% of the mean H_o of the FS, respectively (Fig. 3). Similarly, the means of H_e of the three generations of selfing were 0.052, 0.026 and 0.004, respectively, which were 36.1%, 18.1% and 2.8% of the FS, respectively. Both H_o and H_e showed significantly negative correlations with the inbreeding coefficients (F) of the materials (Fig. 4). The F value of the FS was assumed to be zero, and thus, F = 0.5, 0.75 and 0.875 for the advancing generations of selfing. Observed heterozygosity (H_o) showed significantly ($P < 0.0001$) higher values than H_e, indicating a higher probability of survival for heterozygous individuals. The difference between H_o and H_e increased with advancing generations of selfing (Fig. 4).

Correlation between heterozygosity and vigour

The correlation between the observed allozyme heterozygosity (H_o) and stem volume (vigour) among fast- and slow-growing families was not significant (Fig. 5). The second-best family had the lowest heterozygosity, whereas one of the slow-growing families had the highest heterozygosity. However, the correlation between H_o and stem volume among a reference FS family and selfed lines (S_1, S_2 and S_3) from the three generations of selfing was positively significant (R = 0.7909, $P < 0.05$) (Fig. 6). In this trial the FS family had the largest volume and the highest heterozygosity. The selfed lines had slow growth

Fig. 4 Correlations between H_o or H_e and inbreeding coefficients (F) among the average of FS families (FS) and averages of the three generations of selfing (S_1, S_2 and S_3)



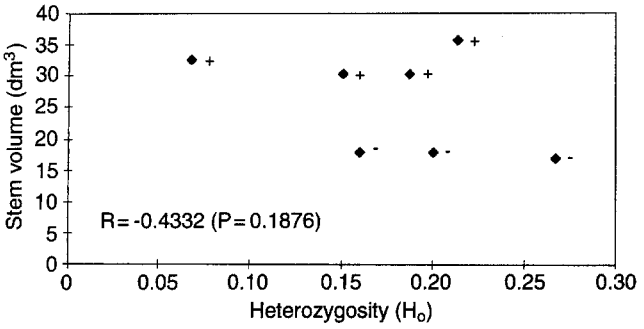


Fig. 5 Relationship between observed heterozygosity (H_o) and stem volume among the fast- and slow-growing families. + = fast, − = slow

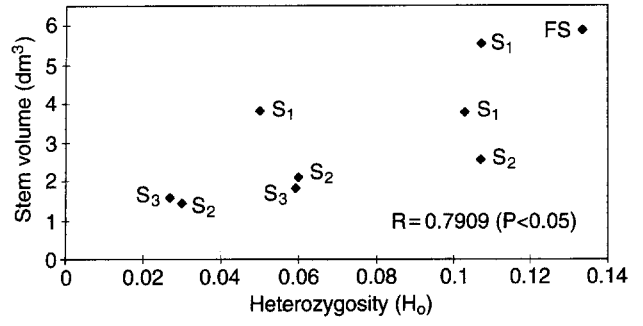


Fig. 6 Relationship between observed heterozygosity and stem volume among a FS family (*FS*) and selfed lines S_1, S_2 and S_3, advancing generation of selfing

and low heterozygosity. Generally, the selfed lines behave in an expected way: the further the selfing generation, the lower the heterozygosity and the slower the growth.

Discussion

The mean percentage of polymorphic loci within populations in *B. pendula* was 55.5%, which was 10% higher than the average found in angiosperm woody plants (45.1%) but consistent with outcrossing wild-pollinated woody plants (53.0%) (reviewed by Hamrick et al. 1992). The number of alleles per locus (1.7) was consistent with the average observed for angiosperm woodly plants (1.68) and slightly lower than that of outcrossing wind-pollinated woodly plants (1.84). However, the expected heterozygosity ($H_e = 0.170$) in this study was higher than the averages of both angiosperm ($H_e = 0.143$) and outcrossing wind-pollinated woody plants ($H_e = 0.154$), but lower than that of other European angiosperm tree species (Müller-Starck et al. 1992).

Seed collected from orchards is usually a mixture of many cross combinations. Thus, the comparison between the pooled FS families and stand origins (populations) for genetic variability parameters may closely reflect the real situation in tree breeding practice. The results of the present study showed a higher percentage of polymorphic loci and a higher allelic number per locus in pooled FS families than in natural populations, but no difference in heterozygosity was observed between the two groups. A higher H_o can be expected if random mating occurs among the pooled families. Nevertheless, the present results indicate that genetic variability of the pooled FS families was at least not reduced through breeding activities. Even the results of pooled stands (populations) ($P = 60.0\%$, $A = 1.9$, $H_o = 0.175$ and $H_e = 0.172$) did not show a higher level of allozyme variability than what was observed in the pooled FS families. However, caution must be exercised with respect to the interpretation of the present results, since the sample size of the stands was small. Therefore, the means of the three stands was used in the comparison. The results from this study support the conclusion proposed by Cheliak, et al. (1988) that there were no dramatic reoganisations of the genetic variation in the pooled selected sample that was used for breeding relative to the random population. Similar observations have been reported by Ellstrand and Marshall (1985) for domesticated populations of radish (*Raphanus sativus* L.) and by Knowles (1985) for comparisons between the clonal seed orchard and natural mature stands of *Picea mariana*. Thus, at the level of overall genetic structure, the phenotypically selected population accurately reflects the random population from which it was sampled (Cheliak et al. 1988). However, contradictory evidence also exists. Mitton and Jeffers (1987) found a higher heterozygosity in superseedlings than in control seedlings of *Picea engelmannii*, and Bergmann and Ruetz (1991) reported a higher average heterozygosity in a clone collection of selected plus trees than in both single stand and pooled stands of *Picea abies*.

A comparison between intra-population and intra-family variability, on the basis of the proportion of polymorphic loci (P), number of alleles per locus (A) and heterozygosity (H_o and H_e), showed no significant difference except for A values, indicating a reduced mean allelic number per locus in FS family average ($\overline{FS}$) with the same level of polymorphic loci and heterozygosity. However, the differences in heterozygosity among families were large.

Comparisons between different generations of selfing and $\overline{FS}$ revealed a significant decrease in genetic variability, especially a significantly linear decrease in heterozygosity with an increase of inbreeding coefficients. Observed heterozygosity (H_o) was always higher than H_e in this study (except in P-FS, which was not actually a true case), indicating that heterozygotes have better survival. The growing devaition of H_o from H_e with increasing inbreeding indicating a stronger overdominant effect with advancing generations of selfing or, alternatively, a stronger selection against deleterious homozygotes.

The observed heterozygosity (H_o) of the S_1 line average ($\overline{S_1}$) was about half that of the FS family, while H_e of S_1 was much less than this proportion, i.e. only 36.1%. Similar situations occurred in the subsequent generations of selfing. The low H_e value can probably be explained as the extinction of rare alleles by eliminating deleterious homozygotes due to inbreeding, thereby resulting in changes in gene frequency and a decrease of genetic diversity or H_e. Reduced allelic number per locus and the percentage of polymorphic loci in selfed lines support this explanation, and we have also found that the natural selection on selfed materials of silver birch is very strong (Wang et al. 1995).

Results of the present study also demonstrate that there is no significant difference in genetic variability, including heterozygosity, between the fast- and slow-growing family groups. Consequently, no correlation was found between observed heterozygosity and stem volume growth of the FS families. However, a positive correlation between H_o and stem volume was revealed based on the material including the three generations of selfing and a reference FS family. Similar results were reported in *Pinus attenuata* by Strauss (1986). In this study there was no relationship between heterozygosity and growth if a few individuals that showed unusually slow growth and low heterozygosity were excluded from the crossbreds, but the correlation was positively significant within the inbreds. Studies on a correlation between allozyme heterozygosity and vigour have given contradictory results ranging from positive to negative (Mitton and Grant 1980; Knowles and Grant 1981; Mitton 1983; Mitton and Grant 1984; Govindaraju and Dancik 1986; Strauss 1986 and 1987; Bush et al. 1987; Bush and Smouse 1992). No generalisation can be made. The present results suggest that among crossbred families, allozyme heterozygosity does not play an important role as an indicator of vigour. However, if severe inbreeding occurs and the heterozygosity of the material decreases to an abnormal level, then heterozygosity becomes crucial to growth. Whether that is due to the allozymes per se or due to loci directly important in determining growth (quantitative trait loci) is an open question. The present study also contributes to further use of the "inbred line option" in forest tree breeding. Heterosis and inbreeding depression are commonly considered to be mirror-images on the basis of current knowledge gained from *Zea mais* and other agricultural crops. Thus, where inbreeding depression is strong, heterosis can be expected when crossing lines. In the breeding *Betula* species this may open the road to hybrid breeding (Wang et al. 1995).

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