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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 (Viheri-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and half-sib families and stand origin controls in progeny tests in southern Finland (Raulo and Koski 1977; Hagqvist unpublished data).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 con- torta* (Knowles and Mitton 1980). A relationship between heterozygosity and the stability of annual trunk growth in *Pinus attenuata* was noted by Strauss (1987).
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 (S1, S2 and S3) 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 served 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 all 20 selected FS control in progeny test series 950/1. Four fast- and three 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, with one standard FS family being used as a control in progeny test series 950/1.

Data analysis

Four genetic parameters – percentage of polymorphic loci (P) (with 0.95 criterion), mean number of alleles per locus (A), observed (H0) and expected heterozygosis (Hs) – were calculated using the BIOSYS-1.7 computer programme (Swofford 1989). The Hs was an unbiased estimate because of the small samples (Nei 1978). The Hs, values of the selfed lines were adjusted by subtracting the corresponding reduction in heterozygosis given by the inbreeding coefficient (F) (Hedrick 1985). At the population level, the parameters were calculated as an 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 (S1, S2 and S3), the average variability over the selfed lines of each generation (S1, S2 and S3) 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 6Pgdl) 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

<table>
<thead>
<tr>
<th>Buffer system</th>
<th>Usage</th>
<th>Composition</th>
<th>pH</th>
<th>Isozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC 7.1</td>
<td>Electrode</td>
<td>0.135 M Tris, 0.043 M citric acid</td>
<td>pH 7.1</td>
<td>DIA, PGM, SDH</td>
</tr>
<tr>
<td></td>
<td>Gel</td>
<td>1:14</td>
<td>pH 7.1</td>
<td>LAP</td>
</tr>
<tr>
<td>TC 7.8</td>
<td>Electrode</td>
<td>0.135 M Tris, 0.039 M citric acid</td>
<td>pH 7.8</td>
<td>6PGD, IDH, ADH</td>
</tr>
<tr>
<td></td>
<td>Gel</td>
<td>1:14</td>
<td>pH 7.8</td>
<td></td>
</tr>
<tr>
<td>Ashton 8.1</td>
<td>Electrode (AI)</td>
<td>0.5 M LiOH</td>
<td>pH 8.1</td>
<td>PGI, EST, FEST</td>
</tr>
<tr>
<td></td>
<td>Gel</td>
<td>0.277 M boric acid, 0.048 M citric acid</td>
<td>pH 8.1</td>
<td></td>
</tr>
</tbody>
</table>

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-aminopeptidase (LAP, EC 3.4.11.1), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), isocitrate dehydrogenase (IDH, EC 1.1.1.42), alcohol dehydrogenase (ADH, EC 1.1.1.1), phosphoglucone isomerase (PGI, 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 mA 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.

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 Solits 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.