

Allozyme Variation in *Abeliophyllum distichum* NAKAI, an Endemic Tree Species of Korea

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Abstract

Abeliophyllum distichum NAKAI is a geographically restricted tree species, found only in Korea. Genetic variation at 11 isozyme loci was analyzed from 120 individuals. Low level of genetic diversity was observed ($A=1.6$, $P=36.4\%$, $H_e=0.098$), which is a typical pattern shown in endemic rare tree species. Most (94%) of the total genetic diversity was found within populations and estimated gene flow was moderately high ($Nm=2.29$). Ecology and life-history characteristics of *A. distichum* coupled with low level of genetic diversity suggested that there should be a special emphasis on the conservation study for this species.

Key words: *Abeliophyllum distichum*, allozymes, genetic variation, endemic.

FDC: 165.3; 165.5; 176.1 *Abeliophyllum distichum*; (519.5).

Introduction

Abeliophyllum distichum NAKAI is a narrowly distributed rare tree species in the family *Oleaceae*, which is in a monotypic genus found only in Korea. The closest relative of the genus *Abeliophyllum* is probably the genus *Forsythia* as suggested by its common name white forsythia, but they can be easily distinguished by several vegetative and floral characters. *A. distichum* is a sexually and asexually reproducing tree species. Based on field observations, however, *A. distichum* appears to predominantly reproduce asexually. The greatest number of *A. distichum* plants occurs in Gwesan, the middle part of Korea (Songdukri and Yulgiri populations in this study). Several natural populations also have been ascertained since the latter part of this century. The narrow geographic range of *A. distichum* and its small population size confer a vulnerability to extinction. Furthermore, several potential threats to the species have been identified, including water development, recreation, collecting for ornamental trees, and loss of habitat. In order to save this species from such dangers, some populations have already been conserved *in situ* and been under restoration program. It should be noted that in some populations, e.g. Songdukri population in this study, there is a possibility of transplantation from other populations into them during the restoration. On the other hand, the Forest Genetics Research Institute of the Republic of Korea has made a plan of the establishment of an *ex situ* conservation stand and has been trying to collect seeds and/or vegetative materials for reproduction.

Conservation biology, especially in rare plants, has been traditionally influenced mainly by ecology (LANDE, 1988; SCHEMSKE et al., 1994). During the last decade, however,

interest also has focused increasingly on population and evolutionary genetics of plants (FOLK and HOLSINGER, 1991). When population size is extremely small, genetic variation may be lost on account of random events (genetic drift). Furthermore, there is an increased probability of mating among relatives (inbreeding), which could result in reduced population fitness (BARRETT and KOHN, 1991). The spatial distribution of alleles may be another important genetic factor in conservation biology. If polymorphisms are distributed evenly over a species' range, then loss of a particular segment of the population may not necessarily increase the chance of extinction. If, however, populations are genetically structured, loss of a particular group of individuals may result in the loss of potentially important alleles. Consequently, understanding the genetic diversity and the structure of a plant species is very crucial to establishing the strategy for the conservation. In fact, recent theoretical approaches in conservation biology have combined the ecology and the genetics of species (VANE-WRIGHT et al., 1991).

In the present study, we analyzed isozymes to estimate the level and the distribution pattern of genetic variation in the geographically restricted endemic tree, white forsythia, *Abeliophyllum distichum*. This information will contribute to a better understanding of the genetic diversity and structure of the endemic rare tree species and could then be used to develop plans to manage this rare species.

Methods and Materials

A. distichum is generally known to be distributed in 5 to 6 locations in Korea. Young leaves were collected from 4 populations that have been known as the central populations of this species (Fig. 1). In preliminary studies of isozyme analysis, enzyme activity in young leaves showed the best results. More than thirty individuals were sampled at each population.

Leaves were stored in refrigerator at 4°C until they were used for isozyme analysis. Leaves were ground in the phosphate-PVP grinding buffer solution of SOLTIS et al. (1983) with 4% PVP and 1% 2-mercaptoethanol. Proteins were separated in 12% starch gels using the buffer systems of KIM et al. (1993). Eleven isozymes were screened, of which 8 isozyme systems were used to resolve 11 putative loci. The isozyme systems stained were aconitase (ACO, E.C. 4.2.1.3), glutamate dehydrogenase (GDH, E.C. 1.4.1.3), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44), phosphoglucoisomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 2.7.5.1) and shikimate dehydrogenase (SKDH, E.C. 1.1.1.25). Genotypes at all loci were inferred based on the known subunit structures and cellular compartmentalization of the enzyme (WEEDEN and WENDEL, 1989).

Genetic diversity statistics (percent polymorphic loci P ; mean number of alleles per locus A ; gene diversity or expected

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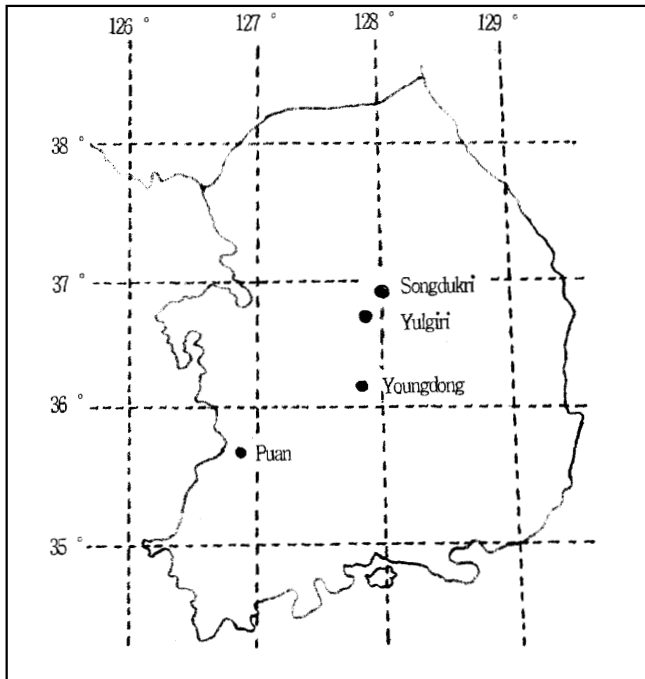


Fig. 1. – Locations of 4 *A. distichum* populations in Korea that were sampled for isozyme analysis.

heterozygosity H_e ; observed heterozygosity H_o , deviations from HARDY-WEINBERG expectations, and NEI's (1978) genetic identities were calculated following HEDRICK (1985) and BERG and HAMRICK (1997) using a computer program BIOSYS-1 developed by SWOFFORD and SELANDER (1989).

WRIGHT's fixation index (F) was calculated for each polymorphic locus in every population (WRIGHT, 1922). Out-crossing rates (t) were estimated using the equation $F_e = (1-t)/(1+t)$, which assumes mating system equilibrium (HEDRICK, 1985). For each population, F_e was estimated by $[(H_e - H_o)/H_e]$, where the calculated values were means over all loci.

The proportion of total gene diversity found among populations (G_{st}) was calculated ($G_{st} = D_{st}/H_t$) by partitioning total gene diversity for each polymorphic locus (H_t) into diversity within populations (H_s) and diversity among populations (D_{st} ; NEI, 1973).

Gene flow was estimated by $Nm = [(1 - G_{st})/4G_{st}] \alpha$ (WRIGHT, 1931; CROW and AOKI, 1984), where Nm is the number of migrants per generation, and $\alpha = [(n-1)/n]^2$ where n is the number of populations.

Results

Approximately 13 isozyme loci were observed from the eight isozyme systems, but only 11 putative loci were consistently interpretable and scorable. Of these, *Idh*, *Mdh-1*, *Mdh-3*, *Pgi-1*, and *Skdh* were monomorphic. Three loci (*Gdh*, *Pgi-2*, and *Pgm-2*) had three alleles, and the remaining loci of *Aco*, *Mdh-2*, and *6Pgdh-1* had two alleles. Allele frequencies at 11 loci for each population are given in table 1.

Estimates of polymorphisms and heterozygosity are given in table 2. Within populations we detected an average of 1.6 alleles per locus, 36.4% of polymorphic loci, 0.077 of observed heterozygosity and 0.098 of expected heterozygosity. Genetic identities between *A. distichum* populations were high, ranging

Table 1. – Allele frequencies at 11 loci for 4 natural populations and χ^2 -tests for deviation from the HARDY-WEINBERG proportions in *A. distichum*.

Locus	Populations			
	Songdukri	Yulgiri	Youngdong	Puan
<i>Acon</i>				
<i>a</i>	.893-	.975-	.808+***	.808+
<i>b</i>	.107	.025	.192	.192
<i>Gdh</i>				
<i>a</i>	.089	.056	.000	.100
<i>b</i>	.911+*	.889-	1.000	.900-
<i>c</i>	.000	.056	.000	.000
<i>Idh</i>				
<i>a</i>	1.000	1.000	1.000	1.000
<i>Mdh-1</i>				
<i>a</i>	1.000	1.000	1.000	1.000
<i>Mdh-2</i>				
<i>a</i>	.125	.075	.019	.000
<i>b</i>	.875+	.925-	.981-	1.000
<i>Mdh-3</i>				
<i>a</i>	1.000	1.000	1.000	1.000
<i>6Pgdh-1</i>				
<i>a</i>	.071	.075	.096	.115
<i>b</i>	.929+***	.925+***	.904-	.885+
<i>Pgi-1</i>				
<i>a</i>	1.000	1.000	1.000	1.000
<i>Pgi-2</i>				
<i>a</i>	.067	.175	.038	.000
<i>b</i>	.767+	.600+	.769-	1.000
<i>c</i>	.167	.225	.192	.000
<i>Pgm-2</i>				
<i>a</i>	.982-	1.000	1.000	.761
<i>b</i>	.018	.000	.000	.065+
<i>c</i>	.000	.000	.000	.174
<i>Skdh</i>				
<i>a</i>	1.000	1.000	1.000	1.000

*, $p < 0.05$; **, $p > 0.01$

The fixation index is given only by a minus sign (heterozygote excess) or by a plus sign (heterozygote deficiency).

from 0.982 to 0.999, with a mean of 0.993 ($SD=0.006$). The relatively low degree of population differentiation was observed (5.8%: see Table 3), which was accompanied by the moderately high estimate of gene flow ($Nm=2.29$).

Four significant deviations from HARDY-WEINBERG expectations were found from the 19 tests (Table 1). These represented deviations at 3 loci scattered over 3 populations. All the significant deviations were positive, indicating a deficit of heterozygotes. The mean outcrossing rate (t) was 0.656 ($SD=0.127$), ranging from 0.541 to 0.837 (Table 2).

Discussion

Several recent reviews of the literature on plant allozymes indicate that endemic and narrowly distributed plant species tend to maintain lower levels of genetic variation than more widespread species (HAMRICK and GODT, 1989; HAMRICK et al., 1992). In the extreme cases, some rare and endangered plant species showed no polymorphism at loci encoding isozymes (WALLER et al., 1987; LESICA et al., 1988; SOLTIES et al., 1992). *A. distichum* maintained similar genetic variation to other endemic plants as well as endemic woody plants. For instance, the mean genetic diversity values for 100 endemic plant species were $P=26.3$ and $H_e=0.063$ (HAMRICK and GODT, 1989), whereas those for *A. distichum* were $P=36.4$ and $H_e=0.098$. On the other hand, those for 26 endemic woody plants were $P=26.3$ and $H_e=0.056$ (HAMRICK et al., 1992). Because *Abelio-*

Table 2. – Estimates of genetic diversity within 4 populations of *A. distichum* (standard errors in parentheses).

Population	<i>A</i>	<i>P</i>	<i>H_o</i>	<i>H_e</i>	<i>t</i>
Songdukri	1,6 (0,2)	45,5	0,073 (0,026)	0,104 (0,039)	0,541
Yulgiri	1,6 (0,2)	36,4	0,079 (0,039)	0,102 (0,052)	0,633
Youngdong	1,5 (0,2)	27,3	0,063 (0,043)	0,083 (0,043)	0,612
Puan	1,5 (0,2)	36,4	0,092 (0,041)	0,101 (0,045)	0,837
Mean	1,6	36,4	0,077	0,098	0,656

A = the mean number of alleles per locus; *P* = the percent polymorphic loci (A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95); *H_o* = observed heterozygosity; *H_e* = the heterozygosity expected under HARDY-WEINBERG; *t* = outcrossing rate estimated from population structure data.

Table 3. – Nei's (1973) gene diversity estimated within and among populations at 6 polymorphic loci in *A. distichum*.

Locus	<i>H_t</i>	<i>H_s</i>	<i>D_{st}</i>	<i>G_{st}</i>
<i>Aco</i>	0,225	0,215	0,010	0,043
<i>Gdh</i>	0,140	0,136	0,004	0,029
<i>Mdh-2</i>	0,104	0,100	0,005	0,046
<i>6Pgdh-1</i>	0,163	0,162	0,001	0,004
<i>Pgi-2</i>	0,359	0,327	0,032	0,089
<i>Pgm-2</i>	0,122	0,105	0,017	0,136
Mean	0,185	0,174	0,011	0,058

phyllum is in a monotypic genus, comparisons of its genetic diversity with either more widespread or less-threatened congeneric species were precluded.

The low level of allozyme variation in *A. distichum* could be explained by several scenarios. Two possibilities might be responsible for the species' evolution. One is the fact that the progenitor(s) of *A. distichum* might contain limited genetic variation, or that very little genetic variation might have been acquired during speciation (e.g., COLE and BIESBOER, 1992). Alternatively, the species might have gone through severe bottlenecks later in its evolutionary history (e.g., ZABINSKI, 1992). Unfortunately, without historical information and detailed phylogenetic inference, the relative likelihood of these scenarios cannot be distinguished. Indeed, both processes might have made some contributions to the low level of genetic diversity observed in this study. Information on the level of genetic diversity in the species of the genus which are assumed to be the closest relatives of *Abeliophyllum* (i.e., *Fraxinus* and *Forsythia*) and the phylogenetic relationships of *A. distichum* with those species may provide some clues for a better understanding of the evolutionary history of *A. distichum*.

The historical range and past population numbers of *A. distichum* are unclear. In contrast to many endangered plant species, whose ranges have been dramatically reduced and whose populations have declined in size and number, the discovery of some populations of *A. distichum* late in this century suggests that it has always been a local endemic. Thus,

it seems unlikely that recent reduction in range or population size is sole cause of the low level of allozyme diversity in this species.

Estimates of outcrossing rates for four populations of *A. distichum* are not high (mean *t*=0.656). This result suggests that some proportion of the seeds may result from selfing or biparental inbreeding. Thus, the small amount of genetic variation found within *A. distichum* may be attributed in part to its mating system; the small amount of genetic variation within populations is associated particularly with selfing species and also with animal-pollinated mixed-mating species (HAMRICK and GODT, 1989). For instance, mean genetic diversity values (*H_e*) are 0.074 for selfers and 0.090 for animal-pollinated mixed-mating species (HAMRICK and GODT, 1989), both slightly lower than that found for *A. distichum*. But comparisons based on indirect estimates of outcrossing should be viewed with some caution because population substructuring will produce a positive inbreeding coefficient. Such substructure is commonly found in clonally reproducing species such as *A. distichum* (BERG and HAMRICK, 1994). In addition, in clonal plants, there is not necessarily a correlation between population size, in terms of ramet numbers, and genetic variation. Regardless of ramet numbers, populations of clonal plants consisting of few genets are subject to similar evolutionary processes of genetic drift and inbreeding (ELLSTRAND and ELAM, 1993). Such processes have potentially negative consequences: random genetic drift may lead to loss of genetic variability in the

absence of gene flow and inbreeding may cause inbreeding depression. If a clonal species is self-incompatible, populations with few genets may face reduced seed set because of mate scarcity (BYERS, 1995). The spatial arrangement of genets, irrespective of genet numbers, may also have important effects on a species' sexual reproduction and mating system. For example, animal pollinators most often move pollens between near neighbors (HEINRICH, 1975). If a population consists of large clones with little or no interdigitation, pollinators may seldom transfer pollens between genets (HANDEL, 1985). If the species also has a genetic incompatibility system, sexual reproduction may be severely limited. Consequently, a clonal plant may have low level of genetic diversity and/or population substructures to an extent. In order to provide evidences for the issues above mentioned, future investigation should be focused on the mating system, the clonal structure and patterns of allozyme diversity of *A. distichum* in a population.

The degree of genetic differentiation among populations of *A. distichum* was much lower ($G_{st}=0.058$) than those reported for other plant taxa which were reviewed by HAMRICK and GODT (1989) and HAMRICK et al. (1992). For example, mean G_{st} estimated from 52 plant species of endemics was 0.27 (mean number of populations per taxon examined=11.9), and that of 18 endemic tree species was 0.14 (mean number of populations examined=8.5). Our estimate of Nm (the number of migrants per generation: 2.29) would explain the current amount of genetic differentiation among populations of *A. distichum*. Although the pollination biology of the species has not been documented, based on its floral morphology and field observations, it appears that pollination is predominantly insect-mediated. As a consequence, the low level of genetic differentiation and the relatively high value of Nm were somewhat surprising, since most of the populations we studied here might be distant enough from each other to make gene flow by pollen or seed dispersal highly unlikely. Only the two populations (Songdukri and Yulgiri) located in Gwesang-gun of Choongbuk province may be in close proximity to allow even low levels of gene flow. The low level of genetic differentiation in *A. distichum* populations may be attributed in part to the artificial gene flow via seeds and/or seedlings. Actually, as described in introduction, some individual trees of populations such as Songdukri were supposed to be transplanted from other populations during the restoration program performed in the 1980s. In addition, the polymorphic loci investigated here might be inappropriate to detect population differentiation. In all polymorphic loci, the frequency of a single common allele was predominantly high in all populations, while the frequencies of remaining alleles were very low (i.e., all the polymorphic loci showed minor polymorphisms) and only two population-specific alleles were investigated at two loci, *Gdh* and *Pgm-2* (refer to Table 1). These distribution patterns of alleles might contribute to making the G_{st} values lower.

A. distichum is a prime candidate for the conservation studies, because it is a monotypic genus. Such taxonomic distinctness has been suggested as an important priority for conservation efforts (HOLSINGER and GOTTLIEB, 1991). Particularly, the deficiency of genetic variation within populations of *A. distichum* observed in this study may preclude adaptation of this species to environmental changes and thus should increase the chance of extinction of this species, although some tree species may have survived from severe population bottlenecks accompanying loss of genetic diversity with few ill effects (SIMON et al., 1986; MOSSELER et al., 1992; ZABINSKI, 1992). Genetic hazards are implicated in the species extinction process at two levels. At the individual level, genetic homozygosity caused by inbreeding could result in a depression of fitness

because deleterious recessive alleles are likely to be exposed (BARRETT and KOHN, 1991). At the population level, loss of alleles could reduce long-term adaptive flexibility under changing environmental circumstances (ALLENDORF, 1986; LANDE and BARROWCLOUGH, 1987). Both short- and long-term changes would reduce the fitness of populations and result in a lowered census size and genetically effective size. These events could increase the likelihood of extinction even in the absence of further external threats to the population. When taken these into consideration, it is an urgent task to establish the proper strategy for the conservation of genetic variation of *A. distichum*. In particular, *ex situ* conservation is of particular importance for this monotypic genus because of its small numbers and limited distribution. Nonetheless, experimental manipulations designed to assess ecological conditions, which is critical for *in situ* persistence, should not be neglected.

Some authors, however, have questioned the utility of genetic studies for conservation purposes. SCHEMSKE et al. (1994) pointed out that parameters such as genetic diversity often are given undue priority in conservation programs. They also suggested that genetic studies are useful for the conservation of rare plants only when there are clearly defined reasons why the study will aid in the implementation of successful management programs. SCHEMSKE et al. (1994) demonstrated the case that demographic studies could provide the most useful information for conservation. Whatever the general merits of their arguments, it is difficult to apply them to clonal and/or partially clonal plants such as *A. distichum*. For the clonal plants, preparation of the detailed demographic data without analyses of the genetic structure of populations may be impractical. For instance, we cannot determine whether a population of ramets consists of single or multiple genets. Thus, for the clonal species, genetic studies should be prerequisite to demographic studies. However, from a management point of view, we should not overlook the importance of basic demographic data such as pollinator requirements for seed-set, numbers of flowering and non-flowering individuals, fruit and seed set, and morph frequencies at the different sites. Any recovery plan for a species requires knowledge of such data to figure out whether a species is already recovering, is declining, or appears to be stable (SCHEMSKE et al., 1994). For *A. distichum*, these basic studies are still lacking. As a consequence, in order to develop more efficient strategy for the conservation of *A. distichum*, we should make an effort to accumulate more data on demographic and/or ecological characteristics.

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Literature

- ALLENDORF, F. W.: Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181–190 (1986). — BARRETT, S. C. H. and KOHN, J. R.: Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: D. A. FALK and K. E. HOLSINGER (editors). *Genetics and Conservation of Rare Plants*. Oxford University Press, New York. pp. 3–30 (1991). — BERG, E. E. and HAMRICK, J. L.: Spatial and genetic structure of two sandhills oaks: *Quercus laevis* WALTER and *Quercus margaretta* (Fabaceae). *American Journal of Botany* **81**, 7–14 (1994). — BERG, E. E. and HAMRICK, J. L.: Quantification of genetic diversity at allozyme loci. *Can. J. For. Res.* **27**, 415–424 (1997). — BYERS, D. L.: Pollen quantity and quality as explanations for low seed set in small populations exemplified by *Equatorium* (Asteraceae). *American Journal of Botany* **82**, 1000–1006 (1995). — COLE, C. T. and BIESBOER, D. D.: Monomorphism, reduced gene flow, and cleistogamy in rare and common species of *Lespedeza* (Fabaceae). *American Journal of Botany* **79**, 567–575 (1992). — CROW, J. F. and AOKI, K.: Group selection for a polygenic behavioral trait: estimating the degree

of population subdivision. Proc. Natl. Acad. Sci. USA **81**, 6073–6077 (1984). — ELLSTRAND, N. C. and ELAM, D. R.: Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics **24**, 217–242 (1993). — FALK, D. A. and HOLSINGER, K. E. (editors): Genetics and Conservation of Rare Plants. Oxford University Press, New York. 283 p. (1991). — HAMRICK, J. L. and GODT, M. J. W.: Allozyme diversity in plant species. In: A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER, and B. S. WEIR (editors). Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates, Sunderland, Massachusetts. pp. 43–63 (1989). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**, 95–124 (1992). — HANDEL, S. N.: The intrusion of clonal growth patterns on plant breeding systems. American Naturalist **125**, 367–384 (1985). — HEDRICK, P. W.: Genetics of Populations. Jones and Bartlett, Boston, Massachusetts (1985). — HEINRICH, B.: Energetics of pollination. Annual Review of Ecology and Systematics **6**, 39–170 (1975). — HOLSINGER, K. E. and GOTTLIEB, L. D.: Conservation of rare and endangered plants: principles and prospects. In: D. A. FALK and K. E. HOLSINGER (editors): Genetics and Conservation of Rare Plants. Oxford University Press, Oxford. pp. 195–208 (1991). — KIM, Z. S., LEE, S. W. and HYUN, J. O.: Allozyme variation in six native oak species in Korea. Ann. Sci. For. **50**(Suppl 1): 253s–260s (1993). — LANDE, R.: Genetics and demography in biological conservation. Science **24**, 1455–1460 (1988). — LANDE, R. and BARROWCLOUGH, G. F.: Effective population size, genetic variation and their use in population management. In: M. E. SOULE (editor): Viable Populations for Conservation. Cambridge University Press, Cambridge, England. pp. 87–121 (1987). — LESICA, P., LEARY, R. F., ALLENDORF, F. W. and BILDERBACK, D. E.: Lack of genetic diversity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology **2**, 275–282 (1988). — MOSSELER, A., EGGER, K. N. and HUGHES, G. A.: Low-levels of genetic diversity in red pine confirmed by random amplified polymorphic DNA markers. Canadian

Journal of Forest Research **22**, 1332–1337 (1992). — NEI, M.: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**, 3321–3323 (1973). — NEI, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**, 583–590 (1978). — SCHEMSKE, D. W., HUSBAND, B. C., RUCKELSHAUS, M. H., GOODWILLIE, C., PARKER, I. M. and BISHOP, J. G.: Evaluating approaches to the conservation of rare and endangered plants. Ecology **75**, 584–606 (1994). — SIMON, J.-P., BERGERON, Y. and GAGNON, D.: Isozyme uniformity in populations of red pine (*Pinus resinosa*) in the Abitibi Region, Quebec. Canadian Journal of Forest Research **16**, 1133–1135 (1986). — SOLTIS, D. E., HAUFLE, C. H., DARROW, D. C. and GASTONY, G. J.: Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal **73**, 9–27 (1983). — SOLTIS, P. S., SOLTIS, D. E., TUCKER, T. L. and LANG, F. A.: Allozyme variability is absent in the narrow endemic, *Bensoniella organa* (Saxifragaceae). Conservation Biology **6**, 131–134 (1992). — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural Survey, IL (1989). — VANE-WRIGHT, R. I., HUMPHRIES, C. J. and WILLIAMS, P. H.: What to protect? Systematics and the agony of choice. Biological Conservation **55**, 235–253 (1991). — WALLER, D. M., O'MALLEY, D. M. and GAWLER, S. C.: Genetic variation in the extreme endemic, *Pedicularis furbishiae* (Scrophulariaceae). Conservation Biology **1**, 335–340 (1987). — WEEDEN, N. F. and WENDEL, J. F.: Genetics of plant isozymes. In: D. E. SOLTIS and P. S. SOLTIS (editors): Isozymes in Plant Biology. Discorides Press, Portland, Oregon. pp. 46–72 (1989). — WRIGHT, S.: Coefficients of inbreeding and relationship. American Naturalist **56**, 330–338 (1922). — WRIGHT, S.: Evolution in MENDELian populations. Genetics **16**, 97–159 (1931). — ZABINSKI, C.: Isozyme variation in eastern hemlock. Canadian Journal of Forest Research **22**, 1838–1842 (1992).

Efficiency of Early Testing in *Pinus sylvestris* L. Grown Under Two Different Spacings in Growth Chamber

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Abstract

An hypothesis in designing early tests was that mimicking the limiting factor occurring later in a rotation would improve the genetic correlations between early and late measurements. Seedlings belonging to 30 open pollinated families of Scots pine (*Pinus sylvestris*) were therefore grown for three growth periods at two spacings, wide and dense (9 and 36 plants per box with an area of 0.188 m²), in the growth chamber to mimic the competition for light. The wide spacing was replicated in another experiment with the same parents, and the seedlings were grown for two growth periods. Eight growth chamber characters were jointly evaluated with 20-year-old tree growth characters in four field trials with the same parents as in the growth chamber trials. The field trials were located in central

Sweden, and established with full-sib families. The additive genetic correlations between growth chamber characters and height in field tests were none or weak. The correspondence between the results from wide and dense spacing in the growth chamber was strong. Moderate genetic correlations between growth chamber characters estimated in two different experiments, established with seed collected in two different years, were probably due to differences in mating in the seed orchard. The additive genetic correlations for height between field test sites were in the range 0.26 to 0.99. The raised hypothesis that mimicking the competition for light by a dense spacing would improve the genetic correlation between early and late measurements was not supported for the tested range of growth chamber conditions. Therefore, this study does not give any support for use of early tests in operational forest tree breeding, although other experiments have resulted in high correlation between field and growth chamber experiments.

Key words: *Pinus sylvestris*, early tests, juvenile-mature correlations, growth trait, genetic correlation.

FDC: 165.3; 165.41; 181.525; 232.13; 174.7 *Pinus sylvestris*; (485).

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