References

BEAVER, J. A., IEZZONI, A. F. and RAMM, C. W.: Isozyme diversity in sour, sweet, and ground cherry. Theor. Appl. Genet. **90**, 847–852 (1995). — BOUSQUET, J., CHELIAK, W. M. and LALONDE, M.: Allozyme variability in natural populations of green alder (*Alnus crispa*) in Quebec. Genome **29**, 345 (1987). — BRADSHAW, D. H. and STETTLER, F. R.: Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars. Theor. Appl. Genet. **86**, 301 (1993). — BUTORINA, A. K.: Cytogenetic study of diploid and spontaneous triploid oaks, *Quercus robur* L., Ann. Sci. For. **50** (Suppl 1), 144–150 (1993). — BUTORINA, A. K., IEVLEV, V. and MURAYA, L. S.: Cytogenetics of the spontaneous triploid of oak (*Quercus robur*). Genetika **19**, 647–659 (1983). — EIFLER, I.: Künstliche Polyploidie-Erzeugung bei *Picea abies* und *Betula verrucosa*. Silvae Genetica **4**, 162–166 (1955). — EIFLER, I.: Beschreibung einer Fixiermethode, die das Auszählen von Birkenchromosomen erleichtert. SD "Der Züchter" **29** (1): 57–59 (1959). — EIFLER, I.: Anwendungsmöglichkeiten der Ploidiezüchtung in der Forstwirtschaft. Arch. Forstwesen **16**, 515–528 (1967). — FINESCHI, S., GILLET, E. and MALVOLTI, E.: Genetics of sweet chestnut (*Castanea sativa* MILL.). III. Genetic analysis of zymograms of single tree offspring. Silvae Genet. **39**, 189–194 (1990). — GEBHARDT, K.: Erzeugung und Nutzung von Ploidie-Mutanten. Allg. Forstzeitg. **49**, 1351–1352 (1988). — HERTEL, H., ZASPEL, I. and EISENHAUER, D.-R.: Investigations about vitality and genetic structure in oak stands. International symposium „Environmental contrains and oaks: ecological and physiological aspects" in Nancy/France August 29 to September 1 1994. p. 153 (1994), — JOHNSSON, H.: Observations on induced polyploidy in some conifers (*Pinus sylvestris, P. contorta, Picea abies, Larix sibirica*). Silvae Genetica **24**, 62–68 (1975). — KIELLANDER, L. C.: Polyploidy in *Picea abies*. Hereditas **36**, 513–516 (1950). — KIM, C. S. and LEE, S. K.: Morphological and cytological characteristics of a spontaneous tetraploid of *Robinia pseudoacacia*. Research Report of the Institute of Forest Genetics, Korea **10**, 57–65 (1973). — MACHON, N., LEFRANC, M., BILGER, I. and HENRY, J.-P.: Isoenzymes as an aid to clarify the taxonomy of french elms. Heredity **74**, 39–47 (1995). — MERTENS, C.: Ökologisch-genetische Untersuchungen von Eichenbeständen der Oberförsterei Chorin. Diplomarbeit, Universität Göttingen (1994). — NAUJOKS, G., ZASPEL, I. und HERTEL, H.: Charakterisierung von Nachkommen tetraploider und diploider Birken. Arbeitstagung d. AG Gehölze d. Gesellsch. f. Pflanzenzüchtung in Berlin, (Poster) (1994). — NIELSSON-EHLE, H.: Über eine in der Natur gefundene Gigasform von *Populus tremula*. Hereditas **21**, 379–393 (1936). — SARVAS, R.: Two triploid aspens and two triploid birches. Comm. Inst. Forest. Fenniae **49**, 1–25 (1958). — SHERALD, J. L., SANTAMOUR, F. S., HAJELA, R. K., HAJELA. N. and STICKLEN, M. B.: A dutch elm disease resistant triploid elm. Can. J. For. Res. **24**, 647–653 (1994). — SPETHMANN, W.: Stecklingsvermehrung von Stiel- und Traubeneiche (*Quercus robur* L. und *Quercus petraea* (MATT.) LIEBL.). Schriften aus der Forstlichen Fakultät der Universität Göttingen und der Niedersächsischen Forstlichen Versuchsanstalt, Band 86. J. D. Sauerländer's Verlag, Frankfurt am Main. pp. 99 (1986).

Isozyme Gene Loci and Their Allelic Variation in *Pinus sylvestris* L. and *Pinus cembra* L.[1])

By F. BERGMANN and H. H. HATTEMER

Abteilung für Forstgenetik und Forstpflanzenzüchtung,
Georg-August-Universität, Büsgenweg 2, D-37077 Göttingen, Germany

(Received 3rd August 1995)

Summary

The genetic structure and variation of 5 Scots pine (*P. sylvestris* L.) and 4 Swiss stone pine (*P. cembra* L.) populations was compared using allele frequency data from the same isozyme-gene-systems. Differences between the 2 pine species belonging to the subgenera *Pinus* and *Strobus*, respectively, concern both the number of controlling gene loci and the number and frequency of alleles. Although Scots pine is characterized by larger populations and a wider natural range than Swiss stone pine, the genetic diversity values did not generally differ to a similar degree or in the direction expected. While Scots pine possesses higher diversity at several enzyme loci, Swiss stone pine reaches the same degree of diversity or even a higher degree at other loci.

Interestingly, the greatest differences in gene diversity between the 2 pine species were detected for isozyme-gene-systems that differ in the number of controlling loci, suggesting that variable allozymes at only 1 (or 2) loci and invariant isozymes at multiple loci are alternate forms of enzyme adaptation in the 2 species. The relationship between such adaptive strategies at the enzyme level and the ecological conditions and life history traits of the 2 species are discussed.

Key words: Pinus sylvestris, Pinus cembra, isozyme loci, genetic diversity, adaptive strategies.

FDC: 165.3; 165.5; 174.7 *Pinus sylvestris;* 174.7 *Pinus cembra.*

Introduction

Most European pine species differ greatly in the sizes and geographic locations of their natural ranges. Whereas Scots pine (*Pinus sylvestris*, section *Pinus*, subgenus *Pinus*) is one of the most widely distributed conifers in central, northern and eastern Europe, Swiss stone pine (*P. cembra*, section *Strobus*, subgenus *Strobus*) is restricted to the high elevations of the Austrian and Swiss Alps, the Eastern Carpathians, and the High Tatra Mountains (HOLZER, 1975). Therefore, it appears to be worthwhile to compare the types and amounts of genetic variation between these 2 pine species.

Extensive studies on the genetic diversity and differentiation of Scots pine have been carried out by, among others, GULLBERG *et al.* (1985), MEJNARTOWICZ and BERGMANN (1985), PRUS-GŁOWACKI and STEPHAN (1994) and GONCHARENKO *et al.* (1994), whereas Swiss stone pine populations have been investigated only by SZMIDT (1982). Russian research groups have compared different stone pine species of the subsection *Cembrae*, section *Strobus*, which occupy a large part of the Eurasian area of the former Soviet Union (GONCHARENKO *et al.*, 1992; KRUTOVSKII, *et al.*, 1995).

[1]) Dedicated to Prof. Dr. G. H. MELCHIOR on his 70th birthday.

The objective of this study, however, was to document the variation patterns of Scots and Swiss stone pine populations and to compare the degree and the structuring of genetic polymorphisms between these two species, which belong to different subgenera (*Pinus, Strobus*) of the genus *Pinus* (CRITCHFIELD and LITTLE, 1966). In order to reliably evaluate the actual differences between the 2 species, we studied the genetic variation at the same isozyme loci (or isozyme systems), so that biochemical, functional, and adaptational similarities (or dissimilarities) can be recognized immediately.

Material and Methods

Five Scots pine populations from Germany (Selb, Fichtel Mts. and Steigerwald), Slovakia (Poprad, Lower Tatra), Poland (Karczma, Beskides) and Russia (Altai region) were included in this study. All populations were represented by bulk seed lots, each collected from more than 30 trees. Seed lots of Swiss stone pine were collected from 36 trees in St. Moritz and 21 trees in the Engadine (Swiss Alps), 15 trees in Morskie Oko (High Tatra, Poland), and 19 trees in Calimari (East Carpathians, Romania). 100 seeds from each bulk seed lot and 6 seeds per single-tree seed lot were analysed.

Haploid endosperm (macrogametophyte) tissue from dormant (stone pine) or soaked seeds (Scots pine) was used for isozyme analysis. For this comparative study, the controlling gene loci of 6 enzyme systems (phosphoglucomutase, PGM; phosphoglucose isomerase, PGI; glutamate oxalacetate transaminase, GOT; malate dehydrogenase, MDH; shikimate dehydrogenase, SKDH; 6-phosphogluconate dehydrogenase, 6PGDH) were chosen, because they function in the primary metabolism and use only 1 native substrate. Details on electrophoretic procedures, buffer systems, and staining solutions were published elsewhere (MEJNARTOWICZ and BERGMANN, 1985; GONCHARENKO *et al.*, 1992; WENDEL and WEEDEN, 1989). Gene frequency data were calculated from the genotype frequencies of stone pine trees or directly from the bulk seed samples of Scots pine. The comparability of the 2 data sets is based on the assumption, that the harvested trees were equally represented in the bulk seed lots. The degree of genetic variation was estimated by the gene diversity measure v (GREGORIUS, 1987), which is also known as the effective number of alleles at a gene locus. The values of this measure are dependent on the sample size, but the increase of a sufficiently large sample will detect only very rare alleles, which do not change the diversity values markedly.

Results

Number of controlling gene loci in the two pine species

In order to accurately evaluate and reliably compare the genetic diversity of the 2 species, enzyme systems of the secondary metabolism or systems with unknown catalytic function *in vivo* were excluded from this study. But even in the primary metabolism, the number of controlling gene loci differs for 2 enzyme systems (see *Table 1*): MDH is encoded by 4 loci in Scots pine (GONCHARENKO *et al.*, 1994) but by 5 loci in Swiss stone pine (HATTEMER and BERGMANN, 1994), whereas 6PGDH is encoded by 2 loci in Scots pine (SZMIDT and YAZDANI, 1984) but by altogether 4 loci in stone pine (BERGMANN and GILLET, in prep.).

For the other systems of the primary metabolism (GOT, PGM, PGI, and SKDH), the number of controlling gene loci does not differ between the 2 pine species (*Table 1*). The data on the mode of inheritance resulted from our own large-scale segregation studies using endosperm of single trees and agree with other results published earlier on Scots and stone pine (GONCHARENKO *et al.*, 1992, 1994; KRUTOVSKII *et al.*, 1995).

Table 1. – The number of controlling gene loci for 6 enzyme systems in Scots pine and Swiss stone pine.

Enzyme system	Number of controlling gene loci	
	P. sylvestris	*P. cembra*
PGM	2	2
PGI	2	2
SKDH	2	2
GOT	3	3
MDH	4	5
6PGDH	2	4

Comparison of the general genetic variation of the two pine species

In order to compare the degree of genetic variation estimated across several populations between 2 species, it is appropriate to use parameters such as *PPP* (the proportion of polymorphic populations). Of the 18 enzyme loci examined in the 4 stone pine populations, 9 were completely monomorphic ($PPP = 0.0$), 5 were polymorphic in 1 ($PPP = 0.25$), 2 ($PPP = 0.50$) or 3 ($PPP = 0.75$) populations, and only 4 loci were polymorphic in all populations (tree samples) studied ($PPP = 1.0$, see *Table 2*). In contrast to this species, only 1 of the 15 gene loci analysed appeared to be monomorphic in all five populations of Scots pine, but 10 gene loci (= 67%) were found to be polymorphic across all populations ($PPP = 1.0$) (*Table 2*). Similar differences could be observed for the allele frequency profiles (for details, see FINKELDEY and GREGORIUS, 1994), since Scots pine populations exhibited major polymorphisms at 7 gene loci, whereas stone pine populations showed this type of polymorphism at only 3 gene loci (*Table 2*).

Table 2. – Proportion of polymorphic populations (*PPP*) and the type of allele frequency profile (*mono* refers to monomorphism, *minor* to minor polymorphism, and *major* to major polymorphism) for 15 gene loci in Scots pine and 18 gene loci in stone pine.

Enzyme gene locus	*P. sylvestris*		*P. cembra*	
	PPP	allelic profile	*PPP*	allelic profile
GOT-A	0.4	*minor*	0.0	*mono*
GOT-B	1.0	*major*	0.0	*mono*
GOT-C	1.0	*major*	0.0	*mono*
PGI-A	0.0	*mono*	0.0	*mono*
PGI-B	1.0	*minor*	0.75	*minor*
PGM-A	0.8	*minor*	1.0	*major*
PGM-B	0.6	*minor*	0.0	*mono*
SKDH-A	1.0	*major*	1.0	*major*
SKDH-B	1.0	*minor*	0.25	*minor*
MDH-A	1.0	*minor*	0.0	*mono*
MDH-B	0.6	*minor*	0.0	*mono*
MDH-C	1.0	*major*	0.0	*mono*
MDH-D	1.0	*major*	0.5	*minor*
MDH-E	–	–	1.0	*major*
6PGDH-A	1.0	*major*	1.0	*minor*
6PGDH-B	1.0	*major*	0.0	*mono*
6PGDH-C	–	–	0.75	*minor*
6PGDH-D	–	–	0.25	*minor*

Although these pronounced differences in the degree of genetic polymorphism at many gene loci may reflect a drastic gene loss in stone pine populations due to isolation, genetic drift or selection (see SZMIDT, 1982), it appears worthwhile to compare the 2 pine species for each isozyme-gene-system separately.

Comparison of the genetic variation of individual isozyme-gene-systems between the two pine species

The 3 loci coding for the GOT system are completely monomorphic in the Swiss stone pine populations (*Table 2*). Similar results have been found for one Swiss and six Siberian stone pine (*P. sibirica*) populations from Russia (GONCHARENKO *et al.*, 1992). In contrast, the 3 GOT loci in Scots pine are partly (*GOT-A*) or completely polymorphic (*GOT-B, GOT-C*) in the 5 populations studied. The gene diversity values v (effective numbers of alleles) for *GOT-B* (range from 1.38 to 2.46) and *GOT-C* (range 1.53 to 2.16) were relatively high, indicating 2 or 3 alleles with intermediate frequencies.

One of the 2 loci coding for the PGI system is monomorphic in both pine species, whereas the other locus (*PGI-B*) is largely polymorphic in stone pine and completely polymorphic in Scots pine (*Table 2*). The values of gene diversity in Scots pine (range 1.06 to 1.38) are somewhat higher than those in stone pine populations (range 1.0 to 1.15), but this locus exhibited a minor polymorphism in both species (*Table 2*). The 2 loci coding for the PGM system were found to show different degrees of polymorphism between the 2 species. While *PGM-B* is completely monomorphic in stone pine, it is partly polymorphic in Scots pine populations, revealing a typical minor polymorphism in all cases (*Table 2*). In contrast to *PGM-B*, the gene diversity at *PGM-A* is, surprisingly, higher in stone pine (range 1.22 to 1.55) than in Scots pine populations (range 1.0 to 1.28) and the allele distribution provides a major polymorphism, whereas the Scots pine populations only exhibited minor polymorphisms (*Table 2*).

Similar differences between the 2 pine species could be found for 1 of the 2 loci (*A*) coding for the SKDH system. The gene diversity at *SKDH-A* is by far higher in stone pine (range 1.88 to 2.46) than in Scots pine populations (range 1.2 to 1.62), although both species revealed a major polymorphism with 3 alleles (*Table 2*). At the second gene locus (*SKDH-B*) most of the stone pine populations were found to be monomorphic, while the Scots pine populations showed a typical minor polymorphism.

The most pronounced differences between the 2 pine species belonging to different sub-genera were observed for the enzyme systems MDH and 6PGDH, where, besides different degrees of polymorphism, different numbers of controlling gene loci could be identified (*Table 1*).

The MDH system in Scots pine is specified by 4 loci (*MDH-A* through *MDH-D*), as is the case for most other conifer species (EL-KASSABY, 1981). The partly complex banding patterns of this system can be resolved by the aid of 2 different staining methods (THORMANN and STEPHAN, 1993). While the gene loci *MDH-A* and *MDH-B* were found to show minor polymorphisms, typical major polymorphisms with 2 or 3 alleles could be observed at *MDH-C* and *MDH-D* (*Table 2*). Accordingly, the gene diversity reached relatively high values for *MDH-C* (range 1.29 to 2.04) and *MDH-D* (range 1.93 to 2.62). In contrast to these data, four MDH loci appeared to be completely or partly (*MDH-D*) monomorphic in stone pine populations, whereas only the 5th locus (*MDH-E*) was found to be highly polymorphic (*Table 2*) with remarkable gene diversity values (range 1.32 to 1.98). When comparing the MDH isozyme patterns of the two species, it becomes obvious that the intensely stained and broad MDH-A zone of Scots pine clearly resembles 2 zones, MDH-B and MDH-C, of stone pine, suggesting a duplication of one of the original MDH loci during the branching process of pine phylogeny. Since *MDH-A* shows only little variability in Scots pine (and other conifer species), it is not surprising that both loci (*MDH-B* and *-C*) are monomorphic in the stone pine populations studied. Another isozyme similarity was found for MDH-D in Scots pine and MDH-E in stone pine, since both isozymes (isozyme zones) produce a characteristic sub-band (secondary isozyme). Again there is agreement in the degree of polymorphism (major polymorphism, see *Table 2*) at these loci and the diversity values are relatively high in both species.

Great differences between the 2 pine species in the degree of genetic polymorphism and in the structuring of gene function in the primary metabolism were observed for the 6PGDH system. This system is encoded by 2 loci in Scots pine (SZMIDT and YAZDANI, 1984), but altogether by 4 loci in Swiss stone pine (and other stone pines, BERGMANN and GILLET, in prep.). Whereas the 2 6PGDH loci are highly polymorphic in all Scots pine populations, the 4 6PGDH loci of stone pine are monomorphic or possess at most a minor polymorphism. The respective gene diversity data are compiled in *table 3*. It can now be speculated that the lack of allelic variation at the stone pine loci may be compensated by a surplus of isozymes. This is particularly the case with the loci 6*PGDH-A* and *-C*, which form an interlocus hybrid enzyme (heterodimer), suggesting that 1 locus is the duplicate of the other. The resulting 3-banded 6PGDH pattern may be considered as a fixed heterozygous phenotype, because it is present in all individuals of the population. The isozymes of 6*PGDH-B* (monomorphic) and 6*PGDH-D* (largely monomorphic) provide additional enzyme activity in the cells of all individuals, although it is not yet established whether these isozymes function in the cytosol or in the plastids.

Table 3. – Gene diversity values v estimated at 2 6*PGDH* gene loci in the Scots pine populations and at 4 6*PGDH* gene loci in the stone pine populations. Since the homology between the 2 sets of loci has not yet been established, the 4 loci of stone pine are marked by primes.

Populations	6*GPDH* gene loci					
	A	*B*	*A'*	*B'*	*C'*	*D'*
Scots pine						
Selb	1.85	1.55				
Steigerwald	1.97	1.98				
Poprad	1.83	1.89				
Karczma	2.08	1.93				
Altai	1.91	2.19				
Swiss stone pine						
St. Moritz			1.18	1.00	1.02	1.00
Engadine			1.18	1.00	1.06	1.00
Morskie Oko			1.15	1.00	1.00	1.13
Calimari			1.10	1.00	1.06	1.00

Discussion

The comparison of genetic structures (and variation) between species belonging to the same genus, but separated by different branches of the phylogenetic tree, raises a lot of problems. These include the migration history after glaciation periods, the population size and structure (mixed or pure stands), the life history traits, and the adaptation to ecological conditions.

Scots and Swiss stone pine belonging to the subgenera *Pinus* and *Strobus*, respectively, of the genus *Pinus* are typical representatives of such species. Although many of the problems mentioned above cannot be solved by genetic studies using a few isozyme gene markers, the results from comparative analyses nevertheless provide some valuable indication of the differential genetic and adaptive systems of the 2 species.

As a tree species with wide distribution in Eurasia, Scots pine possesses considerable allelic variation at nearly all of the investigated enzyme coding gene loci (GONCHARENKO *et al.*, 1994). The intrapopulation diversity is relatively high throughout its natural range, and many enzyme loci exhibit a typical major polymorphism which is assumed to be maintained by some sort of balancing selection (LEWONTIN, 1985). In contrast to Scots pine, Swiss stone pine is restricted to a few high elevation areas in Europe and consists of relatively small and scattered populations (HOLZER, 1975), so that gene flow is largely or completely prevented due to isolation by distance. A previous isozyme study of several stone pine populations seemed to demonstrate the consequences of the species' limited distribution, since the gene diversity at eight loci, most of which code for unspecific enzymes, was found to be lower in comparison with other conifer species (SZMIDT, 1982). At first glance, the results of our comparative study confirm these earlier data, especially when the degree of genetic polymorphism at the GOT, MDH and 6PGDH loci is considered. On the other hand, the genetic diversity estimated at *PGM-A* and *SKDH-A* equals or even exceeds the values obtained for the Scots pine populations, indicating that genetic drift and isolation cannot be the only relevant factors influencing the genetic variation pattern in stone pine populations.

Generally, monomorphic enzyme loci or loci with extreme minor polymorphism (1 prevalent and 1 or 2 very rare alleles) may not always be the result of limited geographic range or particular life history traits (HAMRICK *et al.*, 1992), but can also be an intrinsic property of the enzyme locus itself. Possibly, mutation events, although as frequent as for other loci, will not become established as allozyme variants at those particular loci because of strong selective constraints. Alternatively, optimal adaptation to (extra- or intracellular) environmental homogeneity will also lead to monomorphism at particular loci, the products of which function at flux controlling sites in the primary metabolism (CLARK and KOEHN, 1992).

Another look at the same data sets may advance an old hypothesis stating that allozyme variation at 1 locus (or 2 loci) and multiple-locus isozyme variation might be alternate forms of enzyme adaptation (MYERS, 1978). Applying this hypothesis to our pine species, we can postulate that MDH enzymes and especially 6PGDH enzymes have adapted differently in the 2 pine species. While Scots pine possesses fewer but highly polymorphic isozyme loci in response to unpredictable variation in intra- or extracellular environmental conditions in a variety of habitats, Swiss stone pine has evolved more (by duplications) but mostly invariant isozyme loci in response to constant or predictable environmental conditions including regular intervals or cyclic environmental variability.

Swiss stone pine populations occupy sites near or above the timber line in the Alps and the High Tatra, where a harsh climate and only a limited number of frost-free days during the vegetation period enforce a particular physiological behavior in the trees (HOLZER, 1975). The annual growth is very slow, because most of the energy gained during assimilation must be used for maintenance of the basic metabolism. These metabolic costs can be reduced by, for example, energy-saving enzymatic reactions (KOEHN and BAYNE, 1989). Probably, fixed heterozygosity or additional isozymes may be capable of saving energy by enzyme complementation and faster adjustment to small changes of the intracellular milieu.

Scots pine, on the other hand, uses different allozymes in homo- or heterozygous state for the adaptation to environmental conditions varying in space and time. The alternation of homo- and heterogeneous situations across microgeographical sites likely requires homogeneous and heterogeneous enzyme complements in different frequency distributions. A disadvantage of this adaptive strategy is the high genetic load, which may be compensated by the yearly reproduction and very large seed crops characteristic of Scots pine.

Acknowledgements

We are very much obliged to E. GILLET for carefully reading the manuscript. We also greatly appreciate the technical assistance of S. KRAKUHN. This study was supported by a grant from the Deutsche Forschungsgemeinschaft.

Literature

BERGMANN, F. and GILLET, E.: Phylogenetic relationships among pine species inferred from different numbers of 6PGDH loci. In preparation. (1995). — CLARK, A. G. and KOEHN, R. K.: Enzymes and adaptation. P. 193–228. In: BERRY, R. J., CRAWFORD, T. J. and HEWITT, G. M. (eds.): Genes in Ecology. Blackwell Scientific Publications, Oxford (1992). — CRITCHFIELD, W. B. and LITTLE, E. L.: Geographic Distribution of the Pines of the World. Miscell. Publ. 991, U.S. Dept. Agric., Washington, D.C. (1961). — EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Heredity **72**, 451–452 (1981). — FINKELDEY, R. and GREGORIUS, H.-R.: Genetic resources: selection criteria and design. P. 322–347. In: KIM, Z. S. and HATTEMER, H. H. (eds.): Conservation and Manipulation of Genetic Resources in Forestry. Kwang Moon Kag Publ. Co., Seoul, Korea (1994). — GONCHARENKO, G. G., PADUTOV, V. E. and SILIN, A. E.: Population structure, gene diversity and differentiation in natural populations of Cedar pines (*Pinus* subsect. *Cembrae, Pinaceae*) in the USSR. Plant Syst. Evol. **182**, 121–134 (1992). — GONCHARENKO, G. G., SILIN, A. E. and PADUTOV, V. E.: Allozyme variation in natural populations of Eurasian pines. III. Population structure, diversity, differentiation and gene flow in central and isolated populations of *Pinus sylvestris* L. in eastern Europe and Siberia. Silvae Genetica **43**, 119–131 (1994). — GREGORIUS, H.-R.: The relationship between the concepts of genetic diversity and differentiation. Theor. Appl. Genet. **74**, 397–401 (1987). — GULLBERG, U., YAZDANI, R., RUDIN, D. and RYMAN, N.: Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden. Silvae Genetica **34**, 193–201 (1985). — 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). — HATTEMER, H. H. and BERGMANN, F.: Abschlußbericht zum DFG-Forschungsprojekt: Vergleichende Untersuchungen von Isoenzym-Polymorphismen und ihrer genetischen Kontrolle in der Gattung *Pinus*. (1994). — HOLZER, K.: Genetics of *Pinus cembra* L. Annales Forestales **6**, 139–158 (1975). — KOEHN, R. K. and BAYNE, B. L.: Towards a physiological and genetical understanding of the energetics of the stress response. Biol. J. Linn. Soc. **37**, 157–171 (1989). — KRUTOVSKII, K. V., POLITOV, D. V. and ALTUKHOV, Y. P.: An isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pines. P. 81–102. In: BARADAT, PH., ADAMS, W. T. and MÜLLER-STARCK, G. (eds.): Population Genetics and Genetic Conservation of Forest Trees. SPB Scientific Publ. bv, The Hague. In press. (1995). — LEWONTIN, R. C.: Population genetics. Ann. Rev. Genet. **19**, 81–102 (1985). — MEJNARTOWICZ, L. and BERGMANN, F.: Genetic differentiation among Scots pine populations from the lowlands and the mountains in Poland. P. 253–255. In: GREGORIUS, H.-R. (ed.): Population Genetics in Forestry. Lecture Notes in Biomath. Vol. 60. Springer-Verlag, Berlin and Heidelberg (1985). — MYERS, J. H.: Isozymes and allozymes: alternate forms of protein adaptation? Canad. J. Genet. Cytol. **20**, 187–192 (1978). — PRUS-GŁOWACKI, W. and STEPHAN, B. R.: Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. Silvae Genetica **43**, 7–14 (1994). — SZMIDT, A.: Genetic variation in isolated populations of stone pine (*Pinus cembra*). Silva Fennica **16**, 196–200 (1982). — SZMIDT, A. and YAZDANI, R.: Electrophoretic studies of genetic polymorphism of shikimate and 6-phospho-gluconate dehydrogenases in Scots pine (*Pinus sylvestris* L.). Arboretum Kórnickie **29**, 63–72 (1984). — THORMANN, R. and STEPHAN, B. R.: Interpretation of isozyme patterns of malate dehydrogenase in Scots pine using two different staining methods. Silvae Genetica **42**, 5–8 (1993). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. P. 5–45. In: SOLTIS, D. E. and SOLTIS, P. S. (eds.): Isozymes in Plant Biology. Chapman and Hall, London (1989).