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Phylogenetic relationships among *Pinus* species (*Pinaceae*) inferred from different numbers of 6PGDH loci

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Abstract: Electrophoretic examination of various *Pinus* species from both subgenera revealed that several taxa differ in the number of loci that control the enzyme system 6-phosphogluconate dehydrogenase (6PGDH). Based on inheritance analyses and published data, it was established that all species of subg. *Pinus* possess only two 6PGDH loci, whereas all stone pines of subg. *Strobus* exhibit four controlling loci. In order to trace the phylogenetic links at which one or two gene duplications occurred during pine evolution, several species of subsect. *Strobi* (section *Strobus*) and two species of sect. *Parrya* were additionally investigated. Based on conclusions about the uniqueness of gene duplications and the different numbers of 6PGDH loci, a phylogenetic tree of the pine taxa was constructed. This tree shows some new features not recognized in earlier studies and supports several novel assignments postulated in very recent pine classifications.

Many phylogenetic studies in recent years attempted to reanalyse plant systematics and evolution using gene frequency data generated from isozyme electrophoresis (CRAWFORD 1990). Hence, quantitative differences among taxa calculated as genetic distances were used for the construction of phylogenetic trees and other descent schemes (see, e.g., WHEELER & al. 1983). Such relationships may, however, be biased by the fact that alleles at particular isozyme loci are not selectively equivalent, and thus their frequencies are also shaped by environmental forces (GOLDING 1994). Consequently, taxa experiencing similar environmental conditions may exhibit similar allele frequency distributions even though they were widely separated phylogenetically. A more reliable characteristic for evaluating phylogenetic relationships is the number of isozymes (or isozyme loci), since changes in the number of loci (e.g. by gene duplication) are rare events in the course of evolution and often mark branching points of divergent speciation. Therefore, it has been suggested that contemporary taxa having the same number of isozyme loci belong to the same systematic assemblage as compared to taxa with different numbers of isozyme loci (GOTTLIEB 1983).

The genus *Pinus* is a very heterogeneous taxon comprising numerous subgenera, sections, subsections and species, and it is not surprising that different taxonomies of this genus have been developed by different plant systematicists (for reviews see MIROV 1967, LITTLE & CRITCHFIELD 1969, KRÜSSMANN 1972). Recent molecular (DNA) marker studies on this genus provided new insights into its phylogeny, which led to some modifications of the earlier classification systems (STRAUSS & DOERKSEN 1990, WANG & SZMIDT 1993). The reliability of these results again depends on the frequency of point mutations during the speciation epochs and their sensitivity to environmental conditions (DOYLE 1991). On the other hand, the number of loci coding for enzymes of the primary metabolism is highly conserved in diploid plants (GOTTLIEB 1982), and a change in this number will be a unique event depending not only on a sequence of complex chromosomal rearrangements (GOTTLIEB 1983) but also on the adaptive advantage of the new isozyme complement.

In the context of a comparative study on different pine species from Eurasia, we have noted that the enzyme 6-phosphogluconate dehydrogenase (6PGDH) functioning in the oxidative pentose phosphate pathway is specified by different numbers of loci in different taxa of the genus *Pinus*. Therefore, the objective of this study is to utilize the different numbers of 6PGDH gene loci for examining the phylogenetic relationships among pine taxa. Since we do not have the capacity to investigate the multitude of all pine species ourselves, we must rely on the genetic data published for other pine species, mostly from subg. *Pinus*.

Materials and methods

Seed sources. Single-tree and bulk seed lots of *Pinus cembra* L. were collected in Poland (Morskie Oko, High Tatra), Romania (Calimari, East Carpathians), Austria (Schmelz, Seetaler Alps) and Switzerland (St. Moritz, Swiss Alps). Bulk seed lots of *P. sibirica* DUTOUR and *P. pumila* (PALL.) REGEL (from Russia), *P. koraiensis* SIEB. & ZUCC. and *P. densiflora* SIEB. & ZUCC. (South Korea), *P. sylvestris* L. (Southern Germany, Poland), *P. nigra* ARNOLD (Austria) and *P. leucodermis* ANT. (Slowenia) were kindly provided by colleagues. In addition, bulk seed lots of *P. peuce* GRISEB., *P. strobus* L., *P. parviflora* SIEB. & ZUCC., *P. griffithii* MCCLELLAND, *P. flexilis* JAMES, *P. edulis* ENGELM. and *P. aristata* ENGELM. were bought in Germany from commercial seed suppliers (Appel, Darmstadt; Steingässer, Miltenberg).

Isozyme analysis. The haploid endosperm (macrogametophyte) tissue of single seeds was used as test material, since it allows for easy species comparisons due to the lack of intralocus hybrid bands in heterozygous individuals. Furthermore, seed endosperms from putative heterozygous trees enable inheritance analysis of isozyme patterns without resorting to breeding (for review see FERET & BERGMANN 1976).

Laboratory procedure. The crude endosperm extracts were subjected to horizontal starch-gel electrophoresis, using a 0.2 M Tris-citrate buffer system pH 7.4. Since the 6PGDH patterns in several pine species could not be resolved with the standard separation procedure, we employed a more concentrated gel buffer type (30% electrode buffer) and an increased current during separation (180 mA per 12 cm distance for 6 hr). Following electrophoretic separation, the activity zones of 6PGDH in the gel slabs were stained with a mixture composed of 100 ml 0.02 M Tris-HCl pH 8.0, 100 mg 6-phosphogluconate (Ba-salt), 20 mg NADP, 20 mg MTT, 2 mg PMS and a few drops of 10% $MgCl_2$. In order to recognize background staining not connected to the 6PGDH system, we stained a replicate slab without the substrate. In many cases some faint bands (sometimes IDH pattern) could be detected in this slab and must be considered when evaluating the 6PGDH patterns.

Genetic interpretation of 6PGDH patterns in seeds from single trees. Inheritance analysis of the 6PGDH banding patterns from a random sample of at least 15 endosperms from each of 80 Swiss stone pine trees was performed with the aid of the computer program HAPLOZYM (GILLET 1996).

Genetic interpretation of 6PGDH patterns in bulk seed lots. Since single-tree seed lots were not available for pine species of sect. *Parrya* and subsect. *Strobi*, the number of controlling loci was inferred from the isozyme banding patterns of many haploid endosperms from bulk seed lots. The genetic interpretation was based on band variation within individual zones, the possible occurrence of interlocus hybrid bands, and the analogy to the genetically analyzed patterns of Swiss stone pine and pines of subg. *Pinus*. This genetic interpretation must, however, be regarded as preliminary.

Results and discussion

Number of 6PGDH loci in species of subg. *Pinus*. The results of our large-scale isozyme studies on European pine species confirm the genetic interpretation of the 6PGDH patterns in Scots pine (*P. sylvestris*) seeds (Fig. 1), that is based on two controlling gene loci (SZMIDT & YAZDANI 1984). Similarly, two loci could also be identified in *P. mugo* and *P. densiflora* (subsect. *Sylvestres*), which were included in our comparative investigation. In order to determine whether this genetic basis of the 6PGDH system is universal to all pine taxa, various publications on the subject were checked. Based on the literature compiled in Table 1, this enzyme system was always reported to be controlled by two gene loci. Most of the species studied belong to various subsections of sect. *Pinus* within subg. *Pinus* (sometimes referred to as "hard pines"), whereas only one species (*P. roxburghii*, subsect. *Canarienses*) was analysed from sect. *Pinea* (or *Ternatae*) (see Table 1). Although data for several pine species assigned to sections of subg. *Pinus* are not available, it is yet reasonable to suggest that all species belonging to subg. *Pinus* possess (or express) only two loci which specify 6PGDH enzymes.

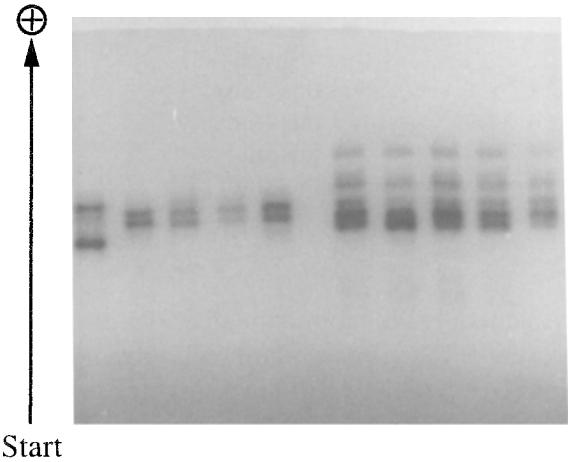

Fig. 1. 6PGDH zymograms of five seed endosperms each of *Pinus sylvestris* (left) and *P. cembra* (right). The second anodal zone of *P. cembra* zymograms represents an interlocus hybrid band (heterodimer) between the most anodal zone (A) and the fourth zone (C). See Fig. 2 for further description

Table 1. Number of 6PGDH loci (isozymes) in various species of the two subgenera *Pinus* and *Strobus* of the genus *Pinus* (*Pinaceae*)

Taxon	No. of 6PGDH loci	References
Subg. *Pinus*		
Sect. *Pinus*		
Subsect. *Sylvestres*		
P. sylvestris	2	SZMIDT & YAZDANI 1984
P. mugo	2	this study
P. densiflora	2	this study
P. nigra	2	SCALTSOYIANNES & al. 1994
P. leucodermis	2	MORGANTE & al. 1993
Subsect. *Australes*		
P. rigida	2	GURIES & LEDIG 1978
P. taeda	2	ADAMS & JOLY 1980
Subsect. *Ponderosae*		
P. ponderosa	2	O'MALLEY & al. 1979
P. jeffreyi	2	FURNIER & ADAMS 1986
Subsect. *Contortae*		
P. contorta	2	WHEELER & al. 1983
P. banksiana	2	WHEELER & al. 1983
P. virginiana	2	WHEELER & al. 1983
P. clausa	2	WHEELER & al. 1983
Subsect. *Oocarpae*		
P. radiata	2	PLESSAS & STRAUSS 1986
P. muricata	2	MILLAR 1985
P. attentuata	2	CONKLE 1981
Sect. *Pinea*		
Subsect. *Canarienses*		
P. roxburghii	2	HUSSAIN 1995
Subg. *Strobus*		
Sect. *Strobus*		
Subsect. *Cembrae*		
P. cembra	4	this study
P. sibirica	4	this study
P. pumila	4	this study
P. koraiensis	4	this study

Two isozymes (or isozyme loci) have generally been found for all enzyme systems which function in the same reaction process of the primary plant metabolism but in two different subcellular compartments (GOTTLIEB 1982). This is also the case for enzymes of the oxidative pentose phosphate pathway, which occurs in both the cytosol and the plastids of plant cells.

Since 6PGDH is an essential enzyme of this pathway, at least two molecular variants must exist in all plant species. In fact, two isozymes of 6PGDH could be detected in many plant species ranging from taxa such as conifers to those of *Onagraceae* and *Poaceae* (GOTTLIEB 1982). This constancy of the minimum number

of isozymes across very diverse plant taxa reflects a remarkable conservation of enzyme function in the primary metabolism, suggesting that whenever mutation events lead to additional isozymes, the mutants are most likely removed by strong purifying selection.

Number of 6PGDH loci in species of subsect. *Cembrae*. Compared to the species of subg. *Pinus*, the stone pines *P. cembra*, *P. sibirica* and *P. koraiensis* (sect. *Strobus*, subsect. *Cembrae*) revealed additional isozymes for several enzyme systems (BERGMANN & HATTEMER 1995). The most pronounced differences in isozyme number between species of the two pine subgenera could be observed for the 6PGDH system. Seed endosperms from Swiss stone pine (*P. cembra*) showed five activity zones after electrophoretic separation using an improved procedure (Figs. 1, 2). An analysis of inheritance is outlined in the following.

A random sample of at least $N = 15$ endosperms from each of 80 *P. cembra* trees was analyzed for 6PGDH. Only four of these trees showed variation in banding pattern among their sampled endosperms. Each of the four samples contained two different banding patterns, one of which was common to all four samples and the other unique to the respective tree, in proportions that do not differ significantly from 1:1 (see Fig. 2). This suggests that each tree is heterozygous at one, but not necessarily the same, 6PGDH-locus. It is supported by the calculation that, if a tree were actually heterozygous at $m > 2$ unlinked loci and each of the 2^m possible haplotypes had equal chances of representation among the endosperm, the probability of detecting only two of the haplotypes in arbitrary proportions in a sample of 15 endosperms would be less than 0.0002. The computer program HAPLOZYM (GILLET 1996) was applied separately to the banding patterns (Fig. 2) from each

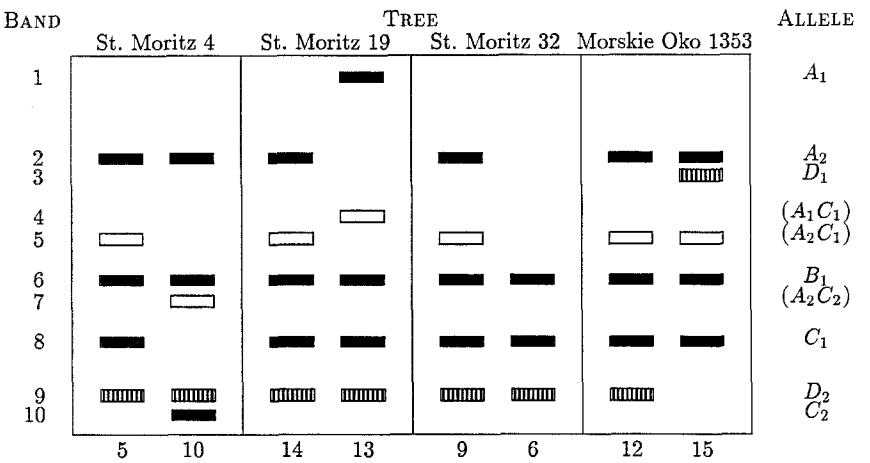

Fig. 2. Diagram of 6PGDH banding patterns segregating among a sample of haploid primary endosperms from each of four *Pinus cembra* trees: 6PGDH appears to be a dimeric enzyme system controlled by four loci, *6PGDH-A,-B,-C,-D*. Each band is encoded by the allele(s) designated at the right. Unfilled bands represent interlocus heterodimers between *6PGDH-A* and *-C*. The range of migration of *6PGDH-D* (hatched bands) overlaps with the ranges of the other loci. Each tree is heterozygous at one locus, with St. Moritz 32 showing a null allele at *6PGDH-A*. Segregation proportions (bottom line) do not deviate significantly from 1:1 for any tree

of the four trees. Compilation of the results yielded the following interpretation:

St. Moritz 4. Bands 8 and 10 are allelic homomeric isozymes defining one locus, band 2 is the homomeric product of a second locus, and bands 5 and 7 are the respective interlocus heterodimers.

St. Moritz 19. Band 8 is the homomeric product of the one locus inferred above, bands 1 and 2 are allelic homomeric isozymes of the second locus, and bands 4 and 5 are the corresponding interlocus heterodimers.

St. Moritz 32. Building on the results of the first two trees, the homomeric band 2 appears to be allelic to a null allele. Accordingly, the heterodimer 5 is only visible together with 2.

Morskie Oko 1353. Bands 3 and 9 are allelic homomeric isozymes. Interlocus heterodimers are not formed. Since homomeric bands 2 and 8 produced by the two loci inferred above are also present, bands 3 and 9 must represent a third locus.

Conclusions from the four trees. Three variable loci were identified. The appearance of band 6 in all endosperms, even in the presence of variation at the other loci, suggests that this band is the product of a fourth locus. Designating the loci alphabetically as *6PGDH-A,-B,-C,-D* according to the position of the band encoded by the allele that is shared by all four trees, the rightmost column of Fig. 2 gives the allele(s) encoding each band. The unusual degree of overlap of the range of migration of *6PGDH-D* with the ranges of the other loci complicates an intuitive interpretation of these patterns. Among these four gene loci, *6PGDH-A* and *-C* form an interlocus hybrid enzyme (heterodimer), indicating that one locus is the duplicate of the other. No polypeptide association was found between the two other loci, *6PGDH-B* and *-D*.

The 6PGDH patterns of Siberian stone pine (*P. sibirica*), mountain stone pine (*P. pumila*) and Korean stone pine (*P. koraiensis*) clearly correspond to those of *P. cembra*. Although additional inheritance analyses with seed lots from single trees of these species have not been performed, it is concluded that all stone pines of subsect. *Cembrae* (sect. *Strobus*) possess four gene loci which specify the 6PGDH system.

Number of 6PGDH loci in species of subsect. *Strobi* and sect. *Parrya*. Based on the data sets compiled by GOTTLIEB (1982) and on the assumption that the minimum number of isozymes of the primary metabolism is highly conserved in diploid plants, two conclusions can be drawn from our results: (1) Two duplications of the original 6PGDH loci must have occurred during the phylogenetic branching process from the ancient pines to the stone pines, and (2) the stone pines of subg. *Strobus* are phylogenetically younger taxa than the pine species of subg. *Pinus*. The latter conclusion is supported by paleobotanical findings which showed that the oldest fossil pine pollen (130–100 million years ago) resembles that of subsect. *Sylvestres, Ponderosae* and *Canariensis*, whereas fossil pollen resembling that of subg. *Strobus* was dated to later epochs (50–40 million years ago) (for review see MILLAR 1993).

Therefore, the question arises which taxa between the ancient pines (probably progenitors of sect. *Parrya*) and the stone pines of subsect. *Cembrae* represent the phylogenetic links, in which one or two duplications of the 6PGDH loci have occurred during pine evolution. In order to elucidate these relationships, five species of sect. *Strobi* subsect. *Strobus (P. strobus, P. peuce, P. flexilis, P. griffithii* and *P.*

Table 2. Number of 6PGDH loci (isozymes) in species of *Pinus* sect. *Strobus* and *Parrya* of subg. *Strobus*. References: [1] BUCHERT & al. 1997, [2] SCHUSTER & al. 1989, [3] BETANCOURT & al. 1991

Taxon	No. of 6PGDH loci	
	This study	Published data
Subg. *Strobus*		
Sect. *Strobus*		
Subsect. *Strobi*		
P. strobus	4	4 [1]
P. parviflora	4	
P. peuce	3	
P. flexilis	3	2 (variable) [2]
P. griffithii	3	
Sect. *Parrya*		
Subsect. *Cembroides*		
P. edulis	2	1 (variable) [3]
Subsect. *Balfourianae*		
P. aristata	2	

parviflora) and two species of sect. *Parrya* (*P. edulis* of subsect. *Cembroides* and *P. aristata* of subsect. *Balfourianae*) were additionally investigated.

The genetic interpretation of the respective 6PGDH patterns must be regarded as preliminary, since no seed lots from single trees were available (see Material and methods). In a few cases, published data on these pine species supplement our genetic interpretation. The results based on these criteria were compiled in Table 2. Surprisingly, the species assigned to subsect. *Strobi* (sect. *Strobus*) differed in the number of 6PGDH loci. Whereas *P. strobus* and *P. parviflora* exhibit four controlling loci in accordance with all stone pines of subsect. *Cembrae*, *P. peuce*, *P. flexilis* and *P. griffithii* appear to have only three active loci. Two variable 6PGDH loci were also described for *P. flexilis* (SCHUSTER & al. 1989), while four loci could be detected in *P. strobus* (BUCHERT & al. 1997; BEAULIEU & SIMON 1994 inferred only one). In contrast to these increased numbers of 6PGDH loci in sect. *Strobus*, the pine species of sect. *Parrya* (*P. edulis*, *P. aristata*) revealed only two active loci, which corresponds to all pine species of subg. *Pinus* (see Table 1).

Phylogenetic relationships among pine taxa derived from 6PGDH duplications. In the present analysis, we attempt to derive phylogenetic relationships from the distribution of 6PGDH gene duplications (Fig. 3). The line of evidence underlying such relationships results from the hypothesis that the duplication of a gene locus is a unique event and that, consequently, all species which possess it represent a monophyletic assemblage.

For the inference of the pine phylogeny, the two duplications of 6PGDH loci were used, although the original and duplicated loci cannot hitherto be dis-

criminated. The root of this scheme of relationships is represented by a progenitor similar to pines of sect. *Parrya* (subsectt. *Balfourianae* and *Cembroides*), since these are assumed to belong to the most primitive pines (see STRAUSS & DOERKSEN 1990). The first recognizable branching event separates a species with three loci (ancestral to pines with three and four loci, i. e. sect. *Strobus* of subg. *Strobus*) from those with primitively only two. The next recognizable event separates a species with four loci (ancestral to all species with four loci, i. e. a few species of subsect. *Strobi* and all stone pines of subsect. *Cembrae*) from those with three loci.

Although this phylogenetic model of the genus *Pinus* shows some features not recognized earlier, it contains several relationships which were already indicated in other studies. The pine species of sect. *Parrya* resemble the "hard pines" (subg. *Pinus*) in the number of 6PGDH loci, which confirms results based on cpDNA (STRAUSS & DOERKSEN 1990, WANG & SZMIDT 1993), where this group of "soft pines" is more closely related to the "hard pines" than all other species of the subg. *Strobus*. Furthermore, the great similarity within sect. *Pinus* based on their cpDNA markers agrees well with the findings of equal numbers of 6PGDH loci across all species (see Table 1).

The grouping of species of sect. *Strobus* inferred from the 6PGDH duplications (see Fig. 3) does not correspond exactly with the current pine taxonomy (LITTLE & CRITCHFIELD 1969). *Pinus strobus* and *P. parviflora* appear to be like the stone pines in having four 6PGDH loci, whereas all the other species of subsect. *Strobi* investigated here possess three active loci (Table 2), which corresponds well with the results on cpDNA (WANG & SZMIDT 1993). This subdivision of the original subsect. *Strobi* is, however, no novel insight into the pine phylogeny, since systematicists recently attempted to reorganize this pine group according to morphological and biochemical (terpene) traits. KINDEL (1995), for instance, did not assign *P. parviflora* to subsect. *Strobi*, but placed this species into a separate subsection closer to subsect. *Cembrae*, which accords with our findings on 6PGDH loci (Table 2).

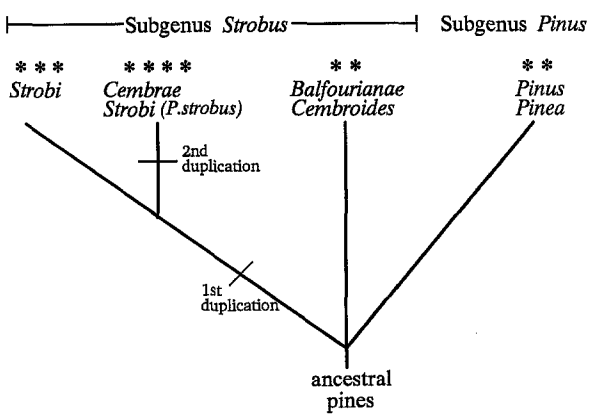

Fig. 3. Scheme of phylogenetic relationships of the genus *Pinus* based on *6PGDH* duplications. Asterisks mark the number of 6PGDH loci. Branches not marked by a duplication event do not necessarily represent monophyla. The position of subsectt. *Balfourianae* and *Cembroides* as most similar to the ancestral pines was adopted from other studies (see STRAUSS & DOERKSEN 1990, WANG & SZMIDT 1993)

Although the model of pine phylogeny developed here on the basis of isozyme gene number reflects a convincing picture including many features already indicated in earlier studies, it should again be emphasized that several parts of this phylogenetic tree must be regarded as preliminary. Additional inheritance analyses using single-tree seed lots from several pine species included in this study must supplement our genetic interpretation of the 6PGDH patterns.

References

ADAMS, W. T., JOLY, R. J., 1980: Genetics of allozyme variants in loblolly pine. – J. Heredity **71**: 33–40.

BEAULIEU, J., SIMON, J.-P., 1994: Inheritance and linkage relationships of allozymes in *Pinus strobus* L. – Silvae Genet. **43**: 253–261.

BERGMANN, F., HATTEMER, H. H., 1995: Isozyme gene loci and their allelic variation in *Pinus sylvestris* L. and *Pinus cembra* L. – Silvae Genet. **44**: 286–289.

BETANCOURT, J. L., SCHUSTER, W. S., MITTON, J. B., ANDERSON, R. S., 1991: Fossil and genetic history of a pinyon pine (*Pinus edulis*) isolate. – Ecology **72**: 1685–1697.

BUCHERT, G. P., RAJORA, O. P., HOOD, J. V., DANCIK, B. P., 1997: Effects of harvesting on genetic diversity in old growth stands of eastern white pine (*Pinus strobus* L.) in Ontario. – Conservation Biology (in press).

CONKLE, M. T., 1981: Isozyme variation and linkage in six conifer species. – In CONKLE, M. T., (Ed.): Proc. Symp. Isozymes of North American Forest Trees and Forest Insects, pp. 11–17. – USDA Gen. Techn. Rep. PSW-48. – Berkeley, CA.

CRAWFORD, D. J., 1990: Plant molecular systematics. – New York: Wiley.

DOYLE, J. J., 1991: The pros and cons of DNA systematic data: Studies of the wild perennial relatives of soybean. – Evol. Trends Pl. **5**: 99–104.

FERET, P. P., BERGMANN, F., 1976: Gel electrophoresis of proteins and enzymes. – In MIKSCHE, J. P., (Ed.): Modern methods in forest genetics, pp. 49–77. – Berlin, Heidelberg, New York: Springer.

FURNIER, G. R., ADAMS, W. T., 1986: Geographic patterns of allozyme variation in Jeffrey pine. – Amer. J. Bot. **73**: 1009–1015.

GILLET, E. M., 1996: Qualitative inheritance analysis of isoenzymes in haploid gametophytes: Principles and a computerized method. – Silvae Genet. **45**: 8–16.

GOLDING, B., 1994: Non-neutral evolution. Theories and molecular data. – New York: Chapman & Hall.

GOTTLIEB, L. D., 1982: Conservation and duplication of isozymes in plants. – Science **216**: 373–380.

– 1983: Isozyme number and phylogeny. – In JENSEN, U., FAIRBROTHERS, D. E., (Eds): Proteins and nucleic acids in plant systematics, pp. 209–221. – Berlin, Heidelberg, New York: Springer.

GURIES, R. P., LEDIG, F. T., 1978: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). – Heredity **40**: 27–32.

HUSSAIN, A., 1995: Untersuchungen zur genetischen Kontrolle von Isoenzym-Polymorphismen und zur genetischen Struktur von *Pinus roxburghii* SARG. – Dissertation, Forstwissenschaftlicher Fachbereich, Universität Göttingen.

KINDEL, K.-H., 1995: Kiefern in Europa. – Stuttgart: G. Fischer.

KRÜSSMANN, G., 1972: Handbuch der Nadelgehölze. – Hamburg: Parey.

LITTLE, E. L., CRITCHFIELD, W. B., 1969: Subdivisions of the genus *Pinus*. – USDA Forest Service, Misc. Publ. **1144**. – Washington, D. C.

MILLAR, C. I., 1985: Inheritance of allozyme variants in bishop pine (*Pinus muricata*). – Biochem. Genet. **23**: 933–946.

– 1993: Impact of the eocene on the evolution of *Pinus* L. – Ann. Missouri Bot. Gard. **80**: 471–498.

MIROV, N. T., 1967: The Genus *Pinus*. – New York: Ronald Press.

MORGANTE, M., VENDRAMIN, G. E., GIANNINI, R., 1993: Inheritance and linkage relationships of isozyme variants of *Pinus leucodermis* ANT. – Silvae Genet. **42**: 231–237.

O'MALLEY, D. M., ALLENDORF, F. W., BLAKE, G. M., 1979: Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. – Biochem. Genet. **17**: 233–250.

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

SCALTSOYIANNES, A., ROHR, R., PANETSOS, K. P., TSAKTSIRA, M., 1994: Allozyme frequency distributions in five European populations of black pine (*Pinus nigra* ARNOLD). – Silvae Genet. **43**: 20–30.

SCHUSTER, W. S., ALLES, D. L., MITTON, J. B., 1989: Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational transect. – Amer. J. Bot. **76**: 1395–1403.

STRAUSS, S. H., DOERKSEN, A. H., 1990: Restriction fragment analysis of pine phylogeny. – Evolution **44**: 1081–1096.

SZMIDT, A., YAZDANI, R., 1984: Electrophoretic studies of genetic polymorphism of shikimate and 6-phospho-gluconate dehydrogenases in Scots pine (*Pinus sylvestris* L.). – Arbor. Kórnickie **29**: 63–72.

WANG, X.-R., SZMIDT, A., 1993: Chloroplast DNA-based phylogeny of Asian *Pinus* species (*Pinaceae*). – Pl. Syst. Evol. **188**: 197–211.

WHEELER, N. C., GURIES, R. P., O'MALLEY, D. M., 1983: Biosystematics of the genus *Pinus*, subsection *Contortae*. – Biochem. Syst. Ecol. **11**: 333–340.

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