desertification of North Africa is relatively recent, so there was not enough time for species differentiation. In the Med-Cecklist (GREUTER et al., 1984), *Cedrus atlantica* is listed as *Cedrus libani* subsp. *atlantica*, implying an uncertainty as to the existence of 2 separate species. The slight differentiation between those two species, could also be attributed to the origin of the material used in this study, which was collected from an arboretum where the trees may have been derived from only a few parental trees.

The heterozygosity (*Table 2*) shows that *C. brevifolia* has high variation (0.3420) while *C. deodara* has no variation at all. *C. libani* and *C. atlantica* are intermediate.

Generally, conifers exhibit high levels of heterozygosity. Up to now a notable exception to this rule was *Pinus resinosa* (FOWLER and MORRIS, 1977), probably as a result of a severe population restriction during the Pleistocene period. The fixation (zero heterozygosity) of *C. deodara* was unexpected, since the species occupies large areas, of about 500.000 ha (M'HIRIT, 1987) in Afganistan and the Himalayas. A possible explanation of this observation is the nature of the material used in the isozyme analysis. As noted above, samples of *C. deodara* were collected from an arboretum established with seeds of unknown origin. The lack of variation can be attributed to fixation of the donor source os the possible limited variation of the "European population".

Comparing the heterozygosity of the *Cedrus* species, excluding *C. deodara*, with that of other coniferous species, it appears that the heterozygosity of *C. brevifolia* is similar to that of *Abies sp.* (SCALTSOYIANNES, PANETSOS and ZARAGOTAS, 1990; SCALTSOYIANNES and PANETSOS, unpublished) while *Cedrus libani* and *Cedrus atlantica* have less heterozygosity.

This interpretation of enzyme variation of *Cedrus* sp. has important implications for future selection and improvement of the species. For *Cedrus deodara* more research is needed to solve the problem of its fixation. Research material should be derived from stands of its natural distribution. Further research on *C. atlantica* and *C. libani*, based on material from natural stands, will elucidate their taxonomic status.

Isozyme studies based on haploid tissue (endosperm) of *C. brevifolia* (unpublished) revealed the same pattern as the one presented in this study, coming from diploid tissue.

References

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. **71**: 33—40 (1980). —— ADAMS, W. T., NEALE, D. B., DOERKSEN, A. H. and SMITH, D. B.: Inheritance and Linkage of Isozyme Variants from seed and vegetative bud tissues in coastal *Douglas-fir* (*Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO). Silvae Genetica **39** (3—4): 153—167 (1990). —— ARBEZ, M.: Les ressources genetiques forestieres en France. Les coniferes 1. INRA, Paris (1987). —— BERGMANN, F.: Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genetica **24** (5—6): 175—177 (1975). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Inf. Rep. PI-X-2, Petawawa Nat. For. Inst., Canadian For. Serv., Agric. Canada (1984). —— CONKLE, M. T.: Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. For. Sci. **17**: 190—194 (1971). —— CONKLE, M. T.: Isozyme variation and linkage in six conifer species. Proc. Symp. on Isozymes of N. Am. Forest Trees and Forest Insects, Barkeley, California (1979). —— CONKLE, W. M., HODGSKISS, P. D., NUNNALLY, L. B. and HUNTER, S. C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA For. Serv. Gen. Tech. Rep. PSW-64, Pac. Southwest For. Range Exp. Stn. Berkeley, CA (1982). —— DAVIS, P. H.: Flora of Turkey and East Aegean Islands. Vol. I. 71—72: Univ. Press, Edinburgh (1965). —— EL-KASSABY, Y. A.: Genetic interpretation of malate dehydrogenase isozymes in some conifer species. The Journal of Heredity **72**, 451—452 (1981). —— EL-KASSABY, Y. A., YEH, F. C. and SZIKLAI, O.: Inheritance of allozyme variants in coastal *Douglas fir* (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. **24**: 325—335 (1982)). —— FAO: Silviculture of Species: *Cedrus spp.* "Silva Mediterranea". FO: SCM/89/6 (1989). —— FOWLER, D. P. and MORRIS, R. W.: Genetic diversity in red pine: evidence for low genetic heterozygosity. Can. J. Forest Res. **7**: 343—347 (1977). —— GREUTER, W., BURDET, H. M. and LONG, G.: Med-Checklist. 1. Pteridophyta (ed. 2) Gymnosperemae Dicotyledonas (Acanthaceae — Gneoraceae). Edition des Conservatoire et jardin botaniques de la ville de Genève (1984). —— GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). Heredity **20**: 27—32 (1978). —— M'HIRIT, O.: Etat actuel des connaissances sur le Cedre. Elements pour un programme de recherche. Rapport Comité C. F. A./C.E.F./C.F.P.O. "Silva Mediterranea" (1987). —— MILLAR, C. I.: Inheritance of allozyme variants in Bishop pine (*Pinus muricata* D. DON.). Biochem. Genet. **23**: 933—946 (1985). —— MITTON, J. B., LINHART, Y. B., STURGEON, K. B. and HAMRICK, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**: 86—89 (1979). —— NEALE, D. B. and ADAMS, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**: 1285—1291 (1981). —— NEALE, D. B., WEBER, J. C. and ADAMS, W. T.: Inheritance of needle tissue isozymes in Douglas-fir. Can. J. Genet. Cytol. **26**: 459—468 (1984). —— NEI, M.: F-Statistics and analysis of gene diversity in subdivided populations. ann. Hum. Genet. **41**: 225—233 (1977). —— NEI, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583—590 (1978). —— SCALTSOYIANNES, A., PANETSOS, K. P. and ZARAGOTAS, D.: Genetic variation of Greek-fir as determined by isozyme analysis and its relation to other Mediterranean Firs. Publ. EEC Cat. No CD-NA-13491-2A-C: 99—117 (1991). —— SCALTSOYIANNES, A. and PANETSOS, K. P.: Isozyme variation of Mediterranean Firs. (unpublished). —— TOTH, J.: Le Cedre dans les pays du pourtour Mediterraneen et dans deux autres pays et son importance forestière. Forests Mediterranneenes **II**, (1), 23—30 (1980).

Inheritance and Linkage of Some Allozymes in Taxus baccata L.

By A. LEWANDOWSKI, J. BURCZYK, and L. MEJNARTOWICZ

Institute of Dendrology, Department of Genetics,
Polish Academy of Sciences, 62—035 Kórnik, Poland

(Received 9th March 1992)

Summary

Eleven enzyme systems coding for 22 loci were assayed in *Taxus baccata*. Mendelian inheritance was confirmed for allozymes at 11 loci by testing the fit of band-pattern segregation in macrogametophytes from heterozygous trees to the expected 1:1 ratio.

Linkage relationships were examined for 54 pairs of allozyme loci showing joint segregation. Five pairs of loci appear to be linked: Pgi2-Sod2 with a recombination frequency (r) = 0.15, Pgi2-Pgm1, with r = 0.24, Pgm1-Sod2, with r = 0.27, Est1-G6p, with r = 0.30, and Est1-Lap2, with r = 0.30.

Key words: *Taxus baccata*, electrophoresis, allozymes, inheritance, linkage.

Introduction

In the last decade electrophoretic variants of enzymes have become very popular genetic markers in forest genetic research. However, the advantages of isozymes for population genetic studies can be fully realized only if the inheritance of isoenzyme band patterns is known.

Simple Mendelian inheritance of allozyme loci has been described for many members of the *Pinaceae* and some species from other conifer taxa (for review see PAULE, 1990).

Data on linkage is especially important when multilocus genotypes are used as genetic markers. The information obtained from linkage maps of various species also proved useful for characterizing phylogenetic relationships among them (CHELIAK and PITEL, 1985). Recently, several studies have been published, demonstrating for conifers some linked allozyme loci (see SZMIDT and MUONA, 1989).

This report describes 11 enzymes extracted from seeds of English yew (*Taxus baccata* L.), a member of the Taxaceae. Mendelian inheritance is inferred using segregation date from analyses of macrogametophytes. Linkages were examined among 54 pairs of allozyme loci.

Material and Methods

Seed samples

Seeds for electrophoresis were collected from 41 trees growing in a natural stand of English yew (*Taxus baccata* L.) in Wierzchlas, Poland. Seeds were collected on November 1990, when the arils were red and the outer surface of seeds dark green. The seeds were immediately cleaned and then stratified following SUSZKA (1985).

At first the seeds were stratified for 6 months at a cyclically alternating temperature 15~20°C (24+24 hours/cycle) after that a cool stratification at 3°C was continued for about 4 months until the seeds started to germinate.

Seven macrogametophytes from each of the 41 studied trees were first sampled to define genotypes. Afterwards, additional 10 to 64 macrogametophytes per tree were analyzed from 19 highly heterozygous trees that were selected to study linkage and to verify the segregation ratio.

Electrophoretic methods

Macrogametophyte tissue and embryo were isolated separately from the seeds and homogenized in 50 μl of TRIS-HCl buffer pH 7.2 with the addition a 0.15% 2-mercaptoethanol.

Homogenates were subjected to horizontal (12%) starch gel electrophoresis by applying the following 2 buffer systems (electrode buffer/gel buffer): System I, 0.06 M lithium hydroxide — 0.3 M boric acid, pH 8.1 / 0.03 M TRIS — 0.005 M citric acid — 1% electrode buffer, pH 8.5 (RIDGEWAY et al., 1970). System II, 0.13 M TRIS — 0.043 M citric acid, pH 7.0 / 1:10 dilution of electrode buffer (SICILIANO and SHAW, 1976).

Gel slices were stained for the activity of 11 different enzymes using recipes described by CHELIAK and PITEL (1984). The enzymes, their abbreviations, enzyme commission codes and buffer systems upon which they were run are listed in *table 1*.

The zone specifying the most anodally migrating variants was designated as 1, the next as 2, and so on. Within each zone, the most frequent variant was assigned the value of 100. Other variants of the zone were described according to their mobility relative to the most frequent variant. Variants lacking stain activity were designated al null (N). For multiple-banded variants, the slowest band was used for calculating relative mobility.

Statistical analysis

The inheritance of alloymze polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the expected 1:1 ratio, using the chi-square test. The chi-square test was also used for the estimation of heterogeneity of results pooled over all trees (MATHER, 1963, p. 13 to 25 and 69 to 90).

The statistical evaluation of linkage relationships was done using the three-way maximum likelihood G-test (SOKAL and ROHLF, 1981, p. 722 to 724). When the several trees studied for an allozyme locus pair were homogeneous both for segregation at single loci and for linkage in a G-test, the data were pooled. Recombination fraction (R) as calculated by the binomial estimator: R = r/n, where r is the number of recombinant types observed and n is the total number of observations. The standard error of this estimate is given by:
$[R(1—R)/n]^{1/2}$ (RUDIN and EKBERG, 1978).

Table 1. — Enzyme and buffer systems used for electrophoretic analyses of *Taxus baccata*.

Enzyme	Abbreviation	Enzyme Commission number	Buffer system
Esterase	EST	3.1.1.1	I
Fluorescent esterase	FEST	3.1.1.1	I
Glutamate-oxaloacetate-transaminase	GOT	2.6.1.1	I
Glucose-6-phosphate dehydrogenase	G6P	1.1.1.49	I
Isocitrate dehydrogenase	IDH	1.1.1.42	II
Leucine aminopeptidase	LAP	3.4.11.1	I
Menadione reductase	MNR	1.6.99.2	II
6-phosphogluconate dehydrogenase	6PGD	1.1.1.44	II
Phosphoglucose isomerase	PGI	5.3.1.9	I
Phosphoglucomutase	PGM	2.7.5.1	I
Superoxide dismutase	SOD	1.15.1.1	II

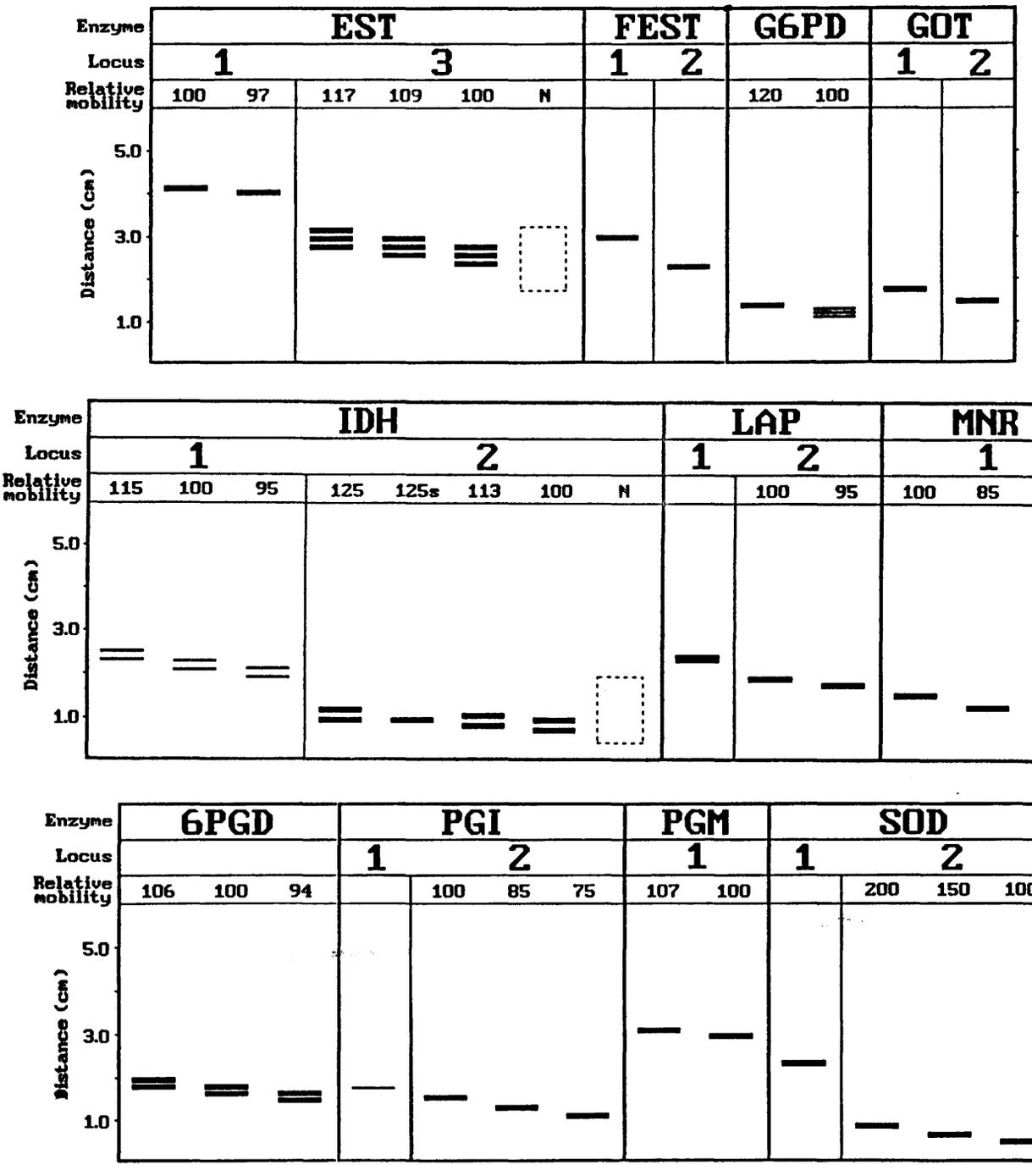

Figure 1. — Enzyme phenotypes found in *Taxus baccata*. Mobility is expressed relative to the most common variant, set to be 100 (see text).

Results and Discussion

Inheritance of isozyme patterns

Twenty two loci were identified from the 11 enzyme systems analyzed, of which four (Est2, Got3, Mnr2 and Pgm2) could not be scored consistently. Zymogram phenotypes for the remaining 18 allozyme loci are presented in *figure 1*. Mendelian inheritance was tested only for the eleven best staining polymorphic allozyme loci. In summary, no significant deviation from the expected 1:1 segregation ratio nor chi-square — heterogeneity tests were observed at any of the loci studied (*Table 2*), indicating that these allozymes exhibited distinct, co-dominant expression and simple Mendelian segregation in their mode of inheritance. We have not found any previous information about the mode of inheritance of allozyme loci in *Taxus baccata*.

Description of the observed isozyme patterns is presented below.

Esterase (EST)

There were 3 zones of activity on gels stained for EST. Est1 and Est3 stained intensively and consistently, while Est2 stained faintly or was absent on many gels thus it was excluded from this study. Two single-banded (97, 100) and three triple-banded (100, 109 and 117) variants were

Table 2. — Observed allozyme segregation in macrogametophytes of heterozygous trees and chi-square tests for goodness of fit to a 1:1 ratio and heterogeneity among employed trees.

Locus	Allelic combination	Observed segregation	Deviation χ^2 test (1 df)	Heterogeneity χ^2 test (df)
Est-1	97/100	88:86	0.02	4.47 (8)
Est-3	100/109	75:86	0.75	1.48 (8)
	100/117	29:33	0.26	1.45 (3)
	109/117	36:45	1.00	1.30 (4)
	109/N	12:8	0.80	-
G6p	100/120	81:80	0.01	3.55 (9)
Idh-1	95/100	21:27	0.75	0.29 (2)
	95/115	7:7	0.00	0.31 (1)
	100/115	142:120	1.85	5.11 (10)
Idh-2	100/113	9:7	0.25	-
	100/125s	9:7	0.25	0.77 (1)
	100/125	87:86	0.01	1.99 (5)
	100/N	33:25	1.10	0.05 (2)
	125s/125	10:17	1.81	-
Lap-2	95/100	92:92	0.00	4.39 (11)
Mnr-1	65/85	28:38	1.52	1.38 (2)
	65/100	80:77	0.06	5.84 (7)
	85/100	89:94	0.14	2.69 (6)
6Pgd	94/100	76:94	1.91	3.32 (7)
	94/106	13:20	1.48	1.00 (2)
	100/106	39:26	2.60	0.06 (2)
Pgi-2	70/85	39:31	0.91	2.05 (4)
	70/100	140:116	2.25	5.56 (8)
	85/100	148:126	1.77	7.41 (8)
Pgm-1	100/107	77:71	0.24	4.38 (8)
Sod-2	100/150	40:26	2.97	-
	100/200	144:120	2.18	5.74 (9)

NOTE: None of the chi-square tests was statistically significant.

observed for Est1 and Est3 regions, respectively. In Est3 we also found a null variant i. e. with no enzyme activity. Among other conifer tree species, the number of esterase loci described, varied from one in *Pinus attenuata* (Strauss and Conkle, 1986) to 5 in *P. torreyana* (Ledig and Conkle, 1983).

Fluorescent esterase (FEST)

Two single-banded invariant zones of activity were found on gels stained for FEST.

Glucose-6-phosphate dehydrogenase (G6PD)

One zone of activity was evident on gels stained for this enzyme. In this zone, two variants: a triple-banded one (100) and a single-banded one (120) were observed. A single zone of activity for this enzyme was reported also for *Larix* species (Cheliak and Pitel, 1985; Fins and Seeb, 1986; Lewandowski and Mejnartowicz, 1990).

Glutamate oxaloacetate transaminase (GOT)

There were 3 zones of activity on gels stained for this enzyme. The 2 most anodal zones (Got1 and Got2) were invariant for all 41 trees. The most cathodal zone Got3 was highly polymorphic, but bands at this zone stained faintly or were absent on many gels thus it was excluded from this study. Three loci for GOT were also reported for many other conifer species (El-Kassaby et al., 1982; Cheliak and Pitel, 1985; Harry, 1986; Müller-Starck and Liu, 1988; Muona et al., 1987; Wang et al., 1990).

Isocitrate dehydrogenase (IDH)

Two zones of activity occurred on gels stained for IDH. The upper, less intensely stained zone (Idh1) had three double-banded variants (95, 100, 115). The lower zone (Idh2) was stained more intensely and had one single-banded variant (125s) and three double-banded variants (100, 113 and 125). In Idh2 we also found a null variant, i. e. with no enzyme activity. Two zones of IDH activity in conifers have been reported by Harry (1986), Muona et al. (1987), Müller-Starck and Liu (1988).

Leucine aminopeptidase (LAP)

Two zones of activity were evident on gels stained for LAP. Only the most cathodal zone (Lap2) showed variants (95 and 100). Genetic control of LAP loci by 2 independent loci has been reported for several other conifer species (Bergmann, 1973; Mejnartowicz, 1976; Rudin, 1977;

Guries and Ledig, 1978; Adams and Joly, 1980; Cheliak and Pitel, 1985; Wang et al., 1990).

Menadione reductase (MNR)

Analysis of MNR electrophoretic patterns revealed two regions, but the most cathodal zone Mnr2 was not consistent enough to score and was not included in the study. Three variants (65, 85 100) were observed for Mnr1. MNR in *Taxus baccata* seems to be equivalent to Diaphorase, similarly as for some other conifer species, see Lewandowski and Mejnartowicz (1990).

6-Phosphogluconate dehydrogenase (6PGD)

Gels stained for 6PGD had two zones of activity. Three double-banded variants (94, 100 and 106) were observed at the more anodal zone. The second (more cathodal) zone stained much less intensely and seems equivalent to Idh2. This zone can also be detected without adding the staining substrate. It is possible that IDH can use citric acid present in the gel as a substrate. For this reason we proposed the control of 6PGD by a single locus in *Taxus baccata.*

Phosphoglucose isomerase (PGI)

Two zones of PGI activity were observed. The faster migrating zone was invariant for all 41 trees. Pgi2 exhibited three variants (70, 85 and 100). In other conifers 2 zones of PGI activity have usually been reported (Guries and Ledig, 1978; Adams and Joly, 1980; El-Kassaby et al., 1982; Harry, 1986; Muona et al., 1987; Wang et al., 1990).

Phosphoglucomutase (PGM)

Two zones of activity were detected on PGM gels. Pgm2 stained faintly or was absent on many gels thus it was excluded from this study. Pgm1 stained much more intensely than Pgm2 and had 2 variants (100 and 107). The fainter staining of the more cathodal zone of Pgm-2 was also reported for some other conifers (Simonsen and Wellendorf, 1975; Guries and Ledig, 1978).

Superoxide dismutase (SOD)

Two zones of activity were detected on SOD gels. The faster region Sod1 was monomorphic. Sod2 containing three well distinguished variants (100, 150 and 200). Two zones of activity for this enzyme were reported for *Larix decidua* (Lewandowski and Mejnartowicz, 1990).

Banding patterns of macrogametophytes and corresponding embryos indicate for IDH, LAP, PGI, PGM and SOD that the same loci are active in both tissues. Other enzymes could not be scored in embryos because of poor and/or blur band resolution.

When the allozyme locus was clearly expressed in the embryo and when heterozygous embryos were found, the quaternary structures of allozymes were inferred. On gels stained for Lap2 and Pgm1, only one or two-banded patterns were found in embryos indicating that these enzymes are probably monomers, which is in accordance with results reported for other conifer taxa (Adams and Joly, 1980; El-Kassaby et al., 1982; Millar, 1985; Harry, 1986). On the other hand, some embryos stained for Idh1, Pgi2 and Sod2 showed an additional third band of intermediate mobility, which suggest dimeric structure of these enzyme. Such a suggestion has also been described for other conifer taxa (Guries and Ledig, 1978; El-Kassaby et al, 1982; Millar, 1985; Harry, 1986; Müller-Starck and Liu, 1988).

Linkage

Out of the 55 possible two-locus combination which can be formed from 11 polymorphic loci, 54 pairs of allozyme loci were compared in at least one tree. Of these 54 pairs 47 pairs were analyzed in more than one tree. *Table* 3 shows the combinations of allozyme loci tested and the number of trees studied. The linkage was found between five pairs of allozyme loci (*Table 4*). Tight blocks were indicated for 3 allozyme loci, in linear order: Pgm1, Pgi2 and Sod2. Pooled estimate of recombination frequencies, over all trees samples, for Pgm1 : Pgi2, Pgm1 : Sod2 and Pgi2 : Sod2 were 0.24, 0.27, and 0.15, respectively. The rest of the linkages detected were less close. Est1 was linked to Lap2, with recombination frequencies 0.30, in the overall data from three trees. Also Est1 and G6p showed 0.30 recombination frequency in total data, but linkage was significant in only one of the 2 investigated trees.

The 5 linked pairs are reported here for the first time for *Taxus baccata.* Comparison of linkage relationships in *Taxus baccata* with those reported for other conifer species is problematical, because of variation in electrophoretic methods and isozyme terminology used by different authors and the possibilities that the same zone of isozyme variation in different species may not be coded by the same gene.

The Pgi2 and one locus of SOD have been found in the same linkage block (designated as A) in *Pinus jeffreyi* and in *P. contorta* (Conkle, 1981). The presence of Pgm1 in this linkage group in *Taxus baccata* is interesting. Some comparable information from other conifer taxa shows independence between Pgi2 and Pgm1 (Stewart and Schoen, 1986; Muona et al., 1987; Geburek and

Table 3. — Number of trees analyzed for linkage for each pair of allozyme loci in *Taxus baccata.*

Loci	1	2	3	4	5	6	7	8	9	10	11
1. Est-1	*	5	2	3	1	3	3	1	5	2	4
2. Est-3		*	1	3	2	4	4	4	4	2	4
3. G6p			*	(-)	2	4	4	3	5	3	1
4. Idh-1				*	2	3	5	1	6	1	3
5. Idh-2					*	2	4	4	7	2	2
6. Lap-2						*	5	6	6	5	1
7. Mnr-1							*	4	10	4	3
8. 6Pgd								*	6	3	2
9. Pgi-2									*	4	7
10. Pgm-1										*	2
11. Sod-2											*

NOTE: (—) indicates not tested two-locus combination.

Table 4. — Significantly linked pairs of allozyme loci in *Taxus baccata* with recombination frequency (R), its stadard devination (SD), G-test for linkage and proportion of trees studied with significant linkage.

Pair of allozyme loci	Total seeds	Recombination frequency R (SD)	G-test	N linked/ /N studied
Est1/G6p	27	0.30 (0.09)	4.61 *	1/2
Est1/Lap2	53	0.30 (0.06)	8.55 **	2/3
Pgi2/Pgm1	112	0.24 (0.04)	31.55 ***	3/4
Pgi2/Sod2	234	0.15 (0.02)	130.42 ***	7/7
Pgm1/Sod2	62	0.27 (0.06)	13.11 **	2/2

NOTE: Significant levels: *) — $P < 0.05$, **) — $P < 0.01$, ***) — $P < 0.001$

WUEHLISCH, 1989). Pgi2 was tightly linked to Got1 in nearly all conifer species studied (for review see SZMIDT and MUONA, 1989). It would have been interesting to check this result in *Taxus baccata* but unfortunately in our material two most anodally migrating zones of GOT were invariant. In some pine species (*Pinus contorta, P. taeda* and *P. jeffreyi*) CONKLE (1981) mapped also Est4, G6p2 and Lap2 in the linked block A, besides the Pgi2 and SOD loci. There is evidence about weak linkage between G6p, Lap2 and Est 1 loci in *Taxus baccata,* however we did not find any evidence for linkage of these loci with Pgi2, Sod2 and Pgm1. Lap2 and Pgi2 appear to be linked on the same block in several species (ECKERT et al., 1981; STRAUS and CONKLE, 1986; SZMIDT and MUONA, 1989). To explain whether differences in linkage relationships among various loci between *Taxus baccata* and other *Pinaceae* species results from phylogenetic differences between them, further studies with other *Taxus* species and involving additional allozyme loci, would be needed.

Literature Cited

ADAMS, W. T. and JOLY, R. J.: Genetics of allozyme variants in loblolly pine. J. Hered. **71**: 199—202 (1980)). —— BERGMANN, F.: Genetische Untersuchungen bei *Picea abies* mit Hilfe der Isoenzym-Identifizierung. II. Genetische Kontrolle von Esterase und Leucinaminopeptidase-Isoenzymen im haploiden Endosperm ruhender Samen. Theor. Appl. Genet. **43**: 222—225 (1973). —— CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Inf. Rep. PI-X-2, Petawawa Nat. For. Inst., Canadian For. Serv., Agric. Canada (1984). —— CHELIAK, W. M. and PITEL, J. A.: Inheritance and linkage of allozymes in *Larix laricina.* Silvae Genet. **34**: 142—148 (1985). —— CONKLE, M. T.: Isozyme variation and linkage in six conifer species. In: Proceed. Symp. Isozymes of North Americ. Forest Tree and Forest Insects. (1979). Berkeley, Calif. Gen. Tech. Rep. PSW-48. Forest Serv., U. S. Dept. Agric., Berkeley, Calif., 11—17 (1981). —— ECKERT, R. T., JOLY, R. J. and NEALE, D. B.: Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For Res. **11**: 572—579 (1981). —— EL-KASSABY, Y. A., YEH, F. C. and SZIKLAI, O.: Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Gen. Cytol. **24**: 325—335 (1982). —— FINS, L. and SEEB, L. W.: Genetic variation in allozymes of western larch. Can. J. For. Res. **16**, 1013—1018 (1986). —— GEBUREK, T. and WUEHLISCH, G.: Linkage analysis of isozyme gene loci in *Picea abies* (L.) KARST. Heredity **62**: 185—191 (1989). —— GURIES, R. P. and LEDIG, F. T.: Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* MILL.). Heredity **40**: 27—32 (1978). —— HARRY, D. E.: Inheritance and linkage of isozyme variants in incense-cedar. J. Hered. **77**: 261—266 (1986). —— LEDIG, F. T. and CONKLE, M. T.: Gene diversity and genetic structure in narrow endemic, Torrey pine (*Pinus torreyana* PARRY ex CARR.). Evolution **37**, 79—85 (1983). —— LEWANDOWSKI, A. and MEJNARTOWICZ, L.: Inheritance of allozymes in *Larix decidua* MILL. Silvae Genet. **39**, 184—188 (1990). —— MATHER, K.: The measurement of linkage in heredity. Chapter 2: 13—26 and chapter 7: 69—90. Methuen and C. Ltd., London (1963). —— MEJNARTOWICZ, L.: Genetic investigations on Douglas-fir (*Pseudotsuga menziesii* MIRB.) FRANCO) populations. Arbor. Kórnickie **21**, 124—187 (1976). —— MILLAR, C. I.: Inheritance of allozyme variants in bishop pine (*Pinus muricata* D. DON.). Biochem. Genet. **23**: 933—946 (1985). —— MUONA, O., YAZDANI, R. and LINDQUIST, G.: Analysis of linkage in *Picea abies.* Hereditas **106**: 31—36 (1987). —— MÜLLER-STARCK, G. and LIU, Y.-Q.: Genetics of *Cuninghamia lanceolata* HOOK. 1. Genetic analysis. Silvae Genet. **37**: 236—243 (1988). —— PAULE, L.: Bibliography: Isozymes and forest trees (1968 to 1989). Report 9, Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå (1990). —— RIDGEWAY, G. J., SHERBURNE, S. W. and LEWIS, R. D.: Polymorphism in the esterase of Atlantic herring. Trans. Am. Fish. Soc. **99**: 147—151 (1970). —— RUDIN, D.: Leucine-aminopeptidases (LAP) from needles and macrogametophytes of *Pinus sylvestris.* Hereditas **85**: 219—226 (1977). —— RUDIN, D. and EKBERG, I.: Linkage studies in *Pinus sylvestris* L. using macrogametophyte allozymes. Silvae Genet. **27**: 1—12 (1978). —— SICILIANO, M. J. and SHAW, C. R.: Separation and visualization of enzymes on gels. Chromatographic and electrophoretic techniques. Heinemann, London, 185—209 (1976). —— SIMONSEN, V. and WELLENDORF, H.: Some polymorphism isoenzymes in the seed endosperm of Sitka spruce (*Picea sitchensis)* (BONG.) CARR.). For. Tree Improv. **9**: 5—20 (1975). —— SOKAL, R. R. and ROHLF, F. J.: Biometry. Second ed. W. H. Freeman, San Francisco (1981). —— STEWARD, S. C. and SCHOEN, D. J.: Segregation at enzyme loci in megagametophytes of white spruce, *Picea glauca.* Can. J. Genet. Cytol. **28**: 149—153 (1986). —— STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483—493 (1986). —— SUSZKA, B.: Conditions for after-ripening and germination of seeds and for seedling emergence of English yew (*Taxus baccata* L.). Arbor Kórnickie **30**: 285—338 (1985). —— SZMIDT, A. E. and MUONA, O.: Linkage relationships of allozyme loci in *Pinus sylvestris.* Hereditas **117**: 91—97 (1989). —— WANG, X.-R., SHEN, X.-H. and SZMIDT, A. E.: The choice of allozyme markers for studies in conifer seed orchards: the case of *Pinus tabulaeformis* CARR. In: Biochemical markers in the population genetics of forest trees. pp. 173—181. Edited by S. FINESCHI, M. E. MALVOLTI, F. CANNATA and H. H. HATTEMER. SPB Academic Publishing, Hague (1990).