Inheritance and Linkage Relationships of Isozymes of *Picea glehnii* (MASTERS.)

By Z. M. WANG[1]), K. NAGASAKA[1])[2]) and K. TANAKA[1])

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Summary

Isozyme variations in 11 enzyme systems were studied for *Picea glehnii* (MASTERS.) by polyacrylamide gel electrophoresis using haploid megagametophyte tissues from 38 plus tree clones. These 11 enzymes were encoded by 21 loci with only one of them (*Dia-4*) monomorphic. A total of 57 alleles were detected. Segregation distortion from the expected 1:1 ratio was found in *Aap-1* (b:c) and *Dia-3* (a:b). Except *Aap-3* and *Aap-4* which were completely linked, linkage relationships were examined for 108 2-locus combinations. Loose linkage was observed between loci *Shd-1* and *G6p, Aap-1* and *Dia-1*, and *Aap-1* and *Dia-2* with recombination frequencies ranging from 0.34 to 0.45, and tight linkage was confirmed between *Shd-2* and *Got-1, Aap-1* and *Got-3,* and *Aap-2* and *Est* with recombination frequencies ranging from 0.19 to 0.26.

Key words: tree, *Picea glehnii,* spruce, genetics, isozyme, polyacrylamide gel electrophoresis, inheritance, linkage.

FDC: 165.3; 165.5; 174.7 *Picea glehnii.*

Introduction

Electrophoresis has been widely used to study forest trees since its introduction to population genetics about 30 years ago (HUDDY and LEWONTIN, 1966). It takes the advantage of allozymes which are gene products and usually codominant, and their mode of inheritance often follows simple Mendelian genetics. It has been found that forest trees share some general genes (CONKLE, 1992) and linkage relationships (CHELIAK and PITEL, 1984; SZMIDT and MUONA, 1989). However, isozyme characteristics do differ among species, and it is important to demonstrate the inheritance of allozymes before using them to study genetics of a species (MILLAR, 1985).

Unlike endosperms in angiosperms, the megagametophyte tissues of conifer seed are haploid. Therefore, they allow direct observation of Mendelian segregation at heterozygous isozyme loci and permit simultaneous study of inheritance and linkage without controlled crosses. However, their utility depends on the inheritance and linkages of electrophoretically detectable polymorphisms at different isozyme loci. If segregation distortion is significant, the loci concerned might bias some genetic analyses (CHELIAK et al., 1984). Knowing linkage relationships among allozymes is essential because various analyses in population genetics require data from independent loci, such as mating systems and genetic distances (SHAW et al., 1981; NEI, 1975). Recombination fractions between loci can be used for constructing linkage maps which have applications in marker-assisted selection, dissection of quantitative trait loci, and map-based gene cloning (TANKSLEY et al., 1989).

Picea glehnii MASTERS. is one of the only 2 native spruce species growing in Hokkaido, Japan. It is widely distributed in Hokkaido, but there is only one small stand found in Mt. Hayachine in northern Honshu in Japan (HORIKAWA, 1972). It grows in various habitats from rocky places to bogs, and up to 2000 m in elevation (HORIKAWA, 1972). Forming pure stands or mixed stands with other tree species, its mature trees can reach 40 m in height and 1.5 m in DBH (SATO, 1990). It is a major forest tree species in Hokkaido and has great economical and ecological significance. It has been planted in large areas in Hokkaido recently (MATSUDA, 1989). Tree improvement programs by plus tree selection, seed orchards and genetic conservation stands have been established for the species in Hokkaido. However, its genetics has not been well studied, and there are only 2 studies reported so far. One (OKADA, 1975) looked at the geographical variation in seedling height growth and bud opening phenology among 12 seed sources of the species in the nursery of Hokkaido Forest Tree Breeding Station, and another studied inheritance of 6 isozymes coded by 10 loci using about 20 seeds from each of 18 trees (KUBOTA et al., 1993). These authors did not analyse linkage relations in their study. Here, we report the inheritance and linkage of 11 isozymes coded by 20 loci for the species.

Materials and Methods

Seeds from 38 open-pollinated *Picea glehnii* trees selected as plus trees over a wide range in Hokkaido were used in this study. They were collected from different seed orchards in different years ranging from 1979 to 1990 and stored at 0 °C until use. Seeds were germinated at room temperature, and 30 to 102 individual megagametophytes were obtained from germinants with visible radicles from each tree and assayed. Tissue preparation and extraction, and polyacrylamide gel electrophoretic techniques followed those of SHIRAISHI (1988a) and TSUMURA et al. (1990). The 11 enzyme systems surveyed and their loci detected were: alanine aminopeptidase (*Aap-1, Aap-2, Aap-3, Aap-4*), diaphorase (*Dia-1, Dia-2, Dia-3, Dia-4*), esterase (*Est*), fumarase (*Fm*), glucose-6-phosphate dehydrogenase (*G6p*), glutamate oxaloacetate transaminase (*Got-1, Got-2, Got-3*), leucine aminopeptidase (*Lap*), menadione reductase (*Mnr-1, Mnr-2*), shikimate dehydrogenase (*Shd-1, Shd-2*), sorbitol dehydrogenase (*Sod*), and 6-phosphogluconic dehydrogenase (*6pg*). If multiple loci occurred for an enzyme, the slowest migrating locus was designated as '1', the next as '2', and so on. Within each locus, the slowest migrating allele was designated as 'a', and the next as 'b', and so on. An allele lacking stain activity (null) was designated as 'n'.

For heterozygous trees, segregation data of each polymorphic locus were pooled to test deviations from 1:1 segregation ratio by log-likelihood G-test (SOKAL and ROHLF, 1981). A G-value was also calculated to test heterogeneity in the observed segregation ratios among trees. Linkage was examined for each possible 2-locus combination by x^2-test (RUDIN and EKBERG, 1978). The expected number for the test was calculated according to BAILEY (1961). The x^2-test for deviation of the observed 2-locus segregation from the expected ratio was partitioned into 3 components with 1 df each, 2 for testing segregation at each locus and a third for testing the independence of loci. BAILEY (1961) has shown that if both loci, not only one of them, deviate significantly from the expected 1:1 ratio,

[1]) Present address: Forest Genetics Laboratory, Forestry and Forest Products Research Institute, Hokkaido Research Center, Hitsujigaoka 7, Toyohira, Sapporo, Japan 062.

[2]) To whom requests for reprints should be sent at: Genetics Analysis Laboratory, Forestry and Forest Products Research Institute, Matsunosato 1, Kukizaki, Ibaraki, Japan 305.

x^2-test for detection of linkage is invalid. For 2-locus pairs with heterogeneous data among trees, a 3-way log-likelihood test was used to test for independence (SOKAL and ROHLF, 1981). The recombination frequencies (r) and their standard errors (SE_r) were estimated using the methods of BAILEY (1961): r=f/n and $SE_r=(r(1-r)/n)^{1/2}$, where f is the number of recombinant types observed in n megagametophytes. For all the tests, 5% significance level was used.

Results and Discussions

Segregation of Alleles at Different Loci

Alanine Aminopeptidase (AAP)

Five zones of activity were observed in AAP gels. One of them between *Aap-3* and *Aap-4* was excluded because of its faint and inconsistent staining, thus only 4 of them were scored for this enzyme (*Fig. 1*). However, the other one stained well in LAP gels, and it became the only one scored for LAP which will be discussed later.

Aap-1 had 4 single-banded phenotypes. Among the 4 segregation allele pairs, only b:c showed significant deviation from the expected 1:1 ratio in pooled G-test with homogeneous segregation between trees (*Table 1*). However, the same allele c in combinations with alleles d and a did not have segregation distortion. Therefore, the distortion of b:c might be due to selection against allele *Aap-1*^{b} or one or more genes tightly linked to this allele.

Table 1. – Log-likelihood G-test on segregation ratios of 19 polymorphic loci in *Picea glehnii* seeds.

LOCUS	Geno-type	Observed ratio	Pooled G[1] Value	Pooled G[1] P(df=1)	Heterogeneity G[2] Value	Heterogeneity G[2] df	Heterogeneity G[2] P
Aap-1	c:d	49:58	0.7579	0.38	0.2434	1	0.62
	b:c	38:66	7.6323	0.01	0.0886	1	0.76
	a:c	133:109	2.3841	0.12	2.0247	4	0.73
	a:d	60:48	1.3361	0.24	0.6005	1	0.43
Aap-2	a:b	270:286	0.4605	0.49	10.8862	10	0.36
	b:c	34:20	3.6714	0.06	-[3]	-	-
	c:d	202:226	1.3465	0.24	15.8579	8	0.04
	a:c	86:73	1.0641	0.30	1.9251	2	0.38
Aap-3	a:b	42:46	0.1819	0.66	0.2898	1	0.59
Dia-1	b:c	288:283	0.0437	0.83	14.5467	10	0.15
	a:b	125:113	0.6053	0.43	8.1122	4	0.08
Dia-2	a:b	136:140	0.0579	0.81	3.2915	5	0.65
Dia-3	a:b	145:111	4.5289	0.03	1.9878	4	0.73
Est	a:b	24:30	0.6681	0.41	-	-	-
	a:n	27:23	0.3203	0.57	-	-	-
Fm	a:b	30:24	0.6681	0.41	-	-	-
G6p	a:c	249:246	0.0182	0.89	5.2323	8	0.73
	a:b	129:115	0.8037	0.37	1.4317	4	0.83
	b:c	23:29	0.6939	0.40	-	-	-
Got-1	b:c	491:472	0.3748	0.54	19.6023	19	0.41
	a:c	24:24	0.0000	1.00	-	-	-
Got-2	b:c	81:63	2.2559	0.13	3.4300	2	0.17
	a:b	38:44	0.4394	0.50	2.1309	1	0.14
Got-3	a:b	59:49	0.9272	0.33	1.8359	1	0.17
Lap	a:n	35:27	1.0351	0.30	-	-	-
Mnr-1	a:b	25:29	0.2966	0.58	-	-	-
Mnr-2	a:b	27:23	0.3203	0.57	-	-	-
	b:c	30:24	0.6681	0.41	-	-	-
6pg	b:c	267:292	1.1184	0.29	11.8491	10	0.29
	a:b	49:55	0.3464	0.55	0.0385	1	0.84
Shd-1	c:e	157:143	0.6536	0.41	5.6080	5	0.34
	c:d	104:95	0.4072	0.52	2.7007	3	0.44
	b:c	70:82	0.9484	0.33	0.1265	1	0.72
	a:c	29:25	0.2965	0.58	-	-	-
	a:b	21:14	1.4095	0.23	-	-	-
	d:n	28:20	1.3396	0.24	-	-	-
Shd-2	b:c	101:106	0.1207	0.72	1.6535	4	0.79
	a:c	137:133	0.0593	0.80	11.3053	4	0.02
	a:b	25:25	0.0000	1.00	-	-	-
Sod	a:b	199:201	0.0100	0.92	4.1503	7	0.76

[1]) Pooled G tests the overall deviation from 1:1 ratio.
[2]) Heterogeneity G tests the heterogeneity in segregation ratios among trees.
[3]) Analysis is based on single tree data.

Four double-banded variants were observed at *Aap-2* (*Fig. 1*). The distance between the 2 bands was the same for alleles a and c, and for alleles b and d. However, it was wider for the first 2 than for the second 2 alleles. Pooled G-test showed no significant deviation from the expected 1:1 segregation for the 4 genotypes (*Table 1*). But, trees had heterogeneous segregations at genotype c:d. For c:d, one of the 9 trees had significant deviations from 1:1 segregation. When data of this tree was removed, both the pooled and heterogeneity G-tests became non-significant with P-values of 0.91 and 0.33, respectively. Thus, the heterogeneity was caused by a single tree, which might be due to chance. Therefore, it was concluded that *Aap-2* is controlled by a single gene locus with 4 alleles.

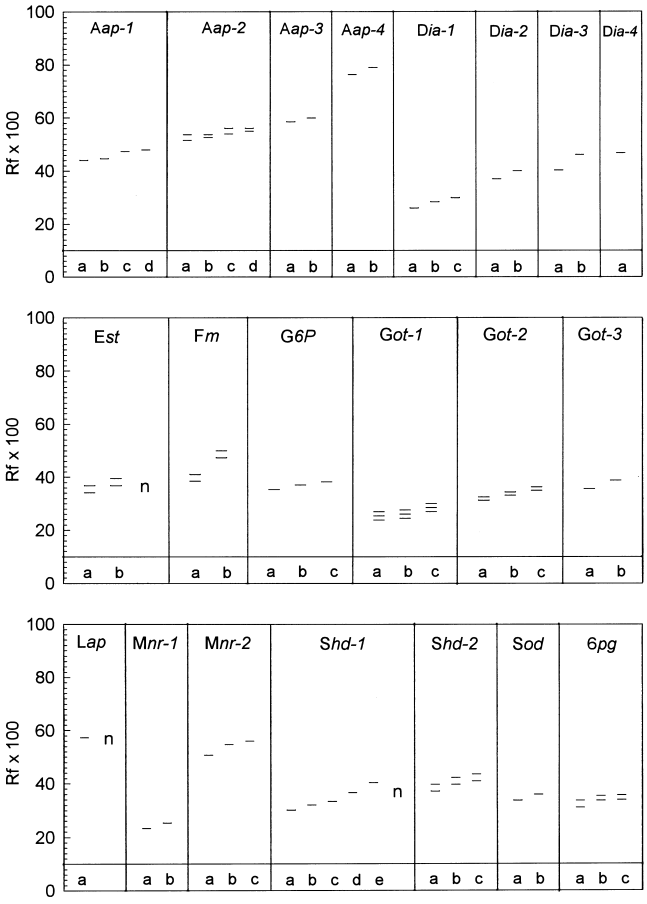

Fig. 1. – Megagametophyte (ln) banding patterns and their allelic designations for 21 allozyme loci in *Picea glehnii*. Rf is the migrational distance relative to that of Brome phenole blue. 'n' indicates a null allele.

Aap-3 and *Aap-4* both were single-banded with 2 alleles each, and the distance between the alleles was larger in locus *Aap-4* than in locus *Aap-3* (*Fig. 1*). Allele b of *Aap-3* and *Aap-4* only appeared in 2 trees, and it always varied identically for both loci. It seems that these 2 loci are completely linked. Thus, only results for *Aap-3* were given in *table 1*. Both the pooled and heterogeneity G values were not significant (*Table 1*), indicating that the mode of inheritance at both loci follows simple Mendelian law.

It seems AAP has not been used in isozyme studies for other spruce species before although it was easy to stain in *Picea glehnii*. However, single- and double-banded phenotypes in

AAP have also been observed in *Pinus densiflora* (NA'IEM et al., 1989) and *P. massoniana* (HUANG et al., 1994).

Diaphorase (DIA)

Two zones of activity were observed for this enzyme for *Picea abies* (MUONA et al., 1987), but 4 for *P. glauca* (KING and DANCIK, 1983). Four zones of activity were also observed in *Pinus massoniana* (HUANG et al., 1994), *Abies mariesii* (SUYAMA et al., 1992) and *A. pinsapo* (PASCUAL et al., 1993). In our gels stained for this enzyme, 4 zones of activity were evident with the fastest migrating one invariant. This zone was referred to correspond to a single locus (*Dia-4*) as it was variable in a very closely related species, *Picea jezoensis* (unpublished data of ours). Three and 2 single-banded variants were detected at *Dia-1* and *Dia-2*, respectively (*Fig. 1*), and the allelic segregation at these 2 loci fitted the expected 1:1 ratio (*Table 1*).

Dia-3 had 2 single-banded alleles (*Fig. 1*) which segregated differently from the expected 1:1 ratio (*Table 1*). One of the 5 heterozygous trees had equal number of a and b alleles, and the rest 4 had more a than b allele although none of them showed significant segregation distortion. This locus seems to be the only one observed by KUBOTA et al. (1993) in their study for the same species. However, they did not detect a significant segregation departure from the 1:1 ratio. A monomeric subunit structure of DIA enzyme has been observed in *Pseudotsuga menziesii* (ADAMS et al., 1990).

Esterase (EST)

There was more than one zone of activity in gels stained for this enzyme, but only one of them provided consistent staining clarity in all the gels. Thus, only this zone was scored for EST. This locus had 3 alleles with 2 of them being double-banded and another without stain activity (*Fig. 1*). There were 2 genotypes observed with only 1 tree each. The pooled G-test indicated that segregation at this locus followed the expected 1:1 ratio (*Table 1*). In other spruce species, different number of loci have been observed for EST (CHAISURISRI and EL-KASSABY, 1993). Single-banded patterns in *P. sitchensis* (CHAISURISRI and EL-KASSABY, 1993), and double- and triple-banded patterns and null alleles in EST have been reported previously for *Picea abies* (LUNDKVIST, 1977; BARTELS, 1971). Null alleles were also observed in *Pinus massoniana* (HUANG et al., 1994), *P. thunbergii* (SHIRAISHI, 1988a), *P. densiflora* (NA'IEM et al., 1989), *P. sylvestris* (RUDIN and EKBERG, 1978), *Calocedrus decurrens* (HARRY, 1986), and *Taxus baccata* (LEWANDOWSKI et al., 1992). A monomeric structure of EST was observed in *Picea abies* (BARTELS, 1971).

Fumarase (FM)

One zone of activity with 2 double-banded variants was stained for this enzyme (*Fig. 1*). Only one tree was heterozygous at this locus, and the G-test indicated that segregation at the locus did not deviate significantly from the expected 1:1 ratio (*Table 1*). A single locus with 4 single-banded phenotypes was observed for this enzyme in *Picea mariana* (BOYLE and MORGENSTERN, 1985), and with 2 in *Larix laricina* (YING and MORGENSTERN, 1990) and *Pinus thunbergii* (SHIRAISHI, 1988a). The molecule of FM is a monomer in *Picea mariana* (BOYLE and MORGENSTERN, 1985).

Glucose-6-phosphate Dehydrogenase (G6P)

Gels stained for G6P showed one zone of activity with 3 single-banded variants (*Fig. 1*). Segregation at this locus did not have significant departure from the expected 1:1 ratio (*Table 1*). The same loci and alleles were observed for the same species by KUBOTA et al. (1993). A single locus for this enzyme has also been reported for *Picea sitchensis* (CHAISURISRI and EL-KASSABY, 1993), *P. glauca* (CHELIAK and PITEL, 1984) and *P. mariana* (BOYLE and MORGENSTERN, 1985). G6P enzyme molecules are dimers in *Pseudotsuga menziesii* (EL-KASSABY et al., 1982).

Glutamate Oxaloacetate Transaminase (GOT)

This enzyme is also called aspartate aminotransferase (AAT). Three zones of activity were observed in gels stained for GOT. *Got-1* appeared as triple-banded phenotypes with 3 alleles, *Got-2* as double-banded with 3 alleles, and *Got-3* as single-banded with 2 alleles (*Fig. 1*). Segregations at all these 3 loci followed the expected 1:1 ratio (*Table 1*).

Three loci were also observed for GOT by KUBOTA et al. (1993) for the same species. However, they only found double- and single-banded phenotypes for *Got-1* and *Got-2,* respectively, and they did not find variation at *Got-3* locus. Three loci for GOT have been reported in *Picea abies* (MUONA et al., 1987), *P. mariana* (PITEL et al., 1987), *P. sitchensis* (CHAISURISRI and EL-KASSABY, 1993), *P. glauca* (CHELIAK and PITEL, 1984), and *P. pungens* and *P. engelmannii* (ERNST et al., 1987) and in many other conifers (FURNIER et al., 1986). Both single- and triple-banded phenotypes have been observed for GOT loci in *Picea mariana* (BOYLE and MORGENSTERN, 1985). In *Picea glauca*, 2 loci with triple-banded variants were observed (KING and DANCIK, 1983). In *Calocedrus decurrens* (HARRY, 1986) and *Pinus muricuta* (MILLAR, 1985), 3 loci with single-, double- and triple-banded phenotypes were observed.

The molecular structure of GOT enzyme has been found to be dimeric in other spruce species, *P. mariana* (BOYLE and MORGENSTERN, 1985; PITEL et al., 1987), *P. glauca* (CHELIAK and PITEL, 1984), *P. pungens* and *P. engelmannii* (ERNST et al., 1987).

Leucine Aminopeptidase (LAP)

There were a few zones of activity in gels stained for this enzyme. However, only one of them was scored for LAP for the reasons discussed in the next paragraph. At this zone, there was only one heterozygous tree. It had 2 alleles, 1 single-banded phenotype with some weakly stained shadow bands and the other without staining activity. The G-test indicated that the segregation at this locus did not deviate significantly from the expected 1:1 ratio (*Table 1*). A null allele has also been observed for LAP in other spruce species, *P. sitchensis* (CHAISURISRI and EL-KASSABY, 1993), *P. mariana* (PITEL et al., 1987), *P. glauca* (KING and DANCIK, 1983), and *P. abies* (LUNDKVIST and RUDIN, 1977). However, KUBOTA et al. (1993) stained LAP for our species and observed 2 variable loci without any null alleles. The *Lap-2* in their study might be the locus we scored for LAP. Two loci have also been reported for LAP in other spruce species, *P. mariana* (PITEL et al., 1987), *P. glauca* (KING and DANCIK, 1983), and *P. abies* (LUNDKVIST and RUDIN, 1977; MUONA et al., 1987). A monomeric molecular structure for this enzyme was observed in *P. abies* (LUNDKVIST and RUDIN, 1977).

Our gels stained for LAP and AAP shared some identical staining activity zones. The LAP locus was also stained in AAP gels although its staining was not as clear and consistent as in LAP gels. In LAP gels, *Aap-1* phenotypes showed up consistently, but the staining was not as sharp as in AAP gels. Furthermore, in LAP gels, *Aap-2* stained faintly or was absent sometimes. Overlapping stain activities of AAP and LAP were also observed in *Pinus densiflora* (NA'IEM et al., 1989), *P. massoniana* (HUANG, et al., 1994), *Calocedrus decurrens* (HARRY, 1986), and *Abies mariesii* (SUYAMA et al., 1992).

Menadione Reductase (MNR)

There were 5 zones of activity in gels stained for MNR. However, only *Mnr-1* (the slowest migrating zone) and *Mnr-2* (the fastest migrating zone) were scored for this enzyme, and the other 3 were not but discussed in the next paragraph. Both *Mnr-1* and *Mnr-2* had single-banded phenotypes with 2 and 3 alleles, respectively (*Fig. 1*). There was only one heterozygous tree for each of the 3 genotypes observed for the 2 loci, and the G-test indicated that segregation at both loci did not deviate from the expected 1:1 ratio (*Table 1*).

The other 3 zones of activity were between *Mnr-1* and *Mnr-2* in MNR gels. However, the stainings of the fastest and slowest of these 3 migrating zones were very faint or not readable at all, and the middle zone stained more clearly but it was absent sometimes. Whenever readable, all the bands in these 3 zones showed exactly the same variations as *Dia-2, Dia-3,* and *Dia-4* in DIA gels. Therefore, they were scored for DIA but not for MNR. Similar stain activities between DIA and MNR enzymes have also been observed in *Picea pungens* and *P. engelmannii* (ERNST et al., 1987) and in other conifers (STRAUSS and CONKLE, 1986; LEWANDOWSKI et al., 1992; LEWANDOWSKI and MEJNARTOWICZ, 1990).

Shikimate Dehydrogenase (SHD)

There were 2 zones of activity in SHD gels. *Shd-1* had 5 single-banded alleles and one without staining activity (*Fig. 1*). Both pooled and heterogeneity G-tests were not significant (*Table 1*), which indicates that segregation at this locus followed the expected 1:1 ratio. A null allele was also observed in *Calocedrus decurrens* (HARRY, 1986), *Abies sachalinensis* (NAGASAKA and KOONO, 1990), and *A. pinsapo* (PASCUAL et al., 1993).

Shd-2 appeared as double-banded with 3 variants (*Fig. 1*). Pooled G-test showed no significant segregation departure from the expected 1:1 ratio at this locus (*Table 1*). Although segregation was heterogeneous among trees for genotype a:c, none of the trees had significant segregation departure from the expected 1:1 ratio. Furthermore, b:c and a:b phenotypes did not show any segregation distortion (*Table 1*). Thus, it was concluded that this zone is controlled by a single locus with 3 alleles.

Two loci with single- and double-banded phenotypes were also observed in *Calocedrus decurrens* (HARRY, 1986). In *Picea mariana* only one locus with single banded phenotypes was observed for SHD (BOYLE and MORGENSTERN, 1985; PITEL et al., 1987). PITEL et al. (1987) also found that this enzyme is a monomer.

Sorbitol Dehydrogenase (SOD)

There was only one zone of activity with 2 single-banded phenotypes observed on gels stained for this enzyme (*Fig. 1*). Nonsignificant pooled and heterogeneity G-tests (*Table 1*) indicated that segregation at this locus followed the expected 1:1 ratio. There seems no other isozyme studies in spruce species have used SOD so far. Nevertheless, it was easy to stain for *Picea glehnii*, and a single locus with single-banded phenotypes has also been observed in *Pinus contorta* (WHEELER and GURIES, 1982), *P. muricuta* (MILLAR, 1985), *P. thunbergii* (SHIRAISHI, 1988a), *P. densiflora* (NA'IEM et al., 1989), and *Ginkgo biloba* (TSUMURA et al., 1987).

6-phosphogluconic Dehydrogenase (6PG)

This enzyme had 2 zones of activity. In the slower migrating zone, all homozygous trees had triple-banded phenotypes. However, all the variants of each of the heterozygous trees could not be interpreted consistently. This zone might correspond to the slower migrating zones observed in *Picea glauca* (KING and DANCIK, 1983), and *P. pungens* and *P. engelmannii* (ERNST et al., 1987) which were controlled by 2 loci forming heterodimers. Two loci with heterodimers seemed occurring in our 6PG gels too but too complex to score. Thus, this zone was excluded from our analyses. The faster migrating zone in our gels for 6PG had 3 double-banded variants (*Fig. 1*) which segregated according to the expected 1:1 ratio (*Table 1*). For the same species, KUBOTA et al. (1993) observed 2 loci for 6PG. Their double-banded *6pg-1* might be the same as our 6PG; however, they only observed 2 alleles at this locus. In other spruce species (*P. mariana, P. glauca, P. pungens, P. engelmannii,* and *P. sitchensis*), 2 or 3 loci with single- and double-banded phenotypes were observed (CHELIAK and PITEL, 1984; BOYLE and MORGENSTERN, 1985; KING and DANCIK, 1983; ERNST et al., 1987; CHAISURISRI and EL-KASSABY, 1993). The functional form of 6PG loci has been reported to be dimeric in *Picea glauca, P. pungens,* and *P. engelmannii* (CHELIAK and PITEL, 1984; ERNST et al., 1987), but monomeric in *P. mariana* (BOYLE and MORGENSTERN, 1985).

Linkage Analysis

We observed that *Aap-3* and *Aap-4* were completely linked. Completely linked loci in AAP have also been observed in *Pinus densiflora* (NA'IEM et al., 1993) and *P. massoniana* (HUANG et al., 1994). Therefore, only *Aap-3* was used in our analyses. The observed 19 polymorphic loci, excluding *Aap-4*, resulted in a total of 108 2-locus combination pairs for linkage tests. Results are given only for pairs with either significant heterogeneity

Table 2. – Linkage test results for pairwise combinations of loci with significant G_i, and/or x^2_L in *Picea glehnii.*

Locus pair (A:B)	Number of trees	Segregation class AB	Ab	aB	ab	Heterogeneity G_i	df	P	Segregation at locus A[1] x^2_A	P	Segregation at locus B x^2_B	P	Joint segregation x^2_L	P	Recombination r	SE(r)
Dia-2:Mnr-1	1	6	16	17	11	-[2]	-	-	0.720	0.39	0.320	0.57	5.432	0.01	-[3]	-
Shd-1:G6p	11	168	137	132	152	35.713	31	0.25	0.748	0.38	0.205	0.65	4.348	0.04	0.456	0.020
Aap-1:Dia-1	5	74	62	38	72	54.020	13	0.00	2.747	0.09	1.967	0.16	9.494	0.00	0.390	0.031
Aap-1:Dia-2	3	29	44	37	24	16.185	7	0.02	1.074	0.29	0.029	0.86	5.776	0.01	0.395	0.042
Aap-1:Got-3	2	49	11	10	38	44.750	4	0.00	1.333	0.24	0.925	0.33	38.990	0.00	0.194	0.038
Aap-2:Est	2	14	39	37	14	25.065	4	0.00	0.038	0.84	0.038	0.84	22.118	0.00	0.269	0.043
Got-1:Dia-2	2	16	27	30	23	15.247	4	0.00	1.041	0.30	0.166	0.68	3.533	0.06	-	-
6pg:Got-2	3	34	26	40	36	14.540	7	0.04	1.882	0.17	1.058	0.30	0.215	0.64	-	-
Shd-1:Aap-2	12	168	146	116	171	53.187	34	0.02	1.212	0.27	1.811	0.17	10.240	0.00	-	-
Shd-2:Got-1	7	54	105	101	57	158.466	19	0.00	0.003	0.95	0.154	0.69	28.450	0.00	0.198	0.022

[1]) There is 1 degree of freedom for x^2_A, x^2_B, and x^2_L.
[2]) Heterogeneity x^2 test was not done as data was from only one tree and thus df is zero for G_i.
[3]) Recombination r was not calculated for the locus pairs if linkages were not confirmed for them.

and/or joint segregation (*Table 2*), and the complete list is available upon request.

Every tree was at least heterozygous at 2 loci. None of the 108 pairs had significant segregation distortion from the expected 1:1 ratio at both loci. Therefore, x^2-test for linkage was valid for all of them (BAILEY, 1961). However, x^2-test detected significant departure from the expected 1:1 segregation ratio at 1 of the 2 loci in 10 of the 108 pairs. Such departure may be due to chance or differential viability of gametes carrying different alleles (ADAMS and JOLY, 1980).

Forty of the 108 pairs had only 1 heterozygous tree each, and only 1 of them (*Dia-2*:*Mnr-1*) showed significant joint segregation distortion (*Table 2*). However, linkage of these 2 loci cannot be confirmed as the data was from only one tree. Among the rest 68 pairs with at least 2 trees each, results were grouped into combinations with homogeneous data and with heterogeneous data among trees and discussed in the following.

Combinations with homogeneous data

This group contained 60 pairs. However, significant linkage was detected for only one of them, *Shd-1*:*G6p* (*Table 2*). Nevertheless, these 2 loci were only very loosely linked with a recombination frequency of 45.6% (*Table 2*).

Combinations with heterogeneous data

There were 8 pairs in this group, and the 3-way log-likelihood test results for them are given in *table 3*. Two (*Got-1*:*Dia-2, 6pg*:*Got-2*) of them did not have significant deviation from joint independence by x^2-test (*Table 2*). The 3-way log-likelihood test for independence for *Got-1*:*Dia-2* (*Table 3*) indicated that the distortion was caused by the different segregations among trees at locus *Got-1.* Thus, linkage for this pair of loci cannot be confirmed. For *6pg*:*Got-2*, the distortion was caused by the interaction between segregations at these 2 loci among trees (*Table 3*). Therefore, linkage for this pair of loci cannot be confirmed either.

Table 3. – Three-way log-likelihood test for independence for combinations with heterogeneous data. Tree x locus A x locus B independence is not given as it is the heterogeneity test in *table 2.*

Locus pairs and hypothesis tested	G_i	df	P
Aap-1:Dia-1 (A:B)			
Tree x locus A independence	2.322	4	0.676
Tree x locus B independence	3.904	4	0.419
Locus A x locus B independence	9.773	1	0.001
Tree x locus A x locus B interaction	38.020	4	0.000
Aap-1:Dia-2			
Tree x locus A independence	1.989	2	0.369
Tree x locus B independence	2.912	2	0.233
Locus A x locus B independence	5.866	1	0.015
Tree x locus A x locus B interaction	5.417	2	0.066
Aap-1:Got-3			
Tree x locus A independence	0.600	1	0.438
Tree x locus B independence	1.835	1	0.175
Locus A x locus B independence	42.496	1	0.000
Tree x locus A x locus B interaction	-0.182	1	0.669
Aap-2:Est			
Tree x locus A independence	1.872	1	0.171
Tree x locus B independence	0.949	1	0.329
Locus A x locus B independence	22.991	1	0.000
Tree x locus A x locus B interaction	-0.748	1	0.386
Got-1:Dia-2			
Tree x locus A independence	9.376	1	0.002
Tree x locus B independence	0.642	1	0.422
Locus A x locus B independence	3.606	1	0.057
Tree x locus A x locus B interaction	1.623	1	0.202
6pg:Got-2			
Tree x locus A independence	2.367	2	0.306
Tree x locus B independence	4.622	2	0.099
Locus A x locus B independence	0.220	1	0.638
Tree x locus A x locus B interaction	7.330	2	0.025
Shd-1:Aap-2			
Tree x locus A independence	9.792	11	0.549
Tree x locus B independence	20.030	11	0.045
Locus A x locus B independence	10.335	1	0.001
Tree x locus A x locus B interaction	13.029	11	0.290
Shd-2:Got-1			
Tree x locus A independence	7.221	6	0.300
Tree x locus B independence	12.252	6	0.056
Locus A x locus B independence	28.913	1	0.000
Tree x locus A x locus B interaction	110.078	6	0.000

The remaining 6 of the 8 pairs in this group showed significant deviation from joint independence by x^2-test (*Table 2*). The 3-way log-likelihood test for independence suggested that distortions in 2 of them (*Aap-1*:*Dia-1, Shd-2*:*Got-1*) were caused by the interaction between segregations of their 2 loci and the 2 loci with trees (*Table 3*). Thus, the 2 loci of *Aap-1*:*Dia-1* and *Shd-2*:*Got-1* are loosely and tightly linked with recombination frequencies of 39% and 19.8%, respectively (*Table 2*). Differences in recombination rates among trees are common in conifers, which might be due to that different trees have different recombination rates or different arrangements of alleles (ADAMS and JOLY, 1980) or due to environmental effects (NIEBLING et al., 1987).

For the rest 4 of the 6 pairs, distortion at *Shd-1*:*Aap-2* was mainly due to segregation differences among trees at locus *Aap-2* (*Table 3*). Thus, linkage for this pair cannot be confirmed. For other 3 pairs (*Aap-1*:*Dia-2, Aap-1*:*Got-3*, and *Aap-2*:*Est*), distortion was due to the interaction between segregation at both loci of each pair (*Table 3*). Therefore, on the basis of the recombination frequencies (*Table 2*), linkage was tight in *Aap-1*:*Got-3* and *Aap-2*:*Est* but loose in *Aap-1*:*Dia-2.*

Among the confirmed linked loci, there seems to have one linkage group involving more than 2 loci, *Dia-1*, Dia-2, *Aap-1,* and *Got-3.* It is possible that in this group *Dia-1* and *Dia-2* are on each side of *Aap-1* with a recombination frequency about 0.39 each. However, a linear arrangement of these 4 loci could not be determined since there was no linkage detected between *Got-3* and *Dia-1* or between *Got-3* and *Dia-2*. If linkage between *Dia-2* and *Mnr-1* could be confirmed by more than one tree, *Mnr-1* would belong to this linkage group.

Loci of AAP, DIA, and GOT were also found to be in one linkage group in *Pinus densiflora* (NA'IEM et al., 1993). In *Pinus contorta, P. taeda, P. jeffreyi* (CONKLE, 1981), *P. densiflora* (NA'IEM et al., 1993), and *P. massoniana* (HUANG et al., 1994), *Est* was also found to be in the linkage group involving loci of AAP and DIA, which was not detected in our study. However, *Est* was tightly linked with one of the AAP loci in our study. We found linkage between loci of SHD and GOT, and such linkage was also detected in *Pinus thunbergii* (SHIRAISHI, 1988b), *P. sylvestris* (SZMIDT and MUONA, 1989) and in *P. densiflora* (NA'IEM et al., 1993). Nevertheless, more than a cursory comparison of linkage relationships in *Picea glehnii* with those reported in other conifer species is nearly impossible for various reasons (ADAMS et al., 1990) unless different species are run on the same gels.

Conclusions

From 38 *Picea glehnii* trees, 21 loci with 57 alleles were detected in 11 enzymes. Only one locus, *Dia-4*, was monomorphic. Among the other 20 polymorphic loci, 2 loci (*Aap-1* and *Dia-3*), or 2 pairs (*Aap-1*, b:c; *Dia-3*, a:b) of the 40 2-allele combinations (*Table 1*), had segregation deviations from the expected 1:1 ratio. Segregation distortion has been found in many conifers (MILLAR, 1985; YING and MORGENSTERN, 1990; ADAMS et al., 1990; GONCHARENKO et al., 1993). This can be due to chance, selection against 1 of the 2 alleles, linkage with lethal genes, isozymes controlled by more than 1 locus, meiotic

distortion, interallelic interaction, or non-genetic influence on the observed variation. No matter what the exact reasons might be, caution should be taken when using *Dia-3* and *Aap-1* with allele b for genetic studies, such as mating system analyses, allele frequencies, and heterozygosity (CHELIAK et al., 1984).

Linkage relationships were examined for all 2-locus pairs observed. It was found that linkage was complete between loci *Aap-3* and *Aap-4*; tight between loci *Shd-2* and *Got-1, Aap-1* and *Got-3,* and *Aap-2* and *Est*; and loose between loci *Shd-1* and *G6p, Aap-1* and *Dia-1,* and *Aap-1* and *Dia-2*. Some population genetic analyses require information from independent loci, such as genetic distance (NEI, 1975) and mating system parameters (SHAW et al., 1981). Therefore, 1 of the 2 loci that are linked should be excluded for such analyses. However, if linkage is not strong, such as with a recombination frequency greater than 0.39 in *Shd-1*:*G6p*, *Aap-1*:*Dia-1, Aap-1*:*Dia-2* 2-locus pairs in our study, linkage should not be a major problem in the use of these loci (SHAW et al., 1981; FURNIER et al., 1986).

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