Inheritance and Linkage of Allozymes in Larix laricina

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Summary

Inheritance and linkage of 29 allozymes, encoded by 13 variable enzyme loci, are reported from a survey of variation in *Larix laricina* using haploid megagametophytic tissue from mature trees across the range of this species. In general, segregation ratios conformed to those expected for traits under single gene Mendelian control. Allozymes of two loci, *G6pd* and *Pgi-2*, could be modified by environmental conditions and, therefore, were not under direct genetic control. It was demonstrated that some collections labelled as single trees by the collection agency were actually composed of seed from at least two different trees.

Linkage was observed between four pairs of genes, three of which have been previously reported in conifers. We have also confirmed that the tight linkage between loci of aspartate aminotransferase and phosphoglucose isomerase, reported in many conifer species, also holds in tamarack. However, it was shown that there has been a change in the apparent loci involved in this linkage group for members of the *Pinus-Picea* group and those of three other genera thus far studied. Some ideas on the evolution of this linkage complex are presented.

Key words: Inheritance, linkage, isozymes, allozymes, *Larix*, evolution, *Pinaceae*.

Zusammenfassung

Bei *Larix laricina* wurden die Vererbung und Kopplung von 29 Allozymen, die von 13 polymorphen Enzymloci kodiert werden, beschrieben. Endospermgewebe von einigen Mutterbäumen des gesamten Verbreitungsgebietes dieser Baumart wurden analysiert. Im allgemeinen unterlag die Gametensegregation einer einfachen Mendelspaltung. Die Allozyme von zwei Loci. *G-6-PD* und *PGI* wurden durch Umwelteffekte modifiziert und stehen somit nicht unter direkter genetischer Kontrolle. Es wurde gezeigt, daß einige, von der Beerntungsagentur als Einzelbaumabsaaten gekennzeichnete Saatgutproben, Mischproben von zwei verschiedenen Bäumen darstellten.

Kopplung wurde zwischen vier Genpaaren beobachtet, von denen über drei bereits früher bei Koniferen berichtet wurde. Die bei vielen Koniferen auftretende enge Kopplung zwischen Loci der Asparat-Aminotransferasen und Phosphoglucose-Isomerasen konnte auch bei der amerikanischen Lärche beobachtet werden. Es wurde gezeigt, daß bei dieser Kopplungsgruppe für Arten der *Pinus-Picea* Gruppe und für drei andere Gattungen eine Veränderung der Genloci auftrat. Einige Hinweise über evolutionäre Prozesse dieser Kopplungsgruppe wurden präsentiert.

Introduction

Gel electrophoresis allows rapid screening and characterization of genetic variation in both natural and artificial populations at the level of single gene traits. These single gene traits, unlike complex quantitative traits, provide more direct measures of genetic variation within, and genetic similarity among, populations (Brown and Moran 1981). However, before the full potential of electrophoretic techniques is realized, genetic control of the allozyme polymorphisms must be demonstrated. Also, it is important to know the linkage relationships, and hence multilocus organization, among the isozyme loci being studied.

Despite the long time required to reach sexual maturity, and in some instances long reproductive mode (e.g. up to 17 months for *Pinus* [Owens *et al.* 1981]), coniferous species are especially attractive organisms for electrophoretic investigations. This derives from the fact that megagametophytic tissue in conifers remains in a haploid condition after fertilization. Therefore testing of inheritance of allozyme polymorphisms can be done by direct observation of segregating haploid gametophytes. Observed segregation is then tested against expected segregation for Mendelian genes (i.e., 1:1 segregation of alternate alleles). More attractive, however, is direct observation of coupling and repulsion forms of gametes in individuals heterozygous at more than one locus. Again, this haploid condition allows formulation of a direct test based upon the expected distribution of gametes under a null hypothesis of no linkage.

In this paper we describe variation patterns of 12 enzyme systems encoded by a total of 21 structural gene loci. Inheritance data are presented for 13 of the loci which were variable in our survey. In addition, tests for pairwise linkage of 47 locus-pairs are reported.

Materials and Methods

Seed Collection

Seeds for this study were assembled for a range-wide provenance test of *Larix laricina* (Du Roi) K. Koch. Because this operation had been underway for over a decade, several different strategies were employed during the cooperative collection phase. The first type of seed collection in the sample consisted of bulked collections, where seeds from several (generally unknown) trees were pooled to provide a provenance sample. Such seeds are of no use for the inheritance and linkage work. However, they do provide an estimate of population parameters within that provenance. These results will be reported at a later date. The second type of seed collection in this sample is that originating from single trees. In this case, cones were collected, generally from 10 trees within a population, and seeds extracted with subsequent single tree identity maintained. It is this sample of seed that will provide the basis for the inheritance and linkage portion of this study. In all, we screened approximately 320 trees for this portion of the survey. The trees represent populations throughout the natural range of tamarack.

Tissue preparation, electrophoresis, and enzyme detection

Mature seeds were allowed to imbibe distilled water for 48 hours prior to electrophoresis. After this time, the seed coats and embryos were removed. The remaining haploid gametophyte from each seed was then placed in a 0.5 ml polystyrene sample cup. One drop (ca 50 μl) of extraction buffer (Yeh and O'Malley 1980) was added to each gametophyte. A total of six gametophytes were examined from each tree. Assuming no meiotic irregularities and no segregation distortion, the probability of incorrectly classifying a heterozygous individual as homozygous is about 3% with this sample size.

Gel and electrode buffers, electrophoretic conditions and starch were as reported by CHELIAK and PITEL (1984). The following enzyme systems were run in the buffers (H = Histidine/Citrate or B = Lithium/Borate) indicated and scored for each of the trees in the study: Aspartate aminotransferase (E.C.2.6.1.1 AAT; B), Aconitase (E.C.4.2.1.3 ACO; H), Aldolase (E.C.4.1.2.13 ALD; H), Glutamate dehydrogenase (E.C.1.4.1.3 GDH; B), Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49 G6PD; H), Isocitrate dehydrogenase (E.C.1.1.1.42 IDH; H), Leucine aminopeptidase (E.C.3.4.11.1 LAP; B), Malate dehydrogenase (E.C.1.1.1.37 MDH; H), Phosphoglucose isomerase (E.C.5.3.1.9 PGI; B), Phosphoglucomutase (E.C.2.7.5.1 PGM; H), Superoxide dismutase (E.C. 1.15.1.1, SOD; H) and 6-Phosphogluconate dehydrogenase (E.C.1.1.1.44 6PGD; H).

Types of Data

Data collected in this portion of the study are in two forms. The first utilizes the observed segregation of allozymes from the initial screening of six gametophytes from each heterozygous tree. From this preliminary survey, a larger sample size of megagametophytes from a subset of trees was run. In general, 50 additional gametes were analyzed from an additional 19 trees. However, in one case, an additional 150 gametes were analyzed. The primary objective of this subsequent sampling was to investigate heterogeneity of segregation ratios and variable linkage among heterozygous trees. Thus, in general, the sub-sample of trees was chosen so as to maximize the number of loci heterozygous per individual.

Data derived from the initial set of 320 trees will be referred to as pooled data, and data derived from the larger sample of gametes from a few selected trees will be referred to as the single tree data. Capital letters refer to a specific enzyme and italic letters refer to the gene locus coding for that enzyme. When enzyme systems are encoded by multiple loci, the loci are numbered sequentially from anode to cathode. Mobility of alleles (R_m) is measured relative to the mobility of a common allele for that gene locus. The mobility of the common allele at a locus (R_f) is measured relative to the buffer front, and is given an R_m of 1.00.

Results and Discussion

Fourty-nine alleles were observed from the 21 loci scored in this survey. One allele at *G6pd* and one allele at *Pgi-2*, which will be discussed in detail elsewhere, were shown to be the result of non-genetic modification of an existing allele. For each variable locus, grouped by enzyme system, we will describe the allozymes observed and present the formal inheritance data. Linkage analyses will be presented in a separate section after the inheritance data.

Inheritance

Monomorphic loci

No variation was observed for the allozyme encoded by the loci Isocitrate dehydrogenase, or Glutamate dehydrogenase.

Aspartate aminotransferase (AAT)

Three zones of activity were evident on gels stained for AAT *(Figure 1A)*. Two zones, AAT-2 and AAT-3, segregated in megametophytes of heterozygous trees for single banded variants. The observed numbers of segregants in both zones, for the pooled sample and for the single trees, were not significantly different from the expected numbers for 1:1 segregation *(Table 1)*. No heterogeneity of segregation ratios was observed among heterozygous single-tree collections. These results indicate that each zone is controlled by a single locus, and that each of these loci, *Aat-2* and *Aat-3*, encodes two different allozymes. Three loci for AAT (also known as glutamate oxaloacetate transaminase [GOT]) are also reported for many other conifer species (ADAMS and JOLY 1980a, GURIES and LEDIG 1978, EL-KASSABY *et al.* 1982, O'MALLEY *et al.* 1979).

Aconitase (ACO)

Two zones of activity were observed on gels stained for ACO *(Figure 1B)*. However, activity was primarily concentrated in the ACO-1 region, suggesting that the zone labelled ACO-2 may in fact be another gene. Furthermore, segregation was observed only for ACO-1. Variants in zone 1 appeared as single-banded phenotypes which did not segregate differently, for either the single tree or the pooled collections, than the expected 1:1 ratio for alternate gametes controlled by a single locus *(Table 1)*. No heterogeneity was observed among the segregation ratios for the nine single trees in this particular survey. These data support the interpretation that *Aco-1* is controlled by a single gene with two alleles. A single zone of ACO activity in conifers has been reported by ADAMS and JOLY (1980a), EL-KASSABY *et al.* (1982), GURIES and LEDIG (1978), KING and DANCIK (1983), YEH and EL-KASSABY (1980), YEH and LAYTON (1979), YEH and O'MALLEY (1980).

Aldolase (ALD)

Gels stained for ALD developed two zones of enzyme activity *(Figure 1C)*. Variation was only detected in the more cathodal zone (ALD-2), where the variants appeared as single-banded phenotypes which migrated slower than the common allele at the locus. ALD was not routinely scored during the major phase of this experiment. Only one tree was observed to be segregating at *Ald-2 (Table 1)*. Since no deviation was observed from the expected 1:1 distribution, we conclude that *Ald-2* is controlled by a single locus in *Larix*.

Two zones of ALD activity have been reported by GURIES and LEDIG (1982) for pitch pine *(Pinus rigida* MILL.), and by CONKLE (1981) for lodgepole pine *(Pinus contorta* DOUGL.) and Jeffrey pine *(Pinus jeffreyi* GREV. et BALF.).

Glucose-6-phosphate dehydrogenase (G6PD)

One zone of enzyme activity was evident on gels stained for G6PD *(Figure 1D)*. In this zone, three phenotypes can be observed: the common allele, a slow variant- and a null allele *Figure 1D)*. Details to be presented elsewhere (CHELIAK and PITEL, in prep.) indicate that the null allele is actually a non-genetic modification of the slow allele. This seems to occur as a result of seed aging, either from prestorage treatment, or from long storage times in the seed bank. Neither of the two forms of the variant allele deviated significantly from the expected distribution in pooled samples of trees *(Table 1)*. There was, however, a significant heterogeneity of the segregation ratio between trees that had the normal 2 allele and those that had apparently lost activity of this allele. Fewer common alleles are recovered when the null is present. In the single tree survey, where all the trees were heterozygous for the common allele and the normal 2 allele, no evidence of deviation from the expected segregation ratio was observed, and no heterogeneity was observed among the segregation ratios of trees surveyed. Therefore, we conclude that *G6pd* in *Larix*

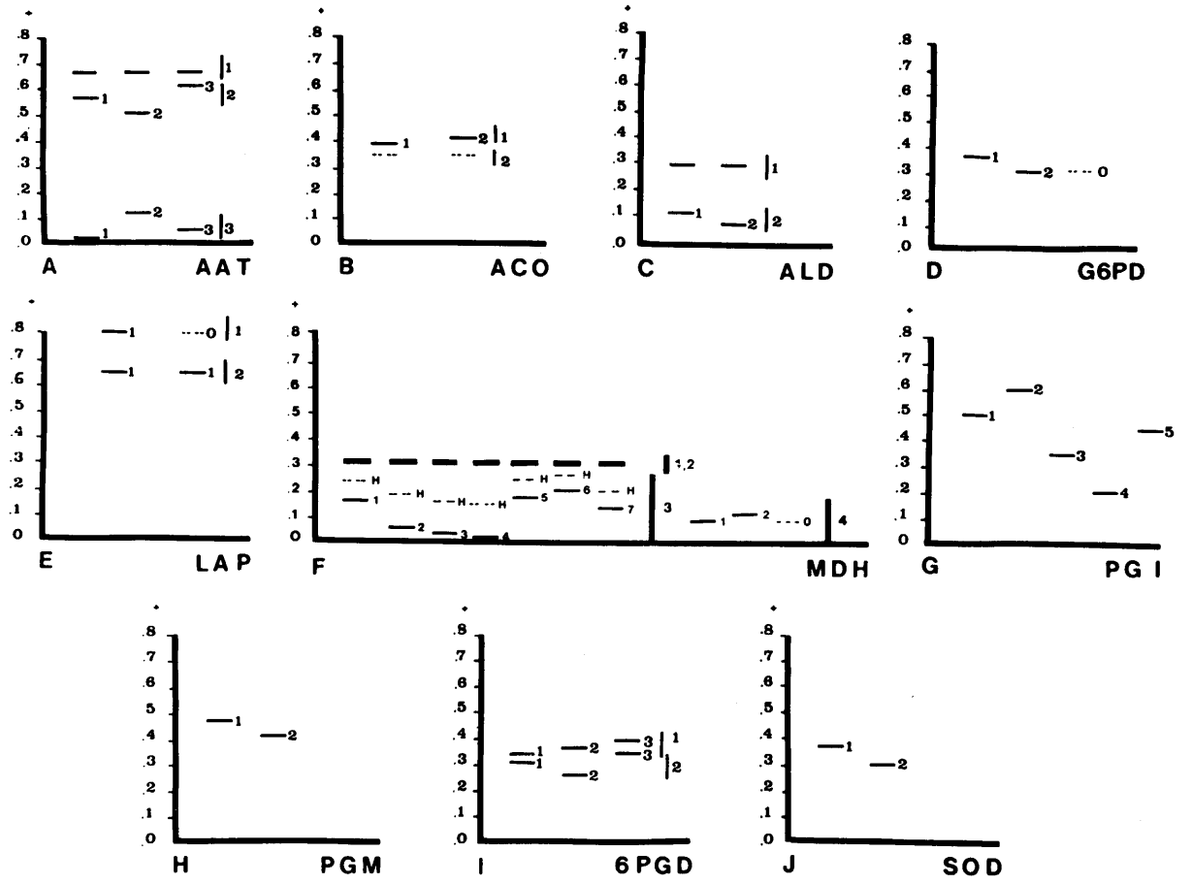

Figure 1. — Megagametophytic (haploid) banding patterns, locus designations, and allozyme numbers for ten variable loci in *Larix laricina*. The horizontal scale is the R_f of the various allozymes. Bands indicated by dashed lines are either null alleles, or interlocus dimerization products (designated as H), see text for details. There are two types of numbering: numbers at the vertical lines on the right of the zymogram indicate the zone of activity (nominally a locus), and numbers to the right of the single bands indicate the allozyme. Representative zymograms of different enzyme systems are designated by different letters.

is coded by a single locus, which has three phenotypes but only two alleles. One of these alleles, the null allele, is a non-genetic modification of the normal 2 allele. The nature of this modification is presently under investigation.

Leucine aminopeptidase (LAP)

Only the more anodal of the two zones of activity observed on gels scored for LAP indicated variation under our electrophoretic conditions *(Figure 1E)*. In this zone, LAP-1, a null allele was observed, which seems to be typical of many conifer species (Adams and Joly 1980a, Allendorf *et al.* 1982, Guries and Ledig 1978, King and Dancik 1983, Lundkvist 1979, O'Malley *et al.* 1979, Rudin 1977). Observed segregation, and lack of heterogeneity among heterozygous single tree collections, supports the interpretation that *Lap-1* is encoded by a single gene with two electrophoretic alleles in the populations surveyed of this species *(Table 1)*.

Malate dehydrogenase (MDH)

Four zones of activity are readily detectable on gels scored for MDH *(Figure 1F)*. We have observed variants in the two more cathodal zones, MDH-3 and MDH-4, in our single tree collections, and variants in MDH-2 in the bulked collections as well. In all, we have detected two alleles at MDH-2, seven alleles at MDH-3, and three alleles at MDH-4. However, many of these alleles are quite rare and have been observed only in the bulked collections.

For MDH-3, three classes of heterozygous trees were observed in both the single tree and pooled collections. No deviations from the expected 1:1 distribution of gametes were observed for the single tree collections, and no heterogeneity was detected among the segregation ratios of these trees *(Table 1)*. However, in the pooled collection, genotype 14 (read one four) seemed to segregate with significantly more of the 1 allele.

Although this may be a real segregation distortion, we have also noted that some collections, labelled by the collector as being single trees, are actually composed of seeds from at least two trees. For example, we have observed three alleles segregating simultaneously from "one tree". Unless the tree is triploid at that locus, the most reasonable conclusion is that the seeds from that collection do not represent the contribution from one tree. In this case, the collection would contribute gametes to the two alternate gamete classes at the parametric population values. Suppose that a particular collection, labelled as from a single tree, was actually represented by two trees: one a homozygote, 11 say, and the other a heterozygote, a 12 for example. When a sample of gametes are surveyed from this collection, and unknowingly assumed to be a single tree, the probability of drawing a 1 allele is 0.75 while the probability of drawing a 2 allele is only 0.25. The net result, when testing for a 1:1 segregation ratio, is segregation distortion. However, the problem is inherent with the collection itself, and not the underlying biology or genetics.

Table 1. — Observed segregation of allozymes from heterozygous trees of *Larix laricina*.

		POOLED TREES			SINGLE TREES			
Variable Locus	Allele Pair	Observed Number 1#	Total	Deviation G_1	Observed Number 1#	Total	Deviation G_1	Heterogeneity G (df)
Aat-2	1,2	11	23	0.043	49	100	0.040	0.360 (1)
Aat-3	1,2	448	904	0.071	219	411	1.775	7.295 (8)
Aco	1,2	325	675	0.926	205	380	2.371	7.365 (7)
Ald					24	48	-	-
G6pd	1,0	527	1108	2.633				
	1,2	142	253	3.808	172	345	0.003	5.840 (6)
Total		669	1361	0.389				
Heterogeneity				6.052*				
Lap-1	1,0	857	1796	3.745	169	321	0.901	3.415 (6)
Mdh-3	1,2	468	906	0.994	121	248	0.145	6.413 (5)
	1,4	344	635	4.429*	77	141	1.200	1.013 (2)
	2,4	127	245	0.331	64	141	1.200	2.626 (2)
Mdh-4	1,0	236	397	14.254*				
Pgi-2	1,2	186	360	0.40	16	44	3.315	-
	1,3	396	735	4.425*	193	384	0.010	4.680 (7)
	1,4	16	38	0.951				
	1,5	226	442	0.226	21	50	1.286	-
	2,3	19	36	0.111				
	2,5	15	36	1.005				
	3,5	62	132	0.485				
	4,5	12	24	0.000				
Pgm	1,2	71	139	0.065	71	143	0.007	1.939 (2)
6Pgd-1	1,2	355	655	4.624*	90	196	1.308	0.178 (3)
	1,3	12	23	0.043				
6Pgd-2	1,3	119	199	7.693*	114	223	0.112	2.507 (4)
Sod	1,2	334	652	0.393	88	180	0.089	11.407 (4)*

refers to the first allozyme listed
* indicates significant departure from the null hypothesis at $P<0.05$.
G_1 = single degree of freedom G test statistic for lack of fit
G (df) = G test statistic (degrees of freedom) for heterogeneity

We can conclude that a collection represents the seeds of more than one individual only when we directly observe three alleles segregating. Therefore, it is difficult to estimate this parameter for the entire collection. We have noticed, however, that these problems tend to be clustered, apparently related to origin of collection.

Therefore, the phenomenon of apparent segregation distortion in the pooled samples could be a real biological problem, or one of incorrect collection techniques. With the information available, it is difficult to assess the relative importance of each cause. However, we conclude that *Mdh-3* in *Larix* is most likely encoded by a single gene which, in the entire sample, likely encodes for a total of seven alleles. In addition, as is common in most conifers, there is an interlocus heterodimer formed between the loci encoding *Mdh-2* and *Mdh-3* (El-Kassaby 1981).

Three phenotypes were observed at the locus encoding MDH-4 *(Figure 1F)*. However, allele 2 was observed only in the bulked collection *(Table 1)*. The other allele, a null, was observed in both the bulked and single tree collections. Common/null heterozygotes at MDH-4, did not segregate according to the expected 1:1 distribution of alternate gametes, due to a significant excess of the common allele as compared to the null. Unfortunately, we did not observe any of these heterozygotes as single tree collections. However, the magnitude of the deviation forces us to conclude that either the null allele represents a significant deleterious selection against the survival of embryos, or that it is tightly linked to a semi-lethal allele. At this point in time, we are unable to favor any of these hypotheses.

Phosphoglucose isomerase (PGI)

In total, five alleles were observed in the one zone of activity which appeared consistently when the gels were stained for PGI activity *(Figure 1G)*. One additional zone of enzyme activity, which migrated anodally relative to the consistent zone was sometimes observed on gels which overstained. However, we did not consider this zone of activity in our survey.

Only one combination, the 13 heterozygote in the bulked collection, deviated significantly from the expected 1:1 distribution of alternate gametes *(Table 1)*. In the single tree collections representing this combination, no deviation from the expected distribution was evident. The same problem of bulked seedlots, mistakenly labelled as single tree collections, could be causing the apparent segregation distortion problems in the pooled samples. Therefore, we conclude that PGI in *Larix* is controlled by at least one gene, *Pgi-2*, which encodes at least five different allozymes.

Phosphoglucomutase (PGM)

Only one zone of activity is evident on gels stained for PGM *(Figure 1H)*. Under different electrophoretic conditions, two zones of PGM activity have been reported in other conifer species (Adams and Joly 1980a, Guries and Ledig 1978, Neale *et al.* 1984, Yeh and El-Kassaby 1980). However, under our conditions, only one zone of activity is evident. Both pooled samples and single tree collections segregated according to Mendelian expectations *(Table 1)*. No heterogeneity was observed in the segregation ratio among the three single tree collections surveyed. We conclude that PGM in *Larix* is controlled by a single gene, *Pgm*, which encodes two allelic forms in the populations surveyed in this species.

6-Phosphogluconate dehydrogenase (6PGD)

Two zones of activity were observed when gels were stained for the activity of 6PGD *(Figure 1I)*. Three variants were observed in both zones of enzyme activity. However, in 6PGD-2, the more cathodal zone, one variant, allele 2, was observed only in the bulked collections.

In both zones of activity, the pooled sample for the heterozygous combination 12 did not segregate according to the expected 1:1 distribution of alternate gametes. However, the observed distribution of gametes from single tree collections was not different from that expected for a Mendelian gene. We suggest that the most likely explanation of these results is the possibility of bulked collections contaminating the single tree collections. However, as previously discussed, without recourse to direct observation of three alleles in a collection labelled as a single tree, we

Table 2. — Significant linkage groups observed in *Larix laricina.*

Locus Pair	G	R[a]	Se[b]
Aat-3:Sod	18.48	0.184	0.063
Aat-3:Sod	7.09	0.300	0.065
Sod:Aco-1	3.90	0.316	0.075
Sod:Aco-1	5.18	0.316	0.075
6Pgd-1:Pgi-2	6.33	0.320	0.060
Aat-2:Pgi-2	56.40	0.030	0.017
Aat-2:Pgi-2	60.37	0.020	0.020

[a] R = recombination value
[b] Se = standard error of the recombination value
G = single degree of freedom G test statistic for lack of fit

really have no way of identifying the problem collections.

Other studies of conifers using 6PGD have reported one (Adams and Joly 1980a, Joly and Adams 1983, Neale and Adams 1981, Yeh 1981), two (El-Kassaby *et al.* 1982, Guries and Ledig 1978, 1982, Moran *et al.* 1980, O'Malley *et al.* 1979, Yeh and El-Kassaby 1980, Yeh and Layton 1979), and three loci (King and Dancik 1983, Krzakowa 1982, Krzakowa and Szweykowski 1979). The number of loci observed seems to be critically dependent upon the pH and composition of the buffer systems used. Under conditions in this study, we conclude that 6PGD is under the control of two genes, *6Pgd-1* and *6Pgd-2,* each of which code for at least three allozymes in *Larix laricina.*

Superoxide dismutase (SOD)

Using the riboflavin staining method of Baume and Scandalios (1979) for visualizing the enzyme SOD, one predominant zone of activity is evident on gels. One slower migrating allozyme variant was detected at this locus *(Figure 1J).* There was no evidence of deviation from the expected 1:1 segregation ratio, in either the pooled or single tree summaries, for individuals heterozygous for these two allozymes *(Table 1).* There was, however, significant heterogeneity among the segregation ratios observed in the single tree summaries.

This heterogeneity indicates that although the various trees in this sample all segregate according to expectations, there are significant differences in the excesses or deficiencies of the various allozymes among the trees. This significant heterogeneity was predominantly attributable to two trees, one of which had an excess of the common allozyme, the other an excess of the variant allele. This could be evidence of some type of lethal or semi-lethal allelism which is in linkage disequilibrium with the alleles of SOD. If this hypothesis were true, the excesses and deficiencies would then be the result of coupling and repulsion phase disequilibrium with the lethal allele. However, we can conclude that this zone of SOD activity in *Larix laricina* is controlled by one gene, *Sod-1,* which codes for at least two different allozymes in this species.

Linkage Analysis

Forty seven, of a possible 55, pairs of simultaneously heterozygous loci were available for a linkage analysis. Results from the combined data are reported as no heterogeneity in joint segregation was detected among trees. Maximum likelihood estimates of the recombination value and associated standard error were calculated using the double backcross method of Bailey (1961 p39). From these analyses, four significant linkage pairs were observed for *Larix laricina (Table 2).*

The association between *Aat-3:Sod* has been previously observed in Douglas-fir by El-Kassaby *et al.* (1982). The mean recombination values for this linkage group are essentially the same in both species (0.242 +/− 0.064 in Larch and 0.22 +/− 0.044 in Douglas-fir). Superoxide dismutase was also observed to be linked to *Aco-1* with a mean recombination value of 0.316 +/− 0.075. *6Pgd-1* and *Pgi-2* were observed to be linked with a mean recombination value of 0.320 +/− 0.066. This is the first report of linkage between these two pairs of loci for conifers. The final linkage group observed was that between *Aat-2* and *Pgi-2.* This group is very tightly linked with a mean recombination value of 0.030 +/− 0.024.

Because of these small recombination values, this is one of the most highly conserved gene blocks known in conifer karyology. However, there is an interesting pattern beginning to emerge as additional data are added to the growing body of conifer genome organization and structure. In studies where members of *Pinus* or *Picea* are involved, this highly conserved linkage group has been observed to exist between *Aat-1:Pgi-2* (or, as in some reports, *Got-1:Gpi-2,* or *Pgi-2).* In contrast, all investigations to date involving species from different genera, such as *Pseudotsuga, Abies,* and the current study with *Larix,* have observed this linkage group to exist between *Aat-2:Pgi-2 (Table 3).* Because all of the zymograms have been characterized in the same way, it is not likely that this is a simple problem of transposed loci. However, to eliminate this possibility, we have characterized a representative of each of these genera by electrophoresing a sample of 10 megagametophytes (singly) from bulked collections of seeds on a single gel using our standard laboratory conditions. The common

Table 3. — Linkage relationships reported between AAT and PGI for several conifer species.

Locus Pair	R[a]	Se[b]	Species	Reference
Aat-1:Pgi-2				
	0.024	0.007	Pinus taeda	Adams and Joly 1980b
	0.038	0.001	Pinus banksiana	Buchart et al (in prep.)
	2.1*	-	Pinus contorta	Conkle 1981
	2.5*	-	Pinus taeda	" "
	2.3*	-	Pinus jeffreyi	" "
	0.048	0.015	Pinus strobus	Ekert et al 1981
	0.030	-	Pinus rigida	Guries and Ledig 1978
	0.043	0.043	Picea mariana	Barrett et al (in prep)
	0.250	0.044	Picea mariana	Boyle and Morgenstern (in prep)
	0.140	-	Picea mariana	Yeh et al (in prep.)
	0.045	0.016	Picea glauca	King and Dancik 1983
Aat-2:Pgi-2				
	0.030	0.017	Larix laricina	Present investigation
	0.020	0.020	Pseudotsuga menziesii	El-Kassaby et al 1982
	0.030	0.020	Abies balsamea	Neale and Adams 1981

[a] R = recombination value
[b] Se = standard error of the recombination value
* linkage values reported in centi-Morgan map units

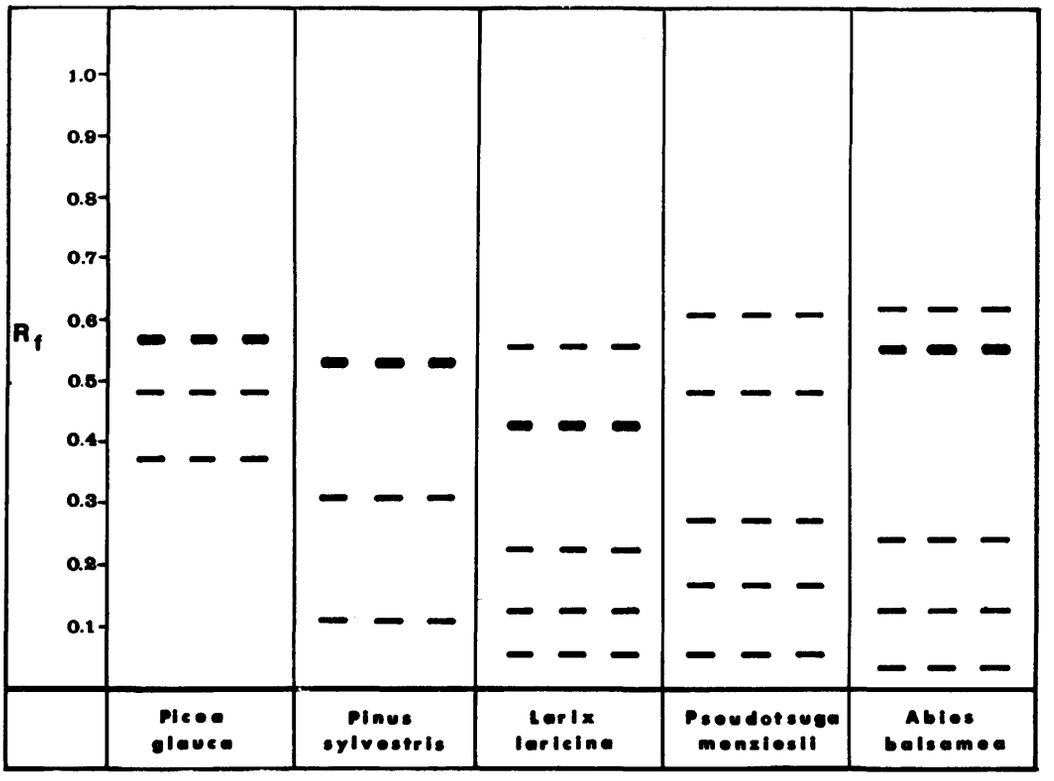

Figure 2. — Representative zymograms of aspartate aminotransferase from bulked seed collections of five different conifer species. The line widths are representative of the staining intensity observed.

allozyme observed for each of these species is shown in *Figure 2*.

These data suggest that evolution from the least common ancestor between the *Pinus-Picea* group, and the other genera investigated, in this likely duplicated gene system, has progressed to the point where accumulated mutations can now be observed as electrophoretic mobility differences. That is, we suspect that what is called *Aat-1* in the *Pinus-Picea* group is functionally *Aat-2* in the other genera. Similarly, we suspect that *Aat-1* in *Pseudotsuga, Abies,* and *Larix,* is functionally *Aat-2* in the *Pinus-Picea* group. We have tried, but withouts success, to screen for substrate specificity and heat sensitivity to try and differentiate AAT loci 1 and 2 in paired runs of *Larix* and *Picea.* In *Figure 2,* however there is a striking concordance in the apparent amount of protein that is transcribed (indexed by staining intensity) at *Aat-1* in the *Pinus-Picea* group and *Aat-2* in members of the other genera. Note also that the third AAT locus in the *Pinus-Picea* group occurs as a single zone of activity (under our conditions), whereas this locus is a three-banded phenotype in the other species.

In summary, we believe that accumulated mutations during the evolution of the AAT enzyme system from the *Pinus-Picea* group to the more advanced conifer genera has resulted in changes which can be observed as differences in electrophoretic mobility. However, we propose that functional homology at these two AAT loci will likely have been maintained in the respective groups. We predict that additional investigations outside of the *Pinus-Picea* group, in the relatively more advanced conifers, will observe linkage groups between *AAT-2:Pgi-2.*

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A Statistical Analysis of Karyotypes of European Black Pine (Pinus nigra Arnold) from Different Sources

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Summary

A comparative, intraspecific analysis of karyotypes was performed on 2-week-old seedlings of European black pine (*Pinus nigra* Arnold) by examining the meristemic cells of root-tips. The metaphase chromosomes were studied by a combination of Feulgen and Aceto-carmine staining methods. Seedlings were categorized according to the country of seed source and geographic locality of collection. There were three collection localities each in France, Turkey, Yugoslavia, and Greece, and two localities in Austria. Several karyotypic variables were determined for each cell: chromosome number, short arm length (SL), long arm length (LL), total chromosome length (TL), arm ratio (AR), relative chromosome length (RL), centromere index (CI), morphological index (MI), presence of secondary constrictions on short arms (S_{sec}) and long arms (L_{sec}), and presence of satellites (SAT). Aneuploidy (2n = 18) was observed in one seedling of each of the Yugoslavian and Greek seed sources.

Analyses of variances for the means of chromosomal variables indicated that there were significant ($p < .05$ or $p < .01$) differences among the seed sources; chromosomes XI and XII were especially variable with significant ($p < .01$) variation found in SL, LL, TL, AR, CI, and MI. The frequency of occurrence for secondary constriction was relatively higher on the long arms of chromosomes and on the longer chromosomes. Satellites were infrequent and only observed in the cells of Yugoslavian and Greek seedlings, especially on the longer chromosomes. French and Austrian seedlings had only one submetacentric chromosome (chromosome XII) whereas the others had two (chromosomes XI and XII). By integrating all the variables measured on all haploid chromsomes, a karyotypic cluster analysis among seed sources was performed. The Austrian and French seedlings clustered closely together, as did the Greek and Yugoslavian. The Turkish seedlings, however, were the least similar to those from any of the other countries. Seed collections within a country always clustered as a group.

Key words: Karyotype, aneuploidy, cluster analysis, *Pinus nigra* Arnold.

Zusammenfassung

Bei 2 Wochen alten Sämlingen von *Pinus nigra* Arnold wurde eine vergleichende intraspezifische Analyse von Karyotypen durchgeführt, indem die Meristemzellen von Wurzelspitzen untersucht wurden. Die Chromosomen wurden in der Metaphase mit Hilfe einer Kombination aus Feulgen- und Aceto-Karminfärbungsmethode untersucht. Die Sämlinge wurden nach den Herkunftsländern und dem geographischen Ort der Einsammlung geordnet. Es gab je drei Sammelorte in Frankreich, der Türkei, Jugoslawien und Griechenland und zwei in Österreich. Für jede Zelle wurden verschiedene karyotypische Variablen bestimmt: Chromosomenzahl, die Kurz- (SL) und Langschenkel-Länge (LL), die Chromosomengesamtlänge (TL), das Schenkelverhältnis (AR), die relative Chromosomenlänge (RL), der Centromer-Index (CI), der morphologische Index (MI), das Vorhandensein sekundärer Einschnürungen an den kurzen (S_{sec}) und langen Schenkeln (L_{sec}) und das Vorhandensein von Satelliten (SAT). In je einem Sämling der jugoslawischen und griechischen Herkünfte wurde Aneuploidie (2n = 18) beobachtet.

Varianzanalysen für die Mittelwerte der Chromosomenvariablen zeigten, daß es dort signifikante Unterschiede zwischen den Saatgut-Herkünften gibt ($p - 0{,}05$ oder $p - 0{,}01$); die Chromosomen XI und XII waren besonders variabel mit einer signifikanten Variation für SL, LL, TL, AR,

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