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Linkage relationships of allozyme loci in *Pinus sylvestris*

ALFRED E. SZMIDT[1] and OUTI MUONA[2]

[1] *Department of Plant Physiology and Forest Genetics, The Swedish University of Agricultural Sciences, Umeå, Sweden*
[2] *Department of Genetics, University of Oulu, Oulu, Finland*

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Linkage relationships among 27 allozyme loci in *Pinus sylvestris* (Scots pine) were analyzed. A total of 266 of the 351 possible two-locus combinations were tested. Four linkage groups could be established. The first group (A) contained the following loci: *Got-2, Lap-2, Adh-2, Adh-1, Pgi-2*. The loci *Got-1* and *Dia-4* probably also belong to this linkage group. The second linkage group (B) included two loci: *Lap-1* and *F-Est*. Three loci (*G6Pd-1, Aco*, and *G6Pd-2*) were assigned to the third linkage group (C). The fourth group (D) included two loci: *Got-3* and *Sdh-2*. It was not possible to determine the location of the other two-locus combinations for which evidence of significant linkage was obtained. These results are compared to earlier linkage maps of *Pinus sylvestris* and other conifers.

Alfred E. Szmidt, Department of Plant Physiology and Forest Genetics, The Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden

A large number of enzyme genetic markers are being used in forest genetic research and information on linkage relationships among them is needed. Such knowledge can be used for several purposes. Information about linkage is of importance for the interpretation of multilocus population data (e.g., EPPERSON and ALLARD 1987). It is also interesting to know to what extent the remarkable evolutionary conservatism of conifers with respect to basic karyotypic features is reflected in linkage relationships (SAX and SAX 1933; SAYLOR 1983). Third, information derived from linkages of marker loci with quantitative characters can be a powerful tool in plant breeding (see, e.g., PATERSON et al. 1988). Even if molecular markers are needed to obtain the necessary number of loci, allozyme locus mapping provides a useful start.

Several reports have been published demonstrating linkage groups of allozyme loci in many conifers, starting with GURIES et al. 1978, RUDIN and EKBERG 1978, LUNDKVIST 1979, CONKLE 1981, etc. In *Pinus sylvestris*, linkage relationships among 12 allozyme loci were studied by RUDIN and EKBERG (1978). We gave a preliminary, unpublished report on additional loci (SZMIDT et al. 1984), and a further set of loci was investigated by NIEBLING et al. (1987). Here, we publish results on linkage relationships between 27 loci, four of which were not studied by RUDIN and EKBERG (1978) or NIEBLING et al. (1987). We also discuss data on linkage in other conifer species.

Material and methods

These linkage data are based on an analysis of seed samples from 18 *P. sylvestris* clones growing in two seed orchards, Brån and Östteg, located in northern Sweden. The clones were chosen because of their high heterozygosity. The enzyme markers were analyzed electrophoretically in crude extracts of haploid macrogametophyte tissue isolated from seeds. A total of 14 different enzyme systems, coded for by 27 loci, were assayed. Electrophoretic procedures and the inheritance patterns of the enzyme markers have been reported earlier (see Table 1). We designate the locus coding for the most anodally migrating allozyme as *1*. For MDH we scored three loci, previously called A, B, and C (SZMIDT et al. 1984). In fact there is another locus between the A and B loci, which did not vary in our material, but forms a dimer with the locus previously designated C (see EL-KASSABY 1981; MÜLLER-STARCK, personal communication). These loci are now designated *Mdh-1, -3*, and *-4*.

A total of 71 to 186 macrogametophytes were analyzed for each heterozygous locus in each clone. Of the 351 possible two-locus combinations among the 27 loci analyzed in this study, 266 combinations could be tested in our material. Of these 266 combinations, 166 combinations were analyzed in more than one clone. For the statistical evaluation of linkage relationships we used Chi-square tests as described by MATHER (1951). This comprised tests

Table 1. Enzyme systems analyzed, their abbreviations, and the Enzyme Commision (E.C.) numbers, number of loci scored and the references for staining and/or genetic interpretation

Enzyme	Abbreviation	E.C. Number	Number of loci scored	Reference
Acid phosphatase	APH	3.1.3.2	1	ADAMS and JOLY 1980a
Aconitase	ACO	4.2.1.3	1	GURIES and LEDIG 1978
Alcohol dehydrogenase	ADH	1.1.1.1	2	RUDIN and EKBERG 1978
Diaphorase	DIA	1.6.4.3	4	YEH and O'MALLEY 1980
Fluorescent esterase	F-EST	3.1.1.1	1	YAZDANI and RUDIN 1982
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	2	YEH and O'MALLEY 1980
Glutamate dehydrogenase	GDH	1.4.1.2	1	ADAMS and JOLY 1980a
Glutamate-oxaloacetate-transaminase	GOT	2.6.1.1	3	RUDIN 1975
Leucineaminopeptidase	LAP	3.4.11.1	2	RUDIN 1977
Malate dehydrogenase	MDH	1.1.1.37	3	RUDIN and EKBERG 1978
6-phosphogluconate dehydrogenase	6PGD	1.1.1.44	2	SZMIDT and YAZDANI 1984
Phosphoglucomutase	PGM	2.7.5.1	2	ADAMS and JOLY 1980a
Phosphoglucose isomerase	PGI	5.3.1.9	2	GURIES and LEDIG 1978
Shikimate dehydrogenase	SDH	1.1.1.25	2	SZMIDT and YAZDANI 1984

for 1:1 segregation at single loci and a third test of independent assortment (1:1:1:1). Chi-square tests were also employed to check the homogeneity of the single clone results. For an initial heterogeneity test, the expected frequencies based on observed overall segregation were used. The results of these heterogeneity tests are given in Table 2. The heterogeneity was further partitioned to that due to single-locus segregation and that due to joint segregation (linkage) (MATHER 1951). The results are given in SZMIDT et al. (1984). Between *Adh-1* and *Pgm-1* overall data were heterogeneous with 0.05 level significance, but neither segregation nor linkage data were heterogeneous over the clones, and the data were pooled over clones (Table 2). Recombination estimates are given for pooled samples. In order to avoid detecting spurious linkages we chose to use the 0.01 significance level for the two-locus tests. For pairs of loci, where single or joint segregation was significantly heterogeneous, single tree data are given. Where possible, the relative order of the loci on chromosomes was determined by comparing recombination frequencies in multiple heterozygotes.

Results

Uneven segregation within a locus

Segregation patterns of allozymes within a locus conformed satisfactorily to the 1:1 proportions expected for a Mendelian character in most cases. However, of the 151 heterozygous allozyme combinations, 16 combinations indicated significantly uneven segregation (only cases where significant linkage was found between the locus pair are included in Table 2). As discussed in several other studies, sampling error or differential viability of gametes carrying different allozymes may account for this (RUDIN and EKBERG 1978; ADAMS and JOLY 1980b). Linkage to lethals may also lead to segregation distortion (SORENSEN 1967). Since no tendency towards uneven segregation for a particular locus or allozyme combination was found, it is likely that the observed deviations from 1:1 proportions were mainly due to chance. As shown by BAILEY (1961), uneven segregation at only one locus does not affect the Chi-squares for linkage. In cases where both loci showed deviations from 1:1 proportions, a modified Chi-square test for linkage was used (BAILEY 1961; RUDIN and EKBERG 1978).

Associations among loci

Because a total of 266 two-locus combinations were tested, detailed presentation of the results is impractical. A table of the complete set of tested loci is found in SZMIDT et al. (1984). Only those results which showed significant linkage are given here. Of the totally 266 two-locus combinations, 41 locus pairs were linked. In nineteen locus pairs analyzed in more than one tree, no significant heterogeneity was detected (Table 2). For thirteen locus pairs the data were heterogeneous over clones, but in only two cases was the heterogeneity due to joint segregation, *Aco/G6Pd-1* and *Dia-2/Dia-3* (Table 3). The evidence of linkage for the remaining 9 pairs of loci was based on single clone data (Table 2). Complete tables for partitioned Chi-square are

Table 2. Chi-square analyses for combinations of loci with significant (P <0.01) linkage. Chi-square test for heterogeneity of data (NS P >0.05, * P <0.05, ** P <0.01), chi-square-tests for segregation at single loci and joint segregation for those pairs where neither joint or single locus segregation was heterogeneous, recombination fraction (R) and its standard deviation (s_R)

Combination	N of	N of	Heterogeneity			Segregation				Joint	Recombination	
						Locus 1		Locus 2		segregation		
Locus 1/locus 2	clones	of seeds	χ^2	df	P	χ^2	P	χ^2	P	χ^2	R	s_R
Aco/Dia-2	5	712	18.5	(12)	NS	0.2	NS	6.9	**	7.7	0.448	0.019
Aco/G6Pd-1	5	724	80.6	(12)	**	–		–		–	–	–
Aco/G6Pd-2	2	233	1.0	(3)	NS	0.0	NS	0.0	NS	20.4	0.352	0.031
Adh-1/Adh-2	2	315	5.6	(3)	NS	2.7	NS	2.0	NS	307.0	0.006	0.022
Adh-1/Dia-1	4	608	8.6	(9)	NS	4.8	*	4.5	*	21.4	0.406	0.020
Adh-1/Dia-2	7	1093	23.6	(18)	NS	3.2	NS	5.1	*	10.5	0.45	0.015
Adh-1/Dia-4	1	173	–			1.0	NS	0.7	NS	124.9	0.075	0.020
Adh-1/Got-1	1	186	–			1.4	NS	0.3	NS	47.5	0.247	0.032
Adh-1/Got-2	6	900	34.5	(15)	**	–		–		–	–	–
Adh-1/Got-3	4	653	9.5	(9)	NS	0.8	NS	0.4	NS	10.0	0.438	0.019
Adh-1/Lap-2	4	625	20.2	(9)	*	–		–		–	–	–
Adh-1/6Pgd-2	6	934	22.1	(15)	NS	–		–		–	–	–
Adh-1/Pgi-2	4	577	15.2	(9)	NS	6.0	*	2.6	NS	95.7	0.296	0.019
Adh-1/Pgm-1	2	351	8.8	(3)	*	0.8	NS	7.4	**	6.8	0.430	0.026
Adh-1/Sdh-1	5	773	7.5	(12)	NS	0.3	NS	0.6	NS	8.9	0.446	0.018
Adh-1/F-Est	1	155	–			0.0	NS	0.3	NS	9.8	0.374	0.039
Adh-2/Got-3	3	470	8.3	(6)	NS	1.2	NS	0.0	NS	7.2	0.432	0.023
Adh-2/Lap-2	2	315	6.9	(3)	NS	–		–		–	–	–
Aph-/Dia-1	1	150	–			2.7	NS	4.5	*	47.0	0.220	0.034
Aph/Got-2	2	271	14.3	(3)	**	–		–		–	–	–
Aph/Mdh-3	1	113	–			2.6		0.0	NS	7.4	0.372	0.045
Dia-1/Dia-2	5	699	13.8	(12)	NS	–		–		–	–	–
Dia-1/Dia-4	1	173	–			4.2	*	0.7	NS	18.8	0.335	0.036
Dia-1/Lap-2	2	302	1.3	(3)	NS	3.4	NS	4.8	*	7.6	0.421	0.028
Dia-1/Pgi-2	2	303	1.9	(3)	NS	1.2	NS	1.5	NS	7.3	0.422	0.028
Dia-2/Dia-3	3	409	30.8	(6)	**	–		–		–	–	–
Dia-2/Mdh-3	6	770	23.0	(15)	NS	–		–		–	–	–
Dia-3/Got-2	3	409	13.3	(6)	*	–		–		–	–	–
Dia-3/Sdh-1	3	398	9.9	(6)	NS	0.2	NS	9.0	**	9.0	0.425	0.025
Dia-3/Sdh-2	2	277	2.2	(3)	NS	4.9	*	0.3	NS	16.2	0.379	0.029
F-Est/G6Pd-2	3	398	8.9	(6)	NS	9.0	**	0.5	NS	7.3	0.432	0.025
F-Est/Lap-1	3	460	5.6	(6)	NS	0.0	NS	0.0	NS	40.2	0.352	0.022
Gdh/6Pgd-2	3	510	7.2	(6)	NS	–		–		–	–	–
Got-2/Lap-2	3	475	12.5	(6)	NS	–		–		–	–	–
Got-2/Pgi-2	5	728	16.1	(12)	NS	0.7	NS	2.4	NS	11.1	0.428	0.018
Got-2/Sdh-2	3	467	15.6	(6)	*	–		–		–	–	–
Got-3/Sdh-2	1	165	–			1.4	NS	2.6	NS	97.8	0.115	0.025
G6Pd-1/G6Pd-2	2	233	1.7	(3)	NS	0.7	NS	0.0	NS	14.9	0.373	0.032
G6Pd-2/6Pgd-2	1	123	–			0.0	NS	1.8	NS	7.8	0.374	0.044
Lap-2/Pgi-2	1	152	–			2.1	NS	2.1	NS	17.8	0.329	0.038
Mdh-3/Pgi-2	4	575	8.4	(9)	NS	0.0	NS	0.0	NS	13.2	0.424	0.021

given in SZMIDT et al. (1984). We concentrate on the firmly established linkage in describing the linkage groups, but all data are given in Tables 2 and 3.

Linkage group A

Fig. 1 shows that we mapped *Got-2, Lap-2, Adh-2,* and *Pgi-2* into linkage group A. This corresponds to the one linkage group established by RUDIN and EKBERG (1978) with four loci, *Adh-1, Adh-2, Lap-2,* and *Got-2*. They found no recombination between the loci *Adh-1* and *Adh-2*, but claimed that these loci are different, because their alleles varied independently. We found 0.6 % recombination between these loci (Table 2). The order of loci and distances between them corresponded to those in RUDIN and EKBERG (1978) (Tables 2 and 3, Fig. 1). NIEBLING et al. (1987) also mapped many of these loci to the same linkage group (*Got-2, Lap-2, Adh-1, Pgi-2*). We found evidence of other loci linked to this group, as based on fairly high recombination rates or single clones. These loci were *Got-1, Dia-4,*

Table 3. Single tree data for combinations with significantly heterogeneous single-locus and/or joint segregation. Source of heterogeneity is indicated below the locus names. 1 – locus 1, 2 – locus 2, J – joint segregation. Single tree χ^2-tests for segregation at single loci and joint segregation (NS P >0.05, * P <0.05, ** P <0.01), recombination frequencies (R) and standard deviations (s_R)

Combination	N of seed	Locus 1	Locus 2	Joint	R	s_R
Aco/G6Pd-1	162	NS	NS	138.9**	0.037	0.015
	71	NS	NS	33.8**	0.155	0.043
(J)	173	NS	NS	34.3**	0.277	0.034
	165	NS	NS	120.5**	0.073	0.020
	153	NS	NS	112.2**	0.072	0.021
Adh-1/Got-2	123	NS	NS	5.1*	0.398	0.044
	158	4.3*	NS	NS	0.424	0.039
(1)	158	NS	12.5*	25.9**	0.297	0.036
	165	NS	NS	5.8*	0.406	0.038
	144	4.7*	6.3*	NS	0.444	0.041
	152	NS	NS	11.6**	0.362	0.039
Adh-1/Lap-2	158	NS	7.3**	34.7**	0.266	0.035
	165	NS	NS	11.2**	0.370	0.038
(2)	150	6.0*	NS	30.8**	0.273	0.036
	152	NS	NS	26.9**	0.289	0.037
Adh-1/6Pgd-2	123	NS	NS	4.3*	0.407	0.044
	173	NS	NS	NS	0.497	0.038
(2)	158	NS	NS	NS	0.437	0.039
	186	NS	NS	NS	0.435	0.036
	150	6.0*	NS	NS	0.467	0.041
	144	4.7*	NS	NS	0.493	0.042
Adh-2/Lap-2	165	NS	NS	13.4**	0.358	0.037
(1)	150	6.0*	NS	30.8**	0.273	0.036
Aph/Got-2	113	NS	NS	NS	0.416	0.046
(2)	158	NS	12.3**	5.7*	0.405	0.039
Dia-1/Dia-2	71	NS	NS	NS	0.451	0.059
	173	4.2*	NS	NS	0.428	0.038
(1)	150	4.5*	NS	NS	0.433	0.040
	153	NS	NS	NS	0.451	0.040
	152	NS	NS	4.4*	0.414	0.040
Dia-2/Dia-3	113	NS	7.4**	NS	0.469	0.047
	144	NS	NS	4.7*	0.410	0.041
(J)	152	NS	NS	48.7**	0.217	0.033
Dia-2/Mdh-3	113	NS	NS	3.9*	0.407	0.046
	71	NS	NS	NS	0.437	0.059
(1)	123	NS	NS	NS	0.423	0.045
	165	9.2**	NS	NS	0.461	0.039
	143	NS	NS	NS	0.455	0.042
	155	NS	NS	NS	0.465	0.040
Dia-3/Got-2	113	7.4	NS	7.4*	0.372	0.045
	144	NS	6.3*	NS	0.451	0.041
(2)	152	NS	NS	NS	0.441	0.040
Gdh/6Pgd-2	173	NS	NS	7.1**	0.399	0.037
	186	NS	NS	9.5**	0.387	0.036
(2)	151	NS	NS	NS	0.424	0.040
Got-2/Lap-2	158	12.3**	7.3**	68.5**	0.171	0.030
	165	NS	NS	57.0**	0.206	0.032
(2)	152	NS	NS	58.1**	0.191	0.032
Got-2/Sdh-2	158	12.3**	NS	4.9*	0.411	0.039
	165	NS	NS	NS	0.467	0.039
(1)	144	6.3*	4.0*	NS	0.438	0.041

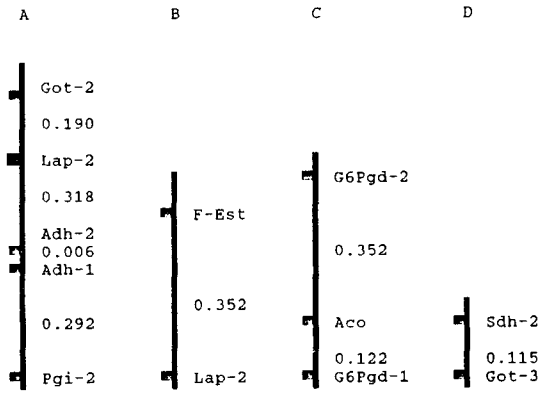

Fig. 1. Proposed location of allozyme loci in the four linkage groups established in this study. Only those loci are shown whose relative orientation could be established. Numerical values indicate recombination frequency.

and they appeared to be tightly linked to *Adh-1* (Table 2). *Dia-1*, *Aph*, and *Dia-2* showed some evidence of belonging to group A. These loci are not included in Fig. 1, either because of the uncertainty of the finding, or inability to find the orientation. RUDIN and EKBERG (1978) also found linkages of these loci to *Got-1* and *Mdh-3*. However, NIEBLING et al. (1978) mapped the *Dia-1*, *Dia-2*, and *Aph* loci to other linkage groups. NIEBLING et al. (1987) also found some additional loci in this linkage group, *Dia-C*, *Peplgg-B* and *Pgk-B*.

Similar linkages have been found in many other conifer species, though not all of these loci have been studied in all species. In particular, linkage between *Pgi-2* and *Got-1* is found in nearly all species studied: *Pinus attenuata* (STRAUSS and CONKLE 1986), *P. contorta* (CONKLE 1981), *P. taeda* (ADAMS and JOLY 1980b; CONKLE 1981), *P. jeffreyi* (CONKLE 1981), *P. ponderosa* (O'MALLEY et al. 1979), *P. rigida* (GURIES et al. 1978; O'MALLEY et al. 1986)), *P. strobus* (ECKERT et al. 1981), *Picea glauca* (KING and DANCIK 1983), *P. mariana* (BOYLE and MORGENSTERN 1985; BARRETT et al. 1987), and *P. abies* (MUONA et al. 1987). In *Pinus sylvestris* these loci belong to the same group A, but linkage was not tested for directly because of lack of an appropriate double heterozygote. Both loci have low variability in *P. sylvestris*. Linkage between the *Pgi-2* and *Got-2* pair has been found in *Pseudotsuga menziesii* (EL-KASSABY et al. 1982), *Abies balsamea* (NEALE and ADAMS 1981) and *Larix laricina* (CHELIAK and PITEL 1985). This linkage was not tested for in *P. lambertiana* (CONKLE 1981), *P. albicaulis* (FURNIER et al. 1986), or *P. thunbergi* (SHIRAISHI 1988), but these species show other linkages similar to group A.

Linkage group B

The linkage group B comprised the *F-Est* and *Lap-1* loci. All three clones indicated significant Chi-square values for linkage and there was no heterogeneity among the single clone data (Table 2). Linkage between *Lap-1* and *F-Est* has also been found in *Pinus contorta, P. taeda, P. jeffreyi* and *P. attenuata* (CONKLE 1981; STRAUSS and CONKLE 1986). NIEBLING et al. (1987) found that *Mdh-C* was linked to *Lap-A*.

Linkage group C

The third linkage group (C) was made up of three loci: *G6pd-1, Aco,* and *G6pd-2*. All possible two-locus combinations among these loci gave evidence of linkage (Tables 2 and 3). However, significant heterogeneity of linkage was found for the *G6pd-1/ Aco* pair (Table 3). These loci were not included in either of the other studies on *P. sylvestris*. According to our results, the *Aco* locus should be placed between the *G6pd-1* and *-2* loci (Fig. 1).

CONKLE (1981) found that the fast migrating *G6pd* locus was linked to *Aco* in *Pinus jeffreyi*. The slower *G6pd* locus was assigned to a separate linkage group including, among others, loci included in the group A in *P. sylvestris*. We did not find evidence of linkage of this locus with the A group. ALTUKHOV et al. (1986) found a linkage group consisting of the *6Pgd, Idh, G6Pd,* and *Gdh* loci in *Picea abies*.

Linkage group D

Evidence of significant linkage between the *Got-3* and *Sdh-2* loci was obtained from a single heterozygote (Table 2). The recombination frequency was quite low, 11.5 %. If our data were confirmed by analysis of additional heterozygotes, this linkage pair could be assigned to a separate group D (Fig. 1). The locus *Sdh-2* also appeared to be loosely linked (r = 0.379) to *Dia-3* in both trees tested (Table 3). *Got-3, Sdh-2* and *Dia-3* may all be in one linkage group D. On the other hand, no linkage was found between *Got-3* and *Dia-3* in the one heterozygote (Table 2). NIEBLING et al. (1987) found a linkage group which included *Got-3, 6Pgd-1, Dia-2,* and *Mdh-1*, which may correspond to this same group.

Other linkages

Loose associations were found for the remaining 15 two-locus combinations tested. However, because of the high recombination rates (all above 40 %),

no conclusive picture as to their possible location could be obtained. *Gdh* showed significant linkage to *G6pd-2*. *6Pgd-2* and *G6pd-2* were found to be linked in a single clone (Table 2). Similarly, no conclusive results were obtained concerning the possible location of the *Dia-2* locus. *Dia-2* was significantly associated with four loci: *Dia-1*, *Mdh-3*, *Aco*, and *Adh-1*. All pairs showed high recombination rates. All loci except *Aco* were included in the study of NIEBLING et al. (1987), but none of these findings were confirmed by their study, and all these three loci were on other linkage groups. *Dia-3* appeared to be linked to the *Sdh-1*, *Got-2*, and *Dia-2* loci (Table 2). NIEBLING et al. (1987) found a linkage to the group A. Significant linkages were also found between *Adh-1/Sdh-1*, *Adh-1/Pgm-1*, *Mdh-3/Pgi-2*, *Got-2/Sdh-2*, and *Got-2/Aph* (Table 2). The high recombination rates observed for these pairs made their mapping very uncertain.

Discussion

We found four different linkage groups among the 27 loci studied. In general, the results agreed well with the studies by RUDIN and EKBERG (1978) and NIEBLING et al. (1987) when fairly low recombination rates were concerned. A comparison of the study of NIEBLING et al. (1987) with ours shows that mapping based on high recombination rates (close to 0.40 or higher) is quite unreliable, even if the results in individual studies are statistically significant.

Recombination rates within species do vary. We found heterogeneity in recombination between the *Aco/G6Pd-1* and the *Dia-2/Dia-3* locus pairs. RUDIN and EKBERG (1978) and NIEBLING et al. (1987) also found heterogeneity for different locus pairs. Several factors could account for this heterogeneity. For instance, meiotic irregularities due to, e.g., an inversion polymorphism, would give rise to variable linkage (SAYLOR and SMITH 1966). Differences in crossing over intensity among trees could also result in inconsistent linkage estimates (ANDERSSON et al. 1969). RUDIN and EKBERG (1978) found differences in recombination rates betwen trees originating from northern and southern parts of Sweden. BARRETT et al. (1987) reported on variation in recombination rate between the *Got* and *Pgi* loci in *Picea mariana*.

Many studies are available on linkage in conifers. The linkage groups established seem to be remarkably well conserved across species. As shown by cytological studies, conifers are conservative with regard to their karyotypic structure (PEDERICK 1970; SAX and SAX 1933; SAX 1960. Indeed, as soon as the first linkage data became available, considerable similarity of various conifers was recognized (see CONKLE 1981). The results of NIEBLING et al. (1987) and our data on Scots pine provide further evidence for similarity of gene arrangement in conifers. However, it is more difficult to provide evidence for clear differences in linkages by comparing published studies. Some discrepancies are due to different designation of loci. Evidence of independent segregation is usually not published, and lack of linkage may be due to independent segregation or to a lack of test for the combination of loci.

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