GENETIC VARIATION OF NORWAY SPRUCE (*PICEA ABIES* [L.] KARST.) POPULATIONS IN AUSTRIA

I. DIGENIC DISEQUILIBRIUM AND MICROSPATIAL PATTERNS DERIVED FROM ALLOZYMES

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ABSTRACT

Allozyme loci were used to study digenic disequilibrium and the spatial pattern within three putatively autochthonous, naturally regenerated, high elevational *Picea abies* stands in Austria. In all populations more cases of digenic disequilibria were found than were to be expected stochastically alone. For all populations together, 24 two-locus combinations resulted in significant deviations from expected random multilocus structure. The measure of disequilibrium, common normalized correlation, was significant for loci *Fest2 – Mdh2* in all populations and for *Aat3 – 6Pgd3* and *Pepca – Mdh3* significant disequilibria were found in two out of three populations. In all other cases disequilibria were population specific. Non-random mating may be the most likely explanation of disequilibrium for unlinked or weakly linked loci. Fourteen two-locus combinations, for which no data on linkage are currently available, were in disequilibria. For this group it remains open whether linkage has been the major deterministic force. In most cases a random distribution of genotypic scores was found within the three populations. Spatial autocorrelation parameters were not significant for nearest neighbors based on the Gabriel-connected net. Spatial correlograms up to a distance of 100 m were computed. In total, 45 out of 700 (6.4 %) standard normal variates (*SND*) were found to be significant. Extensive pollen and seed dispersal may have accounted for the randomness of genes in space. In single loci, such as *Aat3–2* in POP-2, MORAN's index (*I*) was significant and positive in short distance classes and decreased to values close to zero in medium and long distances. On the other hand, significant negative MORAN's *I* values were also found in short distance classes for some loci (*e.g.*, *6–Pgd2–2* in POP-3).While variation in the breeding system (*e.g.*, non-random mating, limited seed and pollen dispersal) cannot be ruled out with certainty to have acted differently at the sites, lack of consistency in significant spatial patterns over the three populations maybe better explained by microselection or rare significant values have resulted by chance alone.

Key words: *Picea abies*, allozymes, correlogram, genetic variation, digenic disequilibria, spatial autocorrelation.

INTRODUCTION

Elucidating the structure of genetic variation in forest tree species is still a prime issue in forest genetics. Especially in the light of an uncertain global future, knowledge of genetic patterns will remain a cornerstone for a better understanding of forest ecosystems. Finally the deep understanding of evolutionary driven processes in space will lead to the improving of forest management including means taken for conservation.

Allozyme studies have indicated that forest tree species are generally highly variable (HAMRICK *et al.* 1992). While in many forest trees the macrospatial pattern of allozyme variation, *i.e.*, the distribution of genetic variants over major parts of the natural range of a species has been thoroughly investigated (see for instance, MÜLLER-STARCK *et al.* 1992), knowledge of the multilocus and spatial structure within single populations is still very limited.

Virtually all evolutionary forces can potentially influence the distribution of genetic variation. Multilocus proportions are influenced among others by linkage relationships, epistatic selection, and restricted gene flow. In forest trees digenic disequilbrium has not been often studied. This may be – at least in part – because inducing forces cannot be easily identified and explanations must often remain speculative. MITTON *et al.* (1980) have analyzed 7 isozyme loci at 3 localities along an elevational transect in *Pinus ponderosa*. They found 5 significant disequilibria out of 30 two-locus combinations and suggested that they resulted from epistatic selection (likely acting on *Pgm1*), although

other reasons could not be ruled out and linkage of loci was unknown. Digenic disequibrium at allozyme loci was also studied in 6 *Liriodendron tulipifera* populations of differing age. Numerous deviations from expected genotype proportions were found in young tree populations, whilst in sexually mature populations only weak evidence for digenic disequilibrium was reported (ROBERDS & BROTSCHOL 1985). A detailed study on disequilibrium was done by EPPERSON & ALLARD (1987) in *Pinus contorta*. They found significant deviations from expected multilocus genotype proportions only at certain but not at all investigated closely linked and unlinked isozyme loci. The authors concluded that neither drift nor population subdivision was responsible for the observed deviations but most probably epistatic selection has caused disequilibrium. In 66 *Pinus contorta* populations disequilibrium was correlated with latitude and restricted populations size and the mixing of different populations during collection (Wahlund effect) were presumed to have substantially contributed to disequilibria (YANG & YEH 1993). More lately, no significant digenic disequilibria were found in 48 specimens of *Picea abies* studied at random amplified polymorphic DNA (RAPD) loci (BUCCI & MENOZZI 1995).

Restricted gene-flow may drive isolation-by-distance processes due to restricted pollen and/or seed dispersal and ultimately may lead within a continuous population to patches of genetic variants which WRIGHT (1946) has termed neighborhoods. Moreover, microselection may also contribute to spatial substructuring within populations. With the emergence of appropriate methods to study genetically such microspatial patterns (SOKAL & ODEN 1978; CLIFF & ORD 1981; SOKAL & JACQUEZ 1991; EPPERSON 1994) several studies have also been made in forest trees. Thus, microspatial analyses were done in conifer species, such as *Larix laricina* (KNOWLES *et al.* 1992), *Picea mariana* (KNOWLES 1991), *Pinus banksiana* (XIE & KNOWLES 1991) and *P. contorta* (EPPERSON & ALLARD 1989). In many cases spatial autocorrelation suggested random distribution of genetic variants in space, while fine-scale genetic structure was reported in broadleaf species with short-distance seed dispersal such as *Quercus macrocarpa* (GEBUREK & TRIPP-KNOWLES 1994) and *Q. laevis* (BERG & HAMRICK 1995). Significant genetic patches were also detected in short-distance classes in the rainforest tree *Atherosperma moschatum* (SHAPCOTT 1995), *Fagus sylvatica* (LEONARDI & MENOZZI 1996), *Gleditsia triacanthos* (SCHNABEL & HAMRICK 1990),and *Maclura pomifera* (SCHNABEL *et al.* 1991). However, spatial substructuring was less pronounced in the broadleaf species *Acer saccharum* (PERRY & KNOWLES 1991; GEBUREK 1994; YOUNG *et al.* 1994) and spatial autocorrelation in *Psychotria nervosa*, a shrub which is pollinated by insects and dispersed by birds, yielded no significant spatial pattern (DEWEY & HEYWOOD 1988). Expectedly, genetic clusters were found in bird-dispersed tree species, such as *Pinus albicaulis* (FURNIER *et al.* 1987, SCHUSTER & MITTON 1991) and *P. flexilis* (CARSEY & TOMBACK 1994).

This is the first paper of a series of genetic investigations in Norway spruce (*Picea abies* [L.] Karst.) in Austria. This species was chosen for the study because of its great ecological and economical importance in Europe. Norway spruce grows naturally from lowland to subalpine altitudes. Valuable information was obtained in many gene marker studies in this conifer species [see KRUTOVSKII & BERGMANN (1995) for references]. However, knowledge of multilocus genetic structure and microspatial pattern in this conifer is incomplete. So far, there was no strong evidence of significant deviations from genotypic equilibrium in *Picea abies* based on RAPD markers and spatial genotypic distribution was also mostly random (BUCCI & MENOZZI 1995). BRUNEL & RODOLPHE (1985) have analyzed the spatial pattern of a single *Picea abies* stand by using allozyme loci. They found a slight but significant correlation between estimated genetic relationship and topographic distance. More recently, LEONARDI *et al.* (1996) have also found mostly random spatial distribution of genotypes in a Norway spruce stand. Main objectives of the present study were to analyze digenic disequilibria and the microspatial pattern within three naturally regenerated, putatively autochthonous populations in Austria.

MATERIAL AND METHODS

Study sites

Three putatively autochthonous and naturally regenerated (according to forest records) *Picea abies* populations (POP–1, POP–2, POP–3) located in the subalpine vegetation zone were studied. POP–1 is situated at Mt. Zirbitzkogel (47° 05' N, 14° 35' E) in the eastern Alpine transitional zone at an elevation of 1,600 m a.s.l.. At this site 70 % of the forest trees are Norway spruces besides European larches and stone pines. Most spruces are approximately 90–100 years old. POP–2 is a pure stand north of the Sengsengebirge (47° 49' N, 14° 22' E) found at an elevation of 1,300 m a.s.l. in the northern limestone Alps. Age of trees at this site may vary between 100 and 300 years. POP–3 is located north of the Proles Alpe at 47° 45' N and15° 29' E at an elevation of 1,300 m a.s.l. Norway spruce trees are roughly 160 years old there and make up 80 % of the

forest besides common beeches and sycamore maples.

At each site, starting from an arbitrarily chosen spruce in the center of the stand, in total 300 specimens with a stem diameter in breast height greater than 5 cm were selected. All 3 × 300 trees were theodoliticaly mapped.

Tissues, electrophoretic methods, and genotyping

Buds were collected from all selected trees by shotgun shooting. Thus, buds were collected from 300 trees at each site. From cone-bearing trees, seeds were additionally sampled by tree-climbing or by shooting. In total, single-tree seed lots from 82 (POP–1), 204 (POP–2), and 189 trees (POP–3) were gathered (Table 1). The diploid material was stored at –80 °C before homogenization. Dewinged seeds were kept separately for each tree and were stored at + 4 °C before usage.

Table 1. Trees genotyped using buds and seeds

Population	Trees genotyped using		
	buds	seeds	both
POP-1	300	82	300
POP-2	300	204	300
POP-3	300	189	300

Enzyme variants were separated by horizontal starch gel electrophoresis. Recipes for extraction buffers, staining solutions and electrophoretic conditions are given in CHELIAK & PITEL (1984). In Norway spruce, zymograms of malate dehydrogenases (MDH, EC 1.1.1.37) and 6-phosphogluconate dehydrogenases (6PGDH, EC1.1.1.44) of diploid material cannot be interpreted doubtless for all genotypes due to overlapping isozyme zones and identical or nearly identical Rm-values of some allozymes. In MDH additionally a modifer gene complicates a thorough interpretation (BREITENBACH-DORFER & GEBUREK 1995). To avoid biased estimates, the genotyping of loci encoding above-mentioned systems was based on segregation patterns of megagametophytes originating from single trees. At least 8 megagametophytes per tree were used for this group of isozymes ('group a'). For all other isozyme systems (cf. Table 2), buds were employed and in total 300 individuals were genotyped at each site. This group of isozymes was called 'group b'. For enzyme description, number and designation of encoding gene loci see Table 2. For linkage relationships of the marker genes see ALTUKHOV *et al.* (1987), MUONA *et al.* (1987); GEBUREK & VON WUEHLISCH (1989), GONCHARENKO *et al.* (1994) and BREITENBACH-DORFER & GEBUREK (1995).

Electrophoretic data analysis

Digenic disequilibrium

Digenic disequilibrium between single allozyme markers was estimated according to COCKERHAM & WEIR (1977) and based on genotypic data for which coupling and repulsion phase cannot be distinguished. Two-locus genotypes for two loci, A, B with alleles A_i, $i = 1,2,..., m$ and B_j, $j = 1,2 ..., n$, respectively, are studied. The frequency of genotypes formed by the union of gametes A_iB_j and A_kB_l is $P_{kl}^{ij} = P_{ij}^{kl}$. Sums of frequencies of genotypes indicated by dots for the indices summed, furnish respective marginal totals. Gametic frequencies of gamete A_iB_j, for instance, are denoted by

$$P_{..}^{ij} = \sum_k \sum_l P_{kl}^{ij} \qquad [1]$$

which equals the gametic frequencies of A_iB_j. Similarly the frequency of genotypes formed by the union of gametes bearing i at A and any other allele at B and any other allele at A and j at locus B is calculated as

$$P_{.j}^{i.} = \sum_k \sum_l P_{lj}^{ik} \qquad [2]$$

which equals the sum frequencies of the recombinants A_iB_j. For convenience, frequencies of alleles have the notation

$$p_i = P_{..}^{i.} = \sum_j \sum_k \sum_l P_{kl}^{ij} \qquad [3]$$

for A_i

$$q_j = P_{..}^{.j} = \sum_i \sum_k \sum_l P_{kl}^{ij} \qquad [4]$$

and for B_j instead. Since all these frequencies are population values and this paper is concerned with samples drawn from populations, tildes denoted sample values from the expected ones, such as

$$\tilde{P}_{..}^{ij} = \sum_k \sum_l \tilde{P}_{kl}^{ij} \qquad [5]$$

and then an estimate of the linkage disequilibrium is given as

$$\tilde{\Delta}_{ij} = \frac{N}{N-1}(\tilde{P}_{..}^{ij} + \tilde{P}_{.j}^{i.} - 2\tilde{p}_i\tilde{q}_j), \qquad [6]$$

Table 2. Enzyme systems, locus designations, and reference of genetic control

Enzyme system, acronym, EC reference	Tissue analysed[1]	Locus	Genetic control
Aspartate aminotransferase (AAT; 2.6.1.1)	b	*Aat1* *Aat2* *Aat3*	ALTUKHOV *et al.* (1987) MUONA *et al.* (1987)
Glutamate dehydrogenase (GDH; 1.4.1.3)	b	*Gdh*	ALTUKHOV *et al.* (1987)
Esterase (fluorescent) (FEST; 3.1.1.1)	b	*Fest1* *Fest2*	unpublished data
Isocitrate dehydrogenase (IDH; 1.1.1.42)	b	*Idh1* *Idh2*	ALTUKHOV *et al.* (1987) MUONA *et al.* (1987)
Malate dehydrogenase (MDH; 1.1.1.37)	m	*Mdh1* *Mdh2* *Mdh3* *Mdh4* *Mmd(2,3)*	ALTUKHOV *et al.* (1987) BREITENBACH-DORFER & GEBUREK (1995)
Phosphoenolpyruvate carboxylase (PEPCA; 4.1.1.31)	b	*Pepca*	unpublished data
6-phosphogluconate dehydrogenase (6PGD; 1.1.1.44)	m	*6pgd1* *6pgd2* *6pgd3*	MORGANTE *et al.* (1989)
Phosphogluconate isomerase (PGI; 5.3.1.9)	b	*Pgi*	MUONA *et al.* (1987)
Phosphoglucomutase (PGM; 2.7.5.1)	b	*Pgm1* *Pgm2*	MUONA *et al.* (1987)

[1] b = buds (groups 'a', see text); m = megagametophytes (group 'b', see text)

where

$$\tilde{P}_{..}^{ij} \qquad [7]$$

and

$$\tilde{P}_{j}^{i.} \qquad [8]$$

cannot be calculated separately, because frequencies of the coupling and repulsion heterozygotes

$$\sum_{k\neq i,j\neq j} P_{kl}^{ij} \qquad [9]$$

and

$$\sum_{k\neq i,j\neq j} P_{il}^{kj} \qquad [10]$$

are unknown. Only the sum of all double heterozygotes can be calculated. Commonly measures of linkage disequilibrium are normalized by products of gene frequencies. Following WEIR (1979), Δ_{ij} was normalized and the resulting correlation coefficient

$$R_{ij}=\tilde{\Delta}_{ij}/[(\tilde{p}_i(1-\tilde{p}_1)+(\sum_j \sum_k \tilde{P}_{ij}^{ik}-\tilde{p}_i^2))(\tilde{q}_i(1-\epsilon$$
$$+(\sum_i \sum_k \tilde{P}_{ij}^{kj}-\tilde{q}_j^2))]^{1/2} \qquad [11]$$

which ranges from −1 to +1 and was tested for significance.

Spatial analysis

Analysis of distribution of alleles in space was carried out by using MORAN's *I*. Allelic data were coded so that each allele was a variable existing in a single specimen. Thus each tree was characterized with respect to a certain allele whether it had 0, 1, or 2 copies. Corresponding genotypes were transformed to 0.0, 0.5 and 1.0. Only allozymes with frequencies less than .95 and greater than .05 were employed. For n alleles per locus, n–1 genotypic scores were spatially analyzed according to SOKAL & ODEN (1978) as

$$I=(N\sum_{l}\sum_{m}(w_{lm}z_l z_m))(\sum_{l}\sum_{m}w_{lm}\sum_{l}z_l^2)^{-1} \quad [12]$$

where: N = number of trees, w_{lm} = join matrix, where w_{lm} is set as 1 if the l^{th} and m^{th} tree are within the distance class and 0 otherwise, $z_l = x_l - x$, and $z_m = x_m - \bar{x}$.

The variables x_l and x_m are the genotypic scores for tree l and m, respectively, and $\bar{x}$ is the mean score for all trees of the respective population. It is common practice to rate autocorrelation by a specific set of paired sample location (CLIFF & ORD 1981). Therefore MORAN's indices were calculated for Gabriel-connected maps and for trees separated by certain distance classes. For the Gabriel-connected maps, trees A and B were considered to be contiguous, if the squared geographic distance between A and B was smaller than the sum of the squared geographic distances between AC and BC for which C is any other tree (GABRIEL & SOKAL 1969). For the correlogram a 5 m class interval was chosen. First and second moments of MORAN's I were calculated under the randomization assumption, the null hypothesis of CLIFF & ORD (1981). Whether or not each spatial coefficient significantly deviated from the expected $E = -1/(N-1)$ was tested by converting coefficient into standard normal deviates (*SND*) (SOKAL & ODEN 1978). According to ODEN (1984) overall significance of the spatial correlogram was assessed by using ŠIDAK's p-value (ŠIDAK 1967). Spatial autocorrelation coefficients greater than the expected value indicate that trees tend to be surrounded by trees that have similar genotypic scores and *vice versa* coefficients smaller than E indicate that trees are likely to have different genotypic scores in the respective distance class.

RESULTS

The allozyme loci studied carried at least two alleles in at least one of the populations. However, based on the 5 % criterion ($P_{0.05}$) only 46 % of the loci were polymorphic. Both average number of alleles (A_p range: 2.8 up to 3.3) and observed heterozygosity (H_o range: .165 up to .170) were very similar among the three populations. At all loci an excess of heterozygotes was observed.

In POP–1, 93 two-locus combinations were tested

Table 3. Significant digenic disequilibria (common normalized Δij) in three *Picea abies* populations.

Two-locus combination		Linkage	POP-1	POP-2	POP-3
Aat	*Aat2*	unlinked	.003	–	.127***[2]
Aat	*Fest2*	no data	.023	.061*	.051
Aat	*6Pgd1*	no data	–	.037	.171***
Aat	*6Pgd2*	unlinked	.073	.060***	.054
Aat	*6Pgd3*	unlinked	.253*	.068***	.037
Aat	*Pgi*	unlinked	.046	.041	.037*
Gdh	*Mdh3*	unlinked	–	.092*	.016
Gdh	*Pepca*	no data	.026	.049	.136**
Gdh	*Pgi*	unlinked	.119**	.015	.057
Fest2	*Idh1*	unlinked	.064	.019	.108**
Fest2	*Idh2*	no data	.115**	.040	.014
Fest2	*Mdh2*	no data	.252***	.113***	.062***
Fest2	*Mdh3*	no data	.084	.074	.041**
Fest2	*6Pgd2*	no data	.038	.032	.081***
Idh1	*Pepca*	no data	.098***	.046	.043
Idh2	*Mdh2*	no data	.014	.173***	–
Pepca	*Mdh3*	no data	.053	.100*	.115***
Pgi	*6Pgd1*	no data	–	.103***	.054
Pgi	*Pgm1*	unlinked	.103*	.025	.074
Pgm1	*6Pgd3*	unlinked	.205*	.038	.024
Mdh2	*Mdh3*	no data	.036	.085***	.038
Mdh2	*6Pgd1*	no data	–	.132***	.006
Mdh3	*6Pgd2*	no data	.055	.049	.084***
6pgd2	*6Pgd3*	unlinked	.169	.143***	.056

1) unlinked loci or no data available from linkage studies (ALTUKHOV *et al.* 1987, MUONA *et al.* 1987, GEBUREK & VON WUEHLISCH 1989, GONCHARENKO *et al.* 1994)

2) * P < 0.05; ** P < 0.01; *** P < 0.001

for disequilibrium and 7 significant ($P < 0.05$) cases were found while on a 5 % level only 5 cases are to be expected by chance alone. In POP–2 86 combinations were tested. Eleven significant cases were detected which is more than double the expected number by chance. Also in POP–3 more significant cases of disequilibrium were found than expected by chance (10 cases out of 110 combinations) (Tab. 3). Pooled over all three populations, 24 two-locus combinations resulted in significant deviations. The common normalized correlation for *Fest2–Mdh2* was significant in all populations and for *Aat3 – 6Pgd3* and *Pepca – Mdh3* significant disequilibrium was found in two out of three populations. Many disequilibrium coefficients for allelic combinations were significant at respective two-locus combinations. However, often significant values were found for rare alleles and chi-squares became inflated (data not shown).

According to the criteria that were imposed, 11 allozymes were eligible for the spatial analysis in POP–1 and POP–2, and 13 allozymes were used for POP–3. In none of the populations, MORAN's *I* resulted in significant values based on the Gabriel-connected nearest neighbor network (POP–1: mean distances 9.95 m for allozymes of group a and 5.30 m for group b, respectively; POP–2:5.85 m (group a) and 4.67 m (group b); POP–3: 7.68 m (group a) and 6.17 m (group b). For the correlograms in total, 45 out of 700 (6.4 %) standard normal variates (*SND*) were found to be significant. Significant associations between genotypic scores as a function of distance class are shown by Fig. 1. In the short distance class (5–10 m) and medium distance classes (15–20 m, 30–35 m, 35–40 m, 40–45 m, 60–65 m) more significant values were detected than could be expected by 5 % chance alone. In single cases, such as *Aat3–2* in POP–2, MORAN's *I* was significantly positive in short distance classes and decreased to significant values close to zero in medium and long distances (ŠIDAK's $p = 0.045$). On the other hand, significant negative MORAN's *I* values were also found in short distance class [*e.g.*, *6-Pgd2–2* in POP–3 (ŠIDAK's $p = 0.019$)]. Figure 2 shows the correlograms for the 3 populations.

DISCUSSION

Digenic disequilibria

Different forces may cause deviations from random multilocus associations: (1) close linkage and/or limited effective population size (OHTA 1982), (2) non-random mating (WEIR & COCKERHAM 1973), (3) population subdivision (SLATKIN 1975),(4) hitch-hiking effects (ASMUSSEN & CLEGG 1981), and (5) epistatic selection

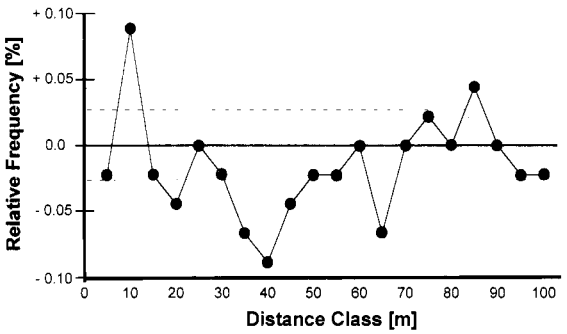

Figure 1. Difference of positive and negative significant associations [standard normal variate (*SND*)] between like genotypic scores and distance class. Points are shown for the upper limits of distance classes.

(HASTINGS 1981).

Significant disequilibrium was detected for several two-locus combinations. By chance alone (5 % level) 15 cases [0.05 x (93 +86 + 110] = 15) were expected, but nearly twice as much, i.e., 28 cases were found. Note that for each population not all 120 two-locus combinations could be tested due to lack of polymorphism at respective loci.

It was expected that digenic disequilibrium would be predominantly be found at closely linked loci. For instance, in *Pinus contorta* disequilibria were found exclusively at linked loci (EPPERSON & ALLARD 1987) while in *Picea abies* predominantly linked loci were prone to deviations from expectations (BUCCI & MENOZZI 1995). Close linkage (recombination frequency $c < 0.08$) in *Picea abies* exists for instance between *Aat1–Pgi*, *Pgm2–Mdh3*, and *Gdh–Idh2* (MUONA *et al.* 1987, GEBUREK & VON WUEHLISCH 1989, GONCHARENKO *et al.* 1994). Therefore, these loci have been of particular interest for this study. However, linkage disequilibrium was unexpectedly not significant for linked loci (*Aat1–Pgi2* and *Gdh–Idh2*), while deviation from digenotypic expectations for *Pgm2–Mdh3* could not be studied due to a lack of locus polymorphism in the three populations. Out of 28 two-locus combinations tested, 10 cases exist for unlinked loci while for the remaining 18 cases linkage relationships are unknown(MUONA *et al.* 1987, GEBUREK & VON WUEHLISCH 1989, GONCHARENKO *et al.* 1994). For the latter group it thus remains unclear whether linkage may be a deterministic force. Disequilibria were not randomly distributed over all loci but mostly involved *Fest2*, *Aat3*, and *Mdh2*, including three two-locus combinations existed *Aat3 –6Pgd3*, *Fest2 – Mdh2*, *Pepca – Mdh3* for which disequilbria were detected in more than one population.

For unlinked loci only very high selection coefficients s (in most cases $0.5 \geq s > 0.3$) following HASTINGS' model (1981) (see Fig. 1 and 2 in his paper)

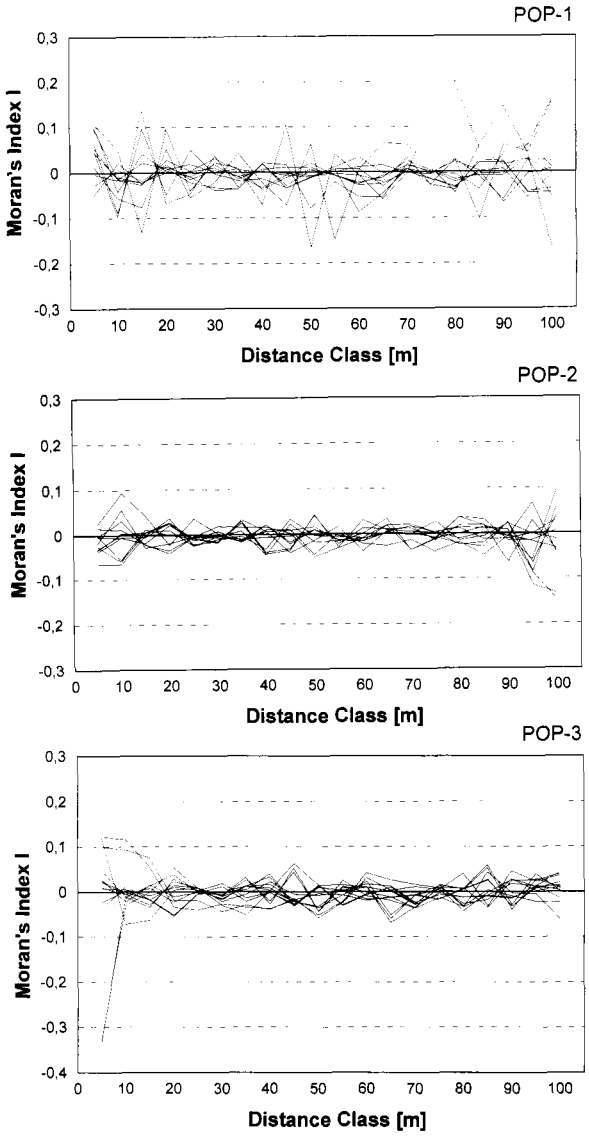

Figure 2. Correlograms for 11 (POP–1 and POP–2) and 13 (POP–3) genotypic scores. MORAN's indices were plotted at the upper limits of distance classes.

would explain disequilibrium values found in this study. Such intensive selection, however, is not very likely for allozymes and it seems therefore more appropriate to exclude epistatic selection as a force for these specific locus combinations. ASMUSSEN & CLEGG (1981) have shown, that the development of disequilibrium is attached to selection coefficients which must exceed the recombination fraction which in turn would result in very high selection on one of the two unlinked loci.

It is also very questionable whether hitch-hiking effects can explain observed deviations from expected multilocus proportions. Such effects cause disequilibrium if selection is acting upon a certain locus while another linked locus is selectively neutral. For allozymes, the impact of hitch-hiking on disequilibrium depends on the vexed question of neutral versus selective action. While certain allozymes are not strictly neutral (BUTLIN & TREGENZA 1998), strong evidence of allozymic adaptiveness within single, local *Picea abies* populations is still missing (*e.g.*, HERTEL & KOHLSTOCK 1996).

Theoretically also limited effective populations size can create disequilibrium and for expected values of normalized correlation can be estimated as $1/(1+4Nc-2c-2Nc^2+c^2)$ (SVED & FELDMAN 1973). In Austria, Norway spruce occurs at high altitudes in large stands which have been occupied the area for approximately 12.000 years after ceasing of glaciation. It cannot be excluded that the populations under study may be the 150th or later generations after postglacial reimmigration into the alpine region. Though historic bottlenecks cannot be ruled out due to biotic or abiotic catastrophes or more lately by intensive human harvests, effective population size (N_e) probably has been high. According to CRAWFORD's (1984) proposal N_e was roughly estimated (see below) and is at least in the order of several thousand specimens. If severe bottlenecks are assumed, N_e still is in the order of several hundred trees. Based on these, even very rough assumptions, it is not very likely that restricted population sizes would have caused disequilibrium for weakly linked or unlinked loci. It seems more likely that disequilibrium detected for instance at *Aat3–6Pgd3* or other unlinked loci has resulted from non-random mating (WEIR & COCKERHAM 1973) and/or has been due to the multilocus Wahlund effect (SINNOCK 1975). If further it is assumed that (1) the populations are of natural origin – as stated in forest records – and (2) an initial disequilibrium was due to mixing of populations (e.g., joining of two immigrating populations after glaciation) a break-down of such a disequilibrium would occur for unlinked or linked loci with high recombination rates within a couple of panmictic generations (see for instance HATTEMER *et al.* 1993, *l.c.* pp. 198–204). This explanation is further supported by the observation that neither strong microspatial structure (see below) nor a pronounced macrospatial structure among the three populations could be found at studied loci. Therefore, the most likely explanation may be non-random mating under the assumptions made for unlinked or weakly linked loci. Fourteen two-locus combinations, for which no data on linkage are currently available, were in disequilibria and thus it would be speculative to exclude one or more factors creating multilocus deviations.

Spatial autocorrelation

Spatial autocorrelation revealed that in most cases genes were randomly distributed in space. Only approximately 7 % of the spatial correlations were significant. Restricted gene-flow in course of limited pollen and/or seed dispersal and microselection can potentially cause patches of like and unlike genotypes in space (SOKAL *et al.* 1989, SOKAL & JACQUEZ 1991).

The reproductive system can strongly effect genetic patchiness. Disaccate pollen of *Picea abies* has a medium to high deposition speed [5.6 up to 8.7 cm /s (STRAKA 1975, *l.c.* p. 170)] and REMPE (1938) showed, that *Picea abies* pollen is much more restricted to vertical transvection than many other forest tree species. Nevertheless, considerable pollen quantities can be transported easily several hundred meters, while exceptionally travels of hundreds of kilometers were reported (REMPE 1938). ANDERSSON (1955) furnished data that pollen density decreased to 30 % and 61 % at a distance of 200 m from the point source under windless and windy conditions (5 m/s wind speed). Expectedly, these features contribute to an extensive gene-flow. In an isolated *Picea abies* population, for instance, 16 % of the pollen effective in fertilizating ovules came from trees at least 120 m away (XIE & KNOWLES 1994). Winged seeds were distributed approximately 50 m from the source depending on horizontal wind speed (KOHLERMANN 1951). Therefore, considerable mechanisms exist in *Picea abies* that favor gene dispersal. However, it is noteworthy in this context that evidence of family structures in *Picea abies* stands has been obtained using phenotypic traits (MALEEV 1986). According to CRAWFORD (1984), N_e can be estimated as $4\pi\delta\ (\sigma_p/2 + \sigma_s)$, when δ equal the density of sexually mature trees, σ_p and σ_s are the standard deviation of pollen and seed dispersal. Density of seed bearing trees for POP–2 and POP–3 (for POP–1 no estimate is available) varied from 160 to 260 trees/ha. If very conservative estimates are made (e.g., calm weather) and it is assumed that within 100 m 91% of the pollen is deposited (ANDERSSON 1955), then 100 m can be taken as standard deviation (see WRIGHT 1976, *l.c.* pp. 74–75). KOHLERMANN (1951) furnished σ_s (40 m , wind speed = 2.7 $m \cdot s^{-1}$). Based on these assumptions, estimate of $N_e > 10^4$and SOKAL *et al.* (1989) have shown that on theoretical grounds even much smaller neighborhood sizes have not resulted in distinct genetic patches. Therefore, significant autocorrelation coefficients are more likely the result of microselection rather than due to restricted gene flow. Restricted gene flow should affect all loci essentially in the same manner at different sites. The small number of significant spatial associations may therefore suggest microselection rather than is due to the breeding system. While the majority of allozymes are probably selectively neutral, some exceptions may exist. For instance, in *Picea abies* selective effects of different site conditions could be traced down by allozymes (BERGMANN & HOSIUS 1996). However, weak effects observed in their study cannot explain the spatial pattern of this study.

Furthermore, epistatic selection may also have contributed to the weak non-random spatial pattern. Based on the data of this study it is impossible to exclude doubtless features related to the breeding system or selection as already was shown by SLATKIN & ARTER (1991) that spatial autocorrelation analysis per se cannot discriminate between selection and processes linked to the reproductive system.

Finally a possible explanation of the findings could also stem from the forest stand history (cf. discussion of digenic disequilibria).Although the populations are presumably of autochthonous origin and, hence, have been naturally regenerated, it must remain unclear whether forest management has had an significant impact on the genetic structure in the past. That different establishment histories shape genetical structures of forest trees species and that this can be detected by spatial autocorrelation analysis has been shown by KNOWLES *et al.* (1992).

CONCLUSIONS

It is impossible to identify single factors causing significant digenic disequilibria or non-random associations of genes in space. However, while non-random mating seems to be the most likely explanation for the deviation from expected random multilocus frequencies, associations of similar genotypic scores in the three *Picea abies* populations are likely the result of microselection or are statistical artefacts. Due to a lack of pronounced genetic clumping, minimum distances between trees used for plus-tree selection or for seed harvests may be not very critical when allozyme variation is tackled.

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REFERENCES

ALTUKHOV, Y.P., KRUTOVSKII, K.V., GAFAROV, N.I., DUKHAREV, V.A. & MOROZOV, G.P. 1987: Allozyme variability in a natural population of Norway spruce (*Picea abies*

(L.) Karst.). I. Polymorphism systems and mechanisms of their genetic control. *Soviet Genetics* **22**: 1028–1040 [Original article was first published in Russian in *Genetica* **22**: 2135–2151 (1986)].

ANDERSSON, E. 1955: Pollenverbreitung und Abstandsisolierung von Forstsamenplantagen. *Zeitschrift für Forstgenetik und Forstpflanzenzüchtung* **4**: 150–152.

ASMUSSEN, M.A. & CLEGG, M.T. 1981: Dynamics of the linkage disequilibrium function under models of gene-frequency hitchhiking. *Genetics* **99**: 337–356.

BERG, E. E. & HAMRICK, J. L. 1995: Fine-scale genetic structure of a Turkey oak forest. *Evolution* **49**: 110–120.

BERGMANN, F. & HOSIUS, B.1996: Effects of heavy-metal polluted soils on the genetic structure ofNorway spruce seedling populations. *Water, Air, and Soil Pollution* **89**: 363–373.

BREITENBACH-DORFER, M. & GEBUREK, Th. 1995: Gene modifies electrophoretic properties of malate dehydrogenase in Norway spruce (*Picea abies* (L.) Karst.). *Hereditas* **122**: 103–108.

BRUNEL, D. & RODOLPHE, F. 1985: Genetic neighbourhood structure in a population of *Picea abies* L.. *Theoretical and Applied Genetics* **71**: 101–110.

BUCCI, G. & MENOZZI, P. 1995: Genetic variation of RAPD markers in a *Picea abies* Karst. population. *Heredity* **75**: 188–197.

BUTLIN, R. K. & TREGENZA, T. (1998): Levels of genetic polymorphism: marker loci versus quantitative traits. *Philosophical Transactions Royal Society London, Series B,* **353**: 187–198.

CARSEY, K. S. & TOMBACK, D. F. 1994: Growth form distribution and genetic relationships in treeclusters of *Pinus flexilis*, a bird-dispersed pine. *Oecologia* **98**: 402–411.

CHELIAK, W. M. & PITEL, J. A. 1984: Techniques for starch gel electrophoresis of enzymes from forest tree species. Agriculture Canada, Can. For. Serv., Inf. Rep. PI–X–42, 49p.

CLIFF, A. D. & ORD, J. K. 1981: Spatial Processes. Models & Applications. Pion Ltd. London, UK, 266 pp.

COCKERHAM, C. C. & WEIR, B. S. 1977: Digenic descent measures for finite populations. *Genetical Research, Cambridge.* **30**: 121–147.

CRAWFORD, T. J. 1984: The estimation of neighborhood parameters for plant populations. *Heredity* **52**: 273–283.

DEWEY, S. E. & HEYWOOD, J. S. 1988: Spatial genetic structure in a population of *Psychotria nervosa.* I. Distribution of genotypes. *Evolution* **42**: 834–838.

EPPERSON, B. K. 1994: Spatial and time-space correlations in systems of subpopulations with stochastic migration. *Theoretical Population Biology* **46**: 160–197.

EPPERSON, B. K. & ALLARD, R. W. 1987: Linkage disequilibrium between allozymes in natural populations of logdepole pine. *Genetics* **115**: 341–352.

EPPERSON, B. K. & ALLARD, R. W. 1989: Spatial autocorrelation analysis of the distribution of genotypes within populations of lodgepole pine. *Genetics* **121**: 369–377.

FURNIER, G. R., KNOWLES, P. CLUDE, M. A. & DANCIK, B. P. 1987: Effects of avian seed dispersal on the genetic structure of whitebark pine populations. *Evolution* **41**: 607–612.

GABRIEL, K. R. & SOKAL, R. R. (1969): A new statistical approach to geographical variation analysis. *Systematic Zoology* **18**: 259–278.

GEBUREK, TH. 1993: Are genes randomly distributed over space in mature populations of sugar maple (*Acer saccharum* Marsh.)? *Annals ofBotany* **71**: 217–222.

GEBUREK, TH. & TRIPP-KNOWLES, P. 1994: Genetic architecture in bur oak, *Quercus macrocarpa* (Fagaceae), inferred by means of spatial autocorrelation analysis. *PlantSystematics and Evolution* **189**: 63–74.

GEBUREK, TH. & VON WUEHLISCH, G. 1989: Linkage analysis of isozyme gene loci in *Picea abies* (L.) Karst.. *Heredity* **62**: 185–191.

GONCHARENKO, G. G., PADUTOV, V. E. & SILIN, A. E. 1994: Construction of genetic maps for some Eurasian coniferous species using allozyme genes. *Biochemical Genetics* **32**: 223–236.

HAMRICK, J. L., GODT, M. W. & SHERMAN-BROYLES, S. L. 1992: Factors influencing levels of genetic diversity in woody plants species. *New Forests* **6**: 95–124.

HASTINGS, A. 1981: Disequilibrium , selection, and recombination: Limits in two-locus, two-allele models. *Genetics* **98**: 659–668.

HATTEMER, H. H., BERGMANN, F. & ZIEHE, M. 1993: Einführung in die Genetik für Studierende der Forstwirtschaft. J.D. Sauerländer's Verlag, Frankfurt a. M., Germany,492 pp.

HERTEL, H. & KOHLSTOCK, N. 1996: Forstpflanzenzüchtung und Biodiversität: Ein Modellversuch zur Untersuchung des Zusammenhangs zwischen genetischer Variation und Stabilität am Beispiel der Fichte (*Picea abies* L. Karst.). *In:* Biodiversität und nachhaltige Forstwirtschaft (ed. G. Müller-Starck). pp. 93–104. Ecomed-Verlagsgesellschaft, Landsberg, Germany.

KNOWLES, P. 1991: Spatial genetic structure within two natural stands of black spruce (*Picea mariana* (Mill.) B.S.P.). *Silvae Genetica* **40**:13–19.

KNOWLES, P., PERRY, D. & FOSTER, H. A. 1992: Spatial genetic structure in two tamarack [*Larix laricina* (Du Roi) K. Koch] populations with differing establishment histories. *Evolution* **46**: 572–576.

KOHLERMANN, L. 1950: Untersuchungen über die Windverbreitung der Früchte und Samen mitteleuropäischer Waldbäume. *Forstwissenschaftliches Centralblatt* **69**: 606–624.

KRUTOVSKII, K. V. & BERGMANN, F. 1995: Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) Karst., and Siberian, *P. obovata* Ledeb., spruce species studied by isozyme loci. *Heredity* **74**: 464–480.

LEONARDI, S. & MENOZZI, P. 1996: Spatial structure of genetic variability in natural stands of *Fagus sylvatica* L. (beech) in Italy.*Heredity* **77**: 359–368.

LEONARDI, S., RADDI, S. & BORGHETTI, M. 1996: Spatial autocorrelation of allozyme traits in a Norway spruce (*Picea abies*) population. *Canadian Journal of Forest Research* **26**: 63–71.

MALEEV, K. I 1986: Possibility of the isolated existence of families in community populations of *Picea abies* × *Picea obovata. Botanicheskii Zhurnal* **71**: 892–903. [Russian]

MITTON, J. B., STURGEON, K. B. & DAVIS, M. L. 1980:

Genetic differentiation in ponderosa pine along a steep elevational transect. *Silvae Genetica* **29**: 100–103.

MORAN, P. A. P. 1950: Notes on continuous stochastic phenomena. *Biometrika* **37**: 17–23.

MORGANTE, M., VENDRAMIN, G. G. & GIANNINI, R. 1989: Genetics of 6PGD and SKDH in Norway spruce (*Picea abies* K.). *Journal of Genetics & Breeding* **43**: 67–72.

MÜLLER-STARCK, G., BARADAT, PH. & BERGMANN, F. 1992: Genetic variation within European tree species. *New Forests* **6**: 23–47.

MUONA, O., YAZDANI, R. & LINDKVIST, G. 1987: Analysis of linkage in *Picea abies*. *Hereditas* **106**: 31–36.

ODEN, N. L. 1984: Assessing the significance of a spatial correlogram. *Geographical Analysis* **16**: 1–16.

OHTA, T. 1982. Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proceedings of the National Academy of Sciences, U.S.A.* **79**: 1940–1944.

PERRY, D. & KNOWLES, P. 1991: Spatial genetic structure within three sugar maple (*Acer saccharum* Marsh.) stands. *Heredity* **66**: 137–142.

REMPE, H. 1938: Untersuchungen über die Verbreitung des Blütenstaubes durch Luftströmungen. *Planta* **27**: 93–147.

ROBERDS, J. H. & BROTSCHOL, J. V. 1985: Linkage disequilibrium among allozyme loci in natural populations of *Liriodendron tulipifera* L.. *Silvae Genetica* **34**: 137–141.

SCHNABEL, A. & HAMRICK, J. L. 1990: Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* **77**: 1060–1069.

SCHNABEL, A., LAUSHMAN, R. H. & HAMRICK, J. L. 1991: Comparative genetic structure of two co-occuring tree species, *Maclura pomifera* (Moraceae) and *Gleditsia triacanthos* (Leguminosae). *Heredity* **67**: 357–364.

SCHUSTER, W. & MITTON, J. B. 1991: Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. *Heredity* **67**: 41–48.

SHAPCOTT, A. 1995: The spatial genetic structure in natural populations of the Australian temperate rainforest tree *Atherosperma moschatum* (Labill.) (Monimiaceae). *Heredity* **74**, 28–38.

ŠIDAK, Z. 1967: Confidence regions for the means of multivariate normal distributions. *Journal of the American Statistician Association* **62**: 626–633.

SINNOCK, P. 1975: The Wahlund effect for the two locus model. *American Naturalist* **109**: 565–570.

SLATKIN, M.1975: Gene flow and selection in a two-locus system. *Genetics* **81**: 787–802.

SLATKIN, M. & ARTER, H. E. 1991: Spatial autocorrelation methods in population genetics. *American Naturalist* **138**: 499–517.

SOKAL, R. R. & ODEN, N. L. 1978: Spatial autocorrelation in biology. 1. Methodology. *Biological Journal of the Linnean Society* **10**: 199–228.

SOKAL, R. R. & JACQUEZ, G. M. 1991: Testing inferences about microevolutionary processes by means of spatial autocorrelation analysis. *Evolution* **45**: 152–168.

SOKAL, R. R., JACQUES, G. M. & WOOTEN, M. C. 1989: Spatial autocorrelation analysis of migration and selection. *Genetics* **121**: 845–855.

STRAKA, H. 1975: Pollen- und Sporenkunde. Gustav Fischer Verlag, Stuttgart, Germany, 238 pp.

SVED, J. A. & FELDMAN, M. W. 1973: Correlation and probability methods for one and two loci. *Theoretical Population Biology* **4**: 129–132.

WRIGHT, S. 1946: Isolation by distance under diverse systems of mating. *Genetics* **31**: 39–59.

WRIGHT, J. W. 1976: Introduction to Forest Genetics. Academic Press, New York, U.S.A.,463 pp.

WEIR, B. S. 1979: Inferences about linkage disequilibrium. *Biometrics* **35**: 235–254.

WEIR, B. S. & COCKERHAM, C. C. 1973: Mixed self and random mating at two loci. *Genetical Research, Cambridge* **21**: 247–262.

XIE, C. Y. & KNOWLES, P. 1991: Spatial genetic substructure within natural populations of jack pine (*Pinus banksiana*). *Canadian Journal of Botany* **69**: 547–551.

XIE, C. Y. & KNOWLES, P. 1994: Mating system and effective pollen immigration in a Norway spruce (*Picea abies* (L.) Karst.) plantation. *Silvae Genetica* **43**: 48–52.

YANG, R.-C. & YEH, F. C. 1993: Multilocus structure in *Pinus contorta* Dougl.. *Theoretical and Applied Genetics* **87**: 568–576.

YOUNG, A. G., WARWICK, S. I. & MERRIAM, H. G. 1993: Genetic variation and structure at three spatial scales for *Acer saccharum* (sugar maple) in Canada and the implications for conservation. *Canadian Journal of Forest Research* **23**: 2568–2578.