Theor. Appl. Genet. 52, 57-64 (1978)

The Allelic Distribution at an Acid Phosphatase Locus in Norway Spruce *(Picea abies)* Along Similar Climatic Gradients

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Summary. The allele frequency distribution at a polymorphic acid phosphatase locus (APH-B) was determined in natural populations of Norway spruce *(Picea abies)* from a latitudinal transect in Finland, an altitudinal transect in the Austrian Alps, and from different locations of the Swiss range. The three independent population groups, selected with respect to similar climatic gradients, were studied to detect the forces that cause the geographic variation at the APH-B locus.

In almost all of the populations investigated, four alleles (APH-B_1 - B_4) could be identified at this enzyme locus, however, the alleles B_1 and B_2, as well as B_3 and B_4, show a great similarity according to their phenotypic appearance after electrophoresis as well as to their frequency distributions along the different transects. With the aid of some theoretical considerations and data comparisons, a selective equivalence of the alleles B_1 and B_2, as well as B_3 and B_4, could be ascertained, thus reducing the number of alleles that can respond differently to natural selection.

After combining the frequencies of the selectively equivalent alleles, similar clinal variation patterns could be observed along the different geographical transects, whereby the frequency of the allele group APH-B_1/B_2 markedly increases with latitudes in Finland and towards higher elevations in the Alps. Correspondingly, the allele group APH-B_3/B_4 predominates in the southern parts of Finland and in the lowlands and foothills of Austria and Switzerland. It is therefore concluded that natural selection causes the geographic variation pattern at the APH-B locus and that one or several temperature variables function as an at least predominant selective force. Possible relationships between this enzyme polymorphism and other tree characters and the physiological role of acid phosphatases in tree adaptation were discussed.

Key words: Enzyme polymorphism – Geographical variation – Gene frequency cline – Climatic gradient – Norway spruce – *Picea abies*

Introduction

The introduction of molecular biological techniques during the last decade has resulted in the discovery of large amounts of genic variation within and among natural populations of many animal and plant species (for review and literature compilation, see Lewontin 1974). It appears that a substantial number of the structural genes coding for enzymes and structural proteins exhibits allelic forms mostly detectable by electrophoretic procedures. The question, however, as to how this high degree of genic variation is maintained in natural populations, can not yet be answered satisfactorily. There is some evidence from empirical data that these genetic polymorphisms are maintained by different types of balancing selection, however, theoretical considerations suggest that the majority of molecular polymorphisms are selectively neutral and possess variation patterns which are only due to mutation rate, genetic drift and migration (see detailed views in Lewontin 1974; Nei 1975; Ayala 1976). One possibility for contributing to the solution of these problems is the presentation of ecological genetic data demonstrating close relationships between the genic variation observed in populations and one or several relevant ecological factors.

Among plant species, forest trees are particularly characterized by their relatively long lifetime and long generation time. Since individuals are forced to stay in one place during their whole lifetime, and since populations are generally located in large heterogeneous areas, forest tree species are particularly adapted to temporally and spatially varying environments (Stern and Roche 1974). Therefore,

0040-5752/78/0052/0057/$ 1.60

it might be assumed that natural forest tree populations contain a relatively large amount of genetic variation consisting of a high proportion of polymorphic loci and considerable heterozygosity per individual. In fact, several studies, although not as extensive as with Drosophila or man, have shown that most or nearly all enzyme and protein loci identified in forest trees are polymorphic with respect to electrophoretic variants (for review see Feret and Bergmann 1976; Rudin 1976). This great genic variation, however, need not be involved in the adaptive strategies of forest tree populations since it may also result from a generally high mutation rate and a large effective population size (Nei 1975). Consequently, it is necessary to examine the adaptive significance of the enzyme polymorphism observed in forest tree populations.

The purpose of the present investigation was to study the distribution of alleles at a gene locus coding for acid phosphatase in two independent geographical transects of Norway spruce *(Picea abies)* populations. One set of populations is located at different latitudes in Finland, whereas the other set grows at different altitudes in the Alps. The investigation should determine whether similar climatic gradients (such as temperature) occurring in the latitudinal as well as in the altitudinal transect have led to similar genic variation patterns. If the allelic forms at this acid phosphatase locus are selectively neutral, the variation patterns along the two transects should reveal no correlation, especially since the two sets of populations are different with respect to the migration rate, population size, and age. The description of the acid phosphatase polymorphism found in specific tissues of Norway spruce and the analysis of its genetic control have been presented in a previous paper (Bergmann 1974).

Materials and Methods

The transect from North to South Finland is represented by eight Norway spruce provenances, which constitute relatively large populations. Although the individual populations are separated by large distances, they are parts of the continuously spread Finnish spruce range. The geographical data for the eight provenances are given in Table 1. The population samples used in our investigation generally consisted of seed lots from individual trees (18-25 trees per population); in two cases (Inari, Kuhmo), only mixed seed samples originating from numerous trees were available. Additionally, mixed seed samples from the other provenances were scored to check the results obtained from the single-tree samples. In a few cases, the data from the mixed seed sample deviate somewhat from those of the single-tree sample so that the frequency data published previously (Bergmann 1975) must be modified.

The altitudinal transect consists of six relatively small populations located within a limited area of the Seetaler Alps in Austria (Fig. 2). Since these populations are linked by several or a few (towards the higher elevations) other spruce stands, they should be termed demes. The geographical data are given in Table 2. From each population, a mixed seed sample originating from 15-20 trees

Table 1. Geographic Data for 8 Finnish Norway-spruce Provenances and Allele Frequencies at the APH-B Locus found in Provenance Samples

Provenance	Region	Geographic Data			Allele Frequencies at APH-B Locus					
Name		Lat.	Long.	Alt.	B_1	B_2	B_1/B_2	B_3	B_4	B_3/B_4
Inari	North F.	68°44′	26°42′	280 m	0.39	0.49	0.88	0.05	0.07	0.12
Kittilä/Pallasjärvi	North F.	68°02′	24°05′	480 m	0.41	0.36	0.77	0.14	0.09	0.23
Kolari	North F.	67°16′	23°51′	150 m	0.34	0.27	0.61	0.23	0.16	0.39
Suomussalmi	Central F.	64°50′	29°35′	220 m	0.23	0.17	0.40	0.33	0.27	0.60
Kuhmo	Central F.	64°15′	29°30′	230 m	0.17	0.19	0.36	0.30	0.34	0.64
Pihtipudas	Central F.	63°17′	25°27′	170 m	0.09	0.12	0.21	0.36	0.43	0.79
Juva	South F.	61°55′	27°58′	100 m	0.08	0.06	0.14	0.39	0.47	0.86
Tuusula	South F.	60°21′	24°59′	50 m	0.03	0.03	0.06	0.48	0.46	0.94

Table 2. Allele Frequencies at the APH-B Locus found in Seed Samples of 6 Populations from the Seetaler Alps (Lat. 47°05′, Long. 14°40′)

Population	Altitude	Allele Frequencies at APH-B Locus					
		B_1	B_2	B_1/B_2	B_3	B_4	B_3/B_4
Murauen	700 m	0.01	0.03	0.04	0.44	0.52	0.96
Eppenstein	850 m	0.05	0.06	0.11	0.39	0.50	0.89
Granitzen	1000 m	0.06	0.09	0.15	0.36	0.49	0.85
St. Wolfgang	1200 m	0.15	0.17	0.32	0.29	0.39	0.68
Schmelz I	1400 m	0.37	0.45	0.82	0.08	0.10	0.18
Schmelz II	1600 m	0.40	0.54	0.94	0.02	0.04	0.06

was used in the analyses. Additionally, four provenances from different locations of the Swiss spruce range were selected for our study with respect to their different elevations (Table 3). From these populations, mixed seed samples were also available.

In all analyses, the endosperm tissue from dormant seeds served as test material. In conifer seeds, this storage tissue results from the haploid macrogametophyte after fertilization and thus represents a gamete of the mother tree. Therefore, it is relatively simple to identify the genotype of individual trees by means of endosperm analyses. Furthermore, the utilization of this tissue provides several methodical and analytical advantages (Bergmann 1973; Feret and Bergmann 1976).

The acid phosphatase system (APH, EC 3.1.3.2.; APH corresponds to the German abbreviation SAP used in previous papers) selected for this study was found to be active in the dry endosperm tissue of dormant seeds, in dormant embryos, and in germinating seedlings during the first weeks. Since the electrophoretic APH pattern of single endosperms indicates the corresponding alleles, only nine seeds per tree were analyzed for genotype identification (the probability for an erroneous classification is $(1/2)^{n-1}$). The allele frequencies were estimated from the genotype frequencies of the respective tree sample. In the cases where no single-tree seed lots were available, the allele frequencies were estimated from a sample of 200 seeds (gametes) per population. This quantity was usually sufficient to provide representative data of the mixed population sample.

The experimental procedures (enzyme extraction, electrophoretic separation, isoenzyme staining) and the evaluation of the phenotypic APH patterns have already been described in detail (Bergmann 1974).

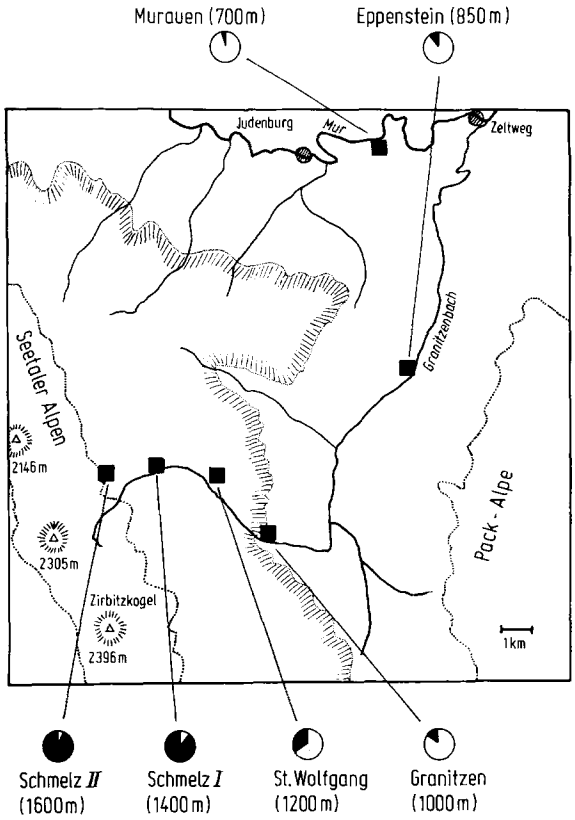

Fig. 2. Map showing the localities of the six Norway spruce populations from the altitudinal transect in the Seetaler Alps (Austria). The circles show the frequencies of alleles APH-B_1/B_2 (black) and APH-B_3/B_4 (white)

Results

The Frequency Distribution of APH-B Alleles Along the Latitudinal Transect in Finland

The APH polymorphism found in Norway spruce seeds is controlled by two unlinked gene loci. While one of these loci (APH-A), coding for the faster migrating APH zone in zymograms (Fig. 1), exhibits only two alleles in our test material, the other gene locus (APH-B) was found to have four different allelic types (APH-B_1 - B_4) (Bergmann 1974). In a few cases, an additional allele without enzyme activity could be observed at the APH-B locus, but this so-called null-allele was neglected in our study since it occurs with very low frequencies in only a few populations. The phenotypes of the alleles APH-B_1 and B_2 appear as single enzyme bands with a relatively high affinity for the substrate (intensely stained in zymograms), whereas the alleles APH-B_3 and B_4 code for double enzyme bands with a lower affinity for the substrate (Fig. 1). In contrast to the APH-A polymorphism, significant differences in allele frequencies of APH-B were found between populations from different environments (Bergmann 1975). In the following, this phenomenon is specified for two characteristic climatic gradients.

The frequencies of the APH-B alleles discovered in the eight spruce populations of the latitudinal transect in Finland are compiled in Table 1. In all populations, the APH-B locus was found to be polymorphic exhibiting all of the

Table 3. Geographic Data for 4 Swiss Norway-spruce Provenances and Allele Frequencies at the APH-B Locus found in Provenance Seed Samples

Provenance		Geographic Data			Allele Frequencies at APH-B Locus					
Name	Region	Lat.	Long.	Alt.	B_1	B_2	B_1/B_2	B_3	B_4	B_3/B_4
Tägerwillen	Thurgau	47°38′	9°07′	520 m	0.0	0.0	0.0	0.44	0.56	1.0
Murg	St. Gallen	47°06′	9°11′	1000 m	0.04	0.07	0.11	0.40	0.49	0.89
Cerniat	Freiburg	46°39′	7°09′	1200 m	0.12	0.17	0.29	0.32	0.39	0.71
Conters	Graubünden	46°53′	9°49′	1700 m	0.39	0.47	0.86	0.05	0.09	0.14

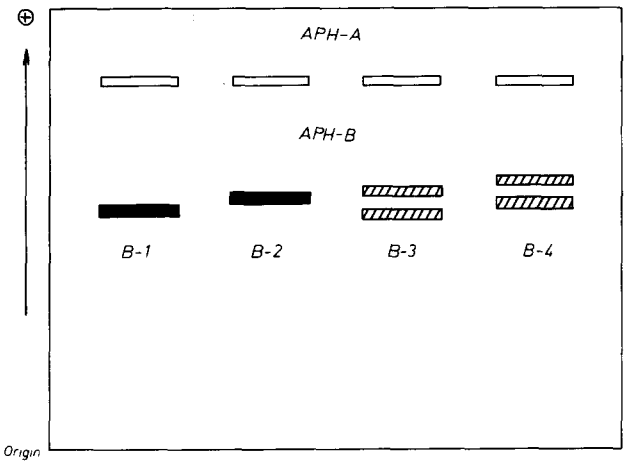

Fig. 1. Schematic drawing of electrophoretic APH patterns found in extracts of Norway spruce endosperm. The phenotypes encoded by the APH-B locus (B1-B4 allozymes) are presented in their typical appearance in zymograms

four allelic types. However, the distribution of allele frequencies among the populations shows several characteristic features. In contrast to various other polymorphic enzyme loci, no generally predominant allele exists at the APH-B locus. The alleles which occur with high frequency in northern provenances are very rare in populations from southern Finland, and vice-versa.

A striking phenomenon of this allele frequency pattern, however, is the significant similarity in frequency of the alleles APH-B_1 and B_2, as well as B_3 and B_4, which could be observed in all of the populations of the transect (Table 1). The frequency of B_1 increases or decreases along the transect with the same order of magnitude as the B_2 frequency. The allele frequencies of B_3 and B_4 behave in the same manner. Since the APH alleles B_1 and B_2 are coding for coincident single-band allozymes and the alleles B_3 and B_4 for coincident double-band allozymes (Fig. 1), it is assumed that B_1 and B_2 as well as B_3 and B_4 specify functionally equivalent acid phosphatases. Therefore, the frequencies of the alleles APH-B_1 and B_2, as well as those of APH-B_3 and B_4, were combined in order to obtain more pronounced data of the genic variation patterns along the transects here investigated. In the following descriptions, the allele combinations APH-B_1/B_2 and APH-B_3/B_4 are regarded as distinct allelic forms (for further analytical treatment see Discussion).

The latitudinal transect in Finland reveals a pronounced clinal variation in allele frequencies of the APH-B system (Table 1). The frequency of APH-B_1/B_2 increases gradually with latitudes ranging from 0.06 in the most southern population to 0.88 in the most northern population. Correspondingly, the frequency of APH-B_3/B_4 decreases from south to north. Although the sample of investigated populations from the Finnish spruce range has been limited, a close relationship already appears between the latitude of the provenance locality and the allele frequencies of this provenance. Tigerstedt (1974) could not detect any clinal variation at an acid phosphatase locus active in spruce seeds even though he had investigated ten provenances from a similar transect in Finland, but Lundkvist and Rudin (1977) were able to find some geographical variation at an acid phosphatase locus among Swedish spruce stands.

The Frequency Distribution of APH-B Alleles Along the Altitudinal Transect in Austria

The six populations (demes) investigated are located on the eastern slope of the Seetaler Alps in Austria. From the valley of the Mur (700 m), the population sites are distributed over altitudes up to 1600 m, close to the tree border (Fig. 2). The smallest distance between adjacent populations (with respect to the transect) is 1.5 km; the distance between the two border populations of this transect is 14 km. The frequencies of the APH-B alleles found in these Norway spruce populations show that all of the four alleles are present in each of the six populations (Table 2). However, the frequencies of APH-B_1 and B_2 are very low in Murauen and those of APH-B_3 and B_4 are low in Schmelz II, indicating a marked tendency of genic variability to decrease towards the borders of the transect.

After combining the allele frequencies of B_1 and B_2 and of B_3 and B_4, which again show the particular covariation, a characteristic clinal variation was observed along this transect (Table 2). The frequency of APH-B_1/B_2 increases slowly up to an elevation of 1200 m (St. Wolfgang), however from these it grows very rapidly, reaching values of 0.8-0.9 (Fig. 2). Correspondingly, the frequencies of the allelic form APH-B_3/B_4 exhibit a reverse pattern. The most significant result from this allele frequency cline is the considerable difference in allele frequencies between the adjacent populations St. Wolfgang and Schmelz I, which are separated by a distance of only 2 km (Fig. 2, Table 2).

In order to ascertain whether this characteristic APH-B allele frequency pattern observed along the altitudinal transect is associated with specific conditions of the Seetaler Alps or is generally dependent on the elevation of the population locality, four additional provenances from different regions and altitudes of the Swiss spruce range were analyzed (Table 3). The striking results show that, independent of the horizontal location, the frequency of APH-B_1/B_2 (or APH-B_3/B_4) is closely correlated with the respective elevation of the population locality. The population located at the highest altitude (Conters – 1700 m) reveals an allele frequency distribution (Table 3) similar to those of the two highest-located populations from the Seetaler Alps (Table 2). On the other hand, the allelic

form APH-B_1/B_2 could not be detected in Tägerwilen, a provenance from the lowland (Table 3). Summarizing these results we can state that the allele frequencies at the APH-B locus are primarily dependent on the elevation of the population locality, whereby APH-B_1/B_2 is absent or very rare in populations from lowlands or foothills and highly predominant in populations from mountainous localities.

Discussion

Almost all of the Norway spruce populations here investigated reveal four alleles (B_1-B_4) at the APH-B locus. These alleles, however, can be partitioned into two groups, one containing B_1 and B_2, the other B_3 and B_4, according to the similarities in their phenotypic appearance in zymograms (Fig. 1) as well as to their characteristic frequency distributions over the respective transects (Tables 1-3). While the marked differences between the phenotypes of the alleles (allozymes) of the two different groups may result from a conformational change of the enzyme molecule possibly involving several amino acid substitutions which need not be connected with charge changes, the minor differences between the allozymes of the same group should be due to a charge change after one particular amino acid substitution (Johnson 1974). If we assume that the very similar, or nearly identical, electrophoretic behavior of the two allozymes of each group corresponds to an identical physiological function in the organism, it can be suggested that the corresponding alleles are selectively equivalent. This hypothesis is supported by the data of the allele frequency distributions along the two transects and in the Swiss provenances. Since this data, however, does not provide sufficient evidence of the selective equivalence of these allelic types, another approach was made to confirm this hypothesis.

Two alleles at a locus are considered selectively equivalent if one allele occurring in an arbitrary genotype can be substituted for the other without changing the fitness value of this genotype. Hence, such a substitution of selectively equivalent alleles generates subsets of selectively equivalent genotypes (with identical fitness values) in a population. Within each such subset of selectively equivalent genotypes, the genotypic structure (genotype frequencies related to the subset) is expected to remain unchanged from the zygotic stage to any other subsequent ontogenetic stage, although the genotype frequencies related to the entire population may change considerably. This fact can be used to prove the selective equivalence of alleles in populations. However, since in general the genotypic structure of a tree population is determinable for only one ontogenetic stage, which mostly does not represent the zygotic stage, an assumption of the genotypic structure in the initial, that is the zygotic stage, has to be made. Examples for such assumptions are Hardy-Weinberg proportions or inbreeding structures.

In order to prove the selective equivalence of the alleles APH-B_1 and B_2 as well as that of APH-B_3 and B_4, the genotypic structure of the corresponding subsets (presented in Table 4) has to be examined in different populations. If p, q, r and s are the frequencies of the alleles B_1, B_2, B_3 and B_4, respectively, the genotypic structure of a population under the assumption of the Hardy-Weinberg law is given by p^2, 2pq, etc. (Table 4). Although this genotypic structure is probably valid only for the initial stage of a population, the genotypic structure within each of the three subsets does not change

Table 4. Genotype Frequencies for a One-Locus-Four-Alleles System in a Population According to the Hardy-Weinberg law and Genotype and Allele Frequencies within Three Subsets of this Population

	First subset			Second subset			Third subset			
Genotypes	B_1B_1	B_1B_2	B_2B_2	B_3B_3	B_3B_4	B_4B_4	B_1B_3	B_1B_4	B_2B_3	B_2B_4
Frequencies in the entire population	p^2	2pq	q^2	r^2	2rs	s^2	2pr	2ps	2qr	2qs
Frequencies within the subsets	$\frac{p^2}{(p+q)^2}$	$\frac{2qp}{(p+q)^2}$	$\frac{q^2}{(p+q)^2}$	$\frac{r^2}{(r+s)^2}$	$\frac{2rs}{(r+s)^2}$	$\frac{s^2}{(r+s)^2}$	$\frac{pr}{C^*}$	$\frac{ps}{C}$	$\frac{qr}{C}$	$\frac{qs}{C}$
Alleles	B_1		B_2	B_3		B_4	B_1	B_2	B_3	B_4
Frequencies within the subsets	$\frac{p}{p+q}$		$\frac{q}{p+q}$	$\frac{r}{r+s}$		$\frac{s}{r+s}$	$\frac{p}{2(p+q)}$	$\frac{q}{2(p+q)}$	$\frac{r}{2(r+s)}$	$\frac{s}{2(r+s)}$

* $C = (p+q)(r+s)$

Table 5. Comparisons Between the Genotype Frequencies Observed within Individual Subsets of Several Finnish Spruce Populations and those Expected Under the Assumption of Initially Existing Hardy-Weinberg Proportions in the Populations.

		Kittilä		Kolari		Suomussalmi		Pihtipudas		Tuusula	
	Genotypes	Frequencies obs.	exp.	Frequencies obs.	exp.	Frequencies obs.	exp.	Frequencies obs.	exp.	Frequencies obs.	exp.
First subset	B_1B_1	0.22	0.25	0.40	0.36	0.60	0.49				
	B_1B_2	0.56	0.50	0.40	0.48	0.20	0.42				
	B_2B_2	0.22	0.25	0.20	0.16	0.20	0.09				
		$\chi^2 = 0.13$		$\chi^2 = 0.14$		$\chi^2 = 1.37$					
Second subset	B_3B_3			0.34	0.33			0.30	0.20	0.32	0.28
	B_3B_4			0.48	0.50			0.30	0.49	0.42	0.50
	B_4B_4			0.18	0.17			0.40	0.31	0.26	0.22
				$\chi^2 = 0.01$				$\chi^2 = 1.54$		$\chi^2 = 0.49$	

during the course of one generation, if the corresponding genotypes are selectively equivalent. In our case, the first two subsets also exhibit Hardy-Weinberg proportions, whereas the genotype frequencies of the third subset result from the product of the respective allele frequencies multiplied by 4 (Table 4). Furthermore, it is noteworthy that the allele frequencies of the third subset are half as high as those of the first two subsets.

Several spruce populations of the Finnish transect were suitable for the study of genotype and allele frequencies within the subsets. However, due to the limited sample sizes in most cases only one of the subsets could be analyzed. The data given in Table 5 does not show any significant deviation between the genotype frequencies observed within the individual subsets and those expected under the assumption of the Hardy-Weinberg law. In a few cases, it was even possible to compare the allele frequencies of the different subsets. In the tree sample from Kolari, the frequencies of B_1 (0.60) and B_2 (0.40) in the first subset and B_3 (0.58) and B_4 (0.42) in the second subset (see Table 5) are double or nearly double the allele frequencies in the third subset (B_1: 0.38, B_2: 0.12, B_3: 0.30, B_4: 0.20). Similar relationships were found in the Tuusula sample (B_3: 0.53, B_4: 0.47 in the second subset and B_3: 0.25, B_4: 0.25 in the third subset). All deviations were found to be not significant when using the χ^2-test.

Although the subset-specific genotypic structure could not be examined in all cases in the Finnish samples, the resulting data still provide good evidence of the selective equivalence of the alleles APH-B_1 and B_2, as well as APH-B_3 and B_4. In particular, the characteristic relationships between the allele frequencies of the three subsets (Table 4), which were ascertained in a few cases, could hardly be meaningfully explained by any other phenomenon. Thus, the combining of the frequencies of these alleles in the tables should now be justified. Finally, the question arises whether such allelic affinities do occur more generally in populations than has as yet been recognized. Possibly, enzyme and protein loci have neither exclusively selectively equivalent (neutral) alleles nor exclusively selectively different alleles, but do have groups of selectively equivalent (within-group neutrality) alleles with marked between-group selective differences.

The frequency distribution of the allelic forms APH-B_1/B_2 and APH-B_3/B_4 along the latitudinal transect in Finland reveals a pronounced clinal variation involving an extraordinarily wide frequency range (Table 1). Since most of the climatic factors vary predominantly from north to south in the Scandinavian countries, it was reasonable to search for a correlation between the systematic allele frequency change and some climatic gradient, especially temperature gradient. Several comparisons indicated good relationships between specific temperature variables (mean annual temperature, mean winter temperature, etc.) and the allele frequency variation, however, a very close correlation could not yet be found. This may result from the lack of climatic data from several provenance localities, or from the inability to trace the relevant climatic variable.

On the other hand, it should not be excluded that the clinal variation observed along the Finnish transect is not caused by selective forces. Such a cline can also be the result of hybridization between previously isolated populations which had differentiated by random genetic drift or founder effects, or may arise from the dispersal of a new allele from one end of the respective range. Although historical data on the Norway spruce immigration into Finland do not support these suggestions (Schmidt-Vogt 1974), the concept based on selective environmental effects remains confounded with that based on drift and migration effects, if the latitudinal transect of Finland is considered alone. Consequently, another transect com-

pletely independent of the Finnish spruce range, but associated with similar climatic gradients, must be investigated to decide upon the existence of selection.

The results obtained from the study of the altitudinal transect in the Seetaler Alps (Table 2, Fig. 2), which represents a typical temperature gradient, clearly show that a) natural selection does in fact cause the variation in allele frequency at the APH-B locus, and b) one or several temperature variables must function as the major selective forces. Then, it is extremely improbable that the great similarity between the latitudinal cline in Finland and the altitudinal cline in the Austrian Alps results from corresponding mixings of twofold occurring pairs of populations. Furthermore, the close association between high frequencies of APH-B_1/B_2 and colder climatic conditions as opposed to very high frequencies of APH-B_3/B_4 and moderate climate both observed in widely separated populations of the Swiss spruce range (Table 3) confirms the assumption that temperature itself operates as one of the selective forces. Additional evidence is provided by the allele frequency data from several pairs of neighboring spruce provenances which only differ in their elevations (Bergmann 1975). Characteristic examples are Istebna (600 m; APH-B_1/B_2: 0.17) and Witów (1400 m; APH-B_1/B_2: 0.69) from Poland; Westerhof (180 m; APH-B_1/B_2: 0.06) and Oderholz (800 m; APH-B_1/B_2: 0.38) from the Harz in Central Germany.

The allele frequency pattern along the altitudinal transect in Austria reveals several additional characteristics. Although gene flow among conifer populations can occur at a considerable rate (Stern and Roche 1974), it is obvious that this genetic parameter does not essentially affect the behavior of the allele frequency cline. In the first part of the transect (from Murauen), the clinal shift is relatively limited, suggesting a marked gene flow effect. However, the cline becomes considerably steeper when the transect reaches the higher elevations (Fig. 2). In particular, the considerable allele frequency differences between the adjacent populations St. Wolfgang and Schmelz I (Table 2, Fig. 2) indicate that selective forces predominantly establish the frequency cline of the APH-B alleles. Therefore, it is concluded that a specific selection gradient, largely based on one or several temperature variables, causes the observed clinal variation: low temperatures favor the genotypes APH-B_1B_1, B_1B_2, B_2B_2 and moderate temperatures, the genotypes B_3B_3, B_3B_4, B_4B_4 (the heterozygotes B_1B_3, B_1B_4, etc. are assumed to have an intermediate fitness in each case). Although a slight gene flow between adjacent populations may be responsible for the maintenance of the APH-B polymorphism especially in the border populations of the transect, its overall effect on this cline must be considered to be relatively weak since in our case selection is acting after gene flow has occurred (for a detailed consideration of migration selection interaction in a cline, see e.g. Karlin and Richter-Dyn 1976).

Although the elevationally-dependent allele frequencies in the populations indicate an advanced stage of adaptation of the spruces in the Seetaler Alps, such a conclusion is primarily valid only for the genotypes at the APH-B locus. However, since these Norway spruce stands have existed in this area for several generations without any effects from man (Holzer 1977), it is reasonable to suggest that the trees, with respect to the whole genome, are largely adapted to their specific environmental conditions. This adaptation is reflected by several other physiological and morphological characters, the variations of which were also found to be dependent on the elevation. In particular, the two complex characters 'bud number' and 'THL' (shoot length related to hypocotyl length), both associated with tree growth and growth cessation, reveal a variation pattern along the altitudinal transect that resembles the APH-B allelic variation to a surprisingly large extent (Holzer and Nather 1974, Holzer 1977). The values of the bud number, for example, increase between the elevations of 1200 m and 1400 m with an order of magnitude similar to that of the APH-B_1/B_2 frequencies (Table 2). An altitudinal cline in these Alpine spruce populations could also be observed for the physiological character 'critical night length for budset', which is important for the height growth of trees (Dormling 1973). Various investigations with Norway spruce from the Scandinavian range demonstrate that numerous tree characters usually used in forest genetics and tree breeding vary clinally from north to south, thus indicating the adaptation to latitudinally-associated climatic factors (for review and compilation of these studies, see Schmidt-Vogt 1977). Corresponding or similar clinal variations in allozyme frequencies over latitudes or altitudes point to possible relationships between individual structural genes and these polygenic characters.

Since selection effects from other loci linked to the APH-B locus can be excluded because of the corresponding allele frequency patterns along several independent transects, the question finally arises as to which role in tree viability is played by the APH-B enzymes. Unfortunately, only poor data on the physiological function of acid phosphatases in plants are available. In several cases, acid phosphatases were found to be associated with the plant cell wall (and the plasmalemma) and assumed to be involved in permeability processes of cell membranes (for review, Woolhouse 1970). One can now imagine that the acid phosphatase allozymes encoded by the APH-B_1 and B_2 alleles are adapted to operate at low temperatures, and those encoded by APH-B_3 and B_4 are adapted to operate at higher temperatures in a certain stage of the tree life cycle. On the other hand, a more indirect adaptive function of these enzymes may be possible. Very low tempera-

tures during particular seasonal periods, for instance, may require a marked change in the permeability of cell membranes (e.g. for increasing the solute concentration within the cells), which can be catalyzed only by the B_1 and B_2 allozymes, whereas minor changes in the membrane permeability only need the action of the B_3 and B_4 allozymes. It may be important in this context that the acid phosphatases encoded by the APH-B locus are found to be active in the very young seedling (besides the endosperm), which is repeatedly exposed to very low night temperatures in the spring, especially in the northern and higher parts of the spruce range. Of course, detailed investigations are necessary to elucidate the relationships between the action of specific acid phosphatases and the physiological adaptedness of particular genotypes to specific environmental conditions.

Acknowledgment

The author wishes to thank Prof. M. Hagman, Finland, Dr. K. Holzer, Austria, Dr. Th. Keller, Switzerland for the supply of seed material and Dr. H.R. Gregorius, Göttingen for his advice in theoretical parts of the manuscript.

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Received November 15, 1977
Communicated by H.F. Linskens

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