

Associations Between Heterozygosity and Growth Rate Variables In Three Western Forest Trees¹

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Abstract: For each of three species, quaking aspen, ponderosa pine, and lodgepole pine, we determined the relationships between a ranking of heterozygosity of individuals and measures of growth rate. Genetic variation was assayed by starch gel electrophoresis of enzymes. Growth rates were characterized by the mean, standard deviation, logarithm of the variance, and coefficient of variation of annual ring widths measured from cores. In aspen, heterozygosity was associated with high mean growth rate, but in ponderosa and lodgepole pines heterozygosity was not associated with mean growth rate. High levels of heterozygosity were associated with high growth variability in aspen and ponderosa pine, and with low growth variability in lodgepole pine.

Several frequently observed phenomena suggest strong genetic influence on growth characteristics of forest trees. When trees from different origins are grown in common gardens they show marked differences in growth traits (Squillace and Silen 1962, Weidman 1939). These interpopulation differences are thought to be primarily the result of natural selection forming adaptations to local environmental conditions. Differences in growth characteristics are seen also between trees within a single population. Genetic differences within a population arise from segregation and a variety of mating types; some individuals are produced by selfing, some by outcrossing between related individuals, and some by outcrossing between unrelated individuals. These matings produce progeny with relatively low, intermediate, or high levels of heterozygosity, respectively. One generation of selfing, for example, reduces heterozygosity by one-half relative to outcrossing. In selfed progeny, an increase in homozygosity of deleterious recessive alleles may result in a lack of vigor (Fowler 1965, Sorensen and Miles 1974). In outbred progeny, however, increased vigor may result from heterosis because of a summation of heterozygote superiority over many loci. Heterosis for growth and viability is commonly observed in crosses between strains of domesticated plants and animals.

This paper considers the relationships between heterozygosity and growth rate variables within populations of three forest tree species. In this study, we used starch gel electrophoresis to determine heterozygosity of several protein genotypes and estimated mean growth rate from measurements of annual radial growth rings taken from increment cores.

THEORETICAL BACKGROUND

Considerable theoretical and empirical evidence suggest that heterozygosity is associated with fitness and morphological variation.

Heterozygosity Associated With Fitness

Heterosis with respect to growth or viability has been reported extensively in crosses between strains or stocks of domesticated plants and animals (Lerner 1954). Offspring from these crosses have levels of heterozygosity higher than those maintained in either parental stock. Experiments have been designed to detect heterosis in individuals that differ in either heterozygosity of a single pair of chromosomes or a few allozyme loci. For example, chromosomes may be made heterozygous or homozygous in several species of *Drosophila*. When heterozygotes were compared to homozygotes, the homozygotes had fitnesses of only 10 to 20 percent that of the heterozygotes (Sved and Ayala 1970, Tracey and Ayala 1974). In three species of marine pelecypods—the blue mussel, *Mytilus edulis*, the ribbed mussel, *Modiolus demissus*, and the California mussel, *Mytilus californianus*—the proportion, of heterozygotes at one or more loci increased with size (Koehn and others 1973, Koehn and others 1976, Tracey and others 1975). Size is highly correlated with age in these animals, so this relationship probably results from higher viability of heterozygotes. In individuals of the common killifish *Fundulus heteroclitus*, heterozygosity of twelve polymorphic loci was monitored for several years in two localities. Fish could be aged accurately by size, so the same cohort of fish was sampled in successive years. In both localities, level of heterozygosity increased with age, and the data were interpreted as evidence for higher viability in individuals with higher heterozygosity (Mitton and Koehn 1975). In the slender wild oat *Avena barbata*, viability differentials favoring heterozygotes were found at each of three esterase loci (Clegg and Allard 1973). In populations

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of *Colias* butterflies, *Colias philodice eriphyle* (Watt 1977) and the sagebrush lizard *Sceloporus graciosus* (Tinkle and Selander 1973), heterozygosity increased steadily throughout the life cycle.

Heterozygosity confers advantages for other aspects of fitness. Growth rates recorded for the American oyster *Crassostrea virginica*, were higher for heterozygotes for several loci than for homozygotes (Singh and Zouros 1978). In the tiger salamander *Ambystoma tigrinum*, growth rates in the field and in the laboratory were associated with heterozygosity of a few allozyme loci. Higher growth rates in highly heterozygous individuals could be detected within the progeny of a single pair cross raised in the laboratory.⁴ A study of the demography of the perennial composite *Liatris cylindracea* revealed that highly heterozygous individuals attained greater size and produced more seeds than highly homozygous individuals (Schaal and Levin 1976). Heterozygosity at a single, highly polymorphic locus in the dark-eyed junco, *Junco hyemalis*, was found to be related to both dominance rank and viability; heterozygotes generally enjoyed higher positions in the dominance order than homozygotes, and also survived stress tests better than homozygotes (Baker and Fox 1978).

The papers cited in this section represent careful studies that gave positive results. Equally careful and comprehensive studies have led some investigators to conclude that allozyme variation is essentially unrelated to either fertility or viability (Christiansen and others 1973, Mukai 1977, Yamazaki 1972).

Heterozygosity Associated With Morphological Variation

Associations between heterozygosity and morphological variation are interesting primarily because variation in morphology may be associated with variation in fitness. To date, the majority of studies of associations between heterozygosity and morphological variation have been conducted utilizing data from several or many populations. In studies comparing a series of island populations, for example, the proportion of polymorphic loci and the level of heterozygosity for a population have been related to the degree of morphological variation in those populations. In both the side-blotched lizard *Uta stansburiana* (Soulé and others 1973) and in macaques *Macaca* sp. (Morris and Kerr 1974), populations with more polymorphic loci and higher levels of heterozygosity showed higher levels or greater ranges of morphological variation. Soulé and others (1973) pointed out that these observations suggest allozyme variation is a reasonable indicator for genetic variation throughout the genome. The

studies just cited agree in that populations with greater numbers of genotypes show greater morphological variation.

We are concerned here with heterozygosity, not as it affects variation between populations but, rather, within populations. Lerner (1954) presented a large body of evidence, primarily from domesticated animals and plants, to support his hypothesis that high levels of heterozygosity enhanced developmental homeostasis among individuals within populations. He proposed that highly heterozygous individuals have lower levels of phenotypic variation than do predominantly homozygous individuals. Lewontin (1956) stressed that homeostasis is most evident for traits directly involved in the determination of fitness. An extension of Lerner's hypothesis is that comparisons of individuals with different levels of heterozygosity, but sampled from the same randomly mating populations, have differences in the levels of developmental homeostasis. The effects of heterozygosity upon developmental homeostasis can be measured when only single chromosomes or a few allozyme loci are used to construct groups with low or high heterozygosity. When viability and fertility in *Drosophila* homozygous or heterozygous for specific chromosomes were compared, heterozygous individuals had lower variance in both traits (Dobzhansky and Wallace 1953). Similarly, when Robertson and Reeve (1952) compared *Drosophila* heterozygous or homozygous for specific chromosomes, they found that heterozygotes had lower variance in wing morphology. By using the level of bilateral asymmetry of side-blotched lizards to estimate their degree of developmental homeostasis, Soulé (1979) found that developmental homeostasis increased with heterozygosity as measured by allozyme loci. When populations were divided into homozygous and heterozygous classes on the basis of single allozyme markers, homozygous classes had higher phenotypic variation for both the common killifish *Fundulus heteroclitus* (Mitton 1978) and the monarch butterfly *Danaus plexippus* (Eanes 1978). These data support the extension of Lerner's hypothesis mentioned earlier: developmental homeostasis can vary with levels of heterozygosity within populations, and the effect of heterozygosity may be detected when only single chromosomes or a few allozymes are used to sort individuals into different levels of heterozygosity.

Relationships between heterozygosity and some aspects of fitness have been found primarily in studies of animals. Long-lived forest trees, however, are subjected to much temporal and spatial heterogeneity during their life cycle and recent reviews indicate that forest trees harbor more genetic variation than other groups of plants or animals (Hamrick 1979, Hamrick and others 1979, Hamrick and others 1981).

This paper discusses the results of recent investigations of forest trees to rank individual trees for levels of heterozygosity and to assess how these ranks relate to mean growth rate and variability of growth rate. Examples are taken from three species native to the Colorado Rocky Mountains: quaking aspen *Populus tremuloides* Michx.,

⁴Pierce, B. A., and J. B. Mitton. *Growth and heterozygosity in the tiger salamander, Ambystoma tigrinum*. (Manuscript submitted for publication.)

ponderosa pine *Pinus ponderosa*, Dougl. ex Laws., and lodgepole pine *Pinus contorta* Dougl. ex Loud.

MATERIALS AND METHODS

Mean growth rate and variability of growth rate were estimated from annual radial growth ring measurements obtained from one core extracted from each tree. A binocular stereoscope fitted with an ocular micrometer was used to measure ring widths. For each tree, three measures of variability in growth were calculated: the variance, the standard deviation, and the coefficient of variation.

Vegetative or gametophytic tissue was subjected to starch gel electrophoresis and heterozygosity scores were obtained directly from the banding patterns on gels. The number of loci heterozygous were summed to obtain an individual heterozygosity score for each clone or tree. If four loci were being scored for each individual, for example, the possible heterozygosity score ranged from 0 to 4.

The analytical techniques of multiple regression and analysis of covariance compared growth characteristics among individuals of different heterozygosity levels. These techniques offer the advantage of removing the confounding effects of independent variables that are strongly correlated.

Aspen

In the Western United States, aspen reproduces predominantly asexually by sending up ramets from lateral roots. The unit of study in this species is, therefore, the clone, and cores from the largest ramets within a proximity of about 10 m were used to estimate growth rate variables. Aspen is dioecious, and the sex of each clone was determined in the spring by examining catkins. Age, sex, elevation, and growth rate variables were recorded for each of 106 clones at elevations from 1700 to 3100 m west of Boulder, Colorado. In addition, genotypes at three loci—peroxidase, phosphohexose isomerase, and glutamate dehydrogenase—were obtained from leaf tissue for each clone (Mitton and Grant 1980).

Ponderosa Pine

Pollen tissue from 14 trees on a ridgetop and a south-facing slope at an elevation of 2590 meters near Glacier Lake was analyzed for four loci—peroxidase, aminopeptidase, phosphohexose isomerase, and phosphoglucumutase (Knowles 1978).

Lodge pole Pine

Needle tissue was sampled from 152 lodgepole pines at the University of Colorado's Mountain Research Station,

Table 1—Distributions of loci heterozygous per individual in quaking aspen, ponderosa pine, and lodgepole pine

Sample	Sample size	Loci studied	Heterozygous loci				
			0	1	2	3	4
Quaking aspen	100	3	40	41	15	4	¹ -
Ponderosa pine	117	4	23	57	30	7	0
Lodgepole pine	152	4	32	69	42	9	0

¹ Dash indicates that this number of loci was not considered.

Ward, Colorado, at an elevation of 2800 m. Needle tissue was prepared for electrophoresis (Mitton and others 1979) to obtain genotypes for four loci—peroxidase, fluorescent esterase, phosphohexose isomerase, and alcohol dehydrogenase.

RESULTS

Results of starch gel electrophoresis were summarized for each population sample as a distribution of the number of loci that were heterozygous per individual (table 1).

Aspen

Correlations among variables complicated the search for an association between heterozygosity and growth rate. Growth rate varied with age, sex, and elevation, and distribution of the sexes was not random with respect to elevation. At low elevations, females outnumbered males slightly, and at high elevations, males outnumbered females by 2:1, or more. Growth rate of females was superior to that of males at all elevations, but female growth rate dropped more sharply with increasing elevation (Grant and Mitton 1979). Radial growth decreases regularly with increasing age of a stem (Fritts 1976). Data were analyzed by multiple regression, with mean growth rate of a clone as the dependent variable, and age of the oldest standing ramet, sex, elevation, and heterozygosity for each clone as independent variables (table 2). The multiple R indicated that the suite of independent variables accounts for about 28 percent ($p < 0.001$) of the variance in mean growth rate among the clones. As expected, growth rate decreased, with age and elevation, but was not affected by sex in this analysis. With

Table 2—Multiple regression analysis of standard deviation of annual ring width in quaking aspen

	Coefficients	Probability
Multiple R	0.530	$P < 0.001$
Independent variables		
Age	-0.064	$P < 0.001$
Heterozygosity	.811	$P < 0.05$
Sex	.593	n.s.
Elevation	-.008	$P < 0.01$

the effects of other variables held constant, the relationship between heterozygosity and growth rate was significant. The range of heterozygosity in this sample was 0 to 3 and mean growth rate increased with the number of heterozygous loci ($p < 0.05$).

Within this sample of clones, mean growth rate and variance of growth rate were highly correlated ($r = 0.92$, $p < 0.001$). This suggests that the average growth rate and the variability of the growth rate over time are intimately associated in some way. A multiple regression performed with the same independent variables but with either variance or standard deviation of growth rate as dependent variables gave similar results; increases in the number of heterozygous loci were associated with increases in growth variability.

Ponderosa Pine

Ponderosa pine sampled near Glacier Lake were distributed over a ridgetop and a south-facing slope. Age structures, growth rates, and gene frequencies for some of the four loci differed between these geographically adjacent but ecologically distinguishable sites (Knowles 1978). Mean growth rate and age were negatively correlated ($r = -0.057$, $p < 0.001$). To eliminate the confounding effects of age, slope aspect, and mean growth, an analysis of covariance was employed. A growth rate variable was the dependent variable, a heterozygosity class was the independent variable, and age, slope aspect, and mean growth rate were covariates. The effect of heterozygosity on mean growth, therefore, could be tested with the effects of age and slope held constant; or, the effect of heterozygosity on variance of growth rate could be tested with the effects of age, slope, and mean growth rate held constant. For this analysis, heterozygosity scores were pooled to create two classes—those predominantly homozygous (0 to 1 loci heterozygous) and those predominantly heterozygous (2, 3, or 4 loci heterozygous).

An analysis of covariance, with mean growth rate as the dependent variable, revealed no differences between trees predominantly heterozygous and those predominantly

homozygous (*table 3*). Analyses of covariance were also done with the standard deviation, the logarithm of the variance, and the coefficient of variation used to characterize growth variability (*table 4*). Results of these analyses were all consistent, and were either statistically significant or at the borderline of significance. Although mean growth rates did not differ between heterozygosity classes, variability of growth rates over time were different for heterozygotes and homozygotes. Growth of heterozygotes was more erratic from year to year than was growth of homozygotes.

Lodge pole Pine

A relationship between genotype and growth rate was tested in lodgepole pine. The pines sampled for this analysis were all from a single relatively homogeneous site. Within this sample of 152 trees, age and mean growth were positively correlated ($r = 0.19$, $p < 0.05$); although within any tree radial increments tended to decrease with age. To correct for the effect of age, analyses of covariance were done with mean growth rate or coefficient of variation as the dependent variable, heterozygosity class as the independent variable, and age as the covariate. The mode for the distribution of individual heterozygosity was at 1, and none of the trees in this sample were heterozygous for all four of the loci (*table 1*). Once again, heterozygosity scores were pooled to create two classes, predominantly homozygous (0 to 1 loci heterozygous) and predominantly heterozygous (2 or 3 loci heterozygous). In this analysis, the contribution of the covariate (age) was not significant, so that similar results could be obtained with a single classification analysis of variance, or with a t-test between means. Mean growth rates did not differ between heterozygosity classes but the classes differed in variability of growth rates. The distribution of coefficients of variation of growth rates (*fig. 1*) shows that, although there was considerable overlap in growth variability between heterozygosity classes, the predominantly heterozygous individuals had a mean of 70.3, in contrast to the predominantly homozygous individuals, which had a

Table 3—Comparisons in ponderosa pine and lodgepole pine of mean growth increment between predominantly homozygous and predominantly heterozygous individuals

Locality	Heterozygosity level	Trees	Unadjusted mean	Adjusted mean	F ¹	Probability
Glacier Lake (Ponderosa pine)	Low	78	14.55	15.21	0.15	P > 0.50
	High	36	16.62	15.11		
Ward (Lodgepole pine)	Low	101	5.30	5.58	1.10	P > 0.50
	High	51	5.58			

¹F statistics for ponderosa pine analyses are from analyses of covariance, with age and slope as covariates. Adjusted values incorporate adjustments for covariates. F statistic for lodgepole pine is based on analysis of variance.

Table 4—Comparisons at two localities in ponderosa pine of annual growth increment variabilities between trees of different heterozygosity labels

Locality	Heterozygosity Level	Trees	Standard Dev.		Probability	Log of Variance		Probability	Coef. of Variation		Probability
			unadj.	adj.		unadj.	adj.		unadj.	adj.	
Glacier Lake	Low	78	9.36	9.68	<0.05	1.87	1.88	<0.05	59.33	58.45	0.10>p 0.05
Glacier Lake	High	36	11.18	11.07		1.01	1.01		63.35	65.26	

Note: Significance levels come from analyses of covariance. Age, slope, and mean growth rate are covariates for standard deviation and logarithm of the variance, and slope and age are covariates for the coefficient of variation. Each probability corresponds to the contrast immediately to its left in this table.

mean of 79.2. The differences between the means were statistically significant. Once again, level of heterozygosity was associated with growth variability, but the direction of the relationship found in lodgepole pine was opposite to those found in ponderosa pine and aspen; that is, in lodgepole pine, high heterozygosity was associated with low variability in growth rate.

DISCUSSION

The data presented here from quaking aspen, ponderosa pine, and lodgepole pine are consistent with data from previous studies in that they find a relationship between heterozygosity and some measure of growth rate. Although we do not find an association between mean growth rate and heterozygosity in either ponderosa pine or lodgepole pine, mean growth rate of highly heterozygous clones of aspen are higher than those of predominantly

homozygous clones, just as the mean growth rates of highly heterozygous American oysters (Singh and Zouros 1978) and tiger salamanders⁵ exceed those of more homozygous individuals. These observations may be considered extensions of Lerner's (1954) hypothesis to smaller and smaller portions of the genome. For example, Lerner established that high heterozygosity in the progeny of crosses between domesticated strains or stocks often bestows vigor in the progeny. The same phenomenon, on a much finer genetic scale, is apparent in our results; aspen clones that are heterozygous for a few loci have growth rates that exceed those of homozygotes. Also, variability in ring width in all species is related to level of heterozygosity, and in this respect, these results are similar to those of Mitton (1978), Eanes (1978) and Soulé (1979).

The results of tests for associations between heterozygosity level and growth variability are consistent in the three species studied in that associations were significant in each species. They are inconsistent, however, in their directions. Predominantly heterozygous individuals show higher growth variability in aspen (*table*

⁵Pierce, B. A., and J. B. Mitton. *Growth and heterozygosity in the tiger salamander, Ambystoma tigrinum*. (Manuscript submitted for publication.)

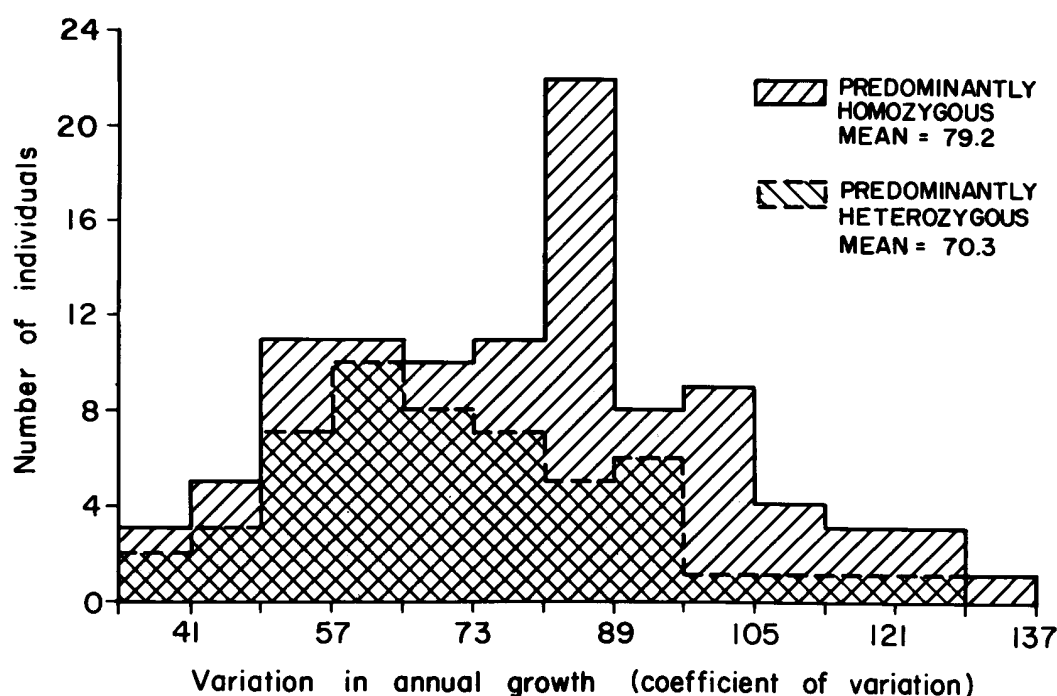


Figure 1—Distribution of the coefficients of variation in annual growth of the predominantly homozygous and predominantly heterozygous lodgepole pine trees ($t = 2.52$, $P > 0.05$).

2) and ponderosa pine (tables 3 and 4), but lower growth variability in lodgepole pine (fig. 1). The sample sizes in each of the studies are substantial, so that we have confidence that the results are real. Although we have no simple, compelling hypothesis to explain the inconsistency, we offer the following possibility. As Lewontin (1956) cautioned, the inverse association between heterozygosity and phenotypic variation would only exist for those phenotypic characters that relate directly to the fitness of the organism. Is it possible that growth characteristics directly contribute to the fitness of lodgepole pine but not to that of ponderosa pine or aspen? Such a situation is doubtful. Rather, we would speculate that growth characteristics are one step removed from a more adaptively significant character, for example, reproductive output. We expect, all else being equal, that annual ring width and seed production are negatively correlated (Fritts 1976). Our measure of growth variability, therefore, may also contain considerable information about variation in seed production. Both quaking aspen and ponderosa pine are known to be highly variable in seed production from year to year (Linhart and others 1979, Linhart and others 1981, Man 1961, Schubert 1974). Years of high seed production are scattered among more common years of low seed production. In contrast, the lodgepole pine in this study has predominantly serotinous cones, and yearly cone production is rather regular. Ponderosa pine and aspen may decrease their growth rates as a result of shunting energy into reproduction during years of heavy seed production. Lodgepole pine, however, may exhibit regular annual growth due to regular reproduction. If high heterozygosity is associated with the extreme of the frequency of reproduction typical for a given species, this could produce the observed association between heterozygosity and growth variability. This conjecture is offered tentatively with the intent of encouraging replication of these studies and initiation of new studies.

Another possible explanation for the discrepancy in direction of relationships between heterozygosity and growth variability is our use of different loci in the different species. If these loci play specific roles in controlling growth rate or growth variability, congruence may occur only when homologous loci are examined in each study.

The mechanisms that produce an association between level of heterozygosity and growth rate variables are of interest both theoretically and practically. Perhaps the first question that must be addressed in consideration of these mechanisms is: When individuals in a population are ranked or placed into groups on the basis of their heterozygosity at a few allozyme loci, just what is being measured? Heterozygosity at a few allozyme loci may estimate heterozygosity (a) of the whole genome, (b) of sections of the chromosomes tightly linked to the allozyme loci, (c) within one or several metabolic pathways, or (d) of only a few allozyme loci. A few loci can be used to reasonably reflect heterozygosity at one or a few dozen loci but it is unlikely that a few loci could accurately rank the members of a population by heterozygosity of the whole

genome.⁶ Perhaps the heterozygosity rankings used here are actually measuring heterozygosity in one of two major metabolic pathways (for example, pentose shunt, or dicarboxylic acid cycle). Heterozygosity levels within a pathway may provide more economic use of energy (Berger 1976), greater degrees of regulation (Johnson 1974), or efficiency for a greater range of temperature or of pH values (Fincham 1972, Gillespie and Langley 1974, Hochachko and Somero 1968). Different levels of heterozygosity within a major pathway could be reflected in growth variability measured from tree cores. Finally, the relationship between heterozygosity and growth rate variables could result solely from the specific loci being monitored. This possibility, however, is incompatible with the hypothesis that protein polymorphisms have little or no effect upon fitness (Kimura and Ohta 1971). A substantial number of kinetic studies of protein polymorphisms now indicate significant differences in *in vitro* performance of different genotypes at a locus (Beckman 1977; Day and others 1974a, 1974b; Merritt 1972; Miller and others 1975; Watt 1979) and these differences may reflect the performance of these genotypes in different environments.

Although the mechanisms for establishing relationships between heterozygosity and growth rate variables are unknown, much is to be gained from this type of study. If these relationships are general and reliable, they can be incorporated into tree improvement programs, because growth rate is likely to affect both quality and quantity of wood being produced. Finally, recent reviews have indicated that forest trees have higher levels of genetic variation than most other groups of animals and plants (Hamrick 1978, Hamrick and others 1979, Hamrick and others 1981). If the associations between heterozygosity and growth rate variables are general, and if differential growth rate and growth variability are adaptive, these associations may help us to understand why so much variation is maintained in forest trees. The influence of allozyme loci upon growth rate could be a fertile field for the joint efforts of geneticists and physiologists.

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