Heterozygosity and Fitness: No Association in Scots Pine

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ABSTRACT

The association of six quantitative traits related to fitness with heterozygosity at 12 allozyme loci has been examined in three populations of Scots pine, *Pinus sylvestris*. Because of several characteristics of this organism and of this extensive data set, it appeared that this study would show a positive association between heterozygosity and these traits if indeed heterozygotes had higher values for these quantitative traits. Using several different statistical techniques including analysis of variance, regression with the scaling recommended from the adaptive distance model, and multiple regression, no evidence of an association was found. For example, only between 7 and 8% of the regression tests were significant at the 5% level and half of these showed a positive association and half showed a negative association. Further, the multiple regression analysis explained on average only 5.8% of the variation observed in the six different traits and only 1.5% of this variation was explained by a positive association. Power analysis was carried out (for the first time on these type of data), both for the single locus heterozygous advantage and the association of individual multiple locus heterozygosity and the quantitative traits. For diameter and height, two traits often used in similar studies, the average power to detect a single locus heterozygous advantage of 0.10 was 0.737 and the average power to detect a mean heterozygote advantage of 0.05 per locus for multiple loci was 0.797. As a result of this study and an examination of the published results from other studies, it appears that what positive associations have been observed are probably not, in large part, due to the presence of intrinsic heterozygote advantage.

THE application of protein electrophoresis to document the large amount of genetic variation in most species was one of the major breakthroughs in evolutionary genetics (see LEWONTIN 1991). However, since this discovery, a heated debate has continued concerning the mechanisms responsible for maintaining this genetic variation. Although the overall pattern of variation for allozymes is generally consistent with expectations of neutrality (*e.g.*, KIMURA 1983; NEI 1987), some studies appear to indicate that different allozyme genotypes may have different fitness consequences (*e.g.*, KOEHN *et al.* 1988; WATT 1992). Further, there have been reports that the extent of allozyme heterozygosity in an individual may be correlated with various quantitative traits, including a number of fitness components and life-history traits, although this finding is far from universal (for reviews, see MITTON and GRANT 1984; ALLENDORF and LEARY 1986; ZOUROS and FOLTZ 1987; HOULE 1989; BUSH and SMOUSE 1992; POGSON and ZOUROS 1994).

Determining how general the correlation between individual allozyme heterozygosity and fitness components is, and the specific cause of any such association, is of great significance in evolutionary genetics as well as for several applied areas in which the research findings of population genetics have been suggested as the basis of breeding programs. For example, both in tree breeding and captive breeding of endangered species, it has been suggested that individual allozyme heterozygosity be measured and genotypes with the highest heterozygosity be selected as breeders. Although these ideas have not been widely adopted in silviculture, there is a general interest in finding a molecular genetic approach to complement the recognized plant breeding protocol generally used (*e.g.*, BUSH and SMOUSE 1992). Similarly, captive breeding decisions in endangered species are still based on minimizing inbreeding levels and retaining population heterozygosity but there has been recurring interest among conservation geneticists in using molecular measures to determine the best individuals for breeding (*e.g.*, RALLS and BALLOU 1986).

There are three major genetic explanations for the positive associations between individual heterozygosity and components of fitness (or other quantitative traits) that have been proposed (*e.g.*, HOULE 1989; CHARLESWORTH 1991). First, heterozygotes may have an intrinsically higher fitness than homozygotes, *i.e.*, there is heterozygote advantage (or overdominance as it is often called) at the allozyme loci being examined. Although intrinsic heterozygote advantage is a commonly cited mechanism for maintenance of genetic polymorphism, its presence has been inordinately difficult to document both in its simplest form (*e.g.*, ALLISON 1964; BISHOP 1981) or as an average over various environments (for reviews, see HEDRICK *et al.* 1976; HEDRICK 1986). Fur-

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ther, TURELLI and GINSBURG (1983) showed that for most stable, multilocus polymorphisms, the fitness of the genotype increases with heterozygosity, suggesting that if the allozyme loci are under a type of selection that leads to a polymorphic equilibrium there should be an association of individual heterozygosity and traits related to fitness.

Second, statistical associations (linkage disequilibria) between the allozyme loci and other selected (and usually linked) loci may result in apparent heterozygote advantage at the allozyme loci. These secondary (unknown) loci may themselves exhibit heterozygote advantage or they may be dominant. In this latter case, several dominant loci together may result in the appearance of marginal heterozygote advantage at the allozyme loci. This general phenomenon was originally called associative overdominance by FRYDENBERG (1963) (see also ZOUROS 1993). When only dominant loci are involved, HOULE (1989) prefers the term "dominance-correlation heterosis" to indicate that none of the loci involved exhibit intrinsic heterozygote advantage. The linkage disequilibria necessary here between the marker loci and selected loci may be caused by such factors as genetic drift in a small population or by population structure (*e.g.,* HEDRICK *et al.* 1978).

Finally, there may be associations of allozyme genotypes and components of fitness due to the presence of nonrandom mating or different levels of inbreeding in the population, a phenomenon that can be called genotypic association and quantified using the concept of identity disequilibrium (WEIR and COCKERHAM 1973; CHARLESWORTH 1991; see also HALDANE 1949). In this case again, neutral alleles may show apparent selective effects, here due to the genotypic associations caused by inbreeding and generally enhanced by linkage. As a simple example of what may occur even when there is no linkage between the allozyme loci and selected loci, if inbred individuals that have both low allozyme heterozygosity and low fitness are pooled in a sample with outbred individuals that have relatively higher values of both heterozygosity and fitness, then a positive association of allozyme individual heterozygosity and a fitness component may occur (*e.g.,* LEDIG *et al.* 1983; LEDIG 1986; HEDRICK 1990).

In the present study, we have attempted to determine if intrinsic heterozygote advantage is likely to be a major factor causing the correlation between allozyme heterozygosity and a number of quantitative traits related to fitness in Scots pine, *Pinus sylvestris.* Notice that for the operation of the two explanations besides intrinsic heterozygote advantage given above, a statistical association between the allozyme loci and other selected loci needs to be generated either by genetic drift, population structure, or inbreeding. In the present study, there are two factors that indicate that if such a correlation is observed, it is very likely to be the result of intrinsic heterozygote advantage. First, Scots pine has a very large population size with extensive pollen flow so that any association caused by linkage disequilibrium generated by genetic drift or population structure is unlikely (*e.g.,* KOSKI 1970; MUONA and SZMIDT 1985; MUONA and HARJU 1989). Second, even though Scots pine is self-compatible, even at the mature seed stage level <5% selfs remain (MUONA and HARJU 1989). While seeds show an excess of homozygosity due to selfing, adult tree genotypes in Scots pine populations are in Hardy-Weinberg proportions (*e.g.,* YAZDANI *et al.* 1985; MUONA and HARJU 1989) and there is no genetic evidence of inbreds surviving. Other data, such as the very high genetic load and extremely high seed-to-adult mortality in Scots pine in Finnish forests, also make it extremely unlikely that there are any inbreds among adults (*e.g.,* SAVOLAINEN and KÄRKKÄINEN 1992). As a result, it is unlikely that we would observe in Scots pine an association generated by any of the nonselective evolutionary factors mentioned above, such as genetic drift, population structure, or inbreeding unless the loci involved were very closely linked.

In addition, three other experimental aspects of this present study may also allow us to document intrinsic heterozygote advantage in Scots pine if it is indeed present. First, we measured six different quantitative traits that are related to fitness, including measures related to viability and to both female and male reproduction. Second, genotypes in two of our populations have been cloned and measurements were taken on multiple ramets of the same clone so that an excellent measure of the intrinsic genotypic value is possible. We have also conducted an analysis to determine the power that we have to detect a given selective difference between heterozygotes and homozygotes and for different multiple locus heterozygotes. Finally, because of the high allozyme heterozygosity in Scots pine, we have 12 polymorphic loci in all our populations so that we have a large range of individual heterozygosity.

MATERIALS AND METHODS

Populations: Three populations were examined, two of which, Viitaselkä and Vilhelminmäki, were seed orchard populations consisting of vegetatively replicated genotypes, while the third, Yllästunturi, was a natural population. The trees in both of the seed orchards are assumed to be random samples of adult Scots pine from the two areas and consisted of individuals from an area wide enough that it is unlikely that there was any inbreeding involved among them. The initial branches were sampled from adult trees in the Finnish forest so their genotypes composition should reflect the composition of the trees that have survived to adulthood. The seed-to-adult survival is perhaps the stage of strongest selection in this environment where less than one seed in a 1000 will mature into an adult tree (MUONA 1990).

The Viitaselkä population (62 15 N, 27 35 E) was 3.2 ha in size and had 25 genotypes, each replicated on the average 20 times in the orchard. The genotypes were originally collected from southern Finland between latitudes 61 and 62 (for details, see MUONA and HARJU 1989). The trees were

planted in 1954 in a grid at 7 m intervals and were all 31 yrs old at the time most of the measurements were taken.

The Vilhelminmäki seed orchard (62 05N, 25 15E) has an area of 3 ha and was planted in 1968. The trees were all 17 yr old at the time most of the measurements were taken, with 5 m intervals between trees. This population had 28 genotypes, each replicated on average 35 times. A complete set of data was available on 23 of these genotypes. The trees in these two seed orchard populations are grafted trees in which the branches were picked from representatives of the two adult wild populations.

The natural population was on the side of the mountain Yllästunturi, close to tree line and above the Arctic Circle (at 450 m above sea level, 67 34N, 24 11E). This population was in an area of about 50 × 100 m in which all adult trees were included, altogether 44 individuals. The age of the trees was measured by core samples at 1.3 m height, and was found to be, on average, 120 yr. However, it is known that in this extreme environment, north of the Arctic Circle, that cores underestimate the age by at least 20 yr (ILVESSALO 1965), making the average of the trees in this population conservatively 140 yr old.

Quantitative traits: The heights and diameters at breast height were measured for Viitaselkä and Vilhelminmäki in 1985 and for Yllästunturi in 1989. In Viitaselkä, five trees per genotype were measured and in Vilhelminmäki, on average six trees per genotype were measured. Female fertility was estimated as the number of liters of cones per tree in Viitaselkä and Vilhelminmäki in 1985, and in Yllästunturi in 1991 as the actual number of female cones. Seed weight per 100 seeds was also measured for Viitaselkä from the 1987 seed crop.

Male fertility was estimated by counting male strobili in all of the populations (for Viitaselkä and Vilhelminmäki in 1985 and for Yllästunturi in 1987). For each tree, the trunk was divided into sections of 1 m, from which one representative branch was sampled. All strobili on this branch were counted. For each genotype, 100 male strobili were measured. SARVAS (1962) has shown that, on average, 1 cm of strobilus corresponds to 0.028 gm pollen. This allowed estimation of pollen weights for each tree (as in MUONA and HARJU 1989).

In Viitaselkä, the timing of pollen shedding was measured for all genotypes. Two trees per genotype were chosen and eight male strobili around the crown were monitored. The data of the start of pollen shedding for each strobilus was recorded, and the mean was used to characterize the tree. For all these variables in the two seed orchard populations, the mean of five or six trees sampled was used to characterize each genotype.

Enzyme loci: The genotypes of all trees were identified for eight enzyme systems (aconitase, fluorescent esterase, glutamate dehydrogenase, glutamate-oxalate-transaminase, leucine amino peptidase (two loci), malate dehydrogenase (two loci), 6-phospho-gluconate dehydrogenase (two loci), and shikimate dehydrogenase (two loci)), making a total of 12 loci. Standard methods of protein electrophoresis were used, specific methods and references are given in MUONA *et al.* (1988). All enzyme genotypes of trees indicated as belonging to the same genotype were verified. For some purposes, the genotypes were pooled to get loci with two alleles. In this case, the most frequent allele was held distinct and the other alleles were pooled into a second class. This pooling occurred in only six heterozygotes out of the 1104 genotypes and is therefore unlikely to have any effect on the statistical analyses described below.

Statistical methods: Allele frequencies, observed heterozygosities, and the fixation index were estimated for all 12 loci in the three populations using standard techniques (*e.g.*, HEDRICK 1985). Analysis of variance was conducted between heterozygotes and homozygotes at each locus for all quantitative traits (genotypes heterozygous for a minor allele, there were six overall, were categorized as heterozygotes in this analysis and others described below). Regression analysis of all variables was done in several ways. First, each locus was analyzed separately, by using the number of the most common allele in the individual (2, 1, or 0) as the dependent variable. Second, following the adaptive distance scaling approach of SMOUSE (1986) and BUSH *et al.* (1987), we scored heterozygotes as 0 and homozygotes as $1 / P_{ii}$ where P_{ii} is the observed frequency of homozygote A_iA_i, and carried out the same regression analysis. Third, an analysis was done by using the number of heterozygous loci out of the 12 polymorphic loci as the dependent variable. Fourth, a multiple regression was done by using 12 independent variables, with heterozygosity at individual loci as independent variables. For all these analyses, age was used as a covariate for the Yllästunturi population. All analyses were done using the SAS 6.1 program package (SAS 1987).

Power analysis: What is the probability that we would have detected a given level of heterozygote advantage with the number of genotypes we have in our three samples? Or more precisely, what is the power of our test (*e.g.*, SOKAL and ROHLF 1981), *i.e.*, what is the probability that we would accept the false null hypothesis that the heterozygotes and homozygotes do not differ in their values for the quantitative traits?

For the two populations grown in seed orchards in which there were multiple copies of each genotype, we can use the following approach to determine the power. Assume a genetic model of the phenotype (P) such that

$$P_{ijk} = \mu + l_i + g_j + e_k \quad (1)$$

where μ is the overall population mean, l_i is the mean deviation of a heterozygote or a homozygote at a given locus, g_j is the genetic deviation due to the other loci, and e_k is the deviation of the environment. The population mean we used is that given in Table 2 for the different traits. For heterozygotes and homozygotes, the mean values were $\mu + l_i$ and $\mu - l_i$, respectively. The selective disadvantage of homozygotes relative to heterozygotes is $s\mu$ so that s is defined as a proportion of the population mean. The values of g_j and e_k were generated using random normal deviates with the genetic and environmental variances estimated for the different traits. The difference between the means of the heterozygotes and homozygotes were tested using a t test (*e.g.*, SOKAL and ROHLF 1981) and compared with the one-tailed t value for $\alpha = 0.05$ with 23 (Viitaselkä) or 21 (Vilhelminmäki) degrees of freedom. The power was the proportion out of 1000 samples in which for a given s value the calculated t exceeded the tabular value.

The same approach was used for the natural population at Yllästunturi except that since there were no separate estimates of the genetic and environmental variance, we defined the phenotypic value as

$$P_{ij} = \mu + l_i + p_j \quad (2)$$

where the estimate of the phenotypic variance was used to determine the size of a random normal deviate, p_j.

The above approach was expanded to determine the power for the individual heterozygosity for when all the loci were considered. First, the genotype of the most heterozygous individual in a sample was initially given a genotypic value of 1.0. The value of the other genotypes was initially given as

$$w_n = (1 - s)^n \quad (3)$$

where n is the number of homozygous loci beyond the number in the most heterozygous individual in the sample. For

TABLE 1

Frequencies of most common allele (p), observed heterozygosities (H_o), and fixation indices (F) at 12 loci in three population of *Pinus sylvestris*

	Population								
	Viitaselkä			Vilhelminmäki			Yllästunturi		
Locus	p	H_0	F	p	H_0	F	p	H_0	F
Aco	0.90	0.20	−0.11	0.80	0.39	−0.24	0.96	0.09	−0.05
F-Est	0.74	0.16	0.69	0.85	0.30	−0.18	0.80	0.36	−0.12
Gdh	0.62	0.56	−0.10	0.61	0.52	−0.10	0.71	0.41	0.02
Got-2	0.58	0.52	−0.07	0.59	0.39	0.19	0.53	0.34	0.40
Lap-1	0.98	0.04	0.04	0.96	0.09	−0.05	0.99	0.02	−0.01
Lap-2	0.88	0.20	0.22	0.96	0.09	−0.05	0.89	0.18	−0.10
Mdh-1	0.92	0.16	−0.09	0.96	0.09	−0.05	0.99	0.23	−0.01
Mdh-3	0.56	0.40	0.19	0.72	0.57	−0.39	0.59	0.36	0.25
6-Pgd-1	0.48	0.32	0.36	0.61	0.70	−0.46	0.55	0.64	−0.28
6-Pgd-2	0.68	0.48	−0.10	0.63	0.30	0.34	0.63	0.43	0.08
Sdh-1	0.92	0.08	0.46	0.94	0.13	−0.07	0.86	0.25	0.04
Sdh-2	0.96	0.08	−0.04	0.96	0.09	−0.05	0.98	0.05	−0.02
Mean	0.77	0.28	0.12	0.80	0.30	−0.09	0.79	0.26	0.03

Samples sizes: Viitaselkä, $N = 25$; Vilhelminmäki, $N = 23$; Yllästunturi, $N = 44$.

example, in Viitaselkä the most heterozygous individual was heterozygous at six loci and the least was heterozygous at one locus so that $n = 0$ to 5. These values were then standardized by the mean value of the trait in the sample so that approximately half had values greater than the mean and approximately half less than the mean. The array of phenotypic values for a sample was generated as above using the estimated genetic and environmental variances. We then calculated the power as the proportion of 1000 such samples that gave a P value <0.05 for regression analysis for a given average heterozygous advantage per locus.

RESULTS

Population genetic and quantitative trait data: The basic population genetic data, *i.e.*, the frequency of the most common allele, the observed heterozygosity, and the fixation index from these three populations for the 12 polymorphic loci are given in Table 1. Notice that each population has a number of loci that have two alleles in substantial frequency. This results in a fairly high average observed heterozygosity of 0.277 over the 12 loci and 3 populations. The value of the fixation index (F) varies considerably with 14 values being positive and 22 negative. However, because of several large positive values, the average F is slightly positive at 0.02 but is not significantly different from 0.0 (using the approach of BROWN 1970, to obtain the variance of the fixation index, the 95% confidence interval width is 0.04). The five largest absolute F values for individual loci, averaged over populations, were *Aco* (−0.13), *F-Est* (0.13), *Got-2* (0.17), *6-Pgd-1* (−0.13), and *Sdh-1* (0.14). Only two of these, *Aco* and *6-Pgd-1*, had negative averages that could possibly indicate heterozygous advantage and only one of these two loci, *Aco*, had negative F values in all three samples. In other words, there is no evidence from these genotypic data of consistent deviations from Hardy-Weinberg proportions, a finding consistent with little selfing or other inbreeding among these adult individuals (and also inconsistent with strong selection for heterozygotes).

The observed number of individuals with different numbers of heterozygous loci (out of the total of 12) for the three populations was compared with those expected if the heterozygote frequencies of the loci were independent of each other. The observed numbers were not significantly different from those expected in all three populations, with all chi-square values not significant. As a result, we can assume that there is a nonsignificant amount of multi-locus association among these electrophoretic loci in these populations.

The basic data for the six quantitative traits are given in Table 2. Comparisons over populations should be made with care, because the trees were of different ages when they were measured, *i.e.*, the trees in Viitaselkä and Vilhelminmäki were 31 and 17 yr old while the average age in the Yllästunturi population was at least 140. Recall that, in Yllästunturi, the number given for cone production is the actual number of cones, not liters of cones, as for the other populations. The estimates of genetic and environmental variation are also given in Table 2 for Viitaselkä and Vilhelminmäki. The overall average broad-sense heritability is 0.415, indicating substantial genetic variation for these quantitative traits within these samples (see also SAVOLAINEN *et al.* 1993).

Association of heterozygosity and fitness: To determine whether there is an association between heterozygosity and these quantitative traits, we used several different approaches. First, for each combination of locus, population, and trait we carried out an analysis of variance (ANOVA) between homozygotes and heterozy-

TABLE 2

Means and standard deviations of quantitative traits in three populations of *Pinus sylvestris*

	Population				
	Viitaselkä		Vilhelminmäki		Yllästunturi
Trait	Mean	v_g, v_e	Mean	v_g, v_e	Mean
Diameter (dm)	22.5 ± 1.57	1.19, 6.50	—	—	26.4 ± 7.3
Height (m)	12.0 ± 0.68	0.23, 1.02	7.4 ± 0.64	0.19, 1.19	9.7 ± 2.0
Pollen weight (g)	509 ± 204	32,687, 56,410	75.7 ± 94	6,900, 12,309	75.4 ± 91
Pollen shedding (da)	18.1 ± 0.8	0.454, 0.382	—	—	—
Cone production (l)	16.1 ± 8.8	73.1, 24.5	5.6 ± 3.5	10.07, 20.33	133[a] ± 107
Seed weight (g/1000)	5.09 ± 0.9	0.749, 0.069	—	—	—

[a] Number of cones.

gotes. Overall, of 156 such comparisons, only 12 or 7.7% were significant, very close to the 5% expected by chance. In addition to looking at individual tests and their significance, we conducted an overall Bonferroni test (see RICE 1989) for the ANOVA values. In this test, the overall level of significance of α is obtained by comparing the individual probability values with α / k where k is the number of tests. If none of the individual P values are below this level, then there is no significance. This test showed nonsignificance for all of the populations tested separately (k = 72, 36, or 48) or jointly (k = 156).

As another approach to determine whether there is an association between the allozymes and the quantitative traits, we calculated a regression of the numbers of the most frequent allele in an individual on the values of the different quantitative traits. Overall, 13 out of 156 of these regressions were significant, or 8.3%, which is again close to the 5% expected by chance.

We also looked to see if any loci showed a greater than 5% proportion of tests significant. None of loci had more than two significant ANOVA tests over the three populations and the six different traits. For the regression analysis, the significant locus-trait-population combinations were generally the same but the locus *Sdh-1* had four of the significant regression values out of the 13 significant tests found. Further, of the 12 significant tests, the heterozygotes had higher values in six and the homozygotes had higher values in the other six. A useful way to illustrate these results is given in Figure 1 in which the level of significance is given for the three populations and 12 different loci for ANOVA. Obviously, there is no pattern for these results, suggesting that there is no locus specific effect on these traits, either favoring heterozygotes or homozygotes.

The Viitaselkä population had the highest number, 15 out of 144 or 10.4%, of the tests significant while Vilhelminmäki had 6 of 72 or 8.3% significant and Yllästunturi had four of 96 or 4.1% significant. These significant tests were evenly divided between ANOVA and regression with 8, 3, and 2 significant ANOVA tests for Viitaselkä, Vilhelminmäkii, and Yllästunturi, respectively. Because the quantitative traits in both Viitaselkä and Vilhelminmäkii were based on means of five or six trees of the same genotype, one might expect if there was any intrinsic association that it would be most likely picked up in these populations. On the other hand, the Yllästunturi population is in a very extreme environment at tree line above the Arctic Circle and one might expect that any associations would be more apparent in such a marginal environment. In any case, the proportion of significant tests in all the populations is quite close to 5%, suggesting that these effects had only marginal, if any, influence.

Finally, we checked to see if any trait had a particularly high number of significant tests. The traits with the highest proportion of significant tests, three out of 24 or 12.5%, were pollen-shedding time and seed weight, both of which we only had measurements on in Viitaselkä. In these cases, two of the significant tests were in one direction and one in the other direction. Because we do not have any measurements on these traits in the other populations, we cannot judge whether these higher proportions are just due to chance or might reflect some real effect on these traits.

Because these traits individually appear to be unrelated to the allozyme variants, we made a simple composite measure of the traits that may give a measure more closely related to overall fitness. Each of the traits were first standardized to 0 mean and unit variance (after LANDE and ARNOLD 1983). We then defined a simple measure of fitness (w) which was equally weighted for viability and reproduction so that

$$w = \frac{viability + reproduction}{2} \qquad (4)$$

As a measure of viability, we then gave equal emphasis to seed weight, as an indicator of early survival, and height, as an indicator of late survival, so that

$$viability = \frac{seed\ wt. + height}{2} \qquad (5a)$$

As a measure of male reproduction, we then gave equal

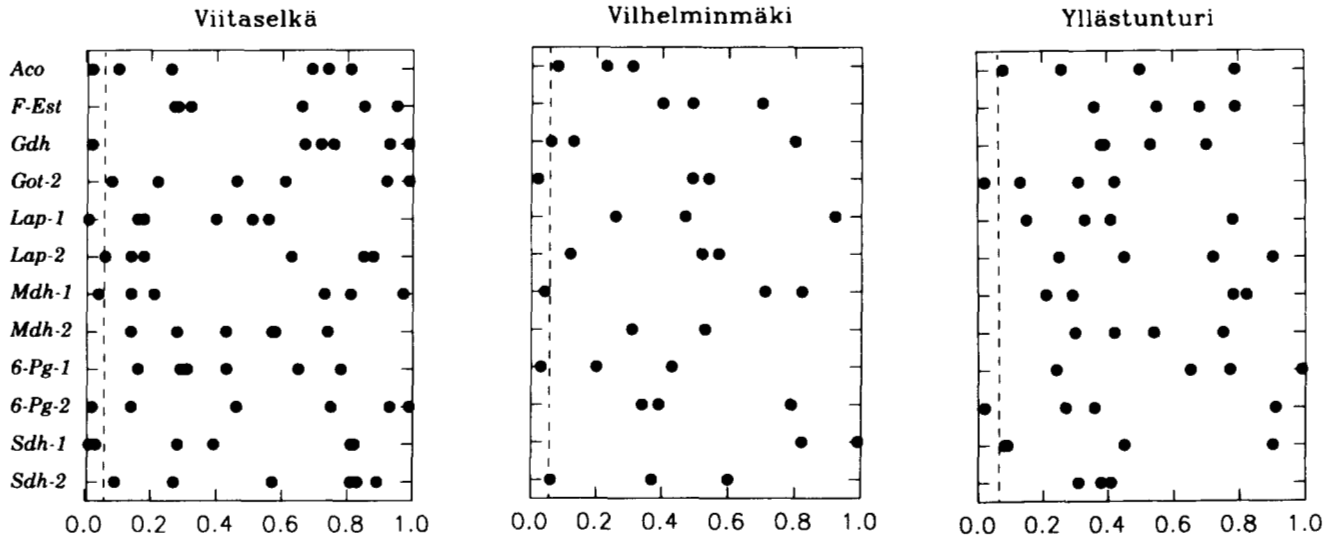

FIGURE 1.—The probability of significance level for a difference between heterozygotes and homozygotes from the ANOVA for the 12 polymorphic loci in the three different populations. The individual points are the probability level for a given quantitative trait. The vertical broken lines indicate significance at the 5% level.

value to pollen weight and pollen-shedding time to indicate male reproductive value. This value can then be equally weighted with cone production, as an indicator of female reproductive value, so that

$$reprod. = \frac{pollen\ wt. + pollen\ shed. + 2(cone\ prod.)}{4}. \quad (5b)$$

This composite measure of fitness can be calculated for each tree and then used in both the ANOVA and regression analysis. None of these tests were significant for the three populations. In other words, this composite measure of fitness does not detect any effects of the allozymes that were missed by examining only one of the quantitative traits related to fitness.

To examine if there are significant associations that are not seen by this approach but are detected by using the adaptive distance scaling suggested by SMOUSE (1986), we also carried out the 156 combinations of regressions using the adaptive distance values. Of these tests, 11 (7%) were significant at the 5% level. For these significant regressions, the coefficient was positive in six cases and negative in the other five. Most of these significant values were for the same combinations as for the previous regressions.

As another approach to examining the association of heterozygosity and the various quantitative traits, we looked at the association of the number of heterozygous loci in individual trees and the value of the six different traits in the three populations using multiple regression (Table 3). Out of 16 combinations of traits and populations, none were significant at the 5% level. The combination of highest significance was diameter at Yllästunturi which was significant at the 7% level. However, association of diameter and heterozygosity at Yllästunturi was negative and, in addition, for Yllästunturi the R^2 values include the effect of age and that the variability of diameter in Yllästunturi was mainly due to age. Over all these combinations, only 5.8% of the variation (average R^2 value) was explained by the multiple regression analysis. In fact, much of this variance was explained by a negative association of heterozygosity and the quantitative traits (see negative values indicated by an asterisk in Table 3) and if these negatives values were set to zero only an average of 1.5% of the variance was explained by a positive relationship of heterozygosity with the quantitative traits.

As a graphical way to illustrate these results, Figure 2 gives for different numbers of heterozygous loci, the mean values of three traits, pollen production, cone production, and height, for the three different populations. Recall that because there is high polymorphism at a number of loci in these populations, the range of individual heterozygosity is high (compared with many other studies), *i.e.*, from 0 to 7 in Yllästunturi and 1 to 6 in the other two populations. Obviously, there is no apparent positive association in Figure 2, *i.e.*, low values for these traits having low individual heterozygosity and high trait values having high heterozygosity. In fact, there appears to be a general lack of pattern between these traits (and the other traits that are not shown here) and individual heterozygosity.

Power analysis: Because there were replicates of the 25 and 23 genotypes grown in Viitaselkä and Vilhelminmäkii, respectively, the power of these tests needs to be calculated to determine how much additional value these replicates were in determining the difference between heterozygotes and homozygotes. As insight into this situation, if there were no environmental variance, then the replicates of the genotypes would give no added power because they would not reduce the phenotypic variance. On the other hand, if there

TABLE 3

Significance of regression (R^2 and P) of quantitative characters on number of heterozygous loci per individual and significance of multiple regression using heterozygosity at individual loci as independent variables in three populations of *P. sylvestris*

	Population					
	Viitaselkä		Vilhelminmäki		Yllästunturi[a]	
	R^2	P	R^2	P	R^2	P
Number of heterozygous loci per individual						
Diameter	0.04[b]	0.33	—	—	0.36[b]	0.07
Height	0.01[b]	0.63	0.09	0.15	0.02[b]	0.43
Pollen weight	0.01	0.69	0.02	0.48	0.03[b]	0.53
Pollen shedding	0.02[b]	0.47	—	—	—	—
Cone production	0.01	0.70	0.09[b]	0.16	0.06	0.42
Seed weight	0.01	0.63	—	—	—	—
Fitness	0.01	0.81	0.02	0.48	0.16[b]	0.11
Heterozygosity at individual loci						
Diameter	0.40	0.75	—	—	0.15	0.04
Height	0.47	0.59	0.63	0.29	0.29	0.49
Pollen weight	0.51	0.47	0.61	0.33	0.24	0.70
Pollen shedding	0.67	0.12	—	—	—	—
Cone production	0.47	0.58	0.58	0.40	0.22	0.81
Seed weight	0.72	0.08	—	—	—	—
Fitness	0.38	0.82	0.56	0.47	0.33	0.36

[a] For the Yllästunturi population, age of the tree was used as a covariate in the multiple regression analysis.
[b] Negative value.

is no genetic variance, then the power is that of a sample that is equal to the number of genotypes times the number of replicates examined per genotype. In the data here, the broad-sense heritability estimates, defined as between genotype variation over total phenotypic variation, average 0.485 for the six traits at Viitaselkä and 0.276 for the three traits at Vilhelminmäkii so that the power appears to be in between these two extremes. An additional factor determining the power is the relative size of the variances to the mean of the trait (see below).

To illustrate the power to detect a difference between heterozygotes and homozygotes of 0.05 and 0.1, we calculated the power for a locus which had 10 heterozygotes and 15 homozygotes and five replicates per genotype (as an example for Viitaselkä) and nine heterozygotes and 14 homozygotes and six replicates per genotype (as an example for Vilhelminmäkii). There were four loci in each of the populations that had this level of polymorphism or greater (equal to or more heterozygotes than this).

As can be seen in Table 4, the power of the tests is greatly dependent on the trait. For diameter and height, the power to determine a selective difference of 0.05 in the seed orchards is substantial (average of 0.564) and a difference of 0.1 is quite high (average of 0.953). We should note that in many of the published reports, it is size or growth rate that is being measured (see DISCUSSION below). Our trees were of even age in the seed orchards (age was used as a covariate in Yllästunturi). In other words, our measures of height and diameter are the data that are most comparable with other studies and they both have good power. For seed weight in Viitaselkä, the power was fairly good for a selective difference of 0.1. On the other hand, for pollen weight and cone production, the power was quite low. This resulted because for these traits, the standard deviation is of the same relative size as the mean, making the detection of a 5 or 10% difference between the heterozygotes and homozygotes very unlikely. The overall power to detect a 10% heterozygous advantage ranged in the seed orchards from 0.062 to 1.0 with a mean value of 0.521.

The power for the Yllästunturi sample (we used 16 heterozygotes and 38 homozygotes) was somewhat lower than for the two orchard populations. In other words, the measurement of the replicates of the genotypes in Viitaselkä and Vilhelminmäkii resulted in the sample size for these populations being effectively larger than the 44 in Yllästunturi sample. To determine what the "effective" sample size, *i.e.*, the equivalent sample size if there were no replicates, is for Viitaselkä and Vilhelminmäkii, we determined the sample size (for the same mean and phenotypic variance) that would give a similar power to that observed. The largest effective sample size was for height, $N > 70$ in Viitaselkä and $N > 80$ in Vilhelminmäkii, and diameter, $N > 70$ in Viitaselkä. For the other combinations, the effect of

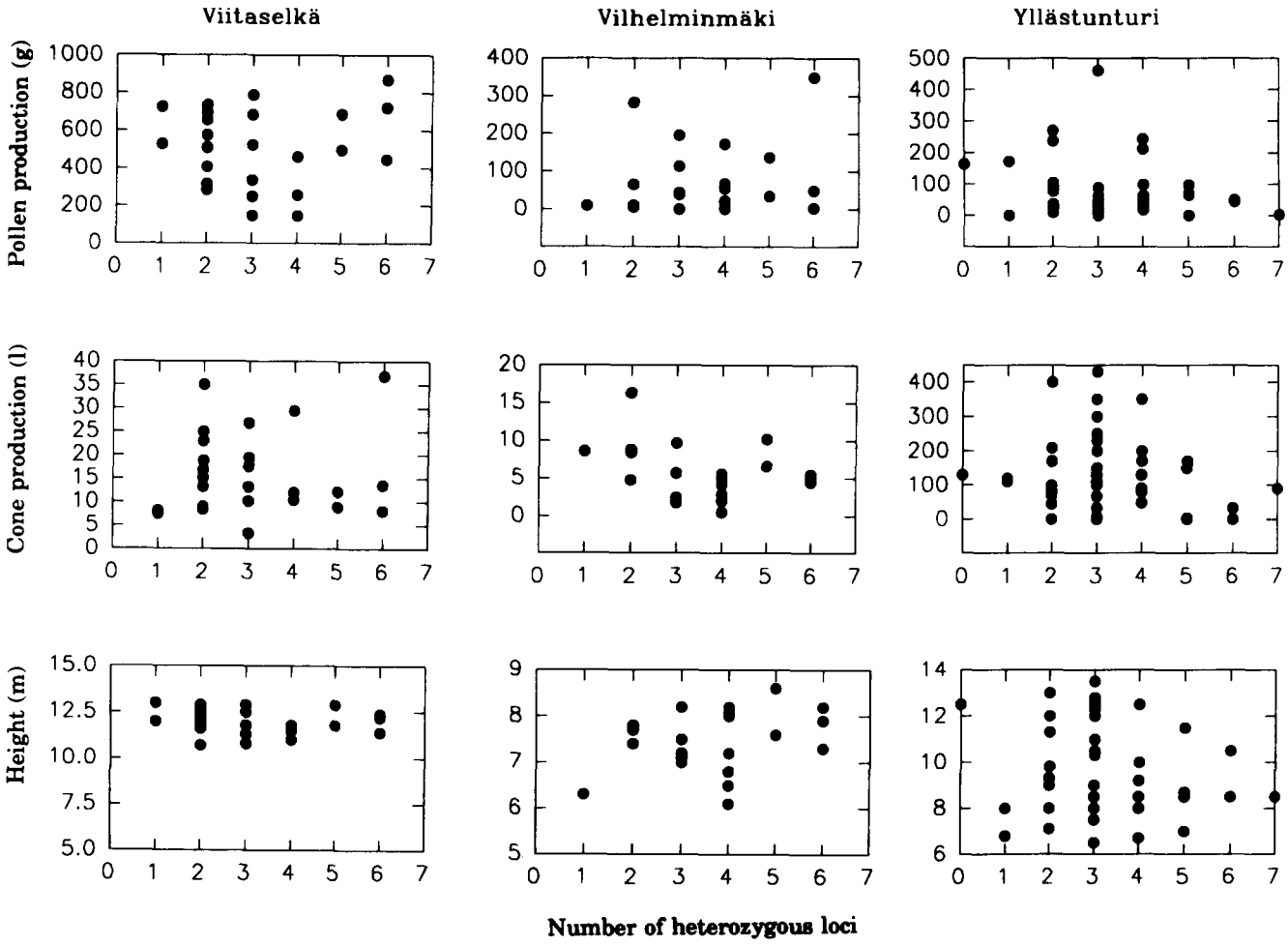

FIGURE 2.—The phenotypic values of three quantitative traits, height, cone production and pollen production, for clonal means (Viitaselkä and Vilhelminmäki) or individuals (Yllästunturi) with different numbers of heterozygous loci.

replicate measurements was marginal with the sample size either $N > 30$ or $N > 40$ for all cases.

Table 5 gives the power for the multiple locus data. Again the power is generally highest for diameter and height, the traits often used in other studies, suggesting that we would have found a significant effect if it had indeed been present. For some of the other traits with high variance-to-mean ratio, again the power was relatively small. The overall power to detect an average heterozygous advantage of 5% ranged from 0.056 to 1.0 for several trait-population combinations with a mean of 0.473.

DISCUSSION

In this study of Scots pine, a number of factors (see Introduction) should have enabled us to document an

TABLE 4

The power to reject the null hypothesis that there is no difference between heterozygotes and homozygotes

	Population					
	Viitaselkä		Vilhelminmäki		Yllästunturi	
Trait	$s = 0.05$	$s = 0.1$	$s = 0.05$	$s = 0.1$	$s = 0.05$	$s = 0.1$
Diameter	0.569	0.976	—	—	0.210	0.329
Height	0.727	0.999	0.397	0.885	0.210	0.497
Pollen weight	0.079	0.155	0.060	0.062	0.062	0.073
Pollen shedding	0.928	1.000	—	—	—	—
Cone production	0.090	0.115	0.069	0.102	0.082	0.103
Seed weight	0.171	0.395	—	—	—	—
Mean	0.427	0.607	0.175	0.350	0.128	0.250

TABLE 5

The power to reject the null hypothesis that there is not a significant regression at the 5% level in a multilocus genotype

	Population					
	Viitaselkä		Vilhelminmäki		Yllästunturi	
Trait	$s = 0.02$	$s = 0.05$	$s = 0.02$	$s = 0.05$	$s = 0.02$	$s = 0.05$
Diameter	0.554	0.998	—	—	0.103	0.385
Height	0.747	1.000	0.283	0.965	0.134	0.639
Pollen weight	0.068	0.154	0.057	0.056	0.060	0.081
Pollen shedding	0.943	1.000	—	—	—	—
Cone production	0.047	0.112	0.062	0.071	0.061	0.089
Seed weight	0.123	0.594	—	—	—	—
Mean	0.414	0.643	0.134	0.364	0.090	0.299

association of allozyme heterozygosity and quantitative traits related to fitness due to intrinsic heterozygote advantage if it had been present. Examining our data using both ANOVA and regression analysis, we found for 312 tests that 8.0% were significant at the 5% level, indicating that there is little obvious effect of allozyme heterozygosity on fitness components in our populations. In fact, in half of these significant cases, the homozygotes were worse than the heterozygotes. Further, using the scaling suggested by SMOUSE (1986) in his adaptive distance approach, we found similar results. Overall, 7% of the adaptive distance regressions were significant at the 5% level and approximately half of these had positive values and half had negative values. Even for a composite measure of fitness that combined the six quantitative traits related to fitness, we found no significant associations out of 72 tests for the three populations tested by both ANOVA and regression analysis. Finally, a multiple regression analysis using all 12 polymorphic loci in an individual heterozygosity measure, explained only a small proportion (<6%) of the variation observed in the six different traits and less than one-third of this variance was due to positive associations of heterozygosity with the quantitative traits.

We carried out an analysis to determine the power we had to detect a difference between heterozygotes and homozygotes and the regression of multiple locus heterozygosity on the various traits in our samples. To our knowledge, this is first time that a power analysis has been carried out on such a data set and it gives insight into where we might have detected heterozygote advantage. For several traits often used in similar studies, diameter and height, the power to detect a single-locus difference of 5 or 10% or a multiple locus effect averaging 2 or 5%, given that it was present, was very high. In carrying out this analysis, it was obvious that the power was quite dependent on the level of the environmental variance for a given trait when there were replicate measurements of genotypes. In addition, it was obvious that the power was very dependent on the relative size of the phenotypic variance and the mean of a given trait. We recommend that any further studies of the association of heterozygosity and fitness include an analysis to determine the power to detect given selective differences.

A number of previous studies have searched for a relationship between individual heterozygosity and traits related to fitness in trees. We do not know how many studies have found no such association and have not reported their results but any such reporting bias would overestimate the ubiquity of the phenomenon (for a discussion of an analogous bias for selection studies, see ENDLER 1986). In addition, a majority of the positive reports in trees have been made by a relative few researchers and their colleagues, suggesting that there may be some bias from these researchers in reporting only results that show a positive association between heterozygosity and a trait related to fitness. Finally, in our opinion, the support for a positive association due to intrinsic heterozygote advantage is not very strong even in the studies reporting a positive relationship if they are critically evaluated (see below).

The initial studies in trees dealt mostly with growth rate in natural populations. For example, MITTON and GRANT (1980) reported a positive relationship between individual heterozygosity and growth rate among 100 clones in *Populus tremuloides* from the Rocky mountains. However, this study included only three polymorphic loci and because of the high degree of asexual reproduction in the species, correlation with other loci is very likely. The number of heterozygous loci was not related to mean growth rate in *P. contorta* and in *P. ponderosa*, "predominately heterozygous" individuals grew more slowly than "predominately homozygous" individuals (KNOWLES and MITTON 1980). In natural population of ponderosa pine, LINHART and MITTON (1985) did not find any relationship between heterozygosity and mean growth or with mean female or male reproduction.

LEDIG *et al.* (1983) found a positive relationship heterozygosity and growth rate in *P. rigida*. These authors concluded that this was due to inbreeding depression from partial selfing, a phenomenon that was found in this species by GURIES and LEDIG (1982). A later reanal-

ysis of the same data led BUSH *et al.* (1987) to conclude that specific loci were responsible for the observed association. Recently, EGUIARTE *et al.* (1992) reported a positive relationship between heterozygosity and growth (but not fecundity) in a tropical palm, *Astrocaryum mexicanum.* In this study, the mean fixation index at the seed level was −0.2 and at the adult stage was −0.4, suggesting that further study is called for to understand the basis of these findings.

Some later studies have included experimental stands. STRAUSS and LIBBY (1987) studied clonal replicates of about 10-yr-old *P. radiata* seedlings, and concluded that overdominance was a very unlikely explanation for an association of allozyme heterozygosity and quantitative traits. STRAUSS (1986) also studied a more controlled situation, where he included selfs and crosses between different trees of *P. attenuata.* Outcrosses did not show consistent relationships, but among the inbreds, heterozygosity was positively correlated with trunk growth and cone production. A study of family mean heterozygosity versus growth rates provided no evidence for heterozygote superiority for growth in *Pseudotsuga menziesii* (BONGARTEN *et al.* 1985). BUSH and SMOUSE (1991) studied selfs and crosses in *P. taeda* and found little evidence for overdominance.

There have also been a large number of studies examining the association of individual heterozygosity and quantitative traits, particularly growth rate, in mollusks (see ZOUROS and FOLTZ 1987). However, in many of the studies that do show a positive relationship between heterozygosity and a quantitative trait, there also appears to be a deficiency of heterozygotes in the early age classes (*e.g.,* GAFFNEY *et al.,* 1990). This suggests that there may be population structure or some other factor influencing genotypic frequencies and also causing an association between the allozyme loci and loci affecting the trait being examined [see the exchange of letters by KOEHN (1990) and ZOUROS (1990)].

In a sophisticated extension to the previous approaches, POGSON and ZOUROS (1994) detected some positive association between multiple locus heterozygotes and height in scallops for allozyme loci but not for other loci identified by DNA markers in the same population. However, only one allozyme locus had a significant positive effect and regression analysis explained only 3% of the variance for the allozyme data. As they state, "the differences observed between the effects of allozyme and RFLP heterozygosity on growth rate provide evidence against the associative overdominance hypothesis, but a strong case against this explanation must await corroboration from similar studies in different species."

SMOUSE (1986) proposed a technique, called the adaptive distance model, to analyze heterozygosity and quantitative trait data. As we have shown above, it does not appear that the adaptive distance model finds more association between allozyme genotypes and quantitative traits in our data set than does ANOVA or the regression model using the number of copies of the most frequent allele. SMOUSE (1986) also suggested that the adaptive distance model could be used to distinguish between effects due to heterozygote advantage and those due to dominance. These conclusions were based on the assumption that the rarer of two homozygotes for a biallelic locus with heterozygote advantage would have a lower fitness and that the population is panmictic and at equilibrium. It has been shown, however, in the absence of panmixia, that the rarer homozygote for a neutral locus (at which alleles are associated with alleles at dominant loci) will also have a lower fitness (OHTA 1971; OHTA and COCKERHAM 1974; CHARLESWORTH 1991). HOULE (1994) has recently shown that when the association is the result of inbreeding or finite population size the adaptive distance model cannot distinguish between heterozygote advantage and associative overdominance caused by dominant loci.

In a large and comprehensive laboratory study in *Drosophila melanogaster,* HOULE (1989) looked for associations of allozyme heterozygosity and size, developmental rate, and fluctuating asymmetry for size. As is the case for Scots pine, *D. melanogaster* is unlikely to exhibit either extensive linkage disequilibrium or genotypic associations because of its large population size and lack of any type of inbreeding. In other words, any observed associations would likely be the result of intrinsic heterozygote advantage. Overall, HOULE found no significant correlation of heterozygosity at eight different loci for four different traits in two different experiments. Based on these results and a review of the literature, he concluded that "Until there is evidence that allozyme heterosis occurs in large, panmictic, natural populations, the hypothesis of functional overdominance must be regarded with considerable skepticism."

Based on these findings and the above discussion, we feel that the use of individual allozyme heterozygosity (or heterozygosity at other marker loci) to select parents in either tree breeding programs or in conservation genetics must be used with extreme caution. Our results, and those of others, indicate that in populations without factors that can cause interlocus correlations, individuals with high allozyme heterozygosity may not be any different for fitness-related traits than individuals with low allozyme heterozygosity. Only when there are high levels of inbreeding, very small population sizes, or extreme population structuring is it likely that an association would be present, and in these cases it would not be due to the intrinsic advantage of heterozygotes. In other words, selection in these cases would be for genotypes that have an ephemeral advantage generated by these secondary factors. Such a breeding or management program may be temporarily effective in some

cases but it would be difficult to support it over traditional techniques in tree breeding (*e.g.*, ZOBEL and TALBERT 1984) or conservation genetics (*e.g.*, HEDRICK and MILLER 1992).

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