THE DISTRIBUTION OF INDIVIDUAL HETEROZYGOSITY IN NATURAL POPULATIONS

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ABSTRACT

Estimation of the distribution of the level of individual heterozygosity within natural populations is explored with both Monte-Carlo simulation studies and data from natural populations. Simulations indicate that heterozygosities estimated from as few as a dozen randomly chosen loci may, to some degree, reflect ($r = 0.35$) heterozygosity determined by 100 independent loci. The shape of the expected distribution of heterozygosity is heavily dependent upon levels of heterozygosity at the loci. Complete genetic data for 12 loci from 997 *Fundulus heteroclitus* are used to describe the distributions of heterozygosity for different localities, for age classes and for sexes. The distributions deviate from normality. Distributions from different localities are not different, but the distributions are heterogeneous among age classes at one of two localities and are heterogeneous between the sexes.

A eukaryotic genome is composed of thousands of loci distributed over a few to a few dozen chromosomes. Thus, a few randomly chosen genes are likely to assort independently, and variation at a randomly chosen set of genes is not expected to be associated with morphological variation at a set of phenotypic characters. Therefore, it is noteworthy that several recent reports suggest that heterozygosity rankings from a few allozyme loci may be related either to morphological variation or to some aspect of growth rate. For example, heterozygotes at five different allozyme loci in *Fundulus heteroclitus* tend to have lower morphological variance than do homozygotes (MITTON 1978). The same relationship is also found in the Monarch butterfly, *Danaus plexippus* (EANES 1978), and heterozygosity for allozyme loci is negatively correlated with bilateral asymmetry in *Uta stansburiana* (SOULÉ 1979). Similarly, individual heterozygosity for allozyme loci has been related to growth rate in the oyster, *Crassostrea virginica* (SINGH and ZOUROS 1978; ZOUROS, SINGH and MILES 1980); highly heterozygous individuals have the highest growth rates. Protein heterozygosity is also positively correlated with both mean growth rate and year-to-year variability of growth rate in quaking aspen, *Populus tremuloides* (MITTON and GRANT 1980). Growth rate increases with protein heterozygosity in both natural and laboratory populations of the tiger salamander (PIERCE and MITTON, in preparation). Protein heterozygosity is not related to mean growth rates of ponderosa pine, *Pinus ponderosa*, or

lodgepole pine, *Pinus contorta*, but it is positively correlated with several measures of growth variability (MITTON *et al.* 1980; KNOWLES and MITTON 1980) in these species. Although the causal mechanism for these associations has not been identified, this matter clearly warrants further investigation.

Associations in natural populations between heterozygosity and either measures of growth rate or morphological variance focus attention upon the distribution of heterozygosity within populations. A problem that first presents itself in this context is the estimation of heterozygosity ranking from a limited number of protein polymorphisms. Surely, a ranking of individuals for heterozygosity of the whole genome cannot be achieved with a few polymorphic loci. We consider here the possibility of estimating the relative heterozygosities of individuals for 100 loci, the number of polymorphic loci that might be found in a few major metabolic pathways. First, we simulate distributions of heterozygosity for 100 loci in a population of 100 individuals and sample a few loci from that data set to examine the estimation of heterozygosity. Second, we present the distribution of heterozygosity for 12 loci as a function of heterozygosities at those loci. Finally, we report the distribution of heterozygosity, based on 12 loci, in a sample of 997 killifish, *Fundulus heteroclitus*, and analyze these data for deviations from expected distributions and for heterogeneity among localities, among age classes, and between the sexes.

MATERIALS AND METHODS

Estimations of heterozygosity: The efficiency of estimating a heterozygosity ranking with a few loci was explored with computer simulation. A population of 100 individuals, each with 100 polymorphic loci, was prepared to represent real data that would subsequently be sampled utilizing a few loci. The 100 loci are not intended to represent the whole genome, but perhaps the polymorphic loci in a few metabolic pathways. Since we are attempting to depict the present capabilities of geneticists and the limitations presented by numerous practicalities, we will examine the efficiency of estimating the real heterozygosity ranking of individuals for 100 loci using 1 to 20 loci. The data are established in different ways to examine some properties of this estimation. One possibility is that the accuracy of estimation of heterozygosity is dependent upon the level of heterozygosity in the sample. Therefore, the original data were simulated with levels of heterozygosity varying from 0.10 to 0.70. In each simulation all 100 loci had the same probability of heterozygosity. This is clearly not a realistic distribution, but we are first interested in the mechanics of estimation, not in accurately depictnig nature. The data are in the form of a 100 $\times$ 100 matrix filled with 0's and 1's. Each row is an individual, each column is a locus. A random-number generator was used to assign heterozygosity (1) or homozygosity (0) for each locus of each individual. The random-number generator was used again to determine which loci of the 100 loci would be used to estimate heterozygosity. For example, when heterozygosity was estimated for all 100 loci with 5 loci, five numbers between 1 and 100 were chosen randomly, and the heterozygosity of the 100 individuals was determined using the loci corresponding to those numbers. This process was repeated 10 times for each level of heterozygosity and for each number of sampled loci chosen. Individual heterozygosity levels based on a few (1 to 20) sampled loci were compared with the individual heterozygosity based on 100 loci with a Pearson product-moment correlation coefficient.

The 100 individuals $\times$ 100 loci data set was then established in a different way to examine an effect of variation of heterozygosity levels across loci. A probability of heterozygosity was assigned to each locus with the aid of a random-number generator with heterozygosities evenly distributed between 0.02 and 0.52. Although this distribution is more realistic (FUERST, CHAK-

RABORTY and NEI 1977), no sincere effort to approach biological reality was made; the interest was in the accuracy of estimating heterozygosity with loci having high or low levels of heterozygosity. This data bank was sampled in several different ways. First, sampled loci were chosen randomly from the 100 loci. Then, sampled loci were chosen randomly from loci with heterozygosities ≤ 0.25 and finally, sampled loci were chosen randomly from loci with heterozygosities ≥ 0.25. Correlations between individual heterozygosities from sampled loci and individual heterozygosities from 100 loci were summarized for 10 iterations with a Pearson product-moment correlation coefficient.

The expected distribution of heterozygosity: An investigation into the distribution of individual heterozygosity in natural populations may consider both observed and expected distributions. Expected distributions were generated with observed genotypic frequencies and the assumption of independent assortment of loci. Expected distributions of individual heterozygosity were generated for 12 loci, the number of loci treated in the empirical data (below). In the first series of distributions, the heterozygosities were equal across loci and distributions were generated for heterozygosities of 0.01, 0.10, 0.30, 0.50 and 0.70. Distributions were then generated for mean heterozygosities of 0.50, but with variation in heterozygosity among the 12 loci.

Empirical data and their analyses: Allozyme data analyzed here are from a study of geographic variation of the common killifish, *Fundulus heteroclitus* (MITTON and KOEHN 1975, 1976). Seven population samples were obtained from 5 localities in Long Island Sound, New York. Samples collected at Northport were taken from an industrial thermal effluent, and individuals taken from Flax Pond, Asharoken, West Meadow Creek and Nissequoque represent natural environments for this species. *F. heteroclitus* is sexually dimorphic, so that the sex of each individual was easily assigned and the age classes were inferred from the frequency distribution of standard length (MITTON 1973). Complete data for 997 fish are available. Observed and expected frequency distributions are summarized and compared using SPSS program #14 (NEI *et al.* 1975), and the skew and kurtosis (SOKAL and ROHLF 1969) of observed distributions of individual heterozygosity are described.

RESULTS

Monte-Carlo simulations were performed by establishing a genetic pool of 100 individuals, each with 100 polymorphic loci, and then estimating the individual heterozygosity with a few (1 to 20) randomly chosen loci. The correlations between individual heterozygosity based on 100 loci and heterozygosity estimated from a subset of loci are summarized with Pearson product-moment correlation coefficients (Table 1). When all loci are given the same probability of heterozygosity, the accuracy of the estimation increases with the number of loci employed, but the accuracy does not increase with the level of heterozygosity. Thus, in a sample of $N = 100$, statistically significant correlations between estimated and real heterozygosity are not achieved until five or more loci are employed in the estimation. When 20 loci are employed in the estimate, the correlation is approximately 0.44. Increasingly accurate estimates are achieved with more sampled loci, a rather intuitive result. The simulation program was modified to allow a flat distribution of heterozygosity with limits of 0.02 and 0.52. Subsets of varying size (1 to 20 loci) were chosen from this distribution on the basis of their heterozygosity. First, loci were chosen randomly to estimate heterozygosity. The results of these simulations are quite similar to those above, despite the difference in the distribution of heterozygosity in the 100 loci. This series of estimations was then conducted twice more, first choosing marker loci randomly from those with heter-

TABLE 1A

Correlations between level of heterozygosity at 100 polymorphic loci and heterozygosity estimated with a randomly chosen subset of loci

No. of loci	Heterozygosity of marker loci 0.1	0.2	0.3	0.5	0.7
1	0.13 (0.09)	0.10 (0.10)	0.04 (0.09)	0.17 (0.14)	0.14 (0.06)
2	0.15 (0.08)	0.09 (0.07)	0.08 (0.10)	0.14 (0.11)	0.09 (0.09)
3	0.15 (0.07)	0.20 (0.11)	0.16 (0.09)	0.21 (0.08)	0.13 (0.06)
5	0.28 (0.11)	0.16 (0.11)	0.21 (0.11)	0.19 (0.10)	0.21 (0.09)
10	0.35 (0.05)	0.28 (0.08)	0.25 (0.10)	0.30 (0.08)	0.29 (0.09)
12	0.34 (0.07)	0.37 (0.12)	0.32 (0.10)	0.32 (0.11)	0.40 (0.07)
20	0.43 (0.11)	0.43 (0.09)	0.44 (0.10)	0.45 (0.11)	0.45 (0.10)

Heterozygosities are equal for all 100 loci and each correlation coefficient is the mean for 10 simulations. Standard deviations of the correlations for the 10 simulations are in parentheses.

ozygosity ≤ 0.25, then from loci with heterozygosity ≥ 0.25. A consistently better estimate of heterozygosity is obtained with highly heterozygous loci (Table 1B), but the estimates obtained from a random sample of loci and from highly heterozygous loci are quite similar. Thus, estimation of heterozygosity for a moderate number of polymorphic loci (100) may be achieved to some degree ($r = 0.20$ to 0.44) with a much smaller sample (5 to 20) of marker loci. This estimation seems to be rather insensitive to the heterozygosity of the whole data set or to the distribution of heterozygosity across loci. Thus, the results presented here may be applicable to a broad range of species and mating systems.

Estimation of the ranking of individuals of a population by their number of heterozygous loci raises questions concerning the shape of the distribution being sampled. The shape of the distribution of heterozygosity is investigated here with 12 polymorphic loci in order to facilitate comparisons with the data that will be presented. Genotypes at the different loci are distributed independently, and the

TABLE 1B

Correlations between level of heterozygosity at 100 polymorphic loci and loci chosen for high or low heterozygosity

No. of loci	Heterozygosity of marker loci 0.02 — 0.52	0.02 — 0.25	0.25 — 0.52
1	0.09 (0.13)	0.08 (0.10)	0.10 (0.08)
2	0.18 (0.08)	0.10 (0.07)	0.13 (0.08)
3	0.18 (0.07)	0.19 (0.09)	0.20 (0.10)
5	0.20 (0.10)	0.21 (0.08)	0.26 (0.05)
10	0.28 (0.11)	0.24 (0.08)	0.38 (0.04)
12	0.37 (0.10)	0.34 (0.12)	0.41 (0.08)
20	0.48 (0.07)	0.32 (0.12)	0.50 (0.08)

The critical value for r at $P = 0.05$ is 0.20, and at $P = 0.01$, $r = 0.25$.

The probabilities of heterozygosity at the 100 loci are evenly distributed between 0.02 and 0.52. Each correlation coefficient is the mean of 10 simulations; standard deviations are in parentheses.

probability of heterozygosity is the same for all loci. Expected distributions for heterozygosities of 0.01, 0.10, 0.30, 0.50 and 0.70 are presented in Figure 1. The degree of skew for these distributions proceeds from 2.822 to 0.716, 0.191, 0.000, and − 0.191. All standard errors are 0.141. Thus, if the probabilities of heterozygosity are equal and are less than 0.50, the distribution is skewed to the right; but if heterozygosities exceed 0.50, the skew is to the left. When sampling a limited number of loci, the distribution of individual heterozygosity is described with a bounded distribution containing a restricted number of discrete classes, and deviations from normality are expected. Distributions of heterozygosity were then generated with a range of heterozygosity among the loci. A series of distributions of heterozygosity, with heterozygosities normally distributed about a mean of 0.50, were examined. As the variance in heterozygosity across loci increased, the distribution of individual heterozygosity narrowed. One distribution with a mean heterozygosity of 0.50, but with a range of heterozygosities of 0.08 to 0.92, is shown (Figure 1) for comparison with a distribution with a mean of 0.50 and a variance of 0.0. This rather large variance in heterozygosity across loci ($s^2 = 0.270$) has a minor effect on the distribution in comparison to differences in mean heterozygosity.

The distribution of heterozygosity for the 12 enzyme loci studied in 997 *Fundulus heteroclitus* sampled from five localities in Long Island Sound is presented in Table 2. This distribution has a mode of 3, its mean is 3.68 and its variance is

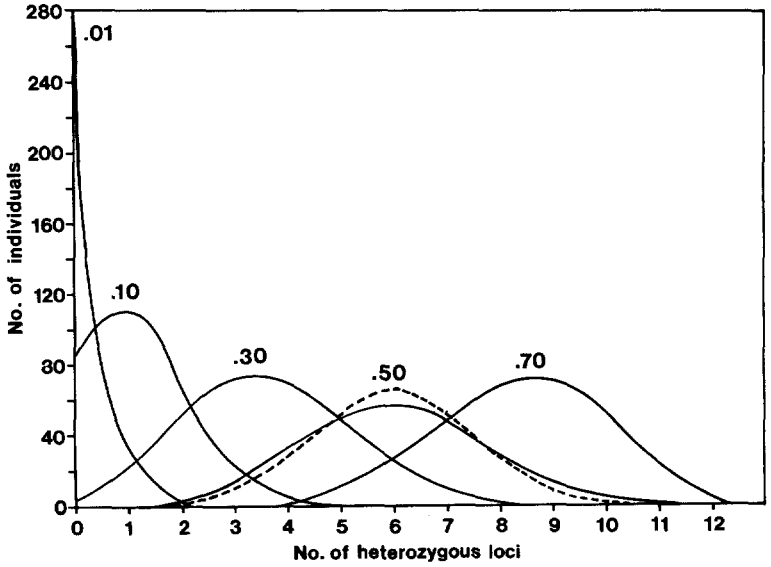

FIGURE 1.—Expected distributions of individual heterozygosity with different per locus levels of heterozygosity (solid lines). The per locus levels of heterozygosity (equal for all 12 loci) are above the appropriate distributions. Skewness progresses from highly positive through zero to negative values as per locus heterozygosity increases. The dotted line is the distribution of individual heterozygosity with a mean heterozygosity of 0.50, but with per locus heterozygosities of 0.08, 0.16, 0.24, 0.32, 0.40, 0.48, 0.52, 0.60, 0.68, 0.76, 0.84 and 0.92.

TABLE 2

The distribution of heterozygosity for 12 loci in 997 Fundulus heteroclitus *and for seven loci in* Crassostrea virginica

Species	Number of heterozygous loci													$Y \pm$ SE	S^2	$g_1 \pm$ SE	$g_2 \pm$ SE
	0	1	2	3	4	5	6	7	8	9	10	11	12				
F. heteroclitus	13	62	160	237	235	166	78	35	8	2	1	0	0	3.68 ± 0.05	2.56	0.29 ± 0.08	0.07 ± 0.16
C. virginica	37	169	296	292	162	40	14	0	—	—	—	—	—	2.54 ± 0.04	1.53	0.22 ± 0.08	−0.13 ± 0.15

Y = mean, SE = standard error, S^2 = variance, g_1 = skewness and g_2 = kurtosis.

2.56. It departs from a normal distribution in that it is skewed to the right. A distribution with a rather similar shape is found in the oyster, *Crassostrea virginica* (from ZOUROS, SINGH and MILES 1980, Table 2). This distribution is based upon seven polymorphic loci, and like the distribution in the marine fish it is skewed to the right.

The fit of the observed distribution of heterozygosity to the expected distribution was tested in seven population samples; none of the observed distributions was significantly different from expectation.

Both the observed and expected distributions reported here depart significantly from normality. The most common cause of this departure is that the distributions are skewed to the right. This is seen for every locality, and it is also found in the expected distributions. An example of the distribution of individual heterozygosity is presented in Figure 2. The skewness, measured by g_1, is 0.233 ± 0.088 for the expected distribution and is 0.397 ± 0.119 for the observed distribution.

The distribution of individual heterozygosity may be used to test for genetic heterogeneity among localities, among age classes or between the sexes. For example, comparison of the distribution of heterozygosity of fish from Northport and fish from all control localities indicates no substantial differences in the frequency distributions. The mode for the distribution at Northport is 3 and the mode for the distribution in control environments is 4; otherwise, the mean, variance, skewness and kurtosis are similar for the two distributions. The differences in frequencies of several enzymes characterizing heated and natural environments (MITTON

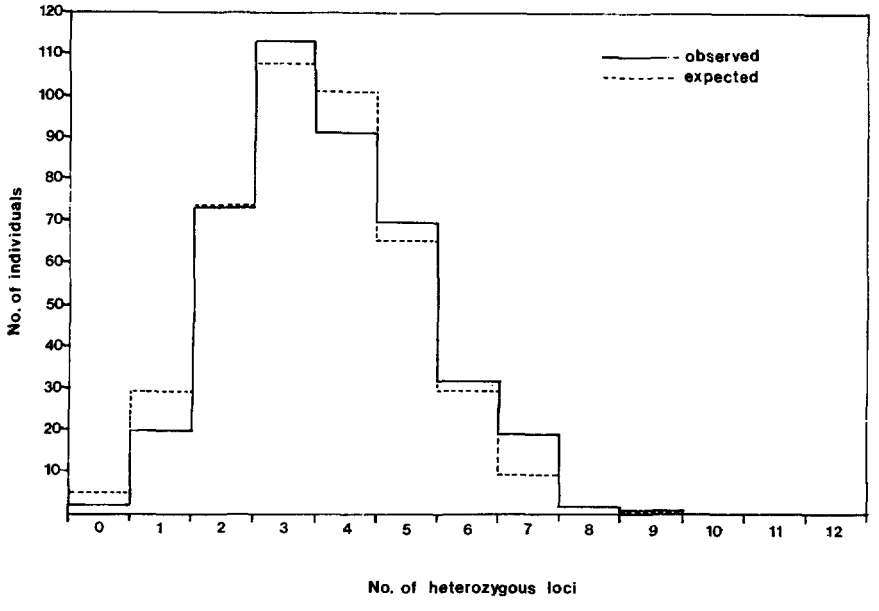

FIGURE 2.—Observed and expected distributions of heterozygosity for Northport. Both distributions deviate from normality due to a positive skew.

TABLE 3

Distributions of heterozygosity for three age classes of F. heteroclitus *from Northport*

Age Class	0,1	2	3	4	5	6 to 12	*N*	χ^2
1	18	18	10	9	3	5	63	
2	14	51	72	69	52	40	298	46.0***
3	4	8	23	12	9	6	62	

Heterozygosity classes at each end of the distribution were pooled to produce cell sizes suitable for statistical analyses. N = sample size, *** = P 0.001 and χ^2 is a row by columns contingency chi-square value.

and KOEHN 1975) have apparently cancelled one another, leaving the frequency distributions of heterozygosity similar.

A test of heterozygosity among age classes was conducted by placing the data from a single locality into a contingency table, with the numbers of loci heterozygous as columns and age classes as rows. Row by columns contingency tests were conducted for individuals captured at Northport (Table 3) and Flax Pond. The age classes are heterogeneous at Northport, but they are homogeneous at Flax Pond ($P > 0.80$).

Comparisons of the distribution of heterozygosity were made between the sexes with the samples collected at Northport, and samples from control environments (Table 4; Figure 3). Neither the means nor the variances for the distribution of heterozygosity differ between the sexes, either in different environments or in the pooled samples. Although there are differences between the sexes for four loci

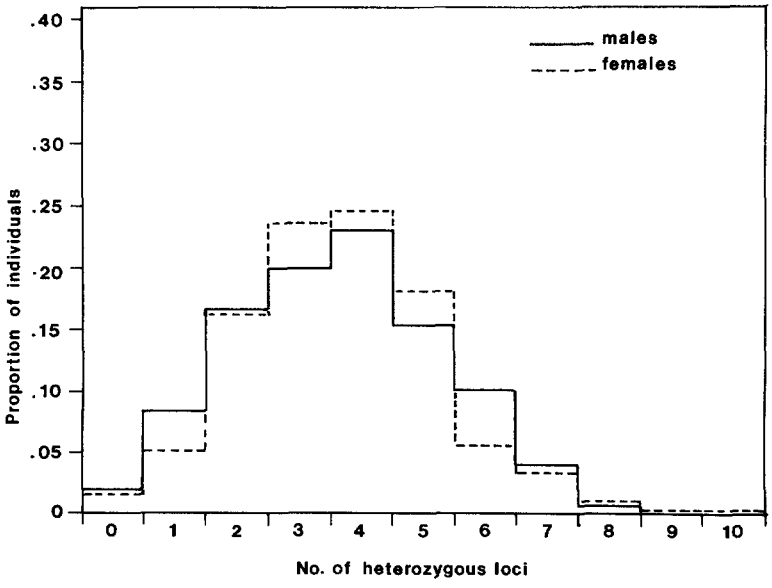

FIGURE 3.—The distribution of individual heterozygosity for males and females from all environments. The distribution of the males is platykurtic with no skew, and the distribution of the females is leptokurtic and positively skewed. See text and Table 4 for further information.

TABLE 4

Descriptive statistics and tests of heterogeneity for distributions of heterozygosity for males and females of Fundulus heteroclitus

Locality and sex	N	$\bar{Y} \pm$ SE	P	S^2	p	$g_1 \pm$ SE	P	$g_2 \pm$ SE	P
Northport ♂♂	165	3.64±0.119		2.33		0.30±0.189		−0.36±0.376	
			NS		NS		NS		*
Northport ♀♀	258	3.75±0.096		2.43		0.46±0.152		0.01±0.302	
Control ♂♂	240	3.57±0.111		2.94		0.08±0.157		−0.47±0.31	
			NS		NS		*		*
Control ♀♀	334	3.72±0.086		2.50		0.38±0.133		0.72±0.266	
All ♂♂	385	3.63±0.085		2.81		0.09±0.124		−0.51±0.248	
			NS		NS		*		*
All ♀♀	592	3.73±0.064		2.46		0.41±0.100		0.41±0.200	

Locality *All* represents the pooled samples from Northport and the four control localities. N = sample size, Y = mean, SE = standard error, P = probability that males and females are drawn from a homogeneous population, g_1 = skewness, g_2 = kurtosis, NS = nonsignificant, * indicates $P < 0.05$.

(MITTON and KOEHN 1975), these differences balance one another to give similar mean values of heterozygosity in the sexes, and a chi-square test reveals no heterogeneity between the sexes in the frequency distribution of heterozygosity ($p. < 0.10$). Yet, there is a difference in the shape of these distributions that is not reflected in these statistics. Skewness and kurtosis differ in the frequency distributions of the sexes. For the males from all localities, the distribution is not skewed, but it is significantly platykurtic (Table 4). The kurtosis of the distribution for the females differs between Northport and and control localities, and the pooling of these localities yields an intermediate but significant degree of kurtosis. The distribution for the females from all localities, however, has a significant skew to the right, and rather than being platykurtotic, is leptokurtic. The pattern of differentiation between the sexes is similar in both the samples from Northport and from the control localities.

DISCUSSION

In the simulations presented here, a statistically significant correlation between the heterozygosity level determined by 100 loci and the estimated level was obtained when five or more loci were employed to rank individuals. But the real interest here is not in statistical significance, but in practical biological relevance. The simulations, in themselves, do not indicate whether something relevant is measured in the ranking of individuals on the basis of 12 polymorphic loci. If the genome of *Fundulus heteroclitus* contains only 100 polymorphic loci, then the rankings obtained and the distributions presented are a reasonable reflection of reality. Yet, it is unknown how many polymorphic loci there are in this genome, and it is not likely that soluble enzymes are a random sample of the genome (but see SOULE, YANG and WEILER 1973; NEI and ROYCHOUDURY 1974). At best, the

distributions presented here may reflect variation within populations for the level of heterozygosity of soluble enzymes.

Given the uncertainty concerninng the relevancy of measuring the level of heterozygosity within a population, one may wonder whether it is worthwhile to continue this line of investigation. Both theoretical considerations and empirical data give some reason for optimism. MILKMAN (1978) and WILLS (1978) have presented models that consider the effect of natural selection on a group of individuals differing in fitness potentials. Both models suggest that natural selection could detect fitness differentials determined by a very large number of loci. Yet, without knowing the number of loci polymorphic in a genome or in a subset of the genome, it is difficult to determine whether the ranking of individuals based on a dozen loci is a profitable investment in time for a population geneticist. Recent evidence suggests that this rationale is worth pursuing. Fitness estimates for several species of Drosophila suggest that individuals homozygous for either chromosome *2* or *3* have fitnesses that are only 10% of that of individuals heterozygous for these chromosomes (SVED and AYALA 1970; MOURAO, AYALA and ANDERSON 1972; TRACEY and AYALA 1974). ROBERTSON and REEVE (1952) demonstrated that Drosophila heterozygous for specific chromosomes exhibited lower phenotypic variation than Drosophila homozygous for those chromosomes. Recent observations indicate that the effect of single gene heterozygosity may influence morphology. A negative correlation between allozyme heterozygosity and fluctuating asymmetry in 15 populations of side-blotched lizard suggests that high heterozygosity enhances developmental stability (SOULÉ 1979). In the killifish, *F. heteroclitus*, and in the monarch butterfly, *Danaus plexippus*, individuals heterozygous for a single enzyme have lower phenotypic variation than do homozygous individuals (MITTON 1978; EANES 1978). In ponderosa pine, *Pinus ponderosa*, lodgepole pine, *Pinus contorta*, and quaking aspen, *Populus tremuloides*, individuals heterozygous for a single enzyme have either different growth variability or different mean growth rates than do individuals homozygous for that enzyme (KNOWLES 1978; KNOWLES and MITTON 1980; MITTON and GRANT 1980).

Multiplicative fitness models, in extreme form, generate either high segregational load or strong linkage disequilibrium (FRANKLIN and LEWONTIN 1970). There is little evidence for extreme segregational load, and investigations in predominantly outbreeding species do not indicate extensive or strong linkage disequilibrium (CLEGG 1978). If one is searching for a selective mechanism to maintain high levels of polymorphism, the models of MILKMAN (1978) and WILLS (1978) have considerable intuitive appeal. But if one were to try to extend the simulations presented here (Table 1) to the number of loci considered in models of threshold or rank order selection, optimism for investigating the models empirically quickly fades. Nevertheless, the few positive results discussed above and the limited success reported here (differences between age classes, sexes; Tables 3 and 4) should not be ignored. It may be that the utility of protein polymorphisms for population biologists is not that they reflect the whole genome, but

that they may reasonably reflect flexibility (BERGER 1976; GILLESPIE and LANGLEY 1974) of one or a few major metabolic pathways.

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LITERATURE CITED

BERGER, E., 1976 Heterosis and the maintenance of enzyme polymorphism. Am. Naturalist **110:** 823–839.

CLEGG, M. T., 1978 Dynamics of correlated genetic systems. II. Simulation studies of chromosonal segments under selection. Theoret. Pop. Biol. **13:** 1–23.

EANES, W. F., 1978 Morphological variance and enzyme heterozygosity in the Monarch butterfly. Nature **276:** 263–264.

FRANKLIN, I. and R. C. LEWONTIN, 1970 Is the gene the unit of selection? Genetics **65:** 707–734.

FUERST, R. A., R. CHAKRABORTY and M. NEI, 1977 Statistical studies on protein polymorphism in natural populations. I. Distribution of single locus heterozygosity. Genetics **86:** 455–483.

GILLESPIE, J. H. and C. H. LANGLEY, 1974 A general model to account for enzyme variation in natural populations. Genetics **76:** 837–848.

KNOWLES, M. H., 1978 Genetic characteristics associated with growth in ponderosa pine. Master's thesis, University of Colorado, Boulder.

KNOWLES, P. and J. B. MITTON, 1980 Genetic heterozygosity and radial growth variability in *Pinus contorta.* Silvae Genetica (in press).

MILKMAN, R., 1978 Selection differentials and selection coefficients. Genetics **88:** 391–403.

MITTON, J. B., 1973 Genetic and morphological adaptation to thermal stress in the coastal marine fish *Fundulus heteroclitus.* Ph.D. thesis, State University of New York at Stony Brook, Stony Brook, New York. (Diss. Abstr. 34, 4846; order no. 78-08943.) ——, 1978 Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. Nature **273:** 661–662.

MITTON, J. B. and M. C. GRANT, 1980 Observations on the ecology and evolution of quaking aspen, *Populus tremuloides,* in the Colorado Front Range Am. J. Botany. **67:** 202–209.

MITTON, J. B., P. KNOWLES, K. B. STURGEON, Y. B. LINHART and M. DAVIS, 1980 Associations between heterozygosity and growth rate variables in western forest trees. In: *Isozymes of North American Forest Trees and Forest Insects.* Edited by M. CONKLE. (In press.)

MITTON, J. B. and R. K. KOEHN, 1975 Genetic organization and response of allozymes to ecological variables in *Fundulus heteroclitus.* Genetics **79:** 97–111 ——, 1976 Morphological adaptation to thermal stress in a marine fish, *Fundulus heteroclitus.* Biol. Bull. **151:** 548–559.

MOURAO, C. A., F. J. AYALA and W. W. ANDERSON, 1972 Darwinian fitness and adaptedness in experimental populations of *Drosophila willistoni.* Genetika **43:** 552–574.

NEI, M. and A. K. ROYCHOUDHURY, 1974 Sampling variances of heterozygosity and genetic distance. Genetics **76:** 379–390.

NIE, N. H., D. H. HULL, J. G. JENKINS, K. STEINBRENNER and D. H. BENT, 1975 *Statistical Package for the Social Sciences,* 2nd Edition. McGraw-Hill, New York.

ROBERTSON, F. W. and E. C. R. REEVE, 1952 Heterozygosity, environmental variation and heterosis. Nature **170:** 286–287.

SINGH, S. M. and E. ZOUROS, 1978 Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). Evolution **32**: 342–353.

SOKAL, R. R. and F. J. ROHLF, 1969 *Biometry*. W. H. Freeman and Co., San Francisco.

SOULÉ, M., 1979 Heterozygosity and developmental stability: Another look. Evolution **33**: 396–401.

SOULÉ, M., S. Y. YANG and M. G. W. WEILER, 1973 Island lizards: The genetic-phenetic variation correlation. Nature **242**: 191–193.

SVED, J. A. and F. J. AYALA, 1970 A population cage test for heterosis in *Drosophila pseudoobscura*. Genetics **66**: 97–113.

TRACEY, M. L. and F. J. AYALA, 1974 Genetic load in natural populations: is it compatible with the hypothesis that many polymorphisms are maintained by natural selection? Genetics **77**: 569–589.

WILLS, C., 1978 Rank-order selection is capable of maintaining all genetic polymorphisms. Genetics **89**: 403–417.

ZOUROS, E., M. SINGH and H. W. MILES, 1980 Growth rate in oysters: an overdominant phenotype and its possible explanations. Evolution (in press).

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