ASSOCIATIONS AMONG PROTEIN HETEROZYGOITY, GROWTH RATE, AND DEVELOPMENTAL HOMEOSTASIS

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INTRODUCTION

In 1954 I. Michael Lerner published *Genetic Homeostasis*, a compendium of observations on the relationships among heterozygosity, growth rate and other measures of performance, and developmental homeostasis. These observations were primarily made on cultivated plants and domesticated animals, and the experiments usually contrasted inbred, predominantly homozygous lines with the highly heterozygous progeny of crosses between inbred lines. Highly heterozygous individuals and lines generally exhibited the traits that breeders strive to fix in their strains. In comparison with inbred, homozygous lines, they usually had superior growth rates; often attained greater size; and generally had more buffered developmental processes, resulting in lower morphological variation (e.g. 8). Contrary results, however, are known (e.g. 10, 89). These results—and similâr studies (20, 21, 88)—have influenced the thinking of plant and animal breeders, but the impact of these studies on population biology and population genetics has, as yet, been slight.

The mechanism underlying the phenomenon of heterosis has received a great deal of attention from empiricists and theoreticians (see 111 for a review). Summarizing briefly, the antithesis of heterosis—inbreeding depression—has been viewed as the consequence of either increased homozygosity at a large
number of overdominant loci or the increased expression of a small number of deleterious, recessive alleles.

The causal mechanisms that underly the general pattern of increased vigor, increased developmental homeostasis, and superior yields in highly heterozygous individuals continues to be the subject of a complex controversy. The term heterosis was coined by G. H. Shull in 1918 specifically for the purpose of reducing confusion about the distinction between the observed phenomenon of hybrid vigor and its underlying mechanisms (90). He offered this terminological change in order to move away from the commonly used term heterozygosis, which explicitly attributed the effect to the level of heterozygosity. He did not intend to downgrade the significance of heterozygosity as an explanatory mechanism, but he did want the phenomenon to be identified and discussed independently of its underlying cause.

A number of early workers specifically studied heterosis by assessing the effect of heterozygosity per se (see reviews in 10, 16, 43, 100, 109; experimental studies in 2, 39). These scholars approached the phenomenon in conceptually distinct, complementary ways by (a) examining the effects on individual genotypes (developmental homeostasis, for example) and (b) studying the effects on populations constructed of genotypic mixtures (stability and genetic homeostasis). The simplest interpretation of the results is that individual organisms with comparatively higher levels of heterozygosity also have comparatively higher levels of individual homeostasis (e.g. stability and consistency), although there are a number of examples in which this general pattern does not hold (1, 10, 91, 78). At an individual level, "yield," in its many facets, is strongly correlated with the level of heterozygosity (8, 77, 89). Indeed, this phenomenon is the foundation upon which the strategy of developing inbred lines and the subsequent hybrid synthesis so common in commercially developed crop plants is primarily based. At the population level, mixtures of genotypes commonly have yields and productivities at approximately the same level as the weighted averages of their individual components, but they are generally superior in stability and consistency of performance (2, 78, 91).

There are three primary hypotheses about the causal genetic properties that account for the observed increases in performance and individual homeostasis: (a) the effect is due to heterozygosity through the mechanism of dominance, (b) the effect is due to heterozygosity through the mechanism of overdominance, and (c) the effect is due to fortuitous combinations of particular genes. Taking applied breeding studies as a whole, we judge that heterozygosity per se is the single most important explanatory factor (91), even though numerous studies have clearly shown it is not a complete explanation; some unexplained variability in performance among lines of equal heterozygosities always remains (e.g. 1, 2). The extent to which dominance or overdominance is the principal mechanism of action of heterozygosity remains a matter of disagreement.
There is additional evidence that contributes to our understanding of the significance of heterozygosity. Polyploidy, a major feature of plant evolution, is thought to be an important factor in many groups largely because of its effects on individual levels of heterozygosity (e.g. 38, 46, 61). The presence of additional chromosome sets permits the establishment, for example, of fixed heterozygosity. Some theoretical work (97, 98) indicates that heterosis due to overdominance could be intimately tied to the evolution of gene duplications. Most students of polyploidy attribute a major role to the increased level of individual genetic diversity produced by polyploidy, although there are studies showing that some of the effects are due simply to the presence of extra chromosomes, not to an increased number of different alleles (e.g. 79). Furthermore, a major group of plants—the conifers—that show little or no polyploidy have the highest known levels of protein heterozygosity (32). There are obviously several evolutionary routes to the creation and maintenance of high levels of heterozygosity.

The positive association between heterozygosity and fitness has also been clearly observed in species that alternate sexual and parthenogenetic reproduction. Minor fitness advantages enjoyed by heterozygous genotypes may go undetected in obligately sexual species, but these advantages can be amplified by an extended period of parthenogenetic reproduction. Heterosis has been observed in crosses between inbred strains of *Daphnia magna* (35), and excesses of heterozygous genotypes are common in natural populations of this species.

Our interpretation of the literature leads us to believe that roughly 70–80% of the effects on growth and developmental stability can be attributed to heterozygosity per se, about 15–20% to the effects of specific gene combinations, and the remainder to as yet unidentified causes. These proportions, of course, vary somewhat from organism to organism.

**PATTERNS IN NATURAL POPULATIONS**

The observation that heterozygosity strongly influences both vigor and stability has been generally accepted in the applied literature for many years. How widespread the phenomenon is in natural populations was still an open question in the early 1960s, largely because the tools needed to assess the degree of heterozygosity were extremely time consuming, cumbersome, and limited to a few, especially tractable organisms.

Within the last two decades, population biologists have made a series of empirical observations that link the heterozygosity of protein polymorphisms with viability, growth rate, developmental stability, and physiological variables such as oxygen consumption. These data are consistent with Lerner's (58) general observations, and they have spurred a critical examination of how the
theory of population genetics accounts for the determination of fitness. Furthermore, these relationships offer a new opportunity to explore the mechanisms underlying heterosis and inbreeding depression. These observations are of particular interest to ecologists and population biologists because they place heterosis and inbreeding depression in the arena of the natural population.

EMPIRICAL OBSERVATIONS

The relationships between heterozygosity and developmental stability were first identified in crosses between inbred strains, but they are also evident within populations when heterozygosity is measured with single chromosomes or single protein polymorphisms. For example, *Drosophila* heterozygous for their second chromosomes develop lower levels of morphological variability in wings than do homozygous individuals (88).

*Protein Heterozygosity and Developmental Stability*

Protein heterozygosity was first related to morphological variability in the marine fish, *Fundulus heteroclitus* (70). A single protein polymorphism was used to divide a population sample into two groups, one heterozygous and the other homozygous for that locus. Meristic variation at 7 scale and fin ray characters was used to quantify the morphological variability of the two groups, which were then compared. This process was repeated for each of 5 loci in 2 populations. In most of these comparisons, the heterozygotes exhibited lower phenotypic variation than the homozygotes.

Protein heterozygosity and developmental stability have been shown to be related in the monarch butterfly, *Danaus plexippus* (22). Six polymorphisms and two morphological characters—length of the forewing and size of the forewing spot—were used in this study. Each of the polymorphisms was used one at a time to separate individuals into classes of homozygotes and heterozygotes, and the classes were then compared. The mean values of the morphological characters did not vary among genotypes, but the variances of the characters were associated with genotypic classes. Overall, heterozygotes had lower variances in 19 of 24 tests, and 5 of these tests were statistically significant. Thus, heterozygous individuals clustered more closely about the means of the character distributions, but the protein genotypes did not influence the direction of deviation from the mean.

Fluctuating asymmetry, the nondirectional imbalance between bilaterally paired characters, is a measure of developmental stability that is correlated with protein heterozygosity. More heterozygous individuals and populations exhibit greater symmetry than more homozygous ones.

The relationship between protein heterozygosity and fluctuating asymmetry has been demonstrated most rigorously in the rainbow trout, *Salmo gairdneri*
The researchers measured bilateral symmetry with 5 characters taken from gill rakers, mandibular pores, and fin rays and estimated heterozygosity with 13 protein polymorphisms. The data consisted of genotypes at each of the 13 polymorphisms and the 5 bilateral characters for each individual. The number of heterozygous loci was negatively correlated with both the number of asymmetric characters and the magnitude of the asymmetry. When each locus was tested individually, 2 of the 13 loci were significantly related to the asymmetry. Even when these 2 loci were removed from the analysis, however, the remaining polymorphisms were still correlated with the number of asymmetric characters. Thus, developmental stability was found to be associated with both the heterozygosity of specific loci and protein heterozygosity in general.

Handford, the author who first tested for relationships between protein heterozygosity and developmental stability in homeotherms, questioned the generality of the preceding observations. He compared morphological variability and protein heterozygosity in the rufous-collared sparrow, *Zonotrichia capensis*, but these sets of characters were independent in this data set (33). Handford noted that all previous positive results had been discovered in poikilotherms, and he postulated that homeotherms would not exhibit relationships between heterozygosity and developmental stability. This study was later criticized for being based on a small sample (23), but a second study on birds (24) directly addressed Handford’s hypothesis. The authors found that enzyme heterozygosity at 4 loci was related to variability in limb size in the house sparrow, *Passer domesticus*, with more heterozygous individuals exhibiting less variability.

Natural selection does not influence all morphological characters with the same intensity, for selection on some characters is extreme but undetectable at others. Strong stabilizing selection modifies the number of caudal fin rays in the guppy *Poecilia reticulata* (5), for example, and this selection is also related to protein heterozygosity (6). More heterozygous individuals are most abundant at the central phenotype (27 caudal fin rays), and more homozygous individuals are more common in fin ray classes above and below 27. Selection on caudal fin rays continues throughout the life cycle of this fish, and differential mortality strengthens this relationship between heterozygosity and the caudal fin ray number. McAndrew, Ward & Beardmore (63) compared heterozygosity and the variability of caudal, anal, and dorsal fin rays in a large study of the plaice, *Pleuronectes platessa*. Despite careful and thorough analyses of the data, no hint of any relationship between heterozygosity and morphological variability was found. The authors compared this negative result with the strong relationship found in the guppy (6). A study is needed to determine whether the relationships between heterozygosity and developmental stability are stronger for characters under stabilizing selection than for those unaffected by selection.
Although the most direct tests for relationships between heterozygosity and developmental stability are conducted within populations, the same relationships can be revealed in comparisons among populations. This fact was first demonstrated in an analysis of geographic variation of 15 populations of the side-blotched lizard on islands in the Gulf of California (95). Four independent scale characters were used to measure fluctuating asymmetry, and genotypes at 18 protein loci were used to measure heterozygosity. Populations with higher levels of heterozygosity had lower levels of asymmetry. A similar result was found in two species of fresh water bivalves (44). Geographically peripheral populations had lower levels of heterozygosity and higher levels of asymmetry.

An unusual phenomenon was used opportunistically to study the relationship between heterozygosity and morphological variability in a sexual diploid species, Poeciliopsis monacha, and a parthenogenetic triploid species of live-bearing fishes, P. 2 monacha-lucida (103). Both species live in the Arroyo de los Platano of Mexico. The triploid species produces unreduced triploid eggs without recombination and uses the sperm of P. monacha to initiate development, but it does not incorporate any of the genetic material from the sperm into the egg. Thus, this species is comprised of a series of clones. Founder events had produced a cline in heterozygosity that was detected with 25 protein loci, with low levels of heterozygosity in small upstream populations and much higher levels in the larger downstream populations. Heterozygosity at these 25 loci ranged from 0.1% to 8.1% in the sexual species, and it was 52% in the triploid clone. Variation in the physical environment was associated with the cline in heterozygosity among the sexual populations. Upstream populations lived in small pools that occasionally dried up and were later recolonized, and downstream populations lived in permanent environments that always had running water. The internal standard in this experiment was the triploid clone, which was a genetic constant replicated in heterogeneous environments. Bilateral symmetry, measured with 8 paired characters (scale counts, fin rays, and premaxillary and dentary tooth counts) did not vary among the populations of the triploid clone, suggesting that the differences in the physical environment did not substantially influence the development of these characters. In contrast to this pattern of homogeneity, the populations of the sexual species exhibited different levels of symmetry, with the more homozygous populations having more asymmetry.

**Protein Heterozygosity and Growth Rate**

The first observations linking protein heterozygosity directly to growth rate were reported in two studies of the American oyster, Crassostrea virginica (93, 112). The design and results of these experiments are quite similar. Oyster spat or larvae were collected and transferred to trays anchored in a bay so that all of
the animals were approximately the same age and in a similar environment with
the same opportunity to feed. One year later, the animals were removed from
the tray, and the largest and the smallest—or the fastest and slowest growing
individuals—were weighed. Genotypes were determined for 5 loci in the first
experiment and 7 loci in the second. In both experiments, a single polymorph-
ism, \textit{GOT-1}, exhibited a pattern of variation distinct from all the rest. Geno-
types at this locus had different weights, but the distribution of genotypes fit
Hardy-Weinberg expectations. At the other loci, the smallest weight classes
had few heterozygotes. Heterozygotes at each of the loci tended to be heavier
than homozygotes, and an individual’s weight was positively correlated with its
number of heterozygous loci. The growth advantage associated with enzyme
heterozygosity occurred in older age classes as well (92).

Similar results were reported for the Pacific oyster, \textit{Crassostrea gigas} (26),
but in this study, animals were collected as adults from natural populations.
Most loci had deficiencies of heterozygotes, and there was a negative correla-
tion—among populations—between the oysters’ mean weight and the inbreed-
ing coefficient. Within populations, heterozygotes clearly tended to weigh
more than homozygotes at each locus, and weight was positively correlated
with individual heterozygosity.

The growth rate of another marine pelecypod, the blue mussel, \textit{Mytilus edulis}, is also associated with protein heterozygosity (52). In this study a
seasonal raft was moored in spring and removed in fall to obtain mussels of
similar age that shared the same environment. Each individual was measured,
and then its genotype was determined for 5 polymorphic enzymes. The shell
lengths ranged from less than 2 mm to more than 20 mm. The mean growth rate
increased with individual heterozygosity, while the variance in growth rate
decreased. A laboratory study of \textit{Mytilus edulis} revealed no association be-
tween protein heterozygosity and growth within progeny of a pair cross (7).

An association between protein heterozygosity and growth was reported for
the tiger salamander, \textit{Ambystoma tigrinum} (80). In 5 of 7 natural populations
sampled for young larvae, there was a positive correlation between heterozy-
gosity at 8 protein loci and snout-vent length, a classical measure of size. An
experiment was conducted in the laboratory to determine whether heterozygous
individuals’ larger size could be attributed to greater growth rates. A single
male and a single female were chosen by genotype, and 462 offspring were
produced by a pair cross. The offspring were arbitrarily assigned to 4 replicate
populations kept in separate aquaria and allowed to grow until a substantial
variance in size among individuals developed. In 2 of the 4 replicates, there
were significant, positive correlations between size and heterozygosity. Thus,
the correlation between heterozygosity and size in natural populations may be
attributable to differential growth rates. The correlation disappears later in the
larval period, however, perhaps owing to differential metamorphosis of
genotypes.
A nexus of relationships involving individual heterozygosity, weight, fecundity, and fetal growth have been reported for the white-tailed deer, *Odocoileus virginianus* (15). Adult females with high levels of enzyme heterozygosity weigh more than more homozygous individuals; they also enjoy an advantage in fecundity (42), with highly heterozygous individuals having a higher rate of twinning. The weight of females is positively correlated with the weight of their fetuses. In addition, fetal growth rates are positively correlated with individual fetal heterozygosity.

There is some evidence of an association between protein heterozygosity and growth rate in domesticated animals. For example, sheep heterozygous for isocitrate dehydrogenase have been reported to grow 10% faster than homozygotes (4). Growth, food consumption, and protein heterozygosity were examined in pigs, and the more heterozygous individuals gained weight more quickly while consuming less food (62). The enzyme glucose-phosphate isomerase is also associated with meat quality and weight gain in pigs (87). This correlation is difficult to interpret, however, because this enzyme is tightly linked to blood group loci that are also consistently associated with meat quality and production.

A relationship has also been reported between individual heterozygosity and fetal growth in man (9). This study was replicated with 93 babies sampled from a hospital in Rome and 98 from a hospital in New Haven. Heterozygosity was recorded for 5 loci in Rome and 4 loci in New Haven, and the babies were placed in three categories: preterm; full term, normal weight; full term, low weight. Preterm babies did not differ from full term normal weight, babies, but the light babies had a significantly lower level of heterozygosity than the other groups.

A relationship between protein heterozygosity and growth rate in plants was first reported in quaking aspen, *Populus tremuloides* (72). The elevation, age of largest ramet, sex, and genotype at three enzyme loci was recorded for 104 clones of aspen in the Front Range of the Colorado Rocky Mountains. The growth rate of each of these clones of aspen was estimated as the average width of the annual rings, which were measured from cores taken from 5 ramets of each clone; the effect of the age of the ramet was removed statistically. There was a significant, positive relationship between the number of heterozygous proteins and the estimated growth rate.

Protein heterozygosity is related to the variability of growth rate in ponderosa pine (*Pinus ponderosa*) and lodgepole pine (*Pinus contorta*) as well, but in neither of these trees is heterozygosity related to the mean growth rates of mature trees (31, 47, 48, 71, 73). In each of these studies, genotypes and growth rates were compared within a single population. Cores were extracted from more than 100 trees, and protein genotypes were obtained from needle tissue for 6 protein polymorphisms in ponderosa pine and 4 in lodgepole pine.
Analysis of covariance was used to control for the effects age and slope aspect. In ponderosa pine, highly heterozygous individuals exhibit higher growth variability than predominantly homozygous individuals, and in lodgepole pine, the opposite occurs. These contrary results do not necessarily indicate that no generalizations can be made from these sorts of studies, for the associations between heterozygosity and growth rate may reflect yet another variable—cone production (73). Further studies on ponderosa pine do reveal a negative relationship between the relative growth rate and relative levels of female cone production. In addition, more heterozygous individuals, as a group, have lower variance in the relative levels of female cone production than more homozygous individuals (Y. B. Linhart & J. B. Mitton, unpublished manuscript).

Protein heterozygosity is also associated with characteristics of seedling growth in ponderosa pine (M. A. Farris & J. B. Mitton, unpublished manuscript). In this greenhouse study of seedling growth, approximately 20 seeds were collected from each of 8 different trees near Boulder, Colorado, and the seeds were weighed and allowed to germinate and grow in perlite-filled, clear plastic tubes. After germination the lengths of the shoot and root were measured every 2 days. At the termination of the experiment, the genotype of each seedling for each of the 11 polymorphisms was determined from needle tissue. Seedling heterozygosity was not related to shoot growth, but a positive association appeared between seedling heterozygosity and root length after the megagametophyte, the nutrient storage tissue, separated from the seedling.

Protein heterozygosity is associated with diameter growth in the pitch pine, Pinus rigida (57). Trees from 8 natural populations were cored in order to estimate the diameter growth, and the genotypes of these trees were inferred for 21 proteins from haploid megagametophytic tissue. The relationship between heterozygosity and diameter growth was highly dependent upon the age of the stand, with the correlation between individual heterozygosity and growth rate becoming increasingly positive as the mean age of the stand increased. The authors speculated that it was in the most mature stands that light, space, nutrients, and water became limiting, and under these conditions of stiff competition, the variation in potential for growth among genotypes was expressed.

Relationships among protein heterozygosity and growth rate have now been reported in marine invertebrates, the tiger salamander, white-tailed deer, pigs, sheep, man, quaking aspen, and several species of conifers. The reports summarized above all compare growth characteristics among individuals within the same population, and in virtually every case, the most highly heterozygous individuals enjoy some advantage. The phenomenon appears to be general.

Now that the generality of the phenomenon has been established, we can focus upon the mechanism underlying the phenomenon. Do the enzymes
employed in these studies influence metabolic rates, or are they simply convenient chromosome markers, providing information on heterozygosity at many other loci or perhaps provide only a ranking of individuals by their degree of inbreeding? To address this problem, we must get closer to the level of enzyme action.

**Protein Heterozygosity and Oxygen Consumption**

If enzyme polymorphisms directly influence such gross measures of fitness as developmental stability and growth rate, then the associations between these measures and heterozygosity must reflect the underlying influences of the enzyme polymorphisms upon metabolism. This line of reasoning led Koehn & Shumway (54) to follow up the studies of the relationships between heterozygosity and growth rate in the American oyster (93, 112) with an examination of the relationship between heterozygosity and oxygen consumption. The animals used in this study were collected in a different year and from a different population than those in the earlier studies, and the five protein polymorphisms in this study only partially overlap those in the earlier ones. Oxygen consumption was measured in the laboratory under both normal temperature and salinity conditions and under a combination of high temperature and salinity conditions that constituted a stress to the oysters. After controlling for the effects of size, oxygen consumption was measured for each individual under normal and stress conditions, and the genotypes at each of the 5 enzyme polymorphisms were determined. Oxygen consumption under both sets of conditions was highly correlated with the number of heterozygous loci. Both regression lines have a negative slope, but the regression line for the stress measurement has a much steeper slope. Under stress, the energy demand of the 5 locus homozygote is 2.5 times as great as that of the 5 locus heterozygote. When the data are analyzed one locus at a time, the results are consistent across loci. In each case, the oxygen demand of the heterozygotes is significantly smaller than that of homozygotes. These data are consistent with the information on growth rate. If all animals consume the same amount of energy, highly heterozygous individuals’ lower oxygen consumption should leave more energy to invest in growth.

The positive relationship between enzyme heterozygosity and growth rate in the tiger salamander prompted laboratory studies of the relationship between heterozygosity and oxygen consumption (J. B. Mitton, C. Carey, and T. D. Kocher, unpublished manuscript). Resting oxygen consumption and active oxygen consumption were measured, and the genotype of each animal was obtained for each of 11 polymorphic loci. The number of heterozygous loci was negatively correlated with resting oxygen consumption, an association analogous to the relationships reported for oysters (54). The number of heterozygous loci was also related to oxygen consumption under forced activity, but this
relationship was positive, i.e. highly heterozygous individuals consumed more oxygen. Thus, highly heterozygous individuals consume less oxygen at rest and more oxygen in vigorous exercise than more homozygous ones.

INTERPRETATIONS AND IMPLICATIONS

Crop Plants

The causes of heterosis have been investigated most thoroughly in the crop plant literature (25). In the opening chapter of the most recent comprehensive review of heterosis, Jinks took perhaps the strongest position and said: “most of the critical evidence from biometrical genetic analyses points to dispersion as the major cause of heterosis [in crop plants]” (40, p. 44). The term dispersion may not be familiar to biologists working with natural populations. Dispersion and association are quantitative genetic terms describing the genome-wide tendency for all alleles that have, say, a positive effect on growth to be associated in one pure bred line (complete association) or to be distributed equally between two pure bred lines (complete dispersion). Jinks asserted that most pure bred lines fall somewhere between these two extremes and that heterosis in crosses between strains is due to the assembly of a “correct gene content” rather than to heterozygosity per se. Therefore, a breeder would do equally well in assembling either a highly homozygous line with the “correct gene content” or a highly heterozygous line containing the same genes. Jinks (40) presents an impressive case, both theoretically and empirically, to support his view that dispersion is the primary cause of heterosis.

How do these results apply to natural populations? We believe that their relevance is limited. First and most importantly, natural populations differ dramatically from pure breeding stocks (32). Second, it seems unlikely that levels of dispersion would be correlated with levels of heterozygosity in natural populations—a necessary condition if dispersion is to explain variation in growth and development. Each generation of sexual reproduction reshuffles the gene pool, randomizing the distribution of alleles at different loci.

Natural Populations

Why is variation in the number of heterozygous proteins related to developmental stability, growth rate, and oxygen consumption in natural populations? Three possibilities are commonly presented in the literature: (a) the enzymes mark blocks of chromosomes and are fortuitously linked to genes directly affecting growth and development; (b) protein polymorphisms constitute a sample of genes whose heterozygosity reflects a continuum between highly inbred (low heterozygosity) and randomly outbred (high heterozygosity) individuals; and (c) the genotypes of enzyme polymorphisms typically exhibit different kinetic characteristics; these differences affect the flow of energy
through metabolic pathways and thereby influence growth, development, and oxygen consumption. Although this issue will not be resolved here, theoretical considerations and other empirical data make some of these possibilities more likely, others less so.

Most of the empirical observations indicate the existence of associations between other variables and the level of individual heterozygosity or the number of heterozygous enzyme polymorphisms. What is heterozygosity really measuring? When gel electrophoresis was introduced to population geneticists (60), proteins were presented as a random sample of the genome. This supposition is clearly incorrect. Different groups of proteins (structural, storage, metabolic, nonmetabolic, and regulatory) have different levels of genetic variation (41, 64, 83), and proteins with different quaternary structures and subunit sizes vary regularly in their levels of genetic variation (34, 51, 104, 105). The genetic variability of proteins is no more representative of the genetic variability of the entire genome than antigenic loci are representative of genes influencing morphological variation. Genetic variation at all loci is subject to mutation and genetic drift—there may be associations among genetic variabilities in these sets of loci, but there do not have to be. Furthermore, individual heterozygosity at a few (or a few dozen) loci may be correlated with individual heterozygosity at a larger number of loci (perhaps 20 to 100), but a small number of loci cannot accurately rank individuals within a population for the individual heterozygosity of the entire genome (13, 75). Heterozygosity at a dozen enzyme polymorphisms is much more likely to estimate enzyme heterozygosity within one or two metabolic pathways than at several thousand variable loci. This line of reasoning suggests that the metabolic pathways sampled in electrophoretic analyses are likely to influence variability in growth rates.

Genes are linked on chromosomes, and natural selection acting upon one gene will influence the frequencies of tightly linked polymorphic genes (59, 101). But linkage is not sufficient justification for attributing the genotypic associations between a marker gene and physiological characteristics to other unseen, undetected loci. Associations will only be evident if the genotypes at the linked genes are not independently distributed, that is, if there is linkage disequilibrium between loci. Linkage is a common phenomenon; linkage disequilibrium is not. Surveys of natural populations generally reveal little or no linkage disequilibrium (76), and studies of linked gene systems reveal that the linkage disequilibrium generated by stochastic processes decays more quickly than expected due to the superior fitness of highly heterozygous individuals (3, 14). The distribution of genotypes at one locus will occasionally reflect selection at other loci, but this is not likely to be the primary explanation of so general a phenomenon (3).
Ledig et al. (57) have recently presented a useful portrayal of a major school of thought on the significance of the empirically observed relationship between heterozygosity and various morphological parameters. As a consequence of their work with pitch pine—in which they observed positive associations between heterozygosity and growth rate in mature stands—they proposed that the primary explanatory factor was the degree of outcrossing. It is well recognized that inbreeding by plants with predominantly outcrossing mating systems results in severe inbreeding depression. Ledig et al. made two assertions: (a) the enzyme loci observed in electrophoretic studies are basically unimportant in determining growth rate or stability and serve only as useful markers for heterozygosity at loci that really do matter, and (b) it is the presence of homozygosity at particular loci that results in the comparative reduction in growth or stability, rather than the general heterozygosity that is the key attribute of the individuals studied. Their argument that protein polymorphisms serve only as markers is significantly weakened by the results described above. The view that the polymorphic enzymes do matter is also supported by the kinetic work described below. Is it simply a matter of semantics to argue over whether it is the degree of heterozygosity or of homozygosity that is most important? The empirical data indicate that the major factor is the level of heterozygosity (or 1 – level of homozygosity). Inbreeding and selfing decrease the level of individual heterozygosity, but individuals with all levels of individual heterozygosity can be produced by complete outcrossing. In addition, the levels of heterozygosity can be varied by mechanisms other than variation in the mating system. For these reasons, we argue that the level of heterozygosity is the more general concept and that the level of inbreeding is an important special case.

Kinetic studies of enzyme polymorphisms generally reveal that proteins produced by different genotypes at a locus perform differently; the kinetic differences are often consistent with patterns of geographic variation or gross physiological measures. The first example of this sort of study was a kinetic analysis of a serum esterase polymorphism in the sucker *Catostomus clarkii*, and a survey of the geographic variation in the allelic frequencies of this polymorphism (49). The esterase genotypes differed in the temperature at which they expressed their maximum velocity, and the temperature of maximum velocity corresponded to the average climatic temperature in the field where that genotype was most common. This study provided the model for a genre of studies that has become increasingly sophisticated, both in the examination of enzyme kinetics and in the field and laboratory tests of predictions taken from kinetic data (11, 12, 17–19, 37, 50, 53, 66, 94, 106–108; reviewed in 55, 65).

Perhaps the most complete story of the influence of a polymorphic enzyme
locus upon geographic distribution, physiology, and demography is that of lactate dehydrogenase (LDH) in *Fundulus heteroclitus*. The LDH-B locus, the LDH most common in the heart, has two common alleles in *F. heteroclitus*. The genotypes have distinct kinetic properties, and the different temperature optima for kinetic variables can be used to describe the latitudinal cline in gene frequencies along the East Coast between Maine and North Carolina (86). LDH is associated with viability differences within the life cycle (74), and heterozygotes at this locus exhibit lower morphological variance than homozygotes, suggesting that LDH exerts an influence upon developmental stability (70). The three LDH genotypes differ in the levels of ATP associated with red blood cells (81, 85), which has led to several testable hypotheses concerning the physiology of whole animals. Varying the level of ATP associated with red blood cells should alter the amount of oxygen delivered to tissues. This hypothesis was tested for both the timing of egg hatching (which is dependent upon oxygen tension) and adult swimming endurance. Homozygotes differed in their swimming endurance, and as predicted, the differences were temperature-dependent (19). Furthermore, the time until hatching of eggs was dependent upon the LDH genotype, and there was a regular progression of hatching times from one homozygote, to the heterozygote, to the other homozygote (18). This study is the most complete picture available of the ways in which a single enzyme locus can influence development, time of hatching, swimming endurance, and viability differentials, but there is no reason to suspect that this enzyme in this species is unique. The differential effect of LAP genotypes on the osmoregulation of the blue mussel, *Mytilus edulis* (50) and the influence of PGI upon the differential survival and flight activity of *Colias eurytheme* (106–108) are also well documented.

**The Paradox of Kinetic Intermediacy and Overdominance**

A general finding of enzyme kinetic studies is the intermediacy of heterozygotes (28). The common result in the empirical studies summarized here is overdominance—heterozygotes enjoy higher growth rates and greater regularity in their development. Is it possible to translate biochemical intermediacy into fitness superiority?

Evolutionary biologists recognize the major significance of environmental variation, in both space and time (36). Let us imagine a life cycle as a series of chained events (for example, germination, seedling establishment, growth, reproduction) in which the fitnesses of genotypes vary but the heterozygote is always intermediate between homozygotes. Let $f_{AA1}$ and $f_{aa1}$ represent the fitnesses of genotypes $AA$ and $aa$ during event 1. In a series of 2 events, if $f_{AA1} = f_{aa2}$, $f_{aa1} = f_{AA2}$, and $f_{Aa}$ is always intermediate, then $f_{Aa} > f_{AA} = f_{aa}$, for all $f$. In the example in Table 1, the fitnesses of homozygotes reverse in
successive chained events within a life cycle, leaving heterozygotes with the highest fitness. For a greater number of chained events, the conditions leading to heterozygote superiority are somewhat less restrictive. This theme is presented in detail by Gillespie (27, 28), who concludes that this fitness configuration and variation can result in overdominance and developmental homeostasis.

The theory of fitness determination from a large pool of polymorphic loci has been undergoing constant revision ever since population geneticists were introduced to electrophoretic data. Initially, attention focused upon the genetic load produced if selection maintained all or most of the polymorphisms. The error in this way of thinking was quickly discovered (45, 68, 99), and the development of rank-order or threshold selection models reduced concern over genetic load and made these discussions more biologically relevant (69, 110). The most recent developments in models of fitness determination are exciting, for they predict associations between components of fitness and individual heterozygosity. For loci that are maintained polymorphic by balancing selection (a most important modifying clause)—in the great majority of cases examined with computer simulation—the fitness of an individual increases with the number of its heterozygous loci (29, 30, 102). This is the general pattern in the empirical data being reviewed here: Among individuals within a population, the more heterozygous individuals exhibit superior growth rates and enhanced developmental stability. These studies do not prove that the theory is correct or that enzymes are maintained by balancing selection, but the congruence between theoretical expectation and empirical observation certainly leaves room for optimism.

Heterosis and overdominance seem to be enhanced in fluctuating environments and most clearly seen when stocks or genotypes are examined across a range of environments. This generalization appears to be consistent with empirical observations on domesticated species (89, 91), theoretical discussions (27, 36), and experimental results (67, 82, 84). Furthermore, it is consistent with the advantages accruing to kinetically intermediate heterozygotes in fluctuating environments. Kinetic and physiological analyses of enzyme polymorphisms have revealed that these polymorphisms can have major physiological effects (55).

**TABLE 1** A chain of intermediate fitnesses can result in superior fitness

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fitness in event I</th>
<th>Fitness in event II</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>1.0</td>
<td>0.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Aa</td>
<td>0.7</td>
<td>0.7</td>
<td>0.49</td>
</tr>
<tr>
<td>aa</td>
<td>0.4</td>
<td>1.0</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Negative Results

The relationships between protein heterozygosity and both growth and development appear to be general, but they are certainly not universal. They have been detected with statistical methods, and they are discovered more easily under some circumstances than others. For example, heterozygosity and mean growth rates are associated in young oysters and pine seedlings, but not in adult ponderosa or lodgepole pines. Virtually all of the surplus energy of larval oysters and pine seedlings is put into growth, while the surplus energy of adult trees in partitioned between reproduction and growth. Such differential apportionment of energy may weaken the relationship between growth and heterozygosity.

The association between heterozygosity and developmental stability is not expected in all characters (96), for some are physically constrained in their development (consider bones in the skull), while others are not, and characters differ in their degree of canalization. Soulé (96) predicts that the relationship between heterozygosity and developmental stability will be strongest in unconstrained characters with low canalization and small coefficients of variation. The negative results reported for plaice (63) may fit this prediction.

We predict, as others have before us, that the advantages of heterozygosity will increase with environmental heterogeneity. There is a positive association between heterozygosity and growth in blue mussels in a natural environment (52), but none was detected in the laboratory (7). The different degrees of environmental variation in these studies may explain these disparate observations.

RECOMMENDATIONS FOR FURTHER RESEARCH

Attempting to chart the course of future basic research is probably foolhardy and maybe even undesirable. Nevertheless, we feel that there are several directions of research that will be particularly fruitful.

First, we believe that analyses within populations that contrast individuals from different sections of the heterozygosity axis will be most informative under stressful conditions. For example, water use efficiency in plants may vary with heterozygosity under conditions of low soil moisture but not otherwise. Thus, it will be easier to distinguish the characteristics of highly heterozygous from those of highly homozygous individuals during certain seasons and life cycle stages.

Second, we believe that the degree of heterozygosity will have the greatest impact on characters that are significant components of fitness. Growth rate, emphasized above, certainly contributes to fitness in most organisms. We recommend that other variables, such as respiration rate, photosynthetic rate,
water use efficiency, fecundity, age and size at first reproduction, and scope for growth, should be examined for their response to different levels of enzyme heterozygosity.

Third, we believe that a comparative search to determine the limits of generality of the phenomenon would be worthwhile. One approach would be to compare the strength of associations with heterozygosity among populations (or species) that differ dramatically in their degree of environmental heterogeneity. We predict that there is a positive correlation between the strength of such correlations and environmental heterogeneity.

Finally, we believe that the generality of associations between heterozygosity and growth justifies placing the phenomenon in an applied and predictive context. For example, we anticipate that the costs of an electrophoretic screening of forest tree seedlings prior to restocking would be more than repaid by the enhanced growth rate of highly heterozygous individuals chosen for planting.

CONCLUDING REMARKS

Enzymes are protein catalysts that control the flow through metabolic pathways. Many enzymes in natural populations are polymorphic, and for those enzymes whose kinetics have been investigated, there are typically differences in performance among genotypes. Such differences can also be detected at the level of the whole animal or plant. Empirical studies have revealed variation among heterozygosity classes in growth rates, developmental stability, and oxygen consumption. These observations cover a remarkably broad taxonomic range: gymnosperms, angiosperms, invertebrates, and vertebrates, including humans. We believe that there is sufficient evidence on hand to state that individual organisms’ level of heterozygosity is a major organizing principle in natural populations of both plants and animals.

Many of the empirical studies summarized here report associations among individual heterozygosity (i.e. the number of heterozygous loci) and measures of performance such as growth rate and developmental stability. While these observations may have been unexpected a decade ago, they are now predicted by fitness determination models in population genetics for those loci whose variation is maintained by balancing selection. Instead of being bewildered by a vast number of multilocus genotypes, we should examine a single axis of genetic variation composed of polymorphic enzymes—the continuum of individual heterozygosity. We are optimistic that these studies will provide fresh insights in population biology and valuable tools for plant and animal breeders.

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