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GENETIC POLYMORPHISM IN HETEROGENEOUS ENVIRONMENTS[1,2]

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INTRODUCTION

The discovery in the last decade, using electrophoresis, of large amounts of genetic polymorphism in natural populations has had a tremendous effect on population genetics. Before then it was not clear how many polymorphic loci there were in a population and the single gene overdominance model generally seemed adequate to explain what polymorphism was documented. In an effort to explain the new-found genetic variation, two opposing camps surfaced in the late 1960s and still exist to some extent today. One group, often called neutralists, believes that most allozymic variants have a minimal effect on fitness and are in a population because of a combination of mutation, finite population size, and migration. In other words, in their view, selection plays little or no role in maintaining different electrophoretic alleles.

The other group, sometimes known as selectionists, believes that some sort of balancing selection is responsible for the maintenance of the majority of electrophoretic alleles. "Balancing selection" is a catch all term for any type of selection that can maintain a stable polymorphism. Among the types of balancing selection that are thought to play a significant role in maintaining genetic polymorphisms are the classical overdominance mode, frequency-dependent selection [see (8) for a recent review], differential selection between the two sexes or between different life stages, and variable selection in time and/or space. This last type of balancing selection is examined here in an effort to understand the importance of selection varying in time and/or space in maintaining genetic polymorphisms.

A significant body of literature demonstrates an association between the genetic attributes of a population and some aspect of the environment. Since much of this material has been reviewed elsewhere (2, 16, 58, 165), we concentrate on two of the

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most studied visible polymorphisms; industrial melanism in moths and shell color and banding in snails, as well as on recent allozyme studies. We then review the use of multivariate statistics and biochemical properties to establish a genetic-environment association for electrophoretic variants, and follow with a discussion of various theoretical models which give the conditions under which temporal and spatial environmental heterogeneity can maintain genetic variation. Next we discuss the experimental evidence which appears to demonstrate that a genetic-environment association accounts for a stable polymorphism of electrophoretic variants. This experimental evidence largely depends on perturbations in the environment and/or the genetic constitution of a population to demonstrate a cause-effect relationship between the environment and the frequency of electrophoretic alleles. Finally an overview of the importance of environmental heterogeneity in maintaining genetic polymorphism is presented.

GENETIC-ENVIRONMENT ASSOCIATIONS

There are a number of examples of associations between particular genotypes and environmental parameters. Such an association of course does not mean that the genetic pattern is the result of the environmental factor unless additional evidence supports a cause-effect relationship. Some efforts have been made (29, 169) to eliminate nonselective causes such as genetic drift or gene flow as explanations for genetic-environmental associations as seen in clines of gene frequency. In general, the examples we discuss are ones in which there is supportive evidence to demonstrate that different selective pressures are actually operating in different environments. Most of the examples are related to spatial heterogeneity in the environment, primarily because fewer polymorphisms associated with temporal differences have been documented. It is not clear whether this is because fewer temporal-based polymorphisms actually exist, or because they are more difficult to document, or both.

General Examples

Perhaps the best example of a genetic polymorphism which is the result of spatial environmental heterogeneity is the phenomenon of industrial melanism. Industrial melanism refers primarily to the spread of black or dark forms of normally light-colored, cryptic moths in areas subject to industrial pollution. Because this phenomenon is such a striking example of both morphological polymorphism and rapid evolutionary change, studies concerning it have been extensive [see (109a) for an excellent review]. In general, genetic control of industrial melanism is unifactorial with the allele for dark or melanic being dominant to that for pale or typical. The spread and maintenance of the polymorphism are due primarily to the fact that melanic individuals are at a cryptic advantage in industrial areas because of darkening of resting backgrounds caused by accumulations of soot and the destruction of lichens. On the other hand, the melanics are at a disadvantage on the lighter resting backgrounds of unpolluted areas (15, 33, 108, 109). The selective agent acting here is differential predation by birds.

Beyond this, the situation is less clear. In some areas the frequency of melanics is apparently much higher than would be expected. An additional advantage for the melanic morph in terms of superior larval viability (56, 57, 109) has been proposed to account for these cases. But in other areas, the melanic frequency is lower than would be expected on the basis of estimated selection coefficients. Here heterozygote advantage has been invoked as a possible cause (33). It has also been shown that melanics and typicals of some species preferentially select appropriate resting backgrounds (107, 166), and this background selection has not been taken into account in attempts to estimate relative fitness in the field.

Even in this classic case of genetic polymorphism where selection is quite strong, it is difficult to relate field observations to a specific genetic model. An example is an excellent study by Bishop (15) where a cline in the melanic frequency in the moth, *Biston betularia,* between Liverpool and rural North Wales was extensively investigated. Estimates were made of selection coefficients, population parameters, gene flow, and reproductive behavior, and these were used in a computer simulation that attempted to reproduce the cline. No amount of manipulation, within the bounds of the estimated parameters, was sufficient to produce a model which mimicked reality. Thus although the relationship of melanism to environmental heterogeneity appears clear, even this relatively simple case of morphological polymorphism is rather complex.

Another example of Mendelian polymorphisms that appears to be maintained by spatial environmental heterogeneity is shell color and banding pattern in the land snail, *Cepaea nemoralis* [for reviews see (32, 58, 98)]. Several studies have indicated that the polymorphism is maintained by differential predation of individuals whose shell color and banding pattern do not appropriately match the background of their habitat (24, 30, 171).

This is not, however, a complete explanation. In some regions morph frequencies are uniform in spite of apparent environmental heterogeneity, and other areas exhibit steep clines in the absence of any discernible environmental gradient (22, 99). It has been suggested that these phenomena, called "area effects," are also due to environmental heterogeneity, the causative agent being variation in climatic factors which differentially affect the various morphs (22, 23, 100, 119). Other authors believe that genetic factors, in the form of different coadapted gene complexes that result in restricted gene flow among different local populations, are important in maintaining area effects (31, 32, 71, 199). The available evidence suggests that each of the viewpoints discussed above is to some extent correct. In all probability, the maintenance of shell polymorphism in *Cepaea nemoralis* is due to a complex interaction of environmental heterogeneity and genetic factors, the relative importance of which varies from locality to locality. Even these two classic cases of single gene polymorphism associated with spatial environmental heterogeneity are not completely understood.

In many situations where adaptive responses to spatial environmental heterogeneity have occurred, the inheritance of the trait involved either has not been precisely determined or has been shown to be polygenic. Several excellent examples of this situation are furnished by studies of heavy metal tolerance in plants (3, 4,

17, 87, 138, 139, 162). These studies have shown that individuals taken from soils contaminated by mine workings have much greater tolerance to the heavy metal in question than do other individuals of the same species taken from closely adjacent areas of uncontaminated soil. A few of the other examples include microdifferentiation with respect to alcohol tolerance in *Drosophila melanogaster* (137), differentiation in banding patterns of island populations of water snakes (*Natrix sipedon*) (25), and modification of vegetative growth form of sea cliff populations of plants (6, 73).

Recent years have seen a large number of surveys of allozymic variation. When populations are sampled over space, one or more loci invariably show a clinal pattern. Usually the cline correlates with some parameter of the physical environment, e.g. temperature, salinity, or rainfall. Although this may indicate an important selective factor, further tests of survival, fecundity, or other fitness components under different environmental regimes should be used to test the hypothesis. Even supportive evidence from fitness tests should be cautiously handled because of the confounding effect of the genetic milieu.

To get around this problem, Wills and his co-workers (195–197) have inbred flies so that only the allozyme locus (and presumably a small region around it) is heterozygous. The inbred flies are then put in a stress environment to measure the relative selective values of the different genotypes. For both loci studied, *octanol dehydrogenase* and *esterase-5,* there was some evidence of selection in flies inbred for 38 generations. Others (110, 202) have criticized this study, but the overall impression is that Wills has demonstrated that selection is acting to some extent on these two allozyme loci in a stress environment. Others have examined components of fitness for different allozymic genotypes in several environments where it is assumed that there is a random association between the allozyme locus and other linked loci. For example, Johnson & Powell (91) found that *Adh* phenotypes had different relative survival when subjected to heat and cold shock, and Marinkovic & Ayala (129, 130), studying five polymorphic loci in *Drosophila pseudoobscura,* found a number of instances where fitness components were reversed in crowded versus optimal conditions.

Several clines for which biochemical support was found are discussed later. Among the well-studied clines are those for the *alcohol dehydrogenase* locus in the crested blenny (96), the *leucine aminopeptidase* and *glucose phosphate isomerase* loci in the blue mussel (116), an *esterase* and a *hemoglobin* locus in the eelpout (29) and several loci in *Avena barbata* (34, 75). The studies in *Avena* have been criticized by Lewontin (126) because *Avena barbata* is highly self-fertilized. Hamrick & Allard (76), however, have recently shown in a common garden experiment that the multilocus xeric and mesic genotypes show differential adaptation. Of interest also are two recent studies of outcrossing perennial plant populations existing in heterogeneous environments (9, 168), where no associations between the gene frequencies at a number of electrophoretic loci and components of the habitat were found.

One good demonstration of heterogeneity in space maintaining a polymorphism is that of a behavioral gene in *Drosophila willistoni.* De Souza, da Cunha, & dos Santos (48) reported the finding of a single gene dominant variant that evolved in a population cage, which exhibited pleiotropic characteristics in that it had "a faster

rate of development, needed less food, and preferred a solid dry environment to pupate." These variants survived outside the food cups in the bottom of the food cage while the normal flies could not. The normal flies of course had greater survival in the food cups. As a result, cage populations with this variant were polymorphic and had both greater numbers and biomass.

Unlike spatial environmental variation, there are very few single gene polymorphisms that have been associated with temporal environmental changes. Most of these cases have been found in organisms with short generation length where the changes in gene frequency corresponded to seasonal changes. Examples of this sort include cyclic changes in the frequency of color morphs of the ladybird beetle *Adalia bipunctata* over seasons (184), seasonal change in enzyme polymorphisms in *Drosophila pseudoobscura* (52), and changes in the frequency of *malate dehydrogenase* alleles in *Daphnia magna* (80). Berger (11) documented an increase in the frequency of the slow allele at the *α-Gpdh* locus from 9 to 18% and 12 to 25% in two different populations of *D. melanogaster* over a season in 1968 and also observed a similar change in two Amherst populations in 1966. He did not find seasonal variation at four other allozyme loci, and Lewontin (126) stated that he found no seasonal variation at a number of loci in the Strawberry Canyon population of *D. pseudoobscura.* Several reports on electrophoretic loci in vole populations (59, 170a, 179) have indicated that gene frequencies may undergo fluctuations related to density. However, Gill (63) has observed seasonal changes in gene frequency for a pelage gene in a vole population which does not undergo the 3–4 year oscillations in density typical of microtine populations. Gershenson (61) also described seasonal changes in the frequency of a melanic morph in Russian populations of the hamster.

Seasonal changes in the frequencies of inversions in *Drosophila* are well known (50, 51, 53), but these inversions, although inherited in a Mendelian fashion, compose a substantial portion of the *Drosophila* genome. The third chromosome of *D. pseudoobscura* has been studied for over three decades and the problems with associating inversion frequency trends with environmental factors have been recently reviewed (1).

If differential selection pressures occur seasonally in an organism that has a generation length of a year or more, there must be some marginal overdominance to maintain a stable polymorphism (see introductory discussion of theoretical models). Otherwise, unless counteracted by migration, such examples as seasonal selection of color polymorphisms in frogs (88, 140), or isopods (79) should lead eventually to fixation of one allele or the other. In these populations, long-term monitoring of the morph frequencies as well as estimates of the number and types of migrants would be valuable.

Lewontin & Krakauer (127) introduced a statistical technique to test whether loci are neutral or are subject to selection over time or space. Their basic assumption is that all alleles in the same population should be exposed to equal doses of genetic drift and migration but if selection is important it should act in different ways on different alleles and loci. A number of studies (72, 126, 127, 136a, 149, 150, 186) appear to have demonstrated selection acting over space, although recently several cautionary comments about this procedure have been made (128, 148, 160, 160a).

The technique seems most appropriate for testing gene frequency changes over time. In the first temporal study, Krimbas & Tsakas (118) suggested that selection was not important in changing the frequencies of alleles at two *esterase* loci. Taylor & Gorman (180) have recently found that changes in allozyme frequencies in an anole introduced to Bermuda were more heterogeneous than expected if genetic drift were the only cause of gene frequency change. In other words, testing data derived from spatially different populations may not be valid in many situations, and there is presently only marginal evidence of selection using the techniques in temporally differentiated populations.

Statistical Associations

To establish that genetic polymorphism is maintained by environmental heterogeneity, one must first demonstrate varying environmental selection with respect to the genotypes in question. Often, no obvious relations exist and an attempt must be made, through the use of some sort of statistical procedure, to determine whether significant gene-environment associations exist. This is particularly true for electrophoretic variation where selection differences are probably relative small.

The techniques generally used in such studies fall into two basic categories. Linear regression and correlation analyses are used in most studies to test for geographic covariation between gene frequencies and environmental variables. A number of papers have also used multivariate analysis procedures to reduce the dimensionality and improve the interpretability of the data.

In the simplest case, linear regression is used to test the hypothesis of linear association between the frequency of a single allele and one or more environmental variables (115, 120). While this sort of analysis may be useful in determining the question of gene-environment association, a maximum likelihood variant of the regression procedure, proposed specifically for problems involving gene frequency data (173) appears to be a preferable approach. It allows one to test three distinct hypotheses: (*a*) Is there interlocality variation in allele frequencies at the locus in question? (*b*) Is interlocality variation in allelic frequencies linearly related to one or more environmental variables? (*c*) Are there substantial deviations from the linear model of (*b*) above? Since the test statistic for each of the hypotheses discussed above is asymptotically distributed as a χ^2 variate, a multilocus analysis involving independently segregating loci can employ the sum of the single locus analyses.

An example of this procedure can be found in the companion paper (117), which assayed the influence of environmental variation on variation of allele frequencies in *Drosophila pavani*. Significant geographic variation in allele frequencies, gene-environment association, and lack of fit components were demonstrated for all loci. While the significance of both the lack of fit and regression components made interpretation of these results somewhat ambiguous, this result is more informative than that of a normal regression procedure.

A somewhat different approach to determining gene-environment associations is taken in a study of the harvester ant (93). In this analysis, the data sets which represent allelic variation at each locus and environmental variation are subjected to separate principal components analyses. [For a discussion of principal compo-

nents analysis see (39, 145).] This results in sets of uncorrelated variables which represent the trends of variation at each locus and major trends in variation of environmental variables. These are used to generate a matrix of interlocus and gene-environment correlations (the intralocus correlation being zero), the individual elements of which are tested for significance.

Procedures similar to the one discussed above have been used in several other studies (92, 161, 185), and in all cases showed significant gene-environment associations. In addition, two of these studies employed the technique of canonical correlation analysis (92, 185) as a "check" on the principal components solution. [For a discussion of canonical correlation see (145).] In both cases this method gave results similar to the principal components solution in terms of demonstrating gene-environment association.

Canonical correlation was also used by Schaffer & Johnson (169), who reanalyzed data from a previous study of gene-environment relationships in *Drosophila melanogaster* (92). The purpose here was to separate chance correlations resulting from patterns of gene flow which coincided with trends in environmental variability from those correlations which actually represented environmental selection. The result again demonstrated significant gene-environment association.

Taylor & Mitton (181) presented a reanalysis of the harvester ant data (93), which used still a different multivariate procedure, factor analysis. [For an account of this somewhat complicated procedure see (163).] The result again indicated gene-environment association, but the resulting factors dictate that no original variable is strongly associated with more than one factor. Taylor & Mitton suggested that this property aids in postulating hypotheses concerning causation.

All of the studies discussed above are concerned with demonstrating linear association between allele frequencies and spatial environmental variation. Bryant (20), on the other hand, tested Levins's (124) hypothesis that increased levels of temporal environmental variability should be reflected by increased levels of genic heterozygosity. His results seem to indicate that environmental variability and heterozygosity are associated.

These studies present a rather consistent picture of gene-environment association. Is there any reason for preferring one technique over another? As was pointed out previously, the regression procedure of Smouse & Kojima (173) seems superior to normal regression analyses in that three distinct hypotheses are tested. Both the principal components approach (93) and canonical correlation analyses (92, 169, 185) apply to one locus–environment associations, but would seem to do little more, and perhaps less than, Smouse & Kojima's methods.

Neither approach takes into account the specific properties of allele frequency data. Such data represent estimates of population parameters, and interpopulation differences can and should be statistically tested for significance. Also, these estimates have associated with them an error variance which is dependent on the frequencies of the alleles and the sample size from which the frequency was estimated. Thus, a study may use data that show no significant geographic variation, or in which different observations represent widely differing degrees of measurement accuracy. In the paper by Rockwood-Sluss, Johnston & Heed (161) on *Drosophila*

pachia, for example, no overall genetic variability was shown, yet significant gene-environment association seemed to be present. It is difficult to judge the reality of these associations on the basis of the methodology employed. Multivariate analyses would however seem to be the only way of approaching multilocus studies, but here too thought should be given to the nature of genetic data. For example, both Taylor & Mitton's (181), and Bryant's (20) analyses employed Studentized genetic variables, thus giving equal weight to both small and large amounts of variability. Small variances in gene frequency or heterozygosity may be as evolutionarily significant as large ones, but the fact remains that one is dealing with estimates and should place the greatest emphasis on the variation that is most likely to be real.

The foregoing criticisms do not question that many of the relationships revealed in the previously discussed studies are real. Replicate analyses of the same data, as well as the consistency of the results, suggest that the techniques employed are fairly robust. The point is rather that the techniques used may at times be inappropriate.

Biochemical Associations

The exact relationship between the biochemical properties of different allozymes and their role with respect to the maintenance of polymorphism is obscure. However, in vitro biochemical differences between different allozymes can add credence to genotype-environment correlations and also elucidate the possible adaptive significance of electrophoretic morphs. For an excellent review of biochemical adaptation and insight into how it may occur, see the recent book by Hochachka & Somero (85).

One of the first demonstrations of a biochemical explanation for a genotype-environment association is the work of Koehn (114) in the freshwater fish, *Catostomus clarkii.* In this fish a cline in an *esterase* locus is associated with latitude so that the *Es-I*^{a} allele frequency varies from 1.0 in southern Arizona to 0.17 in northern Nevada. Extracts of esterases from the three genotypes *Es-I*$^{a/a}$, *Es-I*$^{a/b}$, and *Es-I*$^{b/b}$ were measured for activity at different temperatures and the activities of the enzymes were put on a relative scale (see Figure 1). At the highest temperature (37°C), the homozygote most common in warmer parts of the range had the highest activity. At the lowest temperature (0°C), the other homozygote—which has the highest frequency in the colder parts of the range—had the greatest activity, while the heterozygote had the highest activity in the middle range. A similar situation was found by Merritt (141) in the fathead minnow, *Pimephales promelas,* where there is a north-south cline in gene frequency at the lactate dehydrogenase locus. Above 25°C the homozygote from the northern allele had a significantly lower substrate affinity, but Merritt did not find an explanation for the maintenance of the southern allele. In other words, in both these studies, in vitro biochemical properties of extracts from different genotypes support the hypothesis that the fitness of the electrophoretic morphs depends on temperature.

A much more intensive investigation in search of a biochemical explanation for an electrophoretic polymorphism has been at the *alcohol dehydrogenase* locus in *Drosophila melanogaster.* The basis for this concentration on the *Adh* locus is primarily because alcohols seem to be an important environmental component for

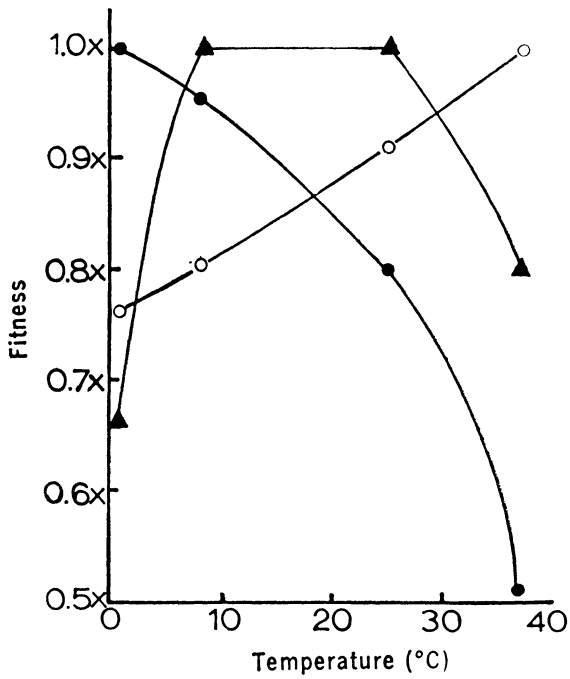

Figure 1 Relationship between temperature-dependent *Es-I* activities of genotypes and "fitness." Open circles indicate values for genotype $Es\text{-}I^{a/a}$, triangles $Es\text{-}I^{a/b}$, and closed circles $Es\text{-}I^{b/b}$.

D. melanogaster, a fly which lives primarily in rotting fruit and vegetables. ADH may, however, have important pleiotropic effects on the juvenile hormone (152) or on the catalysis of retinene or glyceraldehyde (95). In addition, there is also only one *Adh* locus in *Drosophila* and ADH has some biochemical advantages since it is a small molecule and easy to purify.

Gibson (62) was the first to examine the biochemical properties of ADH in an effort to determine the adaptive significance of the morphs. Two electrophoretic morphs, Adh^F and Adh^S, are generally found to be polymorphic in most populations. Gibson tested the activity of extracts from the three genotypes from larvae without treatment, with heat treatment, and with ethanol in the media. The fast homozygote had the highest activity in untreated and ethanol larval extracts. In the heat-treated extracts, the heterozygote had the highest activity. Further analysis of the biochemical properties of *Adh* alleles has generally substantiated and extended Gibson's findings (41–43, 91, 144, 190). These studies have shown significant differences in the ADH allozymes for a number of properties including activity, quantity, substrate specificity, turnover, influence of pH, and heat stability. It is difficult to know, however, which of these factors may be related to selection in natural popula-

tions and how they might give an overall balance of selective forces which could maintain a stable polymorphism. McDonald & Avise (135) have shown, however, that ADH activity and survivorship of adults of nine *Drosophila* species on media treated with isopropanol are correlated. J. F. McDonald (unpublished) has also demonstrated that flies subjected to 28 generations of selection for alcohol tolerance in an experiment by David & Bocquet (40) have a higher ADH activity than unselected flies.

A critical question that must be asked is whether the differences in activity and other biochemical properties found for different *Adh* genotypes are the result of the differences in the structural gene. Some information on this point comes from a survey by Ward (193) of a number of homozygous lines from a natural population. Although the mean activity of the fast strains was approximately twice as high as that of the slow strains, there was substantial overlap between the two types of strains. Birley & Barnes (14) also found that, in a series of inbred lines which had undergone 42 generations of sibmating, the lines having the slow allele had lower enzyme activity. But they also noted a great deal of variation in enzyme activity among the slow and fast lines, as well as some overlap between the two groups.

Perhaps the most telling experiment is one where Ward & Hebert (194) selected both up and down for ADH activity in populations which were homozygous for the slow allele at the structural locus. The results are given in Figure 2, and it is apparent that selection can operate very quickly on enzyme activity without any variation at the structural locus. The up line obtains the activity of a typical fast allele line and the down line reaches a very low activity in just two generations. Of course, the quick plateauing of the response may indicate that only one or a few other genes are affecting ADH activity in these populations. Two other studies suggest that some of the genes that affect ADH activity are on the X chromosome (10, 84).

Furthermore, adaptation to high alcohol levels in nature may not involve genetic change at the *Adh* locus. For example, McKenzie & Parsons (137) have shown that flies in a wine cellar had a much greater resistance to alcohol than those just outside. They found no differentiation with respect to the frequencies of the *Adh* alleles, and assumed that adaptation had taken place through changes at other loci. However, Thörig, Schoone & Scharloo (183) have found a second *Adh* allele with the same electrophoretic mobility as the fast allele, but with different biochemical properties including activity on ethanol.

ADH has also been investigated in plants where the enzyme functions in anaerobic respiration. A polymorphism in cultivated maize is widespread (170), and two common alleles have different biochemical properties (167). Marshall, Broué & Pryor (131) demonstrated that fast homozygotes have a faster growth rate in normal conditions, but that slow homozygotes have a faster growth rate under flooded conditions. The slow homozygote also had lower specific activity in flooded conditions. The lower activity presumably results in less ethanol accumulation, a factor which inhibits growth; as a result there is faster relative growth under anaerobic conditions. In soft bromegrass, however, no differentiation between wet and dry sites was found for the *Adh* alleles, while three other loci apparently unrelated to anaerobiosis did show variation between sites (18).

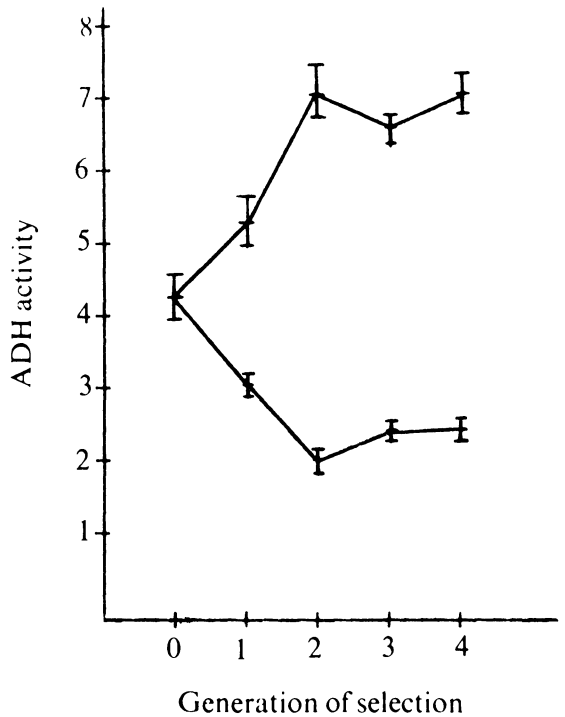

Figure 2 Divergent directional selection for ADH activity from a strain homozygous for the Adh^S allele.

Another enzyme for which biochemical properties of allozymes appear to be related to environmental patterns in gene frequency is α-GPDH in two insects, *Drosophila melanogaster* and the butterfly, *Colias meadii.* α-GPDH has an important role in the metabolism of insect flight muscle and has been widely studied. Alleles at the *α-Gpdh* locus in Drosophila vary in gene frequency both over space and time (11, 92). The general pattern is that the *α-Gpdh* slow allele has a higher frequency in areas with a low mean annual temperature and within populations during the autumn months. Recently, Miller, Pearcy & Berger (142) have found biochemical properties which correlate with this gene frequency pattern; for low and intermediate temperatures, the *S/S* homozygote has a low K_m, and the *F/S* heterozygote has a high specific activity and a low K_m. At high temperatures the *F/F* genotype has a high specific activity and low K_m.

The *α-Gpdh* locus in *C. meadii* showed a sharp cline in allele frequency. The fast allele had the highest frequency in alpine habitats and the slow allele at the greatest frequency at lower altitudes (94). This cline was found at three different locations and, in the one population which was examined in time, it persisted in different years. Evidence supporting a biochemical basis for the cline was found when extracts

from different butterflies were examined. That is, the enzyme from the fast variant has the greatest affinity for the substrate at 10°C and 20°C, while the slow allele has the greatest affinity at 30°C.

It is now apparent that most allozymes differ in some in vitro biochemical properties (126), but it is not clear which properties are selectively important in natural populations. Also, demonstrations that changes in biochemical properties of allozymes may be controlled in part by other loci further complicate the picture. But, as with statistical associations, biochemical associations can give additional support to the selective importance of allozymes.

THEORETICAL MODELS

There are a number of examples where it is thought that differential selection either over time or in space is the mechanism maintaining a genetic polymorphism. It is not generally appreciated, however, that differential selection over time or over space in many cases does not lead to a stable polymorphism (126, 158). A prime example is where different selection affecting viability occurs in the life cycle of an organism and there are just two morphs, the dominant (consisting of the homozygote and the heterozygote) and the recessive morph. Assume, for example, that survival is lower for the dominant morph in the larval stage but that this morph has a higher pre-mating adult survival. On the surface it appears that balancing selection is operating and that it can maintain a stable polymorphism. In fact, these fitness components collapse, so that the overall survival of the morphs is the product of the relative survival in the different life stages or $S_L S_A$, where S_L is the survival in the larval stage and S_A is the survival in the pre-mating adults. Because of this property (and assuming there are no other fitness differences at the gene), the overall fitness of the dominant form is either less than, equal to, or greater than the fitness of the recessive morph. Only in the unlikely case where the fitnesses are exactly equal will the two morphs be maintained in the population. The other two results will lead to the eventual fixation of one allele or the other.

Variation in fitness patterns over time or space can in some cases result in a stable genetic polymorphism. A number of studies have investigated such models and delimited the situations where this type of selection may be important in maintaining polymorphisms. From these theoretical studies it appears that temporal variation in selection may be of limited importance in maintaining genetic polymorphism, while spatial variation in selection may play a much more significant role in maintaining genetic polymorphism. The models we consider are generally coarse-grained, i.e. the whole life of an organism is spent in a particular environment.

Temporal Models

Various theoretical aspects of temporal variation in selection were considered by Fisher & Ford (54), Wright (198) and Kimura (111). Dempster (47), however, was the first to examine the conditions under which a polymorphism can exist. Haldane & Jayakar (74) independently examined the same genetic models several years later.

The first model that Dempster investigated was of a haploid population with discrete generations where the two genotypes had fitnesses which varied in different

generations. He showed that this type of selection will not lead to a stable polymorphism. The only exception is the improbable event that the product of the fitnesses over time is identical for the different alleles. Otherwise the gene frequency will eventually approach 1.0 for the allele for which the product of fitnesses over time is highest. Haldane & Jayakar (74) and Gillespie (65) also obtained this result using somewhat different approaches.

Another model Dempster discussed is in a diploid population where there is complete dominance [termed absolute dominance by Prout (159)] of one allele in all environments and where the recessive genotype is sometimes at a selective advantage and sometimes at a selective disadvantage. This model is of particular interest since there is no marginal overdominance as defined by Wallace (191) either of the arithmetic or geometric means of the fitnesses. Although there are only two phenotypes, as in the haploid model, selection operates so that a stable polymorphism can result. The exact conditions necessary for polymorphism were first given by Haldane & Jayakar (74). They showed that in an infinite population where the relative fitnesses of genotypes A_1A_1 and A_1A_2 are equal to 1.0 and the fitness of A_2A_2 varies, to maintain a genetic polymorphism the geometric mean of the fitness of A_2A_2 must be less than 1.0 and the arithmetic mean must be greater than 1.0. The limits which permit a stable polymorphism with two environments are given in Figure 3, where x_1 is the fitness of A_2A_2 in environment 1 and x_2 is the fitness of A_2A_2 in environment 2. If the fitness of A_2A_2 is close to 1.0, then the potential for polymorphism is very restricted. For example if $x_1 = 1.1$, then x_2 must be between 0.9 and 0.909. However, if there is stronger selection as when $x_1 = 2.0$, then x_2 may be anywhere between 0.0 and 0.5 and can still satisfy the conditions for polymorphism.

The last model Dempster investigated assumes that the heterozygote is exactly intermediate in fitness and that one homozygote is favored in one environment and the other homozygote in the other. As he pointed out this model leads to "cumulative overdominance." Haldane & Jayakar generalized this to any level of dominance and found that the conditions for polymorphism in this case are that the geometric means of the homozygotes must be less than the geometric mean of the heterozygote. These conditions permit polymorphism when there is additive gene action (the heterozygote exactly intermediate between the homozygotes) and selection favors one homozygote part of the time and the other homozygote part of the time, making this an example where sex allows a qualitatively different result. Gillespie (66) found the same conditions using a different approach, and furthermore has shown that the conditions for a stable polymorphism are independent of the autocovariance (autocorrelation) of environments. Using a graphical approach and a frequency-dependent model (35), Hoekstra (86) has recently extended the conditions found by Haldane & Jayakar for the diploid models.

Another type of model used to study temporal variation is one where the fitnesses of the genotypes are random variables (64, 77). The results of these models are generally similar to that of Haldane & Jayakar (74) in that marginal overdominance of the geometric means of the heterozygote leads to a stable polymorphism. The basis for the polymorphism can be given in different biological terms, that is, the heterozygote is more homeostatic than the homozygotes. Karlin & Lieberman (103)

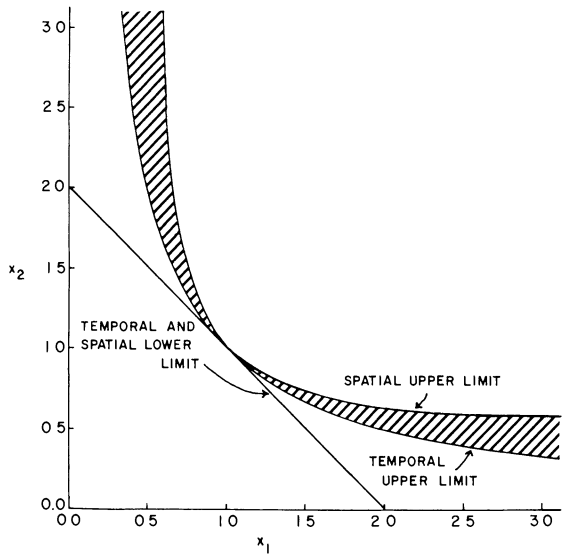

Figure 3 The regions for a stable equilibrium when the relative fitness of genotypes A_1A_1, A_1A_2, and A_2A_2 are 1, 1, and x_2 in environment 1 and 1, 1, and x_2 in environment 2. The crosshatched area is the region where spatial variation gives stability and temporal variation does not.

in their recent general mathematical treatment of random temporal models suggested that in some cases marginal overdominance may not be necessary to maintain a polymorphism if there is enough variance in the selection pattern and the mean selection is not too strong.

Even though the conditions for a stable polymorphism in an infinite population are unrelated to environmental pattern (65), the distribution of gene frequencies over populations is strongly affected by the autocorrelation between subsequent environments (78). For example, positive correlations increase the rate of pseudofixation and consequently reduce the amount of polymorphism in a population. In finite populations, Hedrick (81, 82) found that the environmental pattern was a very important factor in determining the maintenance of genetic variation. For example, using the complete dominance model, the maintenance of genetic variation is maximal when there is an environmental switch every generation (as spring and fall generations in a bivoltine insect). Even in this case, the amount of selection necessary to maintain variation is an order of magnitude greater than that for the overdominance model. Furthermore, when there is zero autocorrelation, the absolute dominance is almost always less effective than neutrality in maintaining genetic variation.

Using Dempster's last model, in which there is additive gene action, and assuming equal but opposite selection in two environments, the effect of environmental pattern

on maintenance of variation in finite populations can be seen in Figures 4 and 5. The parameter used here, the retardation factor, measures the ability of a particular model to maintain genetic variation. If the retardation factor is greater than 1.0, then selection maintains variation more effectively than neutrality does for the same population size. Figure 4 gives values where the environment varies stochastically and Figure 5 gives values for a cyclical environmental pattern. When there is stochastic selection, only when there is a negative autocorrelation between generations is genetic variation maintained more effectively than with neutrality. In fact, when the autocorrelation is positive, then variation is lost faster than if there were no selection at all. For the cyclical model, the amount of maintenance of genetic variation declines with cycle length. These last two observations are related, since a positive autocorrelation results in runs in a particular environment.

To understand the basis for a lower maintenance of genetic variation in both cases, an examination of the gene frequency of unfixed populations for different cycle lengths is helpful. For the longer cycles, selection causes larger oscillations in gene frequency. At the extremes of these oscillations, fixation occurs much more often than at the more intermediate gene frequencies. As a result, both long cycles and positive autocorrelations reduce the amount of variation maintained in a finite population. In infinite populations, genetic tracking of an environment which has a positive autocorrelation results in an increase in fitness (19, 123). In a finite population, however, genetic tracking leads to a loss of genetic variation and a consequent reduction in adaptedness (82).

There has been a sizable degree of interest in random temporal selection models in both finite and infinite populations where there is no mean difference in selection between the genotypes (37, 38, 89, 90, 102, 104, 111, 112, 146, 151, 178). In finite populations, although the retardation factor is the same as for neutrality (82), other parameters not related to the stable gene frequency distribution differ as a function of the initial gene frequency. For example, the probability of fixation is not equal to the initial gene frequency (except when the initial gene frequency is 0.5) when there is variable selection. It is higher for initial gene frequencies below 0.5 and lower for those above (82, 89, 102). Also the time to fixation is not the same as for neutrality, but is shorter for the selection model when the initial gene frequency is near 0.5 and longer for the selection model when the initial gene frequency is near 0.0 or 1.0. Karlin & Levikson (102) and Cook & Hartl (38) observed a similar phenomenon using different models.

Spatial Models

Levene (122) was the first to consider theoretically how differential selection in space could maintain a genetic polymorphism. The model Levene used was a diploid one which assumed that "after fertilization the zygotes settle down at random in large numbers into each of the niches. There is then differential mortality ending with a fixed number of individuals in each niche." Using this model, Levene then showed that if the weighted harmonic means of the homozygotes are less than one (the standardized fitness of the heterozygote in all niches), then a balanced polymorphism is possible. Since the harmonic mean is always less than the geometric mean,

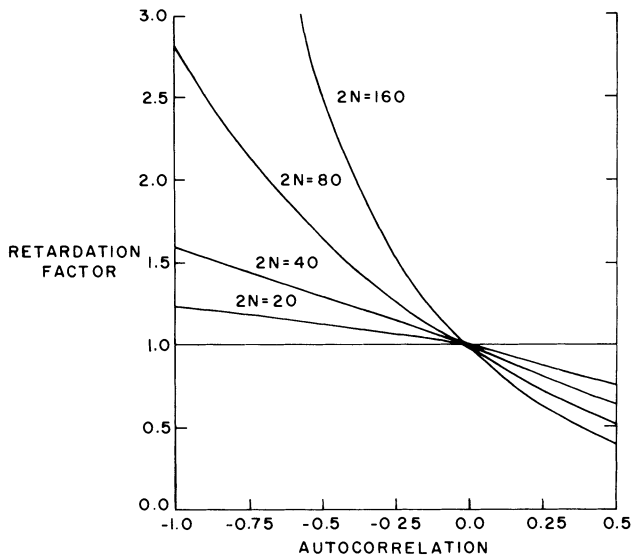

Figure 4 The retardation factor when there are different amounts of environmental autocorrelation and population size. The fitnesses of genotypes, A_1A_1, A_1A_2, and A_2A_2 are 1, 0.75, and 0.5 in environment 1 and 0.5, 0.75, and 1 in environment 2.

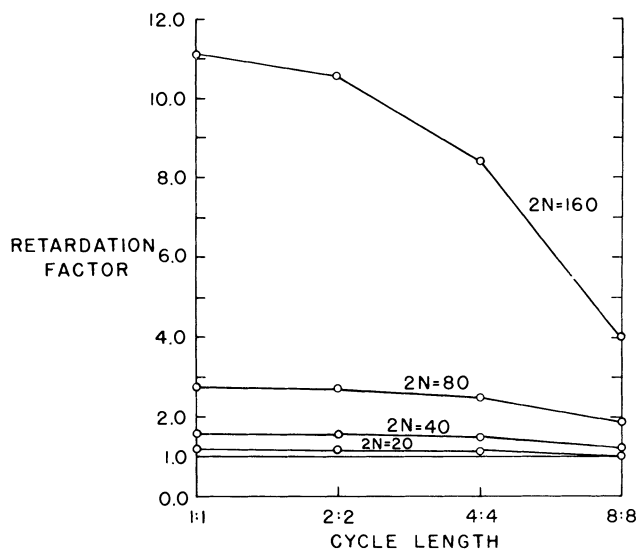

Figure 5 The retardation factor when cycle length and population size are variable. Fitnesses as in Figure 4.

the basic conditions for a stable polymorphism are broader when environments vary in space than when they vary in time.

One assumption implicit in Levene's model is that each niche contributes a constant proportion to the mating pool each generation, independent of the genotypes in the niche. This assumption was first pointed out by Dempster (47), who called Levene's model the "constant-fertile-adult number hypothesis" since Levene assumed the proportion of adults contributed from a niche is constant. [This type of selection has been termed "soft selection" by Wallace (191, 192).] A contrary view, the "constant-zygote-number hypothesis" was suggested by Dempster. In this model a constant proportion of zygotes is present in each niche every generation before selection, but selection subsequently modifies this proportion. So, for example, if two niches received the same numbers of zygotes, but one genotype was lethal only in niche 1, then the proportion of adults which niche 1 contributes to the mating pool would be less than 50%. For this type of selection, which has been called "hard selection" by Wallace, the conditions for a stable polymorphism as noted by Dempster and shown by Wright (200) and Christiansen (27) are that the arithmetic mean of the heterozygote must be greater than that of the homozygotes, in other words, overdominance. However, Arnold & Anderson (5) have shown that by making fitnesses logistic functions and regulating input (instead of output, as Levene has done) to a niche the conditions for a stable polymorphism are similar to those postulated by Levene. In most natural populations, there is probably a mixture of hard and soft selection, although specific examples of both can be given (191).

Another diploid model that has been examined is that of absolute dominance. Maynard Smith (132) mentioned that there could be a stable polymorphism for this model and then discussed the possibility further (133, 134). Prout (159), however, first showed, using Levene's assumptions, that for a stable polymorphism the arithmetic mean fitness of the variable homozygote must be greater than 1.0 and the harmonic mean fitness must be less than 1.0. These limits are also given in Figure 3, which assumes equal proportions in two niches. As with temporal variation, when the fitness of the homozygote is near 1.0, the conditions are quite restrictive. However, since the harmonic mean leads to an asymptote at 0.5 with stronger selection the conditions are much less restrictive than for temporal variation. Of course with hard selection no stable polymorphism is possible with this model.

Recently, Gliddon & Strobeck (70) showed that the same conditions apply to a haploid model. That is, if the fitness of one genotype is normalized to 1.0, then there can be a stable polymorphism if the arithmetic mean of the other genotype is less than 1.0 and the harmonic mean greater. This is in striking contrast to the temporal model for which there is no stable polymorphism with haploidy. Furthermore, C. Strobeck (unpublished) has shown that multiple alleles may be maintained in multiple environments for the haploid model.

When selection varies in space, both limited migration out of a niche and habitat selection may make the conditions less restrictive. Maynard Smith (133) investigated the effect of habitat selection in females using the absolute dominance model. Complete habitat selection by females does make the conditions less restrictive. For

example, if $x_2 = 1.1$, then x_1 may be between 0.9 and 0.9285 and still give a polymorphism and, instead of an asymptote occurring at 0.5, the maximum value of x_1 is asymptotic at 0.75.

Deakin (44, 45) investigated a model where a proportion $(1-k)$ of every subpopulation consists of those which neither ever left nor actively returned to their birthplace, and another proportion (k), which is a random mixture from other subpopulations. Using this approach Maynard Smith (134) and Christiansen (26) have shown that the general sufficient condition for the maintenance of allele A_1 is that $1 - w_i \geq k$ for some subpopulation i, where w_i is the fitness of A_2A_2. Hedrick (unpublished) has carried out Monte Carlo simulations that demonstrate that limited migration is very effective in maintaining a global polymorphism in a finite population. The impact of perturbations when there is a small amount of migration between subdivisions has been treated mathematically by Karlin & McGregor (105, 106). Dickinson & Antonovics (49) have used simulation to examine what conditions may lead to sympatric speciation in infinite populations as well as to examine the effects of finite population size on heterozygosity and gene frequency in complex environments (49a).

There have also been other modifications of the Levene theme. For example, Strobeck (176) demonstrated that Levene's model can be extended to m mating groups within each subdivision. The role of environmental grain involving a stochastic element has been explored by Gillespie (67, 68) who found that, in a patchy or coarse-grained environment, sufficient subdivision can cause a stable polymorphism; this is more likely to occur when the effective number of patches is large and the spatial correlation is small. So an increase in the effective number of patches or a decrease in the spatial correlation will increase the likelihood of polymorphism. It is also possible to maintain polymorphism in fine-grained environments, although this is less likely to occur. Two recent papers (177, 182) have discussed the limitations of the fitness set model of Levins (124).

The question of the interaction of two loci in a subdivided environment with respect to the protection of polymorphism has recently been considered by Christiansen & Feldman (28). When two alleles A_1 and B_1 are rare, subdivision and/or linkage increase the probability of polymorphism in the two-locus case. Allele A_1 will be protected when both A_1 and B_1 are rare in the two-locus case in a manner analogous to the one-locus case discussed by Prout (159).

Other Models

A number of general (verbal) models have been suggested as explanations for the maintenance of genetic variation in relation to environmental parameters. One model is that ecological and/or geographical central populations should be more polymorphic than marginal populations. In one instance—in a survey of 20 allozymes by Prakash (157) in *D. robusta*—this was not true.

Another group of models, the niche variation models, suggests that more genetic variation should be present in those organisms existing in most variable niches and that there is a correlation between the genetic heterozygosity and the relevant physical and biological parameters. Several workers have gathered data to test these

hypotheses (97, 125, 164, 175). A good discussion of these models is given by Soulé (174) and Valentine (187).

One of the difficulties with these models is ascribing a reasonable cause-effect relationship. This is of course related to the difficulty of designing a "critical" experiment (153). For example, in a recent paper, Valentine & Ayala (188) suggested that the higher heterozygosity of a tropical species of krill as compared to temperate and antarctic species is due to a greater stability of resources in the tropics. Species diversity and perhaps other factors also show trends in different latitudes. If both resource stability and species diversity are involved, it is not clear whether the stability of resources causes high species diversity, which in turn causes high heterozygosity or, alternatively, stability of resources causes both the high diversity and the high heterozygosity.

This particular situation may be discussed in terms of the Levene, Dempster, and Haldane & Jayakar models. The high heterozygosity in the tropical krill may be due to large spatial biotic heterogeneity as seen in the species diversity measures. Earlier we suggested that models with spatial heterogeneity and limited migration or habitat selection are very effective at maintaining heterozygosity. On the other hand, the antarctic krill lives in a species-depauperate and spatially homogeneous environment. In addition, it is subjected to seasonal selection pressures due to changes in available resources. As pointed out before, temporal selection is not very effective in maintaining genetic variation and may actually drive variation out of a population.

Another type of model, which is an extension of Lerner's (121) genetic homeostasis model, suggests that heterozygosity per se is advantageous because heterozygotes are more flexible. In this model, the variance in fitness of the heterozygote over different environments is less than that of the homozygotes. This model can again be related to the traditional models but has been extended by Gillespie & Langley (69) and discussed by Bryant (20, 21).

PERTURBATION STUDIES

The associations between environmental parameters and genotypes discussed earlier constitute a substantial body of evidence suggesting that different environments cause different selective pressures. However, they do not conclusively demonstrate that the selection pressures related to different environments are the cause or the only cause of the genetic patterns observed. For example, other environmental factors may cause the same genetic effects, or a suite of factors may cause the same or perhaps different unpredictable effects.

A cause-effect relationship could be demonstrated by genetically monitoring over time a series of populations initiated at different genetic combinations and kept in different environments. From this information, it could then be inferred that a specific environmental factor caused a specific genetic effect. Even this type of experimentation has some basic problems. For example, when an environmental parameter is altered, it may affect another environmental parameter, e.g. raising the temperature may alter the humidity if the humidity is not separately controlled. It

may not be clear in this case whether temperature or humidity is the factor causing an observed genetic change. Or different environmental factors may interact to cause unforeseen genetic responses, e.g. selection may act differently in high temperatures depending upon the relative humidity. Establishing populations with differences in the initial genetic constitution at some marker locus also leads to a difficult problem. As a result of the initiation procedure, particularly from a small number of founders, other linked loci may also be changed in frequency. If this leads to linkage disequilibrium, then it is difficult to determine whether a change in the genetic constitution is the result of selection acting at loci linked to the marker locus or acting at the marker locus itself.

Genetic Perturbations

Genetic perturbation studies are very much in vogue, but by themselves they cannot tell much about the mode of selection that is operating or whether selection is operating at the locus being perturbed. If the gene frequency is perturbed above and below an "equilibrium" gene frequency and returns from both sides, then one can say that in that particular environment there is balancing selection operating in the region of the marker locus. Nei (147) has shown that an overdominant locus in linkage disequilibrium with a marker locus may result in the same response as if balancing selection were operating on the marker locus. Of course, several genes, which are themselves balanced, in linkage disequilibrium with the marker locus, i.e. A_1+- and A_2-+, where the A locus is the marker locus, will appear as overdominance (pseudo-overdominance) even though the linked genes only show partial or complete dominance. In fact, some of the results of studies initiated with small numbers of founders may be due to initial linkage disequilibrium.

There are several approaches to understanding whether a response is due to linked genes. These approaches assume that the linkage is not so tight that it cannot be broken up in a reasonable amount of time and that the locus is not in a chromosomal arrangement such as an inversion. The first approach is an experiment that uses different numbers of independently derived founders. When smaller numbers of founders are used, there should be greater variation in response at the marker locus. Experiments demonstrating this effect have been carried out in *Drosophila* by Jones & Yamazaki (101) for *esterase-5* in *Drosophila melanogaster* and by Powell & Richmond (156) in *D. paulistorum* for the *tetrazolium oxidase* locus. Figure 6 gives the data of Jones & Yamazaki. At both high and low initial gene frequencies, greater variation over replicate lines was observed when two founders, as compared to when larger numbers of founders, were used. The implication is that when a small number of founders is used to initiate a perturbation experiment, it is highly likely that linkage disequilibrium between the marker locus and loci undergoing substantial selection will occur. [See (101, 172) for more discussion.]

Another approach to testing whether linked genes may be responsible for the results of perturbation experiments is to restart the perturbation experiment one or more times with organisms that have reached the equilibrium state. If there was initial linkage disequilibrium, and particularly if the gene frequency equilibrium is

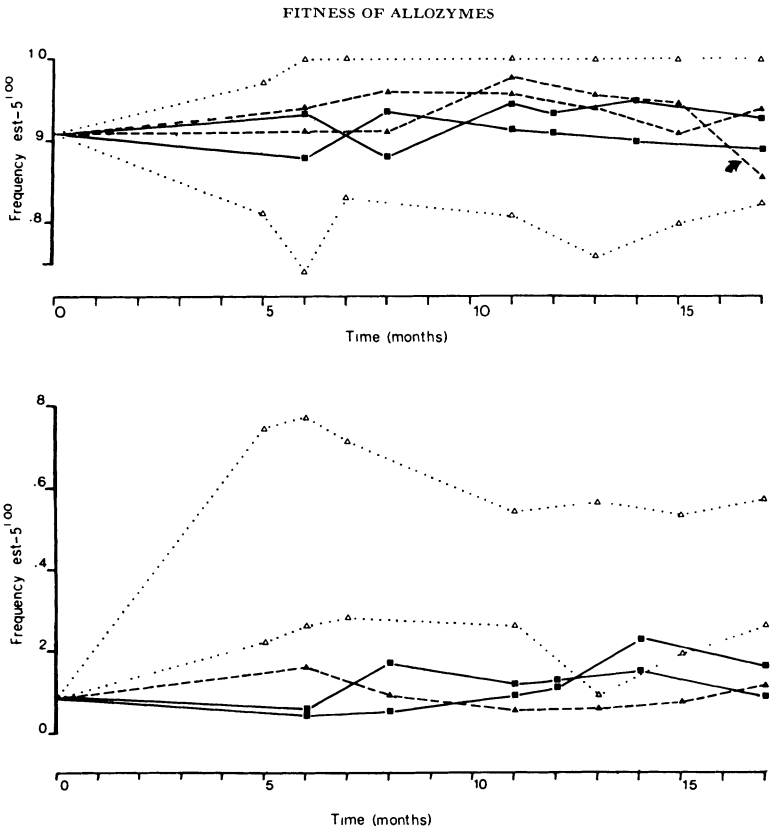

Figure 6 Changes in the frequency of the *Est-5*$^{1.00}$ allele in cages containing different numbers of founding homozygous lines. Open triangles indicate 2 founding lines, closed triangles 10 or 20 founding lines, and squares 44 founding lines.

intermediate, then recombination may have reduced the amount of linkage disequilibrium and the restart may give results more indicative of the amount of selection operating solely on the marker locus. Of course, if the linkage is very tight or if there are very few heterozygotes in which recombination can take place, then the restart may behave much like the initial experiment.

A demonstration of this technique is part of a study of X-linked genes in which Hedrick (83) restarted three replicates of an experiment to test whether a temporary gene frequency equilibrium was due to linked genes. All the restarts behaved quite differently from the initial experiment, demonstrating that something had occurred in all the replicates. Presumably recombination had reduced the amount of linkage

disequilibrium between the marker locus and other loci undergoing selection. A similar explanation may account for the lack of gene frequency change in a restart experiment involving the *esterase-5* locus in *D. pseudoobscura* (55).

A number of genetic perturbation studies have suggested that the allozyme polymorphisms studied are stable polymorphisms (7, 11, 12, 55, 155, 203). Generally these experiments have been carried out in standard laboratory cultures. Although a laboratory environment is simplified as compared to a natural setting, selection may still be acting through heterogeneity in the environment since even a *Drosophila* cage can be quite diverse with respect to such ecological factors as oviposition or pupation site, yeast flora, etc. One field perturbation experiment (60) supports a stable polymorphism for a *transferrin* locus in voles, and the monitoring of several allozyme loci in barley in two populations suggests the operation of some type of balancing selection (34a). One of the most complete studies of a genetic perturbation is that of Yamazaki (201) who found that the *esterase-5* locus in *D. pseudoobscura* was neutral both in a genetic perturbation analysis and from estimates of fitness components.

Environmental Perturbations

Genetic perturbation studies may show that balancing selection is operating to maintain a stable polymorphism at an allozyme locus (or the region marked by the allozyme locus), but do not distinguish different types of balancing selection such as overdominance, frequency-dependent selection, etc. By manipulating the environment one can see if the overall fitness or various components of fitness of the different genotypes are related to environmental factors.

For example van Delden, Kamping & van Dijk (189) found that changes in the gene frequency of the Adh^F allele in *Drosophila melanogaster* were related to the type of alcohol added to the regular food media. Starting with a series of populations obtained from crosses between five lines homozygous for the fast allele and five homozygous for the slow allele, the gene frequency of the fast allele increased for nearly all the populations kept on alcohol (see Figure 7). The concentrations of alcohol were chosen so that no drastic reduction in population size, and therefore little genetic drift effect, recurred. Gibson (62) and Bijlsma-Meeles & van Delden (13) also found that the frequency of the *Adh* alleles changed in response to the addition of alcohol to the media.

Another example of an environmental perturbation apparently resulting in a genetic change is seen in an *amylase* locus in *D. melanogaster* (46). Cage populations from four different geographic regions were kept either on cornmeal or sucrose media for several years. For all four comparisons the changes in frequency of the amylase phenotypes were in the same direction. Furthermore, it is thought that a major function of amylase in *Drosophila* is in the digestion of starch, and the phenotype having the highest frequency on the media containing starch (cornmeal media) also has the highest biochemical activity.

Human activity can extensively change the environment, thus causing an environmental perturbation. For example, as discussed earlier, industrial pollution in Britain resulted in an increase in melanistic forms of *Biston betularia.* In the past decade,

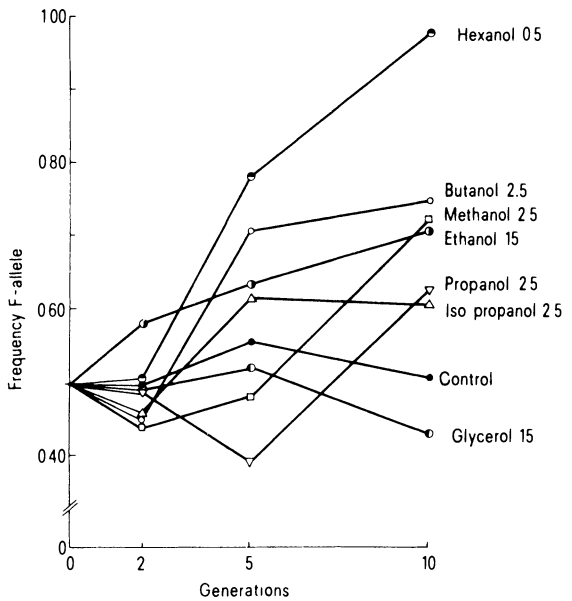

Figure 7 Change in the frequency of the Adh^F allele on different substrates. The numbers following the alcohols refer to volume percentages.

as smoke control measures have been instituted and the effects of pollution reduced, the frequency of melanic forms is also declining (36). Another instance of an environmental perturbation due to human activity resulting in a genetic change was found by Mitton & Koehn (143) in a marine fish, *Fundulus heteroclitus.* One particular population was taken from a cooling pond fed by effluent from a power plant. The water in the pond was 25°C as compared to the mean surface temperature in Long Island Sound of 12°C. Mitton & Koehn surveyed several allozyme loci and found a sizable frequency difference at a *malate dehydrogenase* locus. The frequency of the *a* allele was 0.720 and 0.739 over two years in the power plant pond, and greater than 0.95 in adjacent Long Island Sound populations. In a warmer location on the New Jersey coast, the frequency was even less, 0.019, as would be expected if the allele frequencies were correlated with temperature.

Bijlsma-Meeles & van Delden (13) used a different approach, and asked whether populations polymorphic at the *Adh* locus in *D. melanogaster* were better adapted to different environments than monomorphic ones. The measure of adaptedness which they utilized was the probability of long-term survival of a vial population. The data for the control and the significant environmental factors are given in Table 1. The lowest survival on ethanol media was for the monomorphic *slow* populations, in keeping with genetic perturbation experiments where the frequency of the *slow* allele decreases on ethanol media. A possible balancing factor is suggested by survival in 100% relative humidity, where monomorphic *slow* populations have the

highest survival. Averaged over all five treatments, the polymorphic populations had approximately a 10% higher survival probability.

Table 1 Probability of survival of vial cultures of *D. melanogaster* in different environment conditions after eight generations

	Environments					
	Control	20% ethanol	29.8°C	15°C	35% R.H.	100% R.H.
Monomorphic						
Adh^F	0.96	0.72	0.06	0.92	0.92	0.72
Adh^S	1.00	0.38	0.08	0.68	0.86	0.98
Polymorphic						
0.2 Adh^F	0.90	0.70	0.46	0.88	0.70	0.90
0.8 Adh^F	0.94	0.80	0.34	0.88	0.80	0.82

Three experiments have attempted to demonstrate directly that environmental heterogeneity and genetic polymorphism are related. Powell (154), McDonald & Ayala (136), and Powell & Wistrand (156a) initiated a number of *Drosophila* population cages in which 0–4 environmental factors were varied. These included variations in the type of yeast, *Drosophila* medium, temperature, illumination, and competition. These data are summarized in Table 2, and it is apparent that the populations in constant environments had a lower heterozygosity than those in variable environments. The results of Powell were questioned by King (113) because of the many inversions in his species *D. willistoni.* As a result, McDonald & Ayala used *D. pseudoobscura* populations that only had inversions on the third chromosome. Only three loci were on this chromosome and they did not differ significantly from the other 17 loci. Even though the populations in a constant environment had a lower heterozygosity, the populations with 1–4 variable environmental factors were fairly homogeneous in heterozygosity. Although it is possible that some of the differences observed were due to different effective population sizes or numbers of generations, this experiment demonstrates that environmental heterogeneity and heterozygosity are correlated. Powell & Wistrand used in inversion-free *D. pseudoobscura* stock. Although they estimated population size in a late generation to be greater than 2000, differences in population size in the early generations may have affected heterozygosity.

Table 2 Proportion of heterozygous loci per individual when different numbers of environmental factors are varied. Twenty-two electrophoretic loci were analyzed by Powell (154), 20 by McDonald & Ayala (136), and 9 by Powell & Wistrand (156a)

	Number heterogeneous environmental factors				
	0	1	2	3	4
Powell	0.078 ± 0.031	0.096 ± 0.084	—	0.134 ± 0.076	—
McDonald & Ayala	0.146 ± 0.029	0.186 ± 0.038	0.201 ± 0.040	0.208 ± 0.045	0.186 ± 0.044
Powell & Wistrand	0.236 ± 0.005	0.292 ± 0.004	0.322 ± 0.004	0.336 ± 0.005	—

OVERVIEW

At this time, a substantial amount of circumstantial evidence has accumulated indicating that genetic polymorphisms are related to environmental heterogeneity. This testimony is becoming more sophisticated as new statistical and biochemical techniques become widespread. There is, however, only a small amount of experimental evidence supporting the hypothesis that environmental heterogeneity is a major factor in maintaining genetic variation. One of the problems in obtaining such information is the difficulty in designing critical experiments (153) that can unequivocally exclude alternative hypotheses.

Population genetics theory in the last two decades has begun to incorporate many biologically important parameters. Perhaps models that involve multiple loci or population structure and size or environmental patterns in a biologically realistic manner will generate predictions that can be falsified or verified by observations. Single locus theory indicates that selection acting differentially in space coupled with limited migration and/or habitat selection will maintain a substantial amount of polymorphism. The present mathematical models predict a stable polymorphism due to environmental heterogeneity in a number of situations. There have been, however, few documented cases of this in laboratory or other situations where the magnitude and type of selection could be ascertained.

The role of environmental heterogeneity in genetic polymorphism is just now becoming understood, and many papers will be written before it is clear what proportion of polymorphic loci is maintained or affected by environmental heterogeneity and how environmental differences result in genetic polymorphisms.

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