GENETIC VARIATION

IN

VARYING ENVIRONMENTS

by

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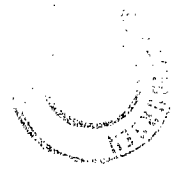

Abstract

In order to assess the relationship between genetic and environmental variability, a large natural population of Drosophila melanogaster was replicated as eight subpopulations which were subjected to four different patterns of environmental variation. The environmental variable imposed was presence of 15% ethanol in the culture medium. Experimental treatments of the populations were intended to simulate constant environmental conditions, spatial heterogeneity in the environment, and two patterns of temporal environmental variation with different periodicity (long- and short-term temporal variation). Additive genetic and phenotypic variation in sternopleural and abdominal chaeta number, and body weight, was estimated in two successive years, and measurements were taken of the genotype-environment correlation of body weight and sternopleural bristle score with medium type. Survivorship, productivity, habitat loyalty, and developmental homeostasis were also measured in each of the populations.

Additive genetic variance of sternopleural chaeta number and of body weight was significantly greater in the three populations experiencing environmental heterogeneity than the control population, but additive genetic variance of abdominal bristle score was not affected by exposing populations to varying environments. Temporal environmental variation was equally, if not more, efficient in promoting the maintenance of genetic variation than spatial heterogeneity, but the "grain" of the temporal variation was of no consequence. Specific genotype-environment interactions were not present, therefore adaptation to heterogeneous environments is by selection of heterozygosity per se, rather than by differential survival of genotypes in

the alternate niches. No habitat loyalty was apparent in any population, but those populations exposed to environmental heterogeneity were more fit with respect to survivorship and productivity than control populations, and hence have a greater probability of evolutionary survival.

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One of the central problems of evolutionary biology today concerns the discovery and description of systematic forces operating to maintain the vast store of genetic variation in natural populations. Extensive electrophoretic surveys in a variety of organisms (e. g. Lewontin and Hubby, 1966; Harris, 1966; O'Brien and McIntyre, 1969; Selander and Yang, 1969; Selander et. al., 1969a, b; and see Lewontin, 1974 for a comprehensive review) have indicated proportions of loci polymorphic in the order of 30%; probably this is a considerable underestimate since electrophoresis only detects a restricted class of enzyme variants. Both variation of electrophoretic conditions and heat denaturation studies have uncovered from 2 to 6 times as many genetic variants at the polymorphic xanthine dehydrogenase (Bernstein et. al., 1973; Coyne, 1976; Singh et. al., 1976), octanol dehydrogenase (Singh et. al., 1975), and α - glycerophosphate dehydrogenase (Johnson, 1976) loci in Drosophila, but the pattern to date is that heterozygosity of monomorphic loci remains little altered by refined techniques (Beckenbach and Prakash, 1977; Coyne and Felton, 1977). Two alternative explanations for this high degree of genetic variability have been advanced: the first argues electrophoretic variants are adaptively neutral, and are maintained by a balance of stochastic processes such as migration and random drift (Shaw, 1965; Kimura, 1968; Kimura and Ohta, 1971a, b; King and Jukes, 1969). Both logical considerations concerning the molecular nature of the protein variants and a considerable body of population genetics theory support this view. The second hypothesis states the polymorphisms are maintained by a form of balancing selection

Polymorphism maintained by balancing selection?

On what basis can we discriminate between the two alternatives?

The first possible approach is to derive expectations of gene frequency distribution under the null hypothesis of selective neutrality and compare this to the observed distribution. The original Kimura - Crow (1964) "infinite alleles" model of selective neutrality, in which an infinite number of novel allelic variants are generated by mutation and their fate determined by random drift in a finite population, predicts that heterozygosity should be related to the product of mutation rate and population size. Clearly any combination of these parameters could generate a given observed heterozygosity, but in general the range of heterozygosity is such that the neutralist hypothesis requires population sizes of all organisms studied to date to be within a factor of four of each other; this is quite unreasonable (Lewontin, 1974). Modification of the model to one more appropriate to the analysis of electrophoretic variants (the "ladder-rung" or "infinite state" model, Ohta and Kimura, 1973, 1974), while tending to make the predicted allele frequencies under neutrality somewhat more uniform, does not qualitatively alter the above conclusion. Derivation of the sampling theory under the infinite alleles neutrality model and subsequent definition of statistical tests by Ewens (1972) and Johnson and Feldman (1973) lead to the rejection of the null hypothesis of neutrality for the published data considered, but neither test is very powerful, nor is the model used applicable to electrophoretic data or in situations in which there is population subdivision and migration. Lewontin and Krakauer (1973) have developed a test based on gene frequency data which embodies the concept that since within a given population different loci share the same evolutionary history such that random events would be expected to have similar effects on all of them, if there is no selection the variances of gene frequencies

over space or time should not differ significantly among loci. Lewontin and Krakauer therefore calculated the expected standardized variance of gene frequencies under the assumption of neutrality as a basis for their test. This test has been fairly widely applied to demonstrate selection, but Ewens and Feldman (1975) and Robertson (1975) have shown that it is only applicable under stringent conditions (such as complete panmixia) which may not be biologically reasonable; departure from these conditions reduces considerably the power of the method. Moran (1976) has compared the distribution of gene frequencies in a finite population in which both the processes of mutation and selection are operative with that derived assuming mutation only - the result is that for any given combination of parameters the two distributions are indistinguishable.

It is unlikely that in the absence of relevant mathematical models and appropriate tests of neutrality further comparisons of observed and expected gene frequency distributions will resolve the controversy. It is necessary therefore to obtain direct experimental evidence of the action of selection; one way in which this may be accomplished is to detect departures from Hardy-Weinberg equilibrium within one generation, or over several stages of the life history. Although this is not a particularly sensitive test, differential survival of heterozygotes over time has been observed at the TO locus in mussels (Koehn et. al., 1973), at the EST-2, EST-3, G6PD, and PGM-1 loci in Fundulus, a marine fish (Mitton and Koehn, 1975), and at the PGI locus in butterflies (Watt, 1977). Marinkovic and Ayala (1975a,b) also found differences in several fitness components for experimentally constructed genotypes at a total of 5 electrophoretic loci. On the other hand, Yamazaki (1971) could not detect genotypic differences at

the EST-5 locus in a series of well-designed experiments, nor could Mukai (1977) at the ADH, αGPDH, or EST-6 loci.

A more sensitive experimental approach is to observe gene frequency changes in independent populations over several generations. If the populations are sufficiently large to exclude drift and the gene frequency changes are consistent in direction and magnitude the evidence would favour support of a selective hypothesis. This type of investigation has been adopted in the form of gene frequency perturbation experiments in laboratory populations of Drosophila (Powell, 1973; Fontdevila et. al., 1975; Bijlsma and van Delden, 1977). In every case initial high and low gene frequencies converged upon an equilibrium value similar to the frequency of the gene in nature. However, Yardley et. al. (1977), in a similar experiment, found results consistent with the interpretation that the α- amylase locus is selectively neutral. There are few natural studies of this sort, but Berger (1971) found allele frequencies at 5 polymorphic loci in 7 populations of Drosophila melanogaster separated both temporally and spatially were remarkably similar, as were allele frequencies at the LAP locus in two species of mussel occupying the same physical and biotic environment (Koehn and Mitton, 1972).

There is now available a large body of evidence demonstrating the existence of correlations between gene or genotype frequencies and environmental parameters over a wide range of species, enzyme loci, and environments. Such associations are indicitive of the action of selection. Application of multivariate statistical analyses to gene frequency in conjunction with climatographic and geographical data has revealed strong genotype-environment associations (Johnson et. al., 1969; Kojima et. al., 1972; Hamrick and Allard, 1972; Johnson

and Schaffer, 1973; Rockwood-Sluss et. al., 1973; Tomaszewski et. al., 1973; Bryant, 1974; Schaffer and Johnson, 1974; Taylor and Mitton, 1974). More recently specific genotype-environment interactions have been reported in which single environmental correlates of particular gene frequency changes are established (Schopf and Gooch, 1971; McNaughton, 1974; McKechnie et. al., 1975; Nevo and Bar, 1975; Koehn et. al., 1976; Corbin, 1977; Saul et. al., 1978).

The detection of persistent linkage disequilibrium is evidence of selection, since under the neutrality hypothesis one would expect any linkage disequilibrium to decay with time. Attempts to detect linkage between electrophoretic markers have been by and large unsuccessful. The utility of the method is at any rate somewhat limited as it requires knowledge of the history of the examined population both in time and numbers.

Correlations between gene frequencies at homologous polymorphic loci in closely related species would not be expected in the absence of selection, and furthermore indicate that organisms adapt to patterns of environmental heterogeneity in the same manner. Borowsky (1977) has developed a relevant test statistic and found that, although appropriate examples are rare, results of this analysis applied to published data are consistent with the hypothesis that natural selection is a determinant of allelic frequency in natural populations.

A major problem is that none of the methods above described are capable of demonstrating direct selection on the enzyme locus concerned; it is always a viable alternative explanation that neutral enzyme loci are linked to a putative selected locus. It is certainly not even clear whether allozyme frequencies are non-randomly associated with inversion polymorphisms known to be selected (Zouros, 1976;

Watanabe, 1977; Voelker et. al., 1978). Demonstration of direct selection depends critically on the relationship of a specific selective agent to the function of a given enzyme. An indication that this might be possible came from the initial observation of Kojima et. al. (1970) that glucose metabolizing enzymes using an internal substrate were substantially less polymorphic than non-glucose metabolizing enzymes utilizing variable external substrates. This finding has been subsequently substantiated and modified (Richmond, 1972; Singh, 1976; Latter, 1975; Myers, 1978), but it is clear that enzymes categorized according to any of several subjective classifications based on function reveal differing degrees of polymorphism in the various classes.

It was Clarke (1975) who first outlined and applied an experimental design relevant to the detection of direct selection, although the basic tenet had been previously recognized (Koehn et al., 1971). One must first comprehensively analyze biochemically gene products of alternate alleles; then, utilizing the combined knowledge of the function of the enzyme, the nature of the observed differences, and the ecology of the organism, postulate a selective factor. The next step is to test experimentally predictions made under a logical hypothesis relating mechanistically the selective factor and gene product; and, finally, the observed pattern in natural populations should be re-examined and interpreted in the light of the experimental results. The alcohol dehydrogenase (ADH) locus in Drosophila melanogaster has been investigated in this manner (Clarke, 1975; Oakeshott, 1976; van Delden et. al., 1978; Cavener and Clegg, 1978). The two electrophoretic alleles, "fast" (F) and "slow" (S) differ in the following biochemical properties : F is nearly twice as active as S,

but is less thermally stable. F and S differ as well in substrate specificity. Given the function of the ADH enzyme, which is the oxidation of environmental alcohols, it is reasonable to hypothesize alcohols, particularly ethanol, as selective agents. One would therefore predict that (1) increasing the concentration of environmental ethanol would favour the F allele, (2) selection favouring F should increase the activity of the population as a whole, (3) heat shock should favour the S, cold shock the F allele, and that (4) the selective effects of different alcohols should be related to their enzyme differences in vitro. All predictions have been confirmed in the laboratory; furthermore, the existence of latitudinal clines of gene frequency in natural populations is consistent with temperature being one relevant component of selection, and the high frequency of the F allele in winery populations of flies indicates the importance of alcohol as a selective agent. A similar series of experiments on the amylase locus (de Jong and Scharloo, 1976) yield the same qualitative conclusion.

Given, then, that the balance of evidence favours the hypothesis that polymorphisms are maintained by "some form of balancing selection", the question arises as to what exactly is the "form", or mechanism, of selective maintenance.

Genetic variance maintained by heterozygote advantage?

Initially heterosis, the well-known phenomenon in which the F_1 hybrid of two inbred lines is unconditionally superior to either parental line, was presumed the major force responsible for sustaining many single-locus polymorphisms. Overdominance at the enzyme level may occur if either the heterozygote properties are unique and outside

the range of homozygote properties, or because the heterozygote contains two and sometimes three different gene products, which provide an intermediate phenotype but bestow a biochemical diversity that becomes adaptive in a varying external or internal environment. The major theoretical problem with this model is that if one assumes loci act independently (Lewontin and Hubby, 1966) then the number of loci that could be under such simultaneous selection is restricted by the magnitude of the segregational load incurred. However, this argument becomes less valid under models of "soft" selection (Wallace, 1975) in which fitness is not an absolute genotypic value but is determined by the relative ranking of organisms with respect to some variable under specified environmental conditions. Genotypic value thus becomes both density and frequency dependent. If one superimposes the fitness function appropriate to the model of soft selection over the ranking of individuals according to their heterozygosities, and propose a threshold value beyond which all individuals are equally fit, then the number of polymorphisms that can be maintained increases as a function of population size; it has been claimed that under this model all the genetic variation currently observed and more can be maintained in a population of moderate size (King, 1967; Milkman, 1967; Sved et. al., 1967; Sved, 1975; Wills, 1978). Effective genetic load is also substantially reduced if one supposes, as does Lewontin and others, that loci respond to selection in correlated blocks (Franklin and Lewontin, 1970; Wills et. al., 1970; Lewontin, 1973; and Wills and Miller, 1976; see Clegg et. al., 1972, for a good example). A further argument against heterosis as a mechanism promoting widespread polymorphism may be the reduction in its effectiveness with extreme equilibrium gene frequencies or small effective population sizes

(Robertson, 1962; Bulmer, 1974).

In spite of considerable effort to detect overdominance at single loci, only a few suspected cases have been reported. In addition to the time-worn example of increased malarial resistance conferred upon individuals heterozygous for the "sickling" haemoglobin variant, and a similar heterozygous advantage associated with the G6PD locus, Richmond and Powell (1970) found a significant excess of heterozygotes at the tetrazolium oxidase locus of Drosophila paulistorum; Koehn et. al. (1973) detected differential survival of heterozygotes at the same locus in mussels; Marshall and Allard (1970) found excess of heterozygotes at a total of six loci in two natural populations of the wild oat, Avena barbata; and Hebert (Hebert et. al., 1972; Hebert and Ward, 1976) established a marked excess of heterozygotes at esterase and malate dehydrogenase loci in large permanent populations of Daphnia magna.

With such findings one is never confident, however, that the excess of heterozygotes is due to selection at the observed enzyme locus, or whether the selected locus is closely linked to the electrophoretic variant. This associative overdominance is almost certainly the explanation of the results of Wills and his colleagues (Wills and Nichols, 1971; Wills, 1972; Wills et. al., 1975), in which Drosophila pseudoobscura were inbred for several generations to make the background genotype as homozygous as possible while remaining heterozygous at either the ODH or EST-5 loci. After 12 generations the animals were tested on stress media (octanol and KCl, respectively), and a significant excess of heterozygotes was observed in ODH males. This effect disappeared after 38 generations, presumably because the continued inbreeding programme had succeeded in breaking down the

close linkage disequilibrium existing between ODH and the putative selected locus. The experiment was, however, instrumental in demonstrating that single-locus heterosis for enzyme variants may well be conditional on presenting the organism with an appropriate stress, and that in outbred organisms many single-gene heterotic systems may have selection coefficients sufficiently small that they are masked by the rest of the genome. These factors, plus the insensitivity of the χ^2 test used to detect departures from Hardy-Weinberg equilibrium, the mixed age structure and/or local inbreeding of populations, the Wahlund effect, and heterosis only involving differences in fecundity may collectively conspire against our power to detect heterosis in nature or in the laboratory (Berger, 1976). Even so, the scarcity of supportative evidence as compared to the total amount of effort expended to detect the phenomenon leads one to seriously doubt the ubiquity of heterotic selective maintenance.

Genetic variance maintained by frequency-dependent selection?

If polymorphism is generally not maintained by heterozygote superiority, then what is the alternative? The theoretical possibility of frequency-dependent selection, in which an allele is at a selective advantage when it is rare but is effectively neutral at equilibrium (or of its close relative, density-dependent selection, in which genotypes have differential competitive abilities at varying population densities), is well established (Clarke, 1972; Cockerham et. al., 1972; Hedrick, 1972; Bulmer, 1974). This mechanism is particularly attractive since a stable equilibrium is possible in the absence of heterosis, and there is no genetic load at equilibrium (Kojima, 1971). [It may be appropriate to specify at this point the precise definitions of

the terms "density-" and "frequency-" dependent; "hard" and "soft" selection. Wallace (1975) has lucidly categorized the various modes of selection as follows: "hard" selection is both density and frequency independent; "soft" selection is density and frequency dependent; "frequency-dependent" selection is frequency dependent, density independent; and "density-dependent" selection is density dependent, frequency independent. Only under hard selection does segregational load impose restrictions on the number of polymorphisms a population can maintain. Clearly the modes of selection are not mutually exclusive within a population - different polymorphisms may be separately influenced by the various selection regimes.]

Kojima and his colleagues have concentrated on detecting frequency-dependent selection for inversion karyotypes and enzyme loci in laboratory populations of Drosophila, with some success. For several pairs of inversions (Tobari and Kojima, 1967), the esterase-6 locus (Yarborough and Kojima, 1967; Kojima and Yarborough, 1967; Huang et. al., 1971) and the alcohol dehydrogenase locus (Kojima and Tobari, 1969), the pattern observed was that as gene frequencies diverged in either direction from the equilibrium value, the corresponding rare homozygous genotype was at a selective advantage for fitness components (egg to adult survivorship, fecundity), whereas at equilibrium values no differences between the genotypes could be detected. However, the generality of the mechanism is again questionable, since the study considered by the authors to be the best demonstration of its action involved a rather unusual direct genotype-environment interaction in the form of larval conditioning of the medium (Bryant, 1974), and the results are not consistently repeatable (Dolan and Robertson, 1975). Factorial experiments to determine the relative

contribution of density and genotypic composition to selection (Birley and Beardmore, 1977; de Benedictis, 1977) find frequency-dependent selection is the stronger force, but may be modified by an interaction with density. Anxolabehère (1976) contends from the results of her experiments using the sepia locus of Drosophila melanogaster that heterosis may even be frequency-dependent!

One final point concerning the detection of frequency-dependent selection has been emphasized by Christiansen et. al. (1977). Fitness estimates derived from genotypic frequencies determined at the same stage of development in two successive generations are only the true total fitnesses if and only if selection is complete at the time of observation. With post-observational selection the fitness estimates will give the impression of frequency-dependent selection favouring the rare genotype, even if the true fitnesses are constant. So the discovery of frequency-dependent fitness estimates may be interpreted as a case in point of post-observational selection as well as true frequency-dependence, and refined experimental analyses of selection are necessary to distinguish the alternatives.

Variance maintained by environmental heterogeneity?

In any discussion of this sort one is inevitably drawn to the intuitively appealing argument that environmental heterogeneity both in space and time is sufficient to maintain genetic variability. The idea is certainly not new and dates back at least to Dobzhansky (1951; see also Cain and Sheppard, 1954). We shall discuss below the theoretical framework which has been constructed about this hypothesis.

Models of spatial variability in selection intensity

It was Levene (1953) who first formally investigated the effects

of spatially varying selection pressures on genetic polymorphism, by analyzing a deterministic, one locus, two allele diploid model in which an infinite population is considered to be distributed among several niches. The model assumes there is initially random mating among the members of the population and random distribution of zygotes among the available habitats, followed by a pattern of differential selection characteristic of each niche; each niche contributes a fixed proportion of survivors to the population (in other words population size is independently regulated in the separate niches). Mating is again at random in the entire population the following generation. Levene was consequently able to derive the conditions under which the trivial equilibrium gene frequencies 0 or 1 are unstable; these are conditions for a protected polymorphism since loss of either allele by selection alone is impossible. A sufficient condition for a stable equilibrium of two alleles is that the weighted harmonic means of the fitness values of the heterozygotes in each niche be greater than that of either homozygote, which requires overdominance in at least one of the niches. However, this condition is not necessary and a stable equilibrium may occur over a restricted range of allelic frequencies for which the marginal overdominance requirement is met with no overdominance in any niche.

Since Levene was by his own admission considering the worst possible case for equilibrium, much of the subsequent work has been directed towards examining the consequences of relaxing the original model restrictions. Prout (1968) has demonstrated that using Levene's model it is possible to state more general sufficient conditions for polymorphism - a protected polymorphism is even possible if one allele is completely dominant in all environments. Introducing a measure of

habitat loyalty (in which the organisms return preferentially to their original habitat) or habitat selection (in which each organism chooses to live in the habitat in which it is most fit) favours the maintenance of genetic polymorphism (Deakin, 1966; Maynard-Smith, 1970; Christiansen, 1974; Taylor, 1976), and relaxes conditions for the existence of equilibria at which there is no segregational load (Taylor, 1975). The original model conditions for polymorphism are less stringent if one restricts migration among niches (Maynard-Smith, 1970; Bulmer, 1972; Christiansen, 1974; Karlin, 1977a), or decreases the spatial correlation among niches (Gillespie, 1974a). However, contrary to Levene's original expectation, random mating within each niche rather than over the entire population does not alter the conditions for the maintenance of heterozygosity (Strobeck, 1974), nor does changing the order of the migration, selection, and mating processes (Bulmer, 1972; Karlin and Kenett, 1977). Increasing the effective number of niches in the Levene population subdivision structure beyond two habitats does not qualitatively or quantitatively change the equilibrium conditions (Karlin, 1977b), but changing the model assumption of soft selection (independent density regulation within each niche) to a model of hard selection (in which the density of the population as a whole is regulated) decreases the likelihood of polymorphism (Christiansen, 1975).

The original Levenemodel and the variations thereof discussed above considered an infinite, deterministic, diploid, one locus, two allele model. The qualitative conclusions remain unaltered in various extensions to haploid (Gliddon and Strobeck, 1975), stochastic (Pollack, 1974), and finite (Hedrick, 1978) models, and may be established in greater generality by a completely different method of analysis

(the "method of small parameters" of Karlin and McGregor, 1972a, b). Furthermore, the multilocus analysis of Gillespie and Langley (1976) of a "Random Levene Model", in which fitnesses at several loci are assigned at random to an effectively infinite number of patches, demonstrates that although there is no linkage disequilibrium, negative correlations across environments give rise to correlations in fitnesses between alleles at two different loci, thus enhancing their overall fitness due to the reduction in the variance of fitness. Large numbers of such loci form correlation groupings which increase the probability of polymorphism over that predicted by single-locus theory.

It is therefore apparent that the conditions for polymorphism in a spatially subdivided population are robust to departures from the assumptions governing their derivation, and are such that they are likely to be met by natural populations.

Models of temporal variation in selection intensity

The family of mathematical models generated by consideration of the effect on the maintenance of genetic variability of temporal variation in selection intensity is more diverse than those previously discussed concerning the effect of population subdivision. There are several approaches to the problem: one may consider either infinite or finite models, and within each of these categories the nature of the environmental variation may be random (stochastic) or cyclical (deterministic), and the genetic system adopted either haploid or diploid. In general the treatment of the infinite models is to derive the conditions for a protected polymorphism, that is, for which the gene frequencies 0 and 1 are unstable; while for the finite models one is concerned rather with "transient" polymorphism and

computes the expected time to fixation and probability of survival of a newly arisen mutant, and compares this to the distribution obtained under the hypothesis of either constant selection or neutrality.

Dempster (1955) initially showed that with random variation in selection intensity quasifixation of a haploid genotype in an infinite population is certain for the allele with higher geometric mean fitness. This conclusion has been examined by Gillespie (1972, 1973a) to include the case of overlapping generations and autocorrelated fitnesses; again the geometric mean fitness of the alleles determines which one will quasifix (i.e. approach a gene frequency of 0 or 1) and temporal variation of fitness has no tendency to maintain polymorphism in the haploid case.

Haldane and Jayakar (1963) first quantified the conditions for protected polymorphism in the case of a large random mating diploid population segregating for two alleles with full dominance to be that the arithmetic mean of the fitnesses of recessives in different generations should be greater than one, and their geometric mean less than one. Gillespie (1973) gives the condition of geometric mean overdominance for the general case, and concluded this was independent of the nature of the autocorrelation of the environment. Subsequent investigation of the latter point, however, revealed that it is contingent upon the environments being weakly autocorrelated; for moderately and strongly autocorrelated environments the tendency is for heterozygosity to decrease, since increasing the autocorrelation has the effect of decreasing the variance such that heterozygote superiority in geometric mean fitness becomes impossible (Gillespie and Guess, 1978). Hartl and Cook (1973, 1975) and Karlin and Leiberman (1974) have restated the geometric mean overdominance

condition, and provided conditions for polymorphism when the relative fitnesses of the two homozygotes are perfectly correlated. Levikson and Karlin (1975) have demonstrated generally, using a diffusion approximation, that when geometric mean overdominance holds, an equilibrium distribution of gene frequencies exists. When the fitnesses recur cyclically rather than at random, the appropriate treatment is a deterministic analysis, but the conditions for protected polymorphism are yet again superiority of geometric mean fitnesses of heterozygotes (Hoekstra, 1975; Nagylaki, 1975). If one considers a pattern of cyclical selection to which both haploid and diploid phases of the life cycle are subject, the conditions for polymorphism are broader than those of the model of selection on the diploid phase only (Ewing, 1977).

Diffusion approximations have traditionally been used to describe the effects of variable selection in finite populations. The first such analysis was by Kimura (1954), who proved that random fluctuations in selection intensity about a mean of zero (a "white noise" environment) facilitates the near fixation or near loss of alleles, thus tending to reduce rather than maintain genetic heterogeneity. Ohta (1972) has shown that the probability of fixation of a mutant gene is reduced by random fluctuations in selection intensity, and that furthermore if the ratio of the mean to the variance of the selection coefficient is small, a mutant gene, even if selected against, becomes fixed in the population like a selectively neutral mutant. Jensen (1973) proved that the ultimate probability of fixation of a rare gene is increased by variability in selection. Karlin and Levikson (1974) more comprehensively formulated a haploid model allowing for variability in the selection coefficients of both alleles as well as for correlation

between the two, and showed that the variance in selection expression reduces and mitigates the mean effects of selection differentials, so that the fixation probability of the abundant allele is diminished. To add further to the confusion, the diploid model analyzed by Avery (1977) in which the selection coefficients of the homozygotes are allowed to vary with equal variance while the fitness of the heterozygote is kept fixed is such that increasing the variance of the selection coefficients of the homozygotes increases heterozygosity, the effect being largest when the selection coefficients of the homozygotes are fully correlated. Here it is found that a small average heterozygote advantage together with a reasonable degree of variance in the coefficients can cause an unexpectedly large amount of heterozygosity to be maintained.

Fortunately the source of the conflicting conclusions has been established by Narain and Pollak (1977). They show that the discrepant results on the fixation probability are due to the difference in the forms of the mean as well as variance functions for the change in gene frequency adopted in the diffusion approximation approach. Exact computations on the finite Markov chain give a general expression for the fixation probability of a gene, in the haploid case, allowing for the variability in selection coefficients as well as for the correlation between the two. The previous results are special cases of this general expression, with the exception of those of Kimura (1954) and Ohta (1972), who chose incorrect expressions for the mean and variance of change in gene frequency. Computer simulations by Hedrick (1974, 1976) are in general agreement with theoretical studies of fixation probabilities: temporal environmental variation is not always effective compared to models of constant selection and neutrality

at maintaining heterozygosity, but ~~that~~ with strong negative autocorrelation or cyclically varying environments polymorphism is retained more readily.

In general, then, the conditions for polymorphic stability through temporal instability of fitnesses are more stringent than those for spatial variability, and are more sensitive to departures from model assumptions. Even small differences in assumptions concerning the nature of the variable selection pressures can radically alter the conclusions, both quantitatively and qualitatively.

Models of spatial and temporal variation in selection intensity

More realistic models encompass those situations in which a population is subdivided into niches, but each niche is subject to temporal environmental fluctuations. Such models have only recently been considered. Gillespie (1975, 1976a) has analyzed the Island Model of Wright, in which the stochastic element derives from random fluctuations in the environment rather than from genetic drift. He found that with temporal fluctuations increasing migration makes polymorphism more likely, whereas for spatially differentiated patches with no temporal variation, reducing migration increases the probability of polymorphic stability. Therefore in populations experiencing simultaneous spatial subdivision and temporal fluctuations, there should be selection for an optimum rate of migration. Hedrick (1978) and Scott and McClelland (1977) find that the combination of the multideme model and cyclical temporal variation greatly increases the likelihood of polymorphism over that predicted if either model is considered separately.

It is interesting in this context that Gillespie (Gillespie and Langley, 1974; Gillespie, 1976b, 1977) has developed a general model

in which tempero-spatial variation can account for the levels of enzyme variability in natural populations. The model assumes complete additivity of enzymatic activity and the existence of a concave function which relates enzyme activity to Darwinian fitness. The enzyme activities are then allowed to fluctuate at random in time and space under a wide variety of models of environmental variation; the interesting conclusion is that the condition for polymorphism - that the variance in the environment must be large enough to override mean difference in activity between homozygous genotypes - is fairly insensitive to assumptions made about the structure of the environment. Since this model also allows the maintenance of an arbitrarily large number of alleles in a randomly fluctuating environment, it demonstrates the generality of the potentially powerful effect simple variation in environmental parameters may have on the maintenance of genetic variation.

Models of environmental "grain"

Adaptation to a heterogeneous environment need not necessarily involve genetic polymorphism at the level of the population, but may equally well be the result of well-developed individual homeostasis or some other mechanism (Lewontin, 1957; Bradshaw, 1965; Levins, 1968), and therefore any theoretical assessment of the generality of this type of selection pressure in maintaining genetic variation must also take account of the circumstances under which one may reasonably expect alternative modes of adaptation. The work of Levins (1962, 1963, 1965, 1968) has been fundamental in this respect. He found he was able to define optimum ecological strategy in terms of a "fitness set" representation, based on the organisms' perception of the environmental variation. If the fitness set is convex (in other words if the envir-

onmental range is small as compared to individual homeostasis), the optimum strategy is a single intermediate generalist phenotype of moderate fitness in each niche; a concave fitness set (in which the difference between niche optima is large compared to individual tolerance) may bring about two distinct patterns of response, dependent on the type of environmental variance. In a spatially heterogeneous (Levins, 1962, 1963) or fine-grained (Levins, 1968) environment the predicted optimal strategy is of a single phenotype specialized to the more frequent niche, whereas temporal variation (Levins, 1962, 1963) or a coarse-grained (Levins, 1968) pattern results in a polymorphic strategy in which fitness is optimum in each niche (i.e. a "mixed" polymorphism of specialized types). The concept of environmental "grain" introduced here refers to the scale on which the organism experiences the environmental variability; all previous models discussed were "coarse-grained" in that each individual spends the selectively relevant part of its life within a single patch. In an environment of the finest possible grain each individual samples all patches in the proportion in which they occur.

There is one further adaptive strategy - that of response to selection in a fluctuating environment, or environmental tracking (Levins, 1965, 1968), which must be viewed as distinct from the mixed strategy polymorphism mentioned above. For such a response to selection to be adaptive it is necessary that the environment be both highly variable and autocorrelated, which implies organisms with a short generation interval will be more likely to depend upon environmental tracking for their adaptation. Levins distinguishes the two kinds of adaptive polymorphism on predicted magnitude of genetic variance, and nature of genetic variance: the optimal genetic variance for

fitness of a mixed polymorphism is approximately equal to the environmental variance on the same scale and is largely epistatic and stable, whereas the genetic variance of "response to selection" polymorphism is at least an order of magnitude lower and is largely additive and easily altered (Levins, 1965).

Further development of the fitness set theory reiterates and extends the model predictions presented above: polymorphism is less likely in fine-grained environments, and long-lived, large, mobile species are more likely to experience their environment as fine-grained (Levins and MacArthur, 1966; Templeton and Rothman, 1974); optimal habitat selection increases the probability that a polymorphic strategy will be adaptive (Bryant, 1973); there should be a positive correlation between average heterozygosity per individual and increasing environmental variance (Bryant, 1973); and that the evolution of short-term homeostatic mechanisms is expected in fine-grained environments with low or negative autocorrelation (or short cycle length) while long term homeostasis should develop in those organisms repeatedly subjected to fine-grained environments in which the autocorrelation is high (or cycle length long) (Templeton and Rothman, 1978).

It is important to recognize that the fitness set approach is not directly applicable to arguments pertaining to the maintenance of genetic polymorphism, since the theory is concerned with optimal adaptive strategy and genetic systems do not optimize. We have previously seen that for the case of temporal variation in an infinite population, the haploid genotype with the highest geometric mean fitness always wins, even though it may have the lowest mean fitness. There is thus no tendency to maintain polymorphism, even though a

polymorphic population would have a higher average fitness (Felsenstein, 1976). However, since the concept of environmental grain is potentially relevant, some work has been done to determine its role in the maintenance of genetic variation - the qualitative conclusion is the same as that from the original fitness set analysis; that is, polymorphism is less likely in a fine- than coarse-grained environment (Gillespie, 1974b; Strobeck, 1975; Templeton, 1977). In fact, Strobeck (1975) has shown that the conditions for polymorphism in a fine-grained environment are the same as for the constant selection model: overdominance.

To summarize, it appears theoretically sound that differing selection pressures caused by environmental variability are sufficient to promote and maintain genetic variance in natural populations, at least in the range of models considered. The major qualitative conclusions of these investigations are presented in Table 1; for a comprehensive review consult Felsenstein (1976). However, one of the main reasons for pursuing this line of research is that the search for experimental evidence demonstrating the relative importance of overdominance as a source of genetic variance has not been particularly successful, despite the equally sound theoretical possibility of its potential as a mechanism promoting polymorphism. We shall now, therefore, consider what evidence has been advanced in support of the hypothesis of selective maintenance of genetic variability through environmental variance. It is not suprising that the majority of the data is in the form of gene frequencies of electrophoretic variants.

"Natural" experiments

The most obvious implication of the thesis is that one should find in nature a correlation between genetic and environmental variance,

TABLE 1

SUMMARY OF QUALITATIVE EFFECTS OF VARIOUS FACTORS ON MAINTENANCE OF GENETIC VARIATION IN POPULATIONS UNDERGOING VARIABLE SELECTION (ADAPTED FROM HEDRICK, 1978)

SPATIAL MODELS	MAINTENANCE OF GENETIC VARIANCE	
CONDITION: HARMONIC MEAN OVERDOMINANCE	LOWER	HIGHER
HABITAT SELECTION	NO	YES
MIGRATION RATE	HIGH	LOW
SPATIAL CORRELATION BETWEEN NICHES	HIGH	LOW
MODE OF SELECTION (SENSU CHRISTIANSEN)	HARD	SOFT
ENVIRONMENTAL GRAIN	FINE	COARSE
TEMPORAL MODELS		
CONDITION: GEOMETRIC MEAN OVERDOMINANCE		
GENETIC MODEL	HAPLOID	DIPLOID
STOCHASTIC ENVIRONMENTAL VARIATION	HIGH AUTOCORRELATION $r \to 1$	LOW AUTOCORRELATION $r \to -1$
DETERMINISTIC ENVIRONMENTAL VARIATION	LONG CYCLE	SHORT CYCLE
MIGRATION RATE	LOW	HIGH
ENVIRONMENTAL GRAIN	FINE	COARSE
SPATIAL-TEMPORAL COMPARISONS		
	TEMPORAL	SPATIAL
	TEMPORAL	TEMPORAL + SPATIAL
	SPATIAL	TEMPORAL + SPATIAL

such that high environmental variability is associated with increased genetic variance, and, conversely, that genetic variance should decrease in a constant environment. Several instances have been reported of low allozyme variability associated with "constant", "stable", or "narrow-niched" environments: Pocket gophers, which inhabit a relatively constant subterranean niche, have an observed heterozygosity (proportion of loci heterozygous per individual) of only 4.7% (Nevo et. al., 1974; see also Selander et. al., 1974); three bee species, which develop and spend much of their adult lives under uniform conditions of temperature and humidity, were monomorphic for 22 enzyme loci sampled (Snyder, 1974); Avise and Selander (1972) found cave dwelling fishes of the genus Astyanax varied in heterozygosity from 0 - 8%; similarly cave dwelling crickets (Ceuthophilus gracilipes) were monomorphic at 80-92% of 26 loci studied; and the heterozygosity of Drosophila busckii, described as occupying a "narrow seasonal and food niche" has a heterozygosity of only 4.4%, as compared to values 2-3 times higher in other Drosophila species (Prakash, 1973b).

Babbel and Selander (1974) examined the relationship between "ecological amplitude" and genetic variability by comparing genetic variability in edaphically restricted and widespread plant species; one pair of species exhibited the requisite inverse relationship between level of genic variability and degree of edaphic restriction, the other did not. Levinton (1973) has demonstrated decrease of genetic variability (measured in terms of both effective and absolute numbers of alleles) in molluscs corresponding to a decrease in environmental variability (depth of burial in sediment, and depth of water). In his analysis of allozymic variation in 4 species of toads arranged

in a graded series from subterranean narrow-habitat specialist to terrestrial broad-habitat generalist, Nevo (1975) found that mean number of alleles per locus, proportion of loci polymorphic per population, and proportion of loci heterozygous per individual correspondingly ranged from 1.13-1.86, .095- .56, and .029- .169 respectively. This genetic variation neither correlates with geographical age nor population size and structure, but is positively correlated with environmental heterogeneity and unpredictability. Steiner (1977) found a significant positive association between heterozygosity and number of oviposition sites utilized by 18 species of Hawaiian Drosophila.

More convincing, perhaps, are the studies which synthesize information gathered from many different groups. Selander and Kaufman (1973) have tabulated observed heterozygosities of a number of vertebrate and invertebrate species, and found a marked difference between them - vertebrates (on the whole large mobile animals) are on the average 2.5 times less genetically variable than the invertebrates (on the whole small and relatively immobile). This was interpreted as supportative of Levins' (1968) contention that small immobile animals are more likely to experience their environment as sets of alternatives (coarse-grained) and hence respond by a strategy of mixed polymorphism; the important point is that environmental uncertainty must be considered in relation to the demographic and other ecological parameters of organisms. Bryant (1974) used principal component analysis to discern relationships between patterns of genetic variation in heterozygosities of statistically correlated ensembles of loci and measures of within-year environmental variability (computed from climatological data) in several groups of animals. 70% of the geographic variation in hetero-

zygosities could be accounted for by the measures of environmental variation. In his recent extensive review of allozymic variation in natural populations of 243 species of plants and animals, Nevo (1978) found that upon classifying the estimates of genic variation among major taxonomic groupings, climatic or life zones, habitat generalists and specialists, and mainland and island populations, the amounts of heterozygosity varied non-randomly among loci, populations, species, habitats, and life zones, and were strongly correlated with ecological heterogeneity. Generalists have consistently significantly higher genetic variation than specialists; this comparison crosses all taxonomic categories, life zones, and breeding systems, and is consistent with the hypothesis that physical and biotic variables are major determinants of genetic variation.

On the other hand, it is now well established that many deep sea invertebrates, supposed to inhabit a highly predictable, stable environment, have amounts of variability in the order of 18% heterozygous loci per individual (Doyle, 1972; Gooch and Schopf, 1972; Ayala et. al., 1975). Indeed, one of the most polymorphic organisms studied so far (with an average heterozygosity of 20.2%), the killer clam, is in fact a specialized organism inhabiting an environment of high trophic stability (Ayala et. al., 1973). Here it is generally argued (Valentine, 1976) that this very physical and biological predictability allows the organisms to perceive their environment as coarse-grained in minor spatial variations and hence they pursue a strategy of genetic specialization, whereas those organisms living in temporally highly seasonal environments adopt a fine-grained strategy such that generalist alleles are selected. One must be cautious in accepting such post hoc explanations; it is perhaps more reasonable

to expect individual cases may fail to show associations between habitat diversity and gene frequency, since we are considering the delicate balance of complex forces in a dynamic system of biological interactions one predicts deviations from generalization under special circumstances. But only by determining the rule can we understand the exceptions.

It is appropriate to mention at this point the idea that variability is associated with habitat diversity ("niche width") is a persistent theme in the ecological literature (e.g. Van Valen, 1965) and has been tested by examining morphological variation. Thus there is no problem in ascertaining that the observed phenotypic variation is subject to natural selection, but the results cannot be interpreted in genetic terms. Nevertheless, it is worthwhile noting that the evidence is not conclusive: the data of Van Valen (1965, 1970) and Rothstein (1973) indicate a positive association between variability of bill characters in several bird species and variety of foods eaten, while Soulé and Stewart (1970) found no such correlation. Sabath (1974) obtained similar negative results (but using enzyme polymorphism) when he plotted genetic variability against niche breadth for 11 species of drosophilid flies.

There are three additional corollaries of the hypothesis that genetic and environmental variability should be positively associated subject to experimental verification. The first is that populations in the centre of a species range should be more polymorphic than marginal populations, the thesis being that peripheral populations have considerably narrower niches (Dobzhansky, 1951). Although certainly true for inversion polymorphism, this pattern is not observed for electrophoretic polymorphisms of Drosophila pseudoobscura (Prakash

et. al., 1969) or Drosophila robusta (Prakash, 1973a) - levels of enzyme variability are similar for both types of populations. However, there is reason to believe chromosomal variability cannot be equated with genic variability as determined by electrophoresis, and in addition one should emphasize the temporal instability of marginal environments - low inversion heterozygosity may allow greater genetic flexibility by permitting constant synthesis of novel genotypes through recombination (Carson, 1959; Lewontin, 1957, 1974; Tabachnick and Powell, 1977). The allozyme data is thus not necessarily inconsistent with the hypothesis. A second prediction which follows from the work of Levins (1968) is that of a negative correlation between degree of genetic differentiation in the population and individual homeostasis; this has been demonstrated in Drosophila (Levins, 1969; see also Beardmore, 1960), and in honeybees (Bruckner, 1976), but Brown and Feldmeth (1971) found no difference in thermal tolerance and ability to acclimate to environmental temperature between populations of desert pupfish from thermally constant springs and thermally fluctuating streams and marshes. The final corollary to the theory of genetic adaptation to spatially heterogeneous environments is that one would predict the development of behavioural preferences of genotypes to different habitat types. The data of Taylor and Powell (1977) of microgeographic and temporal genetic differentiation in natural populations of Drosophila persimilis with respect to chromosome inversion and enzyme polymorphism are consistent with this interpretation.

Even if it could be said at this point that the balance of evidence appears to favour acceptance of the hypothesis, would we then be in a position to do so? Unfortunately, the answer is no, since

inherent in the above approach are many ambiguities which necessitate more caution in interpretation. The first objection is that in any given situation an observed lack of variance cannot be unequivocally assigned to constancy of environmental factors; variance reduced by stochastic processes can never be eliminated as an alternative hypothesis since generally little or nothing is known of the past history or breeding structure of the community. Under the hypothesis of selective neutrality of electrophoretic variants the amount of genetic variance present in a natural population will depend critically on the effective population size (N) and the time since last bottlenecking (T). The previously mentioned case of genetic variation in pocket gophers is illustrative. Pocket gophers of the Thomomys "talpoides" complex are genetically depauperate, the low heterozygosity of approximately 5% superficially indicating adaptation to a constant subterranean niche. However, further investigation of the related Thomomys bottae, inhabiting the same niche, revealed an average 33% of loci polymorphic, a level of variability greater than the average rodent (Patton and Yang, 1977). The explanation here is that while the "talpoides" group have undergone recent population size bottlenecks with the consequent development of small reproductively isolated groups, the pattern is one of lack of severe bottlenecking, and gene flow between adjacent T. bottae populations. The high level of genetic variability observed in the deep sea invertebrates may similarly be accounted for by the exceptionally long time these populations have been stable. It is clearly impossible to discriminate the two explanations in the absence of estimates of the critical parameters; this will only be possible under very special circumstances.

A second problem is that the use of electrophoretic markers is

in some ways a questionable technique with which to investigate this question, for although the evidence generally indicates a form of balancing selection maintains these polymorphisms, specific instances of high isozyme variability can always be argued to be adaptively neutral. On the other hand, if selection is operating it is never clear whether it is for electrophoretic alleles or at some other level.

A further problem associated with the study of natural populations in situ are the inadequate measures of environmental variability employed. Environments are usually described qualitatively as "constant" or "stable" (which may be interpretated to mean significant temporal fluctuations are absent), or "wide-niched" or "narrow-niched" (in reference to the spatial heterogeneity), but appropriate measures are rarely taken (but see daCunha and Dobzhansky, 1954; Bryant, 1974). More importantly, the effects of spatial and temporal heterogeneity are of necessity completely confounded in any natural situation; thus the evidence of variability in the deep sea as being inconsistent with the hypothesis is less convincing when one realizes the existence of temporal stability does not preclude the possibility of spatial heterogeneity.

Even allowing that the demonstrated associations are true, such correlations give no indication of causality (Cain and Sheppard, 1954): are polymorphic populations, by virtue of their polymorphism, more adapted to exploit a greater variety of niches, or does the variability of niches enable the survival of different types? Is the population polymorphic because each individual is a generalist or because it is composed of a mixture of specialist types? Expressed in genetic terms, is heterozygosity per se selected in a fluctuating environment, or

does diversifying selection for alternate alleles increase polymorphism in a spatially heterogeneous habitat? Only if the latter is true does the data support the original hypothesis, although if the alternative is correct the implication is certainly equally as interesting. On what basis can the alternatives be distinguished? If disruptive selection is indeed operating in the majority of situations, one would expect excess of homozygotes and microdifferentiation, such that genetically different types survive differentially in the available habitats. Thoday (1959) initially demonstrated the potential of disruptive selection as a force maintaining genetic variation in laboratory populations of Drosophila. That rapid microdifferentiation can occur in the field over suprisingly short distances even in the face of considerable gene flow has been repeatedly demonstrated by Antonovics, Bradshaw, and their colleagues (see Antonovics, 1971, for relevant references); by Snaydon (Snaydon, 1970; Snaydon and Davies, 1972, 1976; Davies and Snaydon, 1976) in his analysis of the effect of sharp environmental discontinuities in soil type on morphological variation in Anthoxanthum; and McKenzie and Parsons (1974) have shown microdifferentiation of Drosophila melanogaster in response to alcohol in the environment. Such studies are encouraging in that they indicate the power of disruptive selection in promoting genetic divergence in nature, but they are representative of only a restricted class of adaptation - that of evolution in a spatially heterogeneous environment with perhaps rather stronger selection pressures than are commonly encountered.

Laboratory studies

Laboratory investigations, while perhaps lacking the generality of the previous type of study, have been attempted on the basis that

they are less subject to ambiguous interpretation. Lewontin (1958) was one of the first to demontstrate experimentally decline of genetic variation through time under constant conditions when he observed that a balanced chromosomal polymorphism of Drosophila was lost after 26 generations of laboratory culture under invariant conditions of food, moisture, and temperature. On the other hand, the electrophoretic survey of O'Brien and McIntyre (1969) revealed amounts of enzyme variability in an established stock of Drosophila comparable to that of natural populations.

There have been several other studies on the effect of experimental environmental manipulation on the genetic structure of laboratory populations : Beardmore (1961) maintained cage populations of Drosophila pseudoobscura under diurnally constant and fluctuating temperature regimes, and found after several years the populations living in the variable environment had significantly higher additive genetic variance for fifth sternite chaeta number than those in the constant environment. A similar experiment set up with populations containing the Arrowhead and Chiricahua gene arrangements gave equivalent results at generation 37; in addition tests of larval viability under a range of constant and fluctuating conditions at generation 19 showed the "variable" populations more fit in all environments (Beardmore and Levine, 1963). The chromosomal polymorphism drifted towards fixation in both sets of populations and thus appeared to have no selective advantage in the diurnally thermally oscillating environment; earlier work (Beardmore et. al., 1960) had shown populations polymorphic for these gene arrangements were superior in Darwinian fitness (measured in terms of constituent components) than the corresponding monomorphic ones. Long (1970) subsequently modified the experimental design to

discern the effects of long and short term periods of thermal oscillations (i.e. coarse- or fine-grained temporal variability), this time using Drosophila melanogaster and measuring fitness components (productivity, competitive ability, egg-adult survivorship) in response to environmental stress; overall population fitness was greatest in the more variable environment (that undergoing the short-term fluctuations). However, it should be noted that in none of these experiments has an attempt been made to follow the change in genetic variance through time, or, more critically, to partition the genetic and non-genetic components of fitness. Failure to do the latter leaves open the possibility that any increase in variability observed is solely attributable to the increased environmental variance.

More recently, Powell (1971) and McDonald and Ayala (1974) subjected populations of Drosophila to different experimentally controlled levels of environmental heterogeneity, and found that the amount of enzyme variability maintained was greater for the populations in the more variable environments - but these experiments suffer from the criticism that selection was for inversion heterozygosity rather than the allozymes themselves. Powell and Wistrand (1978) subsequently repeated the experiment using an inversion-free stock of Drosophila pseudoobscura and obtained the same qualitative results: populations in the variable environments maintained a higher level of heterozygosity than those under constant conditions, and furthermore the transition from environmental constancy to 1 variable factor, whether it be physical (variation of medium or temperature) or biotic (presence or absence of a competing Drosophila species) has a greater effect on the maintenance of genetic variation than the addition of further variables. On the other hand, a similar experiment by Minawa

and Birley (1978) failed to detect a difference in heterozygosities among three different environmental treatments, although there did appear to be directional selection for some of the enzyme loci. Gibson and Bradley (1974) subjected Drosophila melanogaster to both constant and fluctuating temperatures, and found that additive genetic variance of sternopleural chaeta number was the same in both environments, but that the environmental variance, logically enough, increased under variable temperature conditions. These contrasting results indicate the potential of environmental heterogeneity promoting maintenance of genetic variability, but it is clear the response of a population may be contingent on additional factors which have yet to be delineated.

An alternative approach is to study the effect of known alterations of the genetic material on habitat diversity. One of the earliest experiments of this type (Waddington et. al., 1954) showed marked differences in behaviour of Drosophila mutant stocks with respect to choice of an environment which varied according to either temperature, humididy, or luminosity - a fairly convincing demonstration of habitat selection, which may lead to the maintenance of stable polymorphism. Shugart and Blaylock (1973) found radiation -induced genetic variability increased the niche width of highly inbred populations of Drosophila simulans, where "niche width" was measured in terms of competition with the similarly treated sibling species, D. melanogaster.

Such laboratory investigations circumvent many of the objections raised concerning the genetic analysis of natural populations in situ - the environmental variability is under control so that there is no possibility of confounding different sources of this variance;

under appropriate conditions one is able to identify the cause of a given change; the genetic constitution of the populations subjected to the differing environmental treatments can be replicated; and since it is not necessary to score electrophoretic characters, the special problems associated with interpretation are not encountered. It should be possible to experimentally create environments in which temporal and spatial, fine- and coarse-grained types of variability are separable, and thus to differentiate the effectiveness of each class of variability in promoting adaptation. One may then measure the response of the population to the environmental heterogeneity by scoring meristic traits and partitioning the observed phenotypic variance by the techniques of quantitative genetics into genetic, environmental, and genotype-environment interaction components.

It is the purpose of this investigation, therefore, to design and conduct an experiment such that the data generated will be appropriate to the analysis of the relevant factors determining the relationship (if any) between genetic and environmental variation. Specifically, we shall attempt to assess the following questions:

-Is genetic variance maintained in a variable environment?

-If so, then what is the relationship between degree and type of genetic variance and pattern of environmental heterogeneity experienced? i.e. What is the effect on genetic variance of fine- and coarse-grained, temporal and spatial environmental variation?

-Is there genotype-environment interaction? The presence of an interaction component would indicate it is disruptive selection and fixation of alternate alleles in different environments, rather than selection for heterozygosity per se,

which is operative.

-Is there development of habitat selection in spatially heterogeneous environments?

-Is there a difference in homeostatic abilities of populations under different environmental treatments?

-Is there a difference in fitness components of populations experiencing different environmental treatments?

Materials and Methods

Population

Professor A. Prevosti of the University of Barcelona kindly provided a sample of 158 Drosophila melanogaster males and 122 females, trapped in an orchard in the Canary Islands. A cage population was established in September, 1975 from the wild-caught females and the eggs and larvae present in the vials containing the flies. Within four generations of arrival in the laboratory the population was characterized for electrophoretic variation (J. McKay, PhD thesis) and for the presence of inversions (S. Tsakas, pers. comm.). Of the 9 enzyme systems analyzed, 5 were polymorphic, with an average heterozygosity of 17%. Inversions were present on the second (break points 33D - 35B) and third (break points 88D - 89A) chromosomes, but covered a very small area and were at low frequency (a total of 7 inversions in 109 chromosomes).

The cage was allowed to attain equilibrium population density, then in March, 1976, 8 replicate population cages were initiated, each with a sample of 500 males and 500 females from the original Prevosti cage, obtained by allowing the animals to lay eggs in large numbers and collecting the emerging adults to populate the replicate cages.

Treatment of population cages

The environmental variable imposed was presence of alcohol in the culture medium. The "control" medium was standard Edinburgh agar-molasses-killed yeast medium (UFAW Handbook, 1967); the alcohol medium contained 15% absolute ethanol by volume, which was added after the control food had cooled sufficiently to prevent undue

evaporation of the ethanol, and then mixed thoroughly. Population cages were maintained by the weekly addition of two half pint milk bottles containing 100ml of the appropriate medium, and the concomitant removal of the two bottles added three weeks previously. All cages were maintained at 25°C, and attained population densities of approximately 3000 - 4000 individuals.

The cages were subjected to four different patterns of environmental variation, with two replicates of each pattern. Thus there were two control cages, which received weekly two fresh bottles of control (C) medium. Two cages intended to simulate spatial variation in the environment received one bottle to which 15% alcohol was added (A medium) and one bottle of C medium weekly. Two further cages received on alternate weeks two bottles of C medium, then two bottles of the A medium - a pattern of short-term temporal variation (i.e. within the life-time of an individual). Finally, long-term temporal variation in the environment was simulated by changing the medium type every four weeks; that is, for four weeks these two cages received C medium, and the following four weeks A medium. It was therefore intended that comparison of the treated cages to the control would indicate whether environmental variability had any effect on genetic variance, whereas comparisons among treatments would show relative efficiencies of temporal and spatial, long- and short-term (or fine- and coarse- grained) patterns in producing the effect. Replicate differences would be the result of genetic drift. (Problems arise in that the treatment definitions are only strictly true for the adults; for example, in the "spatial variation" cages females have a choice of two habitats in which to lay their eggs, but the larvae develop subsequently in only that niche). After one year a sample of

500 males and 500 females was taken from one of the control cages to initiate a new cage, which was then treated as a short-term temporally varying environment. The purpose of this was to generate some information relevant to understanding whether environmental variability maintains genetic variability at the level initially present in the population, or whether genetic variability can actually be increased under these conditions.

For experimental purposes adult flies were not removed directly from the cages, rather the population was sampled by allowing animals to lay eggs for 24 hours in fresh bottles; thus all population comparisons were made on animals which had developed under the same environmental conditions. Both males and females were collected as virgins from these bottles, and allowed to mature for three days in vials to which a paste of live yeast had been added. As far as possible the age of the animals was controlled to be three days at the time of scoring for a character and subsequent mating.

Sternopleural bristle number

The mean, phenotypic variance, and additive genetic variance of this character was calculated for each of the populations in two successive years. In the first series of measurements, a sample of approximately 140 males and 140 females from each cage and on each type of medium was scored for the sum of sternopleural chaeta number on right and left sides; the 20 highest scoring and the 20 lowest scoring males and females were selected and mated assortatively. These 40 pairs of flies laid eggs for 72 hours on both C and A medium; 40 offspring (10 males and 10 females from each of the two medium types) from each mating were scored for the character. This design gives

the most efficient estimate of heritability, calculated from the regression of mean offspring score on midparental value (Hill, 1970). The standard error of the heritability estimate is simply the standard error of the regression coefficient (see Sokal and Rohlf, 1969). An estimate of the genotype-environment correlation is obtained using the "cross-regressions" of offspring raised on one substrate on parents raised on the alternate medium. If b_{cc} and b_{aa} are the offspring-parent regressions on control and alcohol media respectively, and b_{ca} and b_{ac} are the "cross-regressions", then the genotype-environment correlation is

$$r_{GE} = \sqrt{\frac{b_{ca}\, b_{ac}}{b_{cc}\, b_{aa}}}$$ (Reeve, 1955)

with approximate standard error

$$SE(r_{GE}) = \frac{1 - r_{GE}^2}{\sqrt{2}} \cdot \sqrt{\frac{SE(b_{cc})\ SE(b_{aa})}{b_{cc}\, b_{aa}}}$$ (Robertson, 1959).

The mean ($\bar{X}$) and phenotypic variance (V_p) can be estimated from the original population sample. Since this estimate is independent of the heritability estimate, the additive gentic variance (V_a) can be estimated from the product of the heritability and phenotypic variance

$$V_a = b\, V_p$$

with standard error

$$SE(V_a) = \sqrt{V_p^2\ VAR(b) \ + \ b^2\ VAR(V_p)}\ , \text{ where } VAR(V_p) = 2V_p^2 / N + 1.$$

The following year an additional series of measurements of sternopleural bristle number were undertaken, utilizing a different design. Here random samples of 200 males and 200 females from each cage were scored for both sternopleural and abdominal (the sum of segments 4 and 5 or 5 and 6 of males and females, respectively)

chaeta number. Of these, a random sample of 50 males and 50 females were mated at random. Ten male and 10 female offspring were scored for both characters in each full-sib family. Only control medium was used throughout this experiment.

Population means and phenotypic variances of the two characters, as well as the phenotypic correlation between them, can be obtained from the original samples from the population cages. As in the previous set of data, information from the offspring-parent regression allows calculation of the heritabilities of both sternopleural and abdominal bristle number, and their genetic correlation; multiplication of the independently obtained phenotypic variance by the heritability gives an estimate of the additive genetic variance. One may also analyze the data as a simple one-way random effects analysis of variance for each character separately; the heritability is then twice the intraclass correlation coefficient. The between family variance component (σ^2_a) is resolvable into half the additive genetic variance, one quarter of the dominance variance, and variance due to common environment and epistatic interactions (Falconer, 1960). Thus we may use σ^2_a and V_a estimated by the regression analysis to estimate an upper limit to the dominance variance (V_d) to be

$$V_d = 4\sigma^2_a - 2V_a ,$$

with standard error

$$SE(V_d) = \sqrt{16\ VAR(\sigma^2_a) + 4\ VAR(V_a)} ,$$

where

$$VAR(\sigma^2_a) = \frac{1}{n^2}\left[\frac{2MS_B}{df_B + 2} + \frac{2MS_W}{df_W + 2}\right] ,$$

MS_B, df_B, MS_W, df_W are the mean squares and degrees of freedom of the between and within family components of the analysis of variance,

respectively, and n is the number of individuals per family.

Abdominal bristle number

A random sample of 400 males and 400 females was obtained from each cage in the first year, and 200 males and 200 females in the second year. Abdominal chaeta number was scored on the fourth and fifth segments of the males, and fifth and sixth segments of the females. Population means and phenotypic variances of the sum of the two segments were obtained from this data; in addition, since it is known that the genetic correlation of bristle number between any two abdominal segments is 1, and the environmental correlation 0 (Reeve and Robertson, 1954), the phenotypic correlation provides an estimate of the heritability of the trait (Frankham, pers. comm.). This is because the phenotypic correlation of two characters, X and Y, is equal to the product of the square root of their heritabilities and genetic correlation, plus the product of the square root of the environmental variances and the environmental correlation.

$$r_p = h_X h_Y r_G + e_X e_Y r_E \qquad \text{(Falconer, 1960)}$$

If r_E is 0 and r_G is 1, as for the case of abdominal bristles, then r_p reduces to $h_X h_Y$, or h^2. The standard error of the estimate is the standard error of a correlation

$$SE(r_p) = \sqrt{\frac{1 - r_p^2}{N - 2}} \qquad \text{(Sokal and Rohlf, 1969).}$$

Body weight

This trait was analyzed in the second year only, utilizing a design appropriate to the detection of genotype-environment interaction. As this character was expected to be influenced more by environmental

conditions than either bristle character, particular care was taken to ensure culture conditions were as similar as possible for parents and offspring. Samples of eggs were collected from the population cages by allowing the flies to lay for several hours in small petri dishes filled with culture medium. Fifty eggs were then transferred using a stylet probe onto the surface of either C or A medium in a vial; animals emerging from these vials were used as parents. One hundred and fifty three-day-old males were weighed to the nearest .05mg - of these the 40 highest scoring and 40 lowest scoring individuals were mated at random to unmeasured females. The females were then allowed to lay approximately 50 eggs in each of 4 vials, 2 of each type of medium. Ten males from each of the 4 vials were then weighed en masse. Twice the regression of son on sire is an estimate of the heritability of the trait on any one of the two alternative media, whereas consideration of the "cross-regression" gives an estimate of the genotype-environment correlation, as described above. Additive genetic variance estimates are again from the product of phenotypic variance estimated from the populations and heritability of the trait.

Survivorship

Samples of eggs were collected from each population cage by allowing the animals to lay for several hours in petri dishes filled with culture medium. Fifty eggs were then transferred into each of 20 vials containing C and 20 vials containing A medium. The proportion of flies emerging is the measure of egg-to-adult survivorship.

Productivity

Several random samples of 10 fertilized females from each of the population cages were introduced into specially constructed "laying chambers", consisting of a small petri dish filled with C medium, over which was placed an inverted 100 ml plastic beaker. The number of eggs laid was counted after 24 hours as a measure of "productivity".

Habitat loyalty

Similar laying chambers were employed for the measurement of habitat loyalty, with the exception that the petri plate was divided into quadrants, 2 of which contained C and 2 of which contained A media. Flies were collected from each cage by introducing simultaneously bottles containing C or A media, and collecting emerging adults from each bottle. The parents of these animals had chosen to lay their eggs on a particular substrate; the question then asked by giving these individuals a choice of substrate in the laying chamber is whether they will preferentially return to the substrate that their parents had chosen and in which they had developed. Specifically, several samples of 10 fertilized females which had developed on C (or on A) media were given a choice of either C or A on which to oviposit, and the number of eggs in each quadrant was counted after 24 hours. Since behavioural phenotypes are notoriously variable, care was taken to ensure all populations were measured on any given day, and over a period of several days. The results were arranged in a 2 x 2 contingency table; actual estimates of degree of habitat loyalty can be obtained by the method of Doyle (1976).

Developmental homeostasis

The measure of developmental homeostasis employed was the variance of the difference in score between left and right sides for sternopleural bristles, and between adjacent terminal segments for abdominal bristles (Reeve, 1960). Two hundred males and 200 females were scored for each population.

Alcohol dehydrogenase phenotype

Samples of at least 192 flies from each of the population cages were electrophoretically typed at the alcohol dehydrogenase (ADH) locus by horizontal starch gel electrophoresis using a Tris-versene-borate continuous buffer system at pH 8.0 (Shaw and Prasad, 1970); staining of gels was according to the procedure outlined in Shaw and Prasad (1970) at room temperature for thirty minutes using ethanol as substrate.

Results

Sternopleural bristle number

Population means and phenotypic variances of sternopleural chaeta score are presented for each year separately in Tables 2 and 3, and are summarized in Table 4. There are no significant differences in mean according to substrate, between replicates, treatments, or successive years, although a consistent sexual dimorphism is observed - males have on the average .9 bristle less than females. Phenotypic variances are not significantly different between substrate, sex, or replicates within treatments, but there is a marked treatment effect in that both temporally varying cages have an increased phenotypic variance compared to either control or spatially heterogeneous cages; this pattern persists over time. Such an increase is attributable to either an increase in genetic variance, or an increase in environmental variance, or both.

Heritability estimates obtained by offspring-parent regression are given, for the first year, in Table 5. Heritabilities are not significantly different between substrates or between replicates within populations, but are significantly higher in the three environmentally varying populations than the control. This could only occur with constant additive genetic variation over all populations if the phenotypic variances were reduced in the variable populations. In fact, we have seen the opposite has happened; therefore the additive genetic variance estimates (V_a) obtained even more clearly indicate increased genetic variance in the treated cages relative to the control - spatially heterogeneous populations have approximately twice, and temporally varying populations approximately 2.6 times the

control V_a; these differences are significant. Furthermore, the comparison of spatial environmental variation to temporal heterogeneity, although not significant, is suggestive that the former is less effective in the maintenance of genetic variability. There is no difference in the level of V_a between the two temporally varying populations with different cycle length. It may be appropriate to mention at this point that the criterion for a decision of significance of a comparison of either heritability or additive genetic variance is whether the upper and lower limits of the smaller and larger estimates, respectively, overlap. As the distributions of these values are not known, no formal test of significance (other than the distribution-free Chebychev's Inequality) is available, so these limits are attached by simply $\pm$ twice the standard error of the estimate. Even though one cannot produce an exact significance level by this method, non-overlapping ranges thus obtained imply an upper limit to the probability of Type I error to be 0.05.

Estimates were obtained of heritability by offspring-parent regression, and also of additive genetic variance in the second year, and the pattern of results described above is again repeated (Table 6). Both heritabilities and additive genetic variances are significantly different in the cages exposed to environmental variation from the control, with spatially heterogeneous cages having twice, and temporally varying cages three to four times the control V_a; additive genetic variation in the populations experiencing spatial environmental heterogeneity is less than the two temporally varying populations, this time significantly so; and still no difference is apparant between the populations undergoing temporal variation of different periodicity. The single cage which had been under control conditions

for the first year, and short-term temporal variation the second, had a heritability and additive genetic variance of sternopleural bristle number equivalent to the two control cages. Thus environmental variation can only maintain genetic variation at a level initially present in the population, and does not have the power to actually increase genetic variation by differentially selecting genotypes specifically adapted to the different niches - at least on the time scale on which we are operating. Comparisons of heritabilities estimated by offspring-parent regression and from the intraclass correlation coefficient obtained by analysis of variance are not significantly different, although the standard errors associated with the latter estimates are understandably much larger (Table 7). Consequently estimates of the dominance variance for this character are not significantly different from zero or from each other in any of the populations.

Estimates of the genotype-environment correlation average .9 overall and are, with one exception, significantly different from one; however, the pattern of variation between replicates, within treatments is greater than the overall between treatment variation in the estimates (Table 8). This is suggestive that although to some extent different genes affecting bristle number are operating in the two environments, they are not instrumental in determining the adaptation of the populations to the different patterns of environmental variation. If microdifferentiation and specific adaptation to each habitat were a major factor promoting the increased genetic variance in the variable environments, the genotype-environment correlation would have decreased, on the average, in the treated populations. Since this is not observed, we have no evidence it is disruptive

Table 2

Population means ($\bar{X}$) and phenotypic variances (V_p) of sternopleural bristle score - Year 1.

Replicate	Medium	Sex	N	$\bar{X}$ (± S.E.)	V_p
Control					
I	C	♀	133	19.158 (.173)	3.998
		♂	133	18.173 (.175)	4.053
	A	♀	129	18.140 (.151)	2.934
		♂	141	17.482 (.168)	3.966
II	C	♀	134	18.866 (.157)	3.320
		♂	133	17.805 (.148)	2.901
	A	♀	133	19.895 (.169)	3.777
		♂	129	18.783 (.177)	4.046
Spatial Variation					
I	C	♀	134	18.925 (.166)	3.679
		♂	133	18.391 (.198)	5.240
	A	♀	111	18.550 (.170)	3.195
		♂	88	17.693 (.205)	3.709
II	C	♀	136	20.757 (.186)	4.689
		♂	135	19.882 (.216)	6.299
	A	♀	92	19.348 (.210)	4.076
		♂	90	18.911 (.249)	5.588
Temporal Variation, Short-term					
I	C	♀	132	20.197 (.234)	7.213
		♂	133	18.263 (.211)	5.938
	A	♀	135	20.430 (.214)	6.202
		♂	133	19.474 (.237)	7.448
II	C	♀	134	19.246 (.187)	4.683
		♂	133	18.414 (.209)	5.805
	A	♀	133	19.850 (.205)	5.614
		♂	134	18.552 (.197)	5.212
Temporal Variation, Long-term					
I	C	♀	135	19.422 (.234)	7.380
		♂	136	18.588 (.214)	6.229
	A	♀	133	18.805 (.207)	5.719
		♂	134	18.425 (.202)	5.464
II	C	♀	134	19.627 (.204)	5.559
		♂	134	18.567 (.189)	4.804
	A	♀	133	21.218 (.225)	6.732
		♂	133	20.211 (.242)	7.789

Table 3

Population means ($\bar{X}$) and phenotypic variances (V_p) of sternopleural bristle score - Year 2.

Replicate	Sex	N	$\bar{X}$ (± S.E.)	V_p
Control				
I	♀	200	19.900 (.139)	3.889
	♂	200	18.365 (.151)	4.545
II	♀	200	19.265 (.125)	3.143
	♂	200	18.420 (.133)	3.551
Spatial Variation				
I	♀	200	18.390 (.163)	5.327
	♂	200	17.610 (.124)	3.093
II	♀	200	20.120 (.132)	3.483
	♂	200	19.410 (.142)	4.042
Temporal Variation, Short-term				
I	♀	200	19.420 (.179)	6.406
	♂	200	18.815 (.208)	8.674
II	♀	200	18.875 (.162)	5.266
	♂	200	18.080 (.158)	5.009
Temporal Variation, Long-term				
I	♀	200	19.615 (.193)	7.464
	♂	200	19.010 (.194)	7.518
II	♀	200	19.715 (.173)	5.964
	♂	200	18.545 (.179)	6.410
Control / Temporal Variation, Short-term				
I	♀	200	19.895 (.149)	4.426
	♂	200	19.125 (.143)	4.080

Table 4

Between year comparison of population means and phenotypic variances of sternopleural bristle score, averaged over replicate and substrate.

	Year 1			Year 2		
Sex	N	$\bar{X}$ (± S.E.)	V_p	N	$\bar{X}$ (± S.E.)	V_p
Control						
♀	529	19.015 (.081)	3.507	400	19.583 (.094)	3.516
♂	536	18.061 (.084)	3.742	400	18.393 (.101)	4.048
Spatial Variation						
♀	473	19.395 (.091)	3.910	400	19.255 (.105)	4.405
♂	446	18.719 (.108)	5.209	400	18.510 (.094)	3.568
Temporal Variation, Short-term						
♀	534	19.931 (.105)	5.928	400	19.148 (.121)	5.836
♂	533	18.676 (.107)	6.101	400	18.448 (.131)	6.842
Temporal Variation, Long-term						
♀	535	19.768 (.109)	6.348	400	19.665 (.130)	6.714
♂	537	18.948 (.106)	6.072	400	18.778 (.132)	6.964

Table 5

Sternopleural bristle score: Genetic parameters obtained by offspring-parent regression. NF is the number of families, and NT the total number of individuals scored. Year 1.

Replicate	Substrate	NF	NT	h^2 (± SE)	V_p	V_a (± SE)
Control						
I	C	31	604	.416 (.030)	4.026	1.675 (.126)
	A	41	815	.422 (.057)	3.450	1.456 (.232)
II	C	35	691	.377 (.044)	3.111	1.173 (.169)
	A	32	617	.385 (.045)	3.912	1.506 (.219)
Spatial Variation						
I	C	34	669	.583 (.035)	4.460	2.600 (.273)
	A	35	700	.628 (.053)	3.452	2.168 (.284)
II	C	36	720	.589 (.041)	5.494	3.236 (.358)
	A	43	859	.699 (.076)	4.832	3.378 (.510)
Temporal Variation, Short-term						
I	C	30	546	.624 (.059)	6.576	4.103 (.527)
	A	40	800	.654 (.044)	6.825	4.464 (.486)
II	C	30	587	.595 (.037)	5.244	3.120 (.333)
	A	32	640	.588 (.037)	5.413	3.183 (.342)
Temporal Variation, Long-term						
I	C	35	690	.637 (.047)	6.805	4.335 (.490)
	A	33	660	.623 (.049)	5.592	3.484 (.407)
II	C	31	601	.673 (.048)	5.182	3.487 (.390)
	A	36	708	.579 (.039)	7.261	4.204 (.460)

Table 6

Sternopleural bristle score: Genetic parameters obtained by offspring-parent regression. NF is the number of families, and NT the total number of individuals scored. Year 2.

Replicate	NF	NT	h^2 (± SE)	V_p	V_a (± SE)
Control					
I	40	787	.287 (.095)	4.217	1.210 (.409)
II	34	662	.487 (.128)	3.347	1.630 (.444)
Spatial variation					
I	42	835	.645 (.072)	4.210	2.716 (.359)
II	46	918	.767 (.072)	3.763	2.886 (.339)
Temporal variation, Short-term					
I	42	833	.747 (.067)	7.540	5.632 (.643)
II	40	785	.751 (.057)	5.138	3.859 (.398)
Temporal variation, Long-term					
I	42	840	.765 (.101)	7.491	5.731 (.858)
II	45	896	.836 (.045)	6.187	5.172 (.458)
Control / Temporal variation, Short-term					
I	36	720	.320 (.121)	4.253	1.361 (.523)

Table 7

Sternopleural bristle score: Comparison of heritability estimated from offspring-parent regression and from the intraclass correlation of analysis of variance. σ^2_a is the added variance between families and V_d the estimate of dominance variance. Year 2.

	From Regression		From Intraclass Correlation		
Replicate	h^2 (± SE)	V_a	h^2 (± SE)	σ^2_a	V_d
Control					
I	.287 (.095)	1.210	.341 (.082)	.693	.352
II	.487 (.128)	1.630	.559 (.115)	1.216	1.604
Spatial variation					
I	.645 (.072)	2.716	.602 (.106)	1.098	-1.040
II	.767 (.072)	2.886	.574 (.100)	1.273	- .680
Temporal variation, short-term					
I	.747 (.067)	5.632	.768 (.116)	3.020	.816
II	.751 (.057)	3.859	.969 (.122)	2.350	1.682
Temporal variation, long-term					
I	.765 (.101)	5.731	1.001 (.119)	4.013	4.590
II	.836 (.045)	5.172	.939 (.115)	2.733	.588
Control / Temporal variation, short-term					
I	.320 (.121)	1.361	.466 (.102)	1.092	1.646

Table 8

Genotype-environment correlations (± standard error) of sternopleural bristle score on control and alcohol media.

Population	Replicate I	Replicate II
Control	.925 (.013)	.785 (.039)
Spatial variation	.999 (.000)	.737 (.035)
Temporal variation, Short-term	.861 (.018)	1.014 (*)
Temporal variation, Long-term	.950 (.007)	.955 (.005)

(*) Standard error undefined

selection which has created the between population differences in additive genetic variance.

Abdominal bristle number

Population means and phenotypic variances of abdominal chaeta score are presented for each year separately in Tables 9 and 10, and are summarized in Table 11. There are no significant differences in mean between replicates, treatments, or successive years, although a sexual dimorphism in score is again observed, males scoring on the average 6.3 bristles less than females. Variances are also not significantly different between replicate, treatment, or year, although it appears the long-term temporal variation cages are somewhat less variable than the others. This pattern is in direct contrast with that observed for the sternopleural bristle scores; here an examination of the phenotypic variances alone indicates that if the populations experiencing environmental heterogeneity have, in fact, significantly higher levels of genetic variation than the control populations, it could only be possible by a concomitant and substantial reduction in the environmental variance, perhaps accomplished by increased individual homeostasis in the variable environments.

Estimates of heritability obtained by correlation of scores in two successive abdominal segments (Table 12) are suggestive that such an hypothesis is unnecessary; heritabilities are remarkably constant between sex, replicate, population, and year. One cannot compute additive genetic variance from this data, as the estimates of phenotypic variance and heritability are not independent; however, the constancy of both heritability and phenotypic variance implies as well no significant difference in additive genetic variance

among populations. That this is indeed so is demonstrated when heritability is independently estimated from the regression of offspring on parent in Year 2 (Table 13) - additive genetic variances are not significantly different between replicates or among populations, although there is a persistent suggestion that the long-term temporally varying populations are less variable than the others (it may be recalled here that long cycle length is theoretically established to be detrimental to the maintenance of genetic variance). The population which was initially treated as a control population, and then subjected to short-term environmental variation in the second year, also showed heritabilities and phenotypic variances of the same order as the other populations.

The close agreement between heritability as measured by correlation and by offspring-parent regression is noteworthy, particularly since the former estimate can be accomplished in a single generation using only one quarter of the animals, and gaining twice the precision of the latter. Estimates of heritability obtained by the intraclass correlation coefficient of analysis of variance are also similar to the regression estimates, and consequently dominance variances are not significantly different from zero or each other (Table 14).

Given the totally different response of the two characters, abdominal and sternopleural bristle number, to pattern of environmental heterogeneity experienced, it is of interest to know to what extent the two traits are controlled by the same genes, or the genetic correlation between them. Phenotypic and genetic correlations between abdominal and sternopleural chaeta score are presented in Table 15, and are for the most part small and positive; not significantly different from zero or each other. The two bristle characters are

Table 9

Population means ($\bar{X}$) and phenotypic variances (V_p) of abdominal bristle score - Year 1.

Replicate	Sex	N	$\bar{X}$ (± SE)	V_p
Control				
I	♀	400	38.965 (.182)	13.282
	♂	400	32.223 (.171)	11.702
II	♀	400	39.088 (.186)	13.850
	♂	400	32.228 (.163)	10.663
Spatial variation				
I	♀	400	42.098 (.189)	14.354
	♂	400	33.710 (.165)	10.828
II	♀	384	40.552 (.214)	17.522
	♂	400	34.338 (.185)	13.733
Temporal variation, Short-term				
I	♀	352	42.219 (.204)	14.570
	♂	387	35.594 (.187)	13.501
II	♀	400	39.333 (.181)	13.145
	♂	400	33.463 (.173)	11.943
Temporal variation, Long-term				
I	♀	400	40.608 (.149)	8.851
	♂	400	34.923 (.158)	10.031
II	♀	400	39.565 (.154)	9.535
	♂	400	34.265 (.157)	9.824

Table 10

Population means ($\bar{X}$) and phenotypic variances (V_p) of abdominal bristle score - Year 2.

Replicate	Sex	N	$\bar{X}$ (± SE)	V_p
Control				
I	♀	200	38.020 (.257)	13.155
	♂	200	31.525 (.246)	12.140
II	♀	200	39.540 (.266)	14.179
	♂	200	33.110 (.223)	9.938
Spatial variation				
I	♀	200	40.300 (.239)	11.417
	♂	200	34.020 (.218)	9.507
II	♀	200	40.465 (.258)	13.356
	♂	200	33.735 (.220)	9.683
Temporal variation, Short-term				
I	♀	200	43.600 (.284)	16.121
	♂	200	36.805 (.239)	11.434
II	♀	200	39.190 (.263)	13.863
	♂	200	33.600 (.238)	11.347
Temporal variation, Long-term				
I	♀	200	39.325 (.239)	11.447
	♂	200	33.760 (.222)	9.892
II	♀	200	40.015 (.220)	9.643
	♂	200	34.585 (.209)	8.767
Control / Temporal variation, Short-term				
I	♀	200	39.605 (.236)	11.145
	♂	200	33.365 (.240)	11.489

Table 11

Between year comparison of population means and phenotypic variances of abdominal bristle score, averaged over replicates.

	Year 1			Year 2		
Sex	N	$\bar{X}$ (± SE)	V_p	N	$\bar{X}$ (± SE)	V_p
Control						
♀	800	39.027 (.130)	13.566	400	38.780 (.185)	13.667
♂	800	32.266 (.118)	11.183	400	32.318 (.166)	11.039
Spatial variation						
♀	800	41.325 (.141)	15.938	400	40.383 (.176)	12.387
♂	784	34.024 (.125)	12.281	400	33.878 (.155)	9.595
Temporal variation, Short-term						
♀	739	40.776 (.137)	13.858	400	41.395 (.194)	14.992
♂	800	34.529 (.126)	12.722	400	35.203 (.169)	11.391
Temporal variation, Long-term						
♀	800	40.087 (.107)	9.193	400	39.670 (.162)	10.545
♂	800	34.594 (.111)	9.928	400	34.173 (.153)	9.330

Table 12

Abdominal bristle score: Heritabilities obtained by correlation of score of adjacent terminal abdominal segments.

Replicate	Sex	Year 1 N	Year 1 h^2 (± SE)	Year 2 N	Year 2 h^2 (± SE)
Control					
I	♀	400	.477 (.044)	200	.521 (.061)
	♂	400	.519 (.043)	200	.464 (.063)
II	♀	400	.552 (.042)	200	.566 (.059)
	♂	400	.471 (.044)	200	.341 (.067)
Spatial variation					
I	♀	400	.522 (.043)	200	.429 (.064)
	♂	400	.478 (.044)	200	.495 (.062)
II	♀	384	.637 (.040)	200	.424 (.064)
	♂	400	.597 (.040)	200	.377 (.066)
Temporal variation, Short-term					
I	♀	352	.512 (.046)	200	.546 (.060)
	♂	387	.569 (.042)	200	.390 (.065)
II	♀	400	.512 (.043)	200	.458 (.063)
	♂	400	.541 (.042)	200	.508 (.061)
Temporal variation, Long-term					
I	♀	400	.270 (.048)	200	.349 (.067)
	♂	400	.416 (.046)	200	.358 (.066)
II	♀	400	.381 (.046)	200	.354 (.067)
	♂	400	.508 (.043)	200	.399 (.065)
Control / Temporal variation, Short-term					
I	♀			200	.439 (.064)
	♂			200	.424 (.064)

Table 13

Abdominal bristle score: Genetic parameters obtained by offspring-parent regression. NF is the number of families, and NT the total number of individuals scored. Year 2.

Replicate	NF	NT	h^2 (± SE)	V_p	V_a (± SE)
Control					
I	40	787	.523 (.107)	12.648	6.615 (1.429)
II	34	662	.418 (.129)	12.059	5.041 (1.594)
Spatial variation					
I	42	834	.492 (.084)	10.462	5.147 (.954)
II	46	918	.463 (.094)	11.520	5.334 (1.144)
Temporal variation, Short-term					
I	42	828	.417 (.092)	13.778	5.745 (1.334)
II	40	782	.473 (.108)	12.605	5.962 (1.427)
Temporal variation, Long-term					
I	42	835	.396 (.085)	10.670	4.225 (.953)
II	45	896	.325 (.113)	9.205	2.992 (1.063)
Control / Temporal variation, Short-term					
I	36	720	.199 (.118)	11.317	2.252 (1.344)

Table 14

Abdominal bristle score: Comparison of heritability estimated from offspring-parent regression and from the intraclass correlation of analysis of variance. σ^2_a is the added variance between families and V_d the estimate of dominance variance. Year 2.

	From Regression		From Intraclass Correlation		
Replicate	h^2 (± SE)	V_a	h^2 (± SE)	σ^2_a	V_d
Control					
I	.523 (.107)	6.615	.526 (.103)	2.782	-2.102
II	.418 (.129)	5.041	.562 (.116)	3.053	2.130
Spatial variation					
I	.492 (.084)	5.147	.428 (.090)	2.131	-1.770
II	.463 (.094)	5.334	.382 (.081)	2.264	-1.612
Temporal variation, Short-term					
I	.417 (.092)	5.745	.473 (.095)	3.418	2.182
II	.473 (.108)	5.962	.523 (.103)	2.774	- .828
Temporal variation, Long-term					
I	.396 (.085)	4.225	.352 (.081)	1.599	-2.054
II	.325 (.113)	2.992	.476 (.092)	2.653	4.628
Control / Temporal variation, Short-term					
I	.199 (.118)	2.252	.333 (.085)	1.593	1.868

Table 15

Phenotypic and genetic correlations (± standard error) of sternopleural and abdominal bristle scores.

Population	Replicate	r_p (± SE)	r_G (± SE)
Control	I	.093 (.050)	.155 (.180)
	II	.082 (.050)	.067 (.201)
Spatial Variation	I	.070 (.050)	.096 (.097)
	II	-.066 (.050)	.140 (.096)
Temporal Variation, Short-term	I	.197 (.049)	.202 (.095)
	II	.081 (.050)	.074 (.093)
Temporal Variation, Long-term	I	.131 (.050)	.014 (.119)
	II	.138 (.050)	.140 (.095)
Control / Temporal Variation, Short-term	I	.186 (.049)	.099 (.332)

thus genetically virtually independent, and free to respond separately to selection in divergent manners.

Body weight

Population means and phenotypic variances are given in Table 16; this character was analyzed in the second year only. Means are not significantly different between treatments or replicates, although there is a non-significant but consistent tendency for the animals which had alcohol as a substrate to be heavier than those developing on control media - "alcohol" flies were on the average .0155 mg, or 2%, heavier than "control" flies. This is consistent with the results of Clarke (1975), who also used alcohol as an experimental variable. Phenotypic variances show no discernable differences between substrate, replicate, or treatment, so if significant differences in additive genetic variances are to be observed among the populations, this must be accompanied by concomitant reductions in environmental variance. That this is indeed the case can be seen upon examination of Table 17, in which heritabilities, phenotypic, and additive genetic variances are presented for each population. Heritabilities do not vary significantly between replicate or substrate within treatments, but spatially varying populations have heritabilities twice as large, and both temporally varying populations three times as large, as the control. Similarly additive genetic variance in each environmentally heterogeneous population is three times that of the control; these differences are significant.

Estimates of the genotype-environment correlation average greater than one (Table 18); although standard errors can not be computed where the estimate exceeds unity, on the whole it must be concluded no

Table 16

Population means ($\bar{X}$) and phenotypic variances (V_p) of body weight (mg). Year 2.

Replicate	Substrate	N	$\bar{X}$ (± SE)	V_p
Control				
I	C	150	.907 (.0065)	.0063
	A	150	.951 (.0070)	.0074
II	C	150	.910 (.0073)	.0081
	A	151	.928 (.0069)	.0072
Spatial variation				
I	C	150	.903 (.0076)	.0086
	A	150	.943 (.0072)	.0078
II	C	150	.992 (.0091)	.0124
	A	150	.911 (.0071)	.0076
Temporal variation, Short-term				
I	C	150	.912 (.0058)	.0050
	A	150	.954 (.0076)	.0087
II	C	150	.939 (.0064)	.0062
	A	150	.891 (.0075)	.0085
Temporal variation, Long-term				
I	C	150	.863 (.0072)	.0078
	A	150	.952 (.0078)	.0092
II	C	150	.888 (.0072)	.0078
	A	150	.908 (.0066)	.0066

Table 17

Body weight: Genetic parameters obtained by offspring-parent regression. NF is the number of families, and NT the total number of individuals scored. Standard errors attached to the estimates of additive genetic variance are all of the order of 10^{-6}; the observed differences between populations are therefore significant. Year 2.

Replicate	Substrate	NF	NT	h^2 (± SE)	V_p	V_a
Control						
I	C	66	1320	.035 (.098)	.0063	.0002
	A	64	1280	.284 (.073)	.0074	.0021
II	C	75	1500	.268 (.065)	.0081	.0022
	A	64	1280	.202 (.119)	.0072	.0015
Spatial variation						
I	C	64	1280	.458 (.114)	.0086	.0039
	A	42	840	.478 (.129)	.0078	.0037
II	C	63	1260	.439 (.113)	.0124	.0054
	A	50	1000	.437 (.119)	.0076	.0033
Temporal variation, Short-term						
I	C	73	1460	.697 (.138)	.0050	.0035
	A	55	1100	.651 (.071)	.0087	.0057
II	C	67	1340	.632 (.117)	.0062	.0039
	A	51	1020	.594 (.140)	.0085	.0050
Temporal variation, Long-term						
I	C	73	1460	.623 (.103)	.0078	.0049
	A	64	1280	.541 (.081)	.0092	.0050
II	C	56	1120	.618 (.091)	.0078	.0048
	A	45	900	.666 (.185)	.0066	.0044

Table 18

Genotype-environment correlations (± standard error) of body weight on control and alcohol media.

Population	Replicate I	Replicate II
Control	1.525 (*)	1.264 (*)
Spatial variation	.934 (.023)	.975 (.009)
Temporal variation, Short-term	.938 (.012)	1.063 (*)
Temporal variation, Long-term	1.065 (*)	1.038 (*)

(*) Standard error undefined

evidence exists for correlations in populations undergoing environmental variation being differentially reduced compared to the control value. The same genes determining the character are operative in each environment; it does not appear diversifying selection maintains genetic variation for this particular character by selecting alternative genotypes in the two environments.

Survivorship

Examination of proportionate egg-to-adult survivorship (Table 19) on each of the two substrates and between populations reveals that survivorship on alcohol medium is reduced on the average by 12% over that on control medium; furthermore, the reduction is greater for the two control populations (16%) than for the average of the three environmentally varying populations (10%). When one compares survivorship of the three treated populations relative to that of the control, it is found that compared to the control population, animals from spatially varying populations are 18% more viable on C, and 29% more viable on A medium; those from short-term temporally variable environments have survivorship on C medium enhanced 27% relative to the control, and 30% on A medium; and the comparable results for the animals experiencing long-term temporal heterogeneity are 14% and 25% for development on C and A medium, respectively. Therefore not only are control populations less fit with respect to survivorship compared to those experiencing different patterns of environmental variation, but there has been adaptation to the presence of alcohol in the environment in those populations regularly exposed to alcohol medium as an alternative habitat.

Table 19

Proportionate survivorship on alcohol and control substrates.

Population	Replicate	Substrate C	Substrate A
Control	I	.702 (.010)	.573 (.011)
	II	.646 (.011)	.556 (.012)
Spatial variation	I	.869 (.008)	.706 (.011)
	II	.724 (.010)	.748 (.014)
Temporal variation, Short-term	I	.855 (.011)	.756 (.014)
	II	.860 (.011)	.715 (.016)
Temporal variation, Long-term	I	.770 (.013)	.703 (.010)
	II	.761 (.014)	.713 (.011)

Productivity

Analysis of variance of the second fitness component studied, productivity, indicates significant variation between populations in number of eggs laid per 24 hour period (Table 20). Computation of the least significant difference ($p = .05$) enables determination of significance of the following 4 contrasts, formulated a priori: control productivity versus that of (1) spatially, (2) short-term temporally, (3) long-term temporally varying environments, and (4) the average of the 3 variable treatments. All but the second comparison are significant. Control populations are thus also less fit with respect to this component of fitness than the average of populations experiencing environmental heterogeneity.

Habitat loyalty

Both absolute numbers of eggs laid on C and A medium by parents who had themselves developed on C or A, and the corresponding loyalty matrices, are given, for each population, in Table 21. Formal X^2 tests of association are not possible given the observed data, as each unit of observation (a single egg) is not independent - putting groups of 10 females into a "laying chamber" for 24 hours does not ensure equal contribution of each individual. In the absence, therefore, of an appropriate measure of the error variance, one is reduced to considering the point estimates of loyalty without attached standard errors; fortunately, the pattern is clear and consistent.

If there is a constant preference for one medium over the other, and this is independent of the medium upon which the parent has developed, then the trace of the loyalty matrix will be 1; this is the

Table 20

Productivity: Analysis of variance.

Source of variation	df	MS	F	
Between populations	3	86097.125	8.606*	$.01 < p < .05$
Between replicates, within populations	4	61176.125	6.115 ns	
Within populations	24	10004.875		

Mean productivity

Population	Replicate	$\bar{X}$	Replicate Average	LSD
Control	I	445.25		
	II	416.50	430.875	145.982
Spatial variation	I	660.50		
	II	506.25	583.375	
Temporal variation, Long-term	I	827.75		
	II	515.50	671.625	
Temporal variation, Short-term	I	495.25		
	II	510.50	502.875	

situation observed for both spatially varying populations. Habitat loyalty comprises the situation in which the trace of the loyalty matrix exceeds unity; substrate preference in this case is conditional upon the parental substrate, such that organisms tend to preferentially choose the medium on which they developed (and which their parents chose). None of the populations exhibit this behaviour; rather, the control and two temporally varying populations show the opposite pattern - in each case the trace of the loyalty matrix is less than unity; animals who had developed upon (and whose parents had chosen) the alcohol substrate show a distinct aversion to this medium when given the choice of C or A on which to oviposit. Therefore, the spatially heterogeneous populations exhibit a different pattern of habitat choice *from* either the control or temporally varying populations, and the direction of the difference is such that the aversion to alcohol medium having experienced it during one stage of the life cycle is overcome, thus implying more efficient resource utilization in the spatially varying environment. However, lack of evidence demonstrating positive habitat loyalty in the treated populations only is consistent with the previous observations that diversifying selection is not here a causative factor maintaining genetic variance in environmentally variable populations. It should be noted that optimum habitat choice, in which the animals preferentially choose to oviposit upon the substrate on which proportionate egg-to-adult survivorship is higher, is operative in each population.

Table 21

Numbers of eggs on C and A medium, and corresponding loyalty matrices.

Replicate	Offspring Substrate	Number of Eggs Parental Substrate C	Number of Eggs Parental Substrate A	Loyalty Matrix Parental Substrate C	Loyalty Matrix Parental Substrate A	Trace of Loyalty Matrix
Control						
I	C	1188	413	.667	.870	.798
	A	593	62	.333	.130	
II	C	1056	530	.634	.731	.903
	A	610	195	.366	.269	
Spatial variation						
I	C	1911	654	.723	.745	.978
	A	731	224	.277	.255	
II	C	1453	588	.718	.721	.997
	A	572	228	.282	.279	
Temporal variation, Short-term						
I	C	1229	506	.620	.808	.812
	A	752	120	.380	.192	
II	C	1219	410	.695	.825	.870
	A	536	87	.305	.175	
Temporal variation, Long-term						
I	C	2372	453	.716	.773	.943
	A	939	133	.284	.227	
II	C	1276	621	.663	.745	.919
	A	648	213	.337	.255	

Developmental homeostasis

An appropriate measure of developmental "noise" for characters which are bilaterally or segmentally repeated is the variance of the difference of the scores on the two sides or segments; a low variance is thus indicative of a developmentally stable, or homeostatic, population. Variances of the difference between left and right sides are given for sternopleural bristles, and between terminal adjacent segments for abdominal bristles, in Table 22. For each character there is no difference in the measure between sex, replicate, or among populations experiencing different patterns of environmental heterogeneity. These findings are not consistent with either Lewontin's (1958) contention that heterozygous populations are more homeostatic than homozygous populations, or with Levins' (1969) hypothesis that there should be an inverse correlation between degree of genetic variation and individual homeostasis.

Alcohol dehydrogenase frequencies

Allelic frequencies at the alcohol dehydrogenase locus were determined since exposure to alcohol was the environmental variable employed. Adaptation to environmental alcohol did not involve gene frequency changes at the ADH locus in the two temporally varying populations compared to the control population, although relative to the initial frequency the "fast" allele approached fixation. The polymorphism was maintained, however, at the frequency initially present, in the populations experiencing a spatially heterogeneous environment - the frequency of the F allele in the Prevosti sample was .88, and the frequency averaged over replicate in the spatially varying populations was also .88 (Table 23).

Table 22

Measurements of developmental "noise". The values given are the mean square differences in score between left and right sides for sternopleural bristles, and between adjacent terminal segments for abdominal bristles.

Population	Replicate	Sex	Sternopleural Bristles	Abdominal Bristles
Control	I	♀	2.070	4.141
		♂	1.522	4.341
	II	♀	1.561	3.959
		♂	1.795	5.256
Spatial variation	I	♀	1.305	4.565
		♂	1.597	3.220
	II	♀	1.734	5.401
		♂	2.139	4.443
Temporal variation, Short-term	I	♀	1.842	4.747
		♂	1.961	5.196
	II	♀	1.781	5.153
		♂	1.858	3.708
Temporal variation, Long-term	I	♀	1.914	5.030
		♂	2.002	4.573
	II	♀	1.909	4.638
		♂	2.067	3.748
Control / Temporal variation, Short-term	I	♀	2.065	4.355
		♂	1.597	4.668

Table 23

Alcohol dehydrogenase frequencies.

Population	Replicate	N	Frequency (F) (± SE)
Prevosti		98	.88 (.033)*
Control	I	323	.97 (.010)
	II	192	.99 (.007)
Spatial variation	I	383	.80 (.020)
	II	288	.95 (.013)
Temporal variation, Short-term	I	288	1.00 (**)
	II	192	.90 (.022)
Temporal variation, Long-term	I	192	.96 (.014)
	II	192	1.00 (**)

(**) Standard error undefined.

* Data of J. McKay, PhD thesis.

Discussion and Conclusions

We are now able to utilize the experimental evidence to empirically assess the questions initially formulated concerning the relationship between genetic and environmental variation. Is genetic variance maintained in a variable environment? The answer to this question is a qualified "yes, sometimes" - three quantitative characters were analyzed, and three patterns of response to environmental heterogeneity were observed. These patterns can be described in terms of the associations between environmental variability and the phenotypic, additive genetic, and environmental variances of the three metric traits (Table 24). The phenotypic and additive genetic variance of sternopleural bristle number is substantially and significantly greater in populations experiencing spatial and temporal environmental variation than control populations, while the environmental variance is equivalent in all populations; for this character genetic variance is certainly maintained under environmentally varying conditions. Additive genetic variance of body weight is similarly three times the level of control populations in the three variable populations; however, the phenotypic variance of body weight is equivalent in each population, so the environmental variance is consequently and contraintuitively reduced in the variable populations. Yet a third pattern is that determined for the second bristle character, abdominal chaeta number - neither phenotypic, additive, nor environmental variances are affected by exposing populations to varying environments. The opposing responses of the two bristle characters is particularly disturbing; although they have been shown to be genetically uncorrelated and thus capable of divergent responses to selection, Drosophila bristles do fulfil the same functional requirement in that they are

Table 24

Summary of phenotypic, additive, and environmental variances, averaged over replicate and substrate, for each of the three characters. Year 2.

Population	V_p	V_a	V_e
Sternopleural bristle number			
Control	3.782	1.420	2.362
Spatial variation	3.987	2.801	1.186
Temporal variation, Short-term	6.339	4.746	1.593
Temporal variation, Long-term	6.839	5.452	1.387
Control / Temporal variation Short-term	4.253	1.361	2.892
Abdominal bristle number			
Control	12.354	5.828	6.526
Spatial variation	10.991	5.241	5.750
Temporal variation, Short-term	13.192	5.854	7.338
Temporal variation, Long-term	9.938	3.609	6.329
Control / Temporal variation, Short-term	11.317	2.252	9.065
Body weight			
Control	.0073	.0015	.0058
Spatial variation	.0091	.0041	.0050
Temporal variation, Short-term	.0071	.0045	.0026
Temporal variation, Long-term	.0079	.0048	.0031

sensory receptors, and therefore intuitively should perceive the same environmental cues irrespective of location on the animal.

What bearing does this information then have on the determination of a biological axiom relating genetic and environmental variation? The problem is that any biological rule is necessarily statistical in nature, and this is particularly true of generalities concerning evolution. Biological systems are complex and interactive; the reductiionist experimental approach is to vary a specific parameter while holding constant other variables, but this does not afford a complete description where it is the interaction between simultaneously varying parameters, most of which are unknown, which is critical. We have here been successful in establishing that simple patterns of environmental variation of only one factor - medium type - can be successful under certain circumstances in the maintenance of additive genetic variation. The fact that the phenomenon appears character-specific and that two apparently functionally related characters could not have been predicted a priori to behave in diametrically opposing manners is a function of our ignorance of potentially relevant factors and their interaction rather than the absence of a general rule. Only by the study of the behaviour of additional characters will it be possible to discern a pattern whereby certain characters can be predicted to maintain genetic variance in the face of environmental variation, whereas others would not. We have therefore demonstrated that a statistical association between degree of genetic and environmental variation does exist, but in the absence of further data cannot derive from this association a rule with specific predictive value.

What is the relationship between genetic variance and pattern of environmental heterogeneity experienced? The two characters for which

an association between genetic and environmental variance has been demonstrated show two different responses to the pattern of environmental variability to which they were exposed. Additive genetic variance for body weight is equivalent when compared among the three environmentally varying populations, whereas additive genetic variance for sternopleural bristle number is significantly less in the spatially heterogeneous populations than in either of the populations experiencing a pattern of long- or short-term temporal variability. This is in contrast to predictions from all theoretical studies, which uniformly agree temporal variation should be much less effective than spatial heterogeneity in promoting the maintenance of genetic variability. Bryant (1976) has, however, argued that spatial variability is an entirely predictable component of environment and that spatially maintained genetic variation may therefore be a transition state toward speciation. Temporal environmental variation, which represents an uncertainty provoking a more general rather than a specific genetic response, should therefore more often be associated with genetic variance than spatial heterogeneity. The experimental results are in accord with this prediction - spatial variation is also expressed temporally over successive stages of the life cycle, but this variation is perhaps not as clearly perceived as experimentally controlled temporal variation; hence, genetic variation is not as readily maintained in the spatially varying populations.

Theory also predicts that periodicity of temporal environmental variation is instrumental in determining its relative efficiency, long cycles being less effective than short-term environmental variation. Environmental grain is also theoretically important, genetic variation being less likely in fine- than coarse-grained

environments. Neither of these appear to be discriminated by the organisms experiencing them, however; additive genetic variance is the same in both long-term (coarse-grained) and short-term (fine-grained) temporally varying populations. It is clearly important to test experimentally theoretical constructs before accepting them solely on the basis of intuitive appeal - the concept of environmental grain has essentially no experimental verification and yet figures prominently in many ecological and genetic theoretical descriptions of evolution in heterogeneous environments.

Is there genotype-environment interaction? The presence of a specific genotype-environment interaction between the environmental variable and the character responding to it indicates the mechanism promoting maintenance of genetic variation in a heterogeneous environment is by selecting alternative genotypes in the different niches. If, however, the genotype-environment correlation does not depart significantly from one, variation in this case is a general and not character-specific response of the population to environmental uncertainty. This is the response observed; it appears selection for heterozygosity per se, rather than specialization to the two environmental states, causes maintenance of genetic variation in populations experiencing environmental heterogeneity. The observations of both a general response to environmental variability and of temporal variation being equally, if not more, efficient than spatial variation in the maintenance of additive genetic variance (despite theoretical considerations) are in accord with Bryant's (1976) suggestion that it is the temporal element of instability to which the populations are responding. The discovery of a general response to a varying environment implies also that functional interaction

of a particular character with the varying environmental parameter is not critical in the determination of the response. While not providing a mechanistic explanation for the different patterns of response of the two bristle characters to the same environmental heterogeneity, this observation does eliminate one source of confusion concerning the outcome - the assumption that because the two characters are responsible for perceiving the same environmental stimuli they should behave similarly to variation in these stimuli has been shown inapplicable; specific interaction of a character with the environmental variable is not a determinant of the maintenance of genetic variation of that character.

Is there development of habitat preferences or habitat loyalty in spatially heterogeneous environments? Theory predicts that a measure of habitat loyalty where animals preferentially return to parental habitats, or optimal habitat preference, where animals preferentially select the habitat in which they are most fit, increases the likelihood of genetic polymorphism in a spatially varying environment. Habitat selection is also a behaviour frequently found in cases where disruptive selection is operating. Animals from all the populations studied exhibit optimal habitat preference, but in no case is positive habitat loyalty practised, a finding consistent with the observation of a general response of the populations to environmental variation rather than diversifying selection and specialized adaptation to alternative habitats. However, in all but the spatially varying populations the animals exhibit a negative habitat loyalty - there is a distinct aversion to the alcohol niche having experienced it during one stage of the life cycle. This aversion is overcome in spatially heterogeneous populations, where both

environments are sampled independently of previous experience or parental choice of habitat, thus implying more efficient resource utilization in the spatially varying environment.

Is there a difference in homeostatic abilities of populations experiencing different environmental treatments? The two opposing theoretical expectations, that there should be an inverse relation between individual homeostasis and genetic variance (Levins, 1969), and that heterozygotes should be developmentally more stable (Lewontin, 1958) both predict an association between homeostatic ability and genetic variation, albeit in different directions. Measurements of developmental noise for the two bristle characters do not vary among any of the populations studied, providing no evidence for either of the contrasting hypotheses. However, it has been suggested that the mean square difference between bilaterally or segmentally repeated characters is not an adequate index of homeostasis in so far as this is a general property of an organism (Reeve, 1960). Comparing environmental variances of the characters studied indicates this view is probably correct. Environmental variance is the sum of a true environmental variance component resulting from the direct effect of environmental differences between individuals, and a component due to local accidents of development which prevent the phenotype of a given genotype being replicated under identical environmental conditions; it is the latter which is an appropriate measure of developmental stability. The total environmental variance of sternopleural and abdominal bristle number remains constant over all patterns of environmental heterogeneity, whereas the environmental variance of body weight actually decreases in the variable environments (Table 24). If one makes the reasonable assumption that the true environmental

variance must necessarily increase in the environmentally varying populations, then these results are only explicable on the grounds that the second, developmental noise, component is concomitantly decreased in the variable environments, and substantially so in the case of body weight. These considerations lead to the conclusion that the genetically more variable populations experiencing environmental variation are in fact more developmentally stable than the control populations, thus supporting the hypothesis of a positive association between homeostatic ability and heterozygosity.

Is there a difference of fitness components of populations experiencing different environmental treatments? With respect to both productivity and survivorship all three variable populations are more fit than the control; no difference in fitness is apparant among the three treated populations. Furthermore, these populations are better adapted relative to the control to the environmental parameter to which they were exposed. These findings are consistent with the idea, prevalent in the literature, that heterozygous or polymorphic populations are more fit than homozygous or monomorphic ones; clearly the populations in the variable environments have greater evolutionary potential than the control populations.

To summarize: Genetic variance is maintained more readily in variable environments, temporal environmental variation being somewhat more effective in promoting this maintenance than spatial variation. The effect of environmental variation is to maintain genetic variance in general; no specific genotype-environment associations are apparent. Variable populations are both developmentally more stable and more fit than the corresponding control populations; the probability of evolutionary survival is thus greater for populations experiencing

environmental heterogeneity. Because these experiments have been conducted under controlled laboratory conditions, it is known that the environmental treatment imposed is the sole agent determining interpopulational differences in level of genetic variability - each population was initiated from a sufficiently large sample of the base population to preclude sampling or drift as a source of the differential response. The generality of the effect, however, cannot be extrapolated from a single laboratory population of Drosophila melanogaster experiencing quite specific alterations of a particular environmental variable. What would be the result if other populations, species, or environmental variables were substituted? Does environmental variation maintain genetic variability in natural populations in situ? Nevo (1978) has summarized a large amount a data from electrophoretic surveys and found species arbitrarily classified as generalists have consistently higher levels of genetic variation than specialists, supporting the hypothesis that physical and biological variables are major determinants of genetic variation in nature. The phenomenon is apparantly ubiquitous.

Several questions remain unanswered, particularly the problem of what exactly is being selected as the environment varies. One feature common to all experimental work on the subject is the generality of the effect variation in environmental parameters has on maintenance of genetic variation. Whether the environmental variable(s) is (are) temporal and / or spatial, long- or short-term, genetic variance of electrophoretic, karyotypic, or quantitative characters is greater in variable populations than controls (Beardmore et. al., 1960; Beardmore, 1961; Beardmore and Levine, 1963; Long, 1970; Powell, 1971; McDonald and Ayala, 1974; Powell and Wistrand, 1978). These results

indicate that selection may be for heterozygotes; at the enzyme level the presence of several active gene products may confer adaptive biochemical diversity in the face of varying environmental conditions. However, one may recall the rationale for investigating selection in variable environments was to circumvent both theoretical problems and the lack of experimental evidence demonstrating heterosis as a cause of selective maintenance of polymorphisms - it appears we have circled back to the original problem. The value of an experiment is not only in the novel contribution synthesis of the results makes to existing theory, but also in positive suggestions which follow from the work concerning the direction further development of the field may take. From the results of this experiment one may speculate heterosis may only be adaptive in variable environments; further research critical to the elucidation of the mechanism of adaptation to heterogeneous environments may be to measure heterozygote advantage, not under constant conditions, but in the face of environmental uncertainty.

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