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NATURAL SELECTION *VS.* RANDOM DRIFT: EVIDENCE FROM TEMPORAL VARIATION IN ALLELE FREQUENCIES IN NATURE

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ABSTRACT

We have obtained monthly samples of two species, *Drosophila pseudoobscura* and *Drosophila persimilis*, in a natural population from Napa County, California. In each species, about 300 genes have been assayed by electrophoresis for each of seven enzyme loci in each monthly sample from March 1972 to June 1975. Using statistical methods developed for the purpose, we have examined whether the allele frequencies at different loci vary in a correlated fashion. The methods used do not detect natural selection when it is deterministic (*e.g.*, overdominance or directional selection), but only when alleles at different loci vary simultaneously in response to the same environmental variations. Moreover, only relatively large fitness differences (of the order of 15%) are detectable. We have found strong evidence of correlated allele frequency variation in 13–20% of the cases examined. We interpret this as evidence that natural selection plays a major role in the evolution of protein polymorphisms in nature.

ANIMAL and plant populations are extremely polymorphic. A typical insect, for example, is heterozygous at 10–15% of the enzyme loci studied by gel electrophoresis. If electrophoretically cryptic variation is taken into account, the average heterozygosity increases to 20–25% of the loci (AYALA 1982). However, it remains largely unsettled what the contribution of natural selection is to the maintenance of these polymorphisms. Proponents of the neutrality theory argue that protein polymorphisms involve mostly alleles that are adaptively equivalent to each other; the polymorphisms and their fluctuations in time and space reflect the vagaries of random sampling from generation to generation in finite populations. Other population geneticists and evolutionists find the neutrality theory wanting and argue that the polymorphisms are adaptive responses to the complexity of the physical and biotic environments.

In an attempt to shed some light on this issue, we started in 1972 a large-scale project involving the sampling of seven enzyme loci in thousands of Drosophila flies of two species over a period of 40 generations. The goal was

to ascertain whether the evolution of allele frequencies in a given population could be explained by random drift alone, or whether natural selection also would need to be implicated.

The criteria used to choose the appropriate species, locality and loci were as follows: (1) The locality had to be one where two Drosophila species could be sampled every month of the year; (2) the locality should be in the temperate zone, where climatic conditions as well as population density would oscillate considerably during the year, so that allele-frequency changes could be observed if the allele frequencies were responding to the oscillating environmental conditions; (3) several enzyme loci, moderately to very polymorphic, should exist that could be easily assayed by electrophoresis; (4) the loci to be studied should be chosen only because of their being polymorphic and because they could be simply and unambiguously assayed, so that they could be considered a random sample of all such loci with respect to the processes maintaining the polymorphisms.

During 1971 we collected Drosophila in several localities of California. McDonald Ranch, in Napa County, was selected because the two sibling species, *D. pseudoobscura* and *D. persimilis*, can be collected every month of the year without undue effort. The site is about 50 miles by road from the Davis Campus of the University of California and consisted of 700 acres of relatively undisturbed oak woodland and chaparral. A survey of about 30 enzyme loci uncovered seven that are polymorphic in both species. The experimental sampling was started in March 1972 and continued monthly until June 1975. More than 30,000 flies of both species were collected and more than 100,000 genes were assayed during that time.

One approach for studying protein polymorphisms in natural populations compares estimates of allele-frequency variation with predictions from particular models. The neutral models of molecular evolution make rather precise predictions concerning the level of heterozygosity, its variance and the allele frequency distribution. These properties of natural populations have been used to test the predictions of one particular neutral model, the infinite-allele model, and fairly good agreement has been obtained (FUERST, CHAKRABORTY and NEI 1977; CHAKRABORTY, FUERST and NEI 1980).

A severe problem with this kind of test is that the predicted results are not unique to the infinite-allele model. GILLESPIE (1979) has developed a model of natural selection in a random environment that yields the same stationary distribution of allele frequencies as the infinite-allele model. The difference between these stationary distributions are the parameters they depend on: $4Nu$ and $2B$, for the infinite allele and Gillespie's model, respectively (where N is the effective population size, u is the mutation rate to electrophoretically detectable neutral alleles and B is a complex parameter that depends on the degree of dominance, the autocorrelation of the environment and the amount of population subdivision). These parameters are usually not known for a given population; hence, $4Nu$ and $2B$ are estimated indirectly from a given quantity, such as the population heterozygosity. The resulting stationary distributions are therefore identical.

A variety of tests of the neutral theory have been described that utilize observations of allele-frequency variation over time (FISHER and FORD 1947; LEWONTIN and KRAKAUER 1973; SCHAFFER, YARDLEY and ANDERSON 1977; WATTERSON 1982; MUELLER *et al.* 1985). All of these tests are either inappropriate or make restrictive assumptions that limit their usefulness in the current study (MUELLER *et al.* 1985).

We describe a new method that relies on time-series statistics that can differentiate between random genetic drift and selection in a variable environment. Qualitatively, the method works as follows. If we examine the allele-frequency variation over time for two unlinked neutral loci, we would expect variation at the first locus to be independent of variation at the second locus; the way in which this independence is examined will be explained later. Consider now the same two loci with selection acting and with the intensity and direction of selection dependent on some environmental variable. Assume further that the environmental variable affects selection at both loci. We then would expect the allele-frequency variation at the two loci to be correlated, because both are responding to the same environmental perturbations.

The statistical methods that we have developed manifest natural selection only under very restrictive conditions; namely, when a pair of loci are responding to the same randomly varying environmental parameters. Yet, we have detected strong evidence of natural selection in 13–20% of the cases, and marginal evidence for 17–27% other cases. We interpret this as evidence that natural selection, in its various forms besides those detectable here, plays a major role in maintaining protein polymorphisms in nature.

MATERIALS AND METHODS

Collection of genetic data: The collecting site is McDonald Ranch, in Pope Valley, Napa County at about 750-feet altitude. Twelve to 16 baits were placed in a linear array along a small creek, about 100 feet from one another, in an area where Oak trees prevail. The baits were 4-gallon buckets with fermenting mashed banana. Flies were collected by net-sweeping over the buckets, and most monthly collections were made in a single afternoon. On a few occasions, during the winter months, collections were made on two or three consecutive days in order to increase the number of flies in the sample.

After arrival in the laboratory, females were placed individually in vials, were allowed to lay eggs for several days and, later, were identified as either *D. pseudoobscura* or *D. persimilis*. The males were separated as to species and occasionally (whenever the female sample was not very large) were used for electrophoresis. Most of the enzyme assays were made on the wild-collected females. The two sibling species can be unambiguously distinguished by the enzyme patterns (AYALA and POWELL 1972). During the first 2 yr of sampling, however, the species of each female was also determined by examination of the polytene chromosomes in F_1 larvae. These determinations were made by the late THEODOSIUS DOBZHANSKY as part of a separate project. Then, his determinations and ours (made by F. J. AYALA) were compared; there was no case of discrepancy.

The methods for gel preparation, electrophoresis and enzyme assay are essentially those described by AYALA *et al.* (1972). Tables 1 and 2 give the number of genes sampled (twice the number of individuals, except for the two sex-linked loci, *Pgm* and *Est-5*, whenever males were assayed). Collections were not made in 5 months: November and December 1972, May and June 1974 and March 1975.

Statistical analysis: Time-series analysis is not a commonly used technique in population biology, although it has been used to describe population dynamics in a variable environment (ROUGHGARDEN 1975) and to examine the spatial correlations of biological populations (SOKAL and ODEN

TABLE 1

Number of genes sampled at each of seven loci in the McDonald Ranch population of D. pseudoobscura

Year and month	Locus							$\bar{N} \pm SE$
	Est-5	*Hk-1*	*Lap*	*Mdh*	*Me-2*	*Odh*	*Pgm*	
1972								
Mar	137	772	266	266	236	268	242	312 ± 79
Apr	162	1086	312	314	306	312	310	400 ± 116
May	144	276	280	230	548	282	520	326 ± 57
June	212	358	392	398	396	398	398	365 ± 26
July	664	396	900	912	396	914	394	654 ± 97
Aug	301	460	576	578	460	574	460	487 ± 38
Sept	563	328	748	772	326	774	324	548 ± 83
Oct	556	306	644	794	302	788	306	529 ± 85
1973								
Jan	21	42	42	42	42	42	37	38 ± 3
Feb	44	88	86	88	88	88	39	74 ± 9
Mar	179	336	332	340	344	344	328	315 ± 23
Apr	384	758	758	760	746	756	632	685 ± 53
May	303	590	576	578	588	568	497	529 ± 39
June	282	558	560	560	510	560	515	506 ± 38
July	318	542	538	544	526	544	431	492 ± 33
Aug	545	630	622	630	600	630	546	600 ± 15
Sept	214	294	286	294	294	294	214	281 ± 11
Oct	348	356	480	490	358	490	216	391 ± 39
Nov	372	456	474	482	358	482	372	428 ± 22
Dec	22	22	22	22	22	22	22	22 ± 0
1974								
Jan	475	536	536	536	536	480	475	511 ± 12
Feb	355	442	436	442	310	442	354	397 ± 21
Mar	230	258	256	258	190	258	230	240 ± 10
Apr	209	252	252	252	154	252	209	226 ± 14
July	97	132	132	132	132	132	97	122 ± 6
Aug	230	300	296	298	300	298	232	279 ± 12
Sept	194	224	224	224	224	224	194	215 ± 6
Oct	158	150	212	212	202	212	158	194 ± 10
Nov	34	68	68	68	68	68	47	60 ± 5
Dec	5	4	6	6	6	6	5	6 ± 0.3
1975								
Jan	4	6	6	6	6	6	5	6 ± 0.3
Feb	116	232	232	232	232	232	204	211 ± 16
Apr	34	68	68	68	68	68	56	61 ± 5
May	95	172	180	184	184	184	165	166 ± 12
June	21	42	42	42	42	42	21	36 ± 4
Mean	229	330	338	344	289	344	264	305
SE	30	42	41	42	33	42	30	17

TABLE 2

Number of genes sampled at each of seven loci in the McDonald Ranch population of D. persimilis

Year and month	Locus							$\bar{N} \pm SE$
	Est-5	*Hk--1*	*Lap*	*Mdh*	*Me-2*	*Odh*	*Pgm*	
1972								
Mar	101	560	176	176	272	174	156	231 ± 58
Apr	98	548	164	166	312	166	166	231 ± 58
May	46	76	80	2	78	80	78	63 ± 11
June	35	52	60	48	152	46	160	79 ± 20
July	91	68	118	122	68	122	68	94 ± 10
Aug	86	100	148	106	100	148	99	112 ± 9
Sept	487	330	716	768	328	768	327	532 ± 80
Oct	821	370	910	1268	368	1312	370	774 ± 158
1973								
Jan	112	200	196	200	200	200	176	183 ± 12
Feb	297	592	584	592	594	592	491	535 ± 42
Mar	255	476	472	512	512	510	468	458 ± 35
Apr	205	396	396	396	396	396	297	355 ± 29
May	176	346	336	340	348	332	278	308 ± 24
June	71	136	140	138	128	130	123	124 ± 9
July	49	84	86	86	84	86	64	66 ± 12
Aug	127	156	154	156	156	156	127	147 ± 5
Sept	522	730	690	730	730	730	522	665 ± 37
Oct	581	516	744	748	516	748	348	600 ± 58
Nov	223	274	276	282	236	282	221	256 ± 11
Dec	46	58	58	58	58	58	46	55 ± 2
1974								
Jan	742	936	924	936	934	812	744	861 ± 35
Feb	338	388	386	388	322	388	338	371 ± 11
Mar	167	178	178	178	178	178	167	174 ± 2
Apr	94	112	112	112	62	112	94	100 ± 7
July	14	18	18	18	18	18	14	17 ± 1
Aug	92	122	122	122	122	122	92	113 ± 6
Sept	202	272	272	272	272	272	202	252 ± 13
Oct	360	302	468	468	422	468	360	407 ± 25
Nov	450	778	778	778	778	778	450	684 ± 60
Dec	80	116	116	116	116	116	80	106 ± 7
1975								
Jan	67	110	110	110	110	110	67	98 ± 8
Feb	284	568	568	568	568	568	430	508 ± 42
Apr	92	184	184	184	184	184	160	167 ± 13
May	110	214	214	214	214	214	190	196 ± 15
June	8	16	16	16	16	16	8	14 ± 1
Mean	215	297	313	325	284	325	228	284
SE	35	40	45	51	39	51	29	17

1978; SOKAL and MENOZZI 1982). Here, we shall summarize the time-series statistics we have used and shall point out the particular patterns that are considered inconsistent with drift alone but are consistent with natural selection in a variable environment. The arguments will be developed in more detail in the next section. Detailed discussions of time-series statistics may be found in JENKINS and WATTS (1968).

The autocorrelation function used measures the correlation between allele frequencies at a single locus separated by k time units (lag). The variance of allele frequencies over time can be partitioned into component sine and cosine waves at different frequencies. The relative contribution of these different periodic functions to the overall variance is measured by the spectral density function.

The correlation between allele frequencies at two different loci separated by k time units is measured by the cross correlation function, $r_{x_1x_2}(k)$. The observed patterns in the cross-correlation function generally fall into three categories: (1) Apparent random fluctuations around $r_{x_1x_2}(k) = 0$. This is the sort of pattern we would expect if only drift would affect allele-frequency variation or if selection were rather weak compared to drift and/or sampling variation. (2) A sharp peak at $r_{x_1x_2}(0)$ that rapidly decays at greater lag times. This pattern can be generated from our selection model (see next section) and certainly represents a substantial departure from the expectations of the drift model. (3) There is a group of observations that shows some evidence of a peak at $r_{x_1x_2}(0)$, but its magnitude is marginal relative to peaks in the second group and to its own fluctuation in $r_{x_1x_2}(k)$ for larger k values. This group might contain loci where an underlying pattern of selection is obscured by sampling variation of one sort or another, or the loci could reflect odd deviations from the drift process. Clearly, our confidence in declaring results in the third group as deviations from the neutral model is substantially lower than it is for the results in the second group.

Just as the variance can be partitioned into frequency components, an analogous procedure can be carried out on the covariance of a bivariate process. The relative weighting of the frequency components in the covariance is summarized by the amplitude spectrum. Essentially the same groups were formed with the amplitude spectra as with the cross-correlation function. For the amplitude spectra we expect to see a peak at low frequencies (w), which gradually decreases to 0 at $w = 0.5$ if selection is acting. Amplitude spectra placed in the second group usually have peaks at the low frequencies and some portion of the spectra where ± two standard deviations did not include 0.

For a large population that has reached an equilibrium with respect to the processes of random drift, mutation and migration, the dynamics of a single allele may be represented as a first order autoregressive process, $X(t) = \alpha\, X(t-1) + z(t)$, where $X(t)$ is the transformed allele frequency and $z(t)$ is a random variable with a mean of 0 and finite variance. We can convert this process to a white-noise stochastic process by prefiltering $X(t)$

$$X'(t) = X(t) - \alpha\, X(t-1)$$

Several tests exist for examining whether two white-noise processes are correlated. Under the neutral hypothesis we, of course, expect the filtered allele-frequency dynamics at different loci to be uncorrelated. The tests described next were all carried out on prefiltered data (that is the $X'(t)$ process). Methods for prefiltering data are described in JENKINS and WATTS (1968, p. 340).

With the prefiltered data, we can again calculate the cross-correlation function. For two uncorrelated white-noise processes we expect the cross correlations to be 0 for all lags. In addition, we can estimate the 95% confidence interval about 0 as $\pm\ 1.96\ \sqrt{1/N}$, where N is the total number of observations. If more than 5% of the observations fall outside of this interval, we take this as evidence of correlation. For this test we examined lags of up to 20 months. Estimates of cross correlations at greater lags were not considered accurate enough to be included in the test.

The cross-correlation test is useful for detecting only certain types of correlation. Periodic components in the cross-correlation function may not be detected by this test. For this reason, we conduct a test of correlation in the frequency domain.

The cross spectrum ($\Gamma_{x_1x_2}(w)$) can be expressed in terms of the cross-amplitude spectrum ($\alpha_{x_1x_2}(w)$) and the phase spectrum $\phi_{x_1x_2}(w)$ as $\Gamma_{x_1x_2}(w) = \alpha_{x_1x_2}(w)\exp[i\phi_{x_1x_2}(w)]$. It can be shown (JENKINS and WATTS 1968, Ch. 9) that if two time series are uncorrelated, then the sample phase

spectrum will be approximately uniformly distributed in the range $-\pi/2$, $\pi/2$. Because the null hypothesis predicts a uniform distribution, we can plot the expected distribution function along with a 95% confidence interval. If more than 5% of the observations lie outside of this confidence interval, we accept this as evidence contrary to the neutral hypothesis.

Data treatment: Alleles used in the study are present in every sample and at an average frequency of 0.05 or greater. Before calculating the time-series statistics, the allele frequencies were transformed with an arcsin $\sqrt{p}$ transformation. Data for all the alleles had up to 5 months of missing data. These points were estimated by interpolation, using the two observations that bracketed the missing points. The mean transformed frequency for each allele was zeroed, and any linear trend in the data was removed.

The spectral analysis was carried out with Parzen's window. Initially, three values of the bandwidth were tried (3.90, 1.95, 0.97) with the spectrum for each allele. The spectra for all alleles showed similar qualitative features for each bandwidth. The spectrum resulting from a bandwidth of 0.97 showed erratic behavior. For this reason the intermediate bandwidth was used because it represented a considerable decrease in variance with, seemingly, only a modest increase in bias. All bivariate spectral analyses were made also with a bandwidth of 1.95. An interval of ± two standard deviations (see JENKINS and WATTS (1968), p. 378) was calculated for the amplitude spectra.

EXPECTATIONS FOR TIME-SERIES STATISTICS

Univariate analysis

If there is a periodic oscillation in allele frequencies (with a 1-yr period, for instance, when samples are taken monthly), this will show up as a peak at $t = 12$ in the autocorrelation function and as a peak at about 0.083 in the autospectrum. Such variation in allele frequencies seems to be more consistent with an environmentally correlated change in selection than with a random drift model.

Bivariate analysis

Neutral models: We assume here that allele frequencies have reached an equilibrium with mutation and/or migration and drift and that the population is fairly large and, thus, the fluctuations from this equilibrium are not severe. With these assumptions (following the argumentation of FELSENSTEIN 1974), the covariance between allele frequencies at linked or unlinked loci will be 0, linkage disequilibrium will be 0 and the correlation between allele frequencies at different loci will be 0. In terms of our time-series statistics this means that we expect the cross-correlation function and the amplitude spectrum lines to have 0 slope and to pass through 0.

Selection in random environments: There are several reasons why the bivariate time-series analysis could produce deviations from neutrality. Two obvious ones are linkage disequilibrium and natural selection. We shall consider linkage disequilibrium generated by random sampling in a later section. In order to analyze the effects of natural selection on the pattern of time-series statistics, it is important to develop specific quantitative models of natural selection. We would also like to make some statement concerning the magnitude of selection necessary to produce patterns significantly different from the neutral model. Time for this model is measured in units of generations. One generation of *D. pseudoobscura* or *D. persimilis* in nature takes about 1 month—the actual

time unit of our samples. Thus, in the following discussion we use time units and generations interchangeably.

We shall assume that there are two, possibly cross-correlated, environmental noise processes, $Y_1(t)$ and $Y_2(t)$ that perturb the fitness of each genotype. We shall further assume that the underlying genetic model is a two locus additive model (KARLIN and FELDMAN 1970). At one locus the genotypes *AA*, *Aa* and *aa* add a constant component to net fitness of u_1, u_2 and u_3, respectively. Likewise, at the second locus, the three genotypes *BB*, *Bb* and *bb* have components v_1, v_2 and v_3 respectively. We further assume that for each *A* or *B* allele $Y_1(t)/4$ is added to the net fitness, while for each *a* or *b* allele $Y_2(t)/4$ is added. The fitnesses of the ten two-locus genotypes are as follows:

$$\begin{array}{lcccc} & AB & Ab & aB & ab \\ AB & u_1 + v_1 + Y_1(t) & u_1 + v_2 + [3Y_1(t) + Y_2(t)]/4 & u_2 + v_1 + [3Y_1(t) + Y_2(t)]/4 & u_2 + v_2 + [Y_1(t) + Y_2(t)]/2 \\ Ab & & u_1 + v_3 + [Y_1(t) + Y_2(t)]/2 & u_2 + v_2 + [Y_1(t) + Y_2(t)]/2 & u_2 + v_3 + [Y_1(t) + 3Y_2(t)]/4 \\ aB & & & u_3 + v_1 + [Y_1(t) + Y_2(t)]/2 & u_3 + v_2 + [Y_1(t) + 3Y_2(t)]/4 \\ ab & & & & u_3 + v_3 + Y_2(t) \end{array} \quad (1)$$

Let the frequencies of the four gamete types (*AB*, *Ab*, *aB*, *ab*) be x_1, x_2, x_3 and x_4, r be the recombination fraction, D the disequilibrium parameter ($D = x_1x_4 - x_2x_3$) and w_{ij} the fitness values from the matrix (1). The dynamics of x_i will then be governed by the recursion,

$$x_i' = \frac{x_i w_i + \epsilon_i r D w_{14}}{\bar{w}}, \quad (2)$$

where $w_i = \Sigma_j w_{ij}x_j$, $\bar{w} = \Sigma w_i x_i$ and $\epsilon_i = -1$ when $i = 1,4$ and 1 when $i = 2, 3$. If p_1 is the frequency of allele *A*, and p_2 is the frequency of allele *B*, then the dynamics of the two-locus genetic system (2) can be expressed in terms of p_1, p_2 and D by noting the following identities: $p_1 = x_1 + x_2$, $p_2 = x_1 + x_3$, $D = x_1 - p_1p_2 = x_4 - (1 - p_1)(1 - p_2) = p_1(1 - p_2) - x_2 = (1 - p_1)p_2 - x_3$.

We know that if $u_2 > u_1$, u_3 and $v_2 > v_1$, v_3, then for the deterministic version of (2) (*i.e.*, when $Y_1(t) = Y_2(t) = 0$), there will be one globally stable equilibrium given by $(\hat{p}_1, \hat{p}_2, \hat{D}) = \left(\frac{u_2 - u_3}{2u_2 - u_1 - u_3}, \frac{v_2 - v_3}{2v_2 - v_1 - v_3}, 0\right)$ (KARLIN and FELDMAN 1970). The dynamics of the environment and the genetic system can be described by the five parameters Y_1, Y_2, p_1, p_2 and D. In order to derive expectations concerning the cross correlation and amplitude spectrum of p_1 and p_2, we must have a linear representation of the dynamics of p_1 and p_2. If we assume that the genetic system is close to the equilibrium, $(\hat{p}_1, \hat{p}_2, \hat{D})$, we can obtain the local linear dynamics around this point. Since the only force perturbing the genetic system away from the equilibrium, $(\hat{p}_1, \hat{p}_2, \hat{D})$, is the environment, the accuracy of this linear approximation will depend on how strong the environmental perturbations are, *i.e.*, on the variance of $Y_1(t)$ and

$Y_2(t)$. For simplicity we assume that the environment can be modeled by a first-order autoregressive process. If we let $\epsilon_1(t) = p_1(t) - \hat{p}_1$, $\epsilon_2(t) = p_2(t) - \hat{p}_2$ and $\epsilon_3(t) = D(t) - \hat{D}$, then the environmental process and genetic system can be represented by the linear equations

$$Y_1(t + 1) = \lambda_1 Y_1(t) + z_1(t + 1), \tag{3a}$$

$$Y_2(t + 1) = \lambda_2 Y_2(t) + z_2(t + 1), \tag{3b}$$

$$\epsilon_1(t + 1) = \alpha_{31} Y_1(t) + \alpha_{32} Y_2(t) + \alpha_{33}\epsilon_1(t), \tag{3c}$$

$$\epsilon_2(t + 1) = \alpha_{41} Y_1(t) + \alpha_{42} Y_2(t) + \alpha_{44}\epsilon_2(t), \tag{3d}$$

$$\epsilon_3(t + 1) = \alpha_{55}\epsilon_3(t), \tag{3e}$$

where $(z_1(t), z_2(t))$ is a bivariate random variable with zero mean and covariance matrix $\begin{bmatrix} \sigma_1^2, & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2, & \sigma_2^2 \end{bmatrix}$, λ_1 and λ_2 are unknown parameters of the environment and the α's are constants given by

$$\begin{aligned}
\alpha_{31} &= \hat{p}_1(1 - \hat{p}_1)/4\hat{\bar{W}}, \\
\alpha_{32} &= -\alpha_{31}, \\
\alpha_{33} &= [\hat{\bar{W}} + (u_2^2 - u_1u_3)/(2u_2 - u_1 - u_3) - u_2]/\hat{\bar{W}}, \\
\alpha_{41} &= \hat{p}_2(1 - \hat{p}_2)/4\hat{\bar{W}}, \\
\alpha_{42} &= -\alpha_{41}, \\
\alpha_{44} &= [\hat{\bar{W}} + (v_2^2 - v_1v_3)/(2v_2 - v_1 - v_3) - v_2]/\hat{\bar{W}}, \\
\alpha_{55} &= 1 - r(u_2 + v_2)/\hat{\bar{W}},
\end{aligned}$$

where

$$\hat{\bar{W}} = (u_2^2 - u_1u_3)/(2u_2 - u_1 - u_3) + (v_2^2 - v_1v_3)/(2v_2 - v_1 - v_3).$$

From equations (3) we can see the $\epsilon_1(t)$ and $\epsilon_2(t)$ are independent of $\epsilon_3(t)$ and that if $|\alpha_{55}| < 1$, then $\epsilon_3 \to 0$, no matter how strong the environmental fluctuations. It is easy to show that, if $u_2 > u_1$, u_3 and $v_2 > v_1$, v_3, then $|\alpha_{55}| < 1$ (KARLIN and FELDMAN 1970). Consequently, the ϵ_3 parameter can be ignored in the following analysis.

The environment can be described qualitatively as follows. We assume $-1 < \lambda_1, \lambda_2 < 1$. When λ_i is greater than zero, environments will tend to resemble most closely their previous environment and, to a lesser degree, the environments of two, three, and so on, time-units ago. The closer λ_i is to 1, the stronger will be the resemblence to previous environments. If λ_i is negative, then the environment will tend to fluctuate between extreme values; for example, if the environmental variable were temperature, hot months would be followed by cold months and vice versa.

Let ρ be the correlation between $z_1(t)$ and $z_2(t)$. $Y_1(t)$ and $Y_2(t)$ are perturbations to the fitness effects of different alleles at the A and B loci. Values of ρ close to 1 imply that $Y_1(t)$ and $Y_2(t)$ are being affected by the same environmental force and that this environmental perturbation affects fitness of alter-

native genotypes in a similar fashion. Thus, under our model of selection, environments that cause an increase in the fitness of *AB/AB* would also cause an increase in the fitness of *ab/ab*. If ρ is close to -1, then the environment would affect these alternative genotypes in an opposite fashion. It would seem, for example, that on a monthly time scale most environmental factors will be positively autocorrelated, *i.e.*, $\lambda_i > 0$.

The process (3) can be compactly written in matrix notation as

$$\mathbf{E}_{t+1} = \alpha\mathbf{E}_t + \mathbf{z}_{t+1}$$

where,

$$\mathbf{E}_t = (Y_1(t),\ Y_2(t),\ \epsilon_1(t),\ \epsilon_2(t))^T,$$

$$\mathbf{z}_t = (z_1(t),\ z_2(t),\ 0,\ 0)^T,$$

$$\alpha = \begin{bmatrix} \lambda_1 & 0 & 0 & 0 \\ 0 & \lambda_2 & 0 & 0 \\ \alpha_{31} & \alpha_{32} & \alpha_{33} & 0 \\ \alpha_{41} & \alpha_{42} & 0 & \alpha_{44} \end{bmatrix}.$$

$\mathbf{V}(k)$ is the covariance matrix of lag k and will satisfy (JENKINS and WATTS 1968, p. 474),

$$\mathbf{V}(k) = \alpha^k\mathbf{V}(0). \tag{4}$$

The entries for $\mathbf{V}(0)$ are determined directly from equation (3) and are as follows:

$$V_{11} = \sigma_1^2/(1 - \lambda_1^2) \tag{5a}$$

$$V_{22} = \sigma_2^2/(1 - \lambda_2^2) \tag{5b}$$

$$V_{12} = \sigma_1\sigma_2\rho/(1 - \lambda_1\lambda_2) \tag{5c}$$

$$V_{13} = [\lambda_1\alpha_{31}V_{11} + \lambda_1\alpha_{32}V_{12}]/(1 - \lambda_1\alpha_{33}) \tag{5d}$$

$$V_{14} = [\lambda_1\alpha_{41}V_{11} + \lambda_1\alpha_{42}V_{12}]/(1 - \lambda_1\alpha_{44}) \tag{5e}$$

$$V_{23} = [\lambda_2\alpha_{31}V_{12} + \lambda_2\alpha_{32}V_{22}]/(1 - \lambda_2\alpha_{33}) \tag{5f}$$

$$V_{24} = [\lambda_2\alpha_{41}V_{12} + \lambda_2\alpha_{42}V_{22}]/(1 - \lambda_2\alpha_{44}) \tag{5g}$$

$$V_{33} = [\alpha_{41}^2V_{11} + \alpha_{32}^2V_{22} + 2(\alpha_{31}\alpha_{33}V_{13} + \alpha_{33}\alpha_{32}V_{23} + \alpha_{31}\alpha_{32}V_{12})]/(1 - \alpha_{33}^2) \tag{5h}$$

$$V_{44} = [\alpha_{41}^2V_{11} + \alpha_{42}^2V_{22} + 2(\alpha_{44}\alpha_{41}V_{14} + \alpha_{44}\alpha_{42}V_{24} + \alpha_{41}\alpha_{42}V_{12})]/(1 - \alpha_{44}^2) \tag{5i}$$

$$V_{34} = [\alpha_{31}\alpha_{41}V_{11} + \alpha_{32}\alpha_{42}V_{22} + \alpha_{33}\alpha_{41}V_{13} + \alpha_{33}\alpha_{42}V_{23} + \alpha_{31}\alpha_{44}V_{14} + \alpha_{32}\alpha_{44}V_{24} + (\alpha_{31}\alpha_{42} + \alpha_{32}\alpha_{41})V_{12}]/(1 - \alpha_{33}\alpha_{44}) \tag{5j}$$

We can use (4) to numerically generate some cross-correlation curves. Typical results for the cross correlation between $p_1(t)$ and $p_2(t)$ are shown in Figures 1 and 2. In these examples we have set $\lambda_1 = \lambda_2 = \lambda$. The three different

curves in Figures 1 and 2 only differ in their value of λ, which is set to 0.8, 0 or -0.8. We see that the strongest correlation occurs between observations made close together in time and gradually decrease for observations farther and farther apart. When $\lambda < 0$, we get the saw-tooth pattern present in both Figures 1 and 2. As mentioned previously, $\lambda \ll 0$ means that the environment is essentially alternating between extreme conditions every generation. Thus, observations that are an even number of units apart will reflect environments that are similar, whereas observations that are an odd number of units apart will reflect environments in opposite states. Consequently, allele frequencies show a higher correlation when separated by an even number of time intervals, but show a small or zero correlation when separated by an odd number of time intervals.

The next qualitative trend worth noting is the relative flatness of the curves in Figure 2 compared with those in Figure 1. This effect is due to the magnitude of the deterministic component of selection relative to the stochastic component. In Figures 1 and 2 the variance and covariance of the environmental parameters are the same; however, the magnitude of the deterministic selection coefficients is much greater in Figure 1 than in Figure 2. When the deterministic components of the fitness matrix (1) produce relatively large differences in fitness, then the sequence of fitness matrices produced by the random environment will largely reflect the underlying deterministic differences. As a consequence, the effect of any one environment will be damped out rapidly by the genetic system. However, if the deterministic differences in fitness are slight, then the same environmental perturbations will almost wholly be responsible for the fitness matrix (1) at each time interval. In this latter case, the deterministic part of the genetic system will be less effective at filtering out environmental fluctuations; hence, the sequence of allele frequencies at both loci will show a stronger "memory" of the environmental fluctuations, which will be translated into strong cross correlations, even at fairly large lags.

For the process (3) we can also determine the matrix of auto- and cross-spectra, $\mathbf{\Gamma}(w)$, at frequency w from JENKINS and WATTS (1968, p. 474),

$$\mathbf{\Gamma}(w) = [\mathbf{I} - \alpha e^{i2\pi w}]^{-1}\mathbf{W}[\mathbf{I} - \alpha' e^{-i2\pi w}]^{-1}, \tag{6}$$

where $\mathbf{I}$ is the identity matrix and $\mathbf{W}$ is the covariance matrix of $\mathbf{z}_t$. We are interested in that one component of this matrix that gives the cross spectra of the A and B alleles. This component is the following:

$$\begin{aligned}\Gamma_{34}(w) = \alpha_{41}\alpha_{31}\left\{\frac{\sigma_1^2}{(1 + \lambda_1^2 - 2\lambda_1 cos\,(w))} + \frac{\sigma_1^2}{(1 + \lambda_2^2 - 2\lambda_2 cos\,(w))}\right\} \Big/ \\ \cdot(1 - \alpha_{33}e^{iw})(1 - \alpha_{44}e^{-iw}) - \alpha_{41}\alpha_{31}\sigma_1\sigma_2\rho\{(1 - \lambda_2 e^{iw})^{-1}(1 - \lambda_1 e^{-iw})^{-1} \\ + (1 - \lambda_2 e^{-iw})^{-1}(1 - \lambda_1 e^{iw})^{-1}\}/(1 - \alpha_{33}e^{iw})(1 - \alpha_{44}e^{-iw}).\end{aligned} \tag{7}$$

Using (7) we have evaluated the amplitude spectrum for the same examples presented in Figures 1 and 2; these are given in Figures 3 and 4. Because the covariances of these examples differ by several orders of magnitude, we have

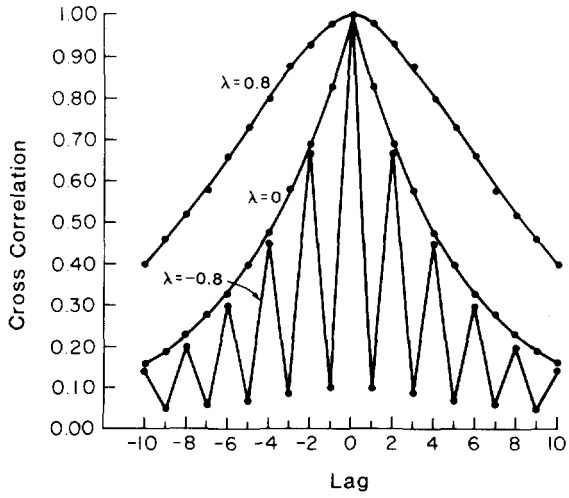

FIGURE 1.—The cross-correlation function for the random-selection model. The three curves are generated from equation (4), using $\lambda = 0.8$, 0 and -0.8. The other parameter values are: $u_1 = u_3 = v_1 = v_3 = 0.5$, $v_2 = u_2 = 1$, $\sigma_1^2 = \sigma_2^2 = 0.0009$, $\rho\sigma_1\sigma_2 = 0.0008$. Lag is the number of time periods between pairs of observations.

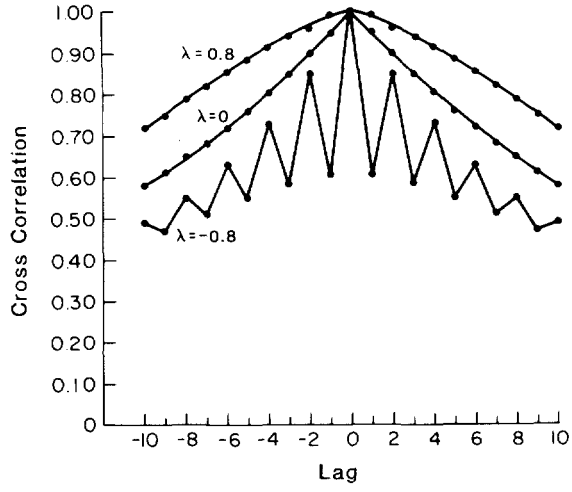

FIGURE 2.—This cross-correlation function is the same as in Figure 1, except that the deterministic components of selection have been reduced to the following values: $u_1 = v_1 = 0.90$, $u_2 = v_2 = 1$, $u_3 = v_3 = 0.90$.

standardized the curves in Figures 3 and 4 by setting the largest peak in each curve to the same value.

The qualitative features of these curves may be explained as follows. When λ is greater than zero, observations close in time tend strongly to resemble each other. As a consequence, periodic components of these processes tend to have long wavelengths and, hence, low frequencies. However, when $\lambda < 0$, we have noticed the saw-tooth appearance of the cross-correlation function. These rapid changes in correlation correspond to periodic components in the sto-

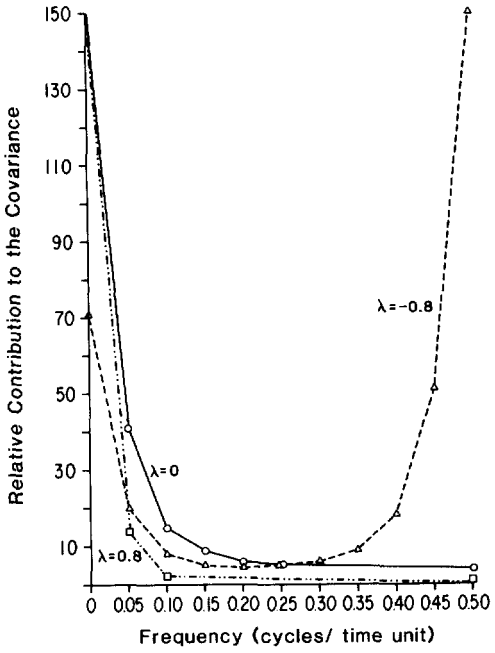

FIGURE 3.—The amplitude spectrum for the random-selection process, with parameter values as in Figure 1.

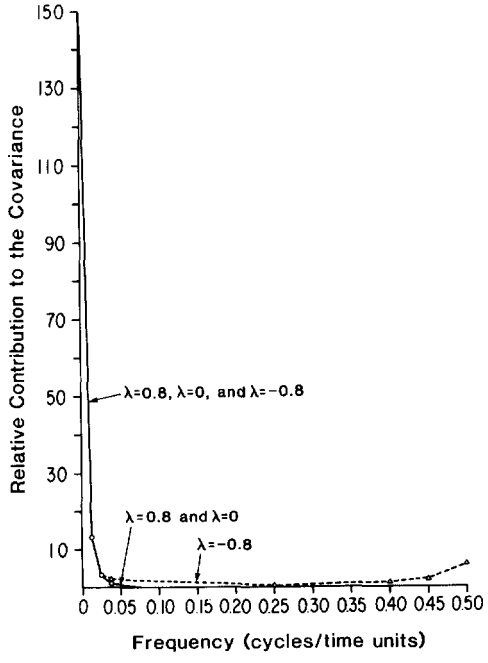

FIGURE 4.—The amplitude spectrum for the random-selection process, with parameter values as in Figure 2.

TABLE 3

The absolute viability of D. pseudoobscura *larvae under competitive conditions at two temperatures*

Locus	*Pgm* locus			Temperature
	FF	*FS*	*SS*	
Mdh				
FF	0.35	0.36	0.34	
FS	0.45	0.44	0.37	18°
SS	0.39	0.41	0.34	
Mdh				
FF	0.21	0.23	0.25	
FS	0.33	0.18	0.12	26°
SS	0.32	0.29	0.30	

From SNYDER and AYALA (1979).

chastic process that oscillate with high frequency. Hence, when $\lambda < 0$, there is a second peak in the amplitude spectrum at high frequencies.

Statistical power of test: The environmental process we have assumed to operate in our selection model perturbs allele frequencies away from their equilibrium and is consequently responsible for the variance in the observed allele frequencies. Even if selection were acting in this manner, there would also be variation in allele frequencies due to drift and to the sampling of individuals. It would seem that if the variance due to drift or sampling were of the same order of magnitude, or greater, than that due to selection, our ability to detect the underlying selection process would be severely impaired.

We will now compute the variance of allele A due to selection in a random environment by using (5h). For each particular example, we compute the sample size, $2N^*$, that would yield the same variance. Our assumption is that there is a reasonable chance to detect the effects of variable selection whenever $2N^*$ is less than our actual sample size.

To carry out this analysis, we need to approximate the environmental variance, σ_1^2, and σ_2^2. We have little information on which to base this approximation. One experiment has been carried out by SNYDER and AYALA (1979) to determine the absolute viabilities of *D. pseudoobscura* at two temperatures as a function of genotype at the *Mdh* and *Pgm* loci. Table 3 shows some relevant results from these experiments. It is clear that our underlying hypothesis that fitness varies with the environment is tenable. For instance, the *SS/SS* genotype has the lowest viability at 18°, but has one of the highest at 26°. In order to estimate the variances of the environmental processes, we have made the following assumptions. First, the absolute viabilities in Table 3 can be described by the additive scheme in (1). Second, we assume that the range of environments presented in Table 3 represents a 95% confidence interval of the typical fluctuations. Third, we have set $\sigma_1^2 = \sigma_2^2$ and $\lambda_1 = \lambda_2$. We have used the resulting estimates of environmental variance in Table 4 to calculate the variance of the selection process, as described previously.

TABLE 4

Variance due to selection (sample size that produces the same variance)

Example	$\lambda = -0.8$	$\lambda = 0$	$\lambda = 0.8$
A	8.5×10^{-5} (1470)	4.3×10^{-4} (294)	2.1×10^{-3} (59)
B	1.7×10^{-3} (75)	1.5×10^{-2} (8)	1.3×10^{-1} (1)

Example A: $u_1 = u_3 = v_1 = v_3 = 0.125$, $u_2 = v_2 = 0.25$
B: $u_1 = u_3 = v_1 = v_3 = 0.2475$, $u_2 = v_2 = 0.25$
$\sigma_1^2 = \sigma_2^2 =$ 4.68×10^{-4} *for* $\lambda = \pm 0.8$
1.3×10^{-3} *for* $\lambda = 0$

We note two important trends in Table 4. As the autocorrelation in the environment changes from positive to negative, the variance due to selection decreases. Thus, our ability to detect the effects of variable selection is much less in a negatively autocorrelated environment than it is in an environment that is positively autocorrelated. It also is clear that the variances for example A are uniformly less than those for example B. This difference is due to the deterministic component of selection. The differences in the deterministic components of fitness are larger in example A than in example B. Just as the deterministic components of fitness damp out the cross correlation between allele frequencies, these components also reduce the overall variance produced by the random environment. In the limit, when all selection is deterministic, the variance in allele frequencies due to selection becomes zero. This underscores the point made earlier that the present analysis is designed specifically to detect variable selection; deterministic selection, no matter how strong, will not be uncovered by these methods.

We can summarize the results of Table 4 as follows: variable selection is most readily detected in environments with positive autocorrelation and when fitness differences are almost wholly due to the variable component of fitness. The magnitude of selection in Table 4 can be expressed as a 95% confidence interval on the absolute fitness of each genotype. Such a confidence interval for the *AB/AB* genotype, for instance, would be $u_1 + v_1 \pm 0.07$. For this example, we can see from Table 4 that, for small differences in the deterministic selection components, the selection process should be discernable from sampling error if most sample sizes are 100 or more. However, when the deterministic components are large, then only in the environments with the strongest positive correlation can we hope to detect the additional effects of variable selection.

Drift alone may mimic variable selection: We have already mentioned that we expect the cross correlation between allele frequencies at linked loci to be 0 if only random genetic drift is acting on the population. Although this will certainly be true for a very large population, it is possible that if the population is sufficiently small then correlations between loci may develop due strictly to chance. We have examined this problem via the computer simulations described next.

We have a sequence of 40 consecutive samples (N_{s1}, N_{s2}, . . ., N_{s40}) that were made to estimate allele frequencies for two populations of Drosophila. We

TABLE 5

The probability that drift alone will produce significant cross correlation

	$N_t = 4n_t$		$N_t = 10n_t$	
Example	$r = 0.5$	$r = 0.01$	$r = 0.5$	$r = 0.01$
		D. persimilis		
A	0.09	0.10	0.040	0.071
B	0.13	0.14	0.040	0.080
		D. pseudoobscura		
A	0.12	0.13	0.11	0.098
B	0.21	0.24	0.12	0.12

Example A: $A_1B_1 = 0.81$, $A_1B_2 = 0.09$, $A_2B_1 = 0.09$, $A_2B_2 = 0.01$

Example B: $A_1B_1 = 0.25$, $A_1B_2 = 0.25$, $A_2B_1 = 0.25$, $A_2B_2 = 0.25$

have modeled random genetic drift by assuming that the effective population size is either four or ten times the sample size. We assume that there are two alleles at each of two linked loci, with recombination fraction r($r = 0.5$ or 0.01). The initial gamete frequencies are given in Table 5. The life cycle is as follows. An infinite zygote pool is formed from the random union of gametes. Zygotes are sampled multinomially from the ten different genotype classes to make a total population size of $4N_{st}$ or $10N_{st}$. From this population a sample of N_{st} is taken without replacement, and allele frequencies are estimated ($p_1(t)$, $p_2(t)$). After 40 generations the cross correlation between $p_1(t)$ and $p_2(t)$ is calculated. If the cross correlations satisfied the following criteria, they were classified as consistent with our selection model:

$$|r_{p_1p_2}(0)| > 0.3$$

$$r_{p_1p_2}(0) > r_{p_1p_2}(1) > r_{p_1p_2}(2) > 0$$

$$\text{or} \qquad r_{p_1p_2}(0) < r_{p_1p_2}(1) < r_{p_1p_2}(2) < 0$$

If any allele was fixed the trial was ignored; at least 100 trials were conducted. The results of these simulations are given in Table 5, where we see that, if the population size is in the range of four to ten times the sample size, then only rarely will random drift mimic the cross-correlation patterns that we expect from our selection model. However, if the population size were less than four times the sample size, then drift could mimic the selection pattern one-third of the time. It must be noted that it seems extremely unlikely that the total population size was this small for the populations of *D. pseudoobscura* and *D. persimilis* under consideration. Last, we observe that the probability that drift alone will mimic selection is the same whether $r = 0.5$ or 0.01. In other words, tight linkage does not appreciably increase the probability of a strong cross-correlation pattern.

RESULTS

D. pseudoobscura and *D. persimilis* are two New World species of the *obscura* group. They are siblings, morphologically indistinguishable from each other except for some inconspicuous but reliable differences in the male genitalia.

At the collecting site, McDonald Ranch, these two species are the most abundant Drosophila species through much of the year, but they are greatly outnumbered by *D. melanogaster* in September and October. From 1971 to mid-1973, *D. pseudoobscura* was more common than *D. persimilis* in nearly all monthly collections. The relative abundance of the two species was reversed from the fall of 1973 to the end of the collections in the summer of 1975.

The seven enzymes assayed for this study are esterase-5 (EC 3.1.1.1), hexokinase-1 (EC 2.7.1.1), leucine amino peptidase (EC 3.4.1.1), cytoplasmic malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), octanol dehydrogenase (EC 1.1.1.1) and phosphoglucomutase (EC 2.7.5.1).

The chromosomal locations of the genes coding for the enzymes are *Est-5* and *Pgm* on the *X*; *Lap, Me* and *Odh* on the 2nd; *Hk-1* on the 3rd; *Mdh* on the 4th. We have mapped the two *X*-chromosome loci in *D. pseudoobscura*: *Est-5* at position 89 and *Pgm* at 115, approximately. The *X* centromere has been located at about position 86 (TAN 1937; STURTEVANT and TAN 1937). Hence, it would seem that both *Est-5* and *Pgm* are on the right arm of the *X* chromosome.

The monthly frequencies of the alleles used in the statistical tests are given in Tables 6 and 7 for *D. pseudoobscura* and *D. persimilis*, respectively. Rare alleles were not used and, thus, are omitted from these tables. All the alleles shown are present in every monthly sample with an average frequency no lower than 0.05. At each locus, the symbol *100* is used to designate the allele most common in *D. pseudoobscura*. The numbers representing the other alleles are obtained by adding to, or subtracting from, 100 the distance in millimeters between the migration positions of the corresponding allozymes in our gels. For example, Me^{98} codes for an enzyme that, under our experimental conditions, migrates 2 mm less than the enzyme encoded by Me^{100}. Alleles are represented by superscripts to the symbol for the gene locus.

The results of the cross-correlation tests are shown in Tables 8 and 9, above the diagonal. If the frequencies of two alleles at two different loci oscillate at random, the cross-correlation function that compares their frequency for samples separated a given number of months is expected to be zero. If the allele frequencies at two loci, however, respond to a varying environmental parameter by changing their frequencies, either in the same direction (both alleles increasing or both decreasing at the same time) or in opposite direction (one allele increasing, the other decreasing in frequency), then the cross correlation will be different from zero.

We have compared for each two alleles their frequencies in samples separated 1 month, 2 months, and so on, up to 20 months. The 95% confidence interval around the value of 0 expected from randomness can be estimated as $\pm 1.96\sqrt{1/N}$, where N is the number of observations (*i.e.*, monthly samples). If

TABLE 6

D. pseudoobscura: *allele frequencies used in the statistical tests*

Year and month	Locus and allele											
	Est-5		*Hk-1*	*Lap*		*Mdh*		*Me*			*Odh*	*Pgm*
	100	*102*	*100*	*100*	*102*	*100*	*112*	*98*	*100*	*102*	*100*	*100*
1972												
Mar	0.329	0.204	0.926	0.812	0.083	0.955	0.045	0.059	0.763	0.131	0.922	0.636
Apr	0.438	0.247	0.948	0.923	0.026	0.965	0.029	0.088	0.788	0.098	0.939	0.664
May	0.542	0.264	0.975	0.900	0.054	0.935	0.065	0.084	0.799	0.099	0.968	0.635
June	0.453	0.245	0.947	0.890	0.077	0.975	0.025	0.053	0.833	0.104	0.955	0.709
July	0.443	0.313	0.970	0.737	0.249	0.966	0.034	0.068	0.788	0.124	0.945	0.692
Aug	0.522	0.226	0.976	0.920	0.061	0.962	0.038	0.076	0.787	0.124	0.970	0.642
Sept	0.535	0.259	0.967	0.917	0.064	0.968	0.031	0.046	0.807	0.120	0.970	0.651
Oct	0.520	0.309	0.974	0.933	0.050	0.965	0.035	0.059	0.840	0.075	0.973	0.623
1973												
Jan	0.381	0.429	0.976	0.905	0.071	0.929	0.071	0.095	0.762	0.143	0.976	0.757
Feb	0.477	0.432	0.989	0.919	0.058	0.977	0.023	0.057	0.875	0.068	0.966	0.692
Mar	0.514	0.291	0.997	0.877	0.090	0.959	0.041	0.044	0.875	0.076	0.922	0.698
Apr	0.505	0.263	0.978	0.898	0.063	0.953	0.047	0.072	0.786	0.129	0.967	0.675
May	0.475	0.251	0.980	0.885	0.063	0.957	0.042	0.049	0.838	0.109	0.958	0.674
June	0.514	0.255	0.968	0.891	0.066	0.939	0.061	0.051	0.855	0.092	0.955	0.693
July	0.516	0.245	0.974	0.881	0.082	0.947	0.053	0.044	0.871	0.084	0.947	0.705
Aug	0.472	0.266	0.991	0.918	0.063	0.956	0.044	0.050	0.908	0.035	0.968	0.687
Sept	0.495	0.248	0.990	0.878	0.073	0.949	0.051	0.014	0.905	0.075	0.929	0.659
Oct	0.466	0.247	0.997	0.869	0.067	0.945	0.055	0.022	0.913	0.050	0.957	0.634
Nov	0.522	0.250	0.972	0.905	0.044	0.956	0.044	0.017	0.908	0.059	0.959	0.656
Dec	0.455	0.273	1.000	0.818	0.136	0.955	0.046	0.136	0.727	0.136	0.955	0.455
1974												
Jan	0.514	0.221	0.976	0.883	0.052	0.946	0.054	0.080	0.851	0.063	0.950	0.630
Feb	0.496	0.228	0.975	0.890	0.060	0.957	0.043	0.036	0.936	0.026	0.964	0.647

Mar	0.526	0.253	0.984	0.894	0.064	0.932	0.068	0.032	0.916	0.037	0.947	0.679
Apr	0.517	0.282	0.882	0.929	0.048	0.968	0.028	0.039	0.896	0.046	0.960	0.684
July	0.608	0.206	0.970	0.909	0.030	0.939	0.061	0.023	0.924	0.046	0.939	0.711
Aug	0.500	0.244	0.893	0.875	0.085	0.980	0.020	0.043	0.913	0.030	0.943	0.681
Sept	0.474	0.201	0.964	0.875	0.067	0.960	0.040	0.031	0.933	0.031	0.955	0.644
Oct	0.443	0.253	0.987	0.868	0.085	0.962	0.028	0.045	0.891	0.045	0.967	0.576
Nov	0.382	0.353	1.000	0.838	0.103	1.000	0.000	0.029	0.912	0.044	0.985	0.787
Dec	0.200	0.600	1.000	0.833	0.000	0.833	0.167	0.000	0.833	0.000	1.000	1.000
1975												
Jan	0.500	0.500	0.833	0.833	0.167	1.000	0.000	0.000	0.833	0.000	1.000	1.000
Feb	0.379	0.259	0.978	0.832	0.091	0.957	0.043	0.009	0.940	0.017	0.983	0.608
Apr	0.324	0.353	0.971	0.912	0.044	0.971	0.015	0.000	0.971	0.015	0.971	0.643
May	0.474	0.284	0.983	0.817	0.122	0.978	0.022	0.005	0.946	0.016	0.984	0.564
June	0.524	0.333	0.952	0.929	0.024	0.952	0.048	0.024	0.952	0.000	1.000	0.667

TABLE 7

D. persimilis: *allele frequencies used in the statistical tests*

Year and month	Locus and allele											
	Est-5			*Hk-1*		*Lap*		*Mdh*	*Me*	*Odh*	*Pgm*	
	106	*107*	*108*	*96*	*100*	*100*	*102*	*100*	*104*	*100*	*100*	*104*
1972												
Mar	0.050	0.723	0.149	0.030	0.711	0.773	0.188	0.994	0.897	0.960	0.253	0.727
Apr	0.082	0.694	0.204	0.040	0.701	0.848	0.079	0.982	0.904	0.982	0.238	0.756
May	0.109	0.630	0.152	0.040	0.961	0.775	0.213	1.000	0.961	1.000	0.263	0.737
June	0.029	0.657	0.200	0.173	0.827	0.700	0.217	0.875	0.855	0.913	0.200	0.788
July	0.077	0.714	0.121	0.044	0.956	0.797	0.203	0.959	0.912	0.992	0.221	0.779
Aug	0.047	0.802	0.140	0.090	0.910	0.838	0.149	1.000	0.833	0.993	0.296	0.704
Sept	0.068	0.741	0.158	0.061	0.930	0.867	0.126	0.986	0.948	0.991	0.275	0.716
Oct	0.072	0.724	0.163	0.003	0.916	0.854	0.135	0.991	0.907	0.988	0.333	0.660
1973												
Jan	0.063	0.652	0.250	0.110	0.890	0.811	0.163	0.990	0.890	0.995	0.239	0.693
Feb	0.094	0.694	0.115	0.076	0.916	0.784	0.200	0.983	0.924	0.985	0.293	0.695
Mar	0.098	0.726	0.122	0.048	0.945	0.824	0.153	0.992	0.906	0.977	0.312	0.675
Apr	0.083	0.737	0.107	0.088	0.912	0.750	0.225	0.992	0.962	0.980	0.246	0.744
May	0.102	0.688	0.148	0.078	0.919	0.753	0.220	0.991	0.940	1.000	0.295	0.691
June	0.113	0.747	0.127	0.096	0.904	0.736	0.243	0.993	0.930	0.992	0.260	0.740
July	0.102	0.776	0.122	0.048	0.952	0.849	0.105	0.988	0.941	0.988	0.375	0.625
Aug	0.087	0.795	0.087	0.071	0.930	0.870	0.123	0.994	0.974	0.994	0.260	0.732
Sept	0.069	0.730	0.159	0.097	0.895	0.800	0.175	0.986	0.974	0.970	0.285	0.703
Oct	0.060	0.719	0.170	0.054	0.938	0.815	0.156	0.988	0.971	0.989	0.333	0.658
Nov	0.045	0.816	0.108	0.095	0.905	0.880	0.105	0.997	0.924	0.997	0.362	0.634
Dec	0.022	0.870	0.087	0.052	0.948	0.879	0.103	1.000	0.948	0.948	0.326	0.674
1974												
Jan	0.051	0.737	0.162	0.069	0.922	0.829	0.145	0.993	0.947	0.983	0.284	0.707
Feb	0.050	0.749	0.148	0.054	0.941	0.816	0.163	0.997	0.978	0.982	0.263	0.683

Mar	0.054	0.784	0.138	0.090	0.905	0.809	0.163	0.983	0.955	0.972	0.329	0.665
Apr	0.043	0.670	0.192	0.071	0.929	0.786	0.179	0.973	0.968	0.991	0.383	0.596
July	0.000	0.927	0.071	0.111	0.889	0.778	0.222	1.000	0.944	1.000	0.214	0.786
Aug	0.044	0.761	0.163	0.098	0.902	0.828	0.172	0.984	0.967	0.984	0.239	0.739
Sept	0.089	0.718	0.129	0.092	0.908	0.802	0.184	0.989	0.967	0.974	0.272	0.723
Oct	0.058	0.797	0.103	0.070	0.931	0.821	0.150	0.985	0.962	0.977	0.311	0.672
Nov	0.074	0.719	0.157	0.105	0.895	0.828	0.149	0.988	0.976	0.986	0.302	0.691
Dec	0.063	0.763	0.100	0.052	0.948	0.871	0.121	1.000	0.948	0.983	0.325	0.675
1975												
Jan	0.134	0.761	0.075	0.072	0.927	0.809	0.155	0.964	0.936	0.964	0.343	0.642
Feb	0.102	0.722	0.127	0.107	0.884	0.796	0.185	0.981	0.961	0.997	0.305	0.619
Apr	0.109	0.717	0.141	0.092	0.908	0.821	0.158	1.000	0.978	0.984	0.288	0.706
May	0.100	0.727	0.146	0.094	0.907	0.790	0.187	0.972	0.963	0.963	0.316	0.674
June	0.000	0.875	0.125	0.063	0.813	0.938	0.063	1.000	1.000	1.000	0.250	0.750

TABLE 8

D. pseudoobscura: *results from the cross-correlation test (upper triangle) and from the cumulative distribution of the phase angle (lower triangle)*

	Alleles											
Alleles	*Est-5*^{100}	*Est-5*^{102}	*Hk-1*^{100}	*Lap*^{100}	*Lap*^{102}	*Mdh*^{100}	*Mdh*^{112}	*Me*^{98}	*Me*^{100}	*Me*^{102}	*Odh*^{100}	*Pgm*^{100}
Est-5^{100}	—[a]	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Est-5^{102}	—	—	NS	NS	NS	*	NS	NS	NS	*	*	NS
Hk-1^{100}	NS	NS	—	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lap^{100}	*	NS	NS	—	—	NS	NS	*	NS	NS	*	NS
Lap^{102}	NS	*	NS	—	—	NS	NS	*	*	NS	NS	NS
Mdh^{100}	*	NS	*	NS	*	—	—	NS	NS	NS	*	NS
Mdh^{112}	*	NS	NS	NS	*	—	—	NS	NS	NS	*	NS
Me^{98}	*	*	*	*	*	*	*	—	—	—	*	*
Me^{100}	NS	NS	*	*	NS	*	NS	—	—	—	*	*
Me^{102}	NS	*	NS	NS	*	*	*	—	—	—	*	*
Odh^{100}	NS	*	NS	NS	NS	NS	NS	NS	NS	*	—	NS
Pgm^{100}	NS	*	NS	*	NS	NS	NS	*	*	*	*	—

[a] — = test not made because alleles at the same locus are involved.
* = significant deviation (see text); NS = nonsignificant deviation.

TABLE 9

D. persimilis: *results from the cross-correlation test (upper triangle) and from the cumulative distribution of the phase angle (lower triangle)*

	Alleles											
Alleles	*Est-5*^{106}	*Est-5*^{107}	*Est-5*^{108}	*Hk-1*^{96}	*Hk-1*^{100}	*Lap*^{100}	*Lap*^{102}	*Mdh*^{100}	*Me*^{104}	*Odh*^{100}	*Pgm*^{100}	*Pgm*^{104}
Est-5^{106}	—[a]	—	—	NS	NS	NS	NS	NS	NS	NS	NS	NS
Est-5^{107}	—	—	—	NS	NS	*	NS	NS	NS	NS	NS	NS
Est-5^{108}	—	—	—	*	NS	NS	NS	*	*	NS	*	*
Hk-1^{96}	NS	NS	NS	—	—	NS	NS	*	NS	*	*	*
Hk-1^{100}	*	NS	*	—	—	NS	NS	NS	NS	NS	*	*
Lap^{100}	*	NS	NS	*	NS	—	—	*	NS	*	NS	NS
Lap^{102}	NS	NS	NS	NS	*	—	—	NS	NS	NS	NS	NS
Mdh^{100}	NS	*	NS	NS	NS	*	NS	—	*	*	NS	NS
Me^{104}	NS	*	*	NS	NS	NS	NS	NS	—	NS	*	NS
Odh^{100}	*	NS	NS	NS	NS	*	*	NS	*	—	*	NS
Pgm^{100}	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	—	—
Pgm^{104}	*	NS	NS	NS	NS	NS	NS	*	*	*	—	—

[a] — = test not made because alleles at the same locus are involved.
* = significant deviation (see text); NS = nonsignificant deviation.

TABLE 10

D. pseudoobscura: *qualitative classification of the results from the cross correlation, the amplitude spectrum and the correlation tests*

	Alleles									
Allele	*Hk-1*^{100}	*Lap*^{100}	*Lap*^{102}	*Mdh*^{100}	*Mdh*^{112}	*Me*^{98}	*Me*^{100}	*Me*^{102}	*Odh*^{100}	*Pgm*^{100}
Est-5^{100}	n	s	n	n	n	b	n	b	s	s
Est-5^{102}	b	n	n	b	n	b	s	s	s	s
Hk-1^{100}		n	n	n	n	n	b	n	n	b
Lap^{100}				n	n	b	b	n	n	b
Lap^{102}				n	n	b	n	b	n	n
Mdh^{100}						n	n	n	b	n
Mdh^{112}						n	n	n	b	n
Me^{98}									b	s
Me^{100}									s	s
Me^{102}									s	s
Odh^{100}										s

s = significant; b = borderline; n = neutral.

more than 5% of the comparisons between a pair of alleles fall outside this interval, the correlation is considered significant. Twenty-five percent (15 of 60) of the cross correlations between allele pairs are significant in *D. pseudoobscura*; 30% (18 of 60) in *D. persimilis*.

If there are periodic components in the cross-correlation function, the cross-correlation test will fail to detect correlated responses between different loci. These may be detected by a test of correlation in the frequency domain. When two time series are uncorrelated, the sample phase spectrum will be approximately uniformly distributed between $-\pi/2$ and $\pi/2$. Observations that fall outside the 95% confidence interval encompassing the expected uniform distribution can then be interpreted as evidence of correlated responses at the two loci compared. The results of this test are shown below the diagonal in Tables 8 and 9. Nearly one-half (29 of 60) of the pairwise comparisons in *D. pseudoobscura* and 30% (18 of 60) in *D. persimilis* are statistically significant.

We want to examine whether linkage may contribute to the correlated response. In *D. pseudoobscura*, seven of the 15 significant cross correlations involve pairs of loci on the same chromosome, namely *Lap*, *Me* and *Odh*, which are all on the second chromosome. If each test is treated as an independent observation, only 3.3 significant tests would be expected on linked loci. In *D. persimilis*, only three (2.7 expected) of the 18 significant correlations are between linked loci, one between *Lap* and *Odh* on the second chromosome and two between *Est-5* and *Pgm* on the *X*. With respect to the cumulative distribution of the phase angle, only six (6.3 expected) of the 29 significant correlations involve loci on the same chromosome in *D. pseudoobscura*, and five (3.3 expected) of 18 in *D. persimilis*.

Thus, of the tests conducted in Tables 8 and 9, only the cross correlations in *D. pseudoobscura* produce an excess of significant tests for linked loci. This bias is not, however, seen in the summary classification in Table 10, which

incorporates the results of all the *D. pseudoobscura* tests. Three of the 12 significant results are for linked loci. Assuming independence of the observations we would expect 1.8 such observations, and this difference is not statistically significant.

We have shown above that the cross-correlation function between two loci responding to the same environmental variable will have a peak (which can be positive or negative) at a time lag of 0 and will rapidly decrease on both sides as the lag between comparisons increases. Similarly, we have shown that the amplitude of the cross spectrum will be flat at value zero, when the allele frequency responses are not correlated, but it will have a peak near the frequency of 0 cycles per unit time and will decrease toward a frequency of 0.5 cycles per unit time (or vice versa) if they are correlated.

We have performed these analyses for all pairs of alleles given in Tables 6 and 7 and have obtained the corresponding sets of 120 graphs for each species. We have, somewhat arbitrarily, classified these graphs into three groups: (1) pairs of alleles that show strong evidence of natural selection, *i.e.*, where the two allele frequencies are changing over time in a correlated fashion and, hence, could be responding to the same environmental variables; (2) pairs of alleles manifesting moderate deviations from the expectations of random allele frequency variation, which we therefore take as marginal evidence of correlated selective responses to the temporal heterogeneity of the environment; and (3) pairs of alleles that show no systematic deviations from the expectations of neutral variation. Representative examples of the three classes are shown in Figures 5–10.

Figure 5 shows three examples of strong cross correlations in *D. pseudoobscura*. The first (Figure 5a) involves alleles $Est\text{-}5^{102}$ and Pgm^{100}, two alleles that did not have a statistically significant cross correlation (Table 8), yet show here a very distinct peak at lag 0, with very rapid decay on both sides. The two loci *Est-5* and *Pgm* are sex-linked; the other two examples in Figure 5 involve unlinked loci. $Est\text{-}5^{102}$ and Odh^{100} show a very sharp positive peak, with the cross correlation decaying as the lag increases and reaching 0 by lag = 4 or 5. Between Me^{102} and Pgm^{100} the cross-correlation peak at lag 0 is negative (Figure 5c). The cross spectrum is positive in the whole range examined for these three pairs of alleles (Figure 5d, e and f). For the first two comparisons, even the lower 95% confidence line is above zero through most of the range.

The three *D. pseudoobscura* examples of moderate deviations from the neutrality expectations are shown in Figure 6. The cross-correlation peaks are negative in 6b and c and are positive in 6a, but the peaks are less extreme than in Figure 5. The cross spectrum is clearly positive for the pair $Est\text{-}5^{100}$, Me^{98} (Figure 6d), but less so for the other two pairs. The Lap^{102}, Me^{102} pair involves two linked loci (chromosome *II*). Figure 7 gives three examples of allele pairs in *D. pseudoobscura* showing no evidence of departure from the neutrality expectations.

The three examples of strong cross correlations in *D. persimilis* are shown in Figure 8. None of the allele pairs involve linked loci. The peaks at lag 0 are not as high as in the *D. pseudoobscura* examples; the cross spectrum (Figure

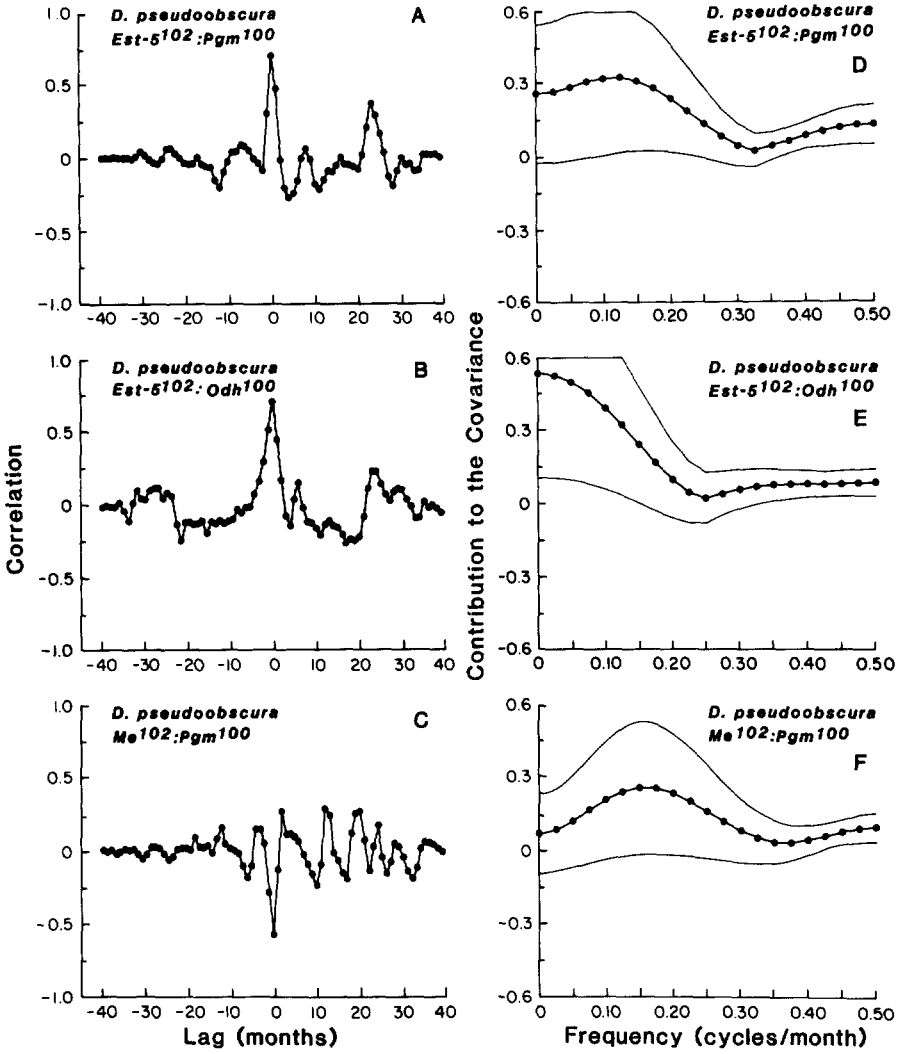

FIGURE 5.—Three pairs of alleles showing strong evidence of correlated responses to environmental variation in *Drosophila pseudoobscura*. Parts a, b and c show the cross-correlation function for allele frequencies sampled at the same time, 1 mo apart, 2 mo apart, and so on. Parts d, e and f show the amplitude of the cross spectrum for the frequencies given in cycles per month. Two standard deviations are delimited by the lines above and below the spectrum curve.

8d, e and f) does not notably depart from the flat distribution at 0 expected from random fluctuations. Examples of cross correlations with lower peaks are shown in Figure 9. The first example involves alleles *Est-1*^{107} and *Pgm*^{104} at linked loci (*X* chromosome). Figure 10 has three *D. persimilis* examples of cross-correlation and spectrum distributions with no evidence of departure from the variations due to white noise.

Comparison of the examples given for the two species (compare particularly Figures 5 and 8) manifests a result that is general for all the alleles in Tables

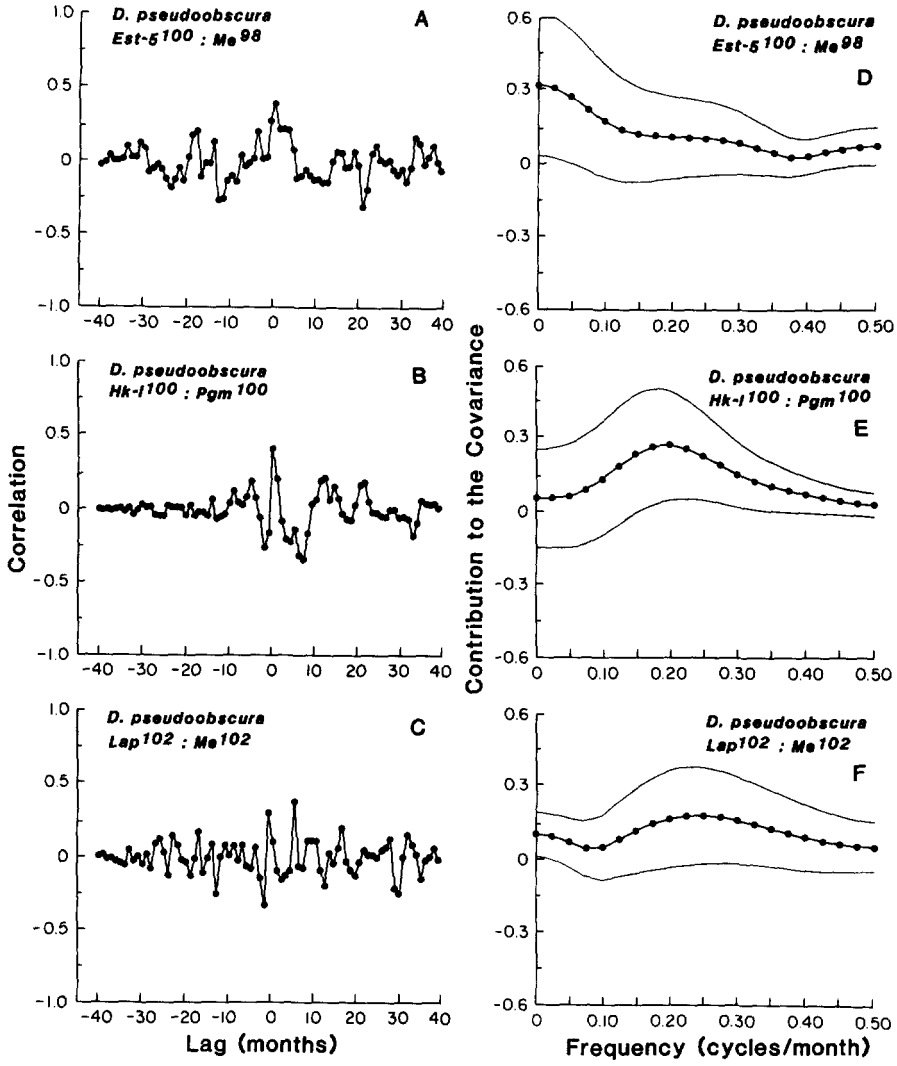

FIGURE 6.—Three pairs of alleles in *Drosophila pseudoobscura* giving marginal evidence of correlated responses to environmental variation. (See legend to Figure 5 for additional details.)

6 and 7; namely, that the cross-correlation function, as well as the amplitude of the cross spectrum, shows stronger departures from the expectations of neutrality in *D. pseudoobscura* than in *D. persimilis*.

We have evaluated the distributions for all pairwise allele comparisons and have qualified such evaluations with the statistical tests given in Tables 8 and 9, in order to produce an overall qualitative classification of the results. The allele pairs are grouped into three classes: (1) those showing significant deviations from neutrality, (2) those considered "borderline" and (3) those showing no deviations from neutrality. The outcomes of these evaluations are in Tables 10 and 11, for *D. pseudoobscura* and *D. persimilis*, respectively. In *D. pseudoobscura*, 20% (12 of 60) of the allele pairs show strong evidence of correlated

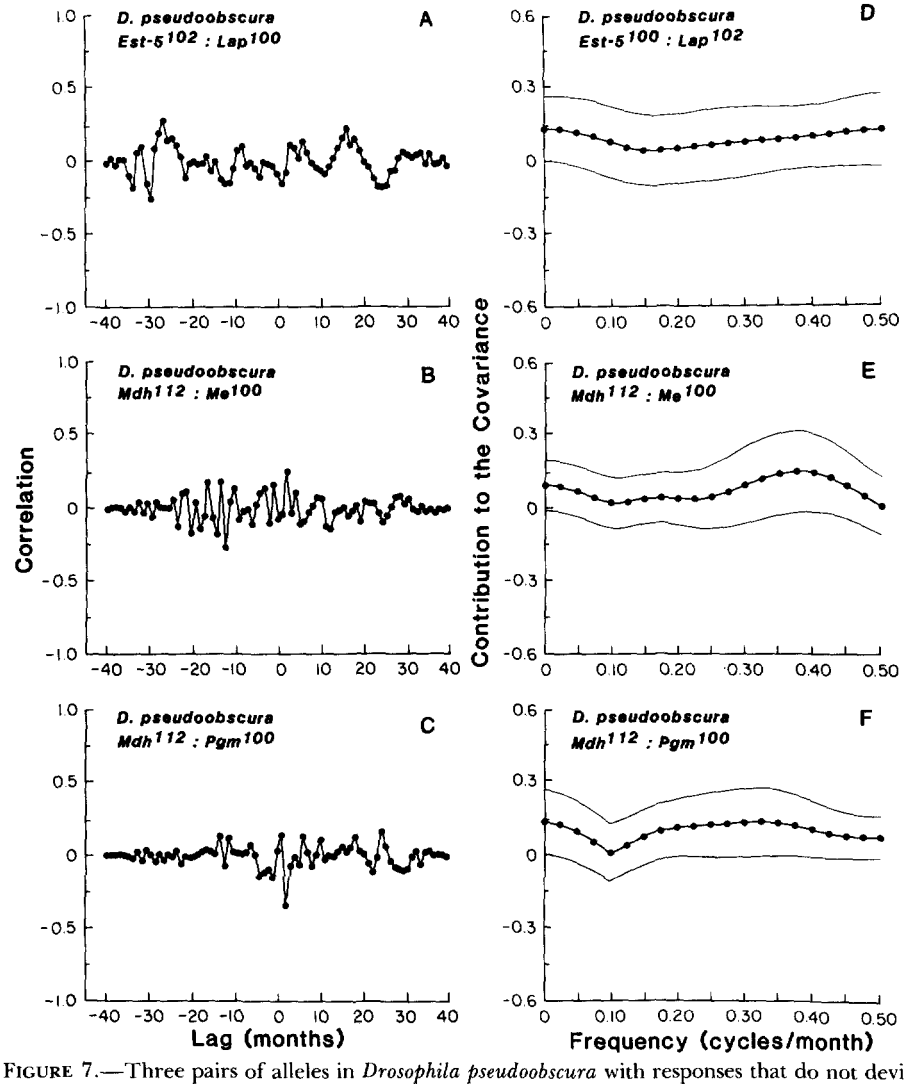

FIGURE 7.—Three pairs of alleles in *Drosophila pseudoobscura* with responses that do not deviate from the neutrality expectations. (See legend to Figure 5 for additional details.)

responses to environmental variations, an additional 27% (16 pairs) show weak evidence, whereas 52% (32 pairs) are not jointly responding to the oscillations of the environment. In *D. persimilis*, 13% (eight pairs) show strong correlations, 17% (ten pairs) show weak correlations, and 70% (42 pairs) give no indication of joint response.

Selection responses to environmental variation can also be detected by means of the autocorrelation between the frequencies observed 1-month apart, 2-months apart, and so on, for each particular allele. Especially interesting would be correlations between samples taken 12-months apart, which would reflect the obvious yearly cycles in temperature and related factors. Yearly cycles in the frequency of third-chromosome arrangements have been repeatedly de-

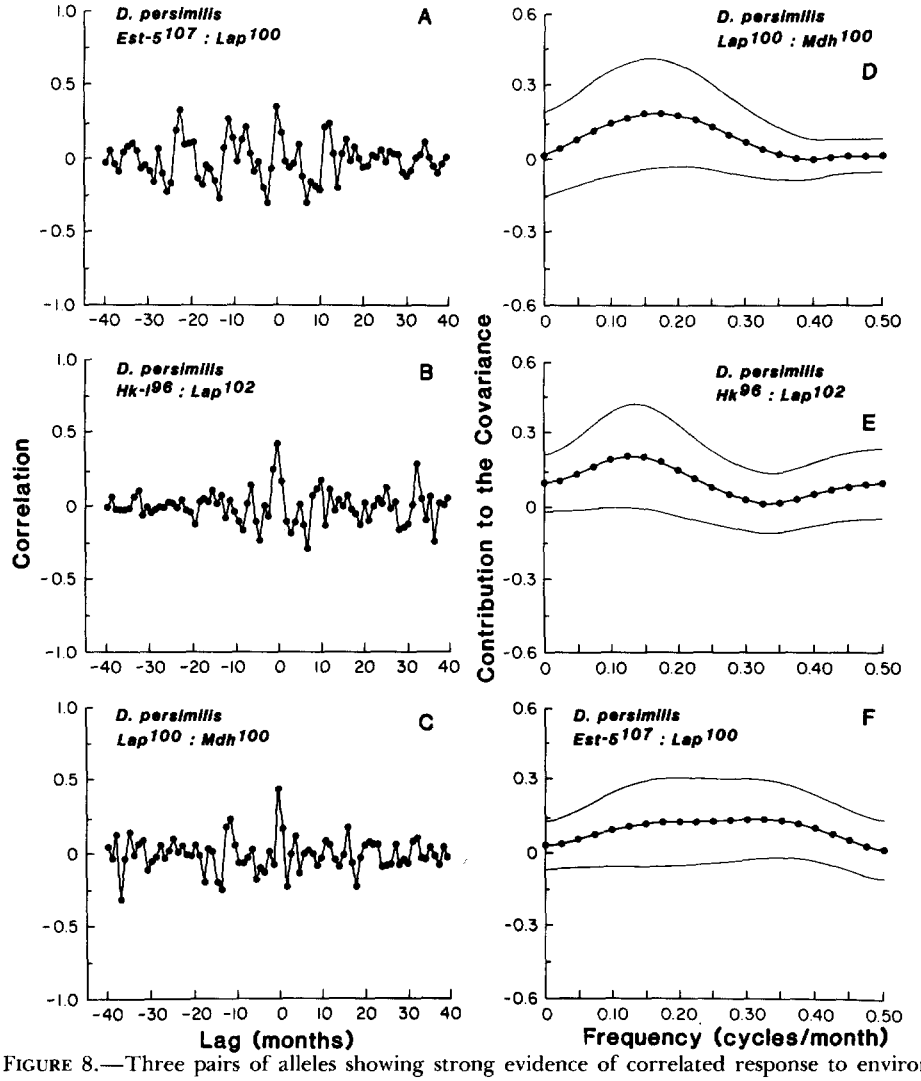

FIGURE 8.—Three pairs of alleles showing strong evidence of correlated response to environmental variation in *Drosophila persimilis*. (See legend to Figure 5 for additional details.)

tected in *D. pseudoobscura* (review in DOBZHANSKY 1971). These yearly cycles occur in *D. pseudoobscura* as well as in *D. persimilis* at McDonald Ranch (DOBZHANSKY and AYALA 1973).

We have examined the autocorrelation function for each one of the alleles listed in Tables 6 and 7. Figure 11 displays the autocorrelation results for three alleles. Peaks for samples taken 12-months apart are clearly apparent for allele Me^{100} in *D. pseudoobscura* (Figure 11a) and allele Lap^{100} in *D. persimilis* (Figure 11c). The most likely interpretation of these 1-yr cycles is that they reflect the response of these alleles to the yearly climatic cycles. The $Est\text{-}5^{102}$ allele shows in *D. pseudoobscura* an autocorrelation peak for samples taken 2-yr apart (Figure 11b). The significance of this peak is less obvious than for

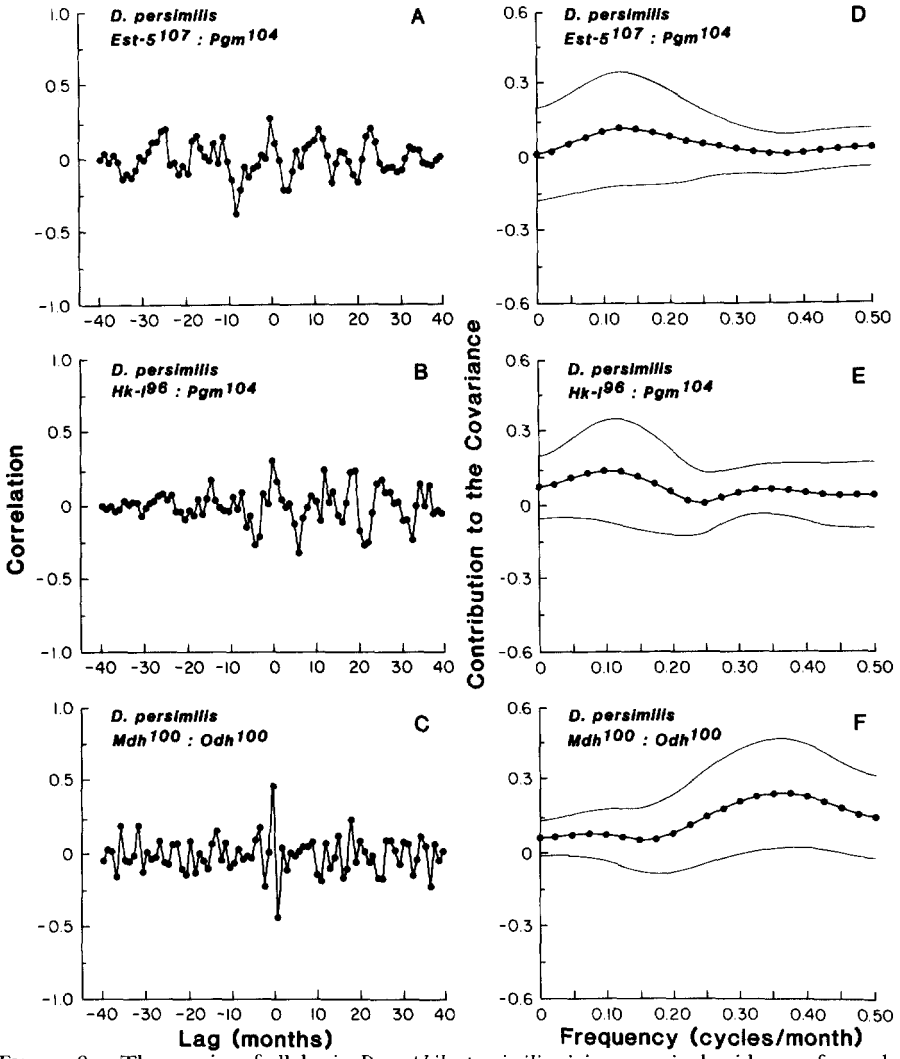

FIGURE 9.—Three pairs of alleles in *Drosophila persimilis* giving marginal evidence of correlated responses to environmental variation. (See legend to Figure 5 for additional details.)

those between the samples 1-yr apart. In general, there is no evidence that most allelic frequencies are strongly responding to the yearly cycles associated with the seasons.

In the absence of cycles we expect the autospectrum to show a peak at the lowest frequencies. The three autospectra shown in Figure 11 (d, e and f) all show peaks at frequencies other than 0. These indicate some underlying periodic component that completes one cycle in 7 (Figure 11d) to 25 months (Figure 11e). The graphs shown in Figure 11d, e and f illustrate as well the effects of the "spectral window" also used in Figures 5–10 (d, e and f). The spectral density uses the autocorrelation estimates, which will be less accurate when the number of months separating two frequencies is large. Therefore, a

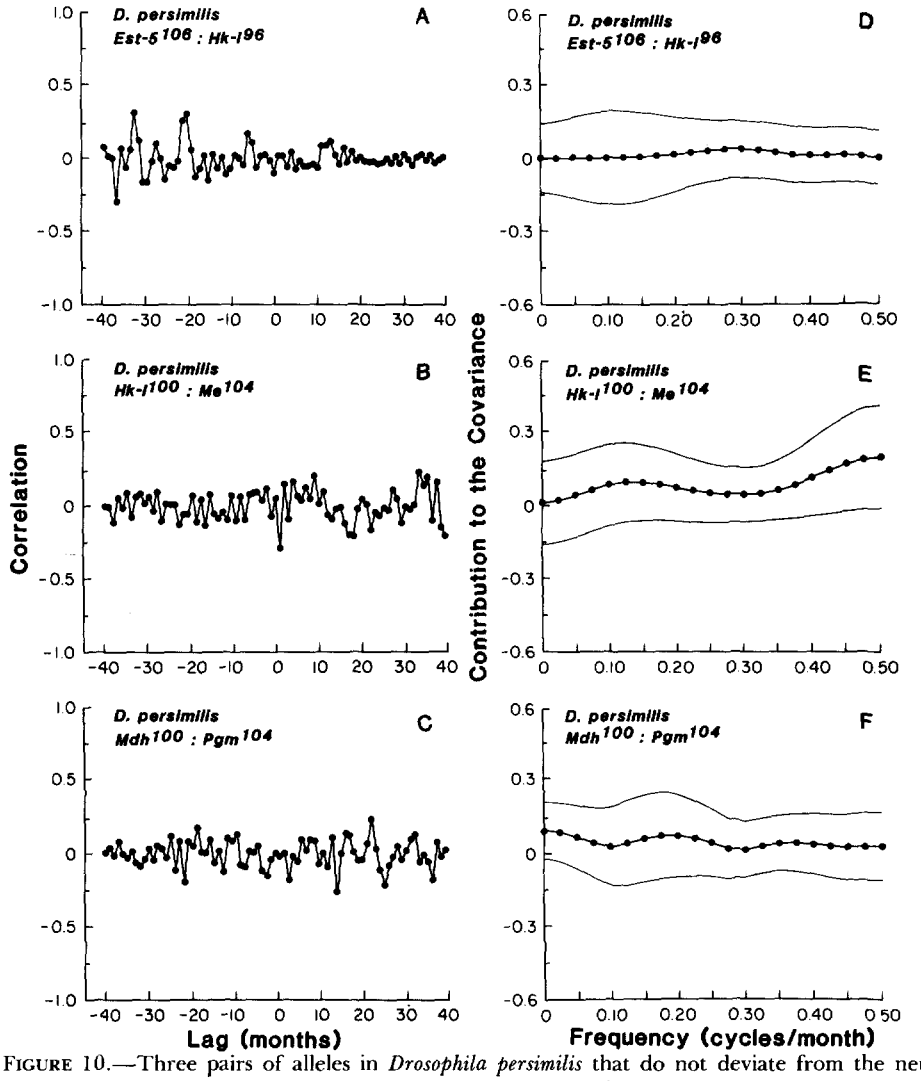

FIGURE 10.—Three pairs of alleles in *Drosophila persimilis* that do not deviate from the neutrality expectations. (See legend to Figure 5 for additional details.)

spectral window (*i.e.*, a weighting function) is used to give greater weight to correlation values between samples close in time. We used three sizes for the spectral window, illustrated in Figure 11, in which it can be seen that the window with intermediate size reduces considerably the variance without much apparent increase in bias. It is this intermediate window that we have used for the spectral distributions depicted in Figures 5–10.

DISCUSSION

Objective criteria are desirable for detecting patterns in experimental data. Statistical tests of hypotheses provide such objective criteria. Unfortunately, in the issue at hand—namely, the relative roles of natural selection and random

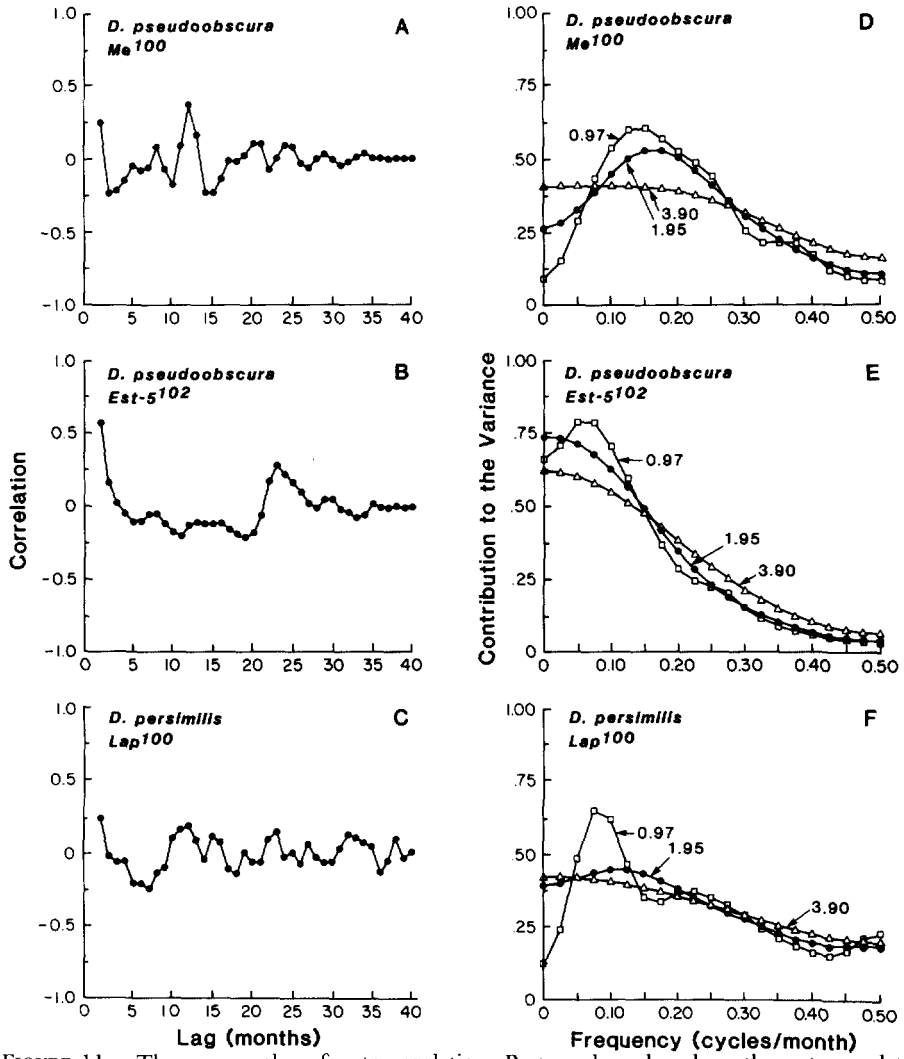

FIGURE 11.—Three examples of autocorrelation. Parts a, b and c show the autocorrelation function. Parts d, e and f show the autospectrum for the first-order autoregressive process. The autospectrum is generated using one of three spectral windows. The spectral window of intermediate magnitude (1.95) is the one used for the spectra displayed in Figures 5–10, parts d, e and f.

drift in causing allele-frequency changes—severe assumptions are usually required in order to derive a precise statistical test (WATTERSON 1977; PERLOW 1979; ROTHMAN and TEMPLETON 1980; MUELLER *et al.* 1985). If such assumptions are not met, the apparent rigor of the statistical tests is only a mirage.

Hence, we have resorted to a variety of tests that are evaluated as a whole in a manner that is, to some degree, subjective. The results of these evaluations are shown in Tables 10 and 11. We have attenuated the implications of sub-

jectivity in various ways. We have displayed a number of cross-correlation functions and amplitude spectra so that others might see the strength of our evaluations and, perhaps, arrive at different conclusions. We have used some objective statistical tests (Tables 8 and 9), which in fact yield a greater number of significant results than those we are willing to classify as such in the summary tables. We also have placed bounds on the strength of selection that can be detected by the methods at hand; and we have determined the frequency with which random genetic drift could mimic results that might appear as strong deviations from neutrality.

The summary classifications given in Table 10 and 11 are guided by four items of information: (1) the cross-correlation function, (2) the amplitude spectrum, (3) the cross-correlation test of the prefiltered data and (4) the phase-distribution test using prefiltered data. We have provided precise expectations for criteria (1) and (2). When examining the cross correlations and the amplitude spectra, we chose only those deviations from the neutral expectations that resembled the expectations of the particular model of natural selection considered; that is, when fitnesses are responding additively to random environmental changes. Criteria (3) and (4) can detect nonindependence between two sets of time-series data when there are periodicities or correlations not consistent with our particular model of natural selection. Thus, we have ignored cases of statistically significant results when the patterns observed with criteria (1) and (2) did not conform to the expectations of the model. In general, we have weighted more heavily criteria (1) and (2) than (3) and (4) in developing the classifications given in Tables 10 and 11. Thus, two instances for each *D. pseudoobscura* and *D. persimilis* appear in the summary tables as strong deviations, even though neither criterion (3) nor (4) indicates statistical dependence. But we have included several examples of each species in the borderline and neutral categories, in spite of strong evidence of selection by criteria (3) and (4), because the cross correlations and amplitude spectra do not conform to our selection model.

The analysis presented in this paper has some limitations. The most important one, in practice, concerns the size of the data set required. The techniques used are applicable to any organism and any number of temporal samples. But they will be most useful when there is a substantial number (>30) of sequential samples, when multiple gene loci are surveyed and when the number of genes in each sample is large. As discussed earlier, the methods are designed to detect selection responses to *variable environments.* Tests of neutrality have often assumed overdominance or directional selection as the alternative hypothesis (SCHAFFER, YARDLEY and ANDERSON 1977; WATTERSON 1977, 1982). These types of selection, or any other deterministic mode, will go undetected by our methods. One particular interest in detecting selection in random varying environments is that this form of selection shares many theoretical properties with particular neutral models (GILLESPIE 1979). Hence, there is a pressing need for methods that discriminate between the two alternative theories.

Despite pronounced seasonal oscillations in inversion frequencies in *D. pseudoobscura* (DOBZHANSKY 1971) there are only a few cases in the present study

of seasonal variation in allele frequency. This result is consistent with other studies with Drosophila. CAVENER and CLEGG (1981) saw no evidence of cycling in 12 polymorphic loci of *D. melanogaster* over a 2-yr period, except possibly for 6*Pgd*. BARKER, EAST and WEIR (unpublished results) also reported no evidence of seasonal patterns in allele frequencies at six loci of *D. buzzatii*; however, they found some significant correlations between alleles at different loci corresponding to $r_{x_1x_2}$ (0) in our study. Thus, there may be in their study correlated movements of allele frequencies over time similar to those observed by us.

The ability to detect selection responses to environmental variation depends on the effective size of the population, the selective magnitude of the deterministic components of fitness, the variance in the stochastic components of fitness, the correlation of the environment, and the sample size for estimating the allele frequencies. The effective size of the population is not an important source of variance if it is much larger, say ten times, than the sample size. In the McDonald population there can be no doubt that the effective population size is several orders of magnitude greater than the number of flies sampled. With respect to the other variables, we have shown that the detection of selection is facilitated when the deterministic components of fitness are small, the stochastic fitness variance is large, the environment is positively autocorrelated and the sample size is large. Only rough estimates of these parameters are possible, except for sample size.

With samples of 100–200 individuals, we roughly estimate that random environmental selection can be detected when the genotype fitnesses oscillate by at least 15%—unless the environment is negatively autocorrelated, in which case greater fitness differences would be required for detection. But there is little hope for detecting selection if the relative fitness of a genotype changes by, say, less than 5% from one environment to another.

A potential source of equivocation is the possibility that random drift would mimic the cross-correlation pattern is variable selection. This, however, will occur infrequently if the effective size of the natural populations is at least ten times greater than the sample sizes—a requirement sufficiently satisfied in our study. The frequency with which random drift might be interpreted as variable selection is further decreased by the use of other criteria besides the cross-correlation function, as we have done it.

Given the restrictive conditions needed to detect variable selection by our methods, there appears to be a sizable number of strong deviations from the neutral expectations: 20% in *D. pseudoobscura* (Table 10) and 13% in *D. persimilis* (Table 11). Analogous methods have been applied by MUELLER *et al.* (1985) to temporal variation of allele frequencies over eight generations (8 yr!) in the butterfly *Ephydryas editha*; more variation was found than expected from random drift alone. Although their methods involved crude estimates of the effective population size, FISHER and FORD (1947) conclude that the *medionigra* allele in the moth *Panaxia dominula* showed more variation over a 6-yr period than was expected from random drift alone. ARNOLD (1982) has developed a model in which estimated allele frequencies are affected by experimental error

TABLE 11

D. persimilis: *qualitative classification of the results from the cross correlation, the amplitude spectrum and the correlation tests*

	Alleles								
Alleles	*Hk-1*^{96}	*Hk-1*^{100}	*Lap*^{100}	*Lap*^{102}	*Mdh*^{100}	*Me*^{104}	*Odh*^{100}	*Pgm*^{100}	*Pgm*^{104}
Est-5^{106}	n	n	n	n	n	b	n	n	b
Est-5^{107}	n	n	s	n	s	b	n	n	b
Est-5^{108}	b	n	n	n	n	b	n	n	n
Hk-1^{96}			s	s	n	n	n	b	b
Hk-1^{100}			n	n	n	n	n	s	s
Lap^{100}					s	n	n	n	s
Lap^{102}					n	n	n	n	b
Mdh^{100}						n	b	n	n
Me^{104}							n	n	n
Odh^{100}								n	n

s = significant; b = borderline; n = neutral.

and migration from an unobserved population. Since migration rates and allele frequencies in the migrant pool are unobserved quantities, this model is flexible enough to account for variation in chromosome frequencies in *D. pseudoobscura* in excess of the sampling variance (ARNOLD 1982). It seems plausible that, if migration was set to an appropriate level and allele frequencies in the migrant pool varied in a particular fashion, we could account for some of the correlated allele frequency changes observed in the present study. Clearly, additional information on the population structure and migration rates at *D. pseudoobscura* and *D. persimilis* would be necessary to evaluate the plausibility of this hypothesis.

Many laboratory studies have also shown that the fitnesses of allozyme genotypes depend on environmental variables such as temperature (*e.g.*, WATT 1977, 1983; PLACE and POWERS 1979; SNYDER and AYALA 1979; GRAVES and SOMERO 1982; WATT, CASSIN and SWAN 1983), salinity (*e.g.*, KOEHN and SIEBENALLER 1981; BURTON and FELDMAN 1983), and pressure (*e.g.*, SIEBENALLER and SOMERO (1978). *In vitro* studies have also shown that kinetics, stability and other properties of enzymes encoded by different alleles are modified by environmental factors such as temperature, salinity, pressure and others (*e.g.*, LEE, MIRSA and AYALA 1981; KOEHN and IMMERMANN 1981; GRAVES, ROSENBLATT and SOMERO 1983). In a similar vein BARKER, EAST and WEIR (unpublished results) identify microspatial environmental heterogeneity coupled with habitat selection as important factors in the maintenance of genetic polymorphisms in *D. buzzatii*. We interpret our present results, together with this preexisting evidence, as strong indication that variable environmental factors significantly affect the fitness, and therefore the evolution, of enzyme polymorphisms.

In the present study the questions arise whether the correlated responses of the alleles might be due to the gene loci examined or to closely linked loci with which they might be associated in linkage disequilibrium. We cannot

answer this question. But it is the case that the alleles examined are responding to the environment and, hence, that selection is contributing to the maintenance and evolution of the polymorphisms, whether or not it is directly affecting the target loci.

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