HETEROSIS AS AN EXPLANATION FOR LARGE AMOUNTS OF GENIC POLYMORPHISM[1]

R. C. LEWONTIN

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

L. R. GINZBURG[2]

Department of Mathematics, Northeastern University, Boston, Massachusetts 02115

S. D. TULJAPURKAR

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT

By using both numerical and analytical approaches, we have shown that heterosis alone is not a mechanism for maintaining many alleles segregating at a locus. Even when *all* heterozygotes are more fit than *all* homozygotes, the proportion of fitness arrays that will lead to a stable, feasible equilibrium of more than 6 or 7 alleles is vanishingly small. More alleles can be maintained if, in addition to heterosis, it is assumed that there is very little variation in fitness from heterozygote to heterozygote, with the ratio of mean heterosis to standard deviation of fitness among heterozygotes in the neighborhood of 10. When such conditions hold, the allelic frequency distribution and equilibrium will be very uniform, with all alleles very close to equal frequency $p = \frac{1}{n}$. It is much more likely that stable equilibria for multiple alleles will be best explained by multiple niche selection.

THE existence of large amounts of genetic polymorphism at many loci in most organisms has been explained either as the result of the accumulation of unselected mutations, modulated by random sampling events (the "neutralist" or "neo-classical" theory) or as the result of the balance of selective forces (the "selectionist" or "balance" theory). Both theories need to cope with the existence of between two and a dozen alleles segregating at a typical polymorphic locus in natural populations. The recent finding by COYNE (1976) and SINGH, LEWONTIN and FELTON (1976) that the xanthine dehydrogenase locus is segregating for 23 alleles out of 60 genomes tested (*Drosophila persimilis*) and for 37 alleles out of 146 genomes tested (*Drosophila pseudoobscura*) shows that genic variation at individual loci may be much greater than we had previously

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[2] Present address: Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, New York 11794.

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imagined. Mutational theories deal explicitly with multiple alleles by assuming that some large number of allelic states can be generated one from another by mutation, different theories putting different restrictions on the kinds of transitions that are allowed (the "infinite alleles" model of KIMURA 1968; the "ladder model" of OHTA and KIMURA 1973). Selective theories, on the other hand, have not dealt explicitly with multiple alleles at a locus, but have depended upon a heuristic extension of the principle that superior heterozygote fitness in the two-allele case will maintain a stable polymorphism. More complex models of selection coefficients varying in time and space, or frequency-dependent fitness models, likewise argue from two-allele cases and assume that some analogous relations among fitnesses in the multiple-allelic case will apply. Although a usable mathematical theory of the stability of multi-allelic polymorphism has existed for some time for the constant fitness model (KIMURA 1956; PENROSE, SMITH and SPROTT 1956; MANDEL 1959) and a less useful one for frequency-dependent fitness (LEWONTIN 1958), no one seems to have asked whether these results make heterosis a reasonable explanation of the observed polymorphism, although as early as 1970 GILLESPIE, confirming a conjecture of KOJIMA, had shown by Monte Carlo simulation that as the number of alleles increases, the proportion of randomly generated fitness arrays that leads to a stable feasible polymorphism drops very rapidly. (See Figure 1 of GILLESPIE 1977). Are the restrictions on the fitnesses implied by the stability analysis so severe as to tax our credulity? That is the question we ask in this paper. We treat only the case of constant fitnesses here. The much more complicated problem of frequency- and density-dependent fitnesses is the subject of a further investigation.

The mathematics of stable equilibrium

KIMURA (1956) originally gave rules for the stability of a n-allelic polymorphism for the continuous time case, but MANDEL (1959) showed that these were exactly equivalent to the discrete time analysis. Thus, we use KIMURA's argument here, but for the discrete model. In any case, there is nothing involved but an application of the standard analysis of stability of linear systems.

Let p_i be the frequency of the allele A_i

W_{ij} be the fitness of genotype A_iA_j

$\overline{W}_i = \sum_{j=1}^{n} p_j W_{ij}$ = marginal fitness of the allele A_i

and $\overline{W} = \sum_{i=1}^{n} p_i \overline{W}_i = \sum_{i=1}^{n} \sum_{j=1}^{n} p_j W_{ij} p_i$ = mean fitness of the population. Then the change in frequency in the ith allele in one generation is

$$\Delta p_i = p_i \, (\overline{W}_i - \overline{W})/\overline{W}. \tag{1}$$

At equilibrium, where $\Delta p_i = 0$ for all i, if all the alleles are to be present so that none of the p_i are zero, we must have that

$$\overline{W}_i - \overline{W} = 0 \text{ for all } i \tag{2}$$

or equivalently

$$\sum_{j=1}^{n} p_j W_{ij} = \overline{W} \text{ for all } i \text{ and } j \tag{3}$$

By subtracting $\overline{W}_n - \overline{W}$ from all the equations and substituting $p_n = 1 - \sum_{i=1}^{n-1} p_i$ these equations can be reduced to a set of $n-1$ independent linear equations.

$$\sum_{j=1}^{n-1} p_j(W_{ij} - W_{in} - W_{nj} + W_{nn}) = W_{nn} - W_{in} \qquad (4)$$

for $i = 1, \; n-1$

Since this is a set of linear equations, there can be only one solution, and this is easily found by the ratio of the determinants

$$\hat{p}_i = \frac{\det D_i}{\det D} \qquad (5)$$

where D is the matrix of coefficients on the left side of the system of equations (4) and D_i is that matrix with the ith column replaced by the column vector on the right hand side of equations (4).

The necessary and sufficient condition for stability of the equilibrium vector $\hat{p}$ is that all the eigenvalues of the matrix D be negative. Because the matrix is symmetric ($W_{ij} = W_{ji}$), there is a simple algorithm for testing this property (GANTMACHER 1960). We denote by the Δ_i a submatrix of D consisting of the upper left hand corner of D down to the ith row and ith column. Then the necessary and sufficient condition for the stability of the allelic frequency equilibrium is that

$$(-1)^i \det \Delta_i > 0 \qquad (6)$$

for all i.

That is, the successive Δ_i must alternate in sign with $\Delta_1 < 0, \Delta_2 > 0, \Delta_3 < 0 \ldots$.

To be biologically relevant, an equilibrium must not only be *stable*, but must also be *feasible*, that is all the allelic frequencies must be between 0 and 1 and they must add up to unity. The conditions for a biologically meaningful equilibrium are then that the solutions (5) all be positive and less than unity, together with conditions (6) for stability. The conditions for stability alone are not enough for our purpose and, as we shall see, many fitness relations give a stable equilibrium, but a meaningless one because some of the allelic frequencies are outside the range of 0 to 1.

Unfortunately, the conditions given by (5) and (6) cannot be restated in terms of a few simple rules on the fitnesses that have any intuitive meaning. For example, it is *not* the case that if all heterozygotes are more fit than all homozygotes, there will necessarily be a stable, feasible equilibrium. Table 1a shows a fitness matrix in which all homozygotes are more fit than all heterozygotes, yet no feasible equilibrium exists. Nor is it the case that if there is a stable feasible equilibrium, all homozygotes will be less fit than heterozygotes. Table 1b shows a stable, feasible equilibrium in which one homozygote, A_3A_3, is more fit than the heterozygote A_2A_3. As already pointed out by KIMURA (1956) and MANDEL (1959), heterosis is neither a mathematically necessary nor sufficient condition for a stable feasible equilibrium of more than two alleles.

Numerical investigations

The problem is to turn expressions (5) and (6) into some interpretable statements about the kinds of fitness relations that lead to stable, feasible equilibria.

TABLE 1

(a) All heterozygotes more fit than all homozygotes but no feasible equilibrium

	A_1	A_2	A_3
A_1	0.6563	0.7462	0.8861
A_2	0.7462	0 2817	0.7654
A_3	0.8861	0.7654	0.6121

$\hat{p} = 0.5517, -0.0132, 0.4615$

(b) Stable feasible equilibrium with one homozygote superior to one heterozygote

	A_1	A_2	A_3
A_1	0.2358	0.8457	0.7482
A_2	0.8457	0.1837	0.3927
A_3	0.7482	0.3927	0.3964

$\hat{p} = 0.4297, 0.1938, 0.3764$

For n alleles there are $n(n+1)/2$ genotypes and we may scale all their fitnesses into the interval 0,1. We may then represent the set of fitnesses of all the genotypes as a point in an $n(n+1)/2$ hypercube of unit dimensions. With such a picture, there are two sorts of questions we may ask. First, what is the *size* of the region within this fitness space which corresponds to stable feasible, equilibria of allele frequency? A moment's reflection shows that for two alleles the answer is precisely $\frac{1}{3}$, because heterosis is the necessary and sufficient condition for a stable, feasible equilibrium of two alleles, and in precisely one-third of all possible fitness relations, the heterozygote will be the most fit of the three genotypes. How will the measure of the stable, feasible region change as the number of alleles is increased? Second, what is the *shape* of the region of stability? Again, for two alleles, we know the answer precisely and can draw the boundaries of the wedge-shaped region in the three-dimensional fitness cube. We cannot delimit these boundaries exactly for higher dimensions, but we will ask a slightly different question. If we break the fitness space into subregions, corresponding to different kinds of constraints on the fitnesses, what will the measure of the stable region within these subspaces be?

One method of investigation would be to make a regular lattice of points in the space and test each one for feasibility and stability. For 10 alleles, there would be a 55-dimensional space, and if we spaced our lattice points even as coarsely as every 0.1 from 0 to 1 for each fitness dimension, there would be 11^{55} such points, many of which would be redundant because of symmetries. This task is obviously impossible. The alternative, which we have chosen, is to throw points onto the fitness space at random and to use the *proportion* of stable, feasible cases to characterize the measures of the stable set in each region of the space. Each fitness is chosen at random from a uniform distribution, and the resultant matrix of fitnesses is tested for stability and feasibility. By generating large numbers of replicate matrices, the density of stable, feasible points in the region can be estimated.

It is essential to understand that although fitnesses are generated at random from a uniform distribution, we are *not* trying to make statements about the

"probability" that stable equilibria will occur. Such probability statements would depend upon assumptions about the *a priori* distribution of fitness values for genotypes in nature as they are formed from the mutational array of alleles. But this is precisely the opposite of what is being done. The random generation of fitnesses is simply a device for obtaining the measure of stable, feasible equilibria in various regions of the parameter space. Once these measures are estimated, they can be used in conjunction with assumptions about the nature of fitness determination to decide whether stable selective equilibria are likely to be found.

RESULTS

(a) *Complete fitness space.* The measure of stable and feasible equilibria in the entire $n(n+1)/2$ fitness simplex was obtained by drawing the W_{ij} independently and identically from the uniform distribution on the interval 0,1. For reasons of computational efficiency, after a matrix was formed it was tested first for stability and then, *if stable*, was tested for feasibility. This procedure was followed in exploring all subspaces as well. As a result we have not only the measures of the stable, feasible equilibria, but also can break down the failures of equilibrium into those cases that were unstable and those that were stable but nonfeasible. The result of 100,000 such random matrices is shown in Table 2a for various numbers of alleles. The observed empirical proportion of stable, feasible equilibria for two alleles does not differ significantly from the expected 0.333 . . . ($\chi_1^2 = 0.796$; $P = 0.40$), and then decreases very rapidly as the number of alleles increases. Indeed, there was not a single stable feasible, equilibrium for 6 alleles out of the 100,000 cases tried: Figure 1 plots the logarithm of the proportion of stable, feasible equilibria against the square of the number of alleles, giving an extremely good fit to a straight line, so we may say as a close

TABLE 2

Proportion of unstable, stable but nonfeasible, and stable feasible equilibria for different numbers of alleles (n) in different regions of the fitness simplex

	a. Total space $N = 100{,}000$			b. Pairwise heterosis $N = 10{,}000$			c. Total heterosis $N = 10{,}000$		
n	Unstable	Stable nonfeasible	Stable feasible	Unstable	Stable nonfeasible	Stable feasible	Unstable	Stable nonfeasible	Stable feasible
2	0.49935	0.16599	0.33466	0	0	1.0000	0	0	1.0000
3	0.87331	0.08432	0.04237	0.0470	0.4306	0.5224	0.0335	0.2545	0.7120
4	0.98516	0.01244	0.00240	0.2954	0.5787	0.1259	0.1918	0.4649	0.3433
5	0.99920	0.00074	0.00006	0.6699	0.3185	0.0116	0.5122	0.3837	0.1041
6	1.00000	0	0	0.9145	0.0852	0.0003	0.8170	0.1693	0.0137
7				0.9895	0.0105	0	0.9589	0.0400	0.0011
8				0.9996	0.0004	0	0.9970	0.0030	0
9				1.0000	0	0	1.0000	0	0

N is the number of replicate matrices generated.

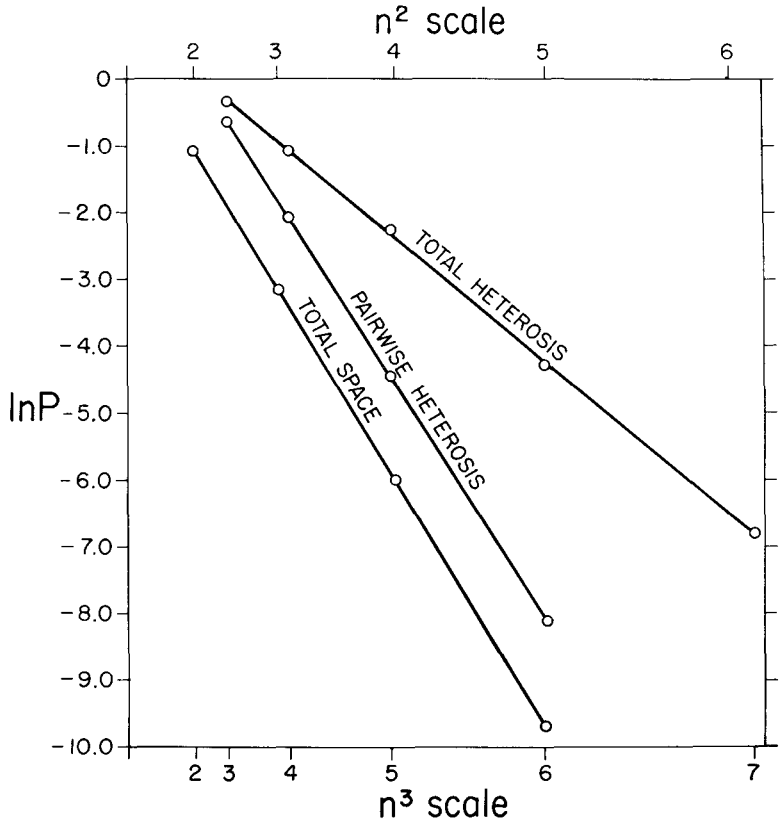

FIGURE 1.—Observed proportions of fitness matrices that yield a stable, feasible equilibrium of allele frequencies for different numbers of alleles. The line for Total Space should be referred to the n^2 scale on the abscissa. The lines for Pairwise Heterosis and Total Heterosis should be referred to the n^3 scale. Ordinate shows logarithm of proportion stable and feasible.

approximation that the measure of stable, feasible equilibria in the total fitness space decreases as e^{-n^2}. The actual empirical relationship is

$$P = 1.723\, e^{-.411n^2},$$

which is not particularly enlightening. This line can be compared directly with the results given in Figure 1 of GILLESPIE (1977).

(b) *Pairwise heterosis.* The fact that the measure of stable feasible, equilibria in the total fitness space is small is perhaps not too surprising. We have gone on to examine smaller regions of this space where intuition would suggest the opposite. The next case is that of pairwise heterosis where each heterozygote is more fit than the homozygotes for its component alleles, *i.e.*, $W_{ii} < W_{ij} > W_{jj}$, but without restriction on the relationship between a heterozygote fitness W_{ij}, and an unrelated homozygote W_{kk}. Table 2b shows the result of 10,000 fitness matrices with this characteristic. (The APPENDIX gives the method used for generating such matrices.) For two alleles, of course, all cases are stable and feasible,

since $W_{11} < W_{12} > W_{22}$ is the necessary and sufficient conditions for stable, feasible equilibrium of two alleles. As the number of alleles increases, the proportion of stable, feasible cases again decreases rapidly so that there are no stable equilibria for 7 or more alleles out of 10,000 cases tried. This result is also plotted in Figure 1, but using an abscissa of n^3 rather than n^2, giving a very good fit to the straight line

$$P = 1.658\, e^{-.0397n^3}.$$

Thus, the measure of stable, feasible equilibria *within* the region of fitness space defined by pairwise heterosis falls off approximately as e^{-n^3}. Clearly pairwise heterosis alone is not a sufficient explanation for the maintenance of large numbers of alleles at a locus, without further restrictions on the fitnesses.

(c) *Total heterosis.* An even greater restriction on fitness relations is the requirement that *all* heterozygotes be more fit than *all* homozygotes, with no fitness overlap between the two classes. The result of generating 10,000 such fitness arrays is shown in Table 2c and Figure 1. (See APPENDIX A for the method.) The measure of stable, feasible equilibria for this region of fitness space falls off at about half the logarithmic rate as for pairwise heterosis,

$$P = 1.630\, e^{-.0194n^3}$$

but still approximately as e^{-n^3}. It appears that even total heterosis, without further restriction, is incapable of maintaining more than a half-dozen alleles in stable equilibrium in a population.

Stability and feasibility

Table 2 shows the loss of biological equilibria with increasing numbers of alleles from two sources: unstable equilibria, and equilibria that, although stable, do not lie in the biologically meaningful range where all alleles frequencies are between 0 and 1. Because stability was tested first and then only the stable residue was tested for feasibility, the values in Table 2 do not make the relative importance of stability and feasibility clear.

TABLE 3

Comparison between the proportion of fitness matrices that are stable and the proportion of stable matrices that give a feasible solution

	Total space		Pairwise heterosis		Total heterosis	
n	Stable	Feasible/ stable	Stable	Feasible/ stable	Stable	Feasible/ stable
2	0.50065	0.66845	1.0000	1.0000	1.0000	1.0000
3	0.12669	0.33444	0.9530	0.5482	0.9665	0.7367
4	0.01484	0.16163	0.7046	0.1787	0.8082	0.4248
5	0.00080	0.07500	0.3301	0.0352	0.4878	0.2134
6	0	——	0.0855	0.0035	0.1830	0.0749
7	0	——	0.0105	0	0.0411	0.0268
8	0	——	0.0004	0	0.0030	0
9	0	——	——	——	0	——

Table 3 shows the proportion of stable equilibria that are also feasible for different numbers of alleles. In all three regions, the proportion of stable equilibria that are feasible decreases with increasing numbers of alleles. This means that feasibility is increasingly more difficult a criterion to satisfy with more and more alleles, even among stable equilibria. In the two heterotic regions of the fitness space, the fall-off in feasible equilibria within the space of stable equilibria is even more rapid than in stable equilibria, although in the total fitness space the reverse is true. This has obvious implications for other models of selection, such as frequency dependent selection, which may have an effect on stability, but not necessarily on feasibility (see below).

The distribution of allele frequencies

In addition to asking about the region of fitness space that leads to stable, feasible equilibria, we may also ask about the distribution of the stable, feasible equilibria in the space of allele frequencies. To what kind of allelic frequency distributions do the stable, feasible fitness matrices lead? The problem is easily solved explicitly for two alleles. If we parameterize the fitnesses of the three genotypes, AA Aa and aa as $1-s$, 1, $1-t$, the frequency of the allele A at equilibrium

$$\hat{p} = \frac{t}{s+t} ,$$

as is well-known. If s and t are uniformly distributed in the interval 0, 1, then the standard textbook method of finding the distribution of a function of two variables yields the density function $\phi(\hat{p})$ as

$$\phi(\hat{p}) = \frac{1}{2(1-\hat{p})^2} \qquad 0 \leq p \leq 0.5$$

$$\phi(\hat{p}) = \frac{1}{2\hat{p}^2} \qquad 0.5 \leq p \leq 1 .$$

This function is shown in Figure 2 and the empirical distribution of $\hat{p}$ from the numerical analysis give an excellent fit when compared at intervals of $p = 0.1$ ($\chi_4^2 = 2.119$, $P = 0.7$) serving as a check on the computer program. Figure 2 shows that allele frequencies will be packed around the center of the gene frequency line, but not drastically so, with 65% of the $\hat{p}$ values falling between 0.25 and 0.75.

For a larger number of alleles, n, we must consider the distribution of the $n-1$ dimensional vector of independent allele frequencies. This can be done by representing the vector as a point in a space of gene frequencies and then asking how the points fill the space. The most convenient representation is an $n-1$ dimensional equilateral tetrahedron so that the frequency of each allele at every point is the distance of the point from one of the $(n-1)$-faces. For three alleles,

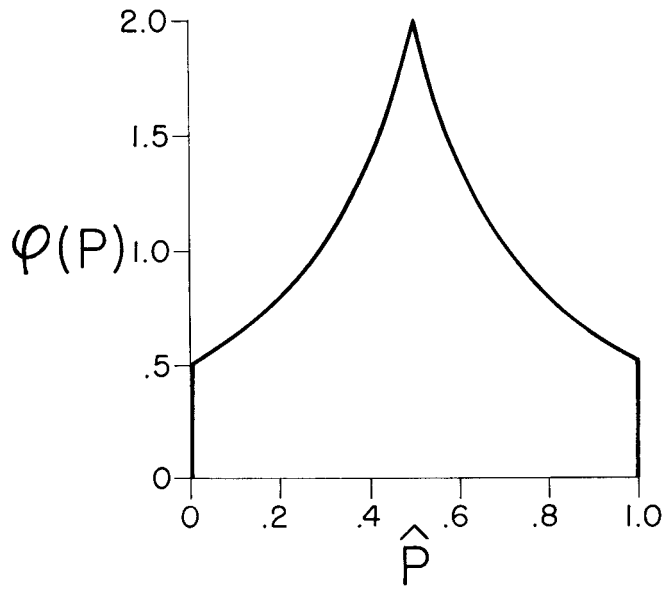

FIGURE 2.—The theoretical distribution of equilibrium allele frequencies in a two allele heterotic model when the fitness of homozygotes are drawn from a uniform distribution.

this is the familiar triangular or de Finetti diagram. To see how such points fill the space we need some measure of location of the point and we have chosen

$$I = \sum_{i=1}^{n} \left(p_i - \frac{1}{n}\right)^2,$$

the sum of squared deviations of the vector components from the centroid of the space, $\frac{1}{n}, \frac{1}{n}, \frac{1}{n} \ldots \frac{1}{n}$, the point of maximum genetic variation. If all the alleles for a given equilibrium are equally frequent, $I = 0$, while if all the alleles are vanishing low in frequency and one allele makes up the entire population $I = \frac{n-1}{n}$. All allelic vectors with equal values of I lie on a spherical surface of radius I around the centroid and are, from the standpoint of genetic variation, identical. At the same time, we can calculate by geometry the proportion of the total volume of the tetrahedral space that lies in a shell between any two spherical surfaces and compare that volume with the proportion of all equilibrium vectors that fall within the shell. In this way we can examine the relative concentrations of the equilibrium points in different regions of the frequency space. Figures 3a, b, c and d make this comparison for $n = 2$, 3, 4 and 5. In each figure there is a continuous curve showing the proportion of the volume of the space that lies in a shell of thickness $\Delta I = 0.01$, and histograms showing the proportion of all stable feasible equilibrium vectors that fall within this shell. For $n = 2$, the histogram is derived analytically, while for $n = 3$, 4 and 5, the empirical results of 5,000 or more computed equilibria are plotted. For $n = 5$, it was not

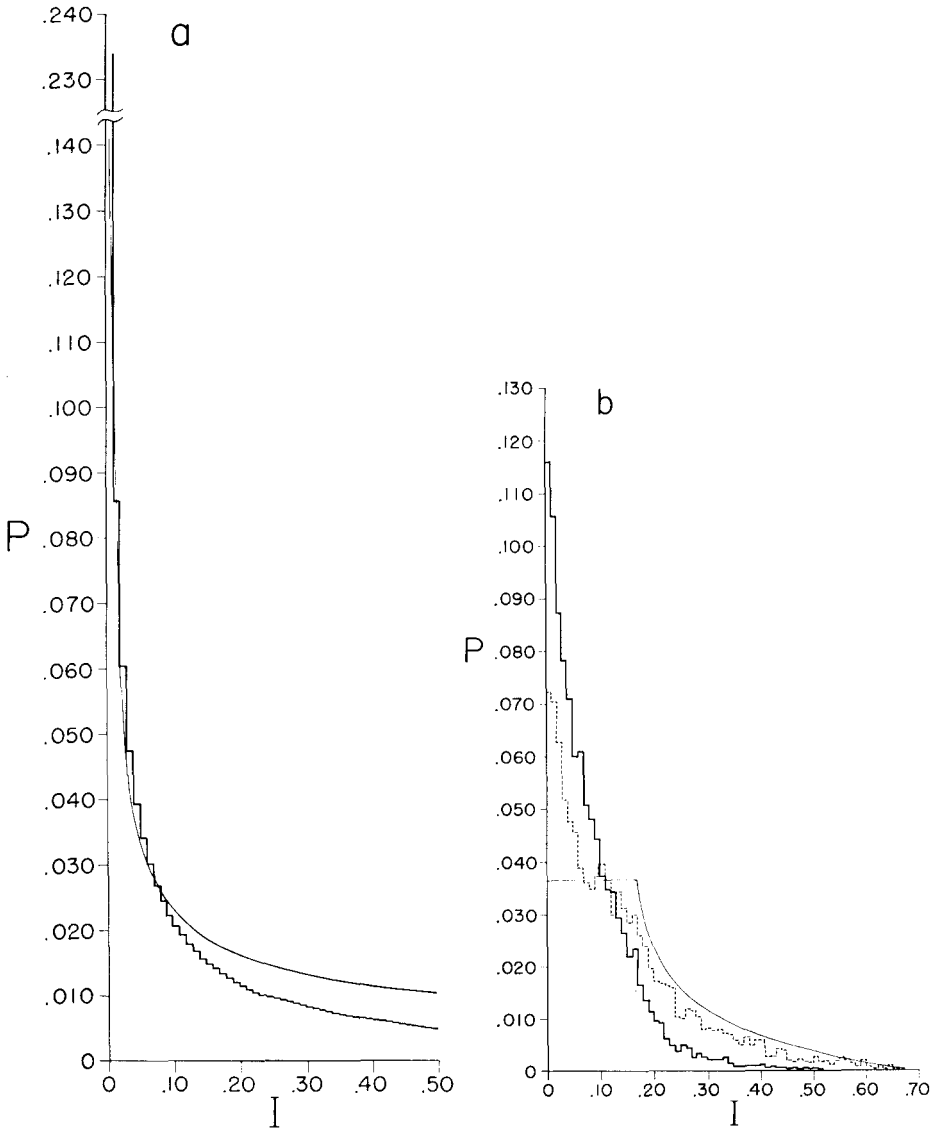

FIGURE 3(a,b).—Comparison of the proportion of equilibrium vectors falling in successive shells around the centroid of the allele frequency simplex (histograms) with the proportion of the volume of the simplex falling in those shells (continuous curve). Heavy histogram is for total heterosis, light histogram for pairwise heterosis. Abscissa: $I = \sum_{i=1}^{n} (p_i - \frac{1}{n})^2$; Ordinate: proportion of cases.

(a) 2 alleles; (b) 3 alleles.

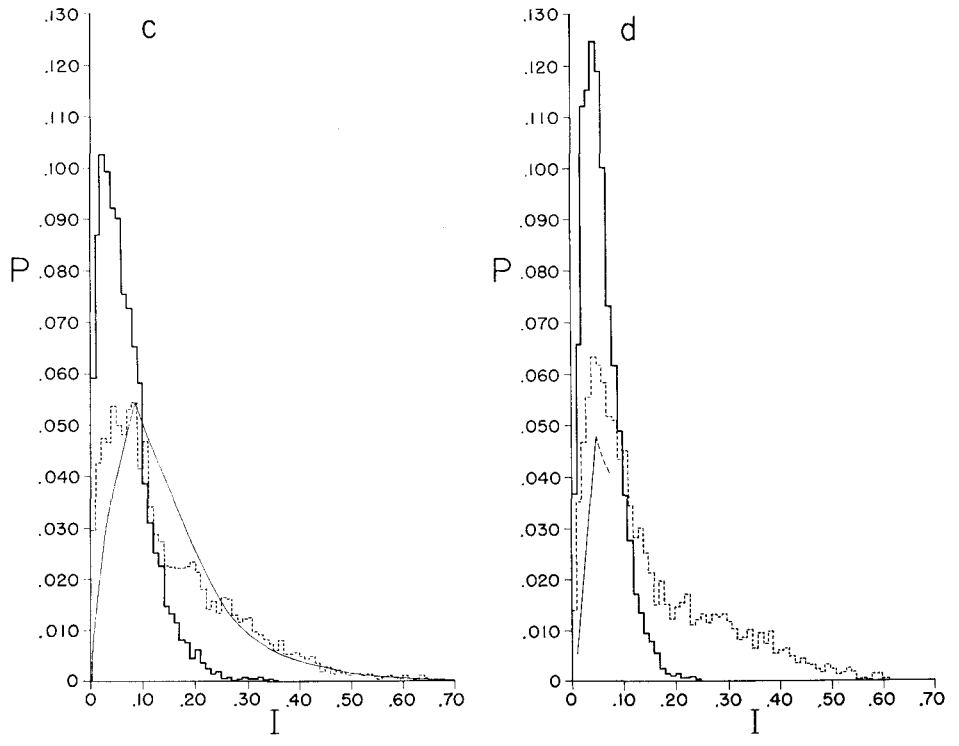

FIGURE 3(c,d).—Comparison of the proportion of equilibrium vectors falling in successive shells around the centroid of the allele frequency simplex (histograms) with the proportion of the volume of the simplex falling in those shells (continuous curve). Heavy histogram is for total heterosis, light histogram for pairwise heterosis. Abscissa: $I = \sum_{i=1}^{n} (p_i - \frac{1}{n})^2$; Ordinate: proportion of cases.

(c) 4 alleles; (d) 5 alleles.

possible to calculate, by geometry, the volumes of shells beyond $I = 0.05$; thus, only a partial comparison is shown. The results in Figure 3 are consistent and clear. The distribution of stable, feasible equilibria is clearly packed near the center of the frequency space, with the packing more pronounced for the totally heterotic model than for pairwise heterosis. Second, the packing becomes less pronounced as the number of alleles increases. For example, we see that there is a consistent deficiency of frequency vectors in the region above $I = 0.13$ and an excess below $I = 0.13$ for pairwise heterosis and 3 alleles. For 4 alleles, however, there is nearly perfect agreement between volume and frequency above $I = 0.25$, the peak of the frequency distribution being shifted only slightly to the left. While only a partial comparison is possible for $k = 5$, the frequency peak now coincides with the volume peak, although more cannot be said. For total heterosis, the larger the number of alleles, the more the peak of the frequency distribution is shifted rightward and the narrower the distribution, but we are unable to compare it with most of the theoretical volume at $k = 5$.

Some analytic results

While we cannot provide simple interpretable conditions on the fitnesses that correspond to our empirical results, there are some easily used conditions on the fitnesses that are necessary conditions for stable equilibrium. If any of these conditions fail on inspection of these fitness matrices, then no stable equilibrium exists.

(a) For every pair of alleles i and j

$$W_{ij} > \frac{W_{ii} + W_{jj}}{2} \tag{7}$$

This comes directly from condition (6) that

$$(-1)^i \Delta_i > 0$$

so that when $i = 1$

$$2W_{ik} - W_{ii} - W_{kk} > 0.$$

But since the order of rows and columns is arbitrary in the fitness matrix, this must be true for any choice of i and k and (7) is proved. It follows immediately from (7) that the weaker condition also holds.

(b) $$\overline{W}_{ij} > \overline{W}_{ii} \tag{8}$$

where $\overline{W}_{ij}$ and $\overline{W}_{ii}$ are the (unweighted) average fitnesses of heterozygotes and homozygotes.

(c) For every pair of different alleles i and j, there must exist a third allele k such that

$$W_{ij} < W_{ik} + W_{jk}, \tag{9}$$

(the "triangle inequality").

The proof depends upon the fact that at stable equilibrium the mean fitness of the population W is a maximum, and that along any two-allele boundary of the allele frequency simplex defined by $p_i + p_j = 1$, there is also a point, p^+, that maximizes fitness along that boundary, because W is a convex function. The full proof is given in APPENDIX B.

Whereas (a) and (b) are requirements on the relation between a heterozygote and its constituent homozygotes, the triangle inequality is a requirement on the relationships among heterozygotes and comes down, essentially, to forbidding any particular heterozygote from having a much greater fitness than any other heterozygote. Indeed, the most fit heterozygote may not be twice as fit as the next best heterozygote, a fact we shall use later. The result of there being one very fit heterozygote will be to drive out the alleles not involved in this heterozygote, irrespective of the homozygous fitnesses.

A region of fitness space with high stability

Although the very strong requirement of total heterosis is insufficient to provide stable polymorphism for even a moderate number of alleles, there is a region of the fitness space that can provide such stability. Consider the degenerate case of n alleles in which all heterozygotes have equal fitness, say $W_{ij} = 1$, and all homozygotes have equal fitness, smaller than the heterozygotes, say $W_{ii} = 1-s$. Clearly, by symmetry, an equilibrium exists with all allele frequencies $p_i = 1/n$. Moreover, this equilibrium is stable because $n-2$ eigenvalues of the matrix of elements $(W_{ij} - W_{in} - W_{jn} + W_{nn})$ are $-s$ and the last is $-ns$, so that are all negative. Beginning with this degenerate case, we can explore the region of fitness space around it by allowing some variation in fitnesses among heterozygotes and among homozygotes. It seems likely that if either variation is not too great, the fitness matrix so produced ought usually be stable.

There is some foreshadowing of this result in the "triangle inequality" of the previous section and in the observation that the empirical distribution of stable vectors in our numerical work was concentrated in the center of the frequency simplex.

Let us assume that

$$W_{ij} = 1 + \delta_{ij}$$
$$W_{ii} = 1-s + \varepsilon_i$$

where δ_{ij} and ε_i are random variables with mean zero and standard deviations σ_δ and σ_ε respectively. What values of s, σ_δ and σ_ε are such as to make a stable, feasible equilibrium very likely? From necessary condition (7) above, s should be large as compared to the sum of the two standard deviations, so that it is extremely unlikely that a heterozygote will be smaller than the average of the two homozygotes that compose it. To prevent much overlap between the homozygote and the heterozygote distributions $s/\sigma > 5$, say. On the other hand, the triangle inequality puts an upper bound on σ_δ. Suppose δ is uniformly distributed. Then provided the lower bound of δ were $-1/3$ and the upper bound $+1/3$, W_{ij} would be bounded between $2/3$ and $4/3$ and the triangle inequality would always be satisfied even in the worst possible sample. This corresponds to

$$\sigma_\delta^2 \leq \frac{(2/3)^2}{12} = 0.037$$
$$\sigma_\delta \simeq 0.20$$

for the variance of a uniform distribution with range $2/3$. This heuristic argument, as we will show, turns out to give a surprisingly good approximation to the polymorphism in this region of the fitness space.

We have generated large numbers of fitness matrices by choosing the heterozygotes from a uniform distribution with mean 1 and standard deviation σ_δ, and heterozygotes from a uniform distribution with mean $(1-s)$ and standard deviation σ_ε. A sampling of the results is given in Tables 4, 5 and 6. Table 4 shows

TABLE 4

Proportion of unstable, nonfeasible and stable feasible equilibria for different numbers of alleles, n, for two cases of equal ratio s/σ

$N = 1000$	$s = 0.1$ $\sigma = 0.01$			$s = 0.5$ $\sigma = 0.05$		
n	Unstable	Stable nonfeasible	Stable feasible	Unstable	Stable nonfeasible	Stable feasible
2	0	0	1.000	0	0	1.000
3	0	0	1.000	0	0	1.000
4	0	0	1.000	0	0	1.000
5	0	0	1.000	0	0	1.000
6	0	0	1.000	0	0	1.000
7	0	0	1.000	0	0.002	0.998
8	0	0.012	0.988	0	0.002	0.998
9	0	0.019	0.981	0	0.011	0.989
10	0	0.028	0.972	0	0.027	0.973
11	0	0.063	0.937	0	0.065	0.935
12	0	0.107	0.893	0	0.096	0.904
13	0	0.178	0.822	0	0.138	0.862
14	0	0.231	0.769	0	0.195	0.805
15	0	0.334	0.666	0	0.281	0.719
16	0	0.402	0.598	0	0.374	0.626
17	0	0.511	0.489	0	0.483	0.517
18	0	0.583	0.417	0	0.564	0.436
19	0	0.676	0.324	0	0.649	0.351
20	0	0.743	0.257	0	0.731	0.269

$\sigma = \sigma_\delta = \sigma_\varepsilon$.

TABLE 5

The effect of changing the ratio s/σ, when $\sigma = \sigma_\varepsilon = \sigma_\delta$, on the proportion of unstable, nonfeasible and stable feasible equilibria

$N = 1000$	$s/\sigma = 10$			$s/\sigma = 6.7$			$s/\sigma = 5$		
n	Unstable	Stable non-feasible	Stable feasible	Unstable	Stable non-feasible	Stable feasible	Unstable	Stable non-feasible	Stable feasible
2	0	0	1.000	0	0	1.000	0	0	1.000
3	0	0	1.000	0	0	1.000	0	0.002	0.998
4	0	0	1.000	0	0.001	0.999	0	0.048	0.952
5	0	0	1.000	0	0.025	0.975	0	0.171	0.829
6	0	0	1.000	0	0.062	0.938	0.001	0.326	0.673
7	0	0	1.000	0	0.131	0.869	0.017	0.530	0.453
8	0	0.012	0.988	0	0.253	0.747	0.072	0.669	0.259
9	0	0.019	0.981	0.001	0.398	0.601	0.165	0.689	0.146
10	0	0.028	0.972	0.001	0.538	0.461	0.315	0.622	0.063
11	0	0.063	0.937	0.014	0.674	0.312	0.499	0.477	0.024
12	0	0.107	0.893	0.039	0.764	0.197	0.679	0.317	0.004
13	0	0.178	0.822	0.088	0.813	0.099	0.845	0.154	0.001
14	0	0.231	0.769	0.180	0.763	0.057	0.931	0.069	0
15	0	0.334	0.666	0.286	0.694	0.020	0.975	0.025	0
16	0	0.402	0.598	0.438	0.551	0.011	0.990	0.010	0
17	0	0.511	0.489	0.602	0.396	0.002	1.000	0	0
18	0	0.583	0.417	0.748	0.251	0.001	1.000	0	0
19	0	0.676	0.324	0.854	0.146	0	1.000	0	0
20	0	0.743	0.257	0.934	0.066	0	1.000	0	0

TABLE 6

Comparison of the effects of changing the variation among the homozygous fitnesses, σ_ε^2, and among heterozygote fitnesses, σ_δ^2, on the stability and feasibility of equilibria

$N = 1000$	$s = 0.1$, $\sigma_\delta = \sigma_\varepsilon = 0.01$			$s = 0.1$, $\sigma_\delta = 0.01$, $\sigma_\varepsilon = 0.04$			$s = 0.1$, $\sigma_\delta = 0.04$, $\sigma_\varepsilon = 0.01$		
n	Unstable	Stable non-feasible	Stable feasible	Unstable	Stable non-feasible	Stable feasible	Unstable	Stable non-feasible	Stable feasible
2	0	0	1.000	0	0	1.000	0	0	1.000
3	0	0	1.000	0	0	1.000	0.011	0.229	0.760
4	0	0	1.000	0	0.010	0.990	0.130	0.480	0.390
5	0	0	1.000	0	0.018	0.982	0.405	0.457	0.138
6	0	0	1.000	0	0.053	0.947	0.700	0.271	0.029
7	0	0.001	0.999	0	0.097	0.903	0.915	0.083	0.002
8	0	0.003	0.997	0	0.159	0.841	0.989	0.011	0
9	0	0.012	0.988	0.001	0.231	0.768	0.999	0.001	0
10	0	0.021	0.979	0.004	0.309	0.687	1.000	0	0
11	0	0.051	0.949	0.009	0.392	0.599			
12	0	0.087	0.913	0.017	0.490	0.493			
13	0	0.137	0.863	0.028	0.570	0.402			
14	0	0.202	0.798	0.049	0.635	0.316			
15	0	0.267	0.733	0.079	0.694	0.227			
16	0	0.358	0.642	0.120	0.718	0.162			
17	0	0.457	0.543	0.185	0.713	0.102			
18	0	0.558	0.442	0.253	0.678	0.069			
19	0	0.650	0.350	0.347	0.621	0.032			
20	0	0.714	0.286	0.433	0.549	0.018			
21	0.001	0.789	0.210	0.516	0.475	0.009			
22	0.005	0.854	0.141	0.591	0.404	0.005			
23	0.009	0.985	0.096	0.673	0.324	0.003			
24	0.014	0.921	0.065	0.749	0.250	0.001			
25	0.029	0.923	0.048	0.814	0.185	0.001			
26	0.057	0.919	0.024	0.870	0.130	0			
27	0.093	0.889	0.018	0.908	0.092	0			
28	0.154	0.837	0.009	0.939	0.061	0			
29	0.229	0.766	0.005	0.955	0.045	0			
30	0.324	0.675	0.001	0.972	0.028	0			

that the measure of stable feasible equilibria is quite high, even for 20 alleles when the ratio $s/\sigma = 10$, and that this is independent of the absolute values of s and σ. Table 5 shows the effect of changing s/σ from 10 to 5. There is a drastic reduction in the measure of stable feasible equilibria. Whereas there are no unstable cases for $s/\sigma = 10$ even for as many as 20 alleles, all the loss being the result of nonfeasible equilibria, at $s/\sigma = 5$, there is a rapid loss of stable equilibria above 10 alleles. Even so, this region of the fitness space has a larger measure of stable, feasible equilibria than the simple totally heterotic region, yet total heterosis does not apply here. The uniform distributions of homozygotes and heterozygote fitnesses have ranges of $\pm\sqrt{12}\,\sigma$ so that if $s/\sigma = 5$, the ranges of the distribution are $\pm 0.69s$ and the overlap between then is nearly 30%. When $s/\sigma = 10$, there is no overlap between homozygotes and heterozygotes so that total heterosis is guaranteed, but in addition there is a very restricted range of

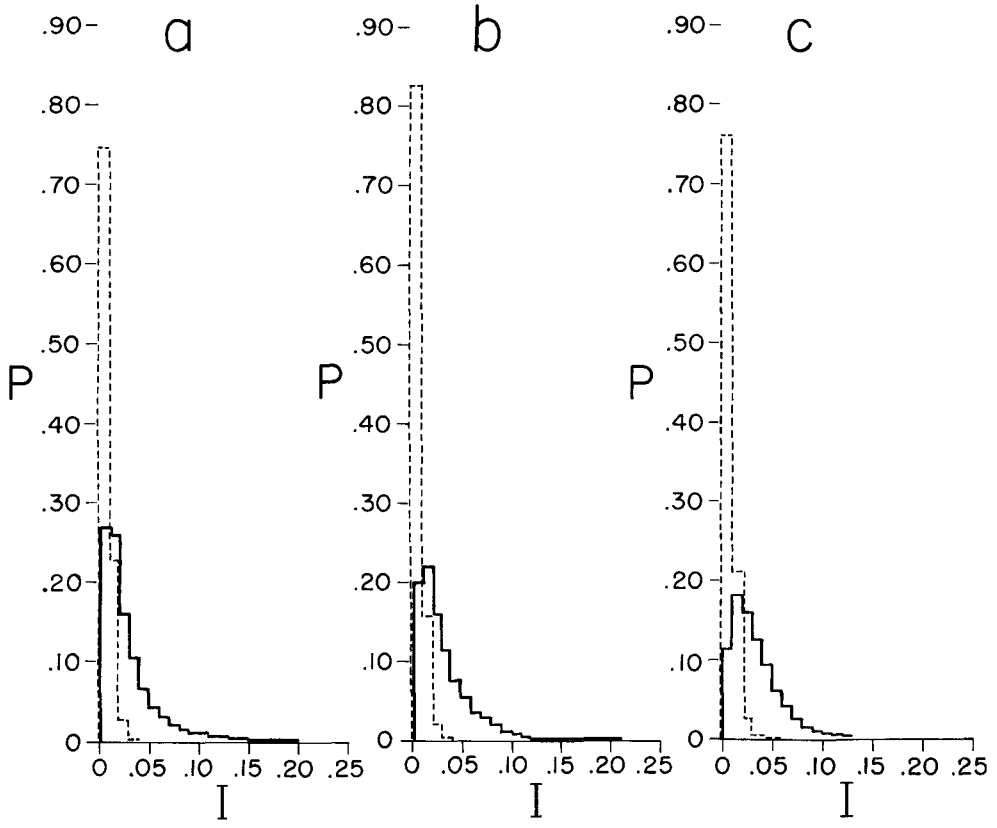

FIGURE 4.—The proportion of equilibrium vectors falling in successive shells around the centroid of the allele frequency simplex for different numbers of alleles and different ratios s/σ. Dashed line: $s/\sigma = 10$; solid line: $s/\sigma = 5$. (a) 3 alleles; (b) 4 alleles; (c) 5 alleles.

homozygous and heterozygous fitnesses. This effect can also be seen in the distribution of stable equilibrium vectors shown in Figure 4. The vectors are extremely tightly packed into the central region of the simplex as compared even with the totally heterotic case of Figure 3.

Table 6 shows the effect of the "triangle inequality". It shows that it is the variation among heterozygote fitnesses, σ_δ, that controls the measure of the stable, feasible equilibria region, rather than the variation among homozygotes, σ_ε^2. Increasing σ_ε from 0.01 to 0.04 has a rather small effect when σ_δ^2 is held at 0.01, but when the reverse change is made, a drastic reduction in the stable, feasible equilibrium occurs.

In summary, there is a region of the fitness space that does have a large measure of stable, feasible equilibria for many alleles, but this region requires a difference between homozygotes and heterozygotes that is quite large compared to the variation in fitness among heterozygotes. Equilibria corresponding to this region of the fitness space are characterized by a nearly uniform distribution of

allele frequencies, with all alleles very close to $p_i = 1/n$. This region will certainly not account for large numbers of segregating alleles in which one or two alleles are rather frequent, while the rest are relatively rare, as in the data of SINGH, LEWONTIN and FELTON (1976) and COYNE (1976).

An analytical characterization of the high stability region

The empirical results of the previous section define a region of high stability in fitness space. We now obtain analytical criteria that support these empirical results and extend their applicability when the number n of segregating alleles becomes large.

In this section it will be convenient to work directly with the matrix of fitnesses (W_{ij}) rather than with the transformed matrix of elements $(W_{ij} - W_{in} - W_{nj} - W_{nn})$. The stability conditions of equation (6) on the latter matrix have been shown by KINGMAN (1961) to be equivalent to the condition that the fitness matrix (W_{ij}) have only one positive eigenvalue.

The region of fitness space explored in the previous section is centered on the case of n alleles in which all heterozygote fitnesses are $W_{ij} = 1$, and all homozygote fitnesses are $W_{ii} = 1 - s$. The matrix (W_{ij}) then has $n - 1$ negative eigenvalues equal to $-s$, and one positive eigenvalue equal to $(n - s)$, in agreement with the stability condition stated above. The region of fitness space around this point consists of points represented by the fitness matirx $(W_{ij} + \delta_{ij})$, where $\delta_{ij} = \delta_{ji}$ are variations in heterozygote fitnesses and $\delta_{ii} = \varepsilon_i$ are variations in homozygote fitnesses. As in the previous section, the elements of the symmetric matrix (δ_{ij}) are taken to be random variables with mean zero and a common standard deviation $\sigma = \sigma_\varepsilon = \sigma_\delta$. The stable region in fitness space may now be defined as that region where the matrix $(W_{ij} + \delta_{ij})$ has only one positive eigenvalue. But the second largest eigenvalue of $(W_{ij} + \delta_{ij})$, λ_2, is always less than or equal to the sum of the second largest eigenvalue of W_{ij} and μ, the largest eigenvalue of (δ_{ij}). Therefore a *sufficient* condition for stability is that $\mu - s \leq 0$.

Therefore we need a description of the eigenvalues of (δ_{ij}). A suitable description is provided by the work of WIGNER (1958). He showed for matrices of the type (δ_{ij}) that, as the dimensionality of n becomes very large, the proportion of eigenvalues of (δ_{ij}) within a unit interval at x is given by

$$f(x) = \frac{1}{2\pi n\sigma^2}\,(4n\sigma^2 - x^2)^{1/2}; \quad \text{for } x^2 < 4n\sigma^2 \ ,$$

and

$$f(x) = 0, \qquad \text{for } x^2 > 4n\sigma^2 \ .$$

It follows at least for large n that the largest eigenvalue of (δ_{ij}) is $\mu \leq 2\sigma\sqrt{n}$.

The stable region of fitness space can therefore be described by the condition $2\sigma\sqrt{n} < s$, which can be written as

$$\frac{s}{\sigma} > 2\sqrt{n} \ . \tag{10}$$

Condition (10) can be used either to characterize the mean difference s between heterozygotes and homozygotes and the variation σ in fitnesses that are required for stability with a given number of alleles, or to define for given s and σ the maximum number of alleles that can be maintained in stable equilibrium.

Although condition (10) should be most accurate for large numbers n of alleles present, it turns out to be very effective even for relatively small numbers. As a test of (10), we give s/σ the values 10, 6.7, 5 respectively and find that the measure of *unstable* states in the resulting fitness space should begin to be observable (*i.e.*, greater than zero) when the number n of alleles equals or exceeds the values of 25, 11, 6 respectively. The empirical results at these three values of s/σ are given in Table 5 and are clearly in excellent agreement with condition (10).

We have assumed so far that the fitness variations δ_{ij} of heterozygotes and $\delta_{ii} = \varepsilon_i$ of homozygotes are drawn from distributions with identical variance σ^2. However WIGNER (1958) pointed out and ARNOLD (1967) has proved that the eigenvalue distribution of the matrix (δ_{ij}) remains unchanged for large n even if the variances of δ_{ij} $(i \neq j)$ and $\delta_{ii} = \varepsilon_i$ are different. In such a case condition (10) remains valid if we replace σ by the standard deviation σ_δ of heterozygote variation. This conclusion is clearly reflected in the empirical observation of the previous section that the measures of stability are very sensitive to changes in σ_δ but insensitive to changes in σ_ε.

It is clear from (10) that in order to achieve stability the mean difference between homozygotes and heterozygotes must be increasingly larger than the variation among heterozygotes as the number of alleles increases. Although (10) does not provide information on the feasibility of equilibria it does define clearly the region of high stability in fitness space.

Other modes of selection

We have shown that large numbers of segregating alleles are not easily accounted for by vague invocations of heterosis, even of the strongest kind, unless that strong heterosis is also accompanied by strong restrictions on the variation in fitness among heterozygotes, and to a lesser degree among homozygotes. It might be argued that there is good reason to suppose that the restricted region of fitness space in which stable equilibrium occur is precisely the region where fitnesses in nature will be found. As new mutations occur, they will be lost to the population if their fitnesses in homozygous and heterozygous condition do not lie in the appropriate region, while the new alleles will be maintained in the population if they have the appropriate fitnesses. Thus, although few new mutations may have the appropriate fitnesses, those that do will be accumulated, and it is these that we seen in nature. The findings on the distribution of allelic frequencies contradicts this hypothesis, however. Those fitnesses that do lead to equilibria create allelic frequency distributions that are nearly uniform, while the problem we are facing is to explain frequency distributions like those at the XDH locus, which are very asymmetrical. All of these conclusions are based

upon fitness values that are (1) independent of genotypic frequencies and (2) constant in time and space. It is a commonplace to suggest that frequency-dependent selection favoring rare genotypes might be responsible for a lot of balanced polymorphism. Like previous arguments about heterosis, this is based on an intuitive extension from the case of two alleles. The problem of frequency-dependent selection must be the subject of a separate study, but we would like to point out one feature of the present work that is relevant. Rare genotype advantage is a mechanism for promoting stability around an equilibrium point, but the actual solution vectors may lie outside the gene frequency simplex, that is be nonfeasible, just as in the constant fitness case. There is nothing *a priori* about frequency-dependent selection that suggests it will lead to feasible equilibria more frequently (although it may, when investigated). But Table 3 shows that for higher numbers of alleles, feasibility is at least as great a problem as stability, and more than 90% of stable equilibria turn out to be nonfeasible. If the same phenomenon should occur for frequency-dependent selection, the situation will not be much improved.

Variable selection in space and time is another question. Recently, GILLESPIE (1977) has shown that if there are many microniches available to a breeding population and if homozygotes differ in fitness from niche to niche, with heterozygotes even slightly more fit than the average of the homozygotes, a large amount of stable polymorphism can be maintained at a locus. Although his exposition contains an error in the conditions for stability (TULJAPURKAR 1977), this error does not affect the conclusion, and it is this kind of model that will probably be needed if a selectionist theory is to be maintained as a plausible explanation of observed allozyme polymorphism.

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APPENDIX A

The generation of random fitness arrays with fitness restrictions

(1) Total heterosis: It is trivial to generate random arrays subject to the restriction that all heterozygotes are more fit than all homozygotes. For n alleles there are $n(n+1)/2$ genotypes, of which n are homozygotes and $n(n-1)/2$ are heterozygotes. Using the usual method of pseudo-random number generation, $n(n+1)/2$ values between 0 and 1 are generated. The nth smallest in the list is identified and then the successive unordered random numbers in the list are assigned without replacement to a homozygote or a heterozygote, depending upon whether each number is less than or equal to, or greater than, the nth smallest.

(2) Pairwise heterosis: A set of fitness matrices that has the same distribution as if random matrices were generated, and then only those in which all heterozygotes were more fit than their component homozygotes were saved, can be efficiently generated as follows: The $n(n+1)/2$ pseudo-random fitnesses are ordered. A list of "eligible" genotypes is created. At the beginning of the cycle, only homozygotes are "eligible". The smallest fitness is assigned to a randomly chosen eligible. Then the next smallest fitness is assigned to another eligible. Since these are both homozygotes, W_{ii} and W_{jj}, they identify a heterozygote W_{ij} as a new eligible, and it is added to the list. The process goes on assigning fitnesses in order to random eligibles. If a heterozygote is chosen, it is simply removed from the list of eligibles. If it is a homozygote it is removed from the list but all heterozygotes between this homozygote and all the homozygotes previously removed from the eligibles list, are now added to the eligible list. The process is continued until all fitnesses have been assigned.

APPENDIX B

Let p = an arbitrary vector of allele frequency
p^+ = point on the i,j boundary that maximizes fitness on that boundary
p^* = equilibrium vector inside the simplex
$W_i = W_i(p) = \sum_j W_{ij}p_j$ = mean fitness of the ith allele at point p

$W = W(p)$ mean fitness of the population at point p

and $W^* = W(p^*)$ mean fitness of the population at equilibrium.

It has been shown by GINZBURG (1972) that

$$\sum_{i=1}^{n} p_i^* W_i(p) = W^* \tag{11}$$

identically for all vectors p.

Subtracting $W(p)$ from both sides of (11) yields

$$\sum_{i=1}^{n} (p_i^* - p_i) W_i(p) = W^* - W(p) \ . \tag{12}$$

We then substitute the particular vector p^+ remembering that at this point $p_i^+ + p_j^+ = 1$ and $W_i(p^+) = W_j(p^+) = W(p^+)$, to find that

$$\sum_{\substack{m=1 \\ m\neq i,\ m\neq j}}^{n} p_m^* W_m(p^+) = W^* - (p_i^* + p_j^*) W(p^+) \ . \tag{13}$$

Subtracting $W(p^+) \sum_{\substack{m\neq i \\ m\neq j}}^{n} p_m^*$ from both sides of (13), we obtain

$$\sum_{\substack{m=1 \\ m\neq i,\ m\neq j}}^{n} p_m^* [W_m(p^+) - W(p^+)] = W^* - W(p^+) \tag{14}$$

But since the right hand side of (14) is positive because the interior equilibrium fitness is a global maximum, at least one of the differences in the brackets on the left hand side must be positive. We then calculate that there must exist at least one $k \neq i,j$, such that

$$W_k(p^+) > W(p^+) \ . \tag{15}$$

If we now evaluate $W_k(p^+)$ and $W(p^+)$,

noting that $p_i^+ = \dfrac{W_{ij} - W_{jj}}{2W_{ij} - W_{ii} - W_{jj}}$,

(15) becomes

$$W_{ki}(W_{ij} - W_{jj}) + W_{kj}(W_{ij} - W_{ii}) > W_{ij}^2 - W_{ii}W_{jj} \tag{16}$$

Solving expression (16) as a quadratic in W_{ij} we obtain

$$W_{ij} < \frac{W_{ki} + W_{kj}}{2} + \sqrt{\left(\frac{W_{ki} + W_{kj}}{2}\right)^2 - Z} \ . \tag{17}$$

where $Z = W_{ki}W_{jj} + W_{kj}W_{ii} - W_{ii}W_{jj}$.

But we have already shown in section (a) that

$W_{ij} > \dfrac{W_{ii} + W_{jj}}{2}$ for any $i \neq j$, and using this in the definition of Z, we find that

$Z > W_{kk}\left(\dfrac{W_{ii} + W_{jj}}{2}\right)$ and so is necessarily positive. As a result (17) can be written as

$W_{ij} < W_{ki} + W_{kj}$ and the triangle inequality is proved.