

GENETIC VARIABILITY MAINTAINED BY MUTATION AND OVERDOMINANT SELECTION IN FINITE POPULATIONS

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ABSTRACT

Mathematical properties of the overdominance model with mutation and random genetic drift are studied by using the method of stochastic differential equations (ITÔ and MCKEAN 1974). It is shown that overdominant selection is very powerful in increasing the mean heterozygosity as compared with neutral mutations, and if $2Ns$ (N = effective population size; s = selective disadvantage for homozygotes) is larger than 10, a very low mutation rate is sufficient to explain the observed level of allozyme polymorphism. The distribution of heterozygosity for overdominant genes is considerably different from that of neutral mutations, and if the ratio of selection coefficient (s) to mutation rate (v) is large and the mean heterozygosity (\bar{h}) is lower than 0.2, single-locus heterozygosity is either approximately 0 or 0.5. If \bar{h} increases further, however, heterozygosity shows a multiple-peak distribution. Reflecting this type of distribution, the relationship between the mean and variance of heterozygosity is considerably different from that for neutral genes. When s/v is large, the proportion of polymorphic loci increases approximately linearly with mean heterozygosity. The distribution of allele frequencies is also drastically different from that of neutral genes, and generally shows a peak at the intermediate gene frequency. Implications of these results on the maintenance of allozyme polymorphism are discussed.

OVERDOMINANT selection is a powerful mechanism for maintaining genetic polymorphism in populations. Although there are only a few examples of authentic overdominant selection (LEWONTIN 1974; NEI 1975), a number of authors (*e.g.*, SVED, REED and BODMER 1967; FRANKLIN and LEWONTIN 1970; SINGH and ZOUROS 1980) have suggested that it is the major factor for maintaining genetic variability in populations at both the phenotypic and molecular levels. Thus, it is important to know the population dynamics of overdominant genes. The theoretical properties of overdominant genes in an infinite population have been studied extensively (FISHER 1922; KIMURA 1956; LEWONTIN, GINZBURG and TULJAPURKAR 1978; and others). However, all natural populations are finite, and the behavior of overdominant genes in finite populations is quite different from that in infinite populations (ROBERTSON 1962). Even overdominant alleles will eventually be fixed in or lost from the population by ran-

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dom genetic drift. On the other hand, new alleles are always produced by mutation and spread through the population. Thus, the level of genetic variability in a population will be determined by the balance of the effect of genetic drift, selection and mutation. Recently, LEWONTIN, GINZBURG and TULJAPURKAR (1978) emphasized the difficulty of maintaining many polymorphic alleles under overdominant selection in an infinite population, but they did not consider mutation. In the presence of mutation, a large number of alleles may be maintained even in a finite population (KIMURA and CROW 1964; WATTERSON 1977; LI 1978).

The study of the allele frequency distribution in finite populations for general overdominant selection with multiple alleles was initiated by WRIGHT (1949). KIMURA and CROW (1964) studied the effective number of alleles that can be maintained in finite populations, presenting an approximate formula for this number. A slightly different approximate formula for this number was also developed by EWENS (1964). Recently, WATTERSON (1977) and LI (1978) conducted a rigorous mathematical study on this subject and showed that KIMURA and CROW's and EWENS's formulae are not accurate under certain conditions. Yet, they did not produce any general formula for the effective number of alleles or expected heterozygosity. More recently, in conjunction to their study of the number of sex-determining alleles that can be maintained in a finite bee population, YOKOYAMA and NEI (1979) presented a general formula for the expected heterozygosity for the WRIGHT model of overdominant selection when population size is larger than a certain level. When population size is small, however, their formula does not work well.

It seems very difficult to derive a general analytical formula for the expected heterozygosity for overdominant selection in finite populations. However, if we use Itô's method of stochastic differential equations, as adapted by MARUYAMA (1980) to population genetics problems, various properties of overdominant selection can be studied numerically. In this paper, we shall present some of our studies in this direction. The problems we have studied are (1) the mean and variance of heterozygosity as a function of population size, mutation rate and selection intensity, (2) the distribution of heterozygosity, (3) the relationship between the mean heterozygosity and proportion of polymorphic loci, (4) the allele frequency distribution (spectrum), and (5) the rate of gene substitution. Unlike previous authors, we have considered asymmetric overdominance as well as symmetric overdominance. All of these properties are important in testing the applicability of overdominant selection in explaining the pattern of protein polymorphism, which has recently been studied in many different organisms.

MATHEMATICAL MODELS AND METHODS

Following KIMURA and CROW (1964), we assume that every new mutation is different from the extant alleles (infinite-allele model) and that the fitness of heterozygotes is the same for all pairs of alleles. We designate the i th allele by A_i and the fitnesses of genotypes A_iA_j and A_iA_i by 1 and $1 - s_i$, respectively. Previous authors have assumed that s_i is the same for all homozygotes. In our ap-

proach, this assumption is not necessary, and we consider two cases, *i.e.*, where s_i is the same for all i (symmetric selection), and where s_i varies with i (asymmetric selection). In the latter case, we assume that $2Ns_i$ is given by $(2N\bar{s} + 4\xi)/5$, where N is the effective population size and ξ is a random variable following the exponential distribution $f(\xi) = (2N\bar{s})^{-1} \exp(-\xi/2N\bar{s})$, in which \bar{s} is the mean of s_i . Note that when s varies randomly with A_i , various types of asymmetric selection are generated. In the first several sections of this paper, we shall consider the case of symmetric selection; then the effect of asymmetric selection will be examined.

Consider a random-mating population of effective size N . We assume that selection and mutation occur deterministically and that, after selection and mutation, $2N$ gametes are randomly chosen for the next generation. As indicated earlier, we shall use Itô's method of stochastic differential equations to study the dynamics of gene frequency changes. Consider the case where $n + 1$ different alleles (A_0, A_1, \dots, A_n) are present in the population, and let $\phi(t; x_1, x_2, \dots, x_n; y_1, y_2, \dots, y_n)$ be the transition probability density that the frequencies of alleles A_1, A_2, \dots, A_n change from x_1, x_2, \dots, x_n to y_1, y_2, \dots, y_n , respectively, in time interval t . Then, the density ϕ satisfies the following Kolmogorov backward equation

$$\frac{\partial \phi}{\partial t} = \frac{1}{2} \sum_{i=1}^n \sum_{j=1}^n x_i (\delta_{ij} - x_j) \frac{\partial^2 \phi}{\partial x_i \partial x_j} + \sum_{i=1}^n \{-2Nvx_i + 2Nsx_i(J - x_i)/(1 - sJ)\} \frac{\partial \phi}{\partial x_i}, \tag{1}$$

where $\delta_{ii} = 1, \delta_{ij} = 0$ if $i \neq j, J = \sum_{i=0}^n x_i^2$ and v is the mutation rate per generation.

The time denoted by t is measured in units of $2N$ generations. It is easy to show that every solution of equation (1) goes to 0 as t becomes infinitely large, because every allele eventually disappears from the population by genetic drift and mutation. Equation (1) does not have any terms for newly arisen mutations. They will be considered when we discuss Itô's equations.

According to the theory of stochastic differential equations, the process governed by (1) can be described by a system of equations of the following type (Itô and McKean 1974, p. 303). Let $x_i(t)$ be the frequency of allele A_i at time t and B_j be an independent Brownian motion variable. The change in $x_i(t)$ in a short time interval dt is then given by

$$dx_i(t) = \sum_{j=1}^n e_{ij} dB_j + 2Nx_i(t) \{-v + s[J - x_i(t)]/(1 - sJ)\} dt \text{ for } i = 1, 2, \dots, n, \tag{2}$$

where $J = \sum_{i=0}^n x_i(t)^2$ and $[e_{ij}]$ is a positive definite square root of the drift matrix $[x_i(\delta_{ij} - x_j)]$.

Equation (2) is a commonly used form of representing the diffusion process given by (1), but there are many other ways of representing it (Watanabe 1971). In this study, we shall use Itô's (1979) form, in which the square root

matrix $[e_{ij}]$ is not involved. Furthermore, in actual computation, we approximate (2) by the corresponding difference equation. The difference equation in ITOH's (1979) form is

$$\Delta x_i(t) = \sum_{\substack{j=0 \\ j \neq i}}^n \sigma(i,j) \sqrt{x_i x_j} B_{ij}(\Delta t) + 2N x_i(t) \{-v + s[J - x_i(t)] / (1 - sJ)\} \Delta t \quad (3)$$

for $i = 1, 2, \dots, n$, where $\sigma(i, j) = 1$ if $i < j$, $\sigma(i, j) = -1$ if $i > j$, and $B_{ij}(\Delta t)$ for $i < j$ is an independent random variable following the normal distribution with mean 0 and variance Δt (white noise); whereas, $B_{ji}(\Delta t) = B_{ij}(\Delta t)$.

In (3), no consideration has been made about the mutant alleles, but they can be introduced in the following way. During the time interval Δt , $2Nv \times 2N\Delta t = 4N^2v\Delta t$ new mutations are introduced on the average with the initial frequency of $1/2N$. Many of the mutant alleles will be lost in the first few generations due to genetic drift, but some will survive and the frequency will reach a specified value, ϵ , which is still small, but larger than $1/2N$. Therefore, if we consider only those mutations whose frequency reaches ϵ , the expected number of mutations introduced during the time interval of Δt is approximately $4N^2v\Delta t \times (1/2N) / \epsilon = 2Nv\Delta t / \epsilon$, since the gene frequency change in early generations is determined almost exclusively by genetic drift. Thus, we assume that on the average $2Nv\Delta t / \epsilon$ mutations are introduced during time interval Δt with an initial frequency of ϵ . Namely,

$$x_{n+i}(t + \Delta t) = \epsilon, \quad i = 1, 2, \dots, k, \quad (4)$$

where k is the number of new mutations introduced and follows the Poisson distribution with mean $2Nv\Delta t / \epsilon$. In practice, $2Nv\Delta t / \epsilon$ was much smaller than 1 in most of our simulations. In the present case, we used either $\epsilon = 0.01$ or $\epsilon = 0.005$. It should be noted that, in our simulation, once new mutations are introduced, their frequencies in subsequent generations are followed even if they are smaller than ϵ . Alleles are considered to have been lost from the population only when $x_i(t)$ becomes 0 or negative. In the above discussion we have neglected $x_0(t)$, but this can be obtained by $1 - x_1(t) - x_2(t) \dots - x_{n+k}(t)$.

It is now clear that the change of $x_i(t)$ in a population (sample path) can be followed by repeatedly applying (3) and (4). Since $B_{ij}(\Delta t)$ can easily be generated by computer, this method greatly facilitates the computation of $x_i(t)$. The usual Monte Carlo simulation requires a large amount of computer time when N is large, but in the present method, the computer time largely depends on the value of Δt and, thus, it is not affected very much by population size. Of course, the accuracy of approximation is higher when Δt and ϵ are small. It is known that the sample paths obtained by simulating (3) converge to the sample paths of the diffusion process governed by (1) as Δt goes to 0 in the sense of ITOH's (not STRATONOVICH's) integral (ARNOLD 1974). In practice, it is important to make Δt smaller than ϵ . We used $\Delta t = 0.1 / (2Ns)$ if this was smaller than 0.001; otherwise we used $\Delta t = 0.001$.

If we use (3) and (4), we can study various properties of both equilibrium and nonequilibrium populations, but in the present paper we shall be mainly concerned with the equilibrium population in which the effects of mutation, selection and random genetic drift are balanced. The equilibrium population was generated by computing $x_i(t)$ repeatedly for the period $t = 1/(Nv)$ or $2N/(Nv) = 2/v$ generations, starting from a monomorphic population. We believe that this is sufficient, since the rate of approach to the equilibrium distribution of allele frequencies for the case of $s = 0$ is $2v + 1/(2N)$ per generation (NEI and LI 1976). After the equilibrium population was established, *i.e.*, after $t = 2/(2Nv)$, we observed allele frequencies at a time interval of 0.1. With this time interval, two consecutive observations are not expected to be completely independent, but nevertheless provide unbiased estimates of parameters we need if a sufficiently large number of observations are made. In each case, we continued our simulation (and observation) until t reached $1000 + 1/(Nv)$ or more. Thus, the total number of observations was 10,000 or more. Once these allele frequency data were obtained, the distribution of allele frequencies, the mean and variance of heterozygosity, *etc.*, were computed.

Since we approximated the diffusion process by (3) and (4), we checked the accuracy of our computation by examining the mean heterozygosity and the allele frequency distribution (spectrum). When there is no selection, the mean heterozygosity is given by $\bar{h} = 4Nv/(1 + 4Nv)$ (KIMURA and CROW 1964). Furthermore, the mean heterozygosity for a small value of $2Ns$ in the case of overdominant selection can be studied analytically by the method of WATTERSON (1977) and LI (1978). The theoretical means of heterozygosities obtained by these methods are presented in Table 1, together with the simulation results. It is clear that the agreement between theory and simulation is satisfactory in all cases. Figure 1 shows the theoretical allele frequency distribution for neutral mutations, together with the results from simulations. The theoretical allele frequency distribution was obtained by KIMURA and CROW's (1964) formula

TABLE 1

Mean heterozygosities for testing the accuracy of the computer simulation used

$+Nv$	$2Ns$	Mean heterozygosity	
		Theoretical	Simulation
0.02	0	0.0196	0.0195
0.1	0	0.0909	0.0897
0.2	0	0.1667	0.1658
0.5	0	0.3333	0.3316
0.004	2	0.0080	0.0079
0.012	6	0.0970	0.0964
0.026	13	0.4548	0.4501
0.060	30	0.6265	0.6360

The time duration (t) for simulation was 2040. The theoretical values for neutral alleles were obtained by $\bar{h} = 4Nv/(1 + 4Nv)$ and those for overdominant alleles were obtained by LI's (1978) method.

$\phi(x) = 4Nvx^{-1} (1 - x)^{4Nv-1}$. The agreement between theory and simulation is again satisfactory except for the values for small x 's ($x < \epsilon$), which tend to be smaller than the expected values. Note that there is no reason to believe that selection makes the agreement worse. Thus, this method of simulation appears to give a quite accurate result for studying the population parameters in which we are interested. Since we used $\epsilon = 0.01$ or 0.005 , our results for the number of rare alleles are not very accurate, but they are not of concern in this paper. Note that rare alleles make little contribution to either heterozygosity or the proportion of polymorphic loci.

HETEROZYGOSITY

Mean: The mean heterozygosity (\bar{h}) is a function of mutation rate (ν), selection coefficient (s) and effective population size (N). However, since we are using the diffusion approximation, there are only two independent parameters, *i.e.*, $N\nu$ and Ns . The relationship among \bar{h} , $N\nu$ and Ns that we obtained is given

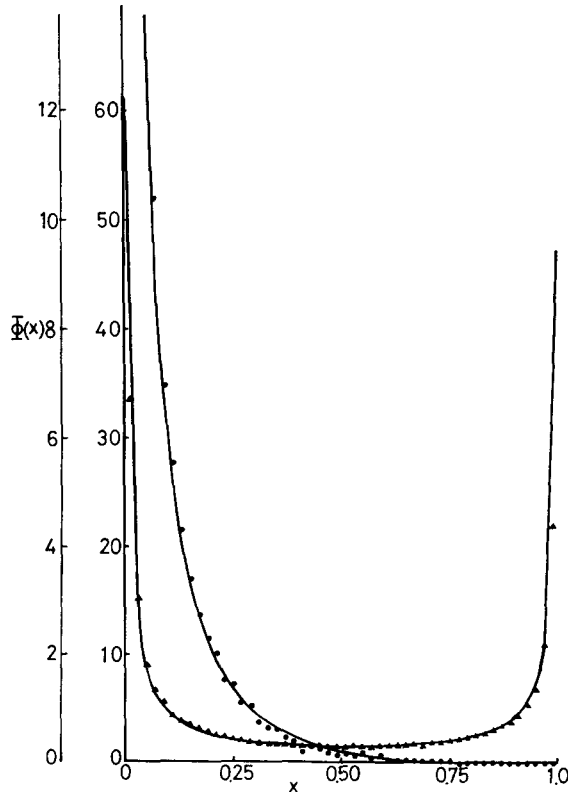


FIGURE 1.—Theoretical allele frequency distributions [$\Phi(x) = 4Nv(1 - x)^{4Nv-1} x^{-1}$] and the distributions obtained by computer simulation for neutral alleles. \blacktriangle and \bullet refer to the cases of $4Nv = 0.08$ and $4Nv = 4$, respectively. The left and right scales of the ordinate refer to the distributions for $4Nv = 0.08$ and $4Nv = 4$, respectively.

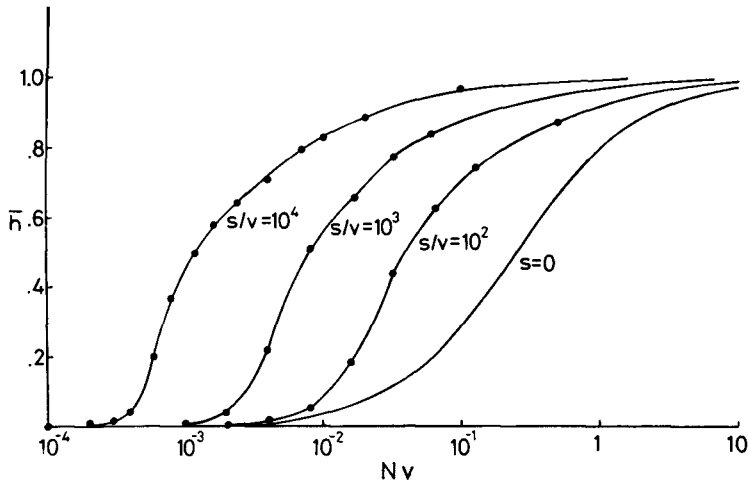


FIGURE 2.—Relationships between Nv and mean heterozygosity (\bar{h}) for overdominant alleles. When s and v remain constant, each curve represents the relationship between N and \bar{h} . The curve for $s = 0$ was obtained by $\bar{h} = 4Nv/(1 + 4Nv)$.

in Figure 2. It is clear that the average heterozygosity under overdominant selection is always higher than that for neutral mutations when $4Nv$ remains the same. For a given mutation rate, the average heterozygosity for neutral mutations increases relatively slowly with increasing N . In the presence of overdominant selection, the average heterozygosity increases rapidly, particularly when the selection intensity (s) is high. However, once the heterozygosity value reaches about 0.8, the rate of increase with increasing N becomes slow and is almost independent of s . The reason for this seems to be that when the average heterozygosity is high, most individuals are equally fit, and thus a further increase of average heterozygosity is attained only by increasing the Nv value. In other words, if most individuals are heterozygous, overdominance provides no advantage.

Figure 2 shows that overdominant selection is a powerful mechanism for maintaining polymorphism as compared with neutral mutations. Thus, if $N = 10^4$ and $v = 10^{-6}$, the expected heterozygosity for neutral alleles is $0.04/1.04 \approx 0.04$. However, if there is slight overdominant selection with $s = 0.001$, \bar{h} becomes 0.55 with the same population size and the same mutation rate. If $s = 0.01$, \bar{h} becomes 0.84.

This problem can be looked at slightly differently. We note that, when a reasonably large number of loci is examined, the average heterozygosity for allozymes is $0 \sim 0.3$ in most natural populations (FURST, CHAKRABORTY and NEI 1977). We also note that the effective population size (N) is at least 10^4 in many species. Thus, under the neutral mutation hypothesis, a level of $\bar{h} = 0.0909$ is maintained if $N = 25,000$ and $v = 10^{-6}$ ($4Nv = 0.1$). On the other hand, if there is slight overdominance with $s = 10^{-4}$ and $N = 25,000$ ($2Ns = 5$), then a mutation rate of about 1.75×10^{-7} ($4Nv = 0.0175$) is sufficient to maintain the

same level of heterozygosity (Table 2). If $s = 5 \times 10^{-4}$ ($2N_s = 25$), the mutation rate required is only about 1.7×10^{-11} ($4Nv = 1.7 \times 10^{-6}$). This indicates that if we invoke overdominant selection for explaining the observed level of heterozygosity for protein loci, we must assume a very low mutation rate. In other words, once an overdominant allele is established in the population, it will stay there for a long time.

Recent advances in molecular biology make it possible to study genetic variability at the nucleotide level. At the nucleotide level, the average heterozygosity per locus is expected to be high. When h is high, overdominant selection is less powerful than when it is low, as mentioned above. Table 2 shows that neutral mutations produce an average heterozygosity of 0.8 when $4Nv = 4$; whereas, overdominant mutations with $2N_s = 25$ require $4Nv = 1.3$ to maintain the same level of heterozygosity. Namely, the mutation rate required for overdominant selection is about one-third of that for neutral mutations. This requirement is much larger than the requirement (1.7×10^{-6}) for the case of $h = 0.0909$.

Variance: NEI and his co-workers (NEI 1975; NEI, FUERST and CHAKRABORTY 1976; FUERST, CHAKRABORTY and NEI 1977) and YAMAZAKI (1977) have used the relationship between the mean (h) and variance [$V(h)$] of heterozygosity among loci for testing the null hypothesis of neutral mutations. The variance of heterozygosity for the case of neutral alleles can be computed by the formula developed by WATTERSON (1974) and STEWART (1976). In the absence of mathematical models for alternative hypotheses, however, they could not determine the power of their test. We have therefore examined this relationship for the overdominance model.

The results obtained are given in Figure 3. It is clear that the relationship depends on the ratio of s/v . When this ratio is 10, the variance is similar to that of neutral genes if h is smaller than 0.2, but substantially lower than the latter if h is large. The difference between neutral genes and overdominant genes becomes conspicuous as the s/v ratio increases. Although the results are not pre-

TABLE 2

Population size (N), selection intensity (s) and mutation rate (v) that are required for maintaining a given level of mean heterozygosity (h)

\bar{h}	$2N_s$	$4Nv$	\bar{h}	$2N_s$	$4Nv$
0.0476	0	0.050	0.500	0	1.0
0.0450	5	0.0075	0.480	5	0.5
0.0457	25	7.5×10^{-7}	0.485	25	0.002
			0.518	50	1.7×10^{-4}
0.0909	0	0.100			
0.0930	5	0.0175	0.8	0	4.0
0.0926	25	1.7×10^{-6}	0.802	5	3.2
			0.796	25	1.3
0.3333	0	0.500	0.794	50	0.5
0.3377	5	0.150	0.808	100	0.2
0.3341	25	1.65×10^{-5}	0.802	200	0.01

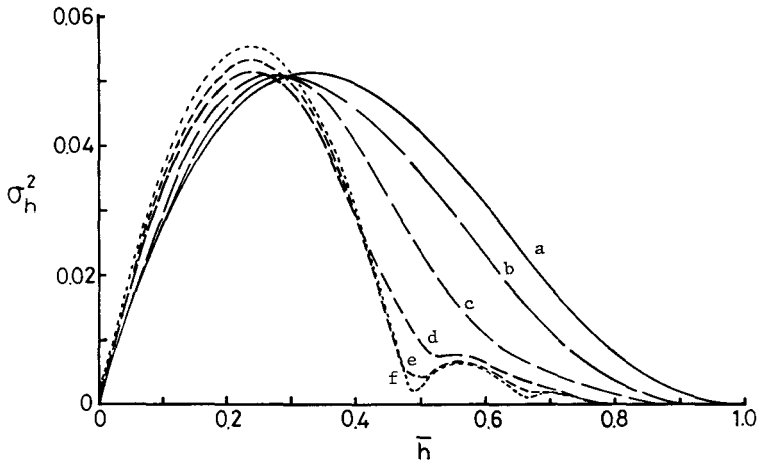


FIGURE 3.—Relationships between the mean (\bar{h}) and variance (σ_h^2) of heterozygosity. (a) $s = 0$; (b) $s/v = 10$; (c) $s/v = 100$; (d) $s/v = 1000$; (e) $s/v = 10^4$; (f) $s/v = 10^5$. The curve for $s = 0$ was obtained by the WATTERSON-STEWART formula. The curves for $s > 0$ were obtained by computer simulation. The number of observations of σ_h^2 was so large that the deviations of the observed values from the smooth curves were very small.

sented, the relationship, when the s/v ratio is larger than 10^5 , is virtually identical with that for $s/v = 10^5$. We first note that in all cases $V(h)$ increases as h increases, reaches a maximum and then starts to decline. When s/v is large, however, there are more than one local maxima. Furthermore, as the s/v ratio increases, $V(h)$ reaches the first maximum at a lower value of h than that for neutral alleles, and the maximum value of $V(h)$ is considerably larger than that for neutral genes. When s/v is 1000 or larger, the first maximum point is located near $h = 0.25$, and as h increases further, $V(h)$ declines rapidly and becomes close to 0 around $h = 0.5$. Namely, in the range of $h = 0 \sim 0.5$, $V(h)$ follows a curve similar to a parabola. This is apparently due to the fact that at individual loci, heterozygosity assumes practically two different values, *i.e.*, 0 and 0.5 when s/v is large, as will be later. At any rate, it is clear from Figure 3 that the relationship between \bar{h} and $V(h)$ is affected considerably by overdominant selection, and thus it can be used to distinguish overdominant selection from neutral mutations, if enough data are available.

Another interesting finding from our simulation is that the relationship between \bar{h} and $V(h)$ is close to the curves given in Figure 3, even if \bar{h} happens to deviate considerably from its true population value. For example, suppose that a particular set of data gives a mean heterozygosity of 0.2 because of the relatively small number of loci examined, but the population mean heterozygosity is 0.25. In this case, the variance of heterozygosity is close to that corresponding to $h = 0.2$ rather than to that corresponding to $h = 0.25$. This indicates that even if the sample size is relatively small, the curves for overdominant genes in Figure 3 can be used for testing alternative hypotheses for maintenance of genetic polymorphism.

Distribution of heterozygosity: The distribution of single-locus heterozygosity for neutral genes has been studied numerically by EWENS and GILLESPIE (1974) and FUERST, CHAKRABORTY and NEI (1977). In the case of overdominant selection, the distribution is determined by Nv and Ns , but when s/v is larger than 1000, the distribution for a given value of h is virtually the same for wide ranges of Nv and Ns . Therefore, we shall again be concerned with the case of $s/v = 1000$.

The distributions of heterozygosity for four different values of h are given in Figure 4. It is clear that, when h is 0.0755 (a), there are two sharp peaks at $h = 0$ and $h = 0.5$, and the frequencies between these two values and those above $h = 0.5$ are very small. This indicates that a locus is virtually either monomorphic or polymorphic, and, if it is polymorphic, the heterozygosity is approximately 0.5. The peak at $h = 0$ is much higher than that at $h = 0.5$. Essentially

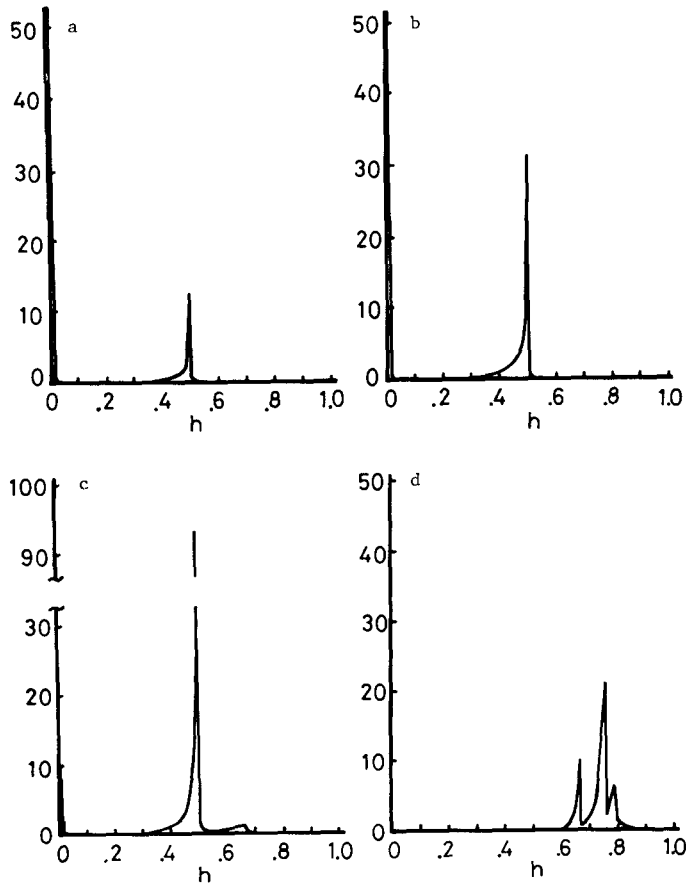


FIGURE 4.—Distributions of heterozygosity for various levels of h . In the computation of these distributions, $s/v = 1000$ was assumed. (a) $2Ns = 10$, $\bar{h} = 0.076$; (b) $2Ns = 12$, $\bar{h} = 0.189$; (c) $2Ns = 20$, $\bar{h} = 0.488$; (d) $2Ns = 80$, $\bar{h} = 0.729$.

the same pattern was obtained for the cases of $\bar{h} < 0.0755$, though the peak at $h = 0.5$ was lower. When \bar{h} is 0.1889 (b), there are still peaks at $h = 0$ and $h = 0.5$, but the peak at $h = 0.5$ is now much higher than that for $\bar{h} = 0.0755$. The frequency of $h > 0.5$ is still negligibly small. In the case of $\bar{h} = 0.4880$ (c), the peak at $h = 0.5$ becomes much higher than that at $h = 0$, and a third peak appears around $2/3$. If \bar{h} further increases, the height of this third peak rises, and fourth and fifth peaks appear around $h = 3/4$ and $h = 4/5$, respectively; whereas, the peak at $h = 0$ gradually disappears. In the case of $\bar{h} = 0.7291$ (d), the fourth peak is highest, and the first and second peaks are invisible. These peaks or spikes apparently occur for a geometric reason and coincide with the peaks conjectured by STEWART (1976) for neutral genes. (The location of the fifth peak is somewhat deviated from $h = 4/5$.)

It should be noted that the pattern of distribution of heterozygosity for neutral alleles is considerably different from that for overdominant alleles, though the locations of the peaks seem to be the same or similar. This can be seen by comparing the present results with those obtained by both EWENS and GILLESPIE (1974) and FUERST, CHAKRABORTY and NEI (1977) for the case of neutral alleles. When \bar{h} is small, the distribution for neutral alleles is essentially L-shaped, with a small peak at $h = 0.5$. When \bar{h} is high, there are apparently a number of peaks, but the frequencies between peaks are always substantial. Clearly, this difference in the distribution pattern can be used for discriminating the two alternative hypotheses in data analysis.

Relationship between average heterozygosity and the proportion of polymorphic loci: We define a locus as polymorphic if the frequency of the most common allele is equal to or less than $1 - q$, where q is a small quantity. In the present study we used $q = 0.01$ and 0.05 . With this definition, the expected proportion of polymorphic loci for neutral alleles is given by $1 - q^{4Nv}$ (KIMURA 1971); whereas, the expected heterozygosity is $4Nv/(1 + 4Nv)$, as mentioned earlier. Therefore, the relationship between these quantities can be obtained analytically. Recently, CHAKRABORTY and YOKOYAMA (1978) studied this relationship for overdominant alleles and showed that the difference between the neutral and overdominant models is small. However, they used selection coefficients of the order of the mutation rate. Actually, if we use a selection coefficient that is much larger than the mutation rate, the relationship for overdominant alleles is significantly different from that for neutral alleles.

Our results on this relationship are presented in Figure 5. This result was obtained by using $4Nv = 0.001 \sim 0.1$ and $2Ns = 0.01 \sim 100$, keeping s/v equal to 1000. The q value used was 0.01. Figure 5 indicates that the proportion of polymorphic loci (P) increases almost linearly with increasing mean heterozygosity (\bar{h}). The relationship is roughly given by $P = 2.13 \bar{h}$ ($\bar{h} \leq 0.47$). This linear relationship is caused by the fact that when $\bar{h} \leq 0.5$, heterozygosity assumes a value of either 0 or 0.5, roughly speaking. At any rate, it is clear from this figure that the relationship for overdominant alleles is considerably different from that for neutral alleles if the selection intensity is sufficiently large. For example, when the mean heterozygosity is 0.2, the expected proportion of poly-

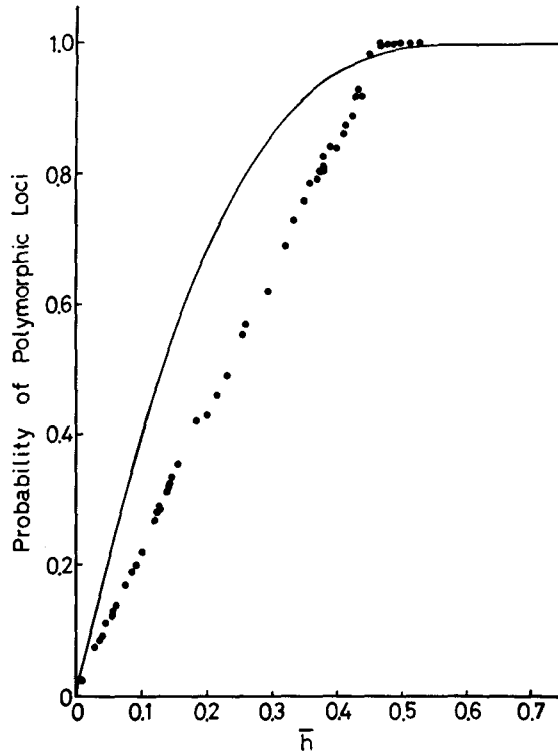


FIGURE 5.—Relationship between mean heterozygosity (\bar{h}) and probability of polymorphic loci (P) when the criterion of $q = 0.01$ is used. The solid line represents the relationship for neutral alleles; whereas, the dots give the relationship for overdominant alleles with $s/v = 1000$. The relationship for overdominant alleles is given approximately by $P = 2.13\bar{h}$ ($\bar{h} \leq 0.47$). In the case of overdominant alleles, essentially the same relationship holds when the criterion of $q = 0.05$ is used.

morphic loci is 0.43 for overdominant alleles, but 0.68 for neutral alleles. Essentially the same result was obtained when $q = 0.05$ was used.

DISTRIBUTION OF ALLELE FREQUENCIES

The distribution of allele frequencies or frequency spectrum for neutral alleles is given by $\Phi(x) = 4Nv x^{-1} (1-x)^{4Nv-1}$ (KIMURA and CROW 1964). LI (1978) studied the allele frequency distribution for relatively small values of $2N_s$ ($2N_s = 10$) for the case of overdominant selection. He showed that when the $4Nv$ value is much smaller than 1, the distribution has a distorted W shape. However, he could not study the case of large $2N_s$ because of technical difficulties. We have therefore studied the allele frequency distributions for various values of $2N_s$ and $4Nv$. Some examples are presented in Figure 6. First, we note that the distribution for neutral genes is always U-shaped when $4Nv < 1$. Compared with this case, the distribution for overdominant alleles has a peak at an intermediate gene frequency even if $4Nv$ is very small, and the peak becomes higher as $2N_s$ increases. The location of the peak is around $x = 0.5$ when $2N_s$

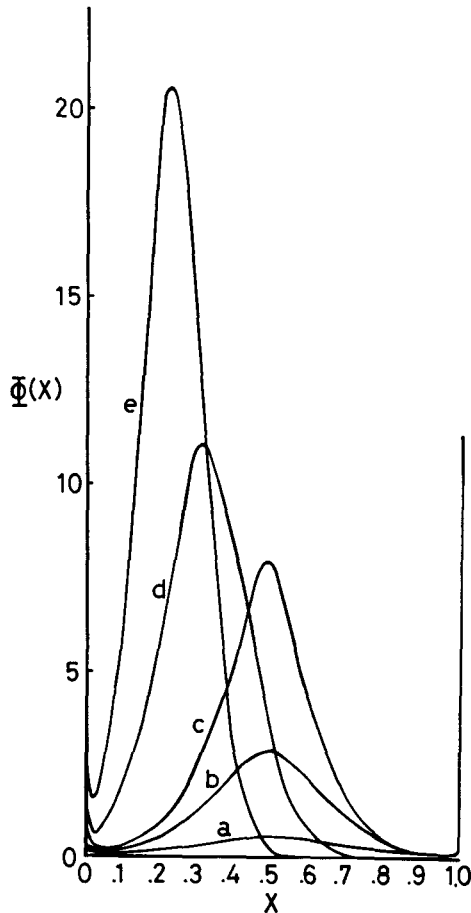


FIGURE 6.—Allele frequency distributions (spectra) for overdominant alleles. (a) $4Nv = 0.0016$, $2N_s = 8$, $\bar{h} = 0.051$; (b) $4Nv = 0.0024$, $2N_s = 12$, $\bar{h} = 0.189$; (c) $4Nv = 0.004$, $2N_s = 20$, $\bar{h} = 0.488$; (d) $4Nv = 0.008$, $2N_s = 40$, $\bar{h} = 0.627$; (e) $4Nv = 0.016$, $2N_s = 80$, $\bar{h} = 0.729$.

is small, but moves down as $2N_s$ increases, because the number of alleles maintained in a population increases. The pattern of the distribution also depends on the value of $4Nv$. If this value increases, the value of $\Phi(x)$ increases, particularly around the peak. When $4Nv$ and $2N_s$ are sufficiently large so that \bar{h} is larger than 0.75, the distribution is approximately given by YOKOYAMA and NEI's (1979) formula.

RATE OF GENE SUBSTITUTION

As emphasized by KIMURA and OHTA (1971), protein polymorphism is a phase of molecular evolution. Any reasonable theory of molecular evolution must be able to explain simultaneously both the level of polymorphism and the rate of gene substitution. In the neutral mutation theory, the rate of gene substitution is equal to the mutation rate. In the case of symmetric overdominant selection, the probability of fixation of a mutant gene when there are only two

alleles is higher than that of neutral mutations (NEI and ROYCHOUDHURY 1973), but no studies have been done about the rate of gene substitution when new mutations are continuously introduced.

We studied the rate of gene substitution for overdominant alleles in the following way. We assumed that a gene consists of a large number of codons, that any mutation produces a new allele and that no intracistronic recombination occurs. We labeled the original allele 1. The first mutation from this allele was designated 11, the second mutation 12, and so forth. When new mutations occur from allele 11, they were designated 111, 112, 113, etc. in the order of occurrence. Similarly, the mutations from 12 were designated 121, 122, 123, etc. This labeling was continued until a particular mutational codon was fixed in the population. This codon fixation was recognized by determining whether or not the number in the second position of allele designation was the same for all genes in the population. For example, when all the genes had number 2 in the second position, codon 2 was regarded as having been fixed. As soon as this fixation occurred, the number in the first position (*i.e.*, 1 in this case) was eliminated to keep the length of the allele designations from becoming too long. This process was followed for a period of $t = 535 \sim 200,000$, depending on the rate of gene substitution, and the total number of gene fixations was recorded.

The results obtained are presented in Table 3, together with the average heterozygosities obtained. In this table, the rate of gene substitution is expressed per $2N$ generations, rather than per generation. It is clear that the rate for neutral mutations ($2N_s = 0$) obtained by simulation is approximately equal to the expected rate of $2Nv$. Furthermore, if $4Nv$ remains the same, overdominant selection with a small value of $2N_s$ accelerates gene substitution, as expected

TABLE 3

Rate of gene substitutions (α) for overdominant mutations compared with those of neutral alleles ($2N_s = 0$)

$4Nv$	$2N_s$	Observed mean heterozygosity \bar{h}	No. of gene substitutions	Substitution rate (α)	\bar{h} obtained from α
0.02	0	0.0195	17	0.0094	0.0185
0.04	0	0.0368	33	0.0183	0.0353
0.08	0	0.0743	82	0.0406	0.0751
0.20	0	0.1658	154	0.0962	0.1614
0.0002	1	0.00030	35	0.00018	0.00036
0.0002	10	0.0102	39	0.00081	0.0016
0.0002	100	0.6704	6	0.00165	0.0033
0.002	1	0.0031	31	0.0015	0.0030
0.002	10	0.0755	33	0.00685	0.0135
0.002	100	0.7193	6	0.00429	0.0085
0.02	1	0.0301	32	0.0154	0.0298
0.02	10	0.2711	128	0.0634	0.1125
0.02	100	0.7489	15	0.0355	0.0663

The rate is measured in units of $2N$ generations and is equal to $2Nv$ if all mutants are neutral. The last column gives the estimate of mean heterozygosity obtained from α under the assumption of neutral mutations.

from NEI and ROYCHOUDHURY's (1973) study. This can be seen by examining the case of $4Nv = 0.02$ in Table 3. However, a large value of $2Ns$ does not necessarily give a higher rate of substitution than does a small value of $2Ns$. Indeed, the rate of gene substitution for a given value of $4Nv$ first increases with increasing $2Ns$, but then declines. This is apparently due to the fact that, as the average heterozygosity increases, the initial advantage of a new mutant allele declines.

The effect of overdominant selection on the rate of gene substitution is, however, much smaller than that on average heterozygosity. In general, overdominant selection increases average heterozygosity more drastically than the rate of gene substitution. This can be seen by computing the average heterozygosity from the observed rate of gene substitution under the assumption of neutral genes. Namely, equating the rate of gene substitution to $2Nv$, we can compute the estimated average heterozygosity by $4Nv/(1 + 4Nv)$ under the assumption of neutral genes. This value is given in the last column of Table 3. It is clear that when $2Ns = 1$, this value is roughly the same as the actual value, because in this case the mutant alleles behave just like neutral alleles. However, when $2Ns$ is large, the estimated average heterozygosity is substantially smaller than the actual value. In other words, for a given rate of gene substitution, overdominant selection produces a higher level of heterozygosity than that for neutral genes. We shall later discuss the implication of this finding for the maintenance of protein polymorphism.

EFFECT OF ASYMMETRIC SELECTION

To see the effect of asymmetric selection, we studied the mean and variance of heterozygosity and the rate of gene substitution for the cases of $2N\bar{s} = 1, 10$ and 100, where \bar{s} is the mean of s_i . Every time a new mutation was introduced, the fitness of the homozygote for the allele was determined by using a random number that followed the exponential distribution mentioned earlier. The mean heterozygosities and the rates of gene substitutions obtained are presented in Table 4. In the case of $2N\bar{s} = 1$, the gene frequency change is dictated by genetic drift, so that the mean heterozygosity and rate of gene substitution are both similar to those for the case of constant selection with $2Ns = 1$, which are in turn similar to those for neutral genes. However, when $2N\bar{s}$ is large, the mean heterozygosity for asymmetric selection tends to be smaller than that for symmetric selection. This is, of course, expected, since asymmetric selection is less efficient in maintaining genetic variation. Compared with the case of neutral genes, however, it is still a very powerful mechanism for maintaining polymorphism. Just as in the case of symmetric selection, the rate of gene substitution for asymmetric selection first increases with increasing $2N\bar{s}$, but then declines. This pattern can be explained by the same principle as that for constant selection.

The comparison of the variances of heterozygosities for symmetric and asymmetric selection is not simple, since the variance is highly dependent on the mean (Figure 3) and the mean is not the same for the two cases even if $\bar{s} = s$. However, our results have shown that the relationship between the mean and

TABLE 4

Mean heterozygosities (\bar{h}) and the rate of gene substitution (α) for the asymmetric overdominant selection

$4Nv$	$2N\bar{s}$	\bar{h}	No. of gene substitutions	α
0.0002	1	0.00046	9	0.00019
0.0002	10	0.00350	50	0.000963
0.0002	100	0.6223	15	0.00115
0.002	1	0.00279	5	0.00104
0.002	10	0.0412	63	0.01255
0.002	100	0.6572	84	0.00514
0.02	1	0.0280	29	0.0143
0.02	10	0.2192	108	0.0535
0.02	100	0.6834	24	0.0462

Selection coefficient s_i for homozygote A_iA_i is assumed to be exponentially distributed. The values of \bar{h} and α for the case of constant s are given in Table 3.

variance is virtually identical with that for symmetric selection, as long as s/v is large.

DISCUSSION

The results obtained in this paper are directly applicable for examining the role of overdominant selection in the maintenance of allozyme polymorphisms. As mentioned earlier, a number of authors used the relationship between the mean and variance of single-locus heterozygosity and showed that it is roughly in agreement with the expected relationship from the neutral mutation hypothesis. The present study has shown that the expected relationship under overdominant selection is considerably different from that predicted by the neutral theory, particularly when the mean is large. Unfortunately, however, most of the estimates of average heterozygosities obtained from natural populations are below 0.2, and in this range the difference between the neutral and overdominant expectations is small. Therefore, this method is not very useful for distinguishing between the two hypotheses.

A more useful method is to examine the distribution of single-locus heterozygosity, the relationship between the proportion of polymorphic loci and average heterozygosity and the allele frequency distribution. FUERST, CHAKRABORTY and NEI (1977) studied the distributions of heterozygosity for 68 different species (or subspecies) and showed that in none of the species examined was the distribution significantly different from the neutral expectation. Their observed distributions are, however, considerably different from those in Figure 4 in this paper. FUERST, CHAKRABORTY and NEI (1977) also examined the empirical relationship between the proportion of polymorphic loci and average heterozygosity in many different species. Comparison of their Figures 8 and 9 with our Figure 5 indicates that the observed relationship is close to the neutral expectation rather than to the overdominant expectation. These results suggest that overdominant selection is not important in the maintenance of allozyme polymorphisms. A similar conclusion can be obtained from an examination of allele

frequency distributions. CHAKRABORTY, FURST and NEI (1980) examined the allele frequency distribution for 138 species and showed that, in all species examined, the distribution was U-shaped and agreed well with the distribution expected under the neutral theory, except those of rare alleles. Their observed distributions are again drastically different from those given in Figure 6.

Our study on the relationship between the average heterozygosity and the rate of gene substitution also suggests the unimportance of overdominant selection. Examining the rate of amino acid substitution in various proteins, KIMURA and OHTA (1971) and NEI (1975) estimated that the rate of amino acid substitutions that are detectable by electrophoresis is roughly 10^{-7} per year per gene. Under the neutral mutation theory, this is equal to the mutation rate per year (KIMURA 1968). Therefore, if we know the generation time, we can compute the mutation rate per generation (ν) under the assumption of neutral mutations. Furthermore, in some species it is possible to get a rough estimate of effective population size (*e.g.*, AYALA 1972). Using this estimate and the mutation rate per generation, we can compute the expected heterozygosity under the neutral theory by $h = 4N\nu/(1 + 4N\nu)$. In most species, however, the observed average heterozygosity is lower than the neutral expectation (NEI 1980). Therefore, there must be some factors that reduce the level of heterozygosity. Obvious candidates for these factors are the bottleneck effect and random fluctuations of selection intensity (NEI 1975), but not overdominance. As seen from Table 3, overdominant mutations increase average heterozygosity tremendously compared with neutral mutations, when the rate of gene substitution is fixed. A detailed discussion on the relationship between average heterozygosity and the rate of gene substitution has been given by NEI (1980).

It should be noted, however, that the above conclusion refers to the general pattern of allozyme polymorphism, and it is still possible that at some specific loci, overdominant selection is operating. This is particularly so in the loci for histocompatibility and immunoglobulins, where a great amount of polymorphism is known to exist. Intensive genetic studies of these loci are now under way, and in the near future more data about the polymorphisms are expected to be collected. To know whether overdominant selection is really involved in these loci, a detailed study is necessary, with various alternative hypotheses being considered.

In the past ten years, the mathematical properties of the neutral theory have been studied extensively (KIMURA and OHTA 1971; NEI 1975; EWENS 1979). On the other hand, the properties of alternative hypotheses are still poorly understood, mainly because of the mathematical difficulties involved. However, in order to know the mechanism of maintenance of genetic variability, we must first know the mathematical properties of the alternative theories. In the study of these properties, we believe that Itô's stochastic differential equations, as used in this paper, will be a valuable tool.

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