Proc. Natl. Acad. Sci. USA
Vol. 75, No. 2, pp. 919–922, February 1978
Genetics

Theoretical study of genetic variability, assuming stepwise production of neutral and very slightly deleterious mutations*

(population genetics/stochastic process/protein polymorphism)

TAKEO MARUYAMA AND MOTOO KIMURA

National Institute of Genetics, Mishima 411, Japan

Contributed by Motoo Kimura, October 28, 1977

ABSTRACT **Mathematical treatments are presented that enable us to compute the amount of genetic variability maintained in a finite population, assuming that mutations occur in stepwise fashion and that both selectively neutral and slightly deleterious alleles are involved. Two numerical examples show that, if very slightly deleterious mutations are prevalent, the amount of genetic variability increases much more slowly as the population number increases than is the case when all the mutations are strictly neutral.**

To analyze theoretically the genetic variability within populations, as detected by electrophoretic methods, Ohta and Kimura (1) proposed a model of stepwise production of neutral alleles. Since then, a number of papers have been published treating the model in various mathematical and statistical contexts (2–12). Of particular importance are papers by Moran (9, 10), who worked out the variance of homozygosity (involving the fourth moment). In addition, the distribution of allelic frequency at equilibrium was derived by Kimura and Ohta (13), assuming linear regression of the frequencies of alleles at the adjacent states. Although this is an approximation, subsequent computer simulations have shown that it must be very close to the true distribution, particularly if $4N_ev$ does not exceed unity (N_e = effective population size, v = mutation rate). In addition, the time-dependent behavior of the second moments was thoroughly worked out recently by one of us (14).

In the present paper, we intend to report our analysis of the step-mutation model, assuming the occurrence of both neutral and slightly deleterious alleles. Such a treatment should be desirable to understand the nature of protein polymorphisms, particularly in view of Ohta's thesis (15–17) that very slightly deleterious mutations as well as neutral ones are playing an important role in the maintenance of genetic variability at the molecular level.

MATHEMATICAL MODEL

Let us assume that all allelic states are expressed by integers, and that each state may be occupied by both neutral and slightly deleterious alleles. We denote by A_i the neutral allele at the ith state. (i = ···, −1, 0, 1, 2, ···), and, similarly, by B_i the deleterious allele at state i. We also assume that if an allele changes state by mutation it moves either one step in the positive direction or one step in the negative direction in the allele space. In addition we assume that mutation occurs between the neutral and deleterious alleles. Fig. 1 illustrates the scheme of mutation and the parameters involved in mutational changes between and among neutral and deleterious alleles.

Note that there are six parameters specifying mutation rates in various directions. To facilitate identification, we put subscript 0 for mutational changes toward neutral alleles and subscript 1 for those toward deleterious alleles. For example, neutral allele A_i changes to deleterious alleles B_{i-1}, B_i, and B_{i+1}, respectively, at the rates $w_1/2$, u_1, and $w_1/2$. Similarly, B_i changes to A_{i-1}, A_i, and A_{i+1} at the rates $w_0/2$, u_0, and $w_0/2$. Mutation rates to and from A_i and A_{i-1} are both $v_0/2$, while those involved between B_i and B_{i-1} are both $v_1/2$.

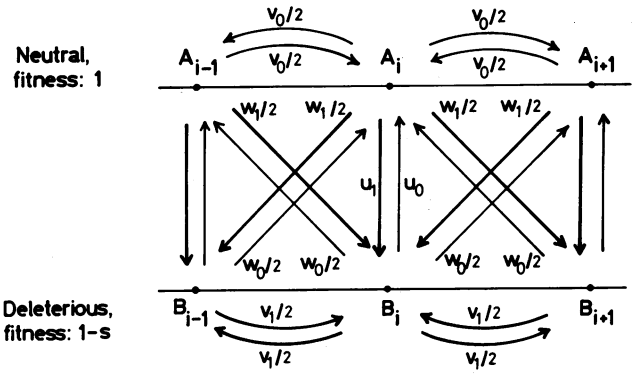

FIG. 1. Scheme of mutation in the model of stepwise production of neutral and deleterious alleles. Parameters that specify the rates of mutations are given together with arrows indicating the directions of mutation.

We consider a random mating population of effective size N_e, and let x_i be the frequency of allele A_i, and similarly let y_i be that of allele B_i. Then, we define the following quantities:

$$\hat{X} = E\left\{\sum_{i=-\infty}^{\infty} x_i\right\}, \quad [1]$$

$$\hat{Y} = E\left\{\sum_i y_i\right\} = 1 - \hat{X}, \quad [2]$$

$$X_k = E\left\{\sum_i x_i x_{i+k}\right\}, \quad [3]$$

$$Y_k = E\left\{\sum_i y_i y_{i+k}\right\}, \quad [4]$$

and

$$Z_k = E\left\{\sum_i x_i y_{i+k}\right\}, \quad [5]$$

in which E stands for the operator for taking expectations. Note that k takes on integer values and that, because of space homogeneity, we have $X_k = X_{-k}$, $Y_k = Y_{-k}$, and $Z_k = Z_{-k}$ so that

$$Z_k = E\left\{\sum_i y_i x_{i+k}\right\}.$$

Let s be the selection coefficient against the slightly deleterious alleles. Then the changes of allelic frequencies due to selection

* Contribution no. 1177 from the National Institute of Genetics, Mishima 411, Japan.

in one generation are

$$\Delta x_i = sYx_i/(1 - sY) \approx sYx_i, \quad [6]$$

$$\Delta y_i = -s(1 - Y)y_i/(1 - sY) \approx -s(1 - Y)y_i = -sXy_i, \quad [6a]$$

in which

$$Y = \sum_i y_i,\ X = \sum_i x_i, \text{ and } X = 1 - Y.$$

Taking into account the changes due to mutation, selection, and random sampling of gametes, we obtain the following set of equations (7–10) in which $\Delta\hat{X}$, ΔX_k, ΔY_k, and ΔZ_k, respectively, denote the changes per generation of $\hat{X}$, X_k, Y_k, and Z_k, and parameters with capital letters denote $2N_e$ times the corresponding quantities represented by lower case letters, i.e., $V_0 = 2N_ev_0$, $V_1 = 2N_ev_1$, $U_0 = 2N_eu_0$, $U_1 = 2N_eu_1$, $W_0 = 2N_ew_0$, $W_1 = 2N_ew_1$, and $S = 2N_es$.

$$2N_e\Delta\hat{X} = -(U_1 + W_1)\hat{X} + (U_0 + W_0)\hat{Y} + S\hat{X}\hat{Y} \quad [7]$$

$$2N_e\Delta X_k = V_0(X_{k-1} + X_{k+1}) - (2U_1 + 2V_0 + 2W_1 - 2S\hat{Y} + 1)X_k + W_0(Z_{k-1} + Z_{k+1}) + 2U_0Z_k + \hat{X}\delta_{0,k} \quad [8]$$

$$2N_e\Delta Y_k = V_1(Y_{k-1} + Y_{k+1}) - (2U_0 + 2V_1 + 2W_0 + 2S\hat{X} + 1)Y_k + W_1(Z_{k-1} + Z_{k+1}) + 2U_1Z_k + \hat{Y}\delta_{0,k} \quad [9]$$

$$2N_e\Delta Z_k = \frac{W_1}{2}(X_{k-1} + X_{k+1}) + U_1X_k + \frac{W_0}{2}(Y_{k-1} + Y_{k+1}) + U_0Y_k + \frac{1}{2}(V_0 + V_1) \times (Z_{k-1} + Z_{k+1}) - [U_0 + U_1 + V_0 + V_1 + W_0 + W_1 + S(\hat{X} - \hat{Y}) + 1]Z_k. \quad [10]$$

In the above equations, $\delta_{0,k}$ stands for Kronecker's delta such that $\delta_{0,k} = 1$ for $k = 0$ and $\delta_{0,k} = 0$ otherwise ($k \neq 0$). These equations involve approximations in treating selection. Namely, we substitute $\hat{X}\hat{Y}$ for $E(XY)$, in which $X = \Sigma_i x_i$ and $Y = \Sigma_i y_i$. Also, such terms as $E\{(\Sigma_i x_i x_{i+k})Y\}$ and $E\{(\Sigma_i y_i y_{i+k})X\}$ are approximated, respectively, by $E\{\Sigma_i x_i x_{i+k}\}\hat{Y}$ ($= X_k\hat{Y}$) and $E\{\Sigma_i y_i y_{i+k}\}\hat{X}$ ($= Y_k\hat{X}$). Therefore, extensive Monte Carlo experiments were performed to check the validity of the solutions that we obtained by solving Eqs. 7–10, assuming the equilibrium condition under which $\Delta\hat{X} = \Delta X_k = \Delta Y_k = \Delta Z_k = 0$. The analytical solutions at equilibrium for X_k, Y_k, and Z_k can be obtained as follows.

Let

$$X_k = \frac{1}{\pi}\int_0^\pi a(\theta)\cos k\theta d\theta \quad [11]$$

$$Y_k = \frac{1}{\pi}\int_0^\pi b(\theta)\cos k\theta d\theta \quad [12]$$

$$Z_k = \frac{1}{\pi}\int_0^\pi c(\theta)\cos k\theta d\theta \quad [13]$$

and noting, for example, $X_{k-1} + X_{k+1} = 1/\pi \int_0^\pi a(\theta)\cdot 2\cos\theta \cos k\theta d\theta$, we have, at equilibrium, the following equation;

$$\begin{bmatrix} a_1 & 0 & a_3 \\ 0 & b_2 & b_3 \\ c_1 & c_2 & c_3 \end{bmatrix}\begin{bmatrix} a(\theta) \\ b(\theta) \\ c(\theta) \end{bmatrix} = \begin{bmatrix} -\hat{X} \\ -\hat{Y} \\ 0 \end{bmatrix}, \quad [14]$$

in which

$$a_1 = 2V_0\cos\theta - 2(U_1 + V_0 + W_1) + 2S\hat{Y} - 1,$$
$$a_3 = 2W_0\cos\theta + 2U_0,$$
$$b_2 = 2V_1\cos\theta - 2(U_0 + V_1 + W_0) - 2S\hat{X} - 1,$$
$$b_3 = 2W_1\cos\theta + 2U_1, \qquad c_1 = W_1\cos\theta + U_1$$
$$c_2 = W_0\cos\theta + U_0,$$
$$c_3 = (V_0 + V_1)\cos\theta - (U_0 + U_1 + V_0 + V_1 + W_0 + W_1) + S(\hat{Y} - \hat{X}) - 1.$$

Then, noting that $c_1 = b_3/2$, $c_2 = a_3/2$ and $c_3 = (a_1 + b_2)/2$ we obtain

$$a(\theta) = \frac{a_3(\hat{X}b_3 - \hat{Y}a_3) - \hat{X}(a_1 + b_2)b_2}{(a_1 + b_2)(a_1b_2 - a_3b_3)}, \quad [15]$$

$$b(\theta) = \frac{b_3(\hat{Y}a_3 - \hat{X}b_3) - \hat{Y}(a_1 + b_2)a_1}{(a_1 + b_2)(a_1b_2 - a_3b_3)}, \quad [16]$$

and

$$c(\theta) = \frac{\hat{X}b_2b_3 + \hat{Y}a_1a_3}{(a_1 + b_2)(a_1b_2 - a_3b_3)}, \quad [17]$$

in which

$$\hat{X} = 1 - \hat{Y} = \{S - (U_0 + W_0 + U_1 + W_1) + \sqrt{\hat{D}}\}/(2S) \quad [18]$$

and

$$\hat{D} = S^2 + 2S(U_0 + W_0 - U_1 - W_1) + (U_0 + W_0 + U_1 + W_1)^2, (S \neq 0).$$

The average homozygosity at equilibrium can then be obtained by

$$\overline{H}_0 = E\left\{\sum_i (x_i + y_i)^2\right\} = X_0 + Y_0 + 2Z_0 = \frac{1}{\pi}\int_0^\pi \{a(\theta) + b(\theta) + 2c(\theta)\}d\theta. \quad [19]$$

We should remark here that although formulae 11, 12, and 13 represent uniquely determined, non-negative solutions of the system of Eqs. 7–10, the approximations involving the selection terms (6, 6a) cause the sum of $X_k + Y_k + 2Z_k$ over k to reduce not exactly to unity. This difficulty can be removed by normalizing them to make the sum equal to unity. However, the deviation from unity is quite small (up to a few percent) even for a large value of S, and it can be neglected without serious error. The numerical values used in Fig. 2 and Table 1 (see below) are normalized, though difference due to the normalization is not large enough to be visible in the figure.

NUMERICAL EXAMPLES

Using the above results, we investigated two numerical examples that may be of interest in understanding the relationship between the population size and the level of heterozygosity.

Example 1: In this example, we assume that mutation rates toward very slightly deleterious alleles are 100 times higher than those toward the neutral alleles so that $u_1/u_0 = v_1/v_0 = w_1/w_0 = 100$. We also assume that mutations causing change in allelic states ("charge states") occur one-fourth as frequently as those causing no change in state so that $w_1 = u_1/4$, $w_0 = u_0/4$. We shall denote the total mutation rate by μ_T, which is assumed to be equal for neutral and deleterious alleles so that $\mu_T = u_1 + v_0 + w_1 = u_0 + v_1 + w_0$. Then mutation rates in all di-

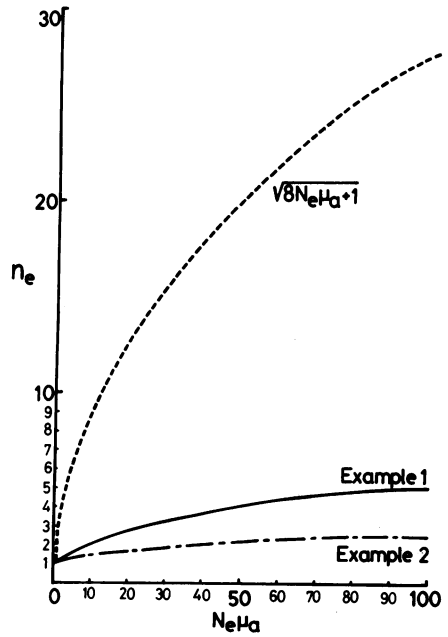

FIG. 2. Relationship between n_e (the effective number of alleles, or the reciprocal of the average homozygosity) and $N_e\mu_a$ (product of the effective population size and "apparent mutation rate"). For details see text.

rections are expressed in terms of the total mutation rate, such as $u_1 = (80/101)\mu_T$, $v_1 = (100/101)\mu_T$, $w_1 = (20/101)\mu_T$, etc. Finally, we assume that the selective disadvantage of slightly deleterious alleles is 10 times as large as the total mutation rate so that $s = 10\mu_T$. To facilitate our illustration for the relationship between the observed variability and the estimated mutation rate, let us define the apparent mutation rate by

$$\mu_a = \hat{X}(v_0 + w_1) + \hat{Y}(v_1 + w_0). \quad [20]$$

This represents the mutation rate that may be estimated by sampling alleles from the population and investigating the rate at which allelic states (charge states) change by mutation. We may take this as the "observed" mutation rate. Note that the total mutation rate μ_T includes mutation rates (u_1 and u_0) that cannot be detected by merely investigating the change of allelic states. To represent the level of genetic variability, we shall use the effective number of alleles n_e, which is the reciprocal of the average homozygosity ($n_e = 1/\bar{H}_0$). In Fig. 2, the effective number of alleles is plotted against $\mu_a N_e$ (the apparent mutation rate multiplied by the effective population size) using a solid line. In the same figure, values of $\sqrt{8N_e\mu_a + 1}$ are plotted by a broken line. This line represents the level of genetic variability that can be attained if all the mutations are selectively neutral.

Example 2: In this example, we assume that selectively neutral alleles can arise by mutation only from very slightly deleterious alleles; none of the neutral alleles can be derived directly from adjacent neutral alleles through a single mutational step so that $v_0 = 0$. Other specifications of conditions among mutation parameters are $w_0 = v_1/100$, $w_1 = u_1/4$, $w_0 = u_0/4$, and $\mu_T = w_1 + u_1 = v_1 + u_0 + w_0$. As before, we assume $s = 10\mu_T$ for very slightly deleterious alleles. The effective number of alleles is plotted against $N_e\mu_a$ in Fig. 2 using a broken line, and this may be compared with the corresponding solid line for the previous example.

SIMULATION STUDIES

To check the validity of the formulae derived in the present report, we have performed Monte Carlo experiments (using the computer TOSBAC 3400) simulating a random mating population of fixed size. In these simulations, in which parameter values were varied greatly, we observed satisfactory agreement between the theoretical predictions and the experimental outcomes. One such example is presented in Table 1. In this experiment (simulating the step mutation model with infinite allelic states), the mutation scheme is like that of *Example 2* discussed above; strictly neutral alleles can arise only from slightly deleterious alleles, i.e., $v_0 = 0$. The experiment consists of a single run extending to 20,000 generations, and the data were collected from the last 10,000 generations at intervals of every 10 generations. In this experiment, very close agreement was obtained with respect to $\hat{X}$; observed $\hat{X} = 0.76760$, predicted $\hat{X} = 0.76942$.

Table 1. Comparison between experimentally observed results and theoretical predictions

	$k = 0$	$k = 1$	$k = 2$	
X_k	0.5143	0.0301	0.0082	(observed)
	0.5462	0.0283	0.0064	(predicted)
Y_k	0.0347	0.0118	0.0034	(observed)
	0.0288	0.0111	0.0030	(predicted)
Z_k	0.0862	0.0311	0.0054	(observed)
	0.0866	0.0318	0.0048	(predicted)

The observed results are taken from one of the Monte Carlo experiments simulating the mutation scheme like that of *Example 2* in *Numerical Examples*. Parameter values used for this simulation are $N_e = 100$, $N_e s = 4$, $N_e\mu_T = 1$, $v_0 = 0$, $w_0 = v_1/10$, $w_1 = u_1/2$, $w_0 = u_0/2.5$, and $\mu_T = w_1 + u_1 = v_1 + u_0 + w_0 = 0.01$.

In our simulation studies, we assumed a small population number; $N_e = 100$. This is based on the consideration that the quantities of our interest such as X_k, Y_k, and Z_k all depend on the product of N_e and other parameters. In other words, they depend on $N_e v_0$, $N_e v_1$, $N_e u_0$, ..., $N_e s$ but not on N_e or other mutation and selection parameters separately. This means that, to the extent that the diffusion approximation is valid, the level of heterozygosity $\bar{H}_e = 1 - 1/n_e$, for example, is not affected by the population size as long as values of $N_e v_0$ etc. remain the same.

DISCUSSION

In the present treatment, we attempted an extension of the stepwise mutation model of Ohta and Kimura (1), by incorporating the idea that very slightly deleterious mutations, as well as strictly neutral ones, are playing an important role in molecular evolution and polymorphism. According to Ohta (15–17), the majority of the "neutral" alleles may be very slightly deleterious, although selection coefficients against them are not excessively large as compared with mutation rates. Such alleles will behave practically as neutral in relatively small populations as in many mammals; the neutral mutation–random drift hypothesis in its simplest form can be used to describe molecular polymorphism in such populations. On the other hand, mutation–selection balance will prevail in very large populations such as those of the neotropical fruit flies studied by Ayala *et al.* (18) and *Escherichia coli* studied by Milkman (19); in such large populations, negative selection becomes effective due to large $N_e s$ values, and this prevents the amount of genetic variability reaching a high level as expected from the strict neutral theory. This is an extended form of the original neutral theory as proposed by one of us (20, 21). Considering the fundamental nature of mutations, this idea of Ohta is sufficiently realistic, and its population genetical consequence should be seriously explored.

In two numerical examples (*Examples 1* and 2), we assumed that very slightly deleterious mutations occur 100 times as frequently as the strictly neutral mutations and that their selective disadvantage is only 10 times as large as the total mutation rates. As shown in Fig. 2, the amount of genetic variability in terms of the effective number of alleles n_e (= reciprocal of the average homozygosity expected under random mating) increases much more slowly as the population number increases in these two examples, compared with $n_e = \sqrt{8N_e\mu_a + 1}$, the value expected if all the mutations are strictly neutral.

Recently, Mukai and Cockerham (22) obtained an estimate of the average mutation rate for electrophoretically detectable alleles (excluding null mutations); $\hat{\mu} = 1.81 \times 10^{-6}$ per locus per generation for *Drosophila melanogaster*. Similar estimates of electromorph mutation rate (per locus per generation) have been obtained by Nei (23) for man (2.4×10^{-6}) and the Japanese macaque (2.3×10^{-6}). Thus, an appropriate value for μ_a in Fig. 2 may be $\mu_a = 2 \times 10^{-6}$. This means that the heterozygosity stays at a fairly realistic level as long as the effective population number N_e does not exceed 5×10^6 in *Example 1* and 2×10^7 in *Example* 2 in Fig. 2.

The observation that species with apparently very large population size such as *D. willistoni* still have the average heterozygosity of around 20% ($n_e = 1.25$) has been used repeatedly to criticize the validity of the neutral theory since the paper of Ayala *et al.* (18). These authors claim that in *D. willistoni* the number of breeding flies per generation is at least 10^9 (with geographic distribution encompassing several million square kilometers) and even if the mutation rate for neutral alleles is as low as $v = 10^{-7}$, $4N_ev$ becomes 400. Thus, if the effective number of alleles is predicted by using Kimura and Crow's (24) formula $n_e = 4N_ev + 1$, it becomes about 400 times larger than the observed value. Discrepancy between theoretical prediction and actual observation still remains if we use Ohta and Kimura's (1) formula $n_e = \sqrt{8N_ev + 1}$, which gives about 28 as the predicted value.

It is clear that the discrepancy is further reduced by using the present model, which can accommodate very slightly deleterious mutations in addition to neutral mutations. In *Example 1* illustrated in Fig. 2, selective disadvantage of these mutations is assumed to be 10 times as large as the total mutation rate, and this amounts to roughly 35 times the apparent mutation rate, so that $s \approx 7 \times 10^{-5}$. In a small population whose effective population number is a few thousand or less, all the mutant alleles would behave as if they were selectively neutral. In a much larger population with $N_e = 3 \times 10^6$, although we have $N_e\mu_a = 6$ so that $\sqrt{8N_e\mu_a + 1} = 7$, the effective number of alleles predicted by this model is still $n_e \doteqdot 1.7$. *Example 2* illustrated in Fig. 2 is more remarkable in that the level of heterozygosity is much less sensitive to the increase of the total population number. For $N_e = 4 \times 10^6$, the effective number of alleles predicted is $n_e \doteqdot 1.28$.

However, if the effective population number is really as large as 10^9 or 10^{10}, even in the present model it is difficult to keep the heterozygosity at a realistic level unless we assume that the fraction of truly neutral mutations is extremely small. Then, if the truly neutral mutation rate is extremely low for such a large population, the rate of evolution in terms of mutant substitutions becomes extremely low, and this seems to give difficulty to the neutral theory, although no data are available on this point for *D. willistoni* and *E. coli*. There is a possibility, however, that the effective population numbers (not the sheer number of individuals) in these species are not as large as 10^9. We would like to point out that if local extinction of colonies and supplanting by adjacent ones occur frequently, we can show that the effective population number of a species becomes much smaller (sometimes two orders of magnitude less) than the total number of the breeding individuals in one generation (detailed treatments of this subject will be published elsewhere). It is indeed likely that local extinction of colonies occurs frequently in *Drosophila* and *E. coli*. Also, Nei *et al.* (25) have emphasized the importance of the bottleneck effect in reducing the heterozygosity. An additional consideration that may be pertinent in the present context is lower physiological homeostasis of these organisms as compared with mammals. The fraction of neutral (not harmful) alleles among newly arisen mutations may be smaller for organisms with lower physiological homeostasis and therefore the role of slightly deleterious mutations will become more prominent in them.

The present model may be made more realistic by letting the selection coefficients of very slightly deleterious alleles follow a certain frequency distribution rather than assigning them only one value (s). Recently, Li (ref. 26; personal communication), using Wright's (27) distribution formula for multiple alleles, made excellent theoretical studies on the amount of genetic variability, assuming the K allele model incorporating two or three classes of mutations with different fitness (including the neutral class). We should emphasize that although natural selection is considered, our point of view is fundamentally different from that of the "selectionist" who resorts to "balancing selection" to explain the maintenance of molecular polymorphisms. It is hoped that we are on the right track to elucidate the mechanism by which genetic variability is maintained at the molecular level.

1. Ohta, T. & Kimura, M. (1973) *Genet. Res.* **22**, 201–204.
2. Avery, P. J. (1975) *Genet. Res.* **25**, 145–153.
3. Brown, A. H. D., Marshall, D. R. & Albrecht, L. (1975) *Genet. Res.* **25**, 137–143.
4. Chakraborty, R. & Nei, M. (1976) *Genetics* **84**, 385–393.
5. Kingman, J. F. C. (1977) *Theor. Popul. Biol.* **11**, 285–290.
6. Li, W.-H. (1976) *Genet. Res.* **28**, 119–127.
7. Li, W.-H. (1976) *Genetics* **83**, 423–432.
8. Marshall, D. R. & Brown, A. H. D. (1975) *J. Mol. Evol.* **6**, 149–163.
9. Moran, P. A. P. (1975) *Theor. Popul. Biol.* **8**, 318–330.
10. Moran, P. A. P. (1976) *Theor. Popul. Biol.* **10**, 145–149.
11. Wehrhahn, C. F. (1975) *Genetics* **80**, 375–394.
12. Weir, B. S., Brown, A. H. D. & Marshall, D. R. (1976) *Genetics* **84**, 639–659.
13. Kimura, M. & Ohta, T. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2761–2764.
14. Maruyama, T. (1977) *Stochastic Problems in Population Genetics* (Springer-Verlag, New York).
15. Ohta, T. (1973) *Nature* **246**, 96–98.
16. Ohta, T. (1974) *Nature* **252**, 351–354.
17. Ohta, T. (1976) *Theor. Popul. Biol.* **10**, 254–275.
18. Ayala, F. J., Powell, J. R., Tracey, M. L., Mourão, C. A. & Pérez-Salas, S. (1972) *Genetics* **70**, 113–139.
19. Milkman, R. (1973) *Science* **182**, 1024–1026.
20. Kimura, M. (1968) *Nature* **217**, 624–626.
21. Kimura, M. (1968) *Genet. Res.* **11**, 247–269.
22. Mukai, T. & Cockerham, C. C. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2514–2517.
23. Nei, M. (1977) *Am. J. Hum. Genet.* **29**, 225–232.
24. Kimura, M. & Crow, J. F. (1964) *Genetics* **49**, 725–738.
25. Nei, M., Maruyama, T. & Chakraborty, R. (1975) *Evolution* **29**, 1–10.
26. Li, W.-H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2509–2513.
27. Wright, S. (1949) in *Genetics, Paleontology and Evolution*, eds. Jepsen, G. L., Simpson, G. G. & Mayr, E. (Princeton University Press, Princeton, NJ), pp. 365–389.