THE AGE OF A NEUTRAL MUTANT PERSISTING IN A FINITE POPULATION*

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ABSTRACT

Formulae for the mean and the mean square age of a neutral allele which is segregating with frequency x in a population of effective size N_e have been obtained using the diffusion equation method, for the case of $4N_e v < 1$ where v is the mutation rate. It has been shown that the average ages of neutral alleles, even if their frequencies are relatively low, are quite old. For example, a neutral mutant whose current frequency is 10% has the expected age roughly equal to the effective population size N_e and the standard deviation $1.4N_e$ (in generations), assuming that this mutant has increased by random drift from a very low frequency. Also, formulae for the mean "first arrival time" of a neutral mutant to a certain frequency x have been presented. In addition, a new, approximate method has been developed which enables us to obtain the condition under which frequencies of "rare" polymorphic alleles among local populations are expected to be uniform if the alleles are selectively neutral. ——It was concluded that exchange of only a few individuals on the average between adjacent colonies per generation is enough to bring about such a uniformity of frequencies.

IN one of our previous papers (KIMURA and OHTA 1969a), we presented a theory on the average number of generations until a mutant gene becomes fixed in a finite population (excluding the cases of loss). The theory can be extended, as outlined in MARUYAMA and KIMURA (1971), to obtain the average number of generations until a mutant gene reaches a certain frequency for the first time starting from a lower frequency (i.e., the mean first arrival time). We need such a theory when we try to understand the evolutionary process consisting of a sequence of mutant substitutions in each of which an originally rare mutant increases its frequency and finally reaches fixation in the population.

On the other hand, in order to understand the nature of extant variations, we need to know the ages of mutant alleles within a population. In other words, we have to consider the problem of how many generations a mutant allele has persisted in the population since it appeared by mutation.

In the present paper, we present a solution to this problem for the case of selectively neutral alleles using the method of diffusion equations. It will be shown that the expected age of such a mutant is quite old; it is much older than one might suppose on the common-sense ground. We shall also discuss the bearing

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of the present finding on our neutral mutation-random drift hypothesis of molecular polymorphisms (KIMURA and OHTA 1971).

BASIC THEORY

We use the diffusion model (KIMURA 1964; see also CROW and KIMURA 1970, p. 371), and denote by $\phi(p, x; t)$ the probability density that the frequency of the mutant allele becomes x at time t (t-th generation) given that it is p at the start ($t = 0$). We first consider the case in which mutations are so rare that further mutations can be neglected. Such a treatment should be realistic if we consider mutants at the molecular level, that is, at each nucleotide site. If the mutant is selectively neutral and if the "variance effective size" of the population is N_e, then the transition probability density satisfies the partial differential equation

$$\frac{\partial\phi(p,x;t)}{\partial t} = \frac{1}{4N_e}\frac{\partial^2}{\partial x^2}\{x(1-x)\phi(p,x;t)\} \tag{1}$$

with the initial condition $\phi(p,x;0) = \delta(x-p)$, where $\delta(\cdot)$ stands for Dirac's delta function.

Our main aim is to evaluate the mean and the variance of the time interval in generations since an allele which now has intermediate frequency x had a lower frequency p.

Let

$$T_i = \int_0^\infty t^i\phi(\mathrm{p},x;t)\,dt \tag{2}$$

be the i-th moment ($i = 0, 1, 2, \ldots$) of t, then the mean time interval is given by

$$\bar{t}(p, x) = T_1/T_0, \tag{3}$$

while the mean square time is given by

$$\overline{t^2(p, x)} = T_2/T_0, \tag{4}$$

from which the variance can readily be obtained. We first derive an equation for T_0 as follows: Integrating both sides of equation (1) with respect to t from $t = 0$ to $t = \infty$, we obtain

$$\int_0^\infty \frac{\partial\phi}{\partial t}\,dt = \frac{1}{4N_e}\frac{\partial^2}{\partial x^2}\{x(1-x)\int_0^\infty \phi dt\}, \tag{5}$$

where ϕ stands for $\phi(p,x;t)$. This yields

$$\phi(p,x;\infty) - \phi(p,x;0) = \frac{1}{4N_e}\frac{d^2}{dx^2}\{x(1-x)T_0\},$$

but the two terms on the left-hand side vanish if we assume $1 > x > p \geqq 0$, because ϕ is asymptotically proportional to $\exp\{-t/(2N_e)\}$ for a large t (cf.

KIMURA 1955) while it is equal to $\delta(x-p)$ for $t=0$. Then, by integrating twice the resulting equation, i. e.,

$$\frac{1}{4N_e}\frac{d^2}{dx^2}\{x(1-x)T_0\}=0,$$

with respect to x, we obtain

$$T_0=\frac{4N_e}{x(1-x)}\{(C_1-1)x+C_2\}, \tag{6}$$

where C_1 and C_2 are constants. These constants can be determined from the consideration that as x approaches unity, T_0, as defined by (2), must approach $4N_ep$, because $\phi(p,1;t)/(4N_e)$ represents the amount of fixation during the t-th generation, and the sum of this quantity over all generations must be equal to p, the probability of ultimate fixation. This leads to $C_1-1=-C_2$ and $C_2=p$.

Thus we obtain

$$T_0=4N_ep/x. \tag{7}$$

The equations for T_1 and T_2 can be obtained in a similar way, so we shall derive a general equation for T_i $(i=1,2,\ldots)$.

Multiplying t^i to both sides of equation (1) and then integrating them with respect to t from $t=0$ to ∞, we obtain

$$\int_0^\infty t^i\frac{\partial\phi}{\partial t}dt=\frac{1}{4N_e}\frac{\partial^2}{\partial x^2}\{x(1-x)\int_0^\infty t^i\phi dt\}. \tag{8}$$

The left-hand side of this equation yields

$$\Big[t^i\phi\Big]_{t=0}^{t=\infty}-i\int_0^\infty t^{i-1}\phi dt,$$

the first term of which vanishes because $t^i\phi$ vanishes both at $t=0$ and $t=\infty$. Thus we obtain the ordinary differential equation for T_i as follows:

$$\frac{1}{4N_e}\frac{d^2}{dx^2}\{x(1-x)T_i\}+iT_{i-1}=0, \tag{9}$$

where $i\geqq 1$.

In the special case of $i=1$, by putting $T_0=4N_ep/x$, equation (9) reduces to

$$\frac{1}{4N_e}\frac{d^2}{dx^2}\{x(1-x)T_1\}+\frac{4N_ep}{x}=0. \tag{10}$$

Then, integrating this equation twice with respect to x, we obtain

$$T_1=\frac{4N_e}{x(1-x)}\{(C_1+4N_ep)x-4N_epx\log_e x+C_2\},$$

where C_1 and C_2 are constants. In determining these constants, we note that as x approaches unity, T_1/T_0 should approach

$$\bar{t}_1(p)=-\frac{1}{p}\{4N_e(1-p)\log_e(1-p)\},$$

the average number of generations until fixation (KIMURA and OHTA 1969a). This leads to

$$C_1 + 4N_e p = -C_2 = 4N_e\{(1-p)\log_e(1-p) + p\}.$$

Thus we obtain the formula for the mean time interval (in generations)

$$\bar{t}(p,x) \equiv \frac{T_1}{T_0} = 4N_e\{-\frac{1-p}{p}\log_e(1-p) - \frac{x}{1-x}\log_e x - 1\}. \qquad (11)$$

Note that this is different from the mean first arrival time which we denote by $\bar{t}_x(p)$ and on which we later present a formula in the discussion. Whereas $\bar{t}_x(p)$ represents the average number of generations until a mutant allele happens to reach a certain frequency x for the first time starting from a lower frequency p, $\bar{t}(p,x)$ represents the average number of generations which an allele having frequency x at present has persisted in the population since it had a lower frequency p in the past.

Similarly, we can obtain the following formula for the mean square age by solving equation (9) for the case of $i = 2$ under the condition that as x approaches unity T_2/T_0 should approach the mean square time until fixation as given by KIMURA and OHTA (1969b).

$$\overline{t^2(p,x)} = \frac{32N_e^2}{p}\{[(1-p)\log_e(1-p) + 2p]\frac{x\log_e x}{1-x} - p\int_0^x \frac{\log_e z}{1-z}dz + 2(1-p)\log_e(1-p) + 2p + p\int_{1-p}^1 \frac{\log_e z}{1-z}dz\}, \quad (x > p). \qquad (12)$$

The variance of the age is then given by

$$\sigma_t^2(p,x) = \overline{t^2(p,x)} - \{\bar{t}(p,x)\}^2.$$

For a mutant allele which is represented only once at the moment of appearance in a population consisting of N individuals, we may put $p = 1/(2N)$ in the above formulae. As N gets large, p approaches zero, and at the limit we have

$$\bar{t}(0,x) = 4N_e\left(-\frac{x}{1-x}\log_e x\right) \qquad (13)$$

and

$$\overline{t^2(0,x)} = 32N_e^2\left(\frac{x}{1-x}\log_e x - \int_0^x \frac{\log_e z}{1-z}dz\right) \qquad (14)$$

respectively for the mean and the mean square ages. These formulae should be valid for molecular mutants which are selectively neutral and which are subject to random frequency drift in a large population.

However, when we consider each gene locus (cistron) as our basic unit, rather than each nucleotide site, we must take into account the possibility that further

mutation occurs before the mutant allele reaches a high frequency. In addition there is the possibility that a mutant allele that has once become fixed in the population eventually has its frequency decreased by further mutation in conjunction with random drift. Also it is possible if the mutation rate is sufficiently high that an allele can never reach complete fixation.

Although the complete treatment taking all these possibilities into account is difficult, we have worked out the average age of the mutant for the case $4N_e v < 1$, where v is the mutation rate per locus per generation. Following KIMURA and CROW (1964), we assume that the number of possible allelic states per locus is so large that whenever mutation occurs it leads to a new (not a pre-existing) allele. The treatment using this model is more complicated than that without mutation, so that we shall only summarize the results.

If $4N_e v < 1$, it can be shown that the probability of a mutant allele's reaching fixation ($x = 1$) is

$$u(p) = 1 - (1 - p)^{1 - 4N_e v},$$

disregarding the possibility that its frequency later decreases by mutation and random drift. It can also be shown that for $4N_e v < 1$, the average age of a mutant having current frequency x is

$$\bar{t}_{\uparrow}(0, x) = \frac{4N_e}{1 - 4N_e v}\left\{ \log_e x + \int_0^1 \frac{1 - (1 - z)^{1-4N_e v}}{z}\, dz \right.$$
$$\left. + \frac{1}{(1 - x)^{1-4N_e v}} \int_x^1 \frac{(1 - z)^{1-4N_e v}}{z}\, dz \right\}. \qquad (13a)$$

It is assumed that the mutant allele increased from a very low frequency sometime in the past rather than decreased from the fixed state. As $4N_e v$ approaches 0, equation (13a) reduces to (13), but in general, numerical integration will be required to compute $\bar{t}_{\uparrow}(0, x)$ from this equation.

On the other hand, if the allele in question decreased from the fixed state—that is, from a frequency of 100%—by mutation and random drift, rather than directly increasing from a low frequency, then the corresponding formula becomes

$$\bar{t}_{\downarrow}(1, x) = \frac{4N_e}{1-4N_e v}\left\{ \frac{(1-x)^{1-4N_e v}}{1-(1-x)^{1-4N_e v}} \int_0^x \frac{[1-(1-z)^{1-4N_e v}]^2}{(1-z)^{1-4N_e v} z}\, dz \right.$$
$$\left. + \int_x^1 \frac{1-(1-z)^{1-4N_e v}}{z}\, dz \right\}. \qquad (13b)$$

This gives the expected "age" of the allele counted from $p = 1 - 1/(2N)$ to the present frequency x assuming that N is large. When $4N_e v = 0$, this agrees with $\bar{t}_{\uparrow}(0, 1-x)$, as it should. It does not include the length of time during which the allele remained fixed in the population before the frequency $1 - 1/(2N)$ was reached. In order to estimate this length of time (the waiting time), let $u_x(1 - 1/2N)$ be the probability that the allele frequency goes down to x from $1 - 1/(2N)$ without previously going back to unity. Then, it can be shown that

$u_x(1 - 1/2N) = \{2N(1 - x)\}^{4N_e v - 1}$. Also, the probability that one mutant is produced during a short time interval of length Δt in the population of fixed state is $2Nv\Delta t$. Combining these two probabilities, we find that for the allele whose current frequency is x and which had once been fixed in the population, the average length of time during which it was fixed is

$$\bar{t}_{fix} = \frac{1}{2Nvu_x(1 - 1/2N)} = \frac{\{2N(1 - x)\}^{1-4N_e v}}{2Nv}.$$

Note that this is the waiting time until a successful mutation first occurs in the population leading to the downward journey reaching x. When $4N_e v = 0$ and $x = 0$, this reduces to $\bar{t}_{fix} = 1/v$.

Finally, we can show that if $4N_e v \geqq 1$, the probability is zero that a new mutant reaches complete fixation (assuming that N_e is large). In other words, complete fixation is prevented by the opposing mutation pressure.

Figure 1 illustrates for several values of $4N_e v$ the relationship between x and $\bar{t}_{\uparrow}$ $(0, x)$ with solid lines, and that between x and $\bar{t}_{\downarrow}$ $(1, x)$ with dotted lines. They were obtained by numerical integration of formulae (13a) and (13b) using a computer. Note that $4N_e v = 0.2$ corresponds to a heterozygosity of about 16%, the value observed in man and Drosophila. Note also that with this level of $4N_e v$, the expected age of the mutant is not much influenced by mutation.

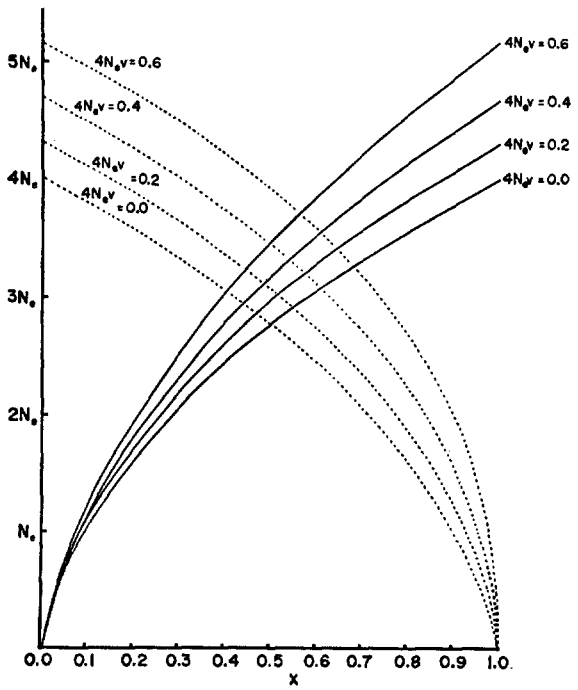

FIGURE 1.—Relationship between x and $\bar{t}^{\uparrow}$ $(0, x)$ as shown by solid lines and that between x and $\bar{t}_{\downarrow}$ $(1, x)$ as shown by dotted lines for some values of $4N_e v$. The abscissae represent the gene frequencies and the ordinates represent the corresponding ages in generations.

MONTE CARLO EXPERIMENTS

In order to check the validity of the above treatment (especially, formulae 13, 13a and 13b), we performed Monte Carlo experiments using TOSBAC 3400 in our institute. The procedure of the experiments follows the one used by HILL and ROBERTSON (1966). Assuming no selection, sampling of gametes is carried out by generating pseudo-random numbers that follow the uniform distribution (RAND 20 in TOSBAC 3400). Namely, if x is the frequency of the mutant allele and if a random number is less than x, one mutant gamete is sampled, while if it is larger than x, one gamete with normal allele is sampled. Sampling is repeated $2N_e$ times to obtain the total of gametes to form the next generation. Each experiment starts by having a mutant allele represented only once in the population and whenever loss or fixation of the mutant occurs, a new mutant is again supplied to continue the experiment. In each generation, the age and the frequency of the mutant allele are recorded.

Figure 2 illustrates the results of the experiments to check formula 13 for the frequency classes up to 0.1. The abscissa represents frequencies of mutant alleles and the ordinate, the corresponding ages. The curve in the figure shows the theoretical prediction for the mean age (assuming no mutation), while the two types of dots represent observed values; the square dots are for the case of $N_e = 100$ and the circular dots for $N_e = 200$. The observed values are the outcome of 10^4 gen-

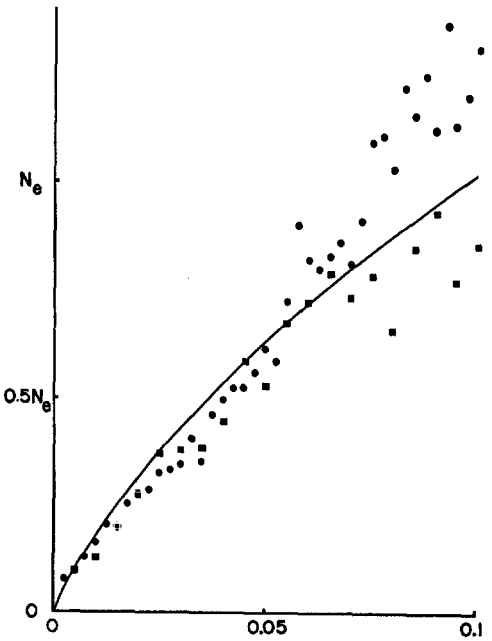

FIGURE 2.—Results of Monte Carlo experiments on the average age of new, neutral mutants. The abscissae represent frequencies of mutant alleles, while the ordinates represent the corresponding ages. In the figure, the curve gives the theoretical values, the square dots give the results for $N_e = 100$ and the circular dots the results for $N_e = 200$.

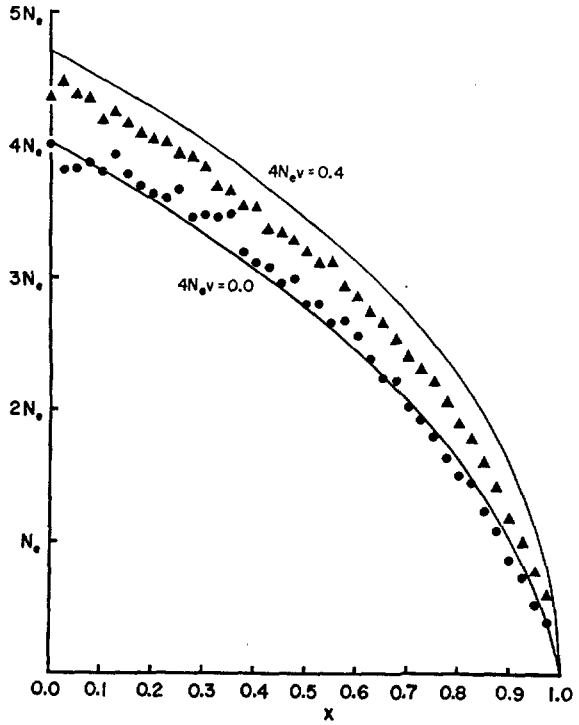

FIGURE 3.—Results of Monte Carlo experiments to check formula 13b for $4N_e\upsilon = 0$ and $4N_e\upsilon = 0.4$. Circular dots represent the outcome of 10,000 runs assuming $4N_e\upsilon = 0$, while triangular dots represent the outcome of 50,000 runs assuming $4N_e\upsilon = 0.4$. The abscissae represent the frequencies and the ordinates represent the corresponding ages. $N_e = 20$ is assumed.

erations of simulation experiments for the case of $N_e = 100$, but 3×10^4 generations for $N_e = 200$. As seen from the figure, the agreement between the theoretical and the experimental results is satisfactory. Figure 3 illustrates the results of the experiments to check formula 13b for the cases of $4N_e\upsilon = 0$ (circular dots) and $4N_e\upsilon = .0.4$ (triangular dots). The observed values are the outcomes of 10,000 runs (rather than generations) for $4N_e\upsilon = 0$ and 50,000 runs for $4N_e\upsilon = 0.4$, assuming $N_e = N = 20$. They corroborate the theoretical prediction that the expected age gets older under mutation pressure irrespective of the direction of mutation. We should remark here that systematic deviation of experimental results from theoretical prediction for $4N_e\upsilon = 0.4$ must be due to a very small population number, i.e., 20, employed in the simulation experiments; the pressure of mutation in preventing fixation does not become sufficiently effective unless the population number becomes much larger. The diffusion treatment is really adequate for such larger numbers.

DISCUSSION

In the present paper we have derived, under the assumption of no further mutation, formula (11) which gives the average number of generations which a

neutral allele segregating in a population with frequency x has persisted in the population since it had the initial frequency p ($< x$) in the past. In this general form, it also gives the average number of generations which a neutral allele takes when its frequency increases from p to x through random genetic drift ($p < x$). Therefore, $\bar{t}(0, x)$ given by formula 13 is appropriate to express the expected age of a neutral allele with frequency x, if the allele has increased its frequency in the population since it first appeared by mutation. On the other hand, if the allele in question has decreased from the previously fixed state, $\bar{t}_{\downarrow}(1, x)$ is appropriate to express its age, where $\bar{t}_{\downarrow}(1, x) = \bar{t}_{\uparrow}(0, 1-x)$ for $4N_e v = 0$. When we try to apply these formulae to actual situations, one difficulty that we encounter is that we cannot know which of these two alternative events has actually occurred. However, we can attach a probability statement to them (as pointed out to us by the referee). Namely, the probabilities of these two alternative events (assuming no further mutation) are $1 - x$ and x, respectively. This follows from the consideration that probability is p/x that a mutant allele with initial frequency p subsequently reaches a higher frequency x before it is either lost from the population or fixed in it. Similarly the probability is $p/(1-x)$ that the frequency of the allele decreases to x.

We have also studied the effect of further mutation on the age of neutral alleles and have found that the effect is relatively minor if $4N_e v$ is small (see Figure 1).

These results should be compared with the mean first arrival time, that is, the average number of generations until a neutral allele reaches frequency x for the first time starting from a lower frequency p. This is given by

$$\bar{t}_x(p) = 4N_e \left\{ \frac{1-x}{x} \log_e(1-x) - \frac{1-p}{p} \log_e(1-p) \right\}. \tag{15}$$

At the limit $p \to 0$, this reduces to

$$\bar{t}_x(0) = 4N_e \left\{ \frac{1-x}{x} \log_e(1-x) + 1 \right\}. \tag{16}$$

When x is much smaller than unity, we have

$$\bar{t}_x(0) \approx 4N_e x. \tag{17}$$

Equation (15) is a special case of a more general equation (A1) which can hold when the mutant is selected as well as when it is neutral (see APPENDIX).

In Figure 4, the mean age $\bar{t}(0, x)$ and the mean first arrival time $\bar{t}_x(0)$ are plotted for frequencies up to 0.1. From the figure it may be seen that expected age of a neutral mutant whose current frequency is 10% is roughly equal to the effective population size, N_e, and it is about five times the corresponding first arrival time. The standard deviation of the age is roughly $1.4N_e$ which is slightly larger than the mean. Since the expected age of a neutral mutant whose frequency is 50% is about $2.8N_e$ generations (see Figure 1), this example suggests that even "rare" polymorphic alleles whose current frequencies are a few percent

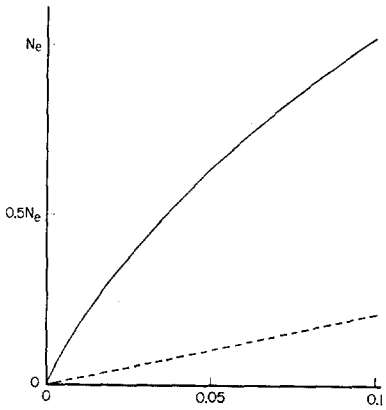

FIGURE 4.—Comparison between the average age (solid line) and the average first arrival time (broken line). The abscissae represent frequencies and the ordinates the ages or times in generations.

have quite old ages if they are neutral and if the population is very large. In fact, a neutral allele whose frequency is only 3.5% has the expected age of about half the population size, i.e. $0.5N_e$ if it has increased from a very low frequency. Furthermore, if this allele happens to be the remnant of a previously fixed allele, expected age becomes still larger. In general, we can make the following probability statement regarding these alternative events (as suggested to us by DR. ALAN ROBERTSON): If $4N_e v$ is small so that there are never more than two alleles segregating simultaneously, the probability that the allele with frequency x has increased from a very small frequency is $1 - x$, while that it has decreased from a previously fixed state is x. This means that if we combine these two alternative possibilities, the average age of the polymorphism with two alleles segregating with respective frequencies of x and $1 - x$ is

$$-4N_e\{x\log_e x + (1-x)\log_e(1-x)\}.$$

One additional property of neutral alleles which may be of interest from a mathematical standpoint is that the average age of a mutant allele having current frequency x (assuming $p = 0$) is equal to the average time until extinction of the same allele (excluding the cases of its eventual fixation). This may be evident by comparing equation (13) of this paper with equation (16) of KIMURA and OHTA (1969a). The same applies to the mean square age, as may be seen by comparing equation (14) of this paper with equation (A7) of KIMURA and OHTA (1969b).

Let us now consider the bearing of the present findings on the spatial pattern of genetic variation. Here we are particularly concerned with the question: how much migration is required to keep the frequencies of a "rare" polymorphic allele essentially uniform among localities when the allele is selectively neutral? First, consider a one-dimensional habitat forming a circle of radius r. Let N_T be the total number of breeding individuals in one generation and assume that they

are distributed uniformly with density δ so that $N_T = 2\pi r\delta$. If we denote by σ^2 the mean square distance of individual migration (assumed to be isotropic) in one generation, then the distance of migration during t generations should follow the normal distribution with mean zero and variance ${\sigma^2}_t = t\sigma^2$ when t is large. If the abscissa of this distribution is wrapped around the circle of radius $2\pi r$, and if the resulting (superimposed) probability distribution on the circle is approximately uniform, then the frequencies among localities of a mutant allele having age t will become essentially uniform. On the other hand, if the superimposed probability distribution on the circle markedly deviates from the uniform distribution, clear local differentiation of allelic frequencies should result. Figure 5 illustrates two such contrasting cases ($\pi r = \sigma_t$ and $\pi r = 3\sigma_t$) together with an intermediate case ($\pi r = 2\sigma_t$).

If we substitute $\bar{t}(0, x)$ for t in the above reasoning and if we note that the superimposed distribution is essentially uniform when

$$\pi r \leqq \sigma_t \,, \tag{18}$$

then we obtain

$$N_T \leqq 2\delta\sigma\sqrt{\bar{t}(0, x)} \tag{19}$$

as a condition for the uniform distribution of allelic frequencies among localities.

Next, we consider a two-dimensional habitat extending over a sphere of radius r. Let us assume that the individuals are distributed uniformly with density δ. Let σ^2 be the mean square distance of individual migration in one generation, and

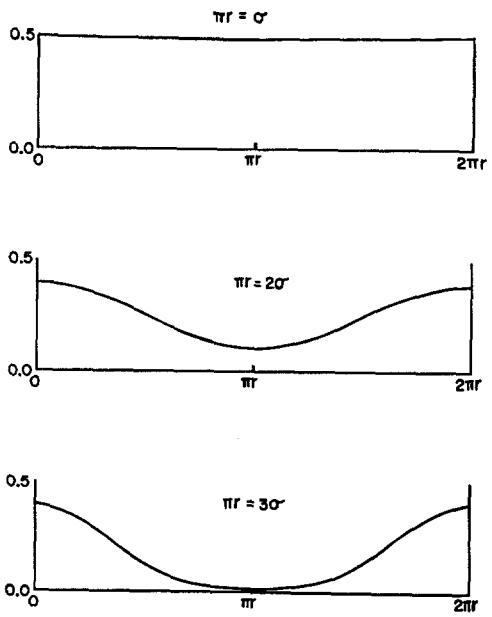

FIGURE 5.—Diagrams illustrating the frequency distribution produced by wrapping one-dimensional normal distribution around a circle of radius r for three cases $\pi r = \sigma$, 2σ and 3σ.

assume that the migration is isotropic. Wrapping the sphere by the two-dimensional normal distribution for the distance of migration in t generations and considering the resulting probability distribution on the sphere similar to the one considered above, the condition (18) leads to

$$\pi N_T \leqq 4\delta\sigma^2 t \tag{20}$$

for the two-dimensional case, since $N_T = 4\pi r^2\delta$ in this case.

For a mutant allele whose average frequency in the whole population happens to be 0.1, the condition for uniform distribution reduces roughly to

$$\sigma^2\delta \geqq 1 \tag{21}$$

if we put $t = \bar{t}(0, 0.1) \approx N_e$ in (20).

If the total population is subdivided into colonies (subpopulations) in each of which the mating is at random, and migration in one generation is restricted to neighboring colonies (two-dimensional stepping stone model, cf. KIMURA and WEISS 1964), condition (21) becomes

$$mN \geqq 1, \tag{22}$$

where m is the rate at which each colony exchanges individuals with four surrounding colonies each generation and N is the effective size of each colony. However, since the age $t(0, x)$ at $x = 0.1$ has a relatively large standard deviation, it may be safer to use the first arrival time $\bar{t}_x$ (0) at $x = 0.1$ for t in (20) to derive the required condition. This leads approximately to

$$mN \geqq 5, \tag{23}$$

These results agree essentially with those obtained by KIMURA and MARUYAMA (1971) based on an entirely different method. For a neutral mutant having $x = 0.05$, the corresponding formula becomes approximately

$$mN \geqq 10. \tag{24}$$

This means that uniform distribution of frequencies among colonies can be attained if exchange of at least 2.5 individuals occurs on the average between two neighboring colonies per generation irrespective of the size of each colony. Thus we conclude that when the average frequency of a "rare" polymorphic allele is a few percent in the whole population, its frequencies among different localities are expected to be essentially uniform if the allele is selectively neutral and if there is migration of a few individuals on the average between adjacent colonies each generation. It is possible that in many Drosophila species, with their enormous population size and with their high individual mobility in addition to the possibility of long range migration by airborne individuals, virtual panmixia are usually attained even if subpopulations are very far apart.

In addition, the associative overdominance at a neutral locus caused by linked selected loci (cf. OHTA and KIMURA 1970, 1971; OHTA 1971) will contribute at least partly to keep the distribution of the neutral alleles uniform among localities. The reason for this is that the associative overdominance creates a sort of inertia so that whenever a local frequency is temporarily disturbed it tends to go

back to the original frequency, although there are no definite equilibrium frequencies for the neutral allele to settle on the long term basis (OHTA 1973).

PRAKASH, LEWONTIN and HUBBY (1969), in their studies on the pattern of genetic variation among subpopulations of *Drosophila pseudoobscura*, rejected the model of neutral isoalleles on the ground that frequencies of rarer alleles at several loci (such as malic dehydrogenase locus) are essentially identical among widely separated subpopulations, and that the isoallelic hypothesis cannot explain such identical allelic configurations. We hope that the above treatment has made it clear that their observations are not incompatible with our neutral mutation-random drift hypothesis of molecular polymorphisms.

Also we would like to point out that if the observed uniformity of the frequencies of rare alleles among localities is due to weak "balancing selection" rather than migration, the effective size of the local population (not the whole species) has to be probably at least the order of a million, not mentioning the fact that the selection coefficients have to be equal among localities. This is because, as first discovered by ROBERTSON (1962) for overdominant alleles, the balancing selection actually accelerates fixation rather than retards it if the equilibrium frequencies lie outside the range 0.2–0.8 unless $N_e\ (s_1 + s_2)$ is very large, where s_1 and s_2 are the selection coefficients against the two homozygotes. In fact, if the equilibrium frequency is 5%, $N_e\ (s_1 + s_2)$ has to be about 2100 in order to retard fixation by a factor of 100 as compared with the completely neutral case (see also CROW and KIMURA 1970, p. 414).

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APPENDIX

A general formula for the average number of generations until a mutant allele first reaches a frequency x starting from a lower frequency p (the mean "first arrival time")

Let $M_{\delta x}$ and $V_{\delta x}$ be, respectively, the mean and the variance of the change in one generation of the frequency of a mutant allele having frequency x. We assume that the stochastic process of change in gene frequency is time homogenous—in other words, the selection coefficient of the mutant remains constant with time even if it may be frequency-dependent.

Then it can be shown using the diffusion equation method that the average number of generations until the mutant reaches frequency x for the first time starting a lower frequency p is given by

$$\bar{t}_x(p) = \int_p^x \psi_x(\xi) u_x(\xi)\{1 - u_x(\xi)\} d\xi + \frac{1-u_x(p)}{u_x(p)} \int_0^p \psi_x(\xi) u_x^2(\xi) d\xi, \qquad \text{(A1)}$$

where

$$\psi_x(\xi) = \frac{2\int_0^x G(\lambda) d\lambda}{V_{\delta\xi} G(\xi)}, \quad u_x(p) = \frac{\int_0^p G(\lambda) d\lambda}{\int_0^x G(\lambda) d\lambda}$$

and

$$G(\xi) = \exp\left\{ -\int_0^{\xi} \frac{2M_{\delta x}}{V_{\delta x}}\, dx \right\} .$$

When $x = 1$, $\bar{t}_x(p)$ reduces to $\bar{t}_1(p)$ (the average time until fixation) given by KIMURA and OHTA (1969a). Also, in the special case of no selection (neutral allele), $M_{\delta x} = 0$ and $V_{\delta x} = x(1-x)/(2N_e)$, so that (A1) reduces to

$$\bar{t}_x(p) = 4N_e \left\{ \frac{1-x}{x} \log_e(1-x) - \frac{1-p}{p} \log_e(1-p) \right\} , \qquad \text{(A2)}$$

where N_e is the "variance" effective size of the population.

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