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Extension of the Haldane–Muller principle of mutation load with application for estimating a possible range of relative evolution rate*

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SUMMARY

The Haldane–Muller principle of mutation load is generalized so as to be applicable to both cases of strong and very weak selection with any time variation. It is proved that in an infinite asexual haploid population, the *average* Malthusian parameter $\overline{m}$ of a population, the evolution rate v, and the total mutation rate μ satisfy the relation $\partial\overline{m}/\partial\mu = v/\mu - 1$, so long as each Malthusian parameter is independent of μ. A similar result is also true in a diploid population under genic selection. It is discussed how the above relation gives a restriction on the possible range of values of relative evolution rate v/μ.

1. INTRODUCTION

Haldane (1937) considered the effect of deleterious mutations on a population in equilibrium with respect to gene frequencies. He showed that the loss of fitness to a population depends almost entirely on the mutation rate and not at all on the effect of the gene upon the fitness of an individual carrying it, provided the effect is large enough to keep the gene rare. Muller (1950) introduced the word *load* in assessing the impact of mutation on the human population, and used Haldane's result in order to circumvent the fact that the effects of individual mutant genes were mainly of unknown magnitude.

Haldane (1957) also considered the deleterious effect of gene substitutions in evolution on the average fitness of a population. He showed that the total cost over the whole period in which the substitution takes place is determined almost entirely by the initial frequency of the mutant and not at all by the selective advantage of the mutant. On the basis of this result he estimated that in horotelic (standard rate) evolution, a new allele may be substituted in a population roughly

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every 300 generations, because a higher substitution rate would result in a cost per generation too large for a population to bear.

Using data of protein evolution, Kimura (1968) estimated that in the history of mammals, nucleotide substitution of a genome was so fast that, on average, one nucleotide pair would have been substituted in the population of a species roughly every 2 years. Comparing his estimate of molecular evolution rate with Haldane's estimate of horotelic evolution rate, he concluded that most mutations produced by nucleotide replacement were almost neutral with respect to natural selection, otherwise the cost of substitution would become intolerable.

In a review of studies undertaken to extend the concept of loads and costs, Crow (1970) examined the range of applicability of the Haldane–Muller principle that the mutation load is almost determined by the mutation rate and rather independent of the selection intensity. In such studies of mutation load it was customary to treat the *equilibrium state* of the *constant fitness* model. When the deleterious effect of a mutation is large enough, such treatments can be meaningful, because in that case the population will soon approach an equilibrium state with respect to gene frequencies, and the effect of changes of environment which causes variation in fitness may be of secondary importance.

However, in the study of molecular evolution it was disclosed that an enormous amount of variability is usually maintained at the molecular level in a population of a species (Lewontin, 1974), and that the selection intensities are often very weak (Mukai, Tachida & Ichinose, 1980). Then, the usual premise of the Haldane–Muller principle that the mutant gene is rare does not necessarily hold. As Crow (1970) pointed out, here a major question arises whether cost and load arguments can still be used to set upper limits on such things as the number of selectively maintained polymorphisms, the mutation rate that can be tolerated, or the rate of gene substitution by natural selection.

During the time since Kimura's proposal of the neutral theory of molecular evolution, his cost argument has been severely criticized by several workers (Maynard Smith, 1968; Felsenstein, 1971; Nei, 1971; Ewens, 1972). The concept of cost is still controversial and has become unpopular among some population geneticists. Even Kimura (1983) does not seem very insistent on the importance of his original cost argument by which he was led to the neutral theory in 1968. However, as for the concept of mutation load, we have noticed that it can be used to set some limits on the possible values of molecular evolution rate if we first extend the Haldane–Muller principle in such a way that it encompasses both strong and very weak selection cases.

In this paper, we show that, at least in an infinite asexual haploid population, even allowing for non-equilibrium state and variable selection of any intensity, the simplicity and generality of the Haldane–Muller principle can be retained by a suitable modification. Namely, we show that the differential increase of the average Malthusian parameter $\bar{m}$ of a population per differental increase of the total mutation rate μ of each gene or gamete is given at any time t as

$$\partial \bar{m}(t)/\partial \mu = v(t)/\mu - 1, \tag{1}$$

so long as the Malthusian parameter of each gene or gamete is independent of μ.

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$v(t)$ is the evolution rate at time t measured by the time rate of increase of *average step numbers* (see below). We give the exact specification of the model and the definition of $v(t)$ relevant to formula (1), proof and extensions of the model, and discuss some biological implications of our results.

2. MODEL OF REPLICON SYSTEM

In population genetics theory it is customary to talk of alleles, chromosomes or gametes as the basic elements constituting a population. From a theoretical point of view, however, there is often no reason to confine the elements to some specific biological objects. They can be anything to which we can associate the notions of genetic state, birth and death and mutation. Since we seek generality, we prefer to use the general term *replicon* for the elements and to clarify the necessary and sufficient properties we must assume on them to obtain a conclusion. For concrete biological application we can easily interpret our somewhat abstract theoretical results with due reservations. In molecular genetics, a replicon is defined as any genetic element that behaves as an autonomous unit of DNA replication. In this paper we use the term replicon extending and specifying it in the following way.

We consider a set of elements called replicons with the following properties. Each replicon starts to exist at the time of its birth and ceases to exist at the time of its death. Each replicon is in a definite genetic state $\sigma \in S$, where S is a set of all possible genetic states. At the initial time $t = 0$ we assume that some replicons called *Adams* are born. Any replicon which is born at a later time ($t > 0$) is not an Adam, and it has one definite replicon as its *parent*. The former replicon is a *child* of the latter. Each replicon may give birth to its child or children while and only while it exists.

Accordingly, any replicon R_m of the mth generation has a definite *phylogeny* (R_0, R_1, R_2, ..., R_m) such that R_0 is an Adam, and R_{j-1} is the parent of R_j ($j = 1, 2, \ldots, m$). Each replicon is either a *mutant* or *non-mutant* according to whether its genetic state is different from or the same as that of its parent. By definition an Adam is non-mutant. We call the number of mutants among the replicons in the phylogeny (R_0, R_1, ..., R_m) of R_m the *step number* of R_m. Let the step number of R_m be n. Let $\sigma^{(0)}$ be the genetic state of an Adam R_0 and let $\sigma^{(j)}$ be the genetic state of replicons with step number j in the phylogeny ($R_0, R_1, \ldots, R_m$) of R_m ($j = 1, 2, \ldots, n$). By definition all replicons with the same step number in the phylogeny of R_m have the same genetic state and $\sigma^{(j)} \neq \sigma^{(j-1)}$ ($j = 1, 2, \ldots, n$). We define the phylogenic state of R_m as $\phi \equiv (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(j)}, \ldots, \sigma^{(n)}) \in S^{n+1} \subset \Phi$, where Φ is the set of all possible phylogenic states of replicons. Thus, each replicon has a certain definite phylogenic state. For any given phylogenic state ϕ, its step number n_ϕ is uniquely determined, that is $n_\phi = n$ for $\phi \in S^{n+1}$.

We call the set of replicons which exist at a given time $t \geqq 0$ a *population*. The step number $\bar{n}(t)$ of a population, or the average step number at time t, is defined by the arithmetic mean of the step numbers of replicons in the population at time t. We define the evolution rate $v(t)$ by

$$v(t) \equiv d\bar{n}(t)/dt. \tag{2}$$

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Along a phylogenic line leading from Adam to each replicon, we say a *substitution* has occurred when the genetic state of a child is different from that of its parent. We define the *substitution rate of a replicon* as the number of substitutions per unit time along the phylogenic line leading to this replicon. Note that the substitution rate of a replicon is generally different from the mutation rate. The mutation rate of a replicon is the probability of giving birth to a child with a different genetic state per unit time. According to our definition (of step numbers and (2)) the evolution rate $v(t)$ is the substitution rate averaged over replicons of the population at time t.

If we knew all the ancestral sequences of a certain population of existing proteins as a function of time, from some previous time 0 up to the present time t, we would be able, according to our definition, to measure the average evolution rate $\bar{n}(t)/t$ of the proteins directly.† At present, this is not feasible. However, the quantity $\bar{n}(t)/t$ can be inferred by comparison of homologous proteins of various organisms and using our knowledge of their divergence times. This $\bar{n}(t)/t$ is the rate of molecular evolution, of which close estimates are attempted by allowing for such effects as back mutations and multiple hits.

Let us introduce a dynamical law in our model. Let $N_\phi(t)$ be the number of replicons of phylogenic state $\phi = (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n)})$ in the population at time t. $N_\phi(t)$ changes with time by the process of birth and death. Let $m_\phi(t)$ be the probability for a replicon of phylogenic state ϕ to give birth to a child, minus the probability of death per unit time. When $m_\phi(t)$ is a function of only $\sigma^{(n)}$ and t, but is independent of ancestral states $\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n-1)}$, it is called the Malthusian parameter of genetic state $\sigma^{(n)}$ at time t.

Let $\mu f_{\phi,\phi'}$ be the probability per unit time for a replicon of phylogenic state $\phi' \equiv (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n-1)})$ to give birth to a mutant child in genetic state $\sigma^{(n)}$ ($\neq \sigma^{(n-1)}$), that is, a mutant child of phylogenic state $\phi = (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n-1)}, \sigma^{(n)})$. Here μ is a positive constant called the total mutation rate and $f_{\phi,\phi'}$ ($\geqq 0$) satisfies

$$\sum_{\sigma^{(n)} \in S - \sigma^{(n-1)}} f_{\phi,\phi'} = 1.\ddagger \tag{3}$$

When $\mu f_{\phi,\phi'}$ is a function of only $\sigma^{(n)}$ and $\sigma^{(n-1)}$, but is independent of ancestral states $\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n-2)}$, it is called the mutation rate of genetic state $\sigma^{(n-1)}$ to $\sigma^{(n)}$.

† For a finite population model of bounded size, where the waiting time until fixation of any replicon's descendants is bounded, the average evolution rate $v_\infty \equiv \lim \bar{n}(t)/t$ ($t \to \infty$) defined through step numbers coincides with the evolution rate defined as the rate at which an *evolutionary event* occurs in a population (Kimura, 1968). Here, the evolutionary event is the occurrence of a mutant whose descendants ultimately replace those of non-mutants (wild types), that is, fix in a population. Without including the size effect there is no fixation, but v_∞ has a well-defined meaning.

‡ Here, we have assumed that the total mutation rate, which is the product of the birth rate of a child and the mutation probability that a child is a mutant, is a constant independent of the phylogenic state ϕ. Since the mutation probability can be reasonably assumed a constant, this assumption is valid at least when the selection acts only through mortality differentials but not through fecundity differentials. Even when the selection acts also through fecundity differentials, the assumption may still hold if the differences are small enough so that the effect of second order terms can be safely neglected.

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We assume that the above process of birth and death occurs to each replicon independently of each other, and that the number of replicons in a population is so large that the time development of $\{N_\phi(t)\}$ can be represented by a dynamical system. Then, we may write:

$$dN_\phi(t)/dt = (m_\phi(t)-\mu)\,N_\phi(t)+\mu f_{\phi,\phi'}\,N_{\phi'}(t),$$
$$\phi = (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n)}), \quad \phi' = (\sigma^{(0)}, \sigma^{(1)}, \ldots, \sigma^{(n-1)}) \in \Phi \quad (n = 0, 1, 2, \ldots) \tag{4}$$

and
$$N_{\phi'}(t) \equiv 0 \quad \text{for} \quad n = 0, \tag{5}$$

with the initial condition

$$N_\phi(0) = \begin{Bmatrix} N_\phi > 0 & (n = 0) \\ 0 & (n \neq 0) \end{Bmatrix}. \tag{6}$$

By definition the parent of a mutant replicon of phylogenic state ϕ should uniquely be of phylogenic state ϕ' in (4).

Let $N(t)$ be the total number of replicons in the population at time t. Then the frequency $x_\phi(t)$ of replicons of phylogenic state ϕ is defined by

$$x_\phi(t) \equiv N_\phi(t)/N(t). \tag{7}$$

We are concerned with how the average Malthusian parameter

$$\overline{m}(t) \equiv \sum_{\phi \in \Phi} m_\phi(t)\, x_\phi(t) \tag{8}$$

of the population is affected by the total mutation rate μ.

Haldane and Muller noted that mutation affects $\{x_\phi(t))\}$, through which $\overline{m}(t)$ should be affected. In nature, the value of the Malthusian parameter must be under the influence of environments which is affected by the genetic structure of a population given by $\{x_\phi(t)\}$. Therefore, in general $m_\phi(t)$ will depend on μ. However, in an asexual haploid population such μ-dependence will be of secondary importance, and we shall prove in the next section formula (1) under a major assumption that $m_\phi(t)$ is independent of μ. Then, we shall give the extension to the case where the Malthusian parameter $m_\phi(t)$ may partially depend on the mutation rate μ, and the extension to a diploid population under genic selection.

(i) *Proof and extensions of formula (1)*

First, let us show that the solution $N_\phi(t)$ of (4)–(6) has a very simple μ-dependence. Introducing a new variable $M_\phi(t)$ instead of $N_\phi(t)$ by

$$N_\phi(t) = M_\phi(t)\, \mu^n \phi\, e^{-\mu t}, \tag{9}$$

we see that (4)–(6) are rewritten in terms of $M_\phi(t)$'s as

$$dM_\phi(t)/dt = m_\phi(t)\, M_\phi(t)+f_{\phi,\phi'}M_{\phi'}(t) \quad (\phi \in \Phi), \tag{10}$$

$$M_{\phi'}(t) \equiv 0 \quad (n_\phi = 0), \tag{11}$$

$$M_\phi(0) = \begin{Bmatrix} N_\phi & (n_\phi = 0) \\ 0 & (n_\phi > 0) \end{Bmatrix}. \tag{12}$$

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(In the derivation of (10), we have used a trivial relation $n_\phi = n_{\phi'} + 1$ about step numbers.) Since the mutation rate μ does not appear explicitly in (10)–(12), it follows that the solution $M_\phi(t)$ of (10)–(12) does not depend on μ so long as the parameters $m_\phi(t)$ and $f_{\phi,\phi'}$ are independent of μ for all t and $\phi, \phi' \in \Phi$. Therefore, differentiating (9) with respect to μ, we have

$$\partial N_\phi(t)/\partial\mu = (n_\phi/\mu - t)\, N_\phi(t) \quad (\phi \in \Phi). \tag{13}$$

As for the μ-dependence of the total number $N(t)$ of replicons, we have

$$\partial \log N(t)/\partial\mu = \bar{n}(t)/\mu - t \tag{14}$$

by summing up (13) over all $\phi \in \Phi$. Then, differentiating (14) with respect to t, we obtain formula (1) only if we notice a relation

$$d \log N(t)/dt = \bar{m}(t) \tag{15}$$

and the definition (2) of the evolution rate $v(t)$. (The relation (15) expresses a trivial fact that the total number $N(t)$ increases with a rate $\bar{m}(t)$ of the average Malthusian parameter. It is derived by summing up (4) over all $\phi \in \Phi$.)

Next, let us extend formula (1) so as to cover the case when the Malthusian parameter $m_\phi(t)$ may depend on the mutation rate μ, but in such a way that it consists of two components as

$$m_\phi(t) = m_\phi^{(0)}(t) - C(\mu, t) \quad (\phi \in \Phi), \tag{16}$$

where $m_\phi^{(0)}(t)$ is the μ-independent part which may depend on ϕ, while $C(\mu, t)$ is the ϕ-independent part which may depend on μ. This is the case when $m_\phi(t)$ has a ϕ-independent μ-derivative $c(\mu, t)$, i.e.

$$\partial m_\phi(t)/\partial\mu = c(\mu, t) \equiv -\partial C(\mu, t)/\partial\mu \quad (\phi \in \Phi). \tag{17}$$

In such a case, (1) is modified as

$$\partial \bar{m}(t)/\partial\mu = v(t)/\mu - 1 + c(\mu, t). \tag{18}$$

In order to obtain (18), we again introduce a new variable $M_\phi(t)$ instead of $N_\phi(t)$ this time by

$$N_\phi(t) = M_\phi(t)\mu^{n_\phi}\phi \exp\{-\mu t - \int C(\mu, t)\, dt\}. \tag{19}$$

Then we find that $M_\phi(t)$ does not depend on μ since $M_\phi(t)$'s are shown to satisfy (10)–(12) with $m_\phi(t)$ replaced by $m_\phi^{(0)}(t)$. Thus, we can obtain (18) from (19) by similar arguments as in (13) and (14).

Finally, let us extend formula (1) to a diploid population whose time development is described by (4) with the Malthusian parameter

$$m_\phi(t) = \sum_{\psi \in \Phi} m_{\phi\psi}(t)\, x_\psi(t) \quad (\phi \in \Phi). \tag{20}$$

Here, $m_{\phi\psi}(t)$ is the Malthusian parameter of an individual with replicons in ϕ and ψ phylogenic states. Assuming genic selection, $m_{\phi\psi}(t)$ is expressed as

$$m_{\phi\psi}(t) = a_\phi(t) + a_\psi(t), \tag{21}$$

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in terms of the genic value $a_\phi(t)$. Then, (20) becomes

$$m_\phi(t) = a_\phi(t) + \sum_{\psi \in \Phi} a_\psi(t)\, x_\psi(t) = a_\phi(t) + \bar{m}(t)/2 \quad (\phi \in \Phi). \tag{22}$$

This is of the same form as in (16) if the genic value $a_\phi(t)$ does not depend on the mutation rate μ. Thus we find that (18) holds with $c(\mu, t)$ replaced by $(1/2)\,\partial\bar{m}(t)/\partial\mu$, i.e.

$$\partial\bar{m}(t)/\partial\mu = 2\{v(t)/\mu - 1\}. \tag{23}$$

If the genic value $a_\phi(t)$ may depend on the mutation rate ψ but has a ϕ-independent μ-derivative $c(\mu, t)$, i.e.

$$\partial a_\phi(t)/\partial\mu = c(\mu, t) \quad (\phi \in \Phi), \tag{24}$$

then formula (23) for the diploid population under genic selection is modified as

$$\partial\bar{m}(t)/\partial\mu = 2\{v(t)/\mu - 1 + c(\mu, t)\}. \tag{25}$$

3. DISCUSSION

The extended Haldane–Muller principle of mutation load (18) and (25) is summarized as

$$\partial\bar{m}(t)/\partial\mu = a\{v(t)/\mu - 1 + c(\mu, t)\}, \tag{26}$$

with $a = 1$ for a haploid population, and $a = 2$ for a diploid population under a genic selection. Here, $\bar{m}(t)$ is the average Malthusian parameter of a population, μ is the total mutation rate per unit time of each gene or gamete, and $v(t)$ is the evolution rate given by (2), while $c(\mu, t)$ is given by (17) or (24), and can be called the differential cost of preventing mutation.

In the derivation of (26), we have allowed for any environmental change and epistatic interaction affecting the Malthusian parameter. But the inter-replicon interaction which may be important in the non-genic selection case is ruled out by the basic assumption that the selection pressure on replicons is independent of the mutation rate. We have also neglected the size effect and the effect of recombination. Although we conjecture that the feature of our results will have a wider range of applicability beyond our restrictions in this paper, it is left for future studies.

As an application of (26), we note that an assumption of optimum mutation rate sets some limit on the possible range of values of relative evolution rate. If the total mutation rate μ of a genome is optimally determined in such a way that the long time average $\bar{m}$ of the average Malthusian parameter $\bar{m}(t)$ of a population should be maximum, we have from (26)

$$v/\mu = 1 - c, \tag{27}$$

where v and c are the long time averages of $v(t)$ and $c(\mu, t)$ of the entire genome respectively.

In the special case $c \equiv 0$, (27) gives $\mu = v$. This relation is essentially the same as that derived by Kimura (1967) based on an assumption that the mutation rate μ is so adjusted as to minimize the sum of mutational and substitutional loads.

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For real organisms we may assume that $c > 0$. In order to reduce the mutation probability that a child is a mutant, it is necessary for organisms to develop a replication system where the replication error is reduced. This necessarily requires more time and free energy for replication, causing a decrease of the Malthusian parameter. The value of c represents this decrease of Malthusian parameter per unit decrease of mutation rate. Therefore, under the hypothesis of optimal mutation rate (27) we must have

$$v/\mu < 1. \tag{28}$$

This property seems to be in accord with the actual data of molecular evolution. The evolution rate per base of DNA seems to be highest for pseudogenes among various genes or parts of DNA, and the evolution rate of a pseudogene is supposed to be close to its total mutation rate because of the lack of selection pressure on it (Miyata, 1982). Therefore, the relation (28) seems to be realized for the entire genome.

Kimura (1979) argued that the fact $v/\mu < 1$ is in favour of the neutral theory of molecular evolution, which assumes that molecular evolution occurs almost wholly by neutral mutation. Since the evolution rate due to neutral mutation is equal to the neutral mutation rate, which is smaller than the total mutation rate μ, the relation $v/\mu < 1$ immediately follows. However, he did not give any causal reason why, almost exclusively, neutral mutation contributes to the molecular evolution rate.

From a general point of view, the total mutation rate μ may include advantageous, deleterious and neutral mutations. Usually, the advantageous mutation rate may be very low as compared to other types of mutation rates, but once a mutant happens to be advantageous its fixation probability should be high. So, it is not obvious whether the contribution to the evolution rate from advantageous mutations is indeed negligible as compared with that from neutral ones.

Kimura showed, on the basis of the fixation probability of a mutant, that if μ represents the advantageous mutation rate, then for large population size we would have $v/\mu > 1$ instead of (28). He argued that in this case the larger the selective advantage the larger the value of v/μ would be, and that it is contrary to the observation at the molecular level. But he did not show why the spread of mutants which were not necessarily advantageous when they arose, but later somehow gained selective advantages, cannot contribute to molecular evolution, that is, why the traditional view of Neo-Darwinian evolution should be dismissed.

Neo-Darwinism by no means assumes that a mutant contributing to evolution should be advantageous from the outset when it arose; an environmental change may make a mutant advantageous which was deleterious when it arose (Fisher, 1958). Such a Neo-Darwinian view of molecular evolution was originally implied by Zuckerkandl & Palling (1965). Later, Van Valen (1974) introduced the term *Red Queen hypothesis* to represent the situation he supposed to be occurring at the molecular level. It is a special case of Neo-Darwinian evolution, where evolution proceeds by a weak positive selection pressure in a constantly changing environment. Ishii, Matsuda & Ogita (1982) introduced a mathematical model of evolution including the case which the Red Queen hypothesis presupposes.

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Although Kimura (1979) argued that the fact $v/\mu < 1$ is in favour of the neutral theory of molecular evolution, it should be stressed that the property (28) holds also for the molecular evolution rate v under a stochastic selection scheme as mentioned in the preceding paragraph. Indeed, under an assumption of optimum mutation rate, (28) holds quite generally for a haploid population under arbitrary selection scheme $\{m_\phi(t)\}$ with the μ-dependence (17) or a diploid population under arbitrary genic selection scheme $\{a_\phi(t)\}$ with the μ-dependence (24). Therefore, the fact that the property (28) is realized for the entire genome cannot alone discriminate between the neutral theory and a Neo-Darwinian theory of molecular evolution.

Since the property (28) is for the entire genome, it does not necessarily guarantee that the same type of inequality always holds as for each locus. It is a relation only about a weighted average of the relative evolution rates of each locus with the mutation rate of each locus as a weight factor. To know the locus dependence of the relative evolution rate v/μ, we must proceed to study how it explicitly depends on the mutation rate and the selection scheme relevant to the locus. A preliminary result of this kind of study was reported in Ishii *et al.* (1982). There it was further discussed that other characteristic features of actual molecular evolution rates can also be successfully explained by a Neo-Darwinian theory invoking a weak positive selection pressure in a constantly changing environment.

As for the assumption of optimum mutation rate, Kimura (1960, 1967) and Leigh (1970) suggested that in an asexual population inter-group selection would optimize the mutation rate, although Leigh (1973) argued that in a sexual population the only effective selection would be that tending to minimize the mutation rate. Here, we only mention a possibility that even in a sexual population, when the migration rate of replicons is sufficiently low, there is room for inter-group selection for mutation rate to operate because of isolation by distance.

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