THE MUTATIONAL LOAD WITH EPISTATIC GENE INTERACTIONS IN FITNESS[1]

MOTOO KIMURA AND TAKEO MARUYAMA

National Institute of Genetics, Mishima, Japan and University of Wisconsin, Madison

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THE mutational load is defined as the proportion by which the population fitness is decreased through the elimination of recurrent harmful mutations (CROW 1958). In a very large population, where the mutant genes are kept in low frequencies by the balance between mutation and selection, it represents the intensity of natural selection at the genotypic level.

The mutational load in a large population was first calculated by HALDANE (1937) without assuming an epistatic component in fitness. Later, a similar but more detailed calculation was carried out by KIMURA (1961). Also, the mutational load in a small population was studied by KIMURA, MARUYAMA and CROW (1963).

The purpose of the present paper is to investigate the effect of epistasis on the mutational load, using a model which assumes that the fitness is a function of the number of mutant genes in an individual. In particular, we will elaborate the case of quadratic interaction in fitness, namely, the deleterious effect of mutant genes to an individual is given by the quadratic expression of the number of mutant genes. This includes a case where the deleterious effect is proportional to the square of the number of mutant genes. Such a model may be realistic if the phenotypic suppression of mutational damage by developmental homeostasis breaks down rapidly as the number of mutant genes increases.

In what follows, we will assume a very large population and investigate first the case of free recombination among mutant genes. Then, in order to clarify the effect of restricted recombination, we will investigate a population of a hypothetical organism having only one pair of chromosomes within which no crossing over takes place. We will also study the mutational load under asexual reproduction. Finally, these results will be compared with other types of epistasis such as threshold character and "diminishing type" epistasis.

Throughout this paper, the senior author (M. K.) is responsible for the theoretical treatments, while the junior author (T. M.) is responsible for the numerical treatments based on a computer.

1. *Free recombination among mutant genes:* Let us consider a very large random mating population of diploid organism and assume that the fitness of an individual having i mutant genes is given by

$$w_i = 1 - h_1 i - h_2 i^2 , \qquad (1.1)$$

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where h_1 and h_2 are non-negative constants. The right side of the above expression becomes negative for a large i. So, we assume that

$$w_i = 0 \qquad \text{for} \qquad i \geqq n\,, \tag{1.2}$$

where n is the smallest number of mutant genes for which the right side of (1.1) becomes negative.

Let f_i be the frequency of individuals having i mutant genes before selection, then the average selection coefficient, h, against each mutant gene is

$$-h = \sum_i f_i(w_{i+1}-w_i)/\sum_i f_i w_i\,. \tag{1.3}$$

Under free recombination between mutant genes, i may be distributed with a Poisson distribution,

$$f_i = e^{-\lambda}\frac{\lambda^i}{i!} \tag{1.4}$$

where λ is the average number of mutant genes per individual before selection. With this distribution,

$$-\sum_i f_i(w_{i+1}-w_i) = (h_1+h_2)(1-\varepsilon_{n-1})+2h_2\lambda(1-\varepsilon_{n-2})+(\varepsilon_{n-1}-\varepsilon_{n-2})w_{n-1}$$

and

$$\sum_i f_i w_i = 1-\varepsilon_n-(h_1+h_2)\lambda(1-\varepsilon_{n-1})-h_2\lambda^2(1-\varepsilon_{n-2}), \tag{1.5}$$

where ε_n represents the tail of the Poisson distribution such that

$$\varepsilon_n = \sum_{i=n}^{\infty}\frac{\lambda^i}{i!}\,e^{-\lambda}\,.$$

As a function of n and λ, ε_n may be evaluated by using "Tables of the Incomplete Γ-Function" by KARL PEARSON (1922), since

$$\varepsilon_n = \gamma(n,\lambda)/\Gamma(n),$$

where $\gamma(n,\lambda)$ is the incomplete gamma function of the first kind, i.e.

$$\gamma(n,\lambda) = \int_0^{\lambda} e^{-t}t^{n-1}dt$$

and $\Gamma(n)$ is the ordinary gamma function. In the present study it turns out that ε's in (1.5) are generally very small and therefore may be neglected. For example, in the case of $M = 0.1$, $h_1 = 0$ and $h_2 = 0.0025$ (see Table 1), we have $n = 21$ and $\lambda \approx 5.76$, giving

$$\varepsilon_n \approx 9\times 10^{-7},\ \varepsilon_{n-1} \approx 3\times 10^{-6} \text{ and } \varepsilon_{n-2} \approx 10^{-5}.$$

This means that individuals having a large enough number of mutant genes, for which $1-h_1 i-h_2 i^2$ becomes negative, are so rare that they may be neglected in the calculation.

Therefore,

$$h = \frac{(h_1+h_2)+2h_2\lambda}{1-(h_1+h_2)\lambda-h_2\lambda^2} \tag{1.6}$$

with good approximation.

Consider a particular locus and let u be the mutation rate and p be the frequency of the mutant gene. Assuming that the gene frequency is very low, its rate of change per generation may be given by

$$\frac{dp}{dt} = u\text{-}hp\,, \tag{1.7}$$

where h is the average selection coefficient against the mutant gene. Multiplying both sides of (1.7) by 2 and summing up over all relevant loci, we have

$$\frac{d\lambda}{dt} = 2M - h\lambda \,, \tag{1.8}$$

where $M = \sum u$ is the number of new mutations produced per gamete per generation and $\lambda = \sum 2p$.

At equilibrium, where mutation and selection balance each other, we have $d\lambda/dt = 0$, or

$$2M = h\lambda \tag{1.9}$$

The above relations (1.7), (1.8) and (1.9) are approximations, but they are satisfactory as long as h is much larger than u. Note also that (1.9) follows from

$$\frac{dp}{dt} = u(1-p) - hp(1-p)$$

which is a more exact expression than (1.7).

Thus, substituting (1.6) in (1.9), we get the following equation for λ:

$$2h_2(M+1)\lambda^2 + (2M+1)(h_1+h_2)\lambda - 2M = 0, \tag{1.10}$$

from which we obtain, as the relevant root,

$$\lambda = \frac{-(h_1+h_2)(2M+1) + \sqrt{(2M+1)^2(h_1+h_2)^2 + 16h_2M(M+1)}}{4h_2(M+1)} \,. \tag{1.11}$$

With this λ, the mutational load may be calculated either from

$$L = (h_1+h_2)\lambda + h_2\lambda^2 \,, \tag{1.12}$$

or, equivalently, from

$$L = \frac{2M + (h_1+h_2)\lambda}{2(M+1)} \,. \tag{1.13}$$

In Table 1, values of λ and L which were computed using (1.11) and (1.13)

TABLE 1

Mutational load under a quadratic gene interaction in fitness with free recombination between mutant genes

Case	M	h_1	h_2	λ		L	
				From equation (1.11)	From numerical analysis	From equation (1.13)	From numerical analysis
1	0.1000	0.00000	0.0025	5.76	5.68	0.0975	0.100
2	0.1000	0.00238	0.00238	5.66	5.57	0.103	0.106
3	0.1000	0.00025	0.00249	5.75	5.66	0.0981	0.101
4	0.1000	0.01666	0.00167	4.97	4.85	0.132	0.145
5	0.1414	0.00000	0.0025	6.76	6.64	0.131	0.136
6	0.1414	0.00238	0.00238	6.68	6.54	0.138	0.143
7	0.1414	0.00025	0.00249	6.75	6.62	0.132	0.137
8	0.1414	0.01666	0.00166	6.08	5.91	0.173	0.177

The average number of mutant genes per individual before selection (λ) and the mutational load (L) are listed for eight different combinations of h_1, h_2 and M, where M is the mutation rate per gamete. In each case, values obtained from the theory are compared with those obtained by purely numerical treatments by a computer.

are listed for various combinations of M, h_1 and h_2. The table also contains, for comparison, the corresponding values of λ and L derived from purely numerical treatments with the help of a computer as detailed in Appendix I. Briefly, the set of zygotic frequencies, $\{f_i\}$, in one generation is transformed into that of the next under a given scheme of mutation, selection and recombination and this operation is repeated until an equilibrium is reached. Then λ and L are computed from the equilibrium distribution. The table shows good agreement between the results obtained from these two different methods. Also, it suggests an interesting fact that if h_1 is zero,

$$L \approx M, \tag{1.14}$$

namely, the mutational load is approximately equal to the mutation rate per gametes, rather than twice this value as expected from the HALDANE-MULLER principle (cf. CROW 1957). The relation (1.14) appears to hold as long as $|h_1|$ is smaller than h_2. Actually, if $\lambda \gg 1$ and $|h_1| \leqq h_2$, the first order terms are less important than the second order terms in (1.10) and (1.12), thus giving $L = M/(M+1)$, or roughly $L = M$, if M is small. On the other hand, if $h_2 = 0$ (no epistasis), we have $L = 2M/(1+2M)$ or roughly $L = 2M$ if M is small. Namely the mutational load is roughly equal to the total mutation rate per zygote. For example, if $M = 0.05$ and no epistatic interaction in fitness, $L = 0.09$. For a larger value of M, the more accurate formula $L = 2M/(1+2M)$ is preferable. For example, if $M = 0.1$, we have $L = 0.167$.

2. *One pair of chromosomes with no crossing over:* In the above treatment, completely free recombination was assumed between mutant genes. In actual situations, however, slight restriction of recombination may occur and even if its effect on the load is negligible, it might be worthwhile to investigate the effect assuming an extreme situation. So, in this section, we will consider a random mating population of a hypothetical organism having only one pair of chromosomes within which no crossing over takes place.

We will denote by g_i the frequency before selection of chromosomes having i mutant genes. Under random mating the frequencies of individuals having various number of mutant genes may be obtained by expanding $(\sum_0^\infty g_i)^2$. As before the selective value of individuals is given by (1.1). Thus the relative selective value, v_i, of chromosomes having i mutant genes is

$$v_i = \sum_{j=0}^{\infty} w_{i+j} g_j = 1 - h_1(i+\mu_1') - h_2(i^2 + 2i\mu_1' + \mu_2'), \tag{2.1}$$

where μ_1' and μ_2' are the first and the second moments of the distribution of the number of mutant genes in a chromosome, namely,

$$\mu_1' = \sum_i i g_i , \qquad \mu_2' = \sum_i i^2 g_i .$$

Let us consider the process by which the frequency distribution changes from one generation to the next: After selection the frequency of chromosomes having i mutant genes changes from g_i to $(g_i v_i)/\bar{v}$, where $\bar{v}$ is the mean selective value

$$\bar{v} = 1 - 2h_1\mu_1' - 2h_2(\mu_1'^2 + \mu_2'). \tag{2.2}$$

The selection is followed by mutation and we assume, as an approximation, that proportion M of the chromosomes having i mutant genes move to the class having

$i+1$ mutant genes. For a more exact treatment, we should assume that the number of new mutations follow Poisson distribution with mean M, but this makes the following treatment much more difficult. However, as will be shown later, the approximation is satisfactory as long as M is small. Thus the frequency of chromosomes in the next generation having i mutant genes is

$$g_0' = \frac{g_0 v_0}{\bar{v}}(1-M) \qquad \text{if } i=0$$

and (2.3)

$$g_i' = \frac{g_i v_i (1-M)}{\bar{v}} + \frac{g_{i-1} v_{i-1} M}{\bar{v}} \text{ if } i \geqq 1 .$$

At equilibrium where $g_i' = g_i$, we may drop the primes in the above set of equations. Let $\phi(\theta)$ be the moment generating function of the distribution defined by

$$\phi(\theta) = \sum_{i=0}^{\infty} \theta^i g_i \ . \tag{2.4}$$

Then, (2.3) at equilibrium gives the following second order differential equation for $\phi(\theta)$:

$$\phi''(\theta) + \frac{A}{\theta}\phi'(\theta) - \frac{B}{\theta(1-M+M\theta)}\phi(\theta) = 0, \tag{2.5}$$

where

$$A = (h_1/h_2)+1+2\mu_1' = S+1+2\mu_1'$$

and (2.6)

$$B = Mv_0/h_2 = Rv_0 ,$$

in which $S = h_1/h_2$, $R = M/h_2$and

$$v_0 = 1 - h_1\mu_1' - h_2\mu_2'. \tag{2.7}$$

If the solution, $\phi(\theta)$, of the above differential equation is obtained, we may use it to calculate

$$\mu_1' = \frac{\phi'(\theta)}{\phi(\theta)}\bigg|_{\theta=1} , \tag{2.8}$$

from which μ_1' may be obtained. For this purpose, we introduce an approximation and substitute (2.5) by

$$\phi''(\theta) + \frac{A}{\theta}\phi'(\theta) - \frac{B}{\theta}\phi(\theta) = 0, \tag{2.9}$$

namely, we omit the term $(1-M+M\theta)$ in (2.5). This should not cause any serious error as long as M is small and θ is very near to unity. We note here that the above approximation equation (2.9) has the same form as the exact equation in the continuous treatment given in Appendix (II.4). Thus, the pertinent solution is

$$\phi(\theta) \propto \sum_{i=0}^{\infty} \frac{B^i\theta^i}{i!\Gamma(A+i)} = (B\theta)^{-(A-1)/2} I_{A-1}(2\sqrt{B\theta}), \tag{2.10}$$

where $I(\cdot)$ stands for the modified Bessel function.
Thus,

$$\mu_1' = \frac{\phi'(\theta)}{\phi(\theta)}\bigg|_{\theta=1} = B^{1/2}\frac{I_A(2B^{1/2})}{I_{A-1}(2B^{1/2})}$$

or, writing $\alpha \equiv A-1 = S+2\mu_1'$ and dividing both side by $B^{1/2}$, we obtain

$$\frac{\alpha-S}{2B^{1/2}} = \frac{I_{\alpha+1}(2B^{1/2})}{I_\alpha(2B^{1/2})} \quad . \tag{2.11}$$

The average number of mutant genes per individual is

$$\lambda = 2\mu_1' \tag{2.12}$$

and this can be obtained by solving the above equation (2.11) for α through iteration by using tables of the modified Bessel functions (cf. SHIBAGAKI 1955). The iteration may be carried out as follows. First, take $v_0 = 1$ so that $B = R = M/h_2$ and solve (2.11) for α, from which we get the first approximation of $\lambda(=\alpha-S)$ and $\mu_1'(=\lambda/2)$. Use this μ_1' to calculate μ_2' from

$$\mu_2' = \frac{R-S(1+M)\mu_1'-2\mu_1'^2}{1+M} \tag{2.13}$$

which is derived from the first equation in (2.3), namely from $\bar{v} = v_0(1-M)$. With these values of μ_1' and μ_2', the values of v_0 and therefore $B = Rv_0$ may be obtained, enabling us to start the second cycle of calculation, from which we get the better approximations of λ and μ_1' by using equation (2.11). The process may be repeated until the desired accuracy is reached. Usually two cycles of iteration were sufficient for our purpose.

The mutational load is calculated from

$$L = 1 - \bar{w} = 2h_1\mu_1' + 2h_2(\mu_1' + \mu_2'). \tag{2.14}$$

In Table 2, values of λ and L thus obtained are listed for five different cases, together with corresponding values of λ and L obtained by the purely numerical treatment by a computer (see Appendix I). The table shows fairly good agreements between the values obtained by the two different methods. For all these five cases, the mutation rate per gamete (M) is 0.1. The load is roughly 0.13 for

TABLE 2

The mutational load under no crossing over in an organism having one pair of chromosomes

				λ		L	
Case	M	h_1	h_2	From theory, eq. (2.11)	From computer	From theory, eq. (2.14)	From computer
1	0.1	0.00000	0.0100	3.31	3.25	0.137	0.130
2	0.1	0.00000	0.0025	6.92	6.76	0.133	0.127
3	0.1	0.00238	0.00238	6.78	6.60	0.137	0.131
4	0.1	0.00025	0.00249	6.90	6.74	0.133	0.127
5	0.1	0.01666	0.00167	5.75	5.57	0.159	0.152

The average number of mutant genes per individual before selection (λ) and the load (L) are listed for five different combinations of values in h_1 and h_2, assuming mutation rate $M=0.1$. In each case, values obtained from the theory are compared with those obtained by the purely numerical treatment by a computer.

the first four cases in which $h_1 \leqq h_2$. In the fifth case, $h_1 \gg h_2$ and the load is larger, being roughly 0.15.

3. *Asexual reproduction:* In the previous section we have considered a random mating population of an organism having only one pair of chromosomes within which no crossing over takes place. In this case, recombination still occurs in the sense that different chromosomes recombine through fertilization. We now proceed to investigate a population of asexually reproducing oragnism, where no recombination takes place.

Let f_i be the frequency before selection of individuals having i mutant genes, whose fitness is w_i and let $2M$ be the average number of mutant genes produced per individuals per generation.

If the number of new mutations follows Poisson distribution with mean $2M$, then the frequency of individuals having i mutant genes in the next generation is

$$f_i' = \sum_{j=0}^{i} \frac{w_{i-j} f_{i-j}}{\bar{w}} \frac{(2M)^j}{j!} e^{-2M} \tag{3.1}$$

where

$$\bar{w} = \sum_{i=0}^{\infty} f_i w_i .$$

The above relation (3.1) is quite general and no restriction is given to the form of w_i's.

In particular, the frequency of individuals having no mutant genes is

$$f_0' = \frac{w_0 f_0}{\bar{w}} e^{-2M} . \tag{3.2}$$

Thus, at equilibrium in which $f_0' = f_0$, we have

$$\bar{w} = w_0 e^{-2M} . \tag{3.3}$$

So, if we assume that mutations are deleterious and the individuals with no mutant genes have the highest fitness, then the mutational load is

$$L = \frac{w_0 - \bar{w}}{w_0} = 1 - e^{-2M} \tag{3.4}$$

For example, if $M = 0.1$ we have $L = 0.181$.
For a smaller M, we have

$$L = 2M$$

approximately. Thus the load is roughly equal to the total mutation rate per individual. The above treatment shows that under asexual reproduction, epistasis has no effect in reducing the mutational load.

DISCUSSION

The model employed in the present paper assumes that the fitness is a function of the number of mutant genes contained in an individual. Such a model may be useful to investigate the following two situations. (1) Mutant genes in each locus are sufficiently rare so that only heterozygotes need to be considered. The

selective elimination is mainly through their deleterious effect in heterozygous condition. (2) Mutant genes are mildly deleterious in homozygous as well as heterozygous states and they are semidominant in fitness, namely, for each mutant gene, the heterozygous condition is only half as deleterious as the homozygous one. This seems to be nearly the case in "viability polygenes" studied by MUKAI (1965a).

The foregoing treatments have shown that under random mating the mutational load is nearly half the total mutation rate per individual if the deleterious effect of the mutant genes to an individual is roughly proportional to the square of its number, unless the mean number of mutant genes per individual is very small. This result may be particularly pertinent for assessing the load due to the viability polygenes, whose total mutation rate is estimated to be at least 70% per individual (cf. MUKAI 1964). If the ordinary HALDANE-MULLER principle (cf. CROW 1957) is applied to such genes, the load becomes at least about 0.5, which may be too high even for Drosophila.

The term mutational load has still been used by some to mean undesirable genes or gene complexes produced by mutation, but we use this strictly in the sense defined by CROW (1958). Namely, the proportional decrease of population fitness through the elimination of recurrent harmful mutations. The elimination may either be carried out by premature death or by sterility of the carriers and it is convenient to call such elimination the genetic death (MULLER 1950). Then, if one mutant gene is eliminated through one genetic death, the proportion of genetic deaths within an equilibrium population should be equal to the mutation rate per individual. In this case, the intensity of natural selection as expressed by the fraction of genetic deaths is equal to the mutation rate per individual but independent of the selection coefficient against individual mutant genes. Such a principle does not hold under epistatic interaction in fitness, but it does suggest that if two mutant genes are eliminated on the average through one genetic death, the mutational load becomes only half as large as the above. The present quadratic model appears to correspond to this latter situation. More generally, if m mutant genes are eliminated through one genetic death, the mutational load may become only $1/m$ as large. This might be expected if the deleterious effect

TABLE 3

The average number (λ) *of mutant genes per individual before selection and the mutational load* (L) *when the viability is a threshold character. Mutant genes act as lethal when* m *or more are present in one individual. The mutation rate per gamete is assumed to be 0.1*

m	λ	L
1	0.200	0.181
2	0.558	0.108
3	1.00	0.0803
4	1.49	0.0654
5	2.03	0.0556
10	5.07	0.0342

is proportional to the mth power of the mutant genes and if the average number of mutant genes per individual is fairly large.

This also might be the case, if viability is a threshold character such that the mutant genes have no deleterious effect when its number is less than m, but produce lethal effect when m or more are present in one individual. In this case, (1.3) gives

$$h = \frac{f_{m-1}}{1-\varepsilon_m} , \tag{4.1}$$

where

$$f_{m-1} = e^{-\lambda} \frac{\lambda^{m-1}}{(m-1)!}$$

and

$$\varepsilon_m = \sum_{i=m}^{\infty} \frac{\lambda^i}{i!} e^{-\lambda} .$$

The mean number of mutant genes (λ) may be obtained by solving (1.9), with h given by (4.1), that is, by solving

$$2M = \frac{\lambda f_{m-1}}{1-\varepsilon_m} . \tag{4.2}$$

With this value of λ, the mutation load is

$$L = \varepsilon_m . \tag{4.3}$$

Table 3 lists λ and L for several values of m when $M = 0.1$. The table shows that L becomes progressively small as m increases, even though the relation $L = 2M/m$ does not hold as might be expected. The above model is rather artificial in assuming that the character is solely determined genetically. Rather, it is likely that most threshold traits are strongly influenced by the environment. The following is a model suggested to us by DR. J. F. CROW: Suppose that, among individuals having no mutant genes, a character (x) is distributed normally with mean 0 and variance σ^2, while for those having i mutant genes, x is distributed normally with mean $-i\alpha\sigma$, ($\alpha>0$), and variance σ^2. The threshold is $c\sigma$ such that only those having x value larger than $c\sigma$ survive. Then the fraction of survivors among those having i mutant genes is

$$W_i = \frac{1}{\sqrt{2\pi}} \int_{c+i\alpha}^{\infty} e^{-x^2/2}\, dx .$$

Let w_i be the relative fitness so that $w_i = W_i/W_0$. If n loci are involved and if p is the frequency of a mutant gene in each locus, then the frequency of individuals having i mutant genes is

$$f_i = \binom{n}{i} (1-p)^{n-i} p^i, \qquad (i = 0, 1, 2, \ldots).$$

Thus,

$$\bar{w} = (1-p)^n + nw_1(1-p)^{n-1}p + \ldots ,$$

$$-h = \{(w_1-1)(1-p)^n + (w_2-w_1)n(1-p)^n p + \ldots\}/\bar{w}\,.$$

If p is small, the higher order terms in p are negligible and we can compute the equilibrium frequency p using the relation $u = hp$.

For example, assuming haploid organism with $n = 4$, $c = -1.5$, $\alpha = 0.05$, $u = 10^{-5}$, we get

$$p = 1.388 \times 10^{-3}$$

$$L = 3.98 \times 10^{-5},$$

showing that the load is very near to $4u$, as expected from the HALDANE-MULLER principle. Table 4 lists results of more exact calculations for several cases.

The good agreement of these examples with the HALDANE-MULLER principle is mainly due to the fact that the individuals having two or more mutant genes are so rare that there is almost no room for epistatic interaction. On the other hand, KING (1966) presented examples of threshold character in which there is a substantial departure from the HALDANE-MULLER principle. He assumed a very high threshold, large effect of a single gene, rather many loci, and an environmental component of variance of the same order of magnitude as the genetic component.

So far, we have considered a type of epistasis in which deleterious effect becomes disproportionately large as the number of mutant genes increases. Such an epistasis may be called the "reinforcing type." On the other hand, under some circumstances, deleterious effect per mutant gene might become progressively small as the number of mutant genes increases. Such an epistasis may be called the "diminishing type." The balance between mutation and selection in this type of epistasis will be somewhat delicate, because the selection against individual mutant genes becomes less intense as their frequencies increase. In

TABLE 4

Mutational load in a haploid organism for a threshold trait determined by four segregating loci. The mutation rate per locus is 10^{-5}

Threshold c	Gene effect α	Gene frequency p	Load	Fraction of survivors W_0
−1.5	0.05	1.39×10^{-3}	3.999×10^{-5}	0.9332
	0.1	6.66×10^{-4}	3.999×10^{-5}	
	0.2	3.11×10^{-4}	3.999×10^{-5}	
	0.3	1.93×10^{-4}	4.000×10^{-5}	
−2.0	0.05	3.486×10^{-3}	4.00×10^{-5}	0.9772
	0.10	1.65×10^{-3}	4.00×10^{-5}	
	0.20	7.45×10^{-4}	4.00×10^{-5}	
	0.30	4.45×10^{-4}	4.00×10^{-5}	
−3.0	0.05	4.08×10^{-2}	3.96×10^{-5}	0.9982
	0.10	1.89×10^{-2}	3.96×10^{-5}	
	0.20	8.17×10^{-3}	3.96×10^{-5}	
	0.30	4.63×10^{-3}	3.97×10^{-5}	

order to treat this case more quantitatively, let us suppose that the fitness of an individual having i mutant genes is given by

$$w_i = 1-s\left(\frac{i}{i+1}\right), \tag{4.4}$$

where $0 \leqq s \leqq 1$. Note that $w_1 = 1-s/2$ and $w_\infty = 1-s$. Under random mating and free recombination among mutant genes, we obtain, by using (1.3) and (1.4), the following expression for the mean selection coefficient.

$$h = \frac{s}{\lambda} \cdot \frac{1-(1+\lambda)e^{-\lambda}}{\lambda-s(\lambda-1+e^{-\lambda})} \tag{4.5}$$

With this h, the value of λ at equilibrium may be obtained from

$$\frac{d\lambda}{dt} = 2M-h\lambda = 0. \tag{4.6}$$

The equilibrium is stable, if

$$\frac{1}{d\lambda}\left(\frac{d\lambda}{dt}\right) < 0 \tag{4.7}$$

and unstable, if

$$\frac{1}{d\lambda}\left(\frac{d\lambda}{dt}\right) > 0\,. \tag{4.8}$$

Table 5 gives some numerical results obtained by assuming $M = 0.1$. For $s = 1.0$, the mean number of mutant genes per individual at equilibrium is 0.431 with load $L = 0.188$. The equilibrium is stable. For $s = 0.5$, there are two equilibrium values of λ, one ($\lambda = 1.66$) is stable and the other ($\lambda = 3.16$) is unstable. The mutational load corresponding to the stable equilibrium is $L = 0.256$ and this is definitely larger than the total mutation rate per individual, that is, $2M = 0.2$. For a slightly smaller value of s, that is for $s = 0.4871$, we have again two equilibrium values of λ; $\lambda = 2.00$ (stable) and $\lambda = 2.60$ (unstable). The load corresponding to the stable equilibrium is $L = 0.276$, which is still larger than in the case of $s = 0.5$. For values of s less than about 0.4845, no equilibrium exists in λ, because $d\lambda/dt = 2M-h\lambda > 0$ for all values of λ and λ tends to increase indefinitely. This means that for $s < 0.4845$ the selection can not check the spread of mutant genes.

These results suggest that the diminishing type epistasis among mutant genes

TABLE 5

Some numerical results for λ *(mean number of mutant genes per individual) and* L *(mutational load) in the case of "diminishing type" epistasis, in which the fitness is given by* $w_i = 1 - si/(i+1)$. *The mutation rate per gamete is assumed to be 0.1*

	λ		
s	Stable	Unstable	L
1.00	0.431	...	0.188
0.50	1.66	3.16	0.256
0.4871	2.00	2.60	0.276
<0.4845	...	...	....

tend to create much larger genetic loads (both expressed and hidden) than in the case of no epistasis and probably this type of epistasis is unfavourable for the evolution of the species. On the other hand, the reinforcing type epistasis tend to reduce the load and appear to be favourable for evolution. Also, this type of epistasis must be more common because of the physiological reason, namely the developmental homeostasis breaks down rapidly as mutant genes accumulate. Therefore, as far as the effect of deleterious mutant genes on fitness is concerned the reinforcing type epistasis will be found more often than the diminishing type in nature.

Recent studies of MATSUDAIRA (1963), SPASSKY *et al.* (1965), MUKAI (1965b) and KITAGAWA (1966) seem to support such a view. In the last study, up to seven lethal genes were accumulated experimentally in one individual to see their effect on fitness in heterozygous condition. It was found that their deleterious effects tend to reinforce each other as their number increases.

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SUMMARY

The effect of epistasis on the mutational load was studied using a model which assumes that fitness is a function of the number of mutant genes in an individual. It was shown that if the deleterious effect of the mutant genes is proportional to the square of their number, the load under random mating becomes roughly half as large as in the case of no epistasis, provided that the average number of such genes per individual is fairly large. Under asexual reproduction, however, the epistasis has no effect in reducing the load. The situation is intermediate for a random mating population of a hypothetical organism having only one pair of chromosomes within which no crossing over takes place.—The mutational load is also reduced under random mating if the fitness is a threshold character such that the mutant genes produce no deleterious effect when their number is less than m but act as lethals when m or more of them are present in one individual.—Epistatic interaction in fitness among deleterious mutant genes is classified into two types, namely, the reinforcing type and the diminishing type. In the former, the deleterious effect becomes disproportionately large as their number in an individual increases. On the other hand, in the latter, the deleterious effect per mutant gene becomes smaller as their number increases.—Reasons are presented to believe that the reinforcing type of epistasis among deleterious mutant genes must be more common than the diminishing type in nature.

APPENDIX I. NUMERICAL TREATMENT OF THE QUADRATIC GENE INTERACTION MODEL WITH A COMPUTER

The process of calculation consists of the following steps: (1) As a starting point, a population of individuals having no mutant genes is assumed so that $f_0 = 1.0$, $f_1 = f_2 = \ldots = 0$. When gametes are formed, segregation takes place before mutation, so that from this initial population, $g_0 = 1$, $g_1 = g_2 = \ldots = 0$. (2) When mutation occurs, mutant genes are produced with their frequencies given by the Poisson distribution with mean M, say with $M = 0.1$, such that from

the above population the frequencies of gametes carrying 0,1,2, ... mutant genes are $e^{-0.1}$, $(0.1)e^{-0.1}$, $(0.1)^2e^{-0.1}/2!$, ... respectively. More generally, if g_0, g_1, g_2, ... are repsectively the frequencies of gametes carrying 0,1,2, ... mutant genes before the production of new mutations, the gametic frequencies after mutation $(g_0', g_1', g_2', \ldots)$ are given by

$$\begin{bmatrix} g_0' \\ g_1' \\ g_2' \\ \cdot \\ \cdot \\ \cdot \\ \cdot \end{bmatrix} = e^{-M} \begin{bmatrix} 1 & 0 & 0 & \cdots \\ M & 1 & 0 & \cdots \\ \frac{M^2}{2!} & M & 1 & \cdots \\ \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \end{bmatrix} \begin{bmatrix} g_0 \\ g_1 \\ g_2 \\ \cdot \\ \cdot \\ \cdot \\ \cdot \end{bmatrix} \tag{I.1}$$

(3) Zygotes are formed assuming random mating, such that $f_0=g_0'^2$, $f_1=2g_0'g_1'$, etc. (4) Selection is practiced with fitness $w_i=1-h_1 i-h_2 i^2$ for a given set of values of h_1 and h_2, for example, $h_1=0.0$, and $h_2=0.0025$. This transforms $\{f_i\}$ into $\{f_i w_i/\bar{w}\}$. (5) Segregation takes place when the gametes are formed, but, the mode of segregation depends on whether crossing over occurs or not. In the case of free recombination between mutant genes, segregation follows binomial distribution in such a way that an individual having i mutant genes produces gametes with various number of mutant genes following the expansion of $(\frac{1}{2}+\frac{1}{2})^i$. In the case of no crossing over, however, homologous chromosomes which formed an individual at fertilization again segregate intact. (6) Mutation follows segregation as described in (2).

The cycle of "zygote formation-selection-segregation-mutation in gametes-zygote formation" is repeated many times until equilibrium is reached with respect to the frequency distribution $\{f_i\}$, and this was carried out using the computer, CDC 1604. Usually 120 cycles (generations) of iteration seemed to be sufficient for our purpose, but sometimes the computations were carried out as many as 500 cycles. When equilibrium is reached, the difference in the average number of mutant genes before and after selection must be equal to twice the number of new mutation per gametes, that is $2M$, and this was checked in all cases.

APPENDIX II. ONE PAIR OF CHROMOSOMES WITH NO CROSSING OVER. TIME CONTINUOUS TREATMENT.

Consider a random mating population of an organism having only one pair of chromosomes within which no crossing over takes place. We will denote by g_i the frequency of chromosomes having i mutant genes. Let $M\Delta t$ be the probability that one mutation occurs in a chromosome during the short time interval of length Δt, i.e. $(t, t+\Delta t)$, and let $w_{i+j}=1-[h_1(i+j)+h_2(i+j)^2]\Delta t$ be the fitness of an individual having $(i+j)$ mutant genes, the fitness being measured during the same time interval, $(t, t+\Delta t)$.

Assuming that the combination of homologous chromosomes occur at random, the relative fitness of chromosomes having i mutant genes is

$$v_i=1-[h_1(i+\mu_1')+h_2(i^2+2i\mu_1'+\mu_2')]\Delta t,$$

where μ_1' and μ_2' are respectively the first and second moment around 0 of the number of mutant genes in a chromosome. The amount of change in g_i during the time interval $(t, t+\Delta t)$ is

$$\Delta g_0 = \frac{g_0 v_0(1-M\Delta t)}{\bar{v}} - g_0 \qquad \text{for } i=0,$$

and

$$\Delta g_i = \frac{g_i v_i(1-M\Delta t)}{\bar{v}} + \frac{g_{i-1}v_{i-1}M\Delta t}{\bar{v}} - g_i \qquad \text{for } i\geqq 1, \tag{II.1}$$

where

$$\bar{v}=1-2[h_1\mu_1'+h_2(\mu_1'^2+\mu_2')]\Delta t.$$

At the limit of $\Delta t \to 0$, the above set of equations reduces to

$$\frac{dg_0}{dt} = g_0\{h_1\mu_1' + h_2(\mu_2' + 2\mu_1'^2) - M\}$$

and (II.2)

$$\frac{dg_i}{dt} = g_i\{h_1(\mu_1' - i) + h_2(\mu_2' + 2\mu_1'^2 - i^2 - 2i\mu_1') - M\} + Mg_{i-1} \ .$$

Let $\sum_{i=0}^{\infty} \theta^i g_i = \phi(\theta)$, $M/h_2 = R$ and $h_1/h_2 = S$, then the above set of equations yield

$$\frac{1}{h_2}\frac{\partial \phi(\theta)}{\partial t} = -\theta^2 \frac{\partial^2 \phi(\theta)}{\partial \theta^2} - (S+1+2\mu_1')\theta \frac{\partial \phi(\theta)}{\partial \theta} + R\theta\phi(\theta) \qquad \text{(II.3)}$$

When the equilibrium is reached with respect to the frequency distribution $\{g_i\}$, $\partial\phi/\partial t = 0$ and we have the following ordinary differential equation,

$$\theta\phi''(\theta) + A\phi'(\theta) - R\phi(\theta) = 0, \qquad \text{(II.4)}$$

where $A = S + 1 + 2\mu_1'$.

The pertinent solution of the above equation is

$$\phi(\theta) = C\sum_{i=0}^{\infty} \frac{R^i\theta^i}{i!\,\Gamma(A+i)} = C(R\theta)^{(1-A)/2} I_{A-1}(2\sqrt{R\theta}), \qquad \text{(II.5)}$$

where C is a constant such that $\phi(1) = 1$, and $I(\cdot)$ stands for the modified Bessel function. Thus the equation for μ_1' is

$$\mu_1' = \frac{\phi'(\theta)}{\phi(\theta)}\bigg|_{\theta=1} = R^{1/2}\frac{I_A(2R^{1/2})}{I_{A-1}(2R^{1/2})} \qquad \text{(II.6)}$$

or

$$\frac{A\text{-}S\text{-}1}{2R^{1/2}} = \frac{I_A(2R^{1/2})}{I_{A-1}(2R^{1/2})}, \qquad \text{(II.7)}$$

where $R = M/h_2$ and $S = h_1/h_2$. Equation (II.7) may be solved numerically for A by using tables of the modified Bessel functions and the average number of mutant genes per individual is then obtained from

$$\lambda = 2\mu_1' = A\text{-}S\text{-}1.$$

The mutational load per short time interval Δt is

$$L = \frac{1-\bar{w}}{\Delta t} = 2[h_1\mu_1' + h_2(\mu_1'^2 + \mu_2')] \ ,$$

which, at equilibrium, reduces to

$$L = 2M - (\frac{h_2}{2})\lambda^2 \ .$$

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