THE GENE INTERACTION COMPONENT OF THE GENETIC LOAD[1]

JACK LESTER KING

Donner Laboratory and Lawrence Radiation Laboratory, University of California, Berkeley

Received September 10, 1965

IT has usually been considered that the principal components of the genetic load are the mutation and segregation loads, plus some presumably smaller components such as the load due to selection for an intermediate optimum of a quantitative character (CROW and KIMURA 1964). I would propose that the interaction of deleterious genes constitutes another distinct component, because of the unique distinguishing characteristics of this source of variation in fitness. The magnitude of the interaction component relative to other components is as yet unknown. It is the purpose of this article to propose further that the interaction component may be a major part of the genetic load, and to explore the implications of such a hypothesis.

Deleterious alleles, with the exceptions of completely dominant and completely recessive lethals, are variable in expression with regard to fitness. They contribute to the reproductive extinction of some carriers (or potential carriers) and not of others. In many, perhaps most, cases this variability of expression probably has both genetic and nongenetic components. When two or more deleterious alleles interact to affect the fitness of a carrier more severely than the sum of their individual average effects, the extra deleterious effect is an expression of a formerly concealed portion of the genetic load.

The deleterious alleles which interact are present in the population because of mutation pressure, and have thus been considered to be a part of the mutation load (KIMURA 1961; CROW and KIMURA 1964). However, the dynamics of their survival in the gene pool and of their expression differs from that of mutations with independent effects. For one thing, the relationship between mutation and "genetic death" is different for genes in interacting systems.

Mutation and the "genetic death" rate: HALDANE (1937) and MULLER (1950) concluded that the reduction in fitness of a population due to mutation was independent of the severity of expression of individual mutations and equal to twice the sum of all mutations with any degree of dominance, plus the sum of all mutations with wholly recessive deleterious effects. MULLER expressed this relationship in terms of "genetic deaths": one individual, on the average, is eliminated for every dominant mutation and for every two fully recessive mutations. Most subsequent estimations of total mutation damage have been based on this relationship. If the interaction component is a major part of the genetic load,

[1] This investigation was supported in part by a postdoctoral fellowship from the National Institute of Arthritis and Metabolic Diseases and in part by the U.S. Atomic Energy Commission.

however, the relationship does not hold. MULLER was aware of this and suggested (1950) that more experimental work on the extent of synergism was needed.

When there is synergistic interaction among deleterious genes, the expected number of genetic deaths per mutation is divided by the average number of the mutant alleles, in excess of the population mean, carried by those individuals selected against. Mutations with slight measurable effects are likely to be expressed and lost only in conjunction with other deleterious alleles, and thus contribute relatively little to the genetic load.

In a genetic system with $2N$ (diploid) loci, $2N\bar{q}$ is the mean number of deleterious mutant alleles carried per adult; $2N\bar{f}$ is the mean number of such alleles carried by individuals eliminated by selection; $2N\overline{\mu(1-q)}$ is the total number of mutations in the system per individual per generation; D is the rate of the selective loss of individuals (MULLER's genetic death rate; HALDANE's mean loss of fitness; the expressed genetic load) attributable to the system. Then at genetic equilibrium

$$2N\bar{q} = \frac{2N\bar{q} - 2ND\bar{f} + 2N\overline{\mu(1-q)}}{1-D} .$$

The expressed genetic load is

$$D = \frac{2N\overline{\mu(1-q)}}{2N\bar{f} - 2N\bar{q}} = \frac{\overline{\mu(1-q)}}{\bar{f} - \bar{q}} .$$

For deleterious effects without interaction, only one locus is involved; $2N = 2$, and $2N\bar{f} = 2$ (for full recessives) or $2N\bar{f} \cong 1$ (for rare dominants). Thus the above formulae are consistent with the HALDANE relationship in the absence of synergistic interaction. D and f_i are partially dependent on q_i, but for a given value of D the equilibrium frequency at the ith locus is

$$\hat{q}_i = \frac{Df_i - \mu_i}{D - \mu_i} , \text{ or } \hat{q}_i = 1 - \frac{D(f_i - q_i)}{\mu_i} .$$

Threshold systems: It is well known that some systems involve the interactions of many genes. Threshold systems are characterized by the stability of the phenotype (or one aspect of it) over a broad range of genetic and environmental variability, with a discontinuity when the genetic and envornmental factors pass beyound a limit of allowable variation. Although almost all empirical studies of threshold systems have dealt with visible morphological characters, other components of fitness must behave genetically in the same way.

Actually, any component of fitness in which gene interaction occurs can be considered as a threshold character. Death or sterility are the transthreshold phenotypes. In cases of reduced fecundity or menternal ability, it can be considered that the threshold applies to the offspring or potential offspring, the transthreshold group being those which fail to survive or which fail to be conceived. The individuals eliminated by selection are the real or potential offspring, of which only a portion may carry the harmful genotypes.

A genetic threshold system was analyzed by WRIGHT (1934b). Following

WRIGHT one may conveniently consider threshold systems in terms of a normally distributed continuous parameter which has nongenetic and additive genetic components of variance. Each of the array of alleles available at each locus contributes additively to the parameter. Those which contribute more than the average value for the locus can be considered to be "plus" alleles; "minus" alleles either contribute nothing, or less than the average value for the locus in the population. Individuals for which the total value of the parameter falls below a minimum threshold are eliminated by selection.

If the threshold is at z standard deviations below the mean of the distribution, the genetic death rate D is equal to the relative area of the unit normal distribution below $-z\sigma$, or $D = A_{(z)}$.

It is important to emphasize that the parameter is an intellectual construction which describes the developmental and population genetic behaviour of interacting systems, and does not necessarily describe any real thing or substance in the developing organism. The normality of the parameter and the additivity of the contributing genes are convenient first order approximations, pending better understanding of developmental mechanisms.

KIMURA (1961) and CROW (CROW and KIMURA 1964) have discounted the likelihood of important amounts of synergistic interaction in natural populations, particularly with respect to the effect of interaction on the relationship between mutation and the expressed genetic load. Their considerations have been restricted to systems with two loci. Before interaction systems can be effective, enough loci with high enough deleterious allele frequencies must be involved that the conjunction of two or more deleterious alleles is not an exceedingly rare event. Tables 2 and 3 in the appendix demonstrate that such conditions are possible, at least in theory.

Synergism in threshold systems: A minus allele of a threshold system should be expected to have a fitness value of less than one. The mean of the continuous parameter in the population of carriers is shifted toward the threshold, which is tantamount to shifting the threshold toward the mean. If e_1 is the effect of the known allele in standard deviations, the rate of transthreshold eliminations among known carriers is equal to the area of the unit normal distribution below $-(z-e_1)$. The fitness value derived by empirical observation would be:

$$1 - s_1 = \frac{1 - A_{(z-e_1)}}{1 - A_{(z)}}.$$

A second allele from the same system, tested separately, would have an empirical fitness value of $1 - s_2$. The two alleles together, under the hypothesis of independent action, would be expected to exhibit a fitness equal to the product of their individual fitnesses: $(1-s_1)(1-s_2)$. Instead, although their direct contributions to the continuous parameter are additive and independent, they act synergistically with respect to fitness because of the shape of the curve (FRASER 1965) to produce a combined fitness value of:

$$1 - s_{1+2} = \frac{1 - A_{(z-e_1-e_2)}}{1 - A_{(z)}}. \qquad \text{(See Figure 1.)}$$

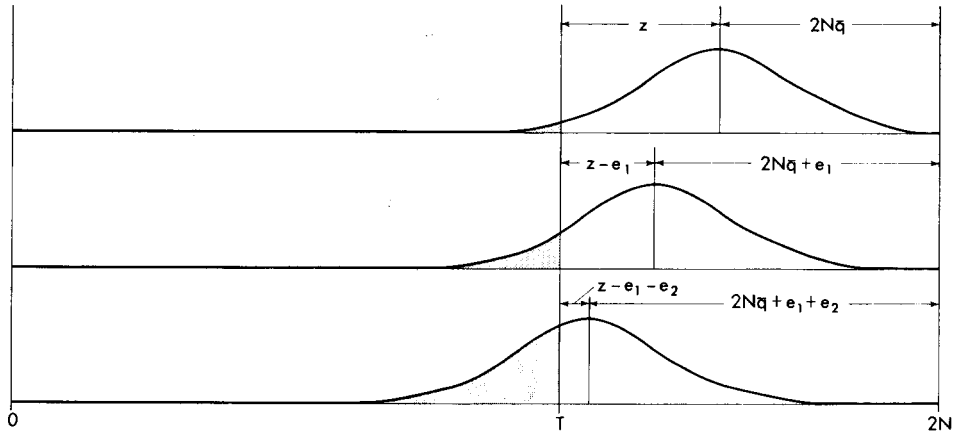

FIGURE 1.—Synergism in a threshold system. Top: Distribution and death rate in an outbred population. Center: Distribution and death rate in the class of individuals carrying an identified deleterious allele, which displaces the mean e_1 standard deviations. Bottom: Distribution and death rate in the class of individuals carrying two identified deleterious alleles, which displace the mean e_1 and e_2 standard deviations respectively. The fundamental action of the two deleterious alleles is additive with respect to the parameter, but the effect on the genetic death rate is synergistic.

Additivity is the simplifying assumption, but individual loci might be dominant or recessive with respect to their contribution to the parameter. Synergism as described above can occur between two homozygous recessive loci. On the other hand, deleterious alleles which are strictly additive in their contributions to the parameter would show a degree of recessivity with regard to fitness, because of synergism between homologues.

Two-threshold systems: In single-threshold systems, the gene equilibrium at each locus is near fixation for the plus allele, at a point where the loss of minus alleles through threshold selection is balanced by mutation pressure. In canalized systems (WADDINGTON 1957; RENDEL 1959), there is an upper as well as a lower threshold, so that selective losses occur for high as well as for low values of the parameter. High-threshold selection removes from the population individuals with fewer minus alleles than the population average, thereby tending to increase the frequency of minus alleles in the surviving population. High-threshold selection is thus added to mutation pressure to increase the equilibrium value of q_i. If mutation from minus to plus is negligible, the mathematical relationship between the mutation and genetic death rates is as previously formulated; however, individuals eliminated at the upper threshold, having on the average fewer than $2N\bar{q}$ minus alleles, make negative contributions to $2Nf$–$2N\bar{q}$. In effect, however, a portion of the genetic load is not due directly to mutation but is a balanced load attributable to the opposing actions of high and low thresholds. The effect does depend on genetic variability and hence directly on mutation (CROW and KIMURA 1964).

Individual loci in a canalized system may stabilize near fixation for either the

plus or the minus allele, so that neither minus nor plus can be considered to be abnormal or mutant in the system. If the system is fairly symmetrical, mutation from minus to plus must be considered. Such a canalized system has two subsets of loci in the population, those near fixation for plus and those near fixation for minus. Selection at the high threshold primarily affects the mostly-minus subset, and balances the mutation pressure of minus-to-plus in that group. Low-threshold selection balances the plus-to-minus mutation pressure of the mostly-plus subset. The subsets could conceivably overlap, with the third group near fixation for alleles with intermediate values and subject to mutation pressure in both directions. In many respects a canalized system near fixation at all loci would behave as two single-threshold systems. Any shift in the mean of the parameter, however, would simultaneously intensify selection at one threshold and relax it at the other.

When q_i has an intermediate value in a two-threshold system, the heterozygote has a selective advantage over either homozygote. This is because heterozygosis is less likely than homozygosis to occur among the transthreshold losses. If mutation pressure, outbreeding, or other factors are able to sustain appreciable polymorphism in canalized systems, this type of heterosis would be quite significant. Polymorphism in canalized systems might be responsible for the observations which led LERNER (1954) to propose the developmental necessity of obligate levels of heterozygosity, and for other indications of the widespread occurrence of heterosis in natural populations.

It has been argued (e.g., CROW 1964) that the number of heterotic loci must be small in any population, since the loss of fitness necessary to keep very many loci polymorphic would be far too great. In canalized polygenic systems, however, the loss of fitness is slight relative to the sum of the selective disadvantages of homozygosis, since a cluster of homozygous alleles is lost with each "genetic death." Furthermore, the heterozygote advantage in such systems is not the cause of such polymorphism that does exist, but rather the result of it.

It might be thought that a heterozygote advantage would automatically assure polymorphism, but this is not the case. Single-locus heterosis tends to favor the increase of the rarer allele and thus to achieve a stable polymorphism, because selection against either allele is directly proportional to the frequency of the homozygote. In polygenic threshold systems, however, the proportion of homozygotes eliminated at each threshold remains stable over the very range of frequencies at which there is a heterozygote advantage. Selection which removes a fixed proportion of plus and minus homozygotes has exactly the same effect as selection *against* heterozygotes: the gene frequency is driven away from an unstable equilibrium point toward fixation at either extreme. Thus two-threshold systems, in the absence of mutation or other disturbing effects, tend toward fixation at every locus (ROBERTSON 1956) at some value which may be either plus or minus relative to former mean values of the locus. However, this tendency is extremely slow even under those pure conditions (FRASER 1962), although random fixation is likely to occur in small populations; with linkage, quasi-equilibria at intermediate frequencies may be maintained for many hundreds of generations. (LEWONTIN 1964).

Allele frequency equilibria: If the mutation rate from minus to plus is ν_i, the change in frequency of the minus allele per generation at the ith locus is Δq_i:

$$q_i + \Delta q_i = \frac{q_i - Df_i + \mu_i(1-q_i) - q_i\nu_i}{1-D};$$

$$\Delta q_i = \frac{q_i(D - \mu_i - \nu_i) + \mu_i - Df_i}{1-D}.$$

At equilibrium $\Delta q_i = 0$ and

$$\hat{q}_i = \frac{Df_i - \mu_i}{D_i - \mu_i - \nu_i}.$$

The values of f_i, q_i, and D are interdependent and dependent on other gene frequencies, but stable equilibria exist somewhere near $q_i = 0$ and $q_i = 1$; there is also an intermediate, unstable equilibrium point. In the absence of mutation, $\hat{q}_i = f_i$, which occurs when both are either one or zero.

Threshold systems do allow for the extended survival of genetic variability arising from mutation pressure and fluctuating environment. Changes in the distribution of the parameter are met with compensating genetic responses which can include the rapid increase in a formerly rare allele. The genetic variability of threshold systems exists in an extremely flexible, responsive state of dynamic disequilibrium, since all gene frequencies in each system are interdependent. If some of the constituent genes are pleiotropic, acting in several threshold systems, the systems are interdependent also.

In a highly relevant study MAGALHÃES *et al.* (1965) introduced recessive lethals in high frequencies into caged *Drosophila willistoni* populations. Sampling at later generations, they found that the "lethal" alleles had persisted in the populations and had acquired linked suppressors, having thus become less deleterious. The populations had made a compensatory genetic response to the presence of the "lethal" alleles.

In the terminology of the present discussion, introduction of the lethals by MAGALHÃES *et al.* shifted the population mean of the continuous parameter toward one threshold; natural selection rapidly shifted it back through the selective elimination not only of the introduced allele but also of other alleles with similar fundamental activities. The result was a selective increase of the alleles acting in an opposing direction. These MAGALHÃES *et al.* quite properly term "suppressors."

This genetic responsiveness, or genetic homeostasis (LERNER 1954), in allowing utilization of concealed variability in prompt responses to changes in the environment or gene pool, could also allow a recurrent mutation with favorable and unfavorable pleiotropic effects to become incorporated into the gene pool even though its net effect on fitness had been negative.

An important corollary is that an upset in the balance of threshold systems by the sudden introduction of a load of induced mutations can be substantially rectified within a very few generations, although the mutations themselves may persist indefinitely. This is extremely pertinent to the empirical estimation of total mutation damage due to radiation and other mutagens.

Inbreeding depression and threshold systems: The concealed genetic loads due

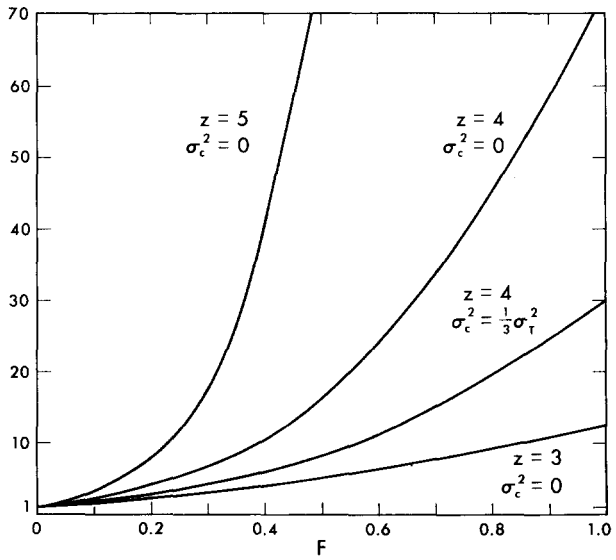

FIGURE 2.—Relative increases in genetic death rate with increases in degree of inbreeding (F). The genetic death rate of each of four hypothetical examples is taken as one with outbreeding. The genetic death rates are nonlinear with F. See Table 1.

to mutation pressure and balanced heterosis are in the form of deleterious recessives, which may be revealed by inbreeding. Although the concealed genetic load of threshold systems does not necessarily involve deleterious recessives, it too can be revealed by inbreeding (NEWCOMBE 1964). Much of the depression of vigor and viability associated with inbreeding is probably due to threshold effects (WRIGHT 1934a; SHELDON *et al.* 1964).

Inbreeding in a previously outbred population increases the genetic component of variance, and therefore the total proportion of tranthreshold values for both high and low thresholds (Figure 2; CROW 1964; NEWCOMBE 1964) is also increased.

The variance of the continuous parameter σ^2_T has two principal components, genetic and nongenetic: σ^2_g and σ^2_e respectively. A degree of inbreeding F increases the additive genetic component of variance by $1 + F$ (ROBERTSON 1952), and raises the total variance of the parameter from σ^2_T to $(\sigma^2_T + F\sigma^2_g)$. The threshold remains at $-z\sigma_T$. Since the standard deviation of the parameter is increased, however, the equivalent threshold on the unit normal distribution is at $-z'$, where

$$-z' = z\left(\frac{\sigma^2_T}{\sigma^2_T + F\sigma^2_g}\right)^{1/2}.$$

If the nongenetic component of variance is negligible, or if it remains proportional to the genetic variance, then

$$z' = z\,(1 + F)^{-1/2}$$

TABLE 1

The effect of inbreeding (F) on the frequencies of lethal or deviant phenotypes due to threshold systems. All frequencies are $\times 10^{-5}$

Threshold distance z	Nongenetic variance* σ_e^2	Degree of inbreeding					
		$F=0$	$F=0.125$	$F=0.25$	$F=0.50$	$F=0.75$	$F=1.00$
$3\sigma_T$	0	135	233	368	734	1170	1700
$4\sigma_T$	0	3.17	9.15	17.9	54.7	123	232
$4\sigma_T$	$\frac{1}{3}\ \sigma_T^2$	3.17	5.91	10.78	26.0	54.7	96.8
$4\sigma_T$	$\frac{1}{2}\ \sigma_T^2$	3.17	5.22	9.15	17.9	31.4	54.7
$5\sigma_T$	0	0.0287	0.118	0.41	2.25	7.84	20.8

* The nongenetic component of variance in each of the first, second, and fifth examples is either negligible or remains proportional to the genetic variance. In the third and fourth examples the nongenetic components of variance are constants equal to one-third and one-half the total variance of the outbred population respectively.

The proportion of lethal or deviant phenotypes due to threshold systems increases rapidly and nonlinearly with F (Table 1; Figure 3). If there is recessivity at any of the loci in a system, the increases and the nonlinearity are even more marked (CROW 1964). Empirical studies have revealed nonlinear relationships between inbreeding depression and F (DOBZHANSKY and SPASSKY 1963; LEVENE *et al.* 1965; MALOGOLOWKIN-COHEN *et al.* 1964).

Inbred lines and hybrid vigor: Continued inbreeding stabilized genotypes and greatly reduces the genetic variance *within* lines. Threshold systems may become stabilized near their optima, so that no further losses occur from them. Some systems, however, will become genetically fixed near enough to one of their

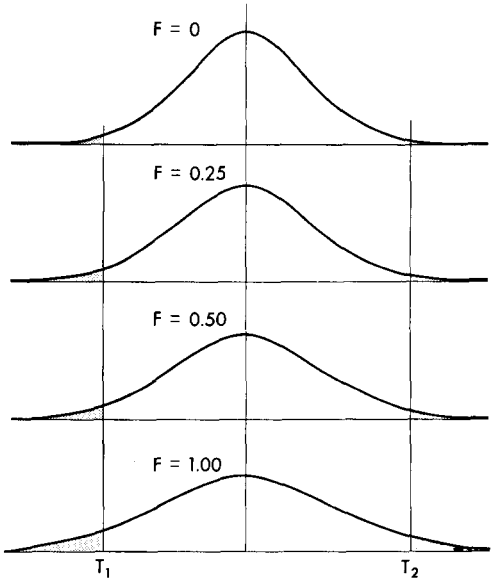

FIGURE 3.—Increased genetic variance causes an increase in the proportion of the distribution which falls above and below the two thresholds of a canalized system (not to scale).

threshold values that nongenetic variation continues to cause a substantial number of subthreshold individuals. Fitness is reduced and phenotypic variability is increased (WRIGHT 1934a), although the total distance between canalized thresholds is not altered (SHELDON *et al.* 1964). Hybrids between separately obtained lines show none of this reduction in fitness unless one or more of the same systems happen to have become fixed near their thresholds in both lines. Because more outbred small populations are subject to random fixation and wide fluctuations in the genetic variability of threshold systems, crosses between different populations are also apt to exhibit hybrid vigor.

I have been privileged to have corresponded with JAMES F. CROW, EVERETT DEMPSTER, THEODOSIUS DOBZHANSKY, F. CLARK FRASER, HOWARD B. NEWCOMBE and SEWALL WRIGHT, receiving suggestions and helpful criticism from each, for which I am thankful.

SUMMARY

The interaction between deleterious genes constitutes a distinct component of the genetic load, with some unique characteristics. The relative magnitude of the interaction component is not known, but it could be of major importance. Deleterious alleles are introduced into the gene pool by mutation and expressed, sometimes in clusters, through chance segregation. Interaction systems can be considered in terms of hypothetical normal parameters, with additive genetic and nongenetic components of variance; the portion of the population represented by the area beyond a selection threshold die or fail to reproduce. This portion is equal to twice the total of the mutation rates for the system divided by the average number of deleterious genes, in excess of the population average, carried by individuals selected against. Canalized systems have thresholds at either extreme of the parameter; a portion of the genetic load attributable to canalized systems is not due directly to mutation, but to the opposing actions of the high and low thresholds. Over an intermediate range of gene frequencies, heterozygotes for loci in canalized systems have a selective advantage over homozygotes, but this does not lead to balanced polymorphism; in the absence of mutation, all loci would tend toward fixation. However, loci in canalized systems may be kept in fluctuating polymorphic disequilibria, or quasi-equilibria, through the interactions of threshold selection, mutation, linkage, pleiotrophy and environmental changes, because gene frequencies in interaction systems are responsively interdependent. This allows for a flexible response to environmental fluctuation and induced mutation.

Inbreeding increases the genetic variance, and thus the proportion of transthreshold individuals. The predicted inbreeding depression due to the interaction component rises rapidly and nonlinearly with the degree of inbreeding, F.

APPENDIX

Mathematical examples of single-threshold systems: If threshold systems, as described, do exist and are important contributors to the variation in fitness of natural populations, it should be possible to construct hypothetical mathematical examples with plausible values. One example is given in detail (Table 2) and others are described briefly (Table 3). Plausible values are

arbitrary; the mutation rates per locus are between 10^{-4} and 10^{-6}, the number of loci between 5 and 20, the nongenetic variance between one fifth and four fifths of the total variance. The distance between the population mean and the threshold is specified to be 4.5 standard deviations of the total variance. This may seem implausibly large, but it must be remembered that the total death rate for each system is of the order of the magnitude of individual mutation rates.

For purposes of simplified calculation it has been assumed that the mutation rates, allele frequencies and additive effects of all the genes in a given system are identical, and that the genetic effect is therefore distributed binomially with a variance of $2Nq(1-q)$ in units of genetic effect. The total distribution in the mathematical examples is a combination of binomial genetic and normal nongenetic distributions.

TABLE 2

A mathematical example of a single threshold genetic system

No. of minus alleles	Binomial frequency	Distance from threshold in σ_e	Normal probability of trans-threshold value	Total frequency of trans-threshold value	Frequency of minus alleles lost
0	.8179	6.80	.000 000	.000 000 0	.000 000
1	.1652	4.56	.000 004	.000 000 6	.000 001
2	.01586	2.31	.010 444	.000 165 6	.000 331
3	.0009610	0.07	.472 097	.000 453 6	.001 361
4	.0000412	—2.18	.985 371	.000 040 6	.000 162
5	.0000014	—4.43	1.000 000—	.000 001 4	.000 007
				.000 661 2	.001 862

$N=10$ loci.
$q=.01$.
$2Nq=0.2$.
Genetic variance $=\sigma_g^2=2Nq(1-q)=.198$.
$\sigma_g=.445$ units of effect.
Genetic and nongenetic components of variance equal.
$\sigma_e^2=\sigma_g^2=0.5\sigma_T^2$; $\sigma_T=\sigma_e\sqrt{2}$.
Threshold at $4.50_T=6.36\ \sigma_e$ from $2Nq$.
Effect of each minus allele: $2.247\ \sigma_e$.
The genetic death rate for the system is $D=6.61\times10^{-4}$. The number of minus alleles per individual selected against is $2N\bar{f}=1862/661=2.82$. $2N\bar{f}-2Nq=2.62$. The mutation rate per locus is $\mu=[(2N\bar{f}-2Nq)(D)]/[2N(1-q)]$. $\mu=8.75\times10^{-5}$.

TABLE 3

Mathematical examples of single threshold genetic systems. The threshold distance is $4.5\sigma_T$ in all examples

No. of loci N	Frequency of minus alleles per locus q	Frequency of minus alleles per individual $2N\bar{q}$	Ratio of genetic to nongenentic variance σ_g^2/σ_e^2	Excess No. of minus alleles per individual selected against $2N\bar{f}-2N\bar{q}$	Death rate of system $\times10^{-5}$ D	Mutations rate per locus $\times10^{-5}$ μ
10	.01	0.2	1.00	2.62	66.1	8.75
10	.01	0.2	0.25	2.44	7.80	0.961
10	.01	0.2	4.00	2.56	137.5	17.8
10	.05	1.0	1.00	4.59	8.58	2.07
20	.05	2.0	1.00	6.57	4.18	0.722
20	.05	2.0	0.25	5.88	0.80	0.124
20	.05	2.0	4.00	9.14	13.37	3.22
20	.10	4.0	4.00	8.86	5.19	1.28
5	.10	1.0	1.00	4.33	5.58	2.68

LITERATURE CITED

CROW, J. F., 1964 Population genetics studies. pp. 314–322. 2nd Intern. Conf. on Congenital Malformations. International Medical Congr. Ltd., New York.

CROW, J. F., and M. KIMURA, 1964 The theory of genetic loads. Proc. 11th Intern. Congr. Genet. **3**: 495–506.

DOBZHANSKY, TH., B. SPASSKY, and T. TIDWELL, 1963 Genetics of natural populations. XXXII. Inbreeding and the muational and balanced genetic loads in natural populations of *Drosophila pseudoobscura*. Genetics **48**: 361–373.

FRASER, A. S., 1962 Simulation of genetic systems. J. Theoret. Biol. **2**: 329–346.

FRASER, F. C., 1965 Some genetic aspects of teratology. Chap. 2. *Teratology*. Edited by J. G. WILSON and J. WARKANY. University of Chicago Press, Chicago, Illinois.

HALDANE, J. B. S., 1937 The effect of variation on fitness. Am. Naturalist **71**: 337–349.

KIMURA, M., 1961 Some calculations on the mutational load. Japan J. Genet. **36**: (suppl.): 179–190.

LERNER, I. M., 1954 *Genetic Homeostasis*. Wiley, New York.

LEVINE, H., I. M. LERNER, A. SOKOLOFF, F. K. Ho, and I. R. FRANKLIN, 1965 Genetic load in Tribolium. Proc. Natl. Acad. Sci. U.S. **53**: 1042–1050.

LEWONTIN, R. C., 1964 The role of linkage in natural selection. Proc. 11th Intern. Congr. Genet. **3**: 517–525.

MAGALHÃES, L. E., A. BRITO DA CUNHA, J. S. DE TOLEDO, S. A. TOLEDO F°, H. L. DE SOUZA, H. J. TARGA, V. SETZER and C. PAVAN, 1965 On lethals and their suppressors in experimental populations of *Drosophila willistoni*. Mutation Res. **2**: 45–54.

MALOGOLOWKIN-COHEN, CH., H. LEVENE, N. P. DOBZHANSKY, and A. S. SIMMONS, 1964 Inbreeding and the mutational and balanced loads in natural populations of *Drosophila willistoni*. Genetics **50**: 1299–1311.

NEWCOMBE, H. B., 1964 Panel discussion. Session on epidemiologic studies. pp. 345–349. 2nd Intern. Conf. on Congenital Malformations. International Medical Congr., Ltd., New York.

RENDEL, J. M. R., 1959 The canalization of the scute phenotype in *Drosophila melanogaster*. Evolution **13**: 425–439.

ROBERTSON, A., 1952 The effect of inbreeding on the variation due to recessive genes. Genetics **37**: 189–207. —— 1956 The effect of selection against extreme deviants. J. Genet. **54**: 236–248.

SHELDON, B. L., J. M. RENDEL, and D. E. FINLAY, 1964 The effect of homozygosity on developmental stability. Genetics **49**: 471–484.

WADDINGTON, C. H., 1957 *The Strategy of the Genes*. Allen and Unwin, London.

WRIGHT, S., 1934a On the genetics of subnormal development of the head (otocephaly) in the guinea pig. Genetics **19**: 471–505. —— 1934b The results of crosses between inbred strains of guinea pigs, differing in number of digits. Genetics **19**: 537–551.