

# Quantitative genetic variability maintained by mutation-stabilizing selection balance in finite populations

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## Summary

Models of variability in quantitative traits maintained by a balance between mutation and stabilizing selection are investigated. The effects of mutant alleles are assumed to be additive and to be randomly sampled from a stationary distribution. With a two-allele model the equilibrium genetic variance in an infinite population is independent of the distribution of mutant effects, and dependent only on the total number of mutants appearing per generation. In a finite population, however, both the shape and standard deviation of the distribution of mutant effects are important. The equilibrium variance is lower when most of the mutational variance is contributed by few genes of large effect. Genes of small effect can eventually contribute substantially to the variance with increasing population size ( $N$ ). The equilibrium variance can be higher in a finite than an infinite population since near-neutral alleles can drift to intermediate frequencies where selection is weakest. Linkage leads to a reduction in the maintained variance which is small unless linkage is very tight and selection is strong, but the reduction becomes greater with increasing  $N$  since more mutants segregate. A multi-allele model is simulated and it is concluded that the two-allele model gives a good approximation of its behaviour. It is argued that the total number of loci capable of influencing most quantitative traits is large, and that the distribution of mutant effects is highly leptokurtic with the effects of most mutants very small, and such mutants are important in contributing to the maintained variance since selection against them is slight. The weakness of the simple optimum model is discussed in relation to the likely consequences of pleiotropy.

## 1. Introduction

Many quantitative characters show considerable heritable variation in natural populations (Falconer, 1981; Mousseau & Roff, 1987; Roff & Mousseau, 1987). Explaining how such genotypic variation is maintained has been one of the most important and controversial problems of population genetics. The problem arises because of the widespread belief that stabilizing selection, in which the fittest individuals have values of the trait near some optimum, is ubiquitous in nature, but selection for an intermediate optimum is expected theoretically to deplete genetic variability (Robertson, 1956) and has been shown to do so experimentally (Gibson & Bradley, 1974; Kaufman, Enfield & Comstock, 1977). There is a certain irresistibility in arguments for the presence of an intermediate optima: for example, the date of egg laying in many northern passerine birds apparently has an optimum dependent on the availability of caterpillars for the young, which are only present for

a brief period in early summer (Lack, 1968). Some of the most compelling evidence for selection for intermediate optima in natural populations comes from comparisons between sibling species of *Drosophila*, where parallel latitudinal clines for various traits have been shown to exist (David & Bocquet, 1975; Hyytia *et al.* 1985). The observation of an intermediate optimum at any single trait considered alone is, however, not in itself evidence of stabilizing selection as Robertson (1973) and others (Falconer, 1981; Rose, 1982; Hill & Keightley, 1988) have emphasized, because negative correlations between characters under directional selection can also generate such optima.

Genotypic mutations are the basic source of all heritable variation, but can a balance between mutation and selection alone explain the maintenance of observed high levels of genetic variation? This is an important question because variation in quantitative traits is believed to be the 'raw material' of evolution. Such variation also provides the basis for responses to

artificial selection, and it is important in understanding the allelic effects and gene frequencies contributing to the variation being utilized.

The work of Clayton & Robertson (1955) suggested that mutation is a weak force in generating quantitative variation. Recent work on directional selection and mutation has, however, indicated that long-term selection responses may in part be due to mutations occurring after commencement of the experiment (Hill, 1982*b*). Lande (1976) focused on mutation-stabilizing selection balance and, by fitting experimentally estimated parameters to a specific model, concluded that high levels of genetic variation can be maintained in the presence of strong stabilizing selection. The assumptions of Lande's 'continuum of alleles' model were based on results of Kimura (1965), i.e. new mutants have effects that differ only slightly from those pre-existing, with the result that the distribution of allelic effects segregating at a locus is approximately normal. Although Lande included an analysis of the effect of linkage, the formulae obtained were essentially the same as those of Kimura (1965).

In a more recent review of the experimental data, Turelli (1984) questioned the appropriateness of the Kimura-Lande (KL) model since, with experimentally measured mutation rates, there are unlikely to be more than two alleles segregating at the loci affecting the trait. Also, the effects of new mutations are likely to be larger than the existing range of variation at the loci, with the consequence that the distribution of allelic effects segregating at the locus is non-normal, an *assumption* critical to Lande's model. With an assumption of lower per-locus mutation rates, Turelli (1984) obtained a formula for the equilibrium variance in the population which contrasts markedly with the KL result, i.e. the equilibrium genetic variance is independent of the effects of mutants on the trait, but depends only on the total number occurring per generation. This result of Turelli with a 'House of Cards' approximation (Kingman, 1978) was obtained earlier for a two-allele model by Latter (1960) and Bulmer (1972), and a similar answer has been subsequently obtained for a five-allele model by Slatkin (1987). Bürger (1986, see also Bürger, Wagner & Stettinger, 1988) has generalized the analysis of the KL continuum of alleles model, and shown that Turelli's 'House of Cards' result is a very good approximation for the KL model over a very wide range of parameters.

In this study we analyse the mutation-stabilizing selection problem for finite populations. Finite population size is likely to be important because, although population sizes in nature can be very large, they are seldom constant and an equilibrium model of the maintenance of genetic variation must consider past fluctuations in effective population number. Also, more importantly, the results of Robertson (1956) showed that the strength of stabilizing selection on an allele is proportional to the square of its effect on the

character. Previously there have been two major analyses of this problem, by Latter (1970) and by Bulmer (1972). Latter's analysis was a finite-population extension of Kimura's (1965) continuum of alleles model and assumed a normal distribution of allelic effects segregating at the loci affecting the trait. Bulmer's model assumed up to two alleles segregating and equal forward and backward mutation rates at each of the loci affecting the trait, with the effects of substitution the same at each locus. A formula was obtained for the equilibrium variance with the following parameters: effective population number, mutant effect on the trait, strength of stabilizing selection and mutation rate.

Here, we investigate how the shape of the distribution of the effects of new mutant alleles influences the variation maintained in the character. The effect of new mutations on quantitative traits varies because they can occur at different places within genes (e.g. flanking sequences, introns, intron-exon boundaries, 'silent' third positions, promoters, active sites, other coding regions), but also because they can occur at genes whose functions vary within the biochemical and developmental system.

Most of the analysis concentrates on a model of two alleles per locus. This is similar to Bulmer's (1972) analysis. Here, we assume that the population size and mutation rate are such that back mutation can be ignored, i.e. a new mutation is unlikely while an existing mutation is segregating, but such a mutation can occur later and its effect is dependent only on the distribution of new mutant alleles and the current values at the locus. The mutation model is therefore step-wise. The consequences of allowing for the possibility of the presence of more than two alleles are investigated using Monte Carlo simulation. The effects of linkage on the equilibrium genotypic distribution are investigated using Monte Carlo simulation and an 'infinite sites' model (Keightley & Hill, 1983). Finally, the results are discussed in relation to the types of mutational distribution likely to be found in nature and the weakness of the model due to its lack of consideration of pleiotropy.

## 2. Model

(a) *Basic assumptions.* The population is assumed to consist of  $N$  diploid individuals with constant population size, random mating and non-overlapping generations. Selection is sufficiently strong or the population size sufficiently small that no more than two alleles segregate at any time at each locus. The frequency of the higher valued allele is  $q$ , and the difference in value between the homozygotes is  $a$ , where  $a$  is a random variable sampled from a distribution of effects of mutant alleles. There is no dominance or epistasis.

(b) *Mutation.* The expected number of mutations appearing per haploid genome per generation is  $\lambda$  and

these occur independently. The increment in variance each generation from mutation is

$$V_M = \lambda E(a^2)/2 \tag{1}$$

(Hill, 1982*a*). Mutational effects are sampled from a time-invariant distribution. For modelling purposes the gamma distribution was chosen since it has a wide range of properties if suitable values are given to its two parameters. The density function of mutants having an increasing effect on the trait (illustrated in Fig. 1) is given by

$$f(a) = \alpha^\beta e^{-\alpha a} a^{\beta-1} / \Gamma(\beta) \quad (0 < a < \infty), \tag{2}$$

where  $\Gamma(\cdot)$  is the gamma function. The parameter  $\alpha$  defines the scale of the distribution and  $\beta$  its shape. In practice, the scale was defined by the parameter  $\epsilon = [E(a^2)/V_E]^{1/2} = [\beta(\beta+1)/\alpha^2]^{1/2}$ , where  $V_E$  is the environmental variance. With shape parameter  $\beta = 1$ ,  $f(a)$  is an exponential distribution; as  $\beta \rightarrow 0$  the distribution becomes increasingly leptokurtic with an increasingly large spike near  $a = 0$  and a long tail; with  $\beta \rightarrow \infty$ , the distribution approaches the limiting case of all effects equal. The distribution is discussed in more detail by Hill & Rasbash (1986) (also see Kimura 1983, ch. 8). Mutants were assumed to have equal probability of increasing or decreasing the trait, with  $f(-a)$  for  $a < 0$  equalling  $f(a)$  given by (2), i.e. a symmetric distribution over  $-\infty < a < \infty$ .

(c) *Selection.* The character is assumed to be under ‘nor-optimal’ stabilizing selection with the optimal phenotype fixed at zero. The phenotypic value of an individual is assumed to be the sum,  $X$ , of the contributions from each locus plus a random independent environmental effect of mean zero and variance  $V_E = 1$ . The relative fitness is given by

$$W(X) = \exp(-X/2w^2), \tag{3}$$

where  $w$  is a measure of the strength of stabilizing selection. Increasing  $w$  implies weaker stabilizing selection. With a multi-locus model where the population mean can vary due to gene frequency changes at any of the loci contributing to the character, Robertson (1956) showed that mutant alleles behave as under-dominant (i.e. the heterozygote is less fit than the homozygotes). The change of gene frequency at one locus under such stabilizing selection is given by

$$\Delta q = a^2(q - \frac{1}{2})q(1-q)/[4(w^2 + \sigma^2)], \tag{4}$$

where  $\sigma^2$  is the phenotypic variance (formally, the phenotypic variance less the genetic variance contributed by the locus). The term  $w^2 + \sigma^2$  is often called the strength of natural selection and referred to as  $V_s$ . This is equivalent to a model of heterozygote inferiority in fitness where  $s = a^2/[8(w^2 + \sigma^2)]$  is the fitness disadvantage of the heterozygote and there is a meta-stable equilibrium at  $q = \frac{1}{2}$ . Importantly, the strength of selection is proportional to the square of the allelic effect. Mutant genes are unconditionally

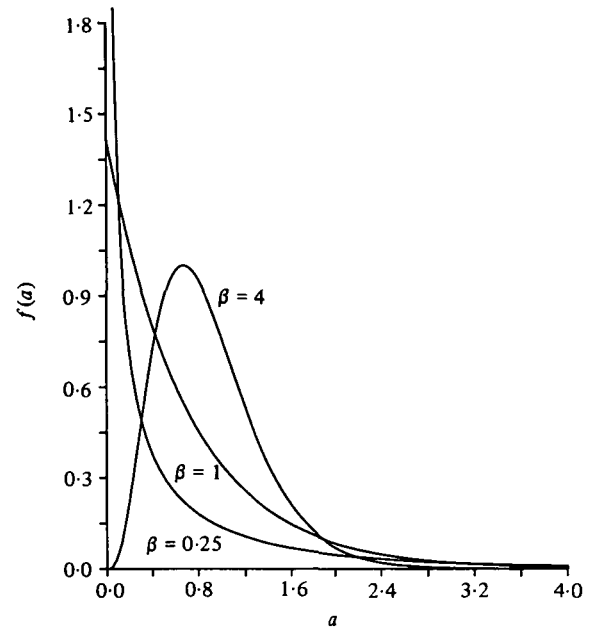


Fig. 1. Examples of the gamma distribution,  $f(a) = \alpha^\beta e^{-\alpha a} a^{\beta-1} / \Gamma(\beta)$  for three values of the shape parameter  $\beta$ . The parameter  $\alpha$  describes scale rather than shape and its value is such that  $E(a^2) = 1$  for each curve.

deleterious and their selection is similar to that of genic selection with  $\Delta q = s^*q(1-q)$ , where

$$s^* = -a^2/[8(w^2 + \sigma^2)]. \tag{5}$$

### 3. Methods

(a) *Transition matrix.* With Robertson’s (1956) result (equation 4) for the change of gene frequency it is possible to model the effects of continued stabilizing selection using a transition matrix. This method allows computation of the expected heterozygosity contributed by a new mutant during its lifetime in a population of  $N$  individuals assuming that no further mutation occurs at the locus while this mutant is segregating. The transition probabilities are defined by the square matrix  $\mathbf{M}$  for the Wright–Fisher stochastic process with values

$$m_{jk} = \binom{2N}{k} (q + \Delta q)^k (1 - q - \Delta q)^{2N-k} \quad (0 \leq j, k \leq 2N),$$

where  $q = j/2N$  and  $\Delta q$  is given by (4). Let  $\mathbf{f}^T(t)$  denote the row vector with elements  $f_j(t)$  which are the probabilities of a population having gene frequency  $j/2N$  ( $0 \leq j \leq 2N$ ) at the generation  $t$ . Thus for a new mutant,  $f_1(0) = 1$  and all other elements are zero. The vector  $\mathbf{f}^T(t)$  at generation  $t$  ( $t > 0$ ) is obtained from

$$\mathbf{f}^T(t) = \mathbf{f}^T(t-1)\mathbf{M}. \tag{6}$$

Let  $\mathbf{h}$  denote a column vector whose elements are the expected heterozygosity at a locus with gene frequency  $j/2N$  ( $0 \leq j \leq 2N$ ); so  $h_j = 2j/(2N)(1 - j/(2N))$ . The expected cumulative heterozygosity,  $H(a)$ , contributed

by a new mutant until it is fixed or lost, is  $H(a) = \sum_i f^T(i) \mathbf{h}$ . This can be computed as

$$H(a) = \mathbf{f}^{T*}(0)(\mathbf{I} - \mathbf{Q})^{-1} \mathbf{h}^*$$

where  $\mathbf{I}$  is the unit matrix,  $\mathbf{Q}$  is the square submatrix of  $\mathbf{M}$  of dimension  $2N - 1$  defined by  $q_{ij} = m_{ij}$  ( $1 \leq i, j \leq 2N - 1$ ), and  $\mathbf{f}^{T*}$  and  $\mathbf{h}^*$  are the corresponding elements of  $\mathbf{f}^T$  and  $\mathbf{h}$  (Kemeny & Snell, 1960).

In order to compute the variance maintained in the population, the expected heterozygosity contributed by mutants over a range of effects  $a$  was computed. The expected genetic variance maintained was then computed from

$$V_g = 2N\lambda \int_{-\infty}^{\infty} (a^2/4) H(a) f(a) da, \tag{7}$$

where  $f(a)$  is the distribution of mutant effects. Results were computed for a range of gamma distributions using different  $\beta$  (shape) and  $\epsilon$  (scale) parameters. Integration over  $f(a)$  was done numerically using Simpson's rule, and convergence of the results was checked by comparing two successive halvings of the  $a$  interval.

(b) *Use of diffusion approximation.* From diffusion theory, the pattern of change in gene frequency in populations of different sizes is approximately the same when the product  $Ns$  is constant. From equation (5)  $Ns \propto Na^2/(w^2 + \sigma^2)$ , so it is unnecessary to compute the variance maintained by new mutants other than for one value of  $N$  and selection strength and a range of  $a$  values. For the results shown, all transition matrix computations were performed with  $N = 80$  and a suitably wide range of  $a$  values. The validity of the approximation was checked for both lower and higher population sizes (i.e.  $N = 20$  and  $N = 160$ ).

Bulmer (1972), using the diffusion approximation (cf. Kimura, 1969), obtained a formula for the asymptotic variance in the character which in our notation is

$$V_g = \frac{na^2\theta M(\frac{1}{2}, \theta + \frac{3}{2}, \Phi)}{8(\theta + \frac{1}{2}) M(\frac{1}{2}, \theta + \frac{1}{2}, \Phi)}, \tag{8}$$

where  $n$  is the number of loci affecting the trait,  $N_e$  the effective population size and  $\theta = 4N_e\mu$  with  $\mu$  the forward and backward mutation rate between the two alleles,  $\Phi = N_e a^2/[8(w^2 + \sigma^2)]$ , and  $M(\cdot)$  is the confluent hypergeometric function (Abramowitz & Stegun, 1965, ch. 13). With the assumption that  $\theta \rightarrow 0$  (i.e. back-mutation is ignored), (8) reduces to

$$V_g = 2N_e V_m \left[ \sum_{i=0}^{\infty} \Phi^i / ((2i + 1) i!) \right] / e^\Phi. \tag{9}$$

This can be evaluated on a computer and converges readily. The results could have been computed by integrating (9) over a density function of  $a$  as in (7), in retrospect a computationally easier method than the transition matrix.

(c) *Monte Carlo simulation.* Using simulation, the effects of simultaneously segregating mutants and

linkage were assessed. The simulation attempts to model mutants affecting the quantitative trait occurring anywhere in the genome and incorporates an infinite number of independently mutable sites, with finite population size, selection and recombination, and is described elsewhere (Keightley & Hill, 1983). In the present study the fitnesses (essentially fertilities) of the  $N$  parents were assigned according to (3) and they were selected for breeding with probability  $w(X)/(N\bar{W})$ . The  $N$  progeny so produced were used as parents in the next generation. The mean additive and genic variances maintained and their standard errors were computed from at least six independent runs.

(d) *Approximate analysis.* Insight into the behaviour of a new mutant allele can be gained from extending Latter's (1960) two-allele treatment. The variance contributed by a mutant allele is given by  $V_g = a^2q(1 - q)/2$ . Assuming the mutant's effect is sufficiently large (strictly large  $Ns$ ) that the mutant is eliminated before reaching appreciable frequency, the variance contributed is approximated by  $V_g \approx a^2q/2$ . The expected variance after selection is therefore  $V'_g \approx a^2(q + \Delta q)/2$ , and substituting (4), this is approximated by

$$V'_g \approx a^2q[1 - a^2/(8(w^2 + \sigma^2))]/2.$$

Thus, the expected change in genetic variance from stabilizing selection is by a factor  $1 - a^2/[8(w^2 + \sigma^2)]$ . The expected proportional change in variance from drift is by a factor  $1 - 1/2N_e$ , where  $N_e$  is the effective population number. Considering mutants of equal effect occurring at rate  $\lambda$  per haploid genome per generation, the expected increment in the variance of the character from mutation is, from (1),  $\lambda a^2/2$ , so a recurrence relation describing the balance between selection, drift and mutation may be written down:

$$V_{g,t+1} = V_{g,t}[1 - a^2/(8(w^2 + \sigma^2))][1 - 1/2N_e] + \lambda a^2/2,$$

where  $V_{g,t}$  is the genic variance at generation  $t$  (i.e. additive variance in the absence of linkage disequilibrium). Ignoring second-order terms, this gives a solution at equilibrium ( $t \rightarrow \infty$ ) of

$$V_g = \lambda N_e a^2/[1 + N_e a^2/(4(w^2 + \sigma^2))]. \tag{10}$$

The same equation has been obtained independently by Bürger *et al.* (1988) using a different, heuristic, argument. In terms of the proportion of variance,  $2N_e V_M = N_e \lambda a^2$ , maintained with no selection, the variance is

$$V_g = 2N_e V_M/[1 + N_e a^2/(4(w^2 + \sigma^2))].$$

As  $N$  becomes infinite, (10) reduces to

$$V_g = 4\lambda(w^2 + \sigma^2), \tag{11}$$

the well-known result of Latter (1960), Bulmer (1972) and Turelli (1984). (An alternative derivation is given by Hill & Keightley (1988).) Where mutant effects are unequal, the equilibrium variance can be computed by

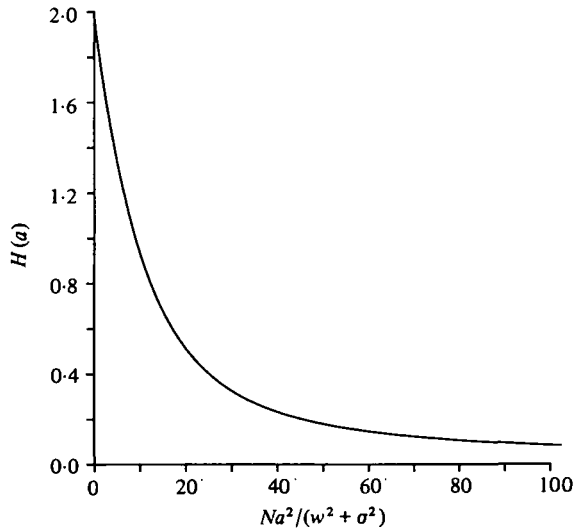


Fig. 2. Heterozygosity maintained during the lifetime of a mutant,  $2 \sum_{i=0}^{\infty} q_i(1-q_i)$ , as a function of  $Na^2/(w^2 + \sigma^2)$ , derived using the transition matrix.

integrating (10) over a density function of mutant effects, but result (11) still holds. Equation (10) also shows that the equilibrium variance is (to first order) a function of  $Na^2/(w^2 + \sigma^2)$ .

4. Results

(i) Two-allele model

(a) Heterozygosity as a function of  $Ns$ . The expected cumulative heterozygosity contributed by a mutant during its lifetime as a function of  $Na^2/(w^2 + \sigma^2) \propto Ns$  is illustrated in Fig. 2, computed using the transition matrix.  $H(a)$  is bounded by the upper value of 2, where drift dominates, and the lower value of zero, where selection causes immediate elimination of the new mutant. The results in the following sections which give examples of  $V_g$  for different types of gamma distribution are all functions of the result in Fig. 2, and were generated by integrating numerically over this function with weighting according to the distribution of mutant effects (equation 7).

(b) Variance maintained with genes of equal effect. Fig. 3 shows the variance maintained as a proportion of that predicted in an infinite population (cf. equation (11)) as a function of  $Na^2/(w^2 + \sigma^2)$ . The graph compares the results from the transition matrix and evaluation of (10). With increasing effects of drift ( $Ns \rightarrow 0$ ), the variance maintained approaches zero; and as the effects of selection become more important (increasing  $N$  or  $a^2/(w^2 + \sigma^2)$ ), the relative variance maintained approaches the asymptote of 1. Interestingly, the results from the transition matrix indicate a maximum greater than the infinite population variance. If the effects of drift and selection are not too strong, the frequency of some mutants can approach the meta-stable point ( $q = 0.5$ ) where the

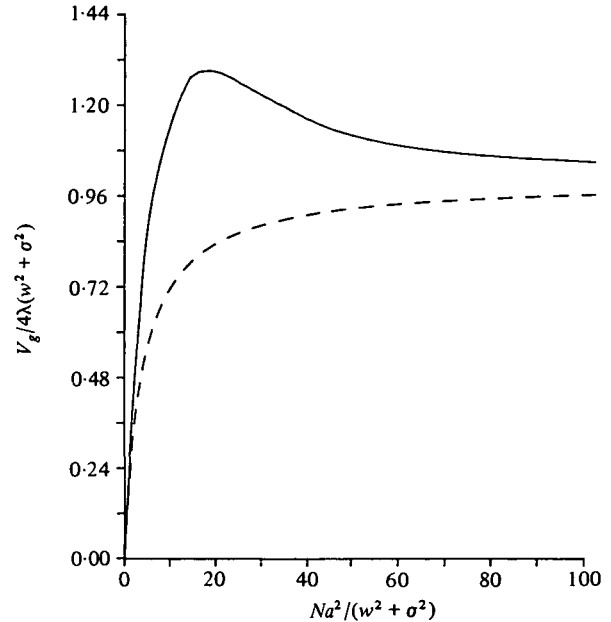


Fig. 3. Predicted equilibrium genetic variance,  $V_g$ , expressed as a proportion of that predicted for an infinite population,  $4\lambda(w^2 + \sigma^2)$ , plotted against  $Na^2/(w^2 + \sigma^2)$ . The curves compare predictions from the transition matrix (exact, —) and from evaluation of equation (10) (approximate, ---).

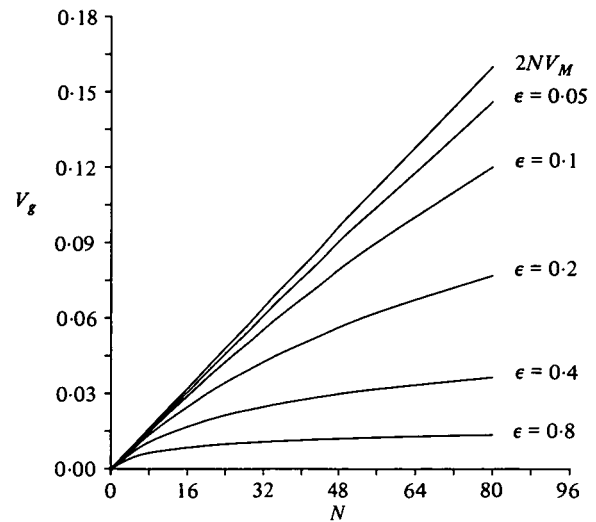


Fig. 4. Predicted equilibrium genetic variance maintained plotted against population size  $N$ . The curves were generated from the transition matrix, assumed a value of  $V_M/V_E = 10^{-3}$ , a gamma distribution of mutant effects, with  $\beta = \frac{1}{2}$  and compare results for a range of  $\epsilon = [E(a^2)/V_E]^{1/2}$  and corresponding mutation rate  $\lambda$ . The strength of stabilizing selection is given by  $w^2 = \sigma^2 = 1$ .

expected change in gene frequency is zero. This possibility is not accounted for by equation (10), which assumes that the selection coefficient is constant and at its maximum. The presence of the maximum in Fig. 3 was confirmed by Monte Carlo simulation with equal mutant effects in a multi-locus model (results not given). Although not shown by Bulmer (1972), the maximum is also obtained by evaluating (9).

(c) The influence of the scale of the mutational

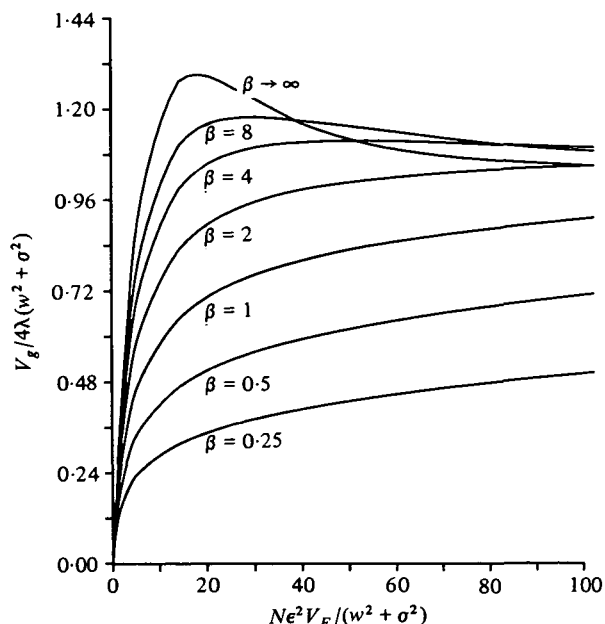


Fig. 5. Predicted equilibrium genetic variance,  $V_g$ , expressed as a proportion of that predicted for an infinite population,  $4\lambda(w^2 + \sigma^2)$ , plotted against  $Ne^2V_E/(w^2 + \sigma^2)$ . The curves were generated by integrating the gamma function for a range of shape parameter,  $\beta$ , from equal effects ( $\beta \rightarrow \infty$ ) to an extremely leptokurtic distribution ( $\beta = 0.25$ ). Results from the transition matrix.

distribution. The influence of the scale of the mutational distribution with varying population size is illustrated in Fig. 4 for the case of a gamma distribution of effects with shape parameter  $\beta = \frac{1}{2}$  (equation 2). The curves were generated by evaluating cumulative heterozygosities using the transition matrix and numerically integrating over (7). The different curves relate  $V_g$  for a fixed value of  $V_M$  with various mutation rates ( $\lambda$ ) and with corresponding values of sizes of effects ( $\epsilon$ ) to satisfy (1). With  $\lambda \rightarrow \infty$  and infinitesimally small effects the expected  $V_g$  is simply  $2NV_M$  as obtained by Clayton & Robertson (1955). This is the upper bound of the maintained variance. The initial trajectory of all the other curves is also  $2NV_M$ , but each slowly approaches the asymptotic value given by (11) as  $N$  increases.

(d) *The influence of the shape of the mutational distribution.* Fig. 5 shows the variance maintained in a finite population as a proportion of that which would be maintained in an infinite population, expressed as a function of  $Ne^2V_E/(w^2 + \sigma^2)$ . The curves relate different gamma distributions of mutant effects ranging from equal ( $\beta \rightarrow \infty$ ) to a highly leptokurtic form ( $\beta = 0.25$ ) (see Fig. 1). The result for equal effects is also shown in Fig. 3. Clearly, the shape of the distribution has a strong influence on  $V_g$ , and with highly leptokurtic forms, the approach to the asymptote is exceedingly slow. Curves for other values of shape parameter  $\beta$  also have maxima. A normal distribution of mutant effects would correspond to a gamma distribution reflected about zero with shape

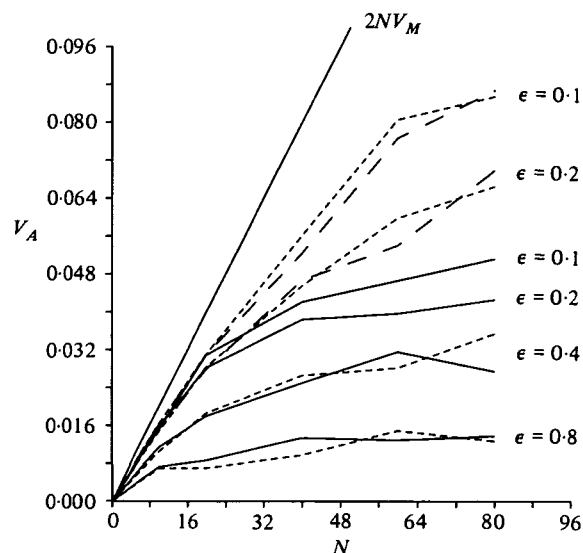


Fig. 6. Equilibrium additive variance plotted against population size,  $N$ , for a gamma  $\frac{1}{2}$  distribution of mutant effects and a range of values of  $\epsilon = [E(a^2)/V_E]^{1/2}$  and corresponding values of  $\lambda$  to give  $V_M/V_E = 10^{-3}$ . The curves are from Monte Carlo simulation and compare three lengths of chromosome,  $L$ , where  $L$  is the number of map units. The strength of selection is given by  $w^2 = \sigma^2 = 1$ . The standard errors of the simulation points are less than 10% of their mean. Modified from Hill & Keightley (1988). ----,  $L \rightarrow \infty$ ; -.-,  $L = 1$ ; —,  $L = 0$ .

parameter  $\beta = 1.75$  (Hill & Rasbash, 1986), so on Fig. 5 its curve would lie between  $\beta = 1$  and  $\beta = 2$ .

(e) *Linkage.* Using the transition matrix method, it is only feasible to work out the expectation of the variance contributed by a single locus. In order to incorporate linkage, Monte Carlo simulation was used. Fig. 6 compares the additive genetic variation maintained from mutations occurring on chromosomes of three different lengths in populations of varying sizes. With free recombination, the simulation results are in good agreement with the predictions from the transition matrix obtainable from Fig. 5, which confirms that mutants can be treated independently to approximate the behaviour of the system. Linkage leads to a reduction in maintained additive variance which is greatest with many mutants of small effect ( $\epsilon \rightarrow 0$ ) and its effects are virtually absent with the larger values. As in the case of directional selection (Keightley & Hill, 1983, 1987), a small amount of recombination eliminates most of the effects of linkage, but is less effective as  $N$  increases.

(ii) *Multi-allele model*

The above analysis is a finite population treatment which assumes that only two alleles can segregate at any locus. In this respect it is similar to the models of Latter (1960) and Bulmer (1972) and to Turelli's (1984) 'House of Cards' approximation (henceforth referred to as the LBT models), with the additional assumption that mutant effects are randomly sampled.

The models of Kimura (1965) and Lande (1976) (KL Gaussian models) assume that mutant effects differ only slightly from those already segregating, with the consequence that the steady-state distribution of allelic effects at the locus is normal. As Turelli (1984) pointed out, there is a fundamental discrepancy between the behaviour of the two types of model. Using Kimura's analysis, the equilibrium variance in an infinite population at a locus ( $V_{g_L}$ ) is

$$V_{g_L} = [2V_{ML}(w^2 + \sigma^2)]^{\frac{1}{2}}, \tag{12}$$

where  $V_{ML}$  is the mutational variance input at the locus. This result can be derived by a different route. Assume at steady state a large number of alleles generates a normal distribution of allelic effects segregating at a locus. The variance maintained at the locus in a finite population can be obtained from the recurrence

$$V_{g_L,t+1} = V_{g_L,t}(1 - 1/2N_e)(1 - V_{g_L}k/2\sigma^2) + V_{ML} \tag{13}$$

because the variance at the locus is reduced each generation by the factor  $(1 - 1/2N_e)$  by drift and  $(1 - V_{g_L}k/2\sigma^2)$  by selection, where  $k$  depends on the strength of selection and is the proportion of the phenotypic variance in the unselected individuals (Bulmer, 1980; Falconer, 1981, p. 180). With stabilizing selection and a normal distribution of phenotypic values,  $k = \sigma^2/(w^2 + \sigma^2)$ . With infinite population size, (13) reduces to Kimura's (1965) formula (12) (ignoring second-order terms). Equation (13) also gives a solution for a finite population which is a quadratic in  $V_{g_L}$ :

$$V_{g_L}^2 k(2N_e - 1) + 2\sigma^2 V_{g_L} - 4N_e V_{ML}\sigma^2 = 0. \tag{14}$$

This formula is similar to that obtained using the same assumptions by Latter (1970).

Fig. 7 compares the equilibrium genetic variance maintained for a range of population sizes for three different models using a gamma distribution of mutant effects with shape parameter  $\beta = \frac{1}{2}$ :

(i) Gaussian: the variance was computed from the solution to (14). This corresponds to the KL prediction.

(ii) 'Two allele': the variance was computed from the transition matrix and numerical integration, for a model of two alleles only per locus.

(iii) 'Multi-allele': the variance was computed by simulation of  $n$  discrete freely recombining loci with no intra-genic recombination, so the number of alleles which can segregate at any locus is not limited.

Also shown is the variance maintained by neutral genes which is simply  $2NV_M$ . The main points to note from this figure are: (a) all three models agree at small population sizes and mutant effects where drift is dominating; (b) the simulation of multiple alleles agrees with the KL Gaussian prediction only when mutant effects are small ( $\epsilon = 0.1$ ) and the mutation rate per locus is high ( $\mu = 2 \times 10^{-3}$ ); (c) otherwise, with decreasing mutation rate, but correspondingly

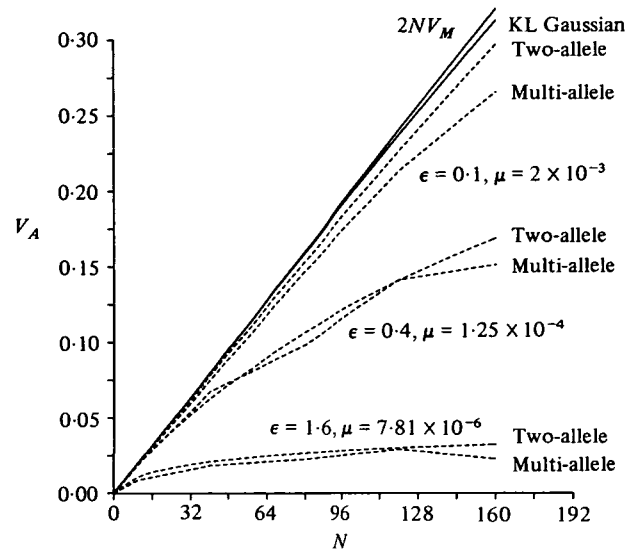


Fig. 7. Equilibrium additive variance plotted against population size,  $N$ , for three different models (see text). Also shown is the variance predicted for no selection,  $2NV_M$ . The simulation used 100 equally mutable freely recombining loci with mutation rates,  $\mu$ , as shown, corresponding values of  $\epsilon$  so that  $V_M = n\mu\epsilon^2/2 = 10^{-3}V_E$  and mutant effects sampled from a gamma distribution with  $\beta = \frac{1}{2}$ . The strength of stabilizing selection is given by  $w^2 = 19$   $\sigma^2 = 19$ . The standard errors of the results from the simulation are less than 5% of their mean.

increased magnitudes of mutant effects (e.g.  $\epsilon = 1.6$  and  $\mu = 7.81 \times 10^{-6}$ ), the simulation agrees better with the two-allele model. The simulation illustrates the difference between the KL approximation and Turelli's (1984) 'House of Cards' approximation. With the number of loci chosen for this example (100), the KL prediction hardly differs from neutrality. A larger number of loci would allow for a smaller standard deviation of the distribution of mutant effects for mutation rates per locus in line with those experimentally measured (Mukai & Cockerham, 1977). In this case, all three models would agree more closely at the population sizes shown, but as population sizes became much larger, would diverge as in Fig. 7 as the effect of selection becomes stronger relative to drift.

### 5. Discussion

(a) *Stabilizing selection and drift.* We have concentrated on a model where the mutation rate is sufficiently low or the population size sufficiently small that two alleles segregate at each locus. The consequences of such a model with infinite population size have been investigated previously (Latter, 1960; Bulmer, 1972) and an important conclusion was that the equilibrium genetic variance,  $V_g$ , is essentially independent of the effects of mutants on the trait, but depends only on the number of new mutants per generation. As a consequence of its independence of the effects of mutants, in an infinite population  $V_g$  is

independent of the shape of the mutational distribution. In a finite population  $V_g$  is also proportional to the mutation rate, but in contrast is also highly dependent on the magnitudes of the effects of mutants. The variance maintained is a function of population size, the effect on the character and strength of stabilizing selection according to  $Na^2/(w^2 + \sigma^2)$ . The variance contributed by a mutant during its lifetime is at a maximum when the combination of parameters  $Na^2/(w^2 + \sigma^2) = 25$ , and is about 30% greater than that in an infinite population, namely  $4(w^2 + \sigma^2)$ . So, for example, a mutant of effect 0.1 under weak stabilizing selection (e.g.  $w^2 + \sigma^2 = 20$ ) would contribute during its lifetime about 30% more variance in a population of  $5 \times 10^4$  than in an infinite population. This effect occurs because near-neutral mutants are able to drift to intermediate frequencies where the strength of selection is weakest, but mutants of larger effect tend to be eliminated almost immediately by selection. The maximum is a consequence of the multi-locus nature of the system. If no other genes were segregating when a mutation occurred, its fate would depend on the relation of the optimum phenotype to the population mean.

(b) *Distribution of mutant effects.* In a finite population  $V_g$  is highly dependent on the shape of the distribution of mutant effects. Using gamma distributions, we have modelled a wide range of possible mutant distributions ranging from all effects equal to a highly leptokurtic form where most mutants are of tiny effect, but most of the mutational variance,  $V_M$ , is contributed by a few genes of large effect. With such a distribution, in contrast to when effects are equal,  $V_g$  increases very slowly with decreasing effect of drift. This slow approach to the asymptote is best understood by considering a fixed mutational distribution and selection, but increasing population size. The mutants of large effect which contribute most to  $V_M$  do not contribute substantially to  $V_g$  since they are quickly eliminated by selection, but the many mutants of small effect eventually contribute substantially to  $V_g$  with increasing population size because they remain nearly selectively neutral until  $N$  becomes very large.

An implicit assumption of the analysis has been a symmetrical distribution of mutant effects. If there are few mutants segregating, or mutant effects are small, asymmetry does not influence the variance maintained, and this was confirmed by simulation (results not shown). The simulations also showed that, in general, slightly less variance is maintained with a skewed distribution of mutant effects, because the population mean is moved away from the optimum and selection is thereby stronger against most new mutants. Also, the genotypic distribution becomes skewed (Keightley & Hill, 1987) and selection tends to remove more variation than it would from a symmetrical distribution.

Results have been presented only for expectation of the genetic variance maintained in the population. As shown by Bürger *et al.* (1988) for stabilizing selection and by Keightley & Hill (1983) for directional selection with mutation, there can be a very high variability between generations in  $V_g$  and a strong autocorrelation of  $V_g$  over successive generations.

(c) *Linkage.* With stabilizing selection the extreme genotypes are less favoured, so there is a tendency for repulsion genotypes to persist and coupling genotypes to be eliminated. The result is that the additive variance is less than the genic variance because there is a 'hidden' negative disequilibrium component. The simulations show that disequilibrium increases with increasing mutation rate and with population size because there are more mutants segregating. Also, for the same reason, linkage has more influence when most of the mutational variance is contributed by many genes of small effect than a few genes of large effect.

The results show that recombination is very efficient at eliminating such influence of disequilibrium. In the examples shown, one crossover is sufficient to give results almost indistinguishable from free recombination. These simulations were, it should be emphasized, extreme cases with relatively strong selection and all the mutants appearing on one chromosome. The results from the simulation are relevant to the appropriateness of the two-allele model (next section), because the simulation is an infinite-sites model with no distinction between alleles and loci. The efficiency of even a small amount of recombination in eliminating linkage disequilibrium (cf. Keightley & Hill, 1983, 1987) implies that the two-allele model is a good enough approximation because mutants occurring close together on the chromosome can be regarded as being either at the same or at different loci.

(d) *Appropriateness of the model.* Most of the analysis has been restricted to segregation of only two alleles per locus. A model has also been investigated which resembles more closely those of Kimura (1965) and Lande (1976), in which the effects of new alleles are assumed to be small relative to the existing variance at the locus at which many alleles segregate, so the asymptotic distribution of allelic effects at a locus is approximately normal. Turelli (1984) has questioned the appropriateness of the KL model since the mutation rate per locus is unlikely to sustain sufficient standing variation at a locus for the assumptions in the model to be valid. Simulation results (Fig. 7) with finite populations support Turelli's objections. The analytical result of Kimura (equal to first order to Lande's) is only in agreement where the mutation rate per locus is exceptionally high and mutant effects small. With mutation rates per locus closer to experimentally obtained estimates, i.e.  $10^{-4}$  to  $10^{-5}$  (Mukai & Cockerham, 1977; Turelli, 1984), the two-allele model provides a good approximation.



### Implications

The maintenance of genetic variation is a central problem in population biology, and the question of whether a mutation-stabilizing selection balance can maintain the observed levels of heritable variation has been frequently addressed (e.g. Lande, 1976, 1980; Turelli, 1984, 1985). The results show that, with finite population size, the shape of the mutational distribution has a strong influence on the genetic variance maintained under mutation-selection balance. There is little information concerning the shape of the distribution of mutational effects for any character, but can an informed guess be made from insights into biochemistry and molecular biology? In principle, all mutants, no matter where they occur in the genome, must have at least some effect on all characters, albeit very small. The interactive nature of metabolism, where the fluxes and metabolite pool concentrations, are systemic properties dependent to a greater or lesser extent on all enzymes in the 'metabolic map' (Kacser & Burns, 1973) tells us that there must be hundreds, if not thousands, of enzymes, variation in the activities of which will affect any character which is in some way controlled by the metabolism of the organism. Evidence for functional constraint in the genome (Kimura, 1983, ch. 7) at such sites as introns, silent (non-replacement) sites within coding sequences, and gene flanking sequences, suggests that there are many places in the genome capable of producing some small phenotypic effect. Thus it can be argued *a priori* that the distribution of mutant effects on complex quantitative characters is highly leptokurtic: most mutants are either of such trivial effect or so 'distant' from the character that they have almost no effect at all, but there is a smaller class of genes, more directly capable of influencing the trait with mutants of relatively large effect. The total number of mutants affecting a character is therefore high, much higher than an experiment designed to count polygenes would detect, but the effect of most of them is very small (see Robertson, 1967).

The difficulties in estimating the number and effects of mutants influencing a quantitative character are highlighted by the following illustrative example. Assume by genetic means only mutants showing an effect on the character of at least one-half of a standard deviation can be detected and the standard deviation of the mutational distribution is 1.6 units. If all effects were equal, then the genetic test would detect all the new mutants. If, however, the mutational distribution were more extreme (for example, gamma with shape parameter 0.25 (see Fig. 1)) then only 21% of the new mutants would be detected but they would contribute most of the variance (96%).

With this consideration in mind, estimates of the number of new mutants per generation affecting various quantitative traits in maize (Sprague, Russell

& Penny, 1960; Russell, Sprague & Penny, 1963) seem rather high. These experiments gave estimates of  $\lambda$  for detectable mutants of about 0.06, implying, with, say, a mutation rate per locus of  $10^{-5}$ , many thousands of loci at which mutations give sufficiently large effects to be detected. Such experiments, however, might now have to take into consideration the possibility of induction of 'mutator' genes (McClintock, 1950) in these crosses caused by movement of transposable elements known to be capable of affecting quantitative traits (Mackay, 1987). The rates of mutation may vary widely between populations as results of T. F. C. Mackay (personal communication) suggest, due to varying transposition rates.

(a) *Predicting maintained heritability – assigning values to parameters.* The important parameters are the mutational variance input per generation, the shape and scale of the mutational distribution, the strength and mode of operation of natural selection, and effective population size. As implied earlier, information is scarce on values of most of these parameters relevant to natural populations. If, however, it is assumed that  $V_M/V_E = 10^{-3}$ , the character is affected by a fairly extreme distribution of effects (i.e. gamma with  $\beta = 0.25$ ); most variance is contributed by mutants of fairly large effect (e.g.  $\epsilon = 0.4$  and therefore the mutation rate per genome  $\lambda = 0.0125$ , implying *c.* 400 loci each mutating at *c.*  $0.3 \times 10^{-5}$ ); and a 'typical' value of  $w^2 = 20 \sigma^2$  (Turelli, 1984), then with  $N_e = 10^3$ , the maintained heritability would be about 21%; with  $N_e = 10^4$ , the maintained heritability would be about 33%, but there would be less than half of the genetic variance that would be present in an infinite population. It is notable that in an infinite population the maintained variance is proportional to  $w^2 + \sigma^2$ , and is very sensitive in a finite population to changes in  $w^2 + \sigma^2$  over a wide range of parameters (flat part of curves in Fig. 5). If many characters are simultaneously subject to stabilizing selection, the value for  $w^2/\sigma^2$  of 20 chosen in the above example may be smaller than typical (i.e. selection strength too strong) due to the genetic load that such selection would impose on the population. Thus, on the face of it, mutation-stabilizing selection balance is an attractive candidate for explaining the observed levels of heritable variation in populations that vary over a wide range in effective population size. The above calculations become less attractive when we consider the problems in estimating the strength of natural selection and in justifying the single character model of stabilizing selection. Such aspects have been discussed in detail by Turelli (1984, 1985).

(b) *Mode of action of natural selection.* The effect of pleiotropy is to reduce the genetic variation maintained since, for example, the selection coefficient against a mutant if stabilizing selection acts independently on each character, *i*, is proportional to  $\sum [a_i^2/(w_i^2 + \sigma_i^2)]$ . Clearly, the analysis could easily be

extended to include pleiotropy and Fig. 2 still applies with the horizontal axis labelled as  $N \sum [a_i^2 / (w_i^2 + \sigma_i^2)]$ , reflecting the selection acting on all the other characters. If mutant effects on each trait are uncorrelated and there are  $k$  traits, then the variance maintained for each is reduced in proportion to  $1/k$  (Turelli, 1985).

Pleiotropic gene action is likely to affect other characters subject to stabilizing selection, or to affect characters such as fertility and viability more closely connected with fitness *per se*. In the former case the selection experienced by a mutant allele will be proportional to the square of the effect on the pleiotropically related character, but in the latter case the pleiotropic selection is directional and is proportional to the allelic effect to first order (see Hill & Keightley, 1988). Thus, especially when allelic effects are small, the selection due to the effect on fitness dominates and even small negative correlations with fitness-related characters are likely to have a large impact on the maintained heritability.

It seems therefore that in order to fully understand the maintenance of variation in a quantitative character the bivariate distribution of mutant effects on that character and on fitness is a critical parameter. Since the present analysis suggests that mutants of small effect are likely to be more important in maintaining variation, the more accessible part of the mutational distribution may be of less interest. We are some way from a satisfactory understanding of the mechanisms of maintenance of variation in polygenic traits.

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