Pleiotropic Model of Maintenance of Quantitative Genetic Variation at Mutation-Selection Balance

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ABSTRACT

A pleiotropic model of maintenance of quantitative genetic variation at mutation-selection balance is investigated. Mutations have effects on a metric trait and deleterious effects on fitness, for which a bivariate gamma distribution is assumed. Equations for calculating the strength of apparent stabilizing selection (V_s) and the genetic variance maintained in segregating populations (V_G) were derived. A large population can hold a high genetic variance but the apparent stabilizing selection may or may not be relatively strong, depending on other properties such as the distribution of mutation effects. If the distribution of mutation effects on fitness is continuous such that there are few nearly neutral mutants, or a minimum fitness effect is assumed if most mutations are nearly neutral, V_G increases to an asymptote as the population size increases. Both V_G and V_s are strongly affected by the shape of the distribution of mutation effects. Compared with mutants of equal effect, allowing their effects on fitness to vary across loci can produce a much higher V_G but also a high V_s (V_s in phenotypic standard deviation units, which is always larger than the ratio V_P/V_m), implying weak apparent stabilizing selection. If the mutational variance V_m is $\sim 10^{-3}V_e$ (V_e, environmental variance), the model can explain typical values of heritability and also apparent stabilizing selection, provided the latter is quite weak as suggested by a recent review.

MOST characters in morphology, behavior, and physiology vary continuously among individuals within populations (Falconer and Mackay 1996). These quantitative or metric traits are usually observed to have abundant genetic variation in natural populations, as reflected directly by the correlation between relatives and indirectly by sustained response to directional selection that takes the phenotype well beyond its original range (Endler 1986; Roff and Mousseau 1987). Such traits are often also found to be under apparent stabilizing selection, as evidenced by the reduced fitness of extreme phenotypes and the constancy of form over geological times and over vast geographic distances (Charlesworth *et al.* 1982; Endler 1986). The existence of genetic variation is thus paradoxical, because stabilizing selection usually depletes genetic variation (Wright 1935; Crow and Kimura 1970) although stabilizing selection in some extreme cases, *e.g.*, in two-locus models with unequal effects, can maintain substantial variation (Bürger 2000, pp. 203–216). How then is genetic variation in metric traits maintained in natural populations? This is a long-standing and fundamental question in evolutionary biology, the answer to which is important for understanding the genetic architecture of metric traits and the selective breeding of domestic animals and crops and comprehending the genetic basis of evolution and adaptation (Barton 1990; Falconer and Mackay 1996; Bürger 2000).

Since the ultimate source of genetic variation is mutation, an intuitively appealing explanation for the maintenance of polygenic variation is that there is equilibrium between the input of new variation by mutation and its erosion by natural selection [*i.e.*, mutation-selection balance (MSB)]. The question is whether mutations affecting a metric trait appear frequently enough and/or have large enough effects to provide sufficient new variation to counterbalance the depletion of variation by stabilizing selection. Empirical studies on various traits in different species show that mutational variance (V_m), the fresh genetic variance of a trait generated by mutation in one generation, is typically $10^{-3}V_e$ (V_e, environmental variance), with a range from 10^{-4} to $10^{-2}V_e$ (Houle *et al.* 1996; Lynch and Walsh 1998). Assuming that the population is in MSB, a review of available data on V_m and standing genetic variance (V_G) showed that the persistence time of deleterious mutants, V_G/V_m, for life history traits is roughly one-half that for morphological traits, consistent with the prediction of MSB that traits more closely related to fitness are under stronger selection and mutations affecting them are eliminated more quickly (Houle *et al.* 1996).

If MSB is accepted as the mechanism for the maintenance of polygenic variation in natural populations, the question remains of how natural selection acts on the trait or on the genes that affect it. Although the observa-

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tion that intermediate phenotypes of a metric trait have the highest fitness indicates apparent stabilizing selection, it could have several distinct causes that lead to different predictions about the genetic architecture of metric traits in equilibrium populations (Robertson 1967).

A straightforward hypothesis is that natural selection acts directly and solely on the metric trait, the value of relative fitness having a quadratic relationship with the trait. This classical hypothesis, called "real" stabilizing selection (Falconer and Mackay 1996), has attracted extensive theoretical analyses, and two main classes of models have been developed. The two models make different extreme assumptions about allele effects and mutation rate per locus (u) and give quite different predictions of the equilibrium variance (Kimura 1965; Turelli 1984; Falconer and Mackay 1996; Bürger 2000): $V_G = (2nV_mV_s)^{1/2}$ for the continuum-of-alleles model and $V_G = 4nuV_s$ for the rare allele model, where n is the number of potentially mutable loci affecting the trait and V_s is the strength of stabilizing selection, the "variance" of the fitness profile in phenotypic standard deviation units. The different approximations are a consequence of assumptions about the variance introduced by new mutations relative to the existing allelic variation (Turelli 1984; Bürger 2000). It is difficult to account for the observed high variance with either model for typical estimates of V_m (*e.g.*, $10^{-3}V_e$) for what are regarded as typical values of V_s (*e.g.*, $20V_e$; Turelli 1984; Falconer and Mackay 1996). In a recent review, however, Kingsolver *et al.* (2001) concluded that the stabilizing selection might be substantially weaker than has been assumed. Further, simple genetic load arguments suggest that real stabilizing selection cannot operate independently on many characters (Robertson 1967; Turelli 1985; Barton 1990).

The hypothesis of the "pleiotropic model" is that natural selection does not act directly on the metric trait in question, but on the alleles affecting it through their pleiotropic side effects on fitness (Robertson 1967; Hill and Keightley 1988). With this model, an allele has a direct effect (a) on a specific trait, with its pleiotropic effects on all other traits condensed into its net effect (s) on fitness. The MSB under this model can also generate apparent stabilizing selection, shown as a negative correlation between fitness and phenotypic deviation from the mean, even for a trait that is purely neutral and irrelevant to fitness (Gavrilets and de Jong 1993). This is because, if unconditionally deleterious alleles can either increase or decrease the value of a specific trait, individuals carrying more deleterious alleles (Barton 1990; Kondrashov and Turelli 1992) or alleles of large deleterious effects if a and s are correlated (Keightley and Hill 1990) will tend to have more extreme phenotypes and low fitness. The model captures the conventional wisdom that essentially all mutations are pleiotropic and deleterious. The pleiotropic model is attractive, because it could explain both the appearance of stabilizing selection and the maintenance of polygenic variation as a consequence of the pleiotropic effects of mutations that are, in agreement with the classical view, unconditionally deleterious and highly pleiotropic.

In addition to the above two hypotheses, many others such as overdominance (Wright 1935; Robertson 1956; Gillespie 1984; Barton 1990), frequency-dependent selection (Slatkin 1979; Barton 1990), genotype-by-environment interaction (Gillespie and Turelli 1989; Gimelfarb 1990; Zhivotovsky and Gavrilets 1992), and epistatic interaction (Zhivotovsky and Gavrilets 1992; Gavrilets and de Jong 1993) have been proposed to explain the maintenance of polygenic variation. All these models have their respective appeal and weakness in explaining the maintenance of polygenic variation, and they and the real stabilizing selection and pleiotropic models are not mutually exclusive. For example, the epistatic model considered by Gavrilets and de Jong (1993) can explain both abundant variation and strong stabilizing selection, but experimental evidence is scanty as to whether such epistasis is common and strong enough (Elena and Lenski 1997; De Visser and Hoekstra 1998).

Given the ubiquity of mutations with deleterious pleiotropic effects, the pleiotropic model inevitably explains some polygenic variation and apparent stabilizing selection for a metric trait. The question is how much. Analytical models of the pleiotropic hypothesis, commonly assuming equal deleterious effect of mutations acting multiplicatively (Barton 1990) or synergistically (Kondrashov and Turelli 1992), can only partly explain the observed levels of polygenic variation and apparent stabilizing selection if $V_m = 10^{-3}V_e$. Relaxing the assumptions makes the models more reasonable. Numerical methods (diffusion approximations and Monte Carlo simulation) can be used instead to tackle complex models (Keightley and Hill 1990; Caballero and Keightley 1994). With $V_m = 10^{-3}V_e$ but allowing deleterious effects to vary among alleles, numerical results indicate that the observed level of polygenic variance can be accounted for (Keightley and Hill 1990; Caballero and Keightley 1994), though the typical strength of stabilizing selection can be only partially explained (Barton 1990; Keightley and Hill 1990).

Here we construct a pleiotropic model on the basis of previous theoretical studies and empirical data available on new mutations and segregating alleles affecting metric traits and fitness to predict the polygenic variance (V_G) and strength of apparent stabilizing selection (V_s) for a metric trait in an equilibrium population. By applying a more general model of the relationship between a and s of mutations, we aim to find out what levels of V_G and V_s could be explained within the pleiotropic model. In particular, a weakness of models in which s has a continuous distribution is the unbounded increase

of V_G with N_e (Keightley and Hill 1990). This was investigated and different assumptions were tried to decrease this dependence so that V_G can asymptote as N_e becomes large. We use both analytical and numerical approaches to obtain predictions from the model and compare them with observations. We hope thus to obtain a better understanding of the genetic properties of metric traits in an equilibrium population under the pleiotropic model. By comparing predictions with empirical observations, we also hope to ascertain how robust is the pleiotropic model as an explanation of polygenic variation.

MODEL AND ANALYSIS

Gene actions and contributions of mutants: Mutations in a diploid individual are assumed to have effects on a neutral metric trait z, with a the difference in value between homozygotes, and pleiotropic effects on fitness, with s the difference in fitness between homozygotes. The gene actions on the metric trait and on fitness within and across loci are assumed to be additive; that is, dominance and epistasis are ignored, which Barton and Keightley (2002) argue is an adequate model. The expected increase in variance in the metric trait each generation, the mutational variance, is given by $V_m = \frac{1}{2}\lambda E[a^2]$ when the expected number of mutations per haploid genome per generation is λ. A population of N diploid individuals, with an effective population size N_e and random mating, is assumed. It is also assumed that the total number of loci per individual is so large and the mutation rate per locus is so low that mutations occurring at the same loci can be ignored.

To assess the genetic variation maintained and the strength of apparent stabilizing selection at MSB, we need to obtain the contribution from all mutant alleles. As the strength of apparent stabilizing selection is a function of the aggregate effects of alleles at all segregating loci in the population (Keightley and Hill 1990; Falconer and Mackay 1996), a multilocus model is analyzed. The results obtained in this subsection are valid in a quite general sense except that linkage disequilibrium between loci is ignored and gene action is additive.

Let the frequencies of the wild-type allele (A) and the mutant allele (a) at a given locus be $1 - x$ and x, respectively. With a one-locus model, the conclusion about the contribution from mutant alleles is quite simple, while within a multi-locus model, the conclusion is not straightforward. If mutations at any of n loci in a diploid individual can affect the neutral trait z and have pleiotropic effects on fitness, then the contribution from all these mutations can be described by the following properties. The genetic variance is

$$V_G = \mathrm{Var}(z - \bar{z}) = \sum_{i=1}^{n} 2x_i(1 - x_i)a_i^2/4, \qquad (1)$$

in which the mean value of the trait is $\bar{z} = \Sigma_{i=1}^{n} x_i a_i$ and x_i is the average frequency of the mutant allele at locus i, the variance of squared deviation is

$$V_{G2} = \mathrm{Var}((z - \bar{z})^2) \equiv (1/N)\sum_{j=1}^{N}(z_j - \bar{z})^4 - \left[(1/N)\sum_{j=1}^{N}(z_j - \bar{z})^2\right]^2$$
$$= \sum_{i=1}^{n}\left\{2x_i(1 - x_i) - 3\left[2x_i(1 - x_i)\right]^2\right\}a_i^4/16 + 2V_G^2, \qquad (2)$$

the covariance of the relative fitness and the squared deviation is

$$\mathrm{Cov}(w, (z - \bar{z})^2) = -\sum_{i=1}^{n} 2x_i(1 - x_i)(1 - 2x_i)(s_i/2)a_i^2/4, \qquad (3)$$

and the variance of relative fitness is

$$V_f = \mathrm{Var}(s - \bar{s}) = \sum_{i=1}^{n} 2x_i(1 - x_i)s_i^2/4. \qquad (4)$$

Bivariate distribution of mutant effects on the metric trait and on fitness and their simulations: To evaluate Equations 1–4, we need to know the properties of mutant effects on the metric trait and on fitness, which vary between alleles. Although much effort has been made to quantify the features of mutant effects (Mackay and Langley 1990; Hill and Caballero 1992; Mackay *et al.* 1992; Davies *et al.* 1999; Elena and Moya 1999; Shaw *et al.* 2000; Imhof and Schlötterer 2001; Wloch *et al.* 2001), detailed information about the marginal and joint distribution of mutant effects on the metric trait and fitness is unknown or indirect. Even for Drosophila, for which there are many studies, the data seem to suggest a highly skewed and leptokurtic distribution of mutation effects (Mackay and Langley 1990; Hill and Caballero 1992; Mackay *et al.* 1992), but the fine-scale information is still lacking. As in Keightley and Hill (1990), the distribution of mutant effects on the metric trait is assumed to be symmetrical about $a = 0$, and only deleterious mutations on fitness are assumed to occur, in accord with the classical view (Falconer and Mackay 1996). For a general model in which a wide relationship between effects and rates of mutations is included, it is assumed that the marginal distribution of effects on fitness, $g(s)$, is a gamma distribution with scale parameter α_s and shape parameter β_s,

$$g(\alpha_s, \beta_s; s) = \alpha_s^{\beta_s} s^{\beta_s - 1}\exp(-\alpha_s s)/\Gamma(\beta_s). \qquad (5)$$

Similarly the marginal distribution of $|a|$ is also a Γ distribution with parameters α_a and β_a. The Γ distribution spans a wide range of possibilities and particularly a small value of the shape parameter β implies that mutant genes of small effects are much more common than those of large effects (see Figure 1). A Γ distribution is said to be leptokurtic if its shape parameter $\beta \leq 1$. The variability of the distributions is defined in terms of $\varepsilon_a = \sqrt{E[a^2]} = \sqrt{\beta_a(\beta_a + 1)}/\alpha_a$ and $\varepsilon_s = \sqrt{E[s^2]} = \sqrt{\beta_s(\beta_s + 1)}/\alpha_s$. The means and variances of the mar-

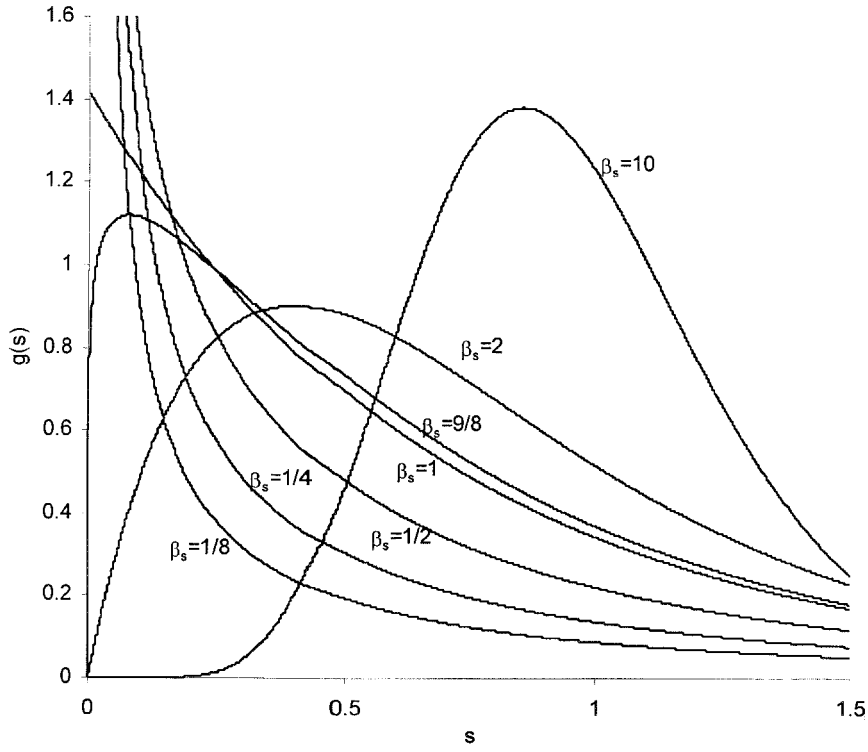

FIGURE 1.—Examples of the gamma distribution $g(s) = \alpha_s^{\beta_s} s^{\beta_s - 1} \exp(-\alpha_s s)/\Gamma(\beta_s)$ for a range of β_s. The parameter α_s describes scale rather than shape and its value is such that $E(s^2) = 1$ for each curve.

ginal distributions are $E[a] = 0$, $V[a] = \varepsilon_a^2$, $E[s] = \varepsilon_s \sqrt{\beta_s/(\beta_s + 1)}$, $V[s] = \varepsilon_s^2/(\beta_s + 1)$. The level of pleiotropy of a mutation presumably changes with its absolute magnitude of phenotypic effect, $|a|$, on the metric trait, mutations with large effect being more likely also to have a serious impact on fitness than those with small effect. This is supported by observations from spontaneous and *P*-element-induced mutation experiments (MACKAY *et al.* 1992; KEIGHTLEY *et al.* 2000). As in KEIGHTLEY and HILL (1990), it is assumed that the effects of mutations on the traits can be correlated, and the correlation of absolute values of mutant effects on the metric trait and on fitness is defined as $\rho = \text{cov}(|a|, s)/\sqrt{V[|a|]\,V[s]}$.

Genetic variances and strength of apparent stabilizing selection were evaluated by Monte Carlo simulation and analytical methods. Effects of mutant alleles were sampled from a bivariate gamma distribution, $h(|a|, s)$ with parameters ε_a, β_a, ε_s, β_s, and ρ using algorithm GTVR (SCHMEISER and LAL 1982; see APPENDIX A) and a from $|a|$ by randomly allocating sign. Since selection acts on fitness, the conditional moments of the mutation effects on the metric trait given s are important in evaluating the properties of the populations at MSB,

$$E[a^2|s] = \varepsilon_a^2\left(b_0 + b_1\frac{s}{\varepsilon_s} + b_2\frac{s^2}{\varepsilon_s^2}\right), \tag{6}$$

$$E[a^4|s] = \varepsilon_a^4\left(c_0 + c_1\frac{s}{\varepsilon_s} + c_2\frac{s^2}{\varepsilon_s^2} + c_3\frac{s^3}{\varepsilon_s^3} + c_4\frac{s^4}{\varepsilon_s^4}\right), \tag{7}$$

where the coefficients b_i and c_i, given in APPENDIX A, are functions of the shape parameters β_s, β_a, and the correlation ρ.

The method of simulating $|a|$ and s employed in this article is different from and more general than that in KEIGHTLEY and HILL (1990) in which only a bivariate Γ distribution with $\beta_a = \beta_s = \frac{1}{2}$ (*i.e.*, Wishart distribution) was assumed for $|a|$ and s.

Genetic variance maintained and strength of apparent stabilizing selection at MSB: KIMURA's (1969) diffusion approximations under the infinite independent loci model were used to obtain the equilibrium frequency distribution $\Phi(x; s)$ and other properties of a mutant with a specific fitness effect s in a large population at MSB. Since $\Phi(x; s)\,dx$ represents the expected number of loci in which the mutants of particular fitness effect s are in the frequency range $x \sim x + dx$ at equilibrium, the total expected number of mutants of fitness effect s is given by $\int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)\,dx$. Thus under the infinite loci model, Σ_i in formulas (1–4) should be transformed to $\int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)\,dx$. Summing over all possible mutants of effects a and s leads to the equilibrium genetic variance from Equation 1,

$$\begin{aligned} V_G &= \int_0^\infty\int_{-\infty}^{\infty}\int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)\,h(a, s)\,2x(1 - x)\,(a^2/4)\,dx\,da\,ds \\ &= \int_0^\infty\int_{-\infty}^{\infty} h(a, s)\,H(s)\,(a^2/4)\,da\,ds, \end{aligned} \tag{8}$$

with the heterozygosity $H(s) \equiv \int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)\,2x(1 - x)\,dx = 8N_e\lambda[1/(2N) - u(s)]/s$, in which the fixation probability of the mutant of initial frequency $1/(2N)$ is given by $u(s) = (1 - \exp(N_e s/N))/(1 - \exp(2N_e s))$ (KIMURA 1962). Similarly, from (2),

$$V_{G2} = \int_0^\infty\int_{-\infty}^\infty h(a, s)\{H(s) - 3K(s)\}\frac{a^4}{16}dads + 2V_G^2 \equiv m_4 + 2V_G^2, \tag{9}$$

with

$$K(s) \equiv \int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)[2x(1-x)]^2dx = \frac{16N_e\lambda}{s}\left\{\frac{1}{2N_es}\left(\frac{1}{N_es}\left[u(s) - \frac{1}{2N}\right] + \frac{1}{2N}(1 - \frac{1}{2N})\right) - \frac{u(s)}{6} + \frac{1}{8N^2}(1 - \frac{1}{3N})\right\}.$$

The expressions for $H(s)$ and $K(s)$ were given by Kimura (1969). Similarly, from (3) and (4), using the same method as Kimura (1969), the covariance of relative fitness and the squared deviation is

$$\text{Cov} = \text{Cov}(w, (z-\bar{z})^2) \equiv \int_0^\infty\int_{-\infty}^\infty h(a, s)\,C(s)\frac{s}{2}\frac{a^2}{4}dads, \tag{10}$$

with

$$C(s) \equiv \int_{1/(2N)}^{1-1/(2N)}\Phi(x; s)2x(1-x)(1-2x)dx = \frac{8N_e\lambda}{s}\left\{\frac{1}{N_es}\left[u(s) - \frac{1}{2N}\right] + \frac{1}{2N}(1 - \frac{1}{2N})\right\}.$$

The variance in fitness is

$$V_f = \int_0^\infty g(s)H(s)(s^2/4)\,ds. \tag{11}$$

As mutant alleles are unconditionally deleterious, the individuals with the more extreme genotypes with respect to the metric trait are less fit and thus selected against, which gives an appearance of stabilizing selection. Stabilizing selection is usually measured as the regression of relative fitness on squared phenotypic deviation from the optimum measured in phenotypic standard deviation units, given as

$$b_{w,P2} = V_P\text{Cov}(w, P2)/V_{P2} = V_P\text{Cov}(w, (z-z_m)^2)/V_{P2}, \tag{12}$$

where the phenotypic variance is $V_P = V_G + V_e$. The variance of squared phenotypic deviations is $V_{P2} = V_{G2} + 2V_e(V_e + 2V_G) = V_{G2} + 2V_P^2 - 2V_G^2$ assuming that environmental effects are normally distributed (Keightley and Hill 1990). Then the strength of stabilizing selection is explicitly expressed by

$$V_s = -1/(2b_{w,P2}) = -(m_4 + 2V_P^2)/(2V_P\text{Cov}). \tag{13}$$

It is worth noting that V_s defined here is different from the conventional one (*e.g.*, Turelli 1984), which is equivalent to V_sV_P. Using the conditional second and fourth moments given in appendix a, we can further reduce the above formulas as

$$V_G = \tfrac{1}{4}\int_0^\infty H(s)E[a^2|s]g(s)\,ds, \tag{14}$$

$$m_4 = \tfrac{1}{16}\int_0^\infty\{H(s) - 3K(s)\}E[a^4|s]g(s)\,ds, \tag{15}$$

$$\text{Cov} = \tfrac{1}{8}\int_0^\infty C(s)\,sE[a^2|s]g(s)\,ds. \tag{16}$$

In the following we first work out analytical results for V_G and V_s for infinite populations, and then numerical results are presented and discussed for finite populations.

ANALYTICAL RESULTS

Let us consider an infinite population such that $N_es \gg 1$, where the heterozygosity and other properties can be simplified to $H = C = 4\lambda/s$ and $K = 0$. It is straightforward to obtain the following properties from (14–16) and (6–7) if the distribution of mutation effects on fitness is not leptokurtic, *i.e.*, $\beta_s > 1$,

$$V_G = (2V_m/E[s])\left(b_0\beta_s/(\beta_s-1) + b_1\sqrt{\beta_s/(\beta_s+1)} + b_2\beta_s/(\beta_s+1)\right) = (2V_m/E[s])(1 - \Omega(\beta_s, \beta_a, \rho))\beta_s/(\beta_s-1), \tag{17}$$

$$m_4 = (V_m\varepsilon_a^2/(2E[s]))\Xi(\beta_s, \beta_a, \rho), \tag{18}$$

$$\text{Cov} = -(b_0 + \sqrt{\beta_s/(\beta_s+1)}\,b_1 + b_2)V_m = -V_m, \tag{19}$$

with

$$\Omega(\beta_s, \beta_a, \rho) = 2\rho(\sqrt{\beta_s/\beta_a} + \sqrt{\beta_a/\beta_s} + \sqrt{\beta_a\beta_s} - \rho)/((\beta_s+1)(\beta_a+1))$$

and

$$\Xi(\beta_s, \beta_a, \rho) = c_0\beta_s/(\beta_s-1) + (c_1 + c_3)\sqrt{\beta_s/(\beta_s+1)} + c_2\beta_s/(\beta_s+1) + c_4(\beta_s+2)/(\beta_s+1).$$

The strength of apparent stabilizing selection V_s can be obtained by putting all these into Equation 13. It is clearly seen that V_G and V_s are dependent on the bivariate distribution of mutation effects on the metric trait and on fitness, whereas Cov is not. These expressions clearly show that, as β_s decreases and approaches one, V_G and V_s tend to infinity (see Figure 2a).

If the distribution of mutation effects on fitness is leptokurtic, *i.e.*, $0 < \beta_s \le 1$, however, simple integration of Equations 13–16 shows that whenever $\rho < 1$, V_G and V_s would be infinite for an infinite population, as shown in simulations of Keightley and Hill (1990). This intrinsic difficulty of the pleiotropic model of continuous fitness effects (Hill and Keightley 1988; Keightley and Hill 1990), which is also a consequence of the assumption of infinite independent loci, is avoided when equal fitness effects are assumed for all mutants (Barton 1990; Kondrashov and Turelli 1992; Tanaka 1996). If variation of the fitness effects of mutation is to be retained, then this difficulty can be avoided by assuming a minimum fitness effect for any mutation, as suggested by Mackay *et al.* (1992). A mutation can be defined as any change in the base sequence of DNA in the genome. Theoretically any such mutation change

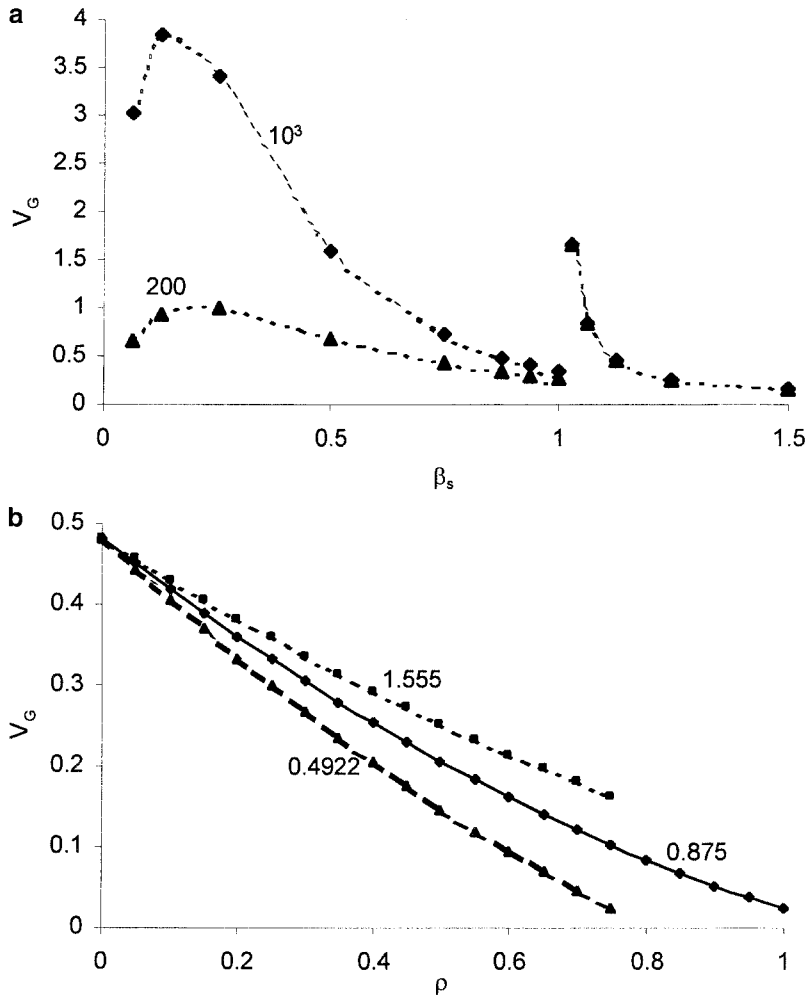

FIGURE 2.—Dependence of the genetic variance maintained in the metric trait on (a) the shape parameter of the fitness effects of mutations when $\rho = 0$ for different values of $E[s]/\Delta s$ and (b) the correlation between the absolute value of the mutation effects on the metric trait and fitness when $E[s]/\Delta s = 10^3$ and $\beta_s = 0.875$ for different values of β_a. The average fitness effect is $E[s] = 0.04$. Dashed lines are interpolations.

at the molecular level can affect the genome and thus the individual (KEIGHTLEY and HILL 1988; NIELSEN 2001). This minimum fitness effect exists in some way although it may be too small to be detected with current available equipment (DAVIES *et al.* 1999).

The relevant fundamental question is whether the distribution of the fitness effect of mutations is continuous or discrete. As KEIGHTLEY (1991) argued, "The assumption of a continuous distribution of allelic deviations is also unrealistic because these are likely to fall into discrete classes." These discrete classes are likely due to the fact that DNA sequence is discrete and that there may be a finite number of alleles and their effects could be discrete. As Max Planck did 101 years ago for avoiding "ultraviolet catastrophe" in black body radiation (*e.g.*, BRANSDEN and JOACHAIN 1992), we assume here that fitness effects of mutations are discretized as $n\Delta s$, where $n = 1, 2, \ldots$, and a "quantum" $\Delta s > 0$ is the possible minimum unit.

With the assumption of discretization of fitness effects of mutants, the properties for an infinite population can then be obtained by substituting (6–7) into (13–16) and (11),

$$V_G = \frac{\lambda}{\Delta s}\sum_{n=1}^{\infty}\frac{E[a^2|s = n\Delta s]}{n}g(n\Delta s)/\sum_{n=1}^{\infty}g(n\Delta s)$$
$$= \frac{2V_m}{\Delta s}\left\{b_0\phi_{-1} + b_1\frac{\Delta s}{\varepsilon_s} + b_2\frac{\Delta s^2}{\varepsilon_s^2}\phi_1\right\}, \quad (20)$$

$$m_4(s) = \frac{\lambda}{4\Delta s}\sum_{n=1}^{\infty}\frac{E[a^4|s = n\Delta s]g(n\Delta s)}{n}/\sum_{n=1}^{\infty}g(n\Delta s)$$
$$= \frac{V_m\varepsilon_a^2}{2\Delta s}\left\{c_0\phi_{-1} + c_1\frac{\Delta s}{\varepsilon_s} + c_2\frac{\Delta s^2}{\varepsilon_s^2}\phi_1 + c_3\frac{\Delta s^3}{\varepsilon_s^3}\phi_2 + c_4\frac{\Delta s^4}{\varepsilon_s^4}\phi_3\right\}, \quad (21)$$

$$\mathrm{Cov} = -\frac{\lambda}{2}\sum_{n=1}^{\infty}E[a^2|s = n\Delta s]g(n\Delta s)/\sum_{n=1}^{\infty}g(n\Delta s)$$
$$= -V_m\left\{b_0 + b_1\frac{\Delta s}{\varepsilon_s}\phi_1 + b_2\frac{\Delta s^2}{\varepsilon_s^2}\phi_2\right\}, \quad (22)$$

and the variance in fitness

$$V_f = \sum_{n=1}^{\infty}\frac{\lambda(n\Delta s)^2}{n\Delta s}g(n\Delta s)/\sum_{n=1}^{\infty}g(n\Delta s) = \lambda\Delta s\phi_1. \quad (23)$$

The definitions of $\phi_{-1}, \ldots, \phi_3$ and their approximations when the minimum fitness effect is very small compared to the standard deviation of fitness effects (*i.e.*, $\Delta s \ll \varepsilon_s$)

TABLE 1

The genetic variance maintained in an infinite population at mutation-selection balance and strength of apparent stabilizing selection under the extreme cases ρ = 0 and 1

β_s	V_G	$\tilde{V}_s$ [b]	V_s
		$\rho = 0$	
Equal[a]	$\frac{2V_m}{E[s]} (\equiv V_G^{eq})$	$\frac{2}{E[s]h^2} (\equiv V_s^{eq1})$	
$0 < \beta_s < 1$[c]	$\Theta(\Delta s) V_G^{eq}$	$\Theta(\Delta s) V_s^{eq1}$	
1	$\ln(\frac{E[s]}{\Delta s}) V_G^{eq}$	$\ln(\frac{E[s]}{\Delta s}) V_s^{eq1}$	$\frac{3\kappa}{4\lambda}h^2 + \tilde{V}_s$
>1	$\frac{\beta_s}{\beta_s - 1} V_G^{eq}$	$\frac{\beta_s}{\beta_s - 1} V_s^{eq1}$	
		$\rho = 1$	
$0 < \beta_s$	$\frac{\beta_s}{\beta_s + 1} V_G^{eq}$	$\frac{\beta_s}{\beta_s + 1} V_s^{eq1}$	$\frac{\beta_s + 2}{4\lambda\beta_s}h^2 + \tilde{V}_s$

[a] The same results as Barton (1990). Here $E[s] = \Delta s$ for equal mutation effect.

[b] $\tilde{V}_s$ is the strength of apparent stabilizing selection when $\lambda \gg 1$ and $\tilde{V}_s = V_P/V_m$. The approximate for $\lambda \ll 1$ is $V_s \approx 3\kappa h^2/(4\lambda)$ for $\rho = 0$. In general, the inequality $V_s > \tilde{V}_s$ holds and thus generally $V_s \geq V_P/V_m$. For a gamma distribution of mutation effects on the metric trait the kurtosis is $3\kappa = (\beta_a + 2)(\beta_a + 3)/(\beta_a(\beta_a + 1))$. The shape parameters β_s and β_a for both marginal Γ distributions can be different.

[c] $\Theta(\Delta s) = -\beta_s/(1 - \beta_s) + (C_0\beta_s^{\beta_s}/(C_1(1 - \beta_s)))(E[s]/\Delta s)^{1-\beta_s}$.

are given in appendix b. It is thus straightforward to get the genetic variance and strength of apparent stabilizing selection. For parameter $\beta_s = 1$,

$$V_G = \frac{2V_m}{E[s]}\left\{\ln(\frac{E[s]}{\Delta s})\, b_0 + \frac{1}{\sqrt{2}}b_1 + \frac{1}{2}b_2\right\}, \quad (17')$$

$$m_4 = \frac{V_m\varepsilon_a^2}{2E[s]}\left\{\ln(\frac{E[s]}{\Delta s})\, c_0 + \frac{1}{\sqrt{2}}(c_1 + c_3) + \frac{1}{2}c_2 + \frac{3}{2}c_4\right\}, \quad (18')$$

while for $0 < \beta_s < 1$,

$$V_G = \frac{2V_m}{E[s]}\frac{\beta_s}{1 - \beta_s}\left\{\frac{C_0\beta_s^{\beta_s - 1}}{C_1}\left(\frac{E[s]}{\Delta s}\right)^{1-\beta_s} b_0 - 1 + \Omega(\beta_s, \beta_a, \rho)\right\}, \quad (17'')$$

$$m_4 = \frac{V_m\varepsilon_a^2}{2E[s]}\left\{\frac{C_0\beta_s^{\beta_s}}{C_1(1 - \beta_s)}\left(\frac{E[s]}{\Delta s}\right)^{1-\beta_s} c_0 + \Xi(\beta_s, \beta_a, \rho)\right\}. \quad (18'')$$

Cov is still given by (19). These equations show clearly that for $\beta_s \leq 1$, V_G and m_4 can be divided into two parts: one is dependent on Δs and the other is not. V_G and V_s are independent of Δs only if $\rho = 1$. This is because the selection in this special case is acting on the absolute value of the trait, which is different from albeit similar to the real stabilizing selection on the trait, which was assumed to act on the squared deviation of the trait (Turelli 1984). Otherwise, if $\rho < 1$, V_G and V_s are dependent on Δs and thus can become infinite in an infinite population. The same results as Equations 17″ and 18″ can also be obtained for $\beta_s > 1$ by this method of discrete approximation. In this situation V_G and V_s remain finite, due to the fact that the numbers of neutral mutations in this case are actually null (see Figure 1). This indicates clearly that the unlimited increase of V_G is due to the accumulation of essentially neutral alleles of large phenotypic effect (Kondrashov and Turelli 1992).

The covariance between the relative fitness and squared deviation is always independent of Δs, the correlation ρ, and shape parameter β_s, being equal to the negative of the mutational variance per generation (*cf.* Bürger 2000, p. 310). Combining (13) and (19) leads to a general constraint on V_P and V_s,

$$V_s \geq V_P/V_m, \quad (24)$$

which in principle makes the pleiotropic model impossible to simultaneously explain high genetic variance and high heritability and strong apparent stabilizing selection. The variance in fitness, $V_f = \lambda\beta_s/\alpha_s = \lambda\varepsilon_s \times \sqrt{\beta_s/(\beta_s + 1)} = \lambda E[s]$, being independent of Δs, decreases as the distribution of mutation fitness effects becomes more leptokurtic. One surprising point is that V_f is proportional to the product $\lambda\varepsilon_s$ rather than $\lambda\varepsilon_s^2$.

It is interesting to compare our results with Barton's (1990), who assumes equal fitness for all mutations and thus no correlation between the absolute values of mutant effects on the metric trait and on fitness. In our notation, this means $s = \Delta s$ (*i.e.*, the minimum effect of mutations is the exclusive effect) and $g(s) = \delta(s - \Delta s)$, where $\delta(\cdot)$ is the Dirac delta function. The same results as Barton (1990) can be obtained from (13–16). If the fitness effects vary across mutations and are distributed as a gamma random variable, then the results thus obtained are different from Barton's (1990; see Table 1). In our results for V_G and V_s, there are two parameters relating to variation of mutation fitness effects: the minimum fitness effect, Δs, and variability of the fitness effects of mutations, ε_s (or equivalently the mean fitness effect $E[s]$). For shape parameter $\beta_s > 1$, the genetic variances are $\beta_s/(\beta_s - 1)$ times that of Barton's (1990) for the same mean fitness effects (see Table 1). As β_s approaches 1, V_G tends to infinity. For $\beta_s = 1$ (*i.e.*, exponential distribution), however, V_G is limited if Δs is finite. If the distribution of mutation effects on fitness is highly leptokurtic (*i.e.*, $\beta_s < 1$), then the genetic variance can also be much larger than that of Barton's (1990) for the same mean fitness effects (see Figure 2a). For instance, if $\beta_s = 1/4$, this increase is ~12-fold if $\Delta s = E[s]/10^2$ and 69-fold if $\Delta s = E[s]/10^3$. The results in Figure 2 show

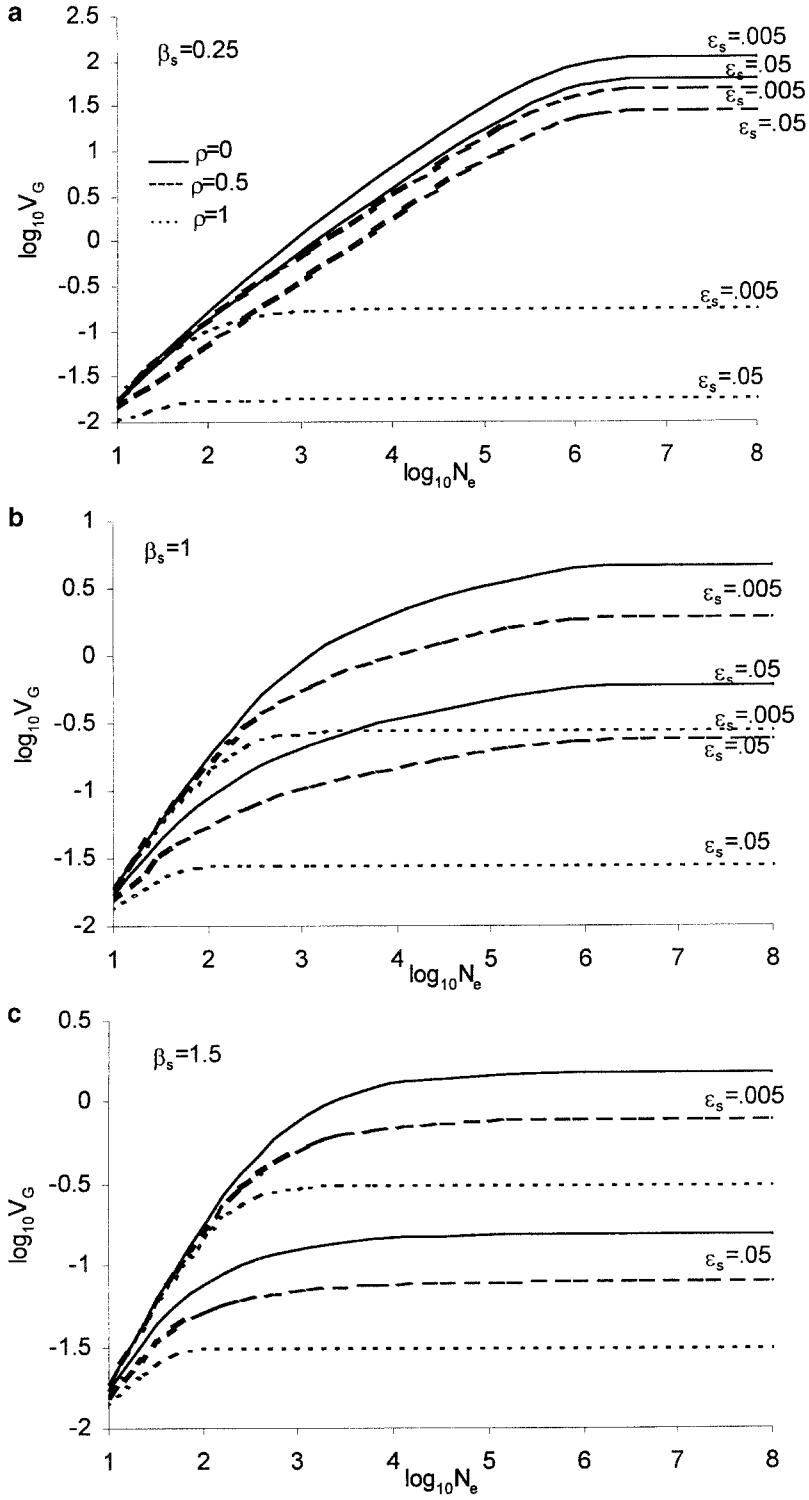

FIGURE 3.—Genetic variance maintained in the metric trait as a function of the effective population size under the assumption of a discrete distribution of mutation fitness effect. The new mutational variance in the metric trait is $V_m = 10^{-3}$ and the minimum fitness effect of mutations is $\Delta s = 10^{-6}$. Three different values of β_s, the shape parameter of the distribution of mutation fitness effects, are investigated. Results are shown for two strengths of fitness selection, ε_s, and three correlations ρ.

that the shape of distribution of fitness effects affects the genetic variance. At the limit $\beta_s \to 0$, almost all mutations have the same effect Δs; whereas when $\beta_s \to \infty$, the kurtosis of the distribution of mutation effects on fitness $3\kappa \to 1$ (*i.e.*, equal effects). Both extreme situations return to BARTON's (1990) results (Table 1 and Figure 2a). Clearly $\beta_s = 1$ is a critical point.

An increasing correlation between $|a|$ and s always reduces V_G (see Figure 2b). The marginal distributions of mutation effects on the trait and on fitness may be different, which does not cause any difference to the genetic variance if $\rho = 0$. If there is some correlation between the absolute values of mutation effects on the trait and on fitness, however, the genetic variance would be affected. The example shown in Figure 2b displays that when the correlation is intermediate, the impact

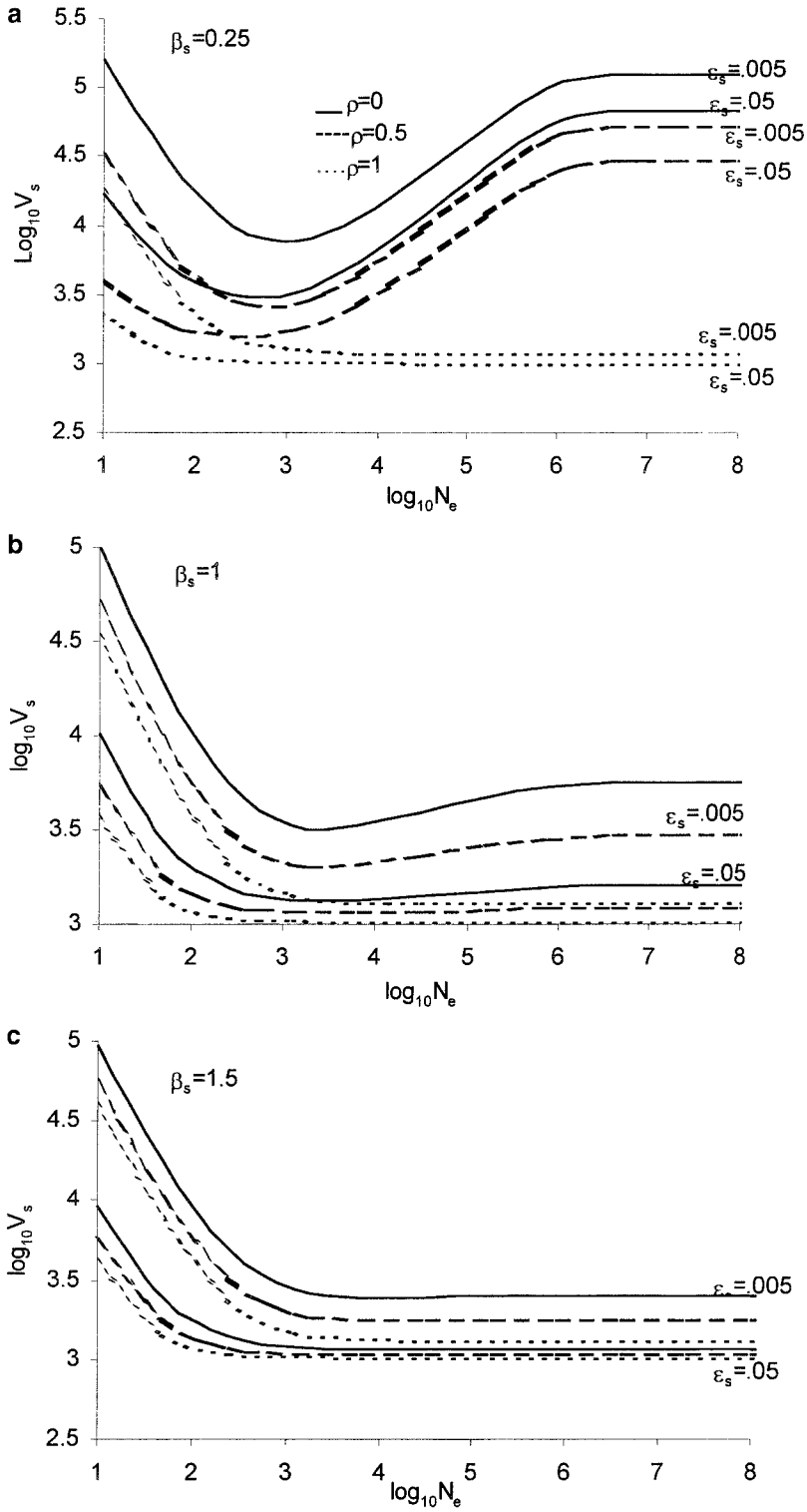

FIGURE 4.—Strength of apparent stabilizing selection as a function of the effective population size, N_e. The mutation rate $\lambda = 0.2$. Other parameters are the same as in Figure 3.

is not large even with a large difference in distributions, *i.e.*, $\beta_s/\beta_a = \frac{1}{2}, 2$. For convenience, we consider only the situations where $\beta_s = \beta_a$ in the following numerical investigation. As there exists a general relation (24), the high variation in the metric trait can be maintained only under weak selection; if mutation effects on fitness vary across loci substantially more genetic variance is induced, but the strong apparent stabilizing selection is still not achieved.

NUMERICAL RESULTS

The effective sizes of some natural populations, especially those of large vertebrates, are unlikely to be large

TABLE 2

The influence of the shape parameter β_s on V_G and V_s under the assumption of discrete mutation fitness effects

β_s	1/8		1/4		1/2		1		1.125		1.5	
$\rho = 0$												
ε_s	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05
V_G	1.1	0.87	1.22	0.762	1.13	0.472	0.90	0.21	0.86	0.18	0.77	0.13
V_s	10010	4580	7449	3091	5054	1936	3454	1352	3263	1298	2877	1204
0.5												
ε_s	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05
V_G	0.61	0.42	0.667	0.357	0.638	0.216	0.55	0.10	0.54	0.091	0.51	0.071
V_s	2551	1864	2653	1691	2423	1367	2151	1163	2112	1144	2032	1111
1												
ε_s	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05	0.005	0.05
V_G	0.139	0.014	0.171	0.018	0.221	0.023	0.27	0.028	0.28	0.029	0.30	0.031
V_s	1118	937	1274	1021	1373	1036	1476	1044	1493	1045	1534	1048

The minimum fitness of mutations was assumed to be $\Delta s = 10^{-6}$ and effective population size $N_e = 1000$. The mutation rate is large enough (*e.g.*, $\lambda > 1$) to ensure that V_s approaches its asymptotes.

and appear to be of the order of 10^3–10^4 (Frankham 1995; Yu *et al.* 2001). Furthermore, the distribution of mutation effects (s) could be leptokurtic, with most mutations having only slightly deleterious effects (Keightley 1994). Therefore $N_e s$ may not always be much larger than unity and the dynamics of most mutations in natural populations may actually be dominated by drift rather than selection, so it is important to consider population size and genetic drift in models on the maintenance of polygenic variation.

The analytical expressions for V_G and V_s are difficult to obtain when $N_e s \sim 1$, which might be typical values for most mutations in natural populations. The calculations using (13–16) when the correlations between $|a|$ and s are not unity show that if the continuous distribution of mutant effects on fitness is assumed, the genetic variance continues to increase as the effective population size increases. This is still true even for other distributions (*e.g.*, normal) of fitness effects in which nearly neutral mutants are not predominant. If the mutant effects on fitness are discretized with the minimum effect Δs, then the genetic variance and the strength of apparent stabilizing selection would approach the asymptotes that were determined by Equations 17″ and 18″ and are shown in Figures 3 and 4. The minimum effect Δs in Figures 3 and 4 was set to 10^{-6}. If it was changed to another value, *e.g.*, 10^{-8}, the trend of V_G and V_s with N_e for $\beta_s \leq 1$ would be the same except that V_G and V_s would asymptote at a population size of $\sim 10^8$ rather than $\sim 10^6$.

Experimental data regarding the joint distributions of both mutation effects on the metric trait and on fitness or even the marginal distributions are scant (*e.g.*, Mackay *et al.* 1992; Keightley *et al.* 2000). An important theoretical question is whether genetic variation and strength of apparent stabilizing selection are sensitive to the shape of distributions of mutation effects on the metric trait and fitness. For this purpose the situations of different β_s have been investigated, and the results are shown in Table 2. The difference is evident. If the correlation $\rho < 1$ and $\varepsilon_s = 0.05$, the genetic variance maintained at MSB decreases (*e.g.*, from 0.87 to 0.13 for $\rho = 0$) and the selection becomes strong (*e.g.*, V_s decreasing from 4580 to 1204 for $\rho = 0$) as the value of the shape parameter β_s increases (*e.g.*, from 0.125 to 1.5). If $\varepsilon_s = 0.005$, the trend of V_G and V_s is the same as that for $\varepsilon_s = 0.05$ except for a maximum between $\beta_s = 0.125$ and 0.25 (*cf.* Figure 2a). If the correlation is equal to unity, the opposite trend occurs; *i.e.*, V_G and V_s increase with β_s (*cf.* Table 1). It is clear in Figures 3 and 4 that V_G increases monotonically and approaches an asymptote as the population size increases for all the values of the shape parameter β_s. If $\beta_s \leq 1$, V_G approaches an asymptote as the population size reaches $\sim 1/\Delta s$. If $\beta_s > 1$, V_G approaches an asymptote independently of the size of the minimum fitness Δs assumed. This, in agreement with the analytical results above for infinite populations (Figure 2 and Table 1), results from the sharp difference between the distribution of mutation fitness effects of $\beta_s > 1$ and $\beta_s \leq 1$. The former has few nearly neutral mutants while the latter has predominately mutants of $s \sim 0$ (see Figure 1). Thus the differences between $\beta_s > 1$ and $\beta_s \leq 1$ are qualitative, while the differences among values of $\beta_s \leq 1$ or among values of $\beta_s > 1$ are only quantitative.

The way in which V_s approaches its asymptote with the effective population size N_e is more complicated (see Figure 4), as V_s can increase or decrease with N_e. For a finite population with a fixed flux of mutations, the strength of apparent stabilizing selection is determined by the interplay of the genetic drift and selection. Because the strength of selection is dependent on $N_e s$

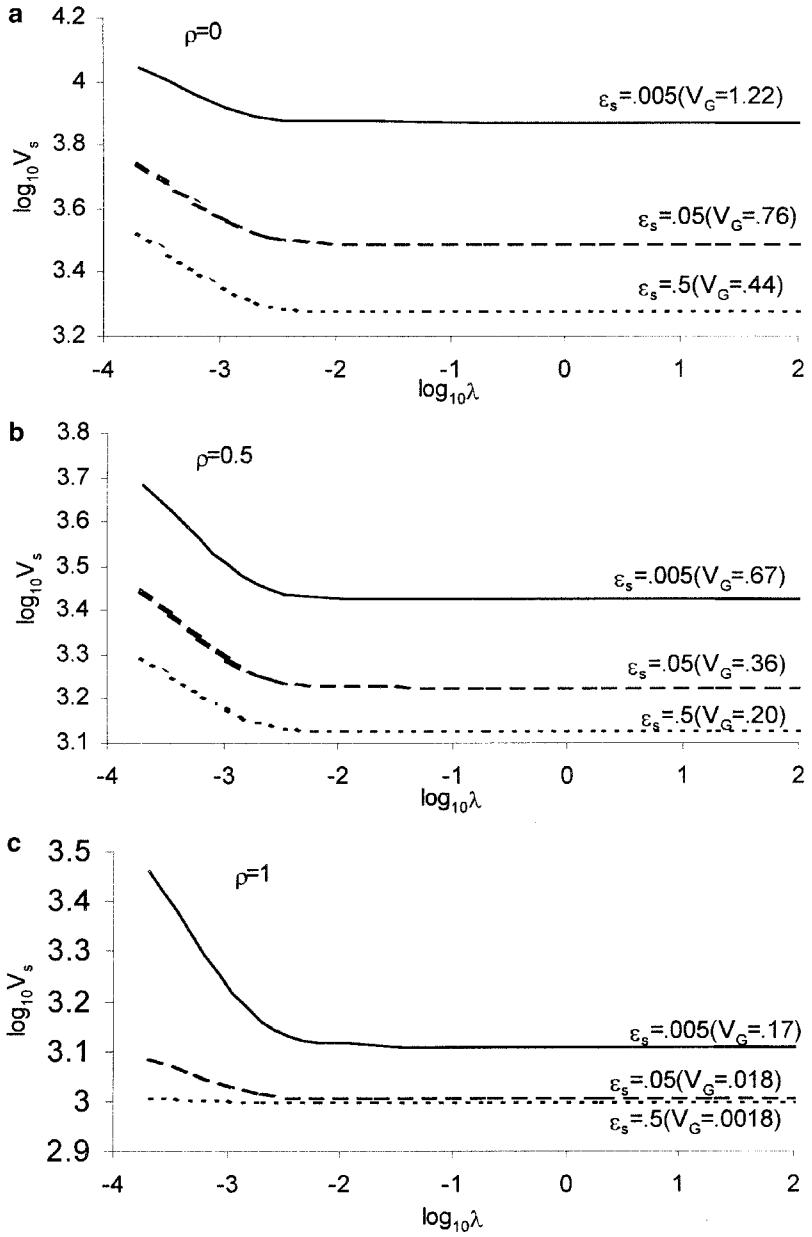

FIGURE 5.—Strength of apparent stabilizing selection at MSB as a function of the mutation rate λ under the assumption of a discrete distribution of mutation effect on fitness. The minimum mutation fitness effect is $\Delta s = 10^{-6}$. Results are shown for three strengths of fitness selection, ε_s, and three correlations ρ between the effects on the metric trait and on fitness. The distribution of mutation effects on fitness is gamma with $\beta_s = 1/4$. Curves for other values of shape parameter β_s (*e.g.*, 0.5, 1, 1.5) are similar.

rather than purely on *s*, selection is predominant and genetic drift can be ignored only when the population size is large. When the population size is not large, the interplay between genetic drift and selection is complicated, which may lead to a different relationship between V_s and N_e for different values of other parameters [*e.g.*, variability of fitness effects of mutations (ε_s), the correlation (ρ), and the mutation rate (λ)]. With $\rho = 1$, V_s decreases to its limiting value as N_e increases. For the situations with $\rho < 1$, the trend of V_s with N_e is complicated. If there are few nearly neutral mutants (*i.e.*, $\beta_s > 1$), V_s decreases and asymptotes as N_e increases, due to the fact that $N_e s$ increases with N_e and soon predominates over the genetic drift. If there are predominantly neutral mutants (*i.e.*, $\beta_s \leq 1$), V_s first decreases and approaches a minimum and then increases to a limiting value with N_e. In this case, $N_e s$ may not significantly increase with N_e because most mutants are neutral or slightly deleterious. If the population size is too small, the dynamics of mutant alleles are controlled mainly by genetic drift. As N_e increases, selection first becomes stronger and then weakens and V_s finally asymptotes as $N_e \Delta s > 1$.

It is also evident in Figures 3 and 4 that V_G and V_s decrease as the correlation, ρ, or ε_s increases, in agreement with analytical results for infinite population (see Figure 2a and Table 1). As $C(s) < 4\lambda/s$, the absolute value of covariance of relative fitness and the squared deviation is $< V_m$ for a finite population (*cf.* Equation 19), which sets up a constraint between V_G and V_s (see Equation 24). Figures 3 and 4 show that V_G can be high enough but V_s is always $>10^3$ (*i.e.*, the inverse of V_m).

As long as the mutational variance increment per generation is given as $V_m = \frac{1}{2}\lambda\varepsilon_a^2 = 10^{-3}V_e$, then the genetic variance is in theory independent of the rate and effects of mutations. This can be seen from (14) and (6). However, different mutation rates may lead to different degrees of apparent stabilizing selection on the population for the same genetic variations retained. In the numerical examples, the effective population size was set to 10^3, and the minimum fitness effect of mutations was set to 10^{-6}. The results shown in Figure 5 show that V_s decreases quickly as the mutation rate λ increases and approaches an asymptote as λ exceeds some value (*e.g.*, 10^{-2} in our examples). In other words, apparent stabilizing selection is weak if the number of mutations segregating in the population becomes few and increases to an asymptote as mutations become numerous (*cf.* Keightley and Hill 1990).

DISCUSSION

Comparison with other models of maintenance of variation: A general pleiotropic model of variation maintained at MSB has been analyzed in this article. The mutants affecting the metric trait of interest also have a deleterious effect on the individual who carries them, and so, because extreme genotypes tend to be less fit, the metric trait appears to be under stabilizing selection. Assuming additive gene action across and within loci and linkage equilibrium, the genetic variance and strength of apparent stabilizing selection have been obtained. The mutation effects on the metric trait and on fitness both vary among loci and are assumed to be distributed as a bivariate gamma with any shape parameter. By employing diffusion approximations under the infinite independent loci model (Kimura 1969), formulas for V_G and V_s have been obtained, and results are in agreement with previous Monte Carlo simulations using a similar multilocus model (Keightley and Hill 1990). Compared to Keightley and Hill (1990) who assumed the heritability of a typical value $\frac{1}{3}$, the heritability in this study varies with the genetic variance and is given by $V_G/(V_G + V_e)$.

Analysis shows that the unlimited increase of V_G and V_s (Keightley and Hill 1990) disappears only when the shape parameter of distribution of the mutation effects on fitness is greater than one, in which there are few nearly neutral mutations. To avoid the unlimited increase of V_G for the highly leptokurtic distribution of mutation effects on fitness (*i.e.*, $\beta_s \leq 1$), fitness effects were assumed to have discrete values, $s = n\Delta s$, $n = 1, 2, \ldots$ with the minimum effect $\Delta s > 0$. Analysis for the infinite population limit reveals that V_G and V_s are proportional to the product of $(\Delta s)^{\beta_s - 1}$ and $E(s)^{-\beta_s}$. Compared with Barton's (1990) results, variation in fitness effects can induce much higher genetic variance at MSB for the population with the same mean fitness effect $E[s]$. If only a few deleterious alleles affecting the metric trait are segregating in the population, the apparent stabilizing selection is weaker than that of the house-of-cards rare allele model (Turelli 1984; Barton 1990). If there are numerous mutant alleles segregating in the population, the constraint on V_G and V_s is similar to that of other pleiotropic models (Kondrashov and Turelli 1992; Gavrilets and de Jong 1993; Tanaka 1996), approximated by $V_s = V_P/V_m$ (in the traditional definition of V_s: $V_s/V_G = V_G/V_m$).

The influence of the correlation between absolute values of mutation effects on the metric trait and fitness on V_G and V_s was assessed. In the extreme case of $\rho = 1$, the selection becomes strongest and correspondingly the variance maintained in the metric trait shrinks. In general, an increase in the correlation leads to reduction in both V_G and V_s, as found by Keightley and Hill (1990). Numerical investigations on the dependence of V_G and V_s on the effective population size N_e show that V_G increases to an asymptote as N_e increases and reaches an order of $1/\Delta s$. This prediction implies that the genetic variances depend greatly on N_e if $N_e < O(1/\Delta s)$. In other words, $1/\Delta s$ would be a measure of the sensitive size of population to the genetic variances if leptokurtically distributed mutations were assumed.

As (13–16) show, the genetic variance and strength of apparent stabilizing selection depend only on the squared deviations of mutation effects on the metric trait from the optimum. Thus the assumption of a symmetrical distribution of the mutation effects on the metric trait about zero is not of significance, confirmed by Monte Carlo simulations, but if the mean effect differs from zero, random genetic drift would lead to a directional change in the population mean. The impact of dominance of mutant alleles is not important if the degrees of dominance of the mutant effects on the metric trait and on fitness are the same. If they differ and if, for example, effects on fitness are purely recessive while effects on the metric trait are partially dominant, the genetic variance would increase and the apparent stabilizing selection would become weaker.

Comparison with observations: Although mutations are important to many phenomena and processes, including the maintenance of variability, estimates of mutation rate (λ) and average mutation effects (ε_s, ε_a) are imprecise. Data suggest that the total deleterious mutation rate is >1 in mammals and ~ 1 in flowering plants (Kondrashov 1998). The scanty data for multicelluar eukaryotes are consistent with any value of λ between 0.1 and 100 (Kondrashov and Turelli 1992). Recent studies on *Caenorhabditis elegans*, however, show that the mutation rate for life history traits is $\ll 1.0$ and is of the order 10^{-3} (Keightley and Caballero 1997; Vassilieva and Lynch 1999). The best estimate of the average selection coefficient against heterozygous mutations is $E[s/2] = 0.02$ (Crow and Simmons 1983). Data for Drosophila bristle traits show that λ is in the range 0.09–1.0 and ε_s in the range 0.01–0.2 (Keightley and

Hill 1990; Caballero and Keightley 1994). Data for competitive viability in Drosophila suggest that $\lambda \geq 0.01$ and $E[s] \leq 0.08$ (Chavarrias *et al.* 2001). Data for yeast *Saccharomyces cerevisiae* show that λ is of the order 10^{-3} and $E[s/2]$ in the range 0.01 and 0.05 (Wloch *et al.* 2001).

Estimates of the strength of natural selection in natural populations have been summarized by Turelli (1984), Endler (1986), Crow (1989), and Kingsolver *et al.* (2001). Endler's (1986) survey indicates that stabilizing selection in natural populations is quite common and strong, with typical estimates of the strength $V_s < 20V_e$ (Turelli 1984). The recent synthesis by Kingsolver *et al.* (2001) shows, however, the distribution of estimated quadratic selection gradients is symmetric about zero, so the mean is nearly zero, with the mean of the absolute values ~0.1. Further, only ~16% of the negative values of quadratic selection gradients are reported as significantly different from zero at $p = 0.05$ and in most cases where there are significant values there is also significant directional selection on the same trait. Thus the estimates provide little evidence for detectable stabilizing selection, given the limited power of the available evidence. Kingsolver *et al.* (2001) thus suggest that stabilizing selection is typically quite weak and not more common than disruptive selection.

To maintain abundant heritability the genetic variance should be of the order of $10^3 V_m$ as experimental data typically show $V_m \sim 10^{-3}V_e$ (Hill 1982; Houle *et al.* 1996; Lynch and Walsh 1998). This requires in Barton's (1990) model that $E[s](=\Delta s) \sim 2 \times 10^{-3}$, which is much smaller than the observed values ~0.04–0.08. However, our model can produce abundant heritability for such values if the minimum fitness effect is assumed to be in the range of 1/1000–1/100 of the average effect (see Figure 2). These values of Δs, albeit being smaller than current experiments can detect (Wloch *et al.* 2001), do not seem unreasonable. Thus allowing deleterious effects to vary among alleles affects the results and gives a possible explanation of abundant heritability.

For a population of size $N_e = 10^3$ and the mutation rate and average mutation effect on fitness in the ranges suggested by the experimental observations, the expectations of V_s (see Table 2 and Figures 4 and 5) are much larger than the typical values suggested by Turelli (1984). Moreover, there exists a general constraint, $V_s \geq V_P/V_m$ for infinite populations. This leads to the same conclusion as other pleiotropic models (Barton 1990; Kondrashov and Turelli 1992; Gavrilets and de Jong 1993; Tanaka 1996) that mutation-selection balance cannot simultaneously explain both abundant heritability and strong stabilizing selection if $V_m \sim 10^{-3}V_e$. Nevertheless, if the suggestion of Kingsolver *et al.* (2001) that the apparent stabilizing selection is "typically quite weak" is true, then this contradiction between the heritability and stabilizing selection disappears. The pleiotropic model studied here has no difficulty in providing a satisfactory explanation to maintenance of variation in the metric trait that is under quite weak apparent stabilizing selection. A "pure" pleiotropic model such as investigated here, in which there is no real stabilizing selection or other force maintaining the variance of the metric trait, would, however, lead to drift in its mean due to random fixation of mutants.

As pointed out by Kondrashov (1998) and Kingsolver *et al.* (2001), the estimates of mutation rates and selection effects are not very reliable. In the theoretical investigations of polygenic variation and stabilizing selection, both variances and strengths at MSB are assessed simultaneously. However, data on mutation rates and effects, heritabilities, and strengths of stabilizing selection that were used to compare with the theoretical predictions are collected, in most cases, from separate experiments. If the rates and effects of the base change in DNA sequences were universal, this might not cause any serious deviation. Although difficult, it would be desirable to design an experiment in which both mutation and selection parameters could be estimated with adequate precision at the same time.

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LITERATURE CITED

Barton, N. H., 1990 Pleiotropic models of quantitative variation. Genetics **124:** 773–782.

Barton, N. H., and P. D. Keightley, 2002 The nature of quantitative genetic variation. Nat. Genet. **3:** 11–21.

Bürger, R., 2000 *The Mathematical Theory of Selection, Recombination, and Mutation.* Wiley, Chichester, UK.

Bransden, B. H., and C. J. Joachain, 1992 *Introduction to Quantum Mechanics.* Longman Scientific & Technical, Harlow, UK.

Caballero, A., and P. D. Keightley, 1994 A pleiotropic nonadditive model of variation in quantitative traits. Genetics **138:** 883–900.

Charlesworth, B., R. Lande and M. Slatkin, 1982 A neo-Darwinian commentary on macroevolution. Evolution **36:** 474–498.

Chavarrias, D., C. López-Fanjul and A. García-Dorado, 2001 The rate of mutation and the homozygous and heterozygous mutational effects for competitive viability: a long-term experiment with *Drosophila melanogaster.* Genetics **158:** 681–693.

Cherian, K. C., 1941 A bivariate correlated gamma-type distribution function. J. Indian Math. Soc. **5:** 133–144.

Crow, J. F., 1989 Fitness variation in natural populations, pp. 91–97 in *Evolution and Animal Breeding*, edited by W. G. Hill and T. F. C. Mackay. C.A.B. International, Wallingford, UK.

Crow, J. F., and M. Kimura, 1970 *An Introduction to Population Genetics Theory.* Harper & Row, New York.

Crow, J. F., and M. J. Simmons, 1983 The mutation load in *Drosophila*, pp. 1–35 in *The Genetics and Biology of Drosophila*, edited by H. L. Carson, M. Ashburner and J. N. Thompson. Academic Press, New York.

Davies, E. K., A. D. Peters and P. D. Keightley, 1999 High frequency of cryptic deleterious mutations in *Caenorhabditis elegans.* Science **285:** 1748–1751.

De Visser, J. A. G. M., and R. F. Hoekstra, 1998 Synergistic epistasis between loci affecting fitness: evidence in plants and fungi. Genet. Res. **71:** 39–49.

Elena, S. F., and R. E. Lenski, 1997 Test of synergistic interaction among deleterious mutations in bacteria. Nature **390:** 395–398.

Elena, S. F., and A. Moya, 1999 Rate of deleterious mutation and

the distribution of its effects on fitness in vesicular stomatitis virus. J. Evol. Biol. **12:** 1078–1088.
Endler, J. A., 1986 *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Longman, Harlow, UK.
Frankham, R., 1995 Effective population size/adult population size in wildlife: a review. Genet. Res. **66:** 95–107.
Gavrilets, S., and G. de Jong, 1993 Pleiotropic models of polygenic variation, stabilizing selection and epistasis. Genetics **134:** 609–625.
Gillespie, J. H., 1984 Pleiotropic overdominance and the maintenance of genetic variation in polygenic characters. Genetics **107:** 321–330.
Gillespie, J. H., and M. Turelli, 1989 Genotype-environment interaction and the maintenance of polygenic variability. Genetics **121:** 129–138.
Gimelfarb, A., 1990 How much genetic variation can be maintained by genotype-environment interactions? Genetics **124:** 443–445.
Hill, W. G., 1982 Predictions of response to artificial selection from new mutations. Genet. Res. **40:** 255–278.
Hill, W. G., and A. Caballero, 1992 Artificial selection experiments. Annu. Rev. Ecol. Syst. **23:** 287–310.
Hill, W. G., and P. D. Keightley, 1988 Interrelations of mutation, population size, artificial and natural selection, pp. 57–70 in *Proceedings of the Second International Conference on Quantitative Genetics*, edited by B. S. Weir, E. J. Eisen, M. M. Goodman and G. Namkoong. Sinauer, Sunderland, MA.
Houle, D., B. Morikawa and M. Lynch, 1996 Comparing mutational variabilities. Genetics **143:** 1467–1483.
Imhof, M., and C. Schlötterer, 2001 Fitness effects of advantageous mutations in evolving *Escherichia coli* populations. Proc. Natl. Acad. Sci. USA **98:** 1113–1117.
Keightley, P. D., 1991 Genetic variance and fixation probabilities at quantitative trait loci in mutation-selection balance. Genet. Res. **58:** 139–144.
Keightley, P. D., 1994 The distribution of mutation effects on viability in *Drosophila melanogaster*. Genetics **138:** 1315–1322.
Keightley, P. D., and A. Caballero, 1997 Genomic mutation rate for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **94:** 3823–3827.
Keightley, P. D., and W. G. Hill, 1988 Quantitative genetic variability maintained by mutation-stabilizing selection balance in finite populations. Genet. Res. **52:** 33–43.
Keightley, P. D., and W. G. Hill, 1990 Variation maintained in quantitative trait with mutation-selection balance: pleiotropic side-effects on fitness traits. Proc. R. Soc. Lond. Ser. B **242:** 95–100.
Keightley, P. D., E. K. Davies, A. D. Peters and R. G. Shaw, 2000 Properties of ethylmethane sulfonate-induced mutations affecting life-history traits in *Caenorhabditis elegans* and inferences about bivariate distributions of mutation effects. Genetics **156:** 143–154.
Kimura, M., 1962 On the probability of fixation of mutant genes in a population. Genetics **47:** 713–719.
Kimura, M., 1965 A stochastic model concerning the maintenance of genetic variability in quantitative characters. Proc. Natl. Acad. Sci. USA **54:** 731–736.
Kimura, M., 1969 The number of heterozygous nucleotide sites maintained in a finite population due to steady flux of mutations. Genetics **61:** 893–903.
Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri *et al.*, 2001 The strength of phenotypic selection in natural populations. Am. Nat. **157:** 245–261.
Kondrashov, A. S., 1998 Measuring spontaneous deleterious mutation process. Genetica **102/103:** 183–197.
Kondrashov, A. S., and M. Turelli, 1992 Deleterious mutations, apparent stabilizing selection and the maintenance of quantitative variation. Genetics **132:** 603–618.
Kotz, S., N. Balakrishnan and N. L. Johnson, 2000 *Continuous Multivariate Distributions, Vol. 1. Model and Applications*, Ed. 2. (Wiley Series in probability and statistics). John Wiley & Sons, New York.
Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.
Mackay, T. F. C., and C. H. Langley, 1990 Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. Nature **348:** 64–66.
Mackay, T. F. C., R. F. Lyman and M. S. Jackson, 1992 Effects of P elements on quantitative traits in *Drosophila melanogaster*. Genetics **130:** 315–332.
Nielsen, R., 2001 Statistical tests of selective neutrality in the age of genomics. Heredity **86:** 641–647.
Robertson, A., 1956 The effect of selection against extreme deviants based on derivation or on homozygosis. J. Genet. **54:** 236–248.
Robertson, A., 1967 The nature of quantitative genetic variation, pp. 265–280 in *Heritage From Mendel*, edited by R. A. Brink. University of Wisconsin Press, Madison, Wisconsin.
Roff, D. A., and T. A. Mousseau, 1987 Quantitative genetics and fitness: lessons from *Drosophila*. Heredity **58:** 103–118.
Schmeiser, B. W., and R. Lal, 1982 Bivariate Gamma random vectors. Oper. Res. **30:** 355–374.
Shaw, R. G., D. L. Byers and E. Darmo, 2000 Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. Genetics **155:** 369–378.
Sheldon, M. R., 2000 *Introduction to Probability Models*, Ed. 7, pp. 60–61. Harcourt Academic Press, New York.
Slatkin, M., 1979 Frequency- and density-dependency selection on a quantitative character. Genetics **93:** 755–771.
Tanaka, Y., 1996 The genetic variance maintained by pleiotropic mutation. Theor. Popul. Biol. **49:** 211–231.
Turelli, M., 1984 Heritable genetic variation via mutation-selection balance: Lerch's Zeta meets the abdominal bristle. Theor. Popul. Biol. **25:** 138–193.
Turelli, M., 1985 Effects of pleiotropy on predictions concerning mutation-selection balance for polygenic traits. Genetics **111:** 165–195.
Vassilieva, L. L., and M. Lynch, 1999 The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. Genetics **151:** 119–129.
Whittaker, J., 1974 Generating gamma and beta random variables with non-integral parameters. Appl. Stat. **23:** 211–214.
Wloch, D. M., K. Szafraniec, R. H. Borts and R. Korona, 2001 Direct estimate of the mutation rate and the distribution of fitness effects in the yeast *Saccharomyces cerevisiae*. Genetics **159:** 441–452.
Wright, S., 1935 The analysis of variance and the correlations between relatives with respect to deviations from an optimum. J. Genet. **30:** 243–256.
Yu, N., Z. Zhao, Y.-X. Fu, N. Sambuughin, M. Ramsay *et al.*, 2001 Global patterns of human DNA sequence variation in a 10-kb region on chromosome 1. Mol. Biol. Evol. **18:** 214–222.
Zhivotovsky, L. A., and S. Gavrilets, 1992 Quantitative variability and multilocus polymorphism under epistatic selection. Theor. Popul. Biol. **42:** 254–283.

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APPENDIX A

Simulation of mutant effects on the metric trait and on fitness: Effects of mutant alleles are sampled from a continuous bivariate gamma distribution, $h(|a|, s)$, with parameters ε_a, β_a, ε_s, β_s, and ρ using algorithm GTVR (Schmeiser and Lal 1982) as $|a| = (Y_1 + Y_3)/\alpha_a$ and $|s| = (Y_2 + Y_3)/\alpha_s$, given three independent gamma random variables (Whittaker 1974): Y_1, Y_2, and Y_3 with shape parameters $\beta_a - \rho\sqrt{\beta_a\beta_s}$, $\beta_s - \rho\sqrt{\beta_a\beta_s}$, $\rho\sqrt{\beta_a\beta_s}$, respectively, and the same scale parameter $\alpha = 1$. Y_3 is a common component of both $|a|$ and $|s|$, and the maximum correlation between $|a|$ and $|s|$ is $\min(\beta_a, \beta_s)/\sqrt{\beta_a\beta_s}$. The joint probability density function of $|a|$ and $|s|$ is

$$h(|a|, |s|) = \frac{\exp(-(|a| + |s|))}{\Gamma(\beta_a - \rho\sqrt{\beta_a\beta_s})\Gamma(\beta_s - \rho\sqrt{\beta_a\beta_s})\Gamma(\rho\sqrt{\beta_a\beta_s})} \times \int_0^{\min(|a|,|s|)} t^{\rho\sqrt{\beta_a\beta_s}-1}(|a| - t)^{(\beta_a-\rho\sqrt{\beta_a\beta_s})-1}(|s| - t)^{(\beta_s-\rho\sqrt{\beta_a\beta_s})-1}\exp(t)\,dt$$

(Cherian 1941; Kotz *et al.* 2000). Although it is not easy to obtain the conditional distribution of the metric trait z given the selective coefficient s, it is possible to get the conditional properties by employing the theorem that, given $Y_2 + Y_3 = y_2 + y_3$, Y_3 is a β random variable with the density function $(\Gamma(\beta_3 + \beta_2)/\Gamma(\beta_3)\Gamma(\beta_2))\nu^{\beta_3-1}(1-\nu)^{\beta_2-1}$ over the range $[0, y_2 + y_3]$, where $\nu = y_3/(y_2 + y_3)$ (Sheldon 2000). The conditional variances and fourth moments of the metric trait given the selective coefficient s can therefore be explicitly expressed in (6) and (7), in which the coefficients b_i, c_i are the functions of the shape parameters β_s, β_a, and the correlation ρ and given as

$$b_0 = (\beta_a - \rho\sqrt{\beta_a\beta_s} + 1)(1 - \rho\sqrt{\beta_s/\beta_a})/(\beta_a + 1)$$

$$b_1 = 2\rho\sqrt{\beta_a(\beta_s + 1)}(1 - \rho\sqrt{\beta_s/\beta_a})/(\beta_a + 1)$$

$$b_2 = \rho(\rho\sqrt{\beta_a\beta_s} + 1)\sqrt{\beta_s/\beta_a}/(\beta_a + 1)$$

and

$$c_0 = (\beta_a - \rho\sqrt{\beta_a\beta_s})(\beta_a - \rho\sqrt{\beta_a\beta_s} + 1)(\beta_a - \rho\sqrt{\beta_a\beta_s} + 2) \times (\beta_a - \rho\sqrt{\beta_a\beta_s} + 3)/(\beta_a^2(\beta_a + 1)^2)$$

$$c_1 = 4(\beta_a - \rho\sqrt{\beta_a\beta_s})(\beta_a - \rho\sqrt{\beta_a\beta_s} + 1)(\beta_a - \rho\sqrt{\beta_a\beta_s} + 2) \times \rho\sqrt{\beta_a\beta_s}\sqrt{(\beta_s + 1)/\beta_s}/(\beta_a^2(\beta_a + 1)^2)$$

$$c_2 = 6(\beta_a - \rho\sqrt{\beta_a\beta_s})(\beta_a - \rho\sqrt{\beta_a\beta_s} + 1)\rho\sqrt{\beta_a\beta_s}(\rho\sqrt{\beta_a\beta_s} + 1)/(\beta_a^2(\beta_a + 1)^2)$$

$$c_3 = 4(\beta_a - \rho\sqrt{\beta_a\beta_s})\rho\sqrt{\beta_a\beta_s}(\rho\sqrt{\beta_a\beta_s} + 1) \times (\rho\sqrt{\beta_a\beta_s} + 2)\sqrt{\beta_s(\beta_s + 1)}/(\beta_a^2(\beta_a + 1)^2(\beta_s + 2))$$

$$c_4 = \rho\sqrt{\beta_a\beta_s}(\rho\sqrt{\beta_a\beta_s} + 1)(\rho\sqrt{\beta_a\beta_s} + 2)(\rho\sqrt{\beta_a\beta_s} + 3) \times \beta_s(\beta_s + 1)/(\beta_a^2(\beta_a + 1)^2(\beta_s + 2)/(\beta_s + 3)).$$

For the special situation that the shape parameters are one-half, then the bivariate Γ distribution is a joint Wishart distribution, and the correlated mutation effects can be sampled as $s = \varepsilon_s u_1^2/\sqrt{3}$ and $|a| = \varepsilon_a[\rho^{1/2}u_1 + (1-\rho)^{1/2}u_2]^2/\sqrt{3}$ (Keightley and Hill 1990), given two pseudorandom independent standardized normal deviates u_1 and u_2. The conditional variances and fourth moments of the metric trait given the effect of the mutant on the fitness are then

$$V[a|s] = E[a^2|s] = \varepsilon_a^2[(1-\rho)^2 + 2\sqrt{3}\rho(1-\rho)\frac{|s|}{\varepsilon_s} + \rho^2\frac{s^2}{\varepsilon_s^2}],$$

$$E[a^4|s] = \varepsilon_a^4\left\{\rho^4\frac{s^4}{\varepsilon_s^4} + \frac{28}{\sqrt{3}}\rho^3(1-\rho)\frac{|s|^3}{\varepsilon_s^3} + 70\rho^2(1-\rho)^2\frac{s^2}{\varepsilon_s^2} + \frac{140}{\sqrt{3}}\rho(1-\rho)^3 \times \frac{|s|}{\varepsilon_s} + \frac{35}{3}(1-\rho)^4\right\},$$

which, except for $\rho = 0$ and 1, are different from the conditional properties from algorithm GTVR (*i.e.*, Equations 6 and 7). This means that both methods can produce the correlated effects with required properties but induce different bivariate distribution density functions (see Kotz *et al.* 2000).

APPENDIX B

The functions ϕ_i of $\alpha_s\Delta s$ are defined as $\phi_i \equiv \Sigma_{n=1}^{\infty} n^i g(n\Delta s)/\Sigma_{n=1}^{\infty} g(n\Delta s)$, $i = -1, 1, 2, 3$. As $g(\cdot)$ is given by (5), $\phi_i = \Psi_i/\Psi_0$, where $\Psi_i = \Sigma_{n=1}^{\infty} n^{\beta_s+i-1}\tau^n$, $i = -1, 0, \ldots, 3$, and $\Psi_{i+1} = \tau d\Psi_i/d\tau$ with $\tau = \exp(-\alpha_s\Delta s)$. For $\beta_s = 1$, for $\Psi_i(i = -1, \ldots, 3)$, we have $-\ln(1-\tau)$, $\tau/(1-\tau)$, $\tau/(1-\tau)^2$, $\tau(1+\tau)/(1-\tau)^3$, and $\tau(1 + 4\tau + \tau^2)/(1-\tau)^4$, which can be approximated by $-\ln(\alpha_s\Delta s)$, $1/\alpha_s\Delta s$, $1/(\alpha_s\Delta s)^2$, $2/(\alpha_s\Delta s)^3$, and $6/(\alpha_s\Delta s)^4$ if $\alpha_s\Delta s \ll 1$. For $0 < \beta_s < 1$, numerical calculations show that when $\alpha_s\Delta s \ll 1$, Ψi $(i = -1, \ldots, 3)$ can be approximated as $C_0 - C_1(\alpha_s\Delta s)^{1-\beta_s}$, $C_1(1-\beta_s)(\alpha_s\Delta s)^{-\beta_s}$, $C_1(1-\beta_s)\beta_s(\alpha_s\Delta s)^{-1-\beta_s}$, $C_1(1-\beta_s)\beta_s(\beta_s+1)(\alpha_s\Delta s)^{-2-\beta_s}$, $C_1(1-\beta_s)\beta_s(\beta_s+1)(\beta_s+2)(\alpha_s\Delta s)^{-3-\beta_s}$. Here C_0 and C_1 are functions of β_s, and some values are given in the following table:

β_s	1/16	1/8	1/4	1/2	3/4	7/8	15/16
C_0	1.71	1.78	1.96	2.61	4.55	8.58	16.59
C_1	16.51	8.60	4.80	3.54	4.75	8.70	16.64

For $\beta_s > 1$, $\Psi_i(i = -1, \ldots, 3)$ approximate $C_1(\alpha_s\Delta s)^{1-\beta_s}$, $C_1(\beta_s - 1)(\alpha_s\Delta s)^{-\beta_s}$, $C_1(\beta_s - 1)\beta_s(\alpha_s\Delta s)^{-1-\beta_s}$, $C_1(\beta_s - 1)\beta_s(\beta_s+1)(\alpha_s\Delta s)^{-2-\beta_s}$, and $C_1(\beta_s - 1)\beta_s(\beta_s+1)(\beta_s+2)(\alpha_s\Delta s)^{-3-\beta_s}$. Finally we have for $\beta_s > 0$, $\phi_1 \approx \beta_s/(\alpha_s\Delta s)$, $\phi_2 \approx \beta_s(\beta_s+1)/(\alpha_s\Delta s)^2$, $\phi_3 \approx \beta_s(\beta_s+1)(\beta_s+2)/(\alpha_s\Delta s)^3$, and $\phi_{-1} \approx [C_0/((1-\beta_s)C_1)](\alpha_s\Delta s)^{\beta_s} - \alpha_s\Delta s/(1-\beta_s)$ $(0 < \beta_s < 1)$; $\phi_{-1} \approx \alpha_s\Delta s \ln(1/\alpha_s\Delta s)$ $(\beta_s = 1)$; $\phi_{-1} \approx \alpha_s\Delta s/(\beta_s - 1)$ $(\beta_s > 1)$.