# Joint Effects of Pleiotropic Selection and Stabilizing Selection on the Maintenance of Quantitative Genetic Variation at Mutation-Selection Balance

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ABSTRACT

In quantitative genetics, there are two basic "conflicting" observations: abundant polygenic variation and strong stabilizing selection that should rapidly deplete that variation. This conflict, although having attracted much theoretical attention, still stands open. Two classes of model have been proposed: real stabilizing selection directly on the metric trait under study and apparent stabilizing selection caused solely by the deleterious pleiotropic side effects of mutations on fitness. Here these models are combined and the total stabilizing selection observed is assumed to derive simultaneously through these two different mechanisms. Mutations have effects on a metric trait and on fitness, and both effects vary continuously. The genetic variance ($V_G$) and the observed strength of total stabilizing selection ($V_{s,t}$) are analyzed with a rare-alleles model. Both kinds of selection reduce $V_G$ but their roles in depleting it are not independent: The magnitude of pleiotropic selection depends on real stabilizing selection and such dependence is subject to the shape of the distributions of mutational effects. The genetic variation maintained thus depends on the kurtosis as well as the variance of mutational effects: All else being equal, $V_G$ increases with increasing leptokurtosis of mutational effects on fitness, while for a given distribution of mutational effects on fitness, $V_G$ decreases with increasing leptokurtosis of mutational effects on the trait. The $V_G$ and $V_{s,t}$ are determined primarily by real stabilizing selection while pleiotropic effects, which can be large, have only a limited impact. This finding provides some promise that a high heritability can be explained under strong total stabilizing selection for what are regarded as typical values of mutation and selection parameters.

THE presence of genetic variation in quantitative traits is important for the selective breeding of domestic animals and crops, evolution, and adaptation (Charlesworth *et al.* 1982; Barton and Turelli 1989; Falconer and Mackay 1996; Barton and Keightley 2002). The existence of genetic variation is, however, paradoxical because stabilizing selection acting on the population usually depletes genetic variation (Wright 1935; Crow and Kimura 1970; Bürger and Gimelfarb 1999; Bürger 2000). As the ultimate source of genetic variation is mutation, an intuitively appealing explanation for the maintenance of polygenic variation is that there is an equilibrium between the input of new variation by mutation and its erosion by natural selection. For *real* stabilizing selection it is assumed that natural selection acts directly and solely on the metric trait, relative fitness having a quadratic relationship with the trait. Under the rare-allele model and the assumption of Gaussian fitness function, predictions for the equilibrium genetic variance are given by the house-of-cards approximation $V_G = 4\lambda_t V_{s,r}$ (Turelli 1984; Bürger 2000), where $\lambda_t$ is the average number of mutations of genes that affect the trait per generation per haploid genome, and $V_{s,r}$ is the strength of real stabilizing selection, the "variance" of the fitness profile, with a large value of $V_{s,r}$ implying weak selection. It is difficult to account for the observed high variance with this model for what are regarded as typical values of $V_{s,r}$ (*e.g.*, $20V_e$), mutation rate per locus, and number of relevant loci (Turelli 1984; Falconer and Mackay 1996). Furthermore, simple genetic load arguments suggest that real stabilizing selection cannot operate independently on many characters (Robertson 1967; Turelli 1985; Barton 1990). In a recent review, however, Kingsolver *et al.* (2001) found that estimates of the strength of stabilizing selection vary greatly, and the typical selection may be much weaker than previously assumed.

In an alternative model, the *pure* pleiotropic model, natural selection is assumed not to act directly on the metric trait in question, but through pleiotropic side effects of mutant alleles on fitness (Robertson 1967; Hill and Keightley 1988). This model can generate apparent stabilizing selection, shown as a negative correlation between relative fitness and phenotypic deviation from the mean (Robertson 1967; Hill and Keightley 1988; Barton 1990; Gavrilets and de Jong 1993): Extreme individuals for the trait tend to carry more harmful mutations. There is observational evidence for apparent selection in nature (Kruuk *et al.* 2002). This

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model can provide an explanation for observed levels of $V_G$ but for only part of the strength of apparent stabilizing selection observed ($V_{s,t}$) and has the further defect that $V_G$ increases without bound as the effective population size increases when the mutational effects are not completely correlated and the distribution of fitness effects is leptokurtic (Keightley and Hill 1990; Caballero and Keightley 1994). Such stabilizing selection induced solely by pleiotropic effects on fitness of mutations is referred to as "pleiotropic selection" in this article. There is a general relationship $V_s \geq V_G^2/V_m$ (Barton 1990; Kondrashov and Turelli 1992; Gavrilets and de Jong 1993; Zhang *et al.* 2002); therefore the pure pleiotropic model cannot in principle explain both the observed levels of genetic variances and typical estimates of strengths of stabilizing selection, provided the mutational variance $V_m$ is of the order $10^{-3}V_e$ as observed (Houle *et al.* 1996; Lynch and Walsh 1998; Lynch *et al.* 1999).

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 weaknesses in explaining the maintenance of polygenic variation.

Nevertheless, the real stabilizing selection and pleiotropic models are not mutually exclusive. Individual mutant alleles can have both deleterious pleiotropic effects on fitness and effects on the metric trait in question (Falconer and Mackay 1996). If the metric trait is not completely neutral, that is, the extreme phenotypes of the metric trait are less fit, natural selection takes place simultaneously through two different mechanisms: the deleterious pleiotropic effects on all other aspects of fitness and real stabilizing selection on the metric trait under study. Individuals that carry mutants are therefore selected against because of both deleterious pleiotropic effects of mutants (*i.e.*, pleiotropic selection) and phenotypic deviations of the trait value from the optimum (*i.e.*, real stabilizing selection). The strength of total stabilizing selection is therefore attributed to both kinds of natural selection. As Kondrashov and Turelli (1992, p. 615) noted, "A complete treatment should consider both direct and indirect selection on the quantitative trait." Tanaka (1996) used a cohort-of-mutations model to combine both pleiotropic and real stabilizing selections and assumed that all mutations had an equal deleterious effect on fitness and a Gaussian distribution of effects on the target trait. However, this cannot readily account for both high heritabilities and strong stabilizing selection (Tanaka 1996, 1998). Although the assumption of an equal fitness effect for all mutations is a convenient way to obtain analytical approximations for $V_G$ and $V_{s,t}$ (Barton 1990; Kondrashov and Turelli 1992; Tanaka 1996), it lacks rigorous support, and experimental data illustrate the highly leptokurtic distribution of mutational effects on fitness (Mackay *et al.* 1992). As shown by Zhang *et al.* (2002), the shape of the distribution of mutational effects does affect the predictions of the pleiotropic model, so that it is necessary to take into account variation in effects of mutations both on the trait and on fitness.

In this study, a compound model of continuously varying effects of mutations on the trait and on fitness is constructed to investigate the maintenance of genetic variance and the observed strength of total stabilizing selection. The interaction between both kinds of selection and their overall impact on genetic variation and strength of total stabilizing selection are explored. We hope thereby to provide a possible explanation for the observations of both high genetic variance and the strong observed stabilizing selection.

## MODEL

We assume additivity of gene action, linkage equilibrium, a random-mating diploid population, and rare mutant alleles. In accordance with the model of real stabilizing selection (Turelli 1984, 1985), the relative fitness of individuals that have a phenotypic value $P$, the sum of the contributions from each locus plus a random independent environmental effect of mean zero, is assumed to be given by $W(P) = \exp(-P^2/2\omega^2)$. The mean fitness of individuals with genotypic value $G = \Sigma_i a_i$ is $W(G) = \exp(-G^2/2V_{s,r}) \approx 1 - G^2/2V_{s,r}$ with $V_{s,r} = \omega^2 + V_e$ measuring the intrinsic strength of real stabilizing selection. $V_e$ is the environmental variance and is scaled as a unit of variance.

It is assumed that there are infinitely many loci on each individual and at each locus there is a continuum of possible mutational effects, but each locus has the same mutation distribution and loci are exchangeable. There are at most two alleles segregating at each locus: the wild type, which is assumed to be at optimum, and the mutant. Mutations have effects on a metric trait ($a$) and pleiotropic deleterious effects on fitness ($s \geq 0$), with a bivariate distribution $h(a, s)$. If the metric trait undergoes real stabilizing selection due to mutations, the observed stabilizing selection would come from these two parts and the equivalent total selection coefficient within each individual is given by $\tilde{s} = s + (1 - 2x)\,a^2/(4V_{s,r})$ (see appendix a), where $x$ is the frequency of the mutant allele. The equivalent total selection coefficient is in general not independent of the frequency of mutant alleles in this compound model. It is therefore less tractable (see appendix a) than the pure pleiotropic model (Barton 1990; Keightley and Hill 1990), in which selection is assumed to act directly on the pleiotro-

pic effect on fitness of each mutant allele and the coefficient is always independent of the frequency of the mutant allele. With the assumption of real stabilizing selection (Turelli 1984; Keightley and Hill 1988), however, selection acts on the total effect of all mutants within individuals and hence depends on the frequency of mutant alleles (Robertson 1956). Such frequency dependence of selection leads to multiple equilibria (Bulmer 1985; Barton 1986) but, unless population size is very small, mutant alleles cannot increase to a high frequency without passing through an intermediate frequency, against which there is selection. The frequency of mutant alleles therefore remains very low (Bulmer 1989). With rare mutant alleles, the equivalent total selection coefficient within each individual organism can therefore be approximated by

$$\tilde{s} = s + a^2/(4V_{s,r}). \quad (1)$$

In an infinite population the equilibrium genetic variance is

$$V_G = 4\lambda V_{s,r}\int_{-\infty}^{\infty}\int_{0}^{\infty} h(a, s)\frac{a^2/(4V_{s,r})}{\tilde{s}}dads = 4\lambda V_{s,r}I_2, \quad (2)$$

in which $I_2$ is determined by the distribution of mutational effects (see appendix b), $\lambda$ is the genome-wide mutation rate over all loci, and the strength of *total* stabilizing selection (*i.e.*, that which would be observed regardless of its source) is

$$V_{s,t} = \frac{V_G^2/V_{s,r} + V_m - \mathrm{Cov}_p}{V_G^2/V_{s,r} + V_m}V_{s,r}. \quad (3)$$

Here $\mathrm{Cov}_p$ is the covariance of relative fitness and squared deviation due to pleiotropic effects on fitness of mutations (see appendix a). When the pleiotropic selection is much stronger than real stabilizing selection, $\mathrm{Cov}_p \to V_m$ (*cf.* Bürger 2000; Zhang *et al.* 2002) and the strongest total selection applies with strength $V_{s,t} = V_G^2/(V_G^2/V_{s,r} + V_m) \equiv V_{s,t}^b$; when the pleiotropic effect is very weak in relation to real stabilizing selection, $\mathrm{Cov}_p \to 0$ and the strength of total stabilizing selection approaches $V_{s,t}$. In general, the following inequality applies for the strength of total stabilizing selection:

$$V_{s,t}^b < V_{s,t} < V_{s,r}. \quad (4)$$

Because the total covariance of relative fitness and squared deviation, $\mathrm{Cov} = V_m + V_G^2/V_{s,r}$, is larger than that both for the pure pleiotropic model, $V_m$ (Bürger 2000; Zhang *et al.* 2002), and for real stabilizing selection, $V_{G2}/(2V_{s,r})$, the total stabilizing selection is certainly stronger than either individual component. The mutational variance on the trait $V_m = \frac{1}{2}\lambda\varepsilon_a^2$, where $\varepsilon_a^2$ is the variance of mutational effects on the trait, is observed to be of the order $10^{-3}V_e$ (Houle *et al.* 1996; Lynch and Walsh 1998; Lynch *et al.* 1999; and this value is used in this study). Therefore $V_{s,r}$ cannot be very large if a high $V_G$ is to be maintained under strong total stabilizing selection (*i.e.*, small $V_{s,t}$).

Although the properties of mutant effects on the metric trait and on fitness are crucial to evaluating $V_G$ and $V_{s,t}$, the distribution of mutational effects is hard to estimate accurately (Mackay and Langley 1990; Hill and Caballero 1992; Mackay *et al.* 1992; Davies *et al.* 1999; Elena and Moya 1999; Keightley *et al.* 2000; Shaw *et al.* 2000; Imhof and Schlötterer 2001; Wloch *et al.* 2001). Even for Drosophila, for which there are many studies, the data seem to suggest a highly skewed and leptokurtic distribution of mutational effects (Mackay and Langley 1990; Hill and Caballero 1992; Mackay *et al.* 1992), but 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 effects of mutations on fitness are assumed to occur, in accord with the classical view (Falconer and Mackay 1996). The variability of the distribution of $a$ is defined in terms of $\varepsilon_a = \sqrt{E(a^2)}$ and for $s$ is $\varepsilon_s = \sqrt{E(s^2)}$. For theoretical comparison, it is assumed in this study that mutational effects on the trait are, in increasing order of leptokurtosis, Gaussian, reflected gamma ($\frac{1}{2}$), reflected gamma ($\frac{1}{4}$), reflected gamma ($\frac{1}{8}$), reflected squared gamma ($\frac{1}{2}$), and reflected quartic gamma ($\frac{1}{2}$); mutational effects on fitness are equal, one-sided Gaussian, gamma ($\frac{1}{2}$), gamma ($\frac{1}{4}$), gamma ($\frac{1}{8}$), squared gamma ($\frac{1}{2}$), and quartic gamma ($\frac{1}{2}$), where gamma ($\beta$) denotes the gamma distribution with shape parameter $\beta$. Those distributions, whose shapes are illustrated in Figure 1, cover a very wide range of all possible mutational effects.

## RESULTS

Analytical approximations are obtained for some special cases for an infinite population and a rare-allele approximation, and numerical calculations were performed to provide support and to extend the results to more general situations. Simple results for some special situations are also presented within Keightley and Hill's (1990) framework using Kimura's (1969) diffusion approximation.

**Pure real stabilizing selection within a finite population, *i.e.*, $s = 0$, thus $\tilde{s} = (1 - 2x)a^2/(4V_{s,r})$:** The observed strength of real stabilizing selection is $V_{s,t} = V_{G2}/(2\,\mathrm{Cov}_r) = V_{s,r}$ and the genetic variance is given by (A2). Numerical calculation shows that, as the effective population size $N_e$ increases, $V_G$ increases and approaches the rare-allele approximation $4\lambda V_{s,r}$ (Turelli 1984; see Figure 2). Theoretically this is because the equilibrium frequencies of mutant genes, $x$, become very small and the heterozygosity can thus be approximated by $H(\tilde{s}) = 4\lambda/\tilde{s}$ as the effective population size $N_e \to \infty$ and thus

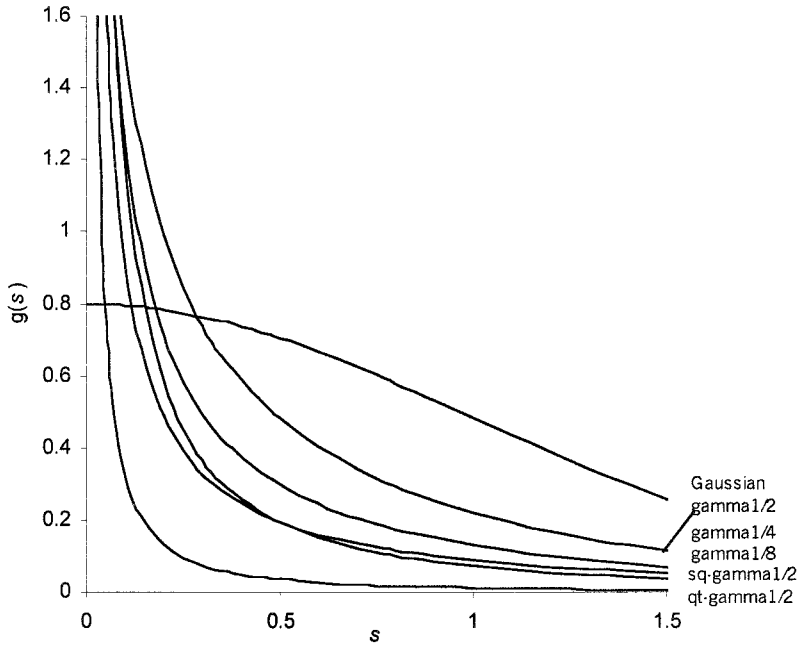


FIGURE 1.—The distribution of mutation effects, $s$, on fitness used in the study with $E(s^2) = 1.0$ in each case. Here sq-gamma (½) and qt-gamma (½) represent squared gamma (½) and quartic gamma (½). The distribution of mutational effects, $a$, on the trait was symmetrical about $a = 0$, but with that of $|a|$ having the same range of distribution as $s$.

$N_e\tilde{s} \gg 1$. Figure 2 also shows that the genetic variance maintained in a finite population depends on the distribution of mutational effects (*cf.* KEIGHTLEY and HILL 1988). Further, if mutational effects on the trait follow a reflected gamma (½), $V_G$ depends little on the mutation rates; whereas for equal mutational effects on the trait, $V_G$ in small populations depends heavily on the mutation rates for given $\lambda V_{s,r}$.

**Pure pleiotropic effects, where the target trait is completely neutral in itself (*i.e.*, $V_{s,r} \to \infty$) and $\tilde{s} = s$:** With all mutants having equal pleiotropic effects, the genetic variance is $V_G = 2V_m/s$ (BARTON 1990), which is too small, given that the estimates of selection coefficients with detectable effects in the laboratory are in the range $s = 0.02$–$0.08$ (CROW and SIMMONS 1983; KEIGHTLEY and HILL 1990; CABALLERO and KEIGHTLEY 1994; CHAVARRAS *et al.* 2001; WLOCH *et al.* 2001). If, however, the pleiotropic effects vary among mutants, substantial variation can occur; indeed $V_G$ becomes unbounded for an infinite population if neutral mutants predominate (see KEIGHTLEY and HILL 1990; CABALLERO and KEIGHTLEY 1994; ZHANG *et al.* 2002). Moreover, the pure pleiotropic model can only partially account for the "typical" strength of stabilizing selection (BARTON 1990; KONDRASHOV and TURELLI 1992; ZHANG *et al.* 2002).

**Joint effects of both pleiotropic and real stabilizing selections within an infinite population, but assuming equal mutational effects on both the trait ($\varepsilon_a$) and fitness ($\bar{s}$):** From Equation 1, $\tilde{s} = \bar{s} + \varepsilon_a^2/(4V_{s,r})$ and the approximation $H(\tilde{s}) = 4\lambda/\tilde{s}$ for an infinite population, the

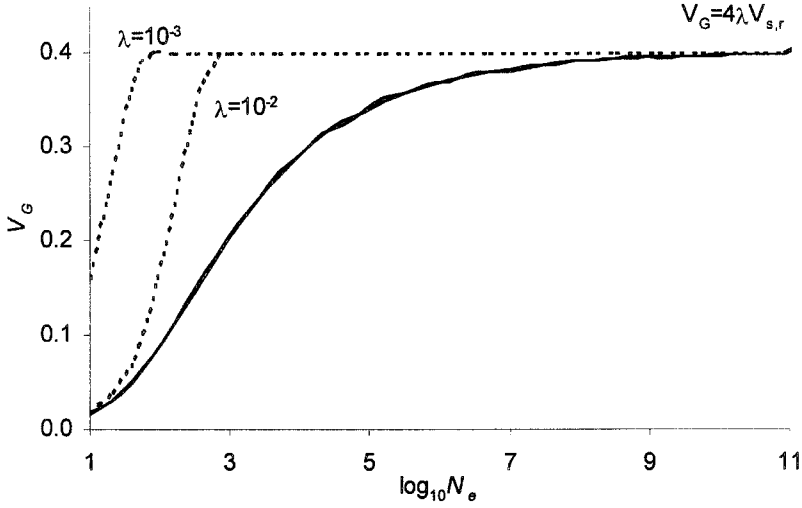


FIGURE 2.—Genetic variance maintained in the metric trait as a function of the effective population size under pure real stabilizing selection (*i.e.*, $s = 0$). Two cases are investigated: $\lambda = 0.01$, $V_{s,r} = 10$ and $\lambda = 0.001$, $V_{s,r} = 100$. Results are shown for two distributions of mutational effects on the trait: gamma (½)-distributed effects (solid lines, essentially superimposed) and equal effects (dashed lines).

genetic variance is given by

$$V_G = H(\bar{s})\varepsilon_a^2/4 = 2V_m/(\bar{s} + \varepsilon_a^2/(4V_{s,r})). \quad (5)$$

As the fourth moment and covariance are $m_4 = V_G\varepsilon_a^2/4$ and $\mathrm{Cov}_p = V_G\bar{s}/2$, the strength of total stabilizing selection (real and apparent) is

$$V_{s,t} = (\varepsilon_a^2/4 + 2V_G)/[\bar{s} + (\varepsilon_a^2/4 + 2V_G)/V_{s,r}] \quad (6)$$

(see APPENDIX A). Equation 6 is a special case of Equation 3. With no pleiotropic effect of mutants (*i.e.*, $s = 0$), selection comes solely from real stabilizing selection on the metric trait, $V_{s,t} = V_{s,r}$; with some pleiotropic effects on fitness, selection becomes stronger (*i.e.*, $V_{s,t}$ decreases). The inclusion of a pleiotropic deleterious effect therefore decreases both the genetic variance and the strength of total stabilizing selection. Equation 5 is the same as that of TANAKA (1996) who assumed equal deleterious effects of mutations on fitness but Gaussian effects on the metric trait. In this case, the population average of the total selection coefficient is simply equal to the sum of the selection coefficients due to both kinds of selection (*cf.* KONDRASHOV and TURELLI 1992; TANAKA 1996, 1998).

**Joint effects, but assuming mutations have an equal pleiotropic effect on fitness ($\bar{s}$) and a continuous distribution $f(a)$ of mutational effects on the metric trait:** In this situation KIMURA's (1969) diffusion theory leads to $H(\bar{s}) = C(\bar{s}) = 4\lambda/\bar{s}$ approximately, and $K(\bar{s}) = 0$ approximately for an infinite population (ZHANG *et al.* 2002). The genetic variance is $V_G = \int_{-\infty}^{\infty}[\lambda a^2/(\bar{s} + a^2/(4V_{s,r}))]f(a)\,da$, and the strength of total stabilizing selection is given by (A7), where the fourth moment is $m_4 = \int_{-\infty}^{\infty}((\lambda a^4/4)/(\bar{s} + a^2/(4V_{s,r})))f(a)\,da$, and the covariance between relative fitness and squared deviation due to pleiotropic effects is $\mathrm{Cov}_p = V_G\bar{s}/2$. In the following we denote the population mean of the selection coefficients arising from real stabilizing selection by $\bar{s}_r \equiv E(a^2/4V_{s,r}) = \varepsilon_a^2/4V_{s,r} = 2V_m/(4\lambda V_{s,r})$, *i.e.*, twice the ratio of mutational variance to the genetic variance maintained in real stabilizing selection. For a neutral trait, $\bar{s}_r = 0$.

If the mean pleiotropic effect on fitness is much weaker than that from real stabilizing selection (*i.e.*, $\bar{s} \ll \bar{s}_r$), the genetic variance approaches the rare-allele approximation $V_G = 4\lambda V_{s,r}$. In general $V_G = 4\lambda V_{s,r}\sum_{i=1}^{\infty}(-1)^{i-1}E(a^{2i})/(4\bar{s}V_{s,r})^i$ (MORAN 1968, p. 296). If $\bar{s} \gg \bar{s}_r$, the genetic variance can be approximated by

$$V_G \approx 2V_m/(\bar{s} + \kappa_4\bar{s}_r). \quad (7)$$

Noting that the kurtosis $\kappa_4 \equiv E(a^4)/E^2(a^2) = 1$, 3, and 35/3 for effects that are equally distributed, normally distributed, and distributed as a gamma (½), respectively, TANAKA's (1996) formula (*i.e.*, Equation 5) is therefore accurate only for equal mutational effects on the trait. Although approximation (7) implies that highly leptokurtically distributed effects of mutations on the trait lead to a low genetic variance (see also Figure 2 for finite populations), TANAKA's (1996) formula gives a good approximation for the situation in which $\bar{s} \gg \bar{s}_r$. The numerical results in Figure 3 show that expression (5) provides a close approximation to $V_G$ when $\lambda$ is either $>10^{-2}$ or $<10^{-6}$ for Gaussian effects of mutations on the trait or when $\lambda > 0.1$ for gamma (½) effects of mutations. For other values of mutation rate, however, TANAKA's (1996) results are much larger than numerical results for both Gaussian and reflected gamma (½) mutational effects. When $\lambda = 10^{-4}$, for example, (5) gives $V_G = 0.028$, which is ~1.5 and 2.3 times as large as the numerical results for Gaussian and reflected gamma (½) mutational effects, respectively.

Figure 3 clearly shows how both effects interfere and contribute to the overall outcome in $V_G$ and $V_{s,t}$. When the mutation rate is very low (*e.g.*, $\lambda < 10^{-5}$) and each mutant has large effects on the trait relative to its effect on fitness, the results approach the house-of-cards approximation (TURELLI 1984). If the mutation rate is high (*e.g.*, $\lambda > 0.1$) and each mutant has a relatively small effect on the trait, the pleiotropic effect must be widespread and becomes the main force of selection, the genetic variance tends to that of BARTON (1990) but the strength of total stabilizing selection approaches $V_{s,t} = (V_G^2/V_m)/[1 + 2V_G/(\bar{s}V_{s,r})]$, which is smaller than that of BARTON (1990). If the mean pleiotropic effect is stronger than that of real stabilizing selection, *i.e.*, $\bar{s} > \bar{s}_r$, expression (7) can give better approximations for $V_G$ than TANAKA's (1996). One interesting phenomenon can be noted by comparing $V_G$ and $V_{s,t}$ in Figure 3: $V_G$ rises as the mutation rate increases while the total stabilizing selection becomes stronger (*i.e.*, $V_{s,t}$ decreases). This is in sharp contrast to both real stabilizing selection, where as $\lambda$ increases $V_G$ increases but $V_{s,t}$ ($= V_{s,r}$) remains unchanged (TURELLI 1984), and the pure pleiotropic model, where as $\lambda$ increases $V_G$ remains unchanged but $V_{s,t}$ decreases (BARTON 1990; *cf.* Figure 5, c and d below).

**General case:** As shown above and by previous work (BARTON 1990; KONDRASHOV and TURELLI 1992; TANAKA 1996), the equal fitness effect assumption cannot provide a simultaneous explanation for the observed high heritability and strong stabilizing selection. If mutational effects on fitness vary across loci in the absence of real stabilizing selection a huge genetic variance can be generated (KEIGHTLEY and HILL 1990; ZHANG *et al.* 2002), so it is important to investigate the influence of variation in fitness effects on $V_G$ and $V_{s,t}$.

The first check is whether the unbounded $V_G$ with increasing population size is avoided with the inclusion of a real stabilizing selection on the trait. The example in Figure 4 shows that with even a weak real stabilizing selection (*e.g.*, $V_{s,r} = 1000$), the genetic variance increases with effective population size $N_e$ when it is small,

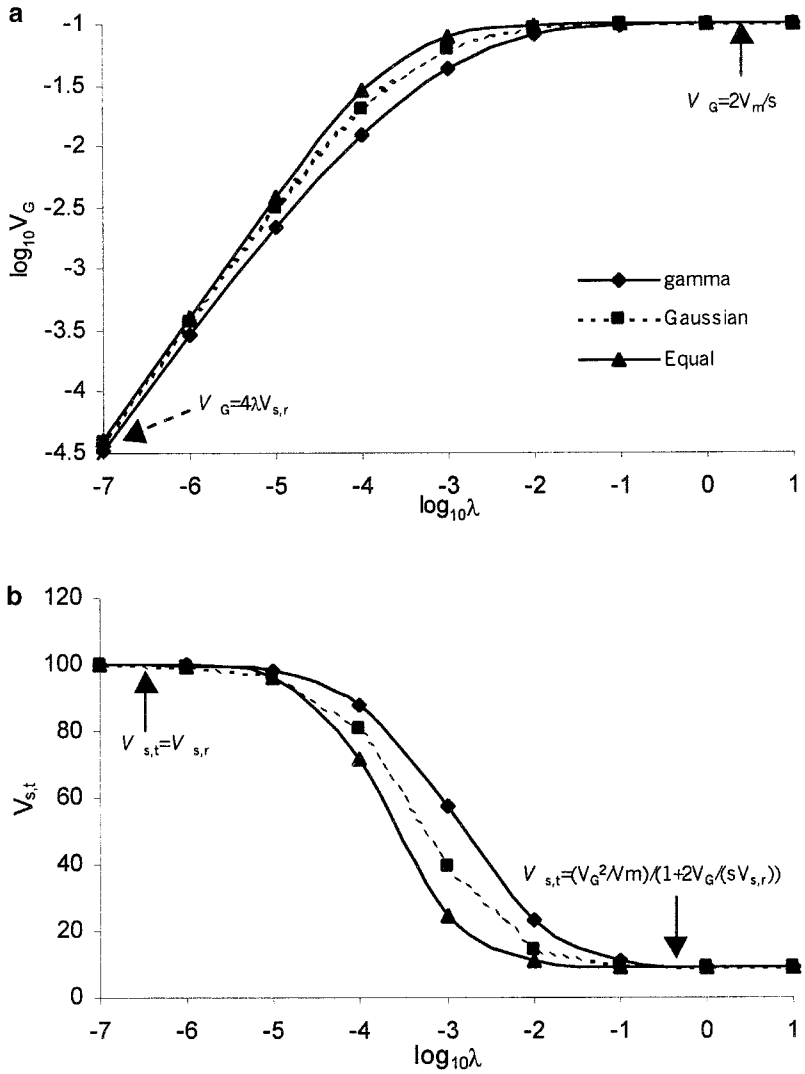


FIGURE 3.—(a) Genetic variance maintained in the metric trait and (b) the strength of stabilizing selection on it as functions of the mutation rate. Mutational effects on the trait follow either a Gaussian or a reflected gamma (½) distribution or are equal ($|a| = \varepsilon_a = \sqrt{2V_m/\lambda}$) and those on fitness are equal ($\bar{s} = 0.02$). The effective population size is infinite and $V_{s,r} = 100$. The curves for equal are given by Equations 5 and 6.

but asymptotes when $N_e$ exceeds some large value. This asymptotic value of $V_G$ depends on the value of $V_{s,r}$, with a high $V_G$ for a weak real stabilizing selection (*i.e.*, a large $V_{s,r}$). At the same time, the value of $V_{s,t}$ also increases and approaches a limit that is less than $V_{s,r}$. This implies that selection becomes weaker as the effective population size increases, but the total stabilizing selection is stronger than the real stabilizing selection.

Suppose that mutational effects on the trait are Gaussian and mutational effects on fitness follow a gamma (½) with mean $\bar{s}_p = E(s) = \varepsilon_s/\sqrt{3}$. If these mutational effects are independent, the genetic variance for an infinite population can be expressed exactly as

$$V_G = 4\lambda V_{s,r}/(1 + \sqrt{\bar{s}_p/\bar{s}_r}) = 2V_m/(\bar{s}_r + \sqrt{\bar{s}_r\bar{s}_p}) \quad (8)$$

(see APPENDIX B), in which $\bar{s}_p$ is the population mean of selection coefficients due solely to pleiotropic effect on fitness and $\bar{s}_r$, as in (7), is due to real stabilizing selection. For an extreme situation where the pleiotropic effect is very weak (*i.e.*, $\bar{s}_p \ll \bar{s}_r$), (8) tends to the house-of-cards approximation (TURELLI 1984), $V_G = 4\lambda V_{s,r}$; while for $\bar{s}_p \gg \bar{s}_r$, the genetic variance reduces to

$$V_G = \sqrt{4\lambda V_{s,r}(2V_m/\bar{s}_p)}. \quad (9)$$

This is the geometric mean of the genetic variance maintained by real stabilizing selection (TURELLI 1984) and for the pleiotropic model with equal fitness effects (BARTON 1990; KONDRASHOV and TURELLI 1992). This genetic variance approaches infinity if the metric trait is neutral (*i.e.*, $V_{s,r} \to \infty$) (*cf.* KEIGHTLEY and HILL 1990), consistent with the results shown in Figure 4a. Equation 8 clearly shows that both kinds of selection reduce $V_G$ but the impact of pleiotropic selection depends on the magnitude of real stabilizing selection. This unequal influence of both kinds of selection on the genetic variation is due to the fact that large pleiotropic effects on fitness can induce only a high fitness deficit whereas large effects on the trait can lead to a high genetic variance as well as a high fitness deficit. If the total selection coefficient were defined as the ratio of mutational variance to the equilibrium genetic variance following BARTON (1990), KONDRASHOV and TURELLI

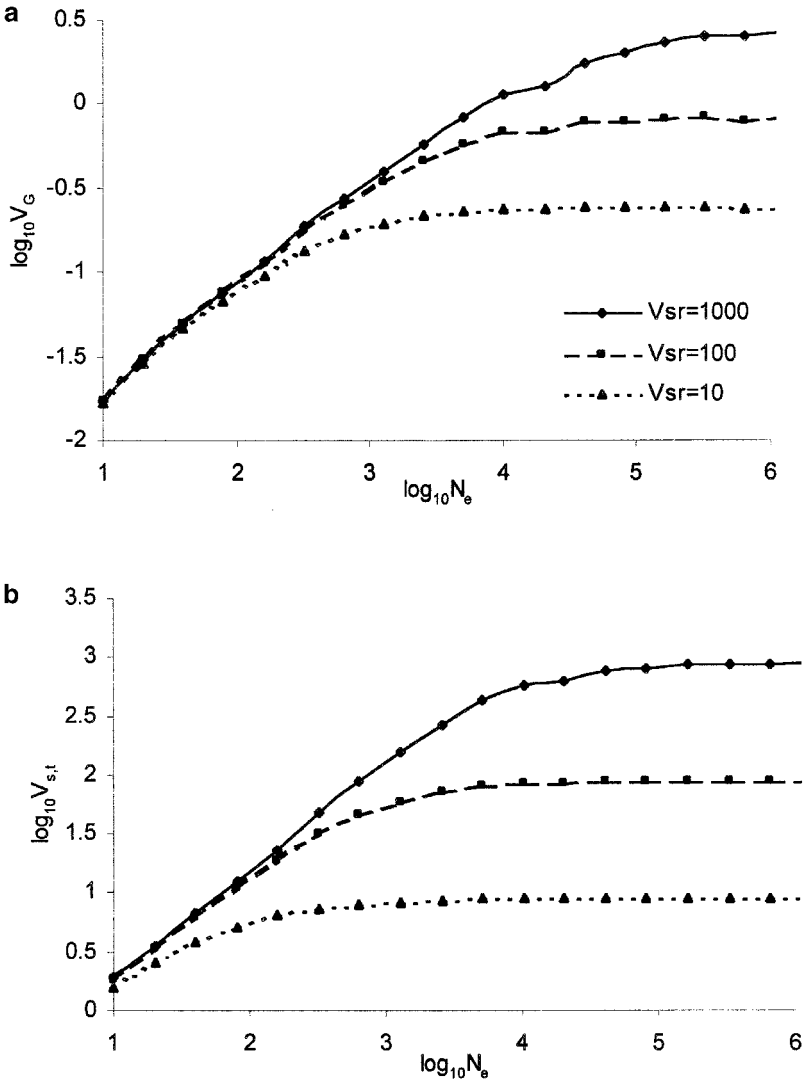


FIGURE 4.—(a) Variation maintained in the metric trait and (b) the strength of real stabilizing selection as functions of the effective population size $N_e$. Both $V_G$ and $V_{s,t}$ are evaluated by Monte Carlo integration. Absolute values of mutational effects on the trait ($|a|$) and on fitness ($s$) are independent and both marginal distributions are gamma (½). Parameters of mutations $\lambda = 0.1$ and $\varepsilon_s = 0.1$. Results are shown for three intrinsic strengths of real stabilizing selection, $V_{s,r}$.

(1992), and TANAKA (1998), (8) implies that the expectation of the total selection coefficient is not simply the sum of both components, but a function, $s_\Gamma = \bar{s}_r + \sqrt{\bar{s}_r \bar{s}_p}$. Further results are listed in Table 1.

Numerical results are shown in Figure 5 for a range of distributions of effects of mutations on the trait and on fitness such as equal, Gaussian, gamma (½), gamma (¼), and gamma (⅛) (except symmetrical for $a$ and one-sided for $s$). With all other properties being the same, TANAKA's (1996) formula (*i.e.*, Equation 5 for equal effects $|a|$ and $s$) predicts the smallest $V_G$ and $V_{s,t}$ (*i.e.*, the strongest selection). Further, the genetic variance maintained increases and total stabilizing selection becomes weaker as mutational effects on fitness become more leptokurtic (see also Table 1). This, albeit in agreement with the conclusion drawn by ZHANG *et al.* (2002), differs from situations when the pleiotropic effect is assumed to be equal (see Figure 3). Comparison of the three curves in Figure 5a in which mutational effects on fitness follow the gamma (½) but effects on the trait are Gaussian, or gamma (½), or gamma (¼), respectively, leads to the conclusion that a more leptokurtic distribution of mutational effects on the trait induces a smaller genetic variance given the same distribution of mutational effects on fitness and the same other properties (*cf.* KEIGHTLEY and HILL 1988). Figure 5b shows that an increase in pleiotropic selection ($\varepsilon_s$) leads to an increase in total stabilizing selection (*i.e.*, decreasing $V_{s,t}$). For equal mutation effects, Figure 5d shows that an increase in mutation rate can induce stronger total stabilizing selection, while for other distributed mutation effects there is a value of mutation rate at which the total stabilizing selection is strongest. This behavior of $V_{s,t}$ may differ from the pure pleiotropic model (KEIGHTLEY and HILL 1990; ZHANG *et al.* 2002).

In a realistic model, mutational effects on the trait and on fitness must be correlated (KEIGHTLEY and HILL 1990). Although analytical treatment is never easy (if possible) when a correlation between mutational effects is included (*e.g.*, TURELLI 1985), it is important to con-

**TABLE 1**

**Exact results of $I_2 = E[\xi^2/(\xi^2 + s)]$, where $\xi = a/\sqrt{4V_{s,r}}$ and the approximations for $V_G$ when $\bar{s}_p \gg \bar{s}_t$ for some distributions of mutational effects on the trait and independently on fitness**

| $g_1(a)^a$ | $g_2(s)^a$ | $I_2$ (where $\theta = \bar{s}_p/\bar{s}_t$) | $V_G$ when $\bar{s}_p \gg \bar{s}_t$ |
|---|---|---|---|
| Gaussian [3] | Exponential [6] [*i.e.*, gamma (1)] | $\frac{1}{2-\theta}\left[2 - \sqrt{\frac{\theta}{2-\theta}}\left(\frac{\pi}{2} - \arctan\sqrt{\frac{\theta}{2-\theta}}\right)\right]$ if $\theta < 2$; $\frac{1}{3}$ if $\theta = 2$; $\frac{1}{\theta-2}\left[\sqrt{\frac{\theta}{\theta-2}}\ln\left(\frac{\theta+\sqrt{\theta(\theta-2)}}{\theta-\sqrt{\theta(\theta-2)}}\right) - 2\right]$ if $\theta > 2$ | $\frac{2V_m}{\bar{s}_p}\ln\left(\frac{8\Lambda V_{s,r}}{2V_m/\bar{s}_p}\right)$ |
| Gaussian [3] | gamma ($\frac{1}{2}$) [11.7] | $1/(1+\sqrt{\theta})$ | $\sqrt{4\Lambda V_{s,r}(2V_m/\bar{s}_p)}$ |
| Reflected exponential [6] | sq-exponential [70] | $\{1 + [\pi/2\sqrt{\theta}(\theta-1) - \theta\ln(\theta)]/[1+\theta]\}/(1+\theta)$ | $1.57\sqrt[4]{4\Lambda V_{s,r}(2V_m/\bar{s}_p)}$ |
| Reflected gamma ($\frac{1}{2}$) [11.7] | sq-gamma ($\frac{1}{2}$) [183.9] | $[1 + (\sqrt[4]{\theta^3} - \sqrt[4]{\theta})/\sqrt{2}]/(1+\theta)$ | $0.707\sqrt[4]{(4\Lambda V_{s,r})^3(2V_m/\bar{s}_p)}$ |
| Reflected sq-gamma ($\frac{1}{2}$) [183.9] | qt-gamma ($\frac{1}{2}$) [46704] | $\{1 + 0.654(\sqrt[8]{\theta^7} - \sqrt[8]{\theta}) - 0.270(\sqrt[8]{\theta^5} - \sqrt[8]{\theta^3})\}/(1+\theta)$ | $0.654\sqrt[8]{(4\Lambda V_{s,r})^7(2V_m/\bar{s}_p)}$ |

[a] sq-exponential, sq-gamma ($\frac{1}{2}$), and qt-gamma ($\frac{1}{2}$) represent squared exponential, squared gamma ($\frac{1}{2}$)-, and quartic gamma ($\frac{1}{2}$)-distributed mutational effects (one sided for fitness and symmetrical for the trait), respectively. The numbers in brackets [ ] are the ratios of the fourth moment to the squared variance, $\kappa_4 = E(a^4)/\varepsilon_m^4$, which describe the leptokurtoses of distributions.

sider the impact of such a correlation on the results for $V_G$ and $V_{s,t}$. Using the method of Keightley and Hill (1990), the mutational effects $|a|$ and $s$ were sampled from a bivariate gamma ($\frac{1}{2}$) distribution. The numerical calculations show that when this correlation is only intermediate ($\rho < 0.5$), its impact on $V_G$ and $V_{s,r}$ is not large (see Figure 6). Unless the correlation between $|a|$ and $s$ is very high, the results based on the assumption of independent mutational effects apply approximately.

## DISCUSSION

The assumptions for the origin of both kinds of selection are distinct. In models of real stabilizing selection, selection is assumed to arise solely from the deviations of the metric traits from their optimum due to mutational effects (*i.e.*, phenotypic selection, selection directly acting on the trait), whereas in pure pleiotropic models the apparent stabilizing selection is assumed to arise as a consequence of direct effects of deleterious mutations on overall fitness, ignoring any effect on the trait itself (*i.e.*, selection acting directly on genes). By assuming that the total stabilizing selection observed on individuals comes simultaneously from both kinds of selection, the joint effect model presented in this article includes the properties of both the real stabilizing selection (Turelli 1984) and the pure pleiotropic models (Keightley and Hill 1990). It is important to know whether new findings about the genetic variation and the stabilizing selection emerge from the analyses of the joint effect model.

The pure pleiotropic model (Keightley and Hill 1990; Kondrashov and Turelli 1992; Zhang *et al.* 2002) can account for substantial quantitative genetic variation, but the apparent stabilizing selection of strength $V_{s,t} > V_G^2/V_m$ seems too weak. Moreover, a defect of such a model with continuously varying and leptokurtic mutational effects is that the genetic variance keeps increasing with the population effective size (Keightley and Hill 1990; Caballero and Keightley 1994), although this can be avoided by assuming there is a minimum mutational effect (Zhang *et al.* 2002). Such divergence of $V_G$ as population size becomes infinite is actually an artifact of allowing a density of mutations that are precisely neutral. Equations 3 and 4 show clearly that when $V_G^2/V_{s,r} \gg V_m$, which might usually be true (Endler 1986; Falconer and Mackay 1996), the strength of total stabilizing selection is significantly less than the constraint $V_G^2/V_m$ imposed by the pure pleiotropic model. Therefore the complete treatment of both pleiotropic effects and real stabilizing selection breaks down the constraint between $V_{s,t}$ and $V_G$ in the pure pleiotropic model (Barton 1990; Kondrashov and Turelli 1992; Zhang *et al.* 2002). With weak real stabilizing selection on the trait, it is easy to produce a high $V_G$ but the total stabilizing selection also appears weak, whatever the pleiotropic effect. For there to be strong

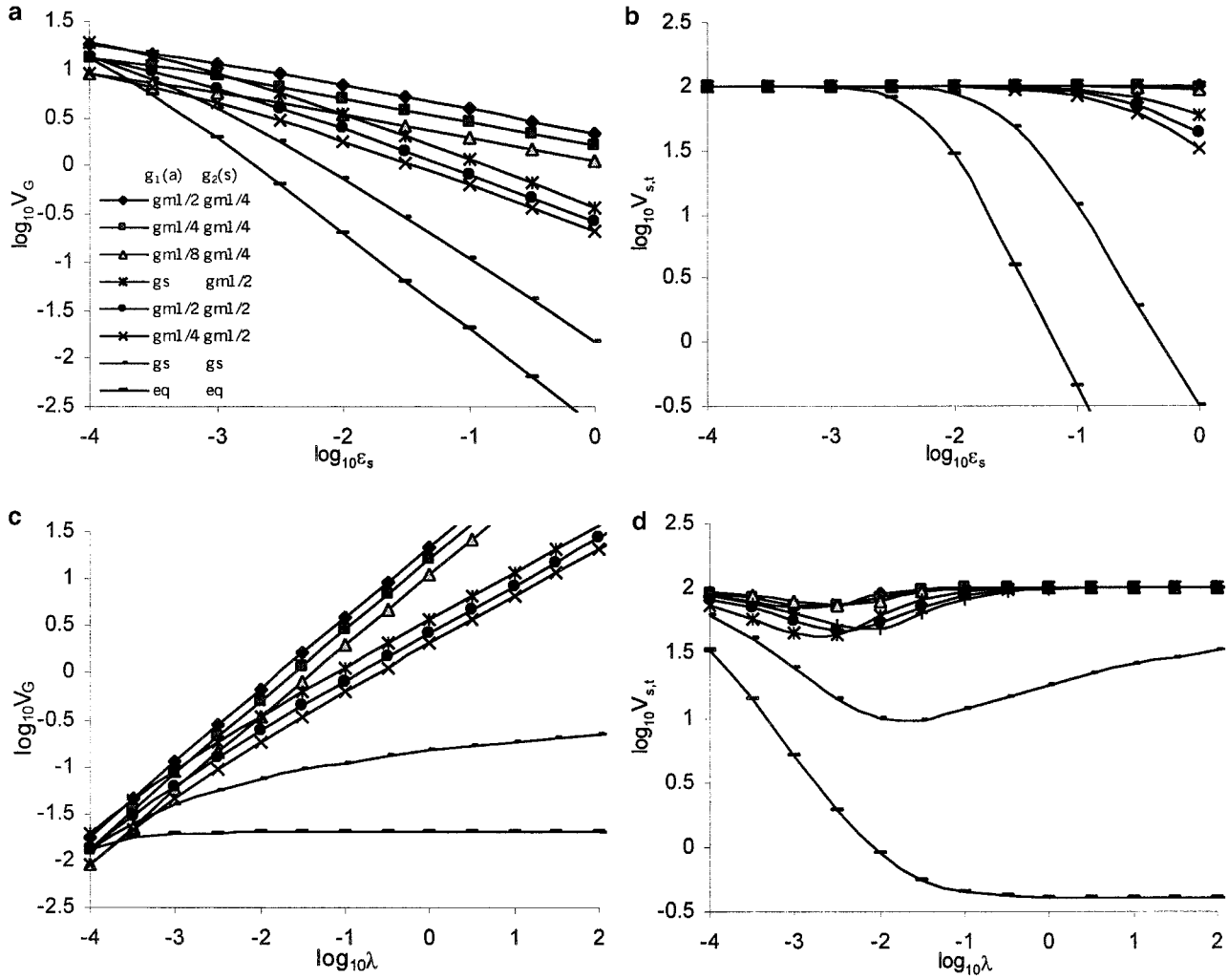


FIGURE 5.—Variation maintained in the metric trait in an infinite population and the strength of real stabilizing selection as functions of (a and b) the pleiotropic effect and (c and d) the mutation rate. The interaction of both effects of selection (pleiotropic effect and real stabilizing selection) has been investigated by either (a and b) fixing the effect of real stabilizing selection (*i.e.*, $\lambda = 0.1$) or (c and d) fixing the pleiotropic effect (*i.e.*, $\varepsilon_s = 0.1$). Results are shown for $V_{s,r} = 100$. For differently distributed mutational effects on the trait and on fitness, the same variablities $\varepsilon_a$ and $\varepsilon_s$ are assumed, and mutational effects are independent. The symbols eq, gs, gm1/2, gm1/4, and gm1/8 represent equally, Gaussian-, gamma (½)-, gamma (¼)-, and gamma (⅛)-distributed mutational effects (one sided for fitness and symmetrical for the trait), respectively.

total stabilizing selection, the real stabilizing selection should be strong enough while the impact of pleiotropic effects is relatively small [see inequality (4)].

In contrast to TANAKA's (1996, 1998) pleiotropic model, which includes both kinds of selection but assumes an equal deleterious effect on fitness for all mutants, the joint effect model presented here, which allows both mutational effects to vary, leads to quite different pictures of how both kinds of selection are responsible for $V_G$. As found by TANAKA (1996) and intuitively argued by KONDRASHOV and TURELLI (1992), the total selection coefficient should be equal to a linear sum of that arising from real stabilizing selection and that solely attributable to pure pleiotropic effect: $s_T = \bar{s}_r + \bar{s}_p$. As in general $\bar{s}_p \gg \bar{s}_r$ (GILLESPIE 1991; KONDRASHOV and TURELLI 1992), the total selection coefficient is approximately equal to the pleiotropic effect $s_T \approx \bar{s}_p$ and KONDRASHOV and TURELLI (1992, p. 615) concluded that real stabilizing selection was "essentially irrelevant to the dynamics of the alleles responsible for variation in the trait." Within the joint effect model, the total selection coefficient, which is a complicated function of both components [see (8) and APPENDIX B], is $> \bar{s}_r$, but $\ll \bar{s}_p$ as well if $\bar{s}_p \gg \bar{s}_r$. This of course leads to a larger $V_G$. Therefore pleiotropic effects on fitness can be large but their impact on $V_G$ is limited.

For a simple explanation of why a distribution of pleiotropic effects allows the model to generate high $V_G$, suppose that new mutations are divided into two equally possible classes: one with equal pleiotropic effect $s_1$, the other with $s_2$, but with both having the same effect on the trait (*i.e.*, $\bar{s}_r$). The two classes contribute to $V_G$ as $2V_m/(s_1 + \bar{s}_r)$ and $2V_m/(s_2 + \bar{s}_r)$, respectively, from TANAKA (1996), and the total genetic variance main-

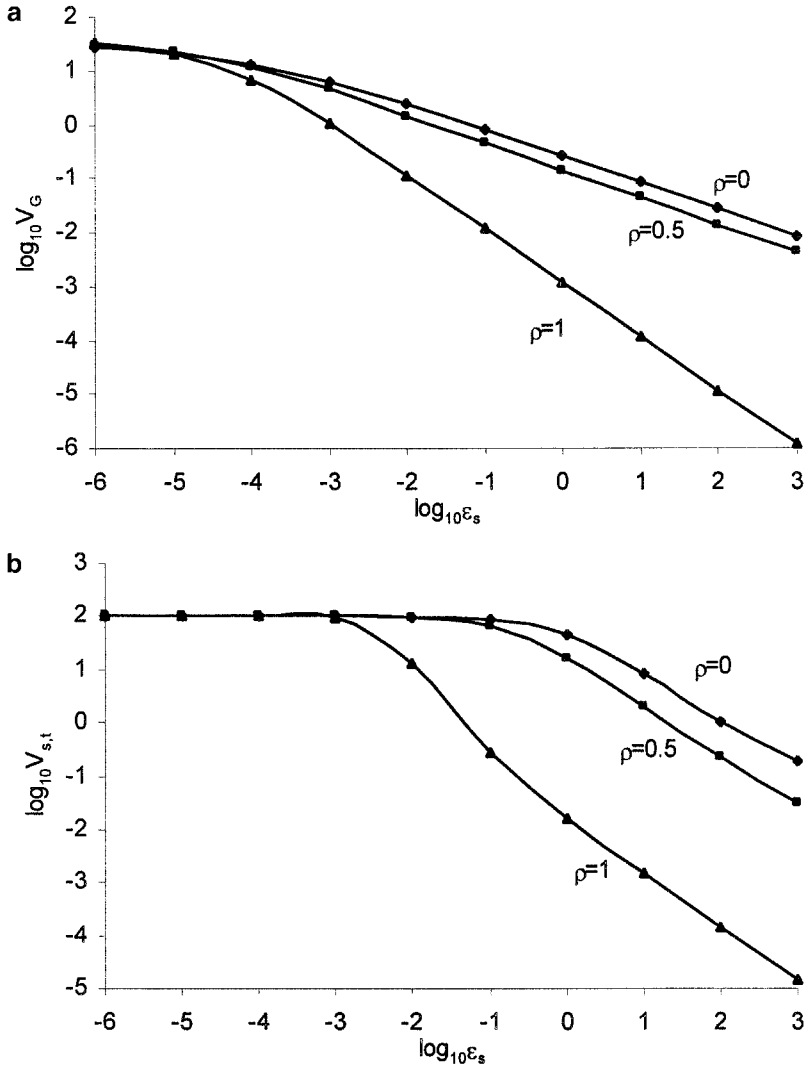


FIGURE 6.—The influence of the correlation (ρ) between absolute values of mutational effects on the metric trait ($|a|$) and on fitness ($s$) on (a) the genetic variance and (b) observed strength of apparent stabilizing selection. Mutational effects on the trait and fitness follow a bivariate gamma (½). The mutation rate is $\lambda = 0.1$ and the intrinsic strength of real stabilizing selection is $V_{s,r} = 100$. Results are shown for three correlations.

tained is then larger than if all mutations have the same mean pleiotropic effect $(s_1 + s_2)/2$ because $[1/(s_1 + \bar{s}_r) + 1/(s_2 + \bar{s}_r)]/2 > 1/[(s_1 + s_2)/2 + \bar{s}_r]$. The numerical results show that if a very small minimum total selection coefficient, say $10^{-10}$, is assumed, the genetic variance maintained is nearly the same as that without such minimum fitness effect. As the mutant alleles of large effects on fitness would be quickly eliminated from the population, the genetic variance is attributable primarily to mildly deleterious mutations. The huge genetic variation generated in the joint effect model of continuously varying pleiotropic effects on fitness, therefore, comes mainly from "a class of alleles with significant effects on the character, but very little effect on fitness" (BARTON 1990, p. 779).

It is also interesting to compare the prediction of the joint effect model with the house-of-cards approximation $V_G = 4\lambda_t V_{s,r}$, where $\lambda_t$ refers solely to the total rate of mutations that affect the metric trait under study (TURELLI 1984, 1985). When the pleiotropic effect is weak in relation to the effect on fitness from real stabilizing selection, the genetic variance can be approximated by the house-of-cards approximation (see Equation 8, Table 1, and Figure 5); but if the pleiotropic effect is large, the genetic variance maintained is given by (9) for Gaussian effects on the trait and gamma (½) effects on fitness of mutations. As the genome-wide mutation rate λ exceeds $\lambda_t$, our prediction of $V_G$ may not be smaller than the house-of-cards approximation (*cf.* TANAKA 1996, 1998). For the typical estimate of strength of real stabilizing selection, $V_{s,r} = 20$ (TURELLI 1984), $V_G = 0.4\sqrt{\lambda/\bar{s}_p}$ from (9) under the condition $\bar{s}_p \gg \bar{s}_r$ (*i.e.*, $\lambda\bar{s}_p \gg 2.5 \times 10^{-5}$). This implies that if both the mutation rate and the mean pleiotropic effect are of similar order, abundant genetic variation can be maintained, and less restrictive conditions are required if the mutational effects are more leptokurtic (see Table 1 and Figure 5).

The mutation rate λ assumed in this study is the genome-wide mutation rate. Although all of the mutations may affect fitness to a varying degree, only a small fraction of them may be considered to appreciably affect the trait under study. It is, however, unrealistic to assume no effect and more appropriate to assume that the distribution of mutational effects on the trait is more lepto-

kurtic than on fitness (see Robertson 1967; Keightley and Hill 1988; Hill and Caballero 1992). The analyses of the joint effect model show that the genetic variance maintained at mutation-selection balance depends not only on the variance of mutational effects but also on their leptokurtosis. For a given distribution of mutational effects on fitness, a more leptokurtic mutational effect on the trait induces a smaller genetic variance, consistent with the results of Keightley and Hill (1988) who studied pure real stabilizing selection in finite populations. Even for this more realistic model, the joint effect model can still generate abundant genetic variation if mutational effects on fitness are sufficiently leptokurtic, say gamma (½), and the genome-wide mutation rate is not $<0.01$ (see Figure 5).

The scanty data for multicellular eukaryotes are consistent with any value of $\lambda$ between 0.1 and 100 (Charlesworth *et al.* 1990; Kondrashov and Turelli 1992; Lynch *et al.* 1999; Kumar and Subramanian 2002). 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; Garcia-Dorado *et al.* 1999; 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 $\lambda$ is in the range 0.09–1.0 and $\varepsilon_s$ 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 $\lambda$ is of the order $10^{-3}$ and $E[s/2]$ is in the range 0.01–0.05 (Wloch *et al.* 2001). Even with such large pleiotropic effects, our joint effect model, which assumes leptokurtic effects both on the trait and on fitness of mutations, predicts high heritabilities under strong total stabilizing selection unless $\lambda$ is very small, say $<0.01$ (see Equation 8, Figure 5, and Table 1). But the estimates of mutation and selection parameters are not very reliable (Kondrashov 1998; Lynch *et al.* 1999; Kingsolver *et al.* 2001). Mutation rates are usually underestimated and mean fitness effects are usually overestimated as the effects of most mutants may be too small to be detected (Kondrashov and Turelli 1992; Davies *et al.* 1999; Lynch *et al.* 1999). The observation of high heritabilities and strong total stabilizing selection may then be interpreted in terms of the joint effect model of continuously varying mutational effects.

In summary, the joint effect model presented here shows that $V_G$ and $V_{s,t}$ are determined primarily by real stabilizing selection while pleiotropic effects, which can be large, have only a limited impact. With an abundant supply of mutations and leptokurtic mutational effects on fitness, the joint effect model can induce a significant amount of stabilizing selection as well as a substantial genetic variance, even with a mutational variance on the trait as low as $V_m = 10^{-3}V_e$ (*cf.* Barton 1990). Combining both kinds of selection and allowing mutational effects on the metric trait and on fitness both to vary change the picture of the mutation-selection model and therefore enable the mutation-selection balance to be a plausible cause of quantitative variation.

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## LITERATURE CITED

Barton, N. H., 1986 The maintenance of polygenic variation through a balance between mutation and stabilizing selection. Genet. Res. **47:** 209–216.

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. Rev. Genet. **3:** 11–21.

Barton, N. H., and M. Turelli, 1989 Evolutionary quantitative genetics: how little do we know? Annu. Rev. Genet. **23:** 337–370.

Bulmer, M. G., 1985 *The Mathematical Theorey of Quantitative Genetics.* Clarendon Press, Oxford.

Bulmer, M. G., 1989 Maintenance of genetic variability by mutation-selection balance: a child's guide through the jungle. Genome **31:** 761–767.

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

Bürger, R., and A. Gimelfarb, 1999 Genetic variation maintained in multilocus models of additive quantitative traits under stabilizing selection. Genetics **152:** 807–820.

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.

Charlesworth, B., D. Charlesworth and M. T. Morgan, 1990 Genetic loads and estimates of mutation rates in highly inbred plant populations. Nature **347:** 380–382.

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.

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.

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.

Garcia-Dorado, G., C. Lopez-Fanjul and A. Caballero, 1999 Properties of spontaneous mutations affecting quantitative traits. Genet. Res. **74:** 341–350.

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., 1991 *The Causes of Molecular Evolution*. Oxford University Press, Oxford.

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., 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 2nd 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., 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 traits 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., 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.

Kruuk, L. E. B., J. Slate, J. M. Pemberton, S. Brotherstone, F. E. Guinness *et al.*, 2002 Antler size in red deer: heritability and selection, but no evolution. Evolution (in press).

Kumar, S., and S. Subramanian, 2002 Mutation rates in mammalian genomes. Proc. Natl. Acad. Sci. USA **99:** 803–808.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz *et al.*, 1999 Perspective: spontaneous deleterious mutation. Evolution **53:** 645–663.

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.

Moran, P. A. P., 1968 *An Introduction to Probability Theory*. Clarendon Press, Oxford.

Robertson, A., 1956 The effect of selection against extreme deviants based on deviation 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 A. Brink. The University of Wisconsin Press, Madison, WI.

Shaw, R. G., D. L. Byers and E. Darmo, 2000 Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. Genetics **155:** 369–378.

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.

Tanaka, Y., 1998 A pleiotropic model of phenotypic evolution. Genetica **102/103:** 535–543.

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.

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.

Zhang, X.-S., J. Wang and W. G. Hill, 2002 Pleiotropic model of maintenance of quantitative genetic variation at mutation-selection balance. Genetics **161:** 419–433.

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

Let us assume that the gene action within and across loci is additive and loci are unlinked and in linkage equilibrium. A random-mating diploid population is assumed. Mutations in a diploid individual have an effect on a metric trait $z$ with $a$ the difference in value between homozygotes and a net effect on fitness that includes pleiotropic effects on all other traits, with $s$ the difference in fitness between homozygotes. There is therefore a bivariate distribution, $h(a, s)$, of $a$ and $s$ for alleles affecting the trait. If there is real stabilizing selection, the total observed stabilizing selection would come from these two parts. Following the method of Falconer and Mackay (1996, p. 27), the mutant allele frequency within a single-locus model is given by $x_1 = \{x - x(1 - x)[s/2 + a^2/(8V_{s,r})]\}/\bar{w}$ with the mean fitness given by $\bar{w} = 1 - x(1 - x)a^2/(4V_{s,r})$ if the previous frequency is $x$. With weak selection (*i.e.*, $\bar{w} \approx 1$), the change in the mutant allele frequency is $\Delta x = x_1 - x \approx -x(1 - x)[s/2 + (1 - 2x)a^2/(8V_{s,r})]$. Thus the equivalent total selection coefficients are

$$\tilde{s} = s + (1 - 2x)a^2/(4V_{s,r}) \tag{A1}$$

(*cf.* Robertson 1956; Bulmer 1985; Keightley and Hill 1988). With Kimura's (1969) diffusion approximations under the infinite independent loci model, the genetic variance can be evaluated by the equation

$$V_G = \int_0^\infty \int_{-\infty}^{\infty} \int_{1/(2N)}^{1-1/(2N)} h(a, s)\Phi(x; \tilde{s})2x(1 - x)\frac{a^2}{4}dxdads \tag{A2}$$

(Zhang *et al.* 2002), the variance of squared deviations is

$$V_{G2} = \int_0^\infty \int_{-\infty}^{\infty} \int_{1/(2N)}^{1-1/(2N)} h(a, s)\Phi(x; \tilde{s}) \times \{2x(1 - x) - 3[2x(1 - x)]^2\}\frac{a^4}{16}dxdads + 2V_G^2 \equiv m_4 + 2V_G^2, \tag{A3}$$

and the covariance of relative fitness (taking positive values because $\bar{s}$ is defined to be positive) and the squared deviation is

$$\mathrm{Cov}(w,\,(z-z_{\mathrm{m}})^2)=\int_0^{\infty}\int_{-\infty}^{\infty}\int_{1/(2N)}^{1-1/(2N)}h(a,\,s)\Phi(x;\,\bar{s})\times 2x(1-x)(1-2x)\frac{s}{2}\frac{a^2}{4}dx\,da\,ds+\frac{V_{\mathrm{G}2}}{2V_{\mathrm{s,r}}}. \tag{A4}$$

Thus the covariance is partitioned into two parts: one due to pleiotropic selection and the other due to stabilizing selection. In the above equations $\Phi(x;\,\bar{s})$ is the equilibrium frequency distribution of mutations, given by

$$\Phi(x;\,\bar{s})=\frac{4N_{\mathrm{e}}\lambda}{x(1-x)\,G(x)}\frac{\int_x^1 G(\xi)\,d\xi}{\int_0^1 G(\xi)\,d\xi},$$

$$\text{with } G(x)=\exp\left\{-2N_{\mathrm{e}}x\left(s+\frac{(1-x)\,a^2}{4V_{\mathrm{s,r}}}\right)\right\}.$$

If a population has a large effective size $N_{\mathrm{e}}$ such that $2N_{\mathrm{e}}a^2/(4V_{\mathrm{s,r}})\gg 1$, numerical calculations show that the distribution function $\Phi(x;\,\bar{s})$ is finite only for very small values of $x$; that is, the equilibrium frequency of mutant alleles is very small, $x\approx 0$. With the assumption that the mutant alleles are very rare, the equivalent total selection coefficient can be approximated by

$$\bar{s}=s+a^2/(4V_{\mathrm{s,r}}). \tag{A5}$$

Thus the equilibrium genetic variance is

$$V_{\mathrm{G}}=\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)H(\bar{s})\,(a^2/4)\,da\,ds, \tag{A6}$$

and the observed strength of total stabilizing selection, *i.e.*, the variance of the total fitness profile as defined by Barton (1990) and Keightley and Hill (1990), is

$$V_{\mathrm{s,t}}\equiv -V_{\mathrm{G}2}/[2\,\mathrm{Cov}(\mathrm{w},\,(z-z_{\mathrm{m}})^2)]=-(m_4+2V_{\mathrm{G}}^2)/[2\,\mathrm{Cov}(w,\,(z-z_{\mathrm{m}})^2)]. \tag{A7}$$

The fourth moment is $m_4=\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)\{H(\bar{s})-3K(\bar{s})\}(a^4/16)\,da\,ds$ and the covariance of relative fitness and squared deviation is $\mathrm{Cov}(w,\,(z-z_{\mathrm{m}})^2)=\mathrm{Cov}_{\mathrm{p}}+\mathrm{Cov}_{\mathrm{r}}$, in which $\mathrm{Cov}_{\mathrm{p}}=\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)\,C(\bar{s})\,(s/2)\,(a^2/4)\,da\,ds$ is the contribution due to pleiotropic effects of mutations and $\mathrm{Cov}_{\mathrm{r}}=V_{\mathrm{G}2}/(2V_{\mathrm{s,r}})$ due to real stabilizing selection. The expressions for the heterozygosity, $H(\bar{s})$, and for $K(\bar{s})$ and $C(\bar{s})$ are given by Zhang *et al.* (2002) by replacing $s$ by $\bar{s}$. In contrast with Keightley and Hill (1990), who assumed that the strength of stabilizing selection is measured in phenotypic standard deviation units and thus is a dimensionless quantity, $V_{\mathrm{s,t}}$ in this article is used in the same way as that in Turelli (1984) and thus has a dimension of genetic variance.

For an infinite population, by using the approximations $H(\bar{s})=C(\bar{s})=4\lambda/\bar{s}$ and $K(\bar{s})=0$, the expressions for the genetic variance and strength of the total stabilizing selection reduce to those given in (2) and (3). Equation 3 is obtained by noting that

$$\begin{aligned}V_{\mathrm{G}2}&=4\lambda\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)[(a^4/16)/\bar{s}]\,da\,ds+2V_{\mathrm{G}}^2\\&=4\lambda V_{\mathrm{s,r}}\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)[(a^2/4)(\bar{s}-s)/\bar{s}]\,da\,ds+2V_{\mathrm{G}}^2\\&=2V_{\mathrm{s,r}}\{V_{\mathrm{m}}-4\lambda\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)[(a^2/4)(s/2)/\bar{s}]\,da\,ds+V_{\mathrm{G}}^2/V_{\mathrm{s,r}}\},\end{aligned}$$

where $\mathrm{Cov}_{\mathrm{p}}=4\lambda\int_0^{\infty}\int_{-\infty}^{\infty}h(a,\,s)[(s/2)(a^2/4)/\bar{s}]\,da\,ds$, and the covariance of relative fitness and squared deviation $\mathrm{Cov}(w,\,(z-z_{\mathrm{m}})^2)=\mathrm{Cov}_{\mathrm{p}}+V_{\mathrm{G}2}/2V_{\mathrm{s,r}}=V_{\mathrm{m}}+V_{\mathrm{G}}^2/V_{\mathrm{s,r}}$.

## APPENDIX B

We consider the evaluation of genetic variance assuming that the population is under stabilizing selection because of the joint effect of pleiotropic and real stabilizing selections and that both mutational effects are independent. If mutational effects on the trait and on fitness follow distributions $g_1(a)$, where $-\infty<a<\infty$, and $g_2(s)$, where $0<s<\infty$, respectively, then evaluation of $V_{\mathrm{G}}=4\lambda V_{\mathrm{s,r}}I_2$ according to (2) is equivalent to the expectation,

$$I_2=\int_{-\infty}^{\infty}\int_0^{\infty}g_1(a)\,g_2(s)[\xi^2/(\xi^2+s)]\,dy_1\,dy_2=E[\xi^2/(\xi^2+s)],$$

in which scaled effects on the trait $\xi=a/\sqrt{4V_{\mathrm{s,r}}}$ are symmetrical about 0 and distributed with mean 0 and variance $\bar{s}_{\mathrm{r}}=\varepsilon_a^2/4V_{\mathrm{s,r}}$. This integral can be obtained exactly for some types of mutational effects and the results are listed in Table 1, showing that $I_2$ depends on only the ratio $\bar{s}_{\mathrm{p}}/\bar{s}_{\mathrm{r}}$, confirmed by numerical calculations on other types of mutational effects. The population mean of the total selection coefficient is thus given by $s_{\Gamma}=\bar{s}_{\mathrm{r}}/I_2$. One example is where mutations have Gaussian effects on the trait and gamma (½) effects on fitness (*i.e.*, a squared Gaussian random variable). Making the transformation $(\xi,\,s)$ to $(\xi,\,v)$, whereby the ratio $v=(s/\bar{s}_{\mathrm{p}})/(\xi^2/\bar{s}_{\mathrm{r}})$ is $F$-distributed with 1 d.f. in both the numerator and denominator, integrating $\xi$ leads to the density function of $v$, $\phi(v)=1/[\pi(1+v)\sqrt{v}]$ (Moran 1968, p. 332). Noting that $\xi^2/(\xi^2+s)=1/[1+(\bar{s}_{\mathrm{p}}/\bar{s}_{\mathrm{r}})v]$, we have $E[\xi^2/(\xi^2+s)]=\int_0^{\infty}\phi(v)[1+(\bar{s}_{\mathrm{p}}/\bar{s}_{\mathrm{r}})v]^{-1}dv=\int_0^{\infty}\{2/[\pi(1+t^2)(1+(\bar{s}_{\mathrm{p}}/\bar{s}_{\mathrm{r}})t^2)]\}dt=1/(1+\sqrt{\bar{s}_{\mathrm{p}}/\bar{s}_{\mathrm{r}}})$.

Thus the expectation is determined only by the ratio of $\bar{s}_{\mathrm{p}}$ to $\bar{s}_{\mathrm{r}}$.