Linkage and the Maintenance of Heritable Variation by Mutation-Selection Balance

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ABSTRACT

The role of linkage in influencing heritable variation maintained through a balance between mutation and stabilizing selection is investigated for two different models. In both cases one trait is considered and the interactions within and between loci are assumed to be additive. Contrary to most earlier investigations of this problem no a priori assumptions on the distribution of genotypic values are imposed. For a deterministic two-locus two-allele model with recombination and mutation, related to the symmetric viability model, a complete nonlinear analysis is performed. It is shown that, depending on the recombination rate, multiple stable equilibria may coexist. The equilibrium genetic and genic variances are calculated. For a polygenic trait in a finite population with a possible continuum of allelic effects a simulation study is performed. In both models the equilibrium genetic and genic variances are roughly equal to the house-of-cards prediction or its finite population counterpart as long as the recombination rate is not extremely low. However, negative linkage disequilibrium builds up. If the loci are very closely linked the equilibrium additive genetic variance is slightly lower than the house-of-cards prediction, but the genic variance is much higher. Depending on whether the parameters are in favor of the house-of-cards or the Gaussian approximation, different behavior of the genetic system occurs with respect to linkage.

THE question of how much heritable variation in quantitative traits can be maintained through a balance between mutation and stabilizing selection has received much attention during recent years. Basically, two kinds of models have been treated analytically, namely diallelic multilocus models (LATTER 1960; BULMER 1972, 1980; BARTON 1986) and continuum-of-alleles models (KIMURA 1965; LATTER 1970; LANDE 1975; FLEMING 1979; TURELLI 1984, 1986; NAGYLAKI 1984; BÜRGER 1986, 1988a,b; FOLEY 1987). TURELLI (1984) and SLATKIN (1987) also considered models with three and five alleles per locus. These analyses, except BULMER's, were primarily devoted to deterministic models not taking into account random drift in finite populations.

For the continuum-of-alleles model two different approximations have been derived, the Gaussian approximation (KIMURA 1965; LANDE 1975) and the house-of-cards approximation (TURELLI 1984). The Gaussian approximation yields $\hat{\sigma}_G^2(G) = 2n\sqrt{\mu}\alpha^2 V_s$ as the equilibrium variance, where n denotes the number of loci affecting the trait, μ the per locus mutation rate, α^2 the variance of mutational effects and V_s the inverse measure of the strength of stabilizing selection (see Equation 9 below). The house-of-cards approximation leads to an equilibrium variance of $\hat{\sigma}_G^2(HC) = 4n\mu V_s$. The latter agrees with the diallelic and triallelic results of LATTER (1960), BULMER (1972), TURELLI (1984) and SLATKIN (1987). The Gaussian and the house-of-cards approximations are, however, extrapolations from the haploid one-locus model under the assumption of global linkage equilibrium. The haploid model has been analyzed rigorously by BÜRGER (1986, 1988a,b), where existence, uniqueness and global stability of a stationary frequency distribution of types have been proved under very general assumptions. In particular, an upper bound for the true equilibrium variance has been derived which is almost identical to the house-of-cards prediction. It follows from these results that the Gaussian approximation is applicable only if $\alpha^2 \leq 4\mu V_s$, but in this case it is lower than the house-of-cards prediction. In fact, LANDE (1975) had noted that the validity of the Gaussian approximation rests on the assumption that $\alpha^2 \ll \mu V_s$ and the numerical results of TURELLI (1984, 1986) suggested that the Gaussian approximation can be correct only if the variance of mutational effects per locus is much smaller than the existing variance at this locus.

It is the aim of the present paper to go beyond extrapolations of haploid models and to investigate the influence of linkage in models of mutation-stabilizing selection balance. LANDE (1975, 1977) investigated the role of linkage and found that the equilibrium expressed genetic variance is independent of the linkage relation of the loci unless linkage is extremely tight. His analysis, however, is based on the assumption of a normal distribution of allelic effects. On the basis of a second order approximation, but based on

the same assumptions concerning the relative magnitudes of the selection and mutation parameters, FLEMING (1979) found that recombination has a very weak influence on the equilibrium variance. These results were confirmed by TURELLI (1984) who performed simulations for two-, four-, and six-locus models. None of these authors reported the existence of multiple stable equilibria. On the other hand BARTON (1986) investigated a diallelic multilocus model assuming linkage equilibrium and a Gaussian distribution of genotypic values and found that stable equilibria may exist where the mean genotypic value deviates from the optimum and the equilibrium variance almost reaches the level of the Gaussian prediction.

Below I will rigorously analyze a diploid two-locus two-allele model of a trait under stabilizing selection and allow for mutation and recombination. No assumptions concerning the distribution of breeding values are imposed. It will be shown that number and position of equilibria depend on the recombination rate, whereas the equilibrium variance is almost independent of it and agrees with the house-of-cards prediction for a wide range of recombination fractions. Only for complete linkage does a slight decrease of the equilibrium variance occur.

The influence of linkage and recombination in finite populations with many loci affecting a trait is investigated using the simulation procedure described in BÜRGER, WAGNER and STETTINGER (1989). Whereas in that paper free recombination and a Gaussian mutant distribution were assumed, the present paper considers linkage between loci and, in addition to a Gaussian mutant distribution, a double γ distribution. The results are qualitatively similar to those of the deterministic two-locus model.

A DETERMINISTIC TWO-LOCUS MODEL

Consider two loci with two alleles each: A and a at the first locus and B and b at the second locus. Suppose that the effects of A, a, B, b on a quantitative character are $c - \alpha/2$, $c + \alpha/2$, $-c - \alpha/2$, $-c + \alpha/2$, respectively. Important special cases are $c = 0$ and $c = \alpha/2$. Assuming additivity within and between loci the effects of the gametes AB, Ab, aB, ab are, independently of c, $-\alpha$, 0, 0, α. From these the effects of all possible genotypes are easily calculated. Throughout we suppose that the trait is under stabilizing selection with optimum at zero such that fitness decreases monotonically from the optimum. This leads to fitness values of the genotypes as displayed in Table 1. Throughout, it is assumed that $0 < b \leq d/2 < \frac{1}{2}$. The assumption $d \geq 2b$ means that the fitness function is concave near the optimum and implies that all equilibria exhibit negative linkage disequilibrium (see APPENDIX). With quadratically deviating fitness

$$m(x) = 1 - s^*x^2, \tag{1}$$

TABLE 1

Fitness values in a diallelic two-locus model for a quantitative trait under stabilizing selection

	BB	*Bb*	*bb*
AA	$1 - d$	$1 - b$	1
Aa	$1 - b$	1	$1 - b$
aa	1	$1 - b$	$1 - d$

where x denotes the genotypic value and s^* is a measure for the strength of stabilizing selection (compare also Equation 9 below), one obtains $d = 4b = 4s$ with $s = s^*\alpha^2$.

This symmetric viability model was first investigated by WRIGHT (1952) and later by BODMER and FELSENSTEIN (1967), KARLIN and FELDMAN (1970) and HASTINGS (1987). These authors investigated the interplay between selection and recombination. I will, additionally, introduce mutation and assume equal forward and backward mutation rates μ at both loci. The frequencies of the gametes AB, Ab, aB and ab are denoted by x_1, x_2, x_3 and x_4, respectively. Linkage disequilibrium is denoted by $D = x_1x_4 - x_2x_3$, the recombination fraction between the loci by r. The marginal mean fitness values of the gametes are then

$$\begin{aligned} m_1 &= 1 - dx_1 - bx_2 - bx_3 \\ m_2 = m_3 &= 1 - bx_1 - bx_4 \\ m_4 &= 1 - bx_2 - bx_3 - dx_4 \end{aligned} \tag{2}$$

and the mean fitness of the population is

$$\begin{aligned} \bar{m} = 1 &- d(x_1^2 + x_4^2) \\ &- 12b(x_1x_2 + x_1x_3 + x_2x_4 + x_3x_4). \end{aligned} \tag{3}$$

Assuming that selection is weak and that the interaction between selection and mutation can be neglected, the gametic frequencies in a large population evolve approximately according to

$$\begin{aligned} \dot{x}_1 = dx_1/dt &= x_1(m_1 - \bar{m}) - rD \\ &\quad + \mu(x_2 + x_3 - 2x_1) \\ \dot{x}_2 = dx_2/dt &= x_2(m_2 - \bar{m}) + rD \\ &\quad + \mu(x_1 + x_4 - 2x_2) \\ \dot{x}_3 = dx_3/dt &= x_3(m_3 - \bar{m}) + rD \\ &\quad + \mu(x_1 + x_4 - 2x_3) \\ \dot{x}_4 = dx_4/dt &= x_4(m_4 - \bar{m}) - rD \\ &\quad + \mu(x_2 + x_3 - 2x_4). \end{aligned} \tag{4}$$

For an exact derivation additional assumptions have to be imposed, like no dominance in the death rates and small differences in the birth rates (NAGYLAKI and CROW 1974). Also in a continuous time model the parameter r is in fact the product of the recom-

bination rate and the birth rate of the double heterozygotes. Hence $r \leq 0.5$ does not necessarily hold. For technical simplicity and since most authors dealing with the problem of mutation stabilizing selection balance have used quadratically deviating fitness the following results are stated under the assumption of Equation 1. Throughout, $\mu > 0$ and $r > 0$ is assumed. As shown in the APPENDIX similar results hold for the more general fitness scheme.

Result 1: System (4) has the following equilibrium points.

(a) F1, given by

$$\hat{x}_1 = \hat{x}_4 = (1/4) + \hat{D}_1, \quad \hat{x}_2 = \hat{x}_3 = (1/4) - \hat{D}_1,$$

$$\hat{D}_1 = \frac{r + 4\mu}{4s} - \frac{1}{4s}\sqrt{s^2 + (r + 4\mu)^2},$$

exists for all admissable parameter values.

(b) If $r(s - 4\mu) > 8\mu^2$ then the following equilibria exist

$$\text{F2:} \quad \hat{x}_1 = \hat{x}_4 = \mu/s, \; \hat{x}_2 = \frac{1}{2} - \frac{\mu}{s} + \sqrt{\frac{1}{4} - \frac{\mu}{s} + \hat{D}},$$

$$\hat{x}_3 = 1 - \hat{x}_1 - \hat{x}_2 - \hat{x}_4$$

and

$$\text{F3:} \quad \hat{x}_1 = \hat{x}_4 = \mu/s, \; \hat{x}_2 = \frac{1}{2} - \frac{\mu}{s} - \sqrt{\frac{1}{4} - \frac{\mu}{s} + \hat{D}},$$

$$\hat{x}_3 = 1 - \hat{x}_1 - \hat{x}_2 - \hat{x}_4$$

where $\hat{D} = -2\mu^2/(sr)$. If $r(s - 4\mu) \leq 8\mu^2$ then F1 is globally asymptotically stable. If $r(s - 4\mu) > 8\mu^2$ then F1 is unstable and F2 and F3 are locally stable. They arise by a pitchfork bifurcation out of F1. The basin of attraction of F2 (F3) is the set where $x_2 > x_3$ ($x_2 < x_3$).

This result shows that the equilibrium gene and genotype frequencies depend on the history of the population and that there will always be negative linkage disequilibrium. It provides a considerable generalization of recent results of HASTINGS (1987). The proof (see APPENDIX) yields some insight into the dynamics of the system.

The aim of the present paper is to investigate the influence of linkage on the genetic variance maintained by mutation-stabilizing selection balance. The subsequent result is an easy consequence of Result 1 and summarizes the (additive) genetic variance $\hat{\sigma}_G^2$ ($= 2\alpha^2(x_1 + 0 + 0 + x_4)$) and the genic variance $\hat{\sigma}_g^2$ ($= 2\alpha^2 p_A (1 - p_A) + 2\alpha^2 p_B(1 - p_B)$, $p_A = x_1 + x_2$, $p_B = x_1 + x_3$) that is maintained at the various equilibria. A departure of these two variances indicates linkage disequilibrium (compare Equation 12).

Result 2: (a) If $r(s - 4\mu) \leq 8\mu^2$ then at the single stable equilibrium F1 the genetic and the genic variances are

$$\hat{\sigma}_G^2 = \alpha^2(1 + 4\hat{D}_1) = \alpha^2 + \frac{r + 4\mu}{s^*} - \sqrt{\alpha^4 + \left(\frac{r + 4\mu}{s^*}\right)^2} \tag{5}$$

and

$$\hat{\sigma}_g^2 = \alpha^2, \tag{6}$$

respectively. $\hat{\sigma}_G^2 \leq \min\{\alpha^2, 4\mu/s^*\}$ always holds and $\hat{\sigma}_G^2$ decreases as r decreases.

(b) If $r(s - 4\mu) > 8\mu^2$ then the equilibrium genetic and genic variances are

$$\hat{\sigma}_G^2 = 4\mu/s^* \tag{7}$$

and

$$\hat{\sigma}_g^2 = \frac{4\mu}{s^*}\left(1 + \frac{2\mu}{r}\right), \tag{8}$$

respectively, independently of which of the two equilibria F2 and F3 is approached.

(c) For fixed but arbitrary μ and s, $\hat{\sigma}_G^2$ is a function of r whose maximal value is $\min\{\alpha^2, 4\mu/s^*\}$ and whose minimal value is attained for $r = 0$. In particular, $\hat{\sigma}_{G,\min}^2/\hat{\sigma}_{G,\max}^2 \geq 2 - \sqrt{2}$. The maximum decrease due to reduction of r is obtained if $4\mu = s$.

It may be noticed that Equation 7 gives just the variance predicted by the house-of-cards model, using an extrapolation from the one-locus haploid case. For an illustration of Result 2, see Figure 1. It is also in good accordance with TURELLI's (1984, Table VII) simulations.

Most authors investigating the maintenance of genetic variation problem have used the fitness function

$$w(x) = \exp\left\{-\frac{x^2}{2V_s}\right\}. \tag{9}$$

If one puts $s^* = 1/(2V_s)$, w corresponds to m (Equation 1), since the logarithm transforms relative fitness to Malthusian fitness if selection is weak. The discrete time model is technically more difficult but leads to almost the same results.

In the case of a more general fitness function satisfying $d \geq 2b$ again equilibria corresponding to F1, F2 and F3 exist. Whereas F2 and F3 can be calculated exactly, F1 is the solution of a third order equation [see APPENDIX, part A]. In the APPENDIX it is also shown that the stability properties of these equilibria are completely analogous to those stated above for the special case. Moreover, the genic and genetic variances maintained at these equilibria are approximately those maintained in the special case with quadratically deviating fitness, if s is replaced by b. If, for example, $d = 2b$ then at F2 and F3 $x_1 = x_4 = (1/4)(1 - \sqrt{1 - 8\mu/b}) \approx \mu/b$ and if $d = 8b$ then $x_1 = x_4 = (1/8)(-1 + \sqrt{1 + 16\mu/b}) \approx \mu/b$.

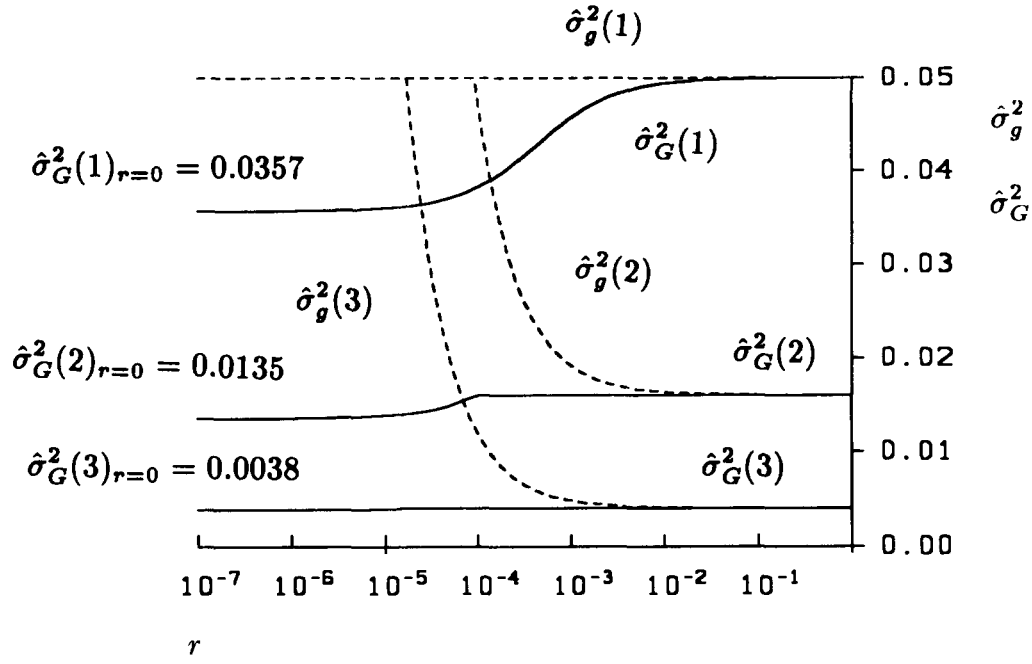

FIGURE 1.—Bold lines illustrate the genetic variance $\hat{\sigma}_G^2$ as a function of the recombination rate according to Result 2. Dashed lines illustrate the genic variance $\hat{\sigma}_g^2$. For the recombination rate, a logarithmic scale is chosen. The parameters are $\alpha^2 = 0.05$, $\mu = 10^{-4}$ and (1) $V_s = 100$, (2) $V_s = 20$ and (3) $V_s = 5$. In the first case F1 is stable for all values of r, in the second case the critical recombination rate where F1 becomes stable is $r_c = 9.4 \times 10^{-5}$ and in the third case $r_c = 1.7 \times 10^{-5}$.

SIMULATION RESULTS IN FINITE POPULATIONS

To study the effects of linkage on the equilibrium variance of a polygenic trait in a finite population, I use a simulation model that does not rely on any numerical evaluation of model equations. Instead it is a direct stochastic simulation of those events which actually occur in natural populations, such as mutation, recombination, survival and death. This model, mainly designed by G. P. WAGNER and F. STETTINGER, was applied in BÜRGER, STETTINGER and WAGNER (1989) to test the ranges of applicability of various models and approximations for predicting the additive genetic equilibrium variance of a quantitative character under mutation-selection balance. In this earlier study free recombination between all loci was assumed.

The simulation model is as follows. Every individual is characterized by n loci which contribute additively to a quantitative character. This produces a genotypic value X. Adding a Gaussian random number E with mean zero and variance 1 yields the phenotype P. On the phenotype stabilizing selection acts according to

$$w_P(P) = \exp\{-P^2/2\omega^2\}. \qquad (10)$$

This yields the mean fitness of individuals with genotypic value X. It is given by Equation 9 with $V_s = \omega^2 + 1$.

The life cycle consists of three stages. (a) From a base population consisting of the surviving offspring of the preceding generation breeding pairs are sampled without replacement. To keep the population size constant a fixed number of "nesting places" that limits the maximal population size N_p was assumed. (b) Each breeding pair produces 10 offspring. The genotype of each descendent is obtained from its parents by recombination according to the specified recombination rate r of adjacent loci without interference. Afterward mutation was performed by adding a Gaussian or a double γ (see Equation 12 below) random number with mean zero and variance α^2 to the current allelic effect. (c) Then viability selection was imposed by assigning fitness values according to (10). The fitness values vary between 0 and 1 and were interpreted as probabilities of survival. More precisely, for each individual a random number (uniformly distributed between 0 and 1) was chosen and the individual survived if its fitness was greater than this random number. The surviving offspring served as the base population for the next parental generation, as described above.

Since each breeding pair produces exactly 10 offspring and sampling is performed without replacement, the effective population size is slightly larger than the number of parents N_p. If $N_p = 20$ then $N_e = 22.5$ and if $N_p = 100$ then $N_e = 111.5$. Some simulations show that N_e is almost independent of the selection intensity. For more details, see BÜRGER, WAGNER and STETTINGER (1989). To produce the statistics, between 40 and 80 different initial populations were

generated and run for 1000 generations if $N_p = 20$ and for 2000 generations if $N_p = 100$. For each run the time average over generations 600 to 1000 and 1400 to 2000, respectively, of the additive genetic variance was taken as an estimator of the variance of the stationary distribution. The mean of these values was taken as the observed equilibrium variance $\hat{\sigma}_G^2$ (Obs). More than 40 runs were performed if the standard deviation of the first 40 runs was larger than 10% of the mean. This was necessary mainly for $N_p = 20$.

BÜRGER, WAGNER and STETTINGER (1989) showed that in finite populations the expected equilibrium variance is very well approximated by the formula

$$\hat{\sigma}_G^2(\text{SHC}) = \frac{4n\mu V_s}{1 + (V_s/N_e\alpha^2)}, \qquad (11)$$

although (11) applies only to equal mutational variances and equal mutation rates at all loci. I will refer to Equation 11 as the stochastic or finite population extension of the house-of-cards prediction, since it interpolates between the house-of-cards prediction $\hat{\sigma}_G^2(\text{HC})$ and the neutral prediction $\hat{\sigma}_G^2(N) = 4n\mu\alpha^2N_e$. The latter may be found in LYNCH and HILL (1986), for example. KEIGHTLEY and HILL (1988) and BARTON (1989) found Equation 11 on the basis of different model assumptions.

To find out the influence of linkage and nonrandom associations between loci the genic variance was calculated. Since in the present model epistasic interactions of effects are neglected the expressed genetic variance can be decomposed according to

$$\hat{\sigma}_G^2 = \hat{\sigma}_g^2 + C_D, \qquad (12)$$

where $\hat{\sigma}_g^2 = \sum_i(\text{Var}(X_i) + \text{Var}(X_i^*))$, X_i and X_i^* being the maternal and paternal contributions from the ith locus, is called the genic variance. C_D represents the covariances between alleles at different gametes and loci due to Hardy-Weinberg and linkage disequilibrium [see also BULMER (1980) Ch. 9; (1988)]. In the simulations the genic variance was measured in the same way as the additive genetic variance and is denoted by $\hat{\sigma}_g^2$(Obs). They were calculated among offspring before selection. Similarly, the kurtosis of the stationary distribution of genotypic values was measured and, at the gene level, mean heterozygosity and percentage of polymorphic loci.

The influence of recombination has been investigated for three different parameter sets, namely Gaussian distribution of mutational effects with variance $\alpha^2 = 0.05$ and 10 loci contributing to the trait with $\mu = 10^{-3}$ on the one hand and 50 loci with $\mu = 10^{-4}$ on the other hand. Additionally, a double gamma distribution (reflected about zero) with $\alpha^2 = 0.05$, 50 loci and $\mu = 10^{-4}$ was investigated. The latter has been suggested by HILL (*e.g.*, KEIGHTLEY and HILL 1988) and is given by

$$u(x) = \frac{a^\beta}{2\Gamma(\beta)} |x|^{\beta-1}\exp\{-a|x|\}, \qquad (13)$$

where $\alpha^2 = [\beta(\beta + 1)/a^2]$ is the variance and a defines the scale. In the present simulations $\beta = 1/2$ was chosen, which gives a highly leptokurtic mutational distribution.

TABLE 2

Influence of the recombination rate on the observed additive genetic variance and genic variance at stochastic equilibrium

V_s	$\hat{\sigma}_G^2$ (SHC)	Variance	r = 0.5	0.1	0.01	0.001	0
100	0.212	Additive	0.202	0.179	0.214	0.174	0.193
		Genic	0.202	0.179	0.230	0.198	0.204
10	0.143	Additive	0.125	0.138	0.117	0.112	0.098
		Genic	0.126	0.142	0.133	0.155	0.152
2	0.059	Additive	0.056	0.058	0.053	0.048	0.042
		Genic	0.057	0.061	0.069	0.097	0.077

The distribution of mutant effects is normal with mean zero and variance $\alpha^2 = 0.05$, the mutation rate is $\mu = 10^{-3}$, the number of loci is $n = 10$ and the population size is $N_p = 100$. The second column contains the values of $\hat{\sigma}_G^2$(SHC), Equation 11, the fourth to eighth the values of $\hat{\sigma}_G^2$(Obs) and of $\hat{\sigma}_g^2$(Obs). Standard errors are less than 8%.

The first choice of parameters is in accordance with LANDE's (1984, 1988) hypothesis that the number of effective loci contributing most additive genetic variance to quantitative traits is small (on the order of 10) and that these are highly mutable. Therefore it is in accordance with the assumptions leading to the Gaussian approximation. The results are presented in Table 2 and show a moderate decrease of expressed variance $\hat{\sigma}_G^2$(Obs) as r becomes very small, unless selection is very weak. Concomitantly, $\hat{\sigma}_g^2$(Obs) is slightly increasing.

The second choice of parameters is in accordance with the assumptions assuring validity of the house-of-cards prediction. Both genetic and genic variances are roughly constant. Since only for $N_p = 100$, $V_s = 2$ and $r = 0$ a statistically significant increase of the genic variance was observed ($\hat{\sigma}_g^2 = 0.042$) its values are not displayed in Table 3.

Both cases are qualitatively in agreement with the two-locus results as discussed below. In particular, $\hat{\sigma}_G^2(\text{Obs})/\hat{\sigma}_G^2$ (SHC) ≥ 0.68 always holds, hence the maximal decrease of variance due to linkage is approximately 30%.

A considerable reduction of variance is found when the Gaussian mutant distribution is replaced by a double gamma distribution, unless selection is very weak (Table 4). This has also been shown by KEIGHTLEY and HILL (1988) on the basis of a different model. Part of their results are extrapolations of a one-locus

TABLE 3

Influence of the recombination rate on the observed additive genetic variance at stochastic equilibrium

	V_s	$\hat{\sigma}_G^2$ (SHC)	r = 0.5	0.1	0.001	0
$N_p = 20$	100	0.022	0.022	0.022	0.022	0.020
	10	0.020	0.020	0.017	0.022	0.019
	2	0.014	0.013	0.016	0.017	0.015
$N_p = 100$	100	0.106	0.097	0.097	0.112	0.103
	10	0.072	0.073	0.075	0.059	0.060
	2	0.029	0.029	0.028	0.029	0.025

The distribution of mutant effects is normal with mean zero and variance $\alpha^2 = 0.05$, the mutation rate is $\mu = 10^{-4}$, the number of loci $n = 50$. The third column contains the values of $\hat{\sigma}_G^2$(SHC), Equation 11, the fourth to seventh the values of $\hat{\sigma}_G^2$(Obs). Standard errors are less than 10%. The genic variances do not significantly deviate from $\hat{\sigma}_G^2$(Obs) except for $N_p = 100$, $V_s = 2$ and $r = 0$, when $\hat{\sigma}_g^2$(Obs) = 0.042.

TABLE 4

Influence of the recombination rate on the observed additive genetic variance at stochastic equilibrium for a double γ distribution of mutant effects with mean zero and variance $\alpha^2 = 0.05$

	V_s	$\hat{\sigma}_G^2$(SHC)	r = 0.5	0.001	0
$N_p = 100$	100	0.106	0.106	0.063	0.091
	10	0.072	0.050	0.040	0.047
	2	0.029	0.016	0.016	0.016

The mutation rate is $\mu = 10^{-4}$, the number of loci $n = 50$. The third column contains the values of σ_G^2(SHC), Equation 11, the fourth to sixth the values of $\hat{\sigma}_G^2$(Obs). Standard errors are less than 10%. The genic variances deviate from $\hat{\sigma}_G^2$(Obs) by more than 10% only if $r = 0$ and $V_s = 10$ and $V_s = 2$ when $\hat{\sigma}_g^2$(Obs) = 0.0600 and $\hat{\sigma}_g^2$(Obs) = 0.020, respectively. However, $\hat{\sigma}_G^2$(Obs) is considerably lower than with a Gaussian mutant distribution, unless selection is very weak.

model with two alleles in which the heterozygote is assumed to be less fit than both homozygotes. This model may be traced back to ROBERTSON (1956), but it is hard to understand how stabilizing selection leads to underdominance at all loci (see also Section 4 below). Table 4 illustrates that a decrease of recombination rate has almost no statistically significant influence on $\hat{\sigma}_G^2$(Obs) and $\hat{\sigma}_g^2$(Obs).

Simulation results not presented here show that the degree of linkage has no statistically significant influence on the kurtosis of the stationary distribution of genotypic values. The double γ mutant distribution leads approximately to twice the kurtosis of the normal mutant distribution. Also mean heterozygosity and polymorphism are somewhat higher if the mutant distribution is double γ instead of normal, although the variance is lower. The reason for this is that with a double γ distribution most mutants have very small effects and those with large effects are quickly eliminated in a small population. This leads to a lower variance. On the other hand most mutants have very small differences in fitness which leads to higher fixation times and higher polymorphism and heterozygosity. Again, recombination rate has no significant influence on these values.

DISCUSSION

Although the present analysis in principle confirms earlier results by LANDE (1975, 1977), FLEMING (1979), BULMER (1980), TURELLI (1984) and KEIGHTLEY and HILL (1988) that the linkage relation between loci has only a moderate influence on the expressed equilibrium variance maintained by a balance between mutation and stabilizing selection, it reveals certain differences and complications, especially at the gene level. In particular, this paper differs methodically from those mentioned above. (i) Contrary to LANDE, FLEMING and BULMER the present results are not based on assumptions leading to a particular distribution of genotypic values. (ii) Whereas, TURELLI simulated two-, four-, and six-locus models numerically, the present paper contains a complete analytic solution and stability analysis of the two-locus model. (iii) The present simulation model seems to be more general and "realistic" than most earlier simulation studies in this field. It assumes that the possible number of alleles per locus is infinite, but it does not rest on any deterministic evolution equations for gene frequencies, or on symmetry assumptions concerning allelic effects. For example, the theoretical and the simulation results of KEIGHTLEY and HILL (1988) are in part based on a transition matrix method involving a one-locus two-allele model with underdominance (this assumption being discussed below) and in part on Monte Carlo simulations using an infinite sites model.

Consider first a quantitative trait, a pair of mutationally equivalent loci contributing to that trait and quadratically deviating fitness as in Equation 1 or Gaussian fitness as in Equation 9. The present analysis shows that the consequences of linkage between these two loci on the genetic variance at mutation-selection balance depend on whether $\alpha^2 < 8\mu V_s$ or $\alpha^2 > 8\mu V_s$ (α^2 is the variance of mutational effects). In the former case the expressed genetic variance decreases as the recombination rate r between the loci decreases, whereas the genic variance remains constant and equal to α^2 (Equations 5 and 6). In the latter case the expressed genetic variance is constant and equal to the house-of-cards prediction $8\mu V_s$ as long as $r \geq r_c = 16\mu^2 V_s/(\alpha^2 - 8\mu V_s)$, whereas the genic variance increases. If $r < r_c$ the genetic variance slightly decreases and the genic variance has reached the value α^2 (see Result 2 and Figure 1). The maximal possible decrease of the genetic variance due to decreasing r is by a factor $2 - \sqrt{2}$. This can be realized only if $\alpha^2 = 8\mu V_s$.

Otherwise the decrease is much less pronounced (Figure 1). As shown in the APPENDIX, a similar behavior occurs for other functions modeling stabilizing selection.

These two cases are in close connection with the distinction of the Gaussian and the house-of-cards approximation. The Gaussian approximation of KIMURA and LANDE can be valid only if at each locus $\alpha^2 \ll \mu V_s$ (LANDE 1975; TURELLI 1984; BÜRGER 1988b). The biological plausibility of this assumption was criticised by TURELLI (1984) who suggested $\alpha^2 \gg \mu V_s$ to be more realistic and based his house-of-cards approximation on it. The above reasoning, based on Result 2, indicates that, depending on which of the inequalities is valid, linkage will effect the equilibrium variances (expressed and hidden) in a different way.

TURELLI (1984) reviewed data from *Drosophila melanogaster* which suggest that lower bounds for the recombination rate for adjacent structural loci in the neighborhood of 10^{-5}–10^{-4} seem reasonable. If this is true then under house-of-cards conditions the equilibria F2 and F3 will always be stable and F1 is always unstable. Hence under house-of-cards conditions the expressed genetic variance is always independent of r but (in the two-locus model) two stable equilibria coexist. In a multilocus model many equilibria will be simultaneously stable. Under assumptions in favor of the Gaussian approximation, however, the expressed genetic variance decreases slightly as r decreases, whereas the dynamics of genotype frequencies are simple since only a single globally stable equilibrium, namely (F1), exists due to mutation pressure.

That Result 2 is not an artifact of the special model is clear from the simulation results of a multilocus model with a possible continuum of allelic effects per locus in a finite population. Table 2 contains the results for the parameter combinations in favour of the Gaussian approximation. In particular they are compatible with LANDE's (1984, 1988) hypothesis that relatively few "effective loci" are responsible for most heritable variation of a trait. Table 3 contains the results for parameter combinations in favour of the house-of-cards prediction. It may be seen that these results agree qualitatively with the analysis of the two-locus model. For example in Table 2 the largest decrease of expressed variance occurs for $V_s = 10$, followed by $V_s = 2$ and $V_s = 100$, which is just the order of increasing $|\alpha^2 - 8\mu V_s|$, as predicted by Result 2. As already shown by KEIGHTLEY and HILL (1988) on the basis of a different model that assumes underdominance at each locus, a leptokurtic mutant distribution leads to a considerably lower equilibrium variance (Table 4). The influence of linkage, however, is again weak under house-of-cards assumptions.

The conformity of the results of both models indicates that they are very robust, since the models are quite different. Although in both models additivity within and between loci is assumed the deterministic model is highly symmetric having alleles with fixed effects, whereas the stochastic is not. In the latter, alleles of arbitrary effects may occur according to the mutant distribution and the number of alleles segregating per locus may vary over time. A short account of the deterministic model with different allelic effects at the two loci may be found in the APPENDIX, part B.

Recently, HASTINGS (1988) investigated disequilibrium in certain two-locus models under mutation-stabilizing selection balance by approximation methods. However, his results do not apply to the model treated in Section 2, since he assumes that at each locus a common allele exists and that the corresponding double homozygote has higher fitness than all other genotypes with two or more of the common alleles. His results may apply, for example, if the effects of the gametes *AB, Ab, aB, ab* are 2α, α, α, 0 and the maximum fitness is attained at 0. Contrary to the present model, in such a model the mean genotypic effect at equilibrium will in general not agree with the fitness maximum. Nevertheless, his expression (11) for the linkage disequilibrium agrees with the linkage disequilibrium at F2 and F3, if the fitness values of Table 1 are plugged into his formula (with the obvious modification that w_{14} is replaced by $w_{14}w_{44}$).

The present results together with many other recent results (BARTON 1989; BULMER 1972, 1980; BÜRGER 1988a,b; BÜRGER, WAGNER and STETTINGER 1989; KEIGHTLEY and HILL 1988; SLATKIN 1987; TURELLI 1984) underline the remarkable robustness of the house-of-cards prediction and its finite population counterpart (11), at least for a Gaussian mutant distribution. They also indicate that extrapolations from one-locus haploid models to multilocus diploid models lead to correct results concerning the equilibrium variance for a wide range of parameters. GABRIEL and WAGNER (1988) and G. P. WAGNER and W. GABRIEL (unpublished data) have shown that parthenogenetic populations may be as effective as sexual populations in adapting to local peaks. All these results indicate that the dynamics of phenotypic means and variances in haploid and diploid models behave very similarly near an adaptive optimum. Additionally, the distribution of genotypic values is in fact nearly Gaussian for a relatively wide range of parameters (compare also BÜRGER, WAGNER and STETTINGER 1989). There is evidence, however, that pleiotropy and epistasis may lead to substantial complications (TURELLI 1985; GOODNIGHT 1988).

The dynamics at the genotype level are much more complicated in the diploid case than in the haploid. In haploid models a uniquely determined, globally stable equilibrium distribution of (geno)types always

exists for (almost) arbitrary stabilizing selection functions and arbitrary mutant distributions (BÜRGER 1986, 1988a,b). This holds independently of whether a finite number of alleles or a continuum of alleles is assumed, or whether generations are discrete or overlapping. On the other hand, already in the diallelic two-locus model up to three equilibria may exist.

If, in the present deterministic model with quadratically deviating fitness, $4\mu/s \geq 1$ then due to mutation pressure F1 is the single stable equilibrium for all recombination values. Although at this equilibrium all gene frequencies are ½ there may be substantial negative linkage disequilibrium. If $4\mu/s < 1$ then F1 is stable only for extremely tight linkage ($r(s - 4\mu) \leq 8\mu^2$) otherwise two stable equilibria F2, F3, with one of the balanced gametes Ab or aB prevailing, exist. Which of the two equilibria is actually approached depends on the initial frequencies of Ab and aB. Linkage disequilibrium at these equilibria is $\hat{D} = -2\mu^2/(sr)$. These results are different from those of BULMER (1972) and BARTON (1986) [see also BULMER (1988) for a review] when their analysis is restricted to two loci. They assumed (in fact for a model with many loci) that the dynamics can be described solely by the gene frequencies, assuming global linkage equilibrium. This assumption is admissible only if $\mu^2/(sr)$ is very small and leads to an "underdominance" like dynamics. Moreover, they assumed a Gaussian distribution of genotypic values. Precise conditions for the validity of this assumption are not yet known, however there is some evidence that sufficiently high gametic mutation rates, and moderate selection intensities are necessary (BÜRGER, WAGNER and STETTINGER 1989).

These assumptions lead to an overdetermination of the BULMER-BARTON model and to a different mean fitness. If their model assumptions are applied to two loci then mean fitness is given by $\bar{m}_B = \bar{m} + 4sD$, whereas $\bar{m}$ is the exact mean fitness (see Equation 3). Of course, they assume linkage equilibrium, i.e. D=0, and as long as this is satisfied there is no difference. But since D does not remain zero and instead becomes negative, as shown in the APPENDIX, the dynamics in their model are slightly different, depending on how large linkage disequilibrium in fact is.

As a consequence, in the BULMER-BARTON model at the equilibrium corresponding to F1 all genotype frequencies are ¼ and the equilibria corresponding to F2 and F3 are lacking the term $\hat{D}$ and are independent of r. Since in their model F2 and F3 are always locally stable (unless $4\mu/s \geq 1$) the impression may emerge that underdominance occurs in stabilizing selection models. This assertion goes back to ROBERTSON (1956) (see also KEIGHTLEY and HILL 1988). The exact analysis shows that these two stable equilibria are not due to underdominance in the strict sense, since their existence, position and stability depends not only on μ/s but in particular on the recombination rate.

BARTON (1986) also showed that for more than three loci additional stable equilibria can exist, where the mean genotypic value deviates from the optimum. It is to be expected that similar phenomena will also occur in the exact model. Whether such equilibria are of much importance if the effects of alleles are asymmetric is questionable, since with increasing asymmetry the range of stability of F1 increases (see APPENDIX B).

In finite populations shifts between the various equilibria in the BULMER-BARTON model can occur. If the population size is large, however (*e.g.* >20,000, depending on the various parameters), the population will be clustered around one of the house-of-cards equilibria and shifts away from the optimum become infrequent (BARTON 1989). Although in small populations such shifts may be rather likely their net effect on the expected variance seems to be small (BÜRGER, WAGNER and STETTINGER 1989). In that paper excursions of variance were frequently observed, but the observed mean variance was approximately $\hat{\sigma}^2_G$(SHC). Probably this is due to the fact that in our simulation model no symmetries are assumed, since BULMER's (1972) analysis of diallelic model including random drift but with symmetry assumptions led to a higher variance than $\hat{\sigma}^2_G$(SHC). Similar effects in diallelic models were observed by BARTON (1989) and KEIGHTLEY and HILL (1988).

To summarize, recent analyses including the present one show a relatively simple dependence of the expressed genetic variance under mutation-selection balance on the gametic mutation rate, the selection intensity, the mutant distribution and the population size. It is very robust with respect to recombination relations among loci and with respect to detailed genetic assumptions like possible number of alleles per locus, symmetry assumptions etc. If, however, the loci contributing much to the variance are tightly linked considerable hidden variance due to negative linkage disequilibrium builds up. Moreover, the detailed genetic composition of a population near an adaptive optimum will strongly depend on genetic parameters like recombination rates and hence on its history due to the existence of multiple stable equilibria at the gene level. COHAN (1984) has shown that uniform selection acting on small isolated populations may lead to genetic divergence between conspecific populations. The present analysis as well as that of BULMER and BARTON allow us to conclude that even stabilizing selection acting on very large populations may lead to genetic divergence between isolated populations. These effects may be of importance if a population experiences directional selection due to changing en-

vironmental conditions and can be one reason for unpredictable long term response.

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APPENDIX

A) To investigate the dynamical properties of the system of differential equations (4) I use an approach based on Ljapunov functions (*cf.* BÜRGER 1983).

Since there is forward and backward mutation at both loci no equilibria at the boundary of the simplex $S_4 = \{(x_i) : x_i \geq 0 \text{ and } \sum x_i = 1, i = 1, 2, 3, 4\}$ can exist and the flow at the boundary points into the simplex. Therefore, it is sufficient to consider the interior of S_4. First we consider the function $Z = x_2x_3/x_1x_4$ and show that $\dot{Z} > 0$ if $Z \leq 1$. Notice that $D = x_1x_4(1 - Z)$. Using (4) an easy calculation shows that

$$\dot{Z} = Z[(d - 2b)(x_1 + x_4) + 2b(x_2 + x_3)] + \frac{1 - Z}{x_1x_4}(rT + \mu(x_1 + x_4(x_2 + x_3)) > 0, \tag{A1}$$

if $Z \leq 1$. Here $T = x_1x_2x_3 + x_1x_2x_4 + x_1x_3x_4 + x_2x_3x_4$. This shows that for arbitrary initial genotype frequencies eventually $D < 0$ hold. Hence the subregion of S_4, where $D < 0$ holds is positively invariant and contains every ω-limit. This argument is no longer valid if $d < 2b$, in which case positive linkage disequilibrium is possible.

Next we show that if $D < 0$ then $\frac{d}{dt}\left(\frac{x_1}{x_4} - 1\right)^2 \leq 0$ holds. Geometrically, $x_1/x_4 = c$ describes a plane in the simplex containing the edge $x_2 + x_3 = 1$ such that this relation is satisfied. Indeed, if $D \leq 0$

$$\begin{aligned}\frac{d}{dt}\left(\frac{x_1}{x_4}-1\right)^2 &= 2\left(\frac{x_1}{x_4}-1\right)\frac{d}{dt}\left(\frac{x_1}{x_4}\right)\\ &= 2\left(\frac{x_1}{x_4}-1\right)\left[\frac{\dot{x}_1x_4-\dot{x}_4x_1}{x_4^2}\right] \qquad \text{(A2)}\\ &= -2\left(\frac{x_1}{x_4}-1\right)^2\left[dx_1-\frac{rD}{x_4}+\mu\frac{x_2+x_3}{x_4}\right]\leq 0\end{aligned}$$

holds. This implies that all orbits converge to the plane $x_1 = x_4$. Additionally, as shown above, $D < 0$ holds as $t \to \infty$.

Subsequently, assume that $x = x_1 = x_4$ and put $y = x_2 - x_3$. Then an easy calculation shows that the dynamics in the plane $x_1 = x_4$ are given by

$$\dot{x} = A(x)(2x - 1) - x(r + 2\mu) + (r/4) - (r/4)y^2 \qquad \text{(A3)}$$

$$\dot{y} = 2yA(x) \qquad \text{(A4)}$$

Here, $A(x) = (d - 4b)x^2 + bx - \mu$. This system has up to three equilibrium points, which have to satisfy $x \leq 1/4$, since we already know that $D < 0$. Simple algebra shows that, if $y = 0$, a unique equilibrium ξ, $0 < \xi < 1/4$ of (A3) exists. This gives fixed point F1. If $d = 4b = 4s$ then F1 can be calculated explicitly and, using $y = x_2 - x_3$, the expression in Result 1 is obtained. All other equilibria have to satisfy $A(x) = 0$. If $2b \leq d < 4b - (b^2/4\mu)$ or $d < 16\mu$ no further equilibria exist, since in this case $A(x) \neq 0$ for $0 < x < 1/4$. If $d > \max(2b, 4b - (b^2/4\mu), 16\mu)$ a unique solution $\hat{x}$, $0 < \hat{x} < 1/4$ of $A(x) = 0$ exists. This yields fixed points F2 and F3. If $d = 4b$ they are given by $\hat{x} = \mu/b$ and

$$\hat{y} = \pm 2\sqrt{\frac{1}{4} - \frac{\mu}{s} - \frac{2\mu^2}{rs}}.$$

If $2b \leq d < 4b$ then F2 and F3 are given by

$$\begin{aligned}\hat{x} &= \frac{1}{2(4b-d)}\left(b - \sqrt{b^2 - 4\mu(4b-d)}\right), \qquad \text{(A5)}\\ \hat{y} &= \pm 2\sqrt{\frac{1}{4} - \hat{x}\left(1 + \frac{2\mu}{r}\right)}.\end{aligned}$$

If $d > 4b$ they are given by

$$\begin{aligned}\hat{x} &= \frac{-1}{2(d-4b)}\left(b + \sqrt{b^2 + 4\mu(d-4b)}\right), \qquad \text{(A6)}\\ \hat{y} &= \pm 2\sqrt{\frac{1}{4} - \hat{x}\left(1 + \frac{2\mu}{r}\right)}.\end{aligned}$$

It follows that F2 and F3 exist only if r is sufficiently large to ensure that $1/4 > \hat{x}(1 + 2\mu/r)$. Some further simple algebra shows that, as r increases from zero, the x-coordinate of F1, ξ, increases and satisfies $\xi < \hat{x}$. For a critical value $r_c = 8\mu(1 - 4\hat{x})$ we have $\xi = \hat{x}$ and $\hat{y} = 0$ and therefore F1, F2 and F3 coincide. In fact, on pitchfork bifurcation occurs at this point.

It remains to prove the stability properties claimed in Result 1. Consideration of $\partial\dot{x}/\partial x$ shows that $\dot{x} < 0$ for all $x > \xi$. Hence each ω-limit lies in the positively invariant subarea $x \leq \xi$ of the plane $x_1 = x_4$. We know already that $r < 8\mu(1 - 4\hat{x})$ is equivalent to $\xi < \hat{x}$, which in turn is equivalent (as is easily shown) to $A(x) < A(\xi) < 0$ if $x < \xi$. Therefore, global stability of F1 follows immediately from Equation A4. Note that this is the case if and only if F2 and F3 do not exist. If $r > r_c$ then F1 is unstable and a linear stability analysis shows that F2 and F3 are locally stable.

To prove that F2 (F3) is globally attractive for the half space $x_2 > x_3$ ($x_2 < x_3$) it suffices to exclude the existence of periodic orbits around F2 (F3) (HOFBAUER and SIGMUND 1988). To this aim I use a Dulac function (due to J. HOFBAUER) in the forward invariant area $x \leq \xi$ and $y \geq 0$ ($y \leq 0$). Indeed

$$\begin{aligned}&\partial(y^{-1}\dot{x})/\partial x + \partial(y^{-1}\dot{y})/\partial y \qquad \text{(A7)}\\ &\quad = y^{-1}(6A(x) + 2(3b - d)x - (b + r - 2\mu))\end{aligned}$$

and $6A(x) + 2(3b - d)x - (b + r - 2\mu) < 0$ in each of these areas. Hence (A7) has constant sign in both areas, which finishes the proof.

B) It is also possible to draw a few conclusions if the effects of mutants are different at the two loci. Assume, for example, that the effects of A, a, B, b are $c - a_1/2$, $c + \alpha_1/2$, $-c - \alpha_2/2$, $-c + \alpha_2/2$. Then, for the general stabilizing selection model the general symmetric fitness model with parameters a, b, c, d, as treated by KARLIN and FELDMAN (1970), emerges. Without further assumptions this is almost intractable. Assuming quadratically deviating fitness, the side assumption $a + d = 2(b + c)$ is obtained. Then it is straightforward to show that an equilibrium with coordinates as F1 (see Result 1) exists, where $s = s^*\alpha^2$ is replaced by $s^*\alpha_1\alpha_2$. Its stability depends on the sign of a (quite complicated) quadratic polynomial in r. However, it can be shown that F1 is locally stable for all $r \geq 0$ if

$$\alpha_1^2\alpha_2^2 < \frac{16}{3}\frac{\mu^2}{s^{*2}} + \frac{8}{3}\frac{\mu}{s^*}\frac{\alpha_1^2 + \alpha_2^2}{2}.$$

That is, if the mean variance of mutation effects $(\alpha_1^2 + \alpha_2^2)/2$ is held constant, F1 is locally stable for all r if the effects are sufficiently different. For example, if $s^* = 10^{-2}$, $\mu = 10^{-4}$, $(\alpha_1^2 + \alpha_2^2)/2 = 0.05$, then F1 is stable if $\alpha_1^2 \geq 0.075$ and $\alpha_2^2 \leq 0.025$. However, in this case the assumptions leading to the house-of-cards approximation are no longer satisfied at locus two. Numerical simulations suggest that in this case F1 is globally stable. If F1 is unstable equilibria similar to F2 and F3 exist, but they do not satisfy $x_1 = x_4$. The variance maintained at these equilibria is slightly higher than in the symmetrical case.