Maintenance of Genetic Variability Under Strong Stabilizing Selection: A Two-Locus Model

Sergey Gavrilets*,† and Alan Hastings*,‡,§

*Division of Environmental Studies, University of California, Davis, California 95616, †N. I. Vavilov Institute of General Genetics, 117809 GSP-1, Moscow B-333, Russia, ‡Institute for Theoretical Dynamics, University of California, Davis, California 95616, and §Center for Population Biology, University of California, Davis, California 95616

ABSTRACT

We study a two locus model with additive contributions to the phenotype to explore the relationship between stabilizing selection and recombination. We show that if the double heterozygote has the optimum phenotype and the contributions of the loci to the trait are different, then any symmetric stabilizing selection fitness function can maintain genetic variability provided selection is sufficiently strong relative to linkage. We present results of a detailed analysis of the quadratic fitness function which show that selection need not be extremely strong relative to recombination for the polymorphic equilibria to be stable. At these polymorphic equilibria the mean value of the trait, in general, is not equal to the optimum phenotype, there exists a large level of negative linkage disequilibrium which "hides" additive genetic variance, and different equilibria can be stable simultaneously. We analyze dependence of different characteristics of these equilibria on the location of optimum phenotype, on the difference in allelic effect, and on the strength of selection relative to recombination. Our overall result that stabilizing selection does not necessarily eliminate genetic variability is compatible with some experimental results where the lines subject to strong stabilizing selection did not have significant reductions in genetic variability.

SINCE the classical papers by WRIGHT (1935) and ROBERTSON (1956) there has been a general belief that in the absence of other factors stabilizing selection on an additive character cannot maintain genetic variability in more than one locus. To explain the maintenance of genetic variability observed in natural population, which presumably are under stabilizing selection, a number of models with additional factors have been proposed. These factors include dominance, mutation, epistasis, pleiotropy, genotype-environment interaction, migration (e.g., KOJIMA 1959; LEWONTIN 1964; BULMER 1973; LANDE 1975; GILLESPIE and TURELLI 1989; GIMELFARB 1989, 1992; HASTINGS and HOM 1990; GAVRILETS and DE JONG 1993; ZHIVOTOVSKY and GAVRILETS 1992). Analytical studies and most of the numerical studies of stabilizing selection on a single additive character that have been done incorporate simplifying assumptions such as weak selection approximation, equal contributions of loci to the character or optimal phenotype equal that of the completely heterozygous individual. Numerical results by GALE and KEARSEY (1968) and KEARSEY and GALE (1968) suggested, however, that relaxation of these assumptions can cause new effects. These authors demonstrated that strong "triangular" stabilizing selection on an additive trait can maintain variability in two and three loci if the effects of the loci are different. Analysis of the stabilizing selection on

an additive character under broader assumptions has been started in several recent papers. Using a weak selection approximation (i.e., assuming no linkage disequilibrium), NAGYLAKI (1989) showed that if the contributions of two diallelic loci to an additive trait are sufficiently different, some forms of stabilizing selection can maintain variability in both loci. HASTINGS and HOM (1990) used the weak selection approximation assuming that the contributions of loci are different and that the optimal phenotype is different from the phenotype of a complete heterozygote. They found that monomorphic equilibria and equilibria with one locus polymorphic can be stable simultaneously. GAVRILETS (1993) applied both the weak selection approximation and a strong selection approximation to a two-locus epistatic viability model with equivalent loci. He showed that moderate changes in the strength of selection relative to recombination can cause significant changes in quantitative and qualitative characteristics of equilibria.

In this paper we consider these effects simultaneously and show that some surprising results emerge. In particular, we show that if stabilizing selection on a single additive character is sufficiently strong relative to recombination, genetic polymorphism can be maintained provided contributions of the loci to the character are not equal. The structure of this paper is as follows. In the next section, we formulate a

general model of stabilizing selection on an additive trait controlled by two diallelic loci. Then we present detailed analysis of the case of quadratic stabilizing selection. We determine exact conditions for existence and stability of monomorphic equilibria and equilibria with one locus polymorphic. We present results of the approximate analysis of equilibria with two polymorphic loci. We demonstrate that such equilibria exist and can be stable. In the final section, we discuss quantitative genetics implications of our results.

GENERAL MODEL

Let an additive quantitative trait be controlled by two diallelic loci. We shall use the notation from HASTINGS and HOM (1990). Assume that the alleles at locus i have effects $\alpha_i/2$ and $-\alpha_i/2$, and that $\alpha_i \neq 0$. We designate the larger of the α_i as α_1 and, without loss of generality, assume that $\alpha_1 = 1$. Let x_1, x_2, x_3 and x_4 be the frequencies of the gametes with the effects $z_1 = (1 + \alpha_2)/2$, $z_2 = (1 - \alpha_2)/2$, $z_3 = (-1 + \alpha_2)/2$ and $z_4 = (-1 - \alpha_2)/2$ on the trait. We assume that the fitness depends only on genotypic value so that the fitness, w_{ij}, of an individual formed by gametes i and j and having phenotype $z_i + z_j$ can be represented as

$$w_{ij} = w(z_i + z_j). \tag{1}$$

We assume that the fitness function $w(z)$ has its optimum at z_0, which in general, is not zero; we scale $w(z)$ so that $w(z_0) = 1$. We suppose that $w(z)$ decreases monotonically from its optimum. Let $w_i = \sum_j w_{ij}x_j$ and $\bar{w} = \sum_i w_i x_i$ be the marginal fitness of gamete i and the mean fitness of the population, respectively. The dynamics of the gamete frequencies under selection and recombination are described by the standard relations

$$\Delta x_i = \frac{w_i - \bar{w}}{\bar{w}} x_i \mp \frac{rw_{14}D}{\bar{w}}, \tag{2}$$

where r is the recombination rate, $D = x_1x_4 - x_2x_3$ is the standard linkage disequilibrium, and w_{14} is the fitness of a heterozygote at both loci, $w_{14} = w(0)$. In (2) the sign is minus for $i = 1$ and 4 and is plus for $i = 2$ or 3.

It has been repeatedly remarked (e.g., HASTINGS 1987; BÜRGER 1989; NAGYLAKI 1989) that if a double heterozygote has the optimum phenotype, i.e., $z_0 = 0$, and fitness function is symmetric, i.e., $w(z - z_0) = w(z_0 - z)$, the model of stabilizing selection on an additive trait reduces to the symmetric viability model analyzed in a number of papers (e.g., BODMER and FELSENSTEIN 1967; KARLIN and FELDMAN 1970). One of the properties of this model is existence of a locally stable polymorphic equilibrium under sufficiently tight linkage (KARLIN and FELDMAN 1970). Restating this in terms of a model of stabilizing selection we have the following result.

If the contributions of the loci to the trait are different and the stabilizing selection fitness function has its optimum at the phenotypic value of a heterozygote and is symmetric about it, then for linkage sufficiently tight relative to selection there always exists a locally stable equilibrium polymorphic at both loci.

In general, for tight linkage it is possible to have more than one stable polymorphic equilibrium, and the equilibria with two loci polymorphic can be stable simultaneously with other equilibria. If $z_0 \neq 0$, then one cannot use the results on the symmetric viability model. Nevertheless, as we shall show below, in some cases the conclusion about existence of a locally stable polymorphic equilibrium under tight linkage is still valid. The result presented in this section assumes that linkage is sufficiently tight relative to the strength of selection; the proof is based on the assumption that the ratio r/s is much less than unity [see KARLIN and FELDMAN (1970)]. In this paper, we argue that the conclusion about the maintenance of genetic variability under stabilizing selection on an additive trait is valid even if selection is not extremely strong or equivalently if linkage is not extremely tight. To demonstrate this we shall analyze the simplest (from an analytical point of view) class of stabilizing selection fitness functions, namely quadratic fitness functions.

QUADRATIC STABILIZING SELECTION

Let the fitness function $w(z)$ be a quadratic

$$w(z) = 1 - s(z - z_0)^2, \tag{3}$$

where s is the parameter measuring the strength of selection, and z_0 is the optimum phenotype which, in general, is not zero. The model (2) has two types of equilibria: equilibria with $D = 0$ and equilibria with $D \neq 0$; we shall consider them separately. At an equilibrium with $D = 0$ either $w_i = \bar{w}$ or $x_i = 0$. The system (2) with fitnesses (3) has eight equilibria with $D = 0$: four equilibria with only a single gamete represented in the population and four equilibria with two gametes present.

Stability of monomorphic equilibria: The equilibria with a single gamete, at which both loci are fixed, always exist. The conditions for stability can be found by excluding one of the gamete frequencies using the relation $x_1 + x_2 + x_3 + x_4 = 1$ and calculating the eigenvalues of the corresponding (3×3)-matrix $V = \{d(\Delta x_i)/dx_j\}$ (cf. BODMER and FELSENSTEIN 1967). That was done using *Mathematica* software (WOLFRAM 1988). An equilibrium is stable if the modulus of all eigenvalues of matrix V lie in the unit circle centered at -1 in the complex plane. In the case studied here, all eigenvalues are real, so this is equivalent to saying that the eigenvalues of V lie between minus two and zero. (This condition is equivalent to the condition that all eigenvalues of matrix $\{d(x_i')/dx_j\}$, where $x_i' =$

$x_i + \Delta x_i$ are less in modulus than unity.) We will designate the equilibria by the vector of gamete frequencies. The conditions for stability of the equilibria with a single gamete present are as follows. For the equilibrium $(1,0,0,0)$, the stability condition is

$$z_0 > \tfrac{1}{2} + \alpha_2 \quad \text{and} \quad z_0 > 1 + \alpha_2/2, \quad (4a)$$

$$-rw_{14} + s(1 + \alpha_2)(1 + \alpha_2 - 2z_0) < 0. \quad (4b)$$

For the equilibrium $(0,1,0,0)$, the stability condition is

$$1 - \alpha_2/2 > z_0 > \tfrac{1}{2} - \alpha_2, \quad (5a)$$

$$-rw_{14} + s(-1 + \alpha_2)(-1 + \alpha_2 + 2z_0) < 0. \quad (5b)$$

For the equilibrium $(0,0,1,0)$, the stability condition is

$$\alpha_2 - \tfrac{1}{2} > z_0 > \alpha_2/2 - 1, \quad (6a)$$

$$-rw_{14} + s(-1 + \alpha_2)(-1 + \alpha_2 - 2z_0) < 0. \quad (6b)$$

For the equilibrium $(0,0,0,1)$, the stability condition is

$$z_0 < -\tfrac{1}{2} - \alpha_2 \quad \text{and} \quad z_0 < -1 - \alpha_2/2, \quad (7a)$$

$$-rw_{14} + s(1 + \alpha_2)(1 + \alpha_2 + 2z_0) < 0. \quad (7b)$$

Note that if selection is very weak relative to recombination, i.e., $s \ll r$, inequalities 4b, 5b, 6b and 7b are satisfied and the stability depends only on the conditions 4a, 5a, 6a and 7a. The latter are exactly those found by HASTINGS and HOM (1990) for the case of weak selection. One can show that if equilibrium $(1,0,0,0)$ is stable under weak selection ($s \ll r$), it is stable under strong selection ($r \ll s$), and vice versa. The same conclusion is true for equilibrium $(0,0,0,1)$. If equilibrium $(0,1,0,0)$ or $(0,0,1,0)$ is stable under strong selection, it is stable under weak selection, but the opposite is not necessarily true. These equilibria can become unstable as the strength of selection relative to recombination decreases.

Existence and stability of equilibria with one locus polymorphic: The equilibria with two gametes with $D = 0$, at which one of the loci is fixed while the other is polymorphic, are given by

$$(x_1^*, x_2^*, 0, 0), \text{ where } x_1^* = \frac{-2 + \alpha_2 + 2z_0}{2\alpha_2}, \quad (8a)$$

$$x_2^* = 1 - x_1^*$$

$$(x_1^*, 0, x_3^*, 0), \text{ where } x_1^* = \tfrac{1}{2} - \alpha_2 + z_0, \quad (8b)$$

$$x_3^* = 1 - x_1^*,$$

$$(0, 0, x_3^*, x_4^*), \text{ where } x_3^* = \frac{2 + \alpha_2 + 2z_0}{2\alpha_2}, \quad (8c)$$

$$x_4^* = 1 - x_3^*,$$

$$(0, x_2^*, 0, x_4^*), \text{ where } x_2^* = \tfrac{1}{2} + \alpha_2 + z_0, \quad (8d)$$

$$x_4^* = 1 - x_2^*.$$

These equilibria are biologically meaningful, if nonzero gamete frequencies lie between zero and one; the corresponding conditions are given in [HASTINGS and HOM (1990), Exps. (8), (12), (6) and (10)). Conditions for stability were found as before (cf. BODMER and FELSENSTEIN 1967). An equilibrium of the form (8) is stable if it is feasible and the following conditions are satisfied.

At the equilibrium $(x_1^*, x_2^*, 0, 0)$ the stability condition is

$$(1 - \alpha_2^2)s + rw_{14}(3 - 2z_0) > 0. \quad (9)$$

At the equilibrium $(x_1^*, 0, x_3^*, 0)$ the stability condition is

$$(-1 + \alpha_2^2)\alpha_2 s + rw_{14}(3\alpha_2 - 2z_0) > 0. \quad (10)$$

At the equilibrium $(0, 0, x_3^*, x_4^*)$ the stability condition is

$$(1 - \alpha_2^2)s + rw_{14}(3 + 2z_0) > 0. \quad (11)$$

At the equilibrium $(0, x_2^*, 0, x_4^*)$ the stability condition is

$$(-1 + \alpha_2^2)\alpha_2 s + rw_{14}(3\alpha_2 + 2z_0) > 0. \quad (12)$$

Note that if selection is very weak relative to recombination, i.e., $s \ll r$, the first terms in (9–12) can be neglected and the stability conditions reduce to those given by HASTINGS and HOM [1990, Exps. (15a), (16–18)]. One can easily see that under strong selection ($r \ll s$) equilibria $(x_1^*, x_2^*, 0, 0)$ and $(0, 0, x_3^*, x_4^*)$ are stable provided they are feasible, while equilibria $(x_1^*, 0, x_3^*, 0)$ and $(0, x_2^*, 0, x_4^*)$ cannot be stable. All these equilibria can change their stability with change in the ratio r/s.

Existence and stability of polymorphic equilibria: At polymorphic equilibria $D \neq 0$. Unfortunately, if $z_0 \neq 0$, these equilibria cannot be found in explicit form. Earlier approximate analysis shows that if selection is very weak relative to recombination, there exists a single equilibrium with two polymorphic loci but it cannot be stable (e.g., HASTINGS and HOM 1990). Here we shall consider the opposite situation when selection is strong relative to recombination. To demonstrate existence of stable polymorphic equilibria, we shall use two different techniques: regular perturbation analysis and bifurcation analysis.

Polymorphic equilibria that exist under very

strong selection: If selection if very strong relative to recombination, one can consider dynamic Equations (2) assuming that $r = 0$ (BODMER and FELSENSTEIN 1967; KARLIN and MACGREGOR 1972). If there is no recombination, dynamics of gamete frequencies are described by

$$x_i' = \frac{w_i - \bar{w}}{\bar{w}} x_i, \qquad (13)$$

Besides eight equilibria with $D = 0$, which were analysed in the preceding section, system (13) with fitnesses (3) has two additional equilibria (cf. BODMER and FELSENSTEIN 1967): an equilibrium of the form

$$(0, x_{2,0}^*, x_{3,0}^*, 0), \text{ where } x_{2,0}^* = \frac{1}{2} + \frac{z_0}{1 - \alpha_2},$$
$$(14a)$$
$$x_{3,0}^* = 1 - x_{2,0}^*,$$

and an equilibrium of the form

$$(x_{1,0}^*, 0, 0, x_{4,0}^*), \text{ where } x_{1,0}^* = \frac{1}{2} + \frac{z_0}{1 + \alpha_2},$$
$$(14b)$$
$$x_{4,0}^* = 1 - x_{1,0}^*.$$

(Here and in what follows, subscripts after commas are used to denote the order of terms in perturbation expansions in terms of r/s.) In general, when the number of loci is two, system (13) can have equilibria with three and four gametes. Such equilibria, however, do not exist if fitnesses are given by (3). Equilibria (14) are biologically meaningful if nonzero gamete frequencies lie between zero and one. The equilibrium $(0, x_{2,0}^*, x_{3,0}^*, 0)$ is feasible if

$$(1 - \alpha_2)/2 > z_0 > -(1 - \alpha_2)/2. \qquad (15)$$

The equilibrium $(x_{1,0}^*, 0, 0, x_{4,0}^*)$ is feasible if

$$(1 + \alpha_2)/2 > z_0 > -(1 + \alpha_2)/2. \qquad (16)$$

Note that D is negative at equilibrium $(0, x_{2,0}^*, x_{3,0}^*, 0)$ and is positive at equilibrium $(x_{1,0}^*, 0, 0, x_{4,0}^*)$. The conditions for stability were found as before. The equilibrium $(0, x_{2,0}^*, x_{3,0}^*, 0)$ is stable if it is feasible, while equilibrium $(x_{1,0}^*, 0, 0, x_{4,0}^*)$ cannot be stable. Figure 1 summarizes the stability results for the case $r = 0$. In Figure 1 we divide parameter space, i.e., possible values for α_2, the relative effect of the locus of smaller effect on the character, and z_0, the optimum phenotype, into regions corresponding to stability of different equilibria [cf. HASTINGS and HOM (1990), Figure 1]. Without loss of generality, we only display the region with z_0 positive since the results for negative z_0 are easily obtained from the results for positive z_0, using the inherent symmetries in the model. This figure shows that in contrast to the weak selection results (HASTINGS and HOM 1990), under very strong
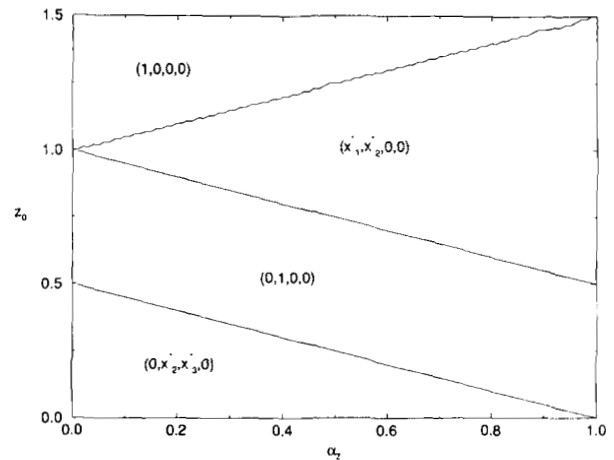

FIGURE 1.—Regions of existence and stability of different equilibria on the (α_2, z_0) plane for very strong selection ($r/s \simeq 0$). The equilibrium that is stable in the corresponding region is designated by the vector of gamete frequencies.

selection different equilibria cannot be stable simultaneously. Note that $|1 - \alpha_2|/2$ is the minimum of the contributions of the gametes to the trait value. Thus, genetic variability can be maintained in both loci under very strong (relative to recombination) quadratic stabilizing selection on an additive trait if the deviation of the optimum phenotype from that of the complete heterozygote is less than the minimum of the contributions of the gametes to the trait value. We know, however, that as selection becomes weaker, the completely polymorphic equilibrium loses stability. It is of interest to know how different characteristics of the polymorphic equilibria depend on the ratio r/s. To answer this question we shall use regular perturbations techniques. See HASTINGS (1985) and GAVRILETS (1992) for other examples of application of this method in analysis of two locus models.

We shall look for polymorphic equilibria of (2) as power series in $\epsilon \equiv r/s$:

$$x_i^* = x_{i,0} + \epsilon x_{i,1} + \ldots, \qquad (17)$$

where as the zero order terms we shall use the equilibrium gamete frequencies from (14). At equilibrium the right-hand sides of equations (2) are zero. Substituting (17) in (2) and equating the terms corresponding to the same power of ϵ, we find algebraic equations from which perturbation terms $x_{i,1}$ can be found. The corresponding formulae for the equilibria that reduce to equilibria $(0, x_{2,0}^*, x_{3,0}^*, 0)$ and $(x_{1,0}^*, 0, 0, x_{4,0}^*)$ as $\epsilon \to 0$ are given in BODMER and FELSENSTEIN (1967). Below we present first order estimates which were found using *Mathematica* software (WOLFRAM 1988). All calculations are simplified if one uses a linear transformation

$$u = x_1 - x_4,$$
$$v = x_2 - x_3,$$
$$t = x_1 + x_4 - x_2 - x_3.$$

Let us consider the equilibrium that reduces to $(0, x_{2,0}^*, x_{3,0}^*, 0)$ as $\epsilon \to 0$. The first order estimates for the equilibrium gamete frequencies are

$$x_1^* = \frac{r}{s} w_{14} \frac{x_{2,0}^* x_{3,0}^*}{\alpha_2}, \tag{18a}$$

$$x_2^* = x_{2,0}^* - \frac{r}{s} w_{14} \left[\frac{x_{2,0}^* x_{3,0}^*}{\alpha_2} - \frac{(x_{2,0}^* - x_{3,0}^*)}{2(1 - \alpha_2)^2} \right], \tag{18b}$$

$$x_3^* = x_{3,0}^* - \frac{r}{s} w_{14} \left[\frac{x_{2,0}^* x_{3,0}^*}{\alpha_2} + \frac{(x_{2,0}^* - x_{3,0}^*)}{2(1 - \alpha_2)^2} \right], \tag{18c}$$

$$x_4^* = \frac{r}{s} w_{14} \frac{x_{2,0}^* x_{3,0}^*}{\alpha_2}. \tag{18d}$$

Using (18) we can find first order estimates for the allele frequencies p_1, p_2, linkage disequilibrium D and the normalized linkage disequilibrium D/D_{max}, where D_{max} is the maximum linkage disequilibrium that is possible at the given allele frequencies, $D_{max} = \min\{p_1 p_2), (1 - p_1)(1 - p_2)\}$. These values are given by:

$$p_1^* = p_{1,0}^* + \frac{r}{s} w_{14} \frac{(x_{2,0}^* - x_{3,0}^*)}{2(1 - \alpha_2)^2}, \tag{19a}$$

$$p_2^* = p_{2,0}^* - \frac{r}{s} w_{14} \frac{(x_{2,0}^* - x_{3,0}^*)}{2(1 - \alpha_2)^2}, \tag{19b}$$

$$D^* = D_0 + \frac{r}{s} w_{14} \left[\frac{x_{2,0}^* x_{3,0}^*}{\alpha_2} + \frac{(x_{2,0}^* - x_{3,0}^*)^2}{2(1 - \alpha_2)^2} \right], \tag{19c}$$

$$D^*/D_{max} = -1 + \frac{r}{s} \frac{w_{14}}{\alpha_2}, \tag{19d}$$

where $p_{1,0} = x_{2,0}^*$, $p_{2,0} = x_{3,0}^*$. These expressions show that an increase in the ratio r/s causes a shift of allele frequencies from $\frac{1}{2}$ and a decrease in the absolute value of linkage disequilibrium. Note that since $|D^*/D_{max}| \leq 1$, the first order analysis gives feasible estimates until

$$r/s \leq \frac{2\alpha_2}{w_{14}} \tag{20}$$

In the APPENDIX we show how the regular perturbation techniques can be used to get rough estimates for the critical value of the ratio r/s under which the polymorphic equilibrium becomes unstable.

Polymorphic equilibria that bifurcate from monomorphic equilibria and equilibria with one locus polymorphic: If selection is very weak relative to recombination (*i.e.*, $\epsilon \gg 1$) there exist equilibria polymorphic at both loci which cannot be stable (HASTINGS and HOM 1990). In the preceding section, we showed that if selection is very strong relative to recombination (*i.e.*, $\epsilon \ll 1$) there exist two polymorphic equilibria one of which can be stable. In this section, we shall

show that for moderately strong selection several polymorphic equilibria exist and at least two of them can be stable simultaneously. As was noted earlier, monomorphic equilibria and equilibria with one locus polymorphic that are stable under weak selection can become unstable as selection becomes stronger. When this happens, a new polymorphic equilibrium bifurcates from the old one. Bifurcation theory provides a means to compute the stability and approximate location of this new equilibrium. General references for bifurcation theory techniques employed here are IOOSS and JOSEPH (1981) and GUCKENHEIMER and HOLMES (1983); for examples of application of these techniques in two locus models see HASTINGS (1981, 1982, 1985b).

Let us consider monomorphic equilibrium $(0,1,0,0)$. To analyze stability of this equilibrium we shall consider dynamic equations (2) for $i = 1, 3, 4$, substituting $1 - x_1 - x_3 - x_4$ for x_2. At this equilibrium the stability matrix V has form

$$\begin{pmatrix} l_1/w_{22} & rw_{14}/w_{22} & 0 \\ 0 & (l_2 - rw_{14})/w_{22} & 0 \\ 0 & rw_{14}/w_{22} & l_3/w_{22} \end{pmatrix}$$

where $l_1 \equiv w_{12} - w_{22} = s\alpha_2(-2 + \alpha_2 + 2z_0)$, $l_2 \equiv w_{14} - w_{22} = s(1 - \alpha_2)(1 - \alpha_2 - 2z_0)$, $l_3 \equiv w_{24} - w_{22} = s(1 - 2\alpha_2 - 2z_0)$. Assume that condition (5a) is satisfied, *i.e.*, that $l_1 < 0$, $l_3 < 0$. That implies that $-2 < l_1/w_{22} < 0$ and that $-2 < l_3/w_{22} < 0$. In this case, equilibrium $(0,1,0,0)$ is stable if $r > r_c$ and unstable if $r < r_c$, where

$$r_c \equiv 1 - w_{22}/w_{14} \tag{21}$$
$$= s(-1 + \alpha_2)(-1 + \alpha_2 + 2z_0)/w_{14}$$

(see Expression 5b). Bifurcation theory implies that as r varies through r_c, a second equilibrium whose value depends on r passes through this fixed monomorphic equilibrium. To approximate this equilibrium and find condition for stability the first step is to change variables (*i.e.*, gamete frequencies) so that the nondiagonal elements of matrix V are zero at the point where $r = r_c$. An appropriate change of variables is

$$x_1 = y_1 - \frac{rw_{14}}{l_1 - l_2 + rw_{14}} y_2,$$

$$x_3 = y_2,$$

$$x_4 = y_3 - \frac{rw_{14}}{l_3 - l_2 + rw_{14}} y_2.$$

Note that negative values of y_2 are not biologically meaningful because then x_3 would be negative. The next step is to find the coefficient f_{22} of y_2^2 in the equation for Δy_2 to use in the bifurcation calculations. Bifurcation theory says that at the new bifurcating equilibrium, to lowest order, the equilibrium values of y_1 and y_3 do not change from their values at equilib-

rium $(0,1,0,0)$, wile $y_2 = -(r_c - r)/f_{22}$. The biologically meaningful positive polymorphic equilibrium solutions occur for $r < r_c$ and are stable if $f_{22} < 0$. Calculating f_{22} value at equilibrium $(0,1,0,0)$, we have

$$f_{22} = \frac{4s(1 - \alpha_2)}{(l_1/s\alpha_2)(l_3/s)} [-(1 - \alpha_2)^3 + z_0(5(1 - \alpha_2)^2$$

$$- \alpha_2 - 8z_0(1 - \alpha_2) + 4z_0^2)]. \quad (22)$$

The last expression can be used to find whether the bifurcating solution is stable (i.e., whether $f_{22} < 0$). In particular this is always true for small deviations of z_0 from 0. Similar techniques can be used to find conditions for stability of equilibria that bifurcate from monomorphic equilibrium $(0,0,1,0)$ and the equilibria with one locus polymorphic. One can show that a pair of polymorphic equilibria that bifurcate from monomorphic equilibria $(0,1,0,0)$ and $(0,0,1,0)$ can exist and be stable simultaneously. The bifurcating equilibria will be discussed further in the section devoted to the case $z_0 = 0$.

Phenotypic effects: In this section we consider how phenotypic variables such as the mean value of the trait, the genotypic variance and its components, and mean fitness of the population at a polymorphic equilibrium depend on the parameters of the model. For simplicity we shall analyze only the polymorphic equilibrium that exists and is stable under very strong selection. At the equilibrium $(0,x_{2,0}^*,x_{3,0}^*,0)$, to zero order the mean value, $\bar{z}$, and the genotypic variance, G, of the trait, the mean fitness of the population and the ratio of G to the genic variance V_g $(= \sum 2\alpha_i^2 p_i (1 - p_i))$ are

$$\bar{z}_{,0} = 2z_0. \quad (23a)$$

$$G_{,0} = (1 - \alpha_2)^2(1 - v_0^2)/2, \quad (23b)$$

$$\bar{w}_{,0} = 1 - s(1 - \alpha_2)^2(2 - v_0^2)/4, \quad (23c)$$

$$(G/V_g)_{,0} = \frac{(1 - \alpha_2)^2}{1 + \alpha_2^2}, \quad (23d)$$

where $v_0 = x_{2,0}^* - x_{3,0}^* = 2z_0/(1 - \alpha_2)$. Expression 23a shows that in general at this polymorphic equilibrium the mean value of the trait is not equal to the optimum value. Polymorphic equilibria with the mean value of the trait different from the optimum value have also been found in the mutation-selection balance models (BARTON 1986; HASTINGS 1990). Deviation of the optimum phenotype from zero decreases the genotypic variance maintained under stabilizing selection (see Expression 23b). Expression 23d shows that a substantial amount of additive genetic variance is "hidden" at this equilibrium. This variation will be "released" by recombination if selection is relaxed. Using first order estimates of the gamete frequencies, one can show that to first order

$$\bar{z} = \bar{z}_{,0} + \frac{r}{s} w_{14} \frac{v_0}{(1 - \alpha_2)}, \quad (24a)$$

$$G = G_{,0} + \frac{r}{s} w_{14}(1 - 2v_0^2), \quad (24b)$$

$$\bar{w} = \bar{w}_{,0} - rw_{14}(1 - v_0^2), \quad (24c)$$

$$G/V_g = (G/V_g)_{,0} + \frac{r}{s} w_{14} \frac{2}{1 + \alpha^2}, \quad (24d)$$

where $\bar{z}_{,0}$, $G_{,0}$ and $\bar{w}_{,0}$ are given by (23). Expression 24b shows that the effect of increase in the ratio r/s on the genotypic variance depends on the location of optimum phenotype; it increases G for small $|z_0|$ and causes the opposite effect for large $|z_0|$. Decrease in recombination rate always causes an increase in the mean fitness (see Expression 24c). Increasing the recombination rate also reduces the fraction of the genetic variance that is "hidden" by linkage disequilibrium.

Analysis of the case $z_0 = 0$: As we noted earlier, if the optimum phenotype z_0 coincides with that of the complete heterozygote, the model considered above reduces to the two locus symmetric viability model analyzed in a number of papers (e.g., BODMER and FELSENSTEIN 1967; KARLIN and FELDMAN 1970). Exact results known for this model can be used to check the validity of our approximations and to get further insight into behavior of our model. If $z_0 = 0$, polymorphic equilibria of both types that were analyzed above can be found in exact form.

Polymorphic equilibrium that exists under very strong selection: In the symmetric viability model there exists a symmetric polymorphic equilibrium with allele frequencies equal one half and gamete frequencies:

$$x_1 = x_4 = \frac{1}{2} + D,$$

$$x_2 = x_3 = \frac{1}{2} - D,$$

where the linkage disequilibrium D is given by

$$D = \frac{r}{4s\alpha_2} - \frac{1}{4} \sqrt{1 + \left(\frac{r}{s\alpha_2}\right)^2} \quad (25)$$

[KARLIN and FELDMAN (1970), Expr. (2.3) with $m = 4s\alpha_2$]. This equilibrium is stable

$$-3r^2 - 2s(1 + \alpha_2^2)r + s^2(1 - \alpha_2^2)^2 > 0 \quad (26)$$

[KARLIN and FELDMAN (1970), Expr. (4.5) with $m = 4s\alpha_2$, $\beta = s\alpha_2^2$, $\gamma = s$, $\alpha = s(1 - \alpha_2)^2$, $\delta = s(1 + \alpha_2)^2$. Note that here $\alpha + \delta = 2(\beta + \gamma)$]. This polymorphic equilibrium reduces to equilibrium $(0,x_{2,0}^*,x_{3,0}^*,0)$ as the ratio r/s tends to zero. BÜRGER (1989, Appendix B) has found the condition for stability of this equilibrium for the case with mutation. If $z_0 = 0$, our first order estimates (19) give equilibrium allele frequen-

cies equal one half and linkage disequilibrium

$$D = -\frac{1}{4} + \frac{r}{4s\alpha_2} \qquad (27)$$

Polymorphic equilibria that bifurcate from monomorphic equilibria and equilibria with one locus polymorphic: If $z_0 = 0$, monomorphic equilibria $(1,0,0,0)$ and $(0,0,0,1)$ cannot be stable (see Expressions 4 and 7). Monomorphic equilibria $(0,1,0,0)$ and $(0,0,1,0)$ are simultaneously stable if

$$\alpha_2 > \frac{1}{2}, \qquad r/s > (1 - \alpha_2)^2 \qquad (28)$$

(Expressions 5 and 6). Equilibria with one locus polymorphic $(x_1^*,x_2^*,0,0)$ and $(0,0,x_3^*,x_4^*)$, are not feasible (see Expressions 8). Equilibria $(x_1^*,0,x_3^*,0)$ and $(0,x_2^*,0,x_4^*)$ are feasible, if $\alpha_2 < \frac{1}{2}$ (Expression 8). These equilibria simultaneously exist and are stable if

$$\alpha_2 < \frac{1}{2}, \qquad r/s > (1 - \alpha_2^2)/3 \qquad (29)$$

(expression 10 and 12). Note that monomorphic equilibria and equilibria with one locus polymorphic cannot be stable simultaneously. When as a result of decrease in the ratio r/s monomorphic equilibria $(0,1,0,0)$ and $(0,0,1,0)$ or equilibria with one locus polymorphic $(x_1^*,0,x_3^*,0)$ and $(0,x_2^*,0,x_4^*)$ become unstable, a new pair of polymorphic equilibria arise. These "unsymmetric" equilibria can be found exactly using formulae given in Appendix 1 of KARLIN and FELDMAN (1970):

$$u = \mp \frac{(1 + \alpha_2)|s(1 - \alpha)^2 - r|}{8r\alpha_2}$$

$$\sqrt{\frac{3r^2 + 2rs(1 + \alpha_2^2) - s^2(1 - \alpha_2^2)^2}{sr}}, \qquad (30a)$$

$$v = -\frac{1 - \alpha_2}{1 + \alpha_2} \frac{s(1 + \alpha_2)^2 - r}{s(1 - \alpha_2)^2 - r} u, \qquad (30b)$$

$$t = \frac{3r^2 - 4rs(1 + \alpha_2^2) + s^2(1 - \alpha_2^2)^2}{4rs\alpha_2}, \qquad (30c)$$

where as before $u = x_1 - x_4$, $v = x_2 - x_3$, $t = x_1 + x_4 - x_2 - x_3$. To see that these "unsymmetric" equilibria bifurcate from monomorphic equilibria $(0,1,0,0)$ and $(0,0,1,0)$ and equilibria with one locus polymorphic $(x_1^*,0,x_3^*,0)$ and $(0,x_2^*,0,x_4^*)$ it is sufficient to substitute the critical values of the recombination rate from (28) and (29) for r in (30). The resulting values of u, v and t exactly describe equilibria $(0,1,0,0)$ and $(0,0,1,0)$ and $(x_1^*,0,x_3^*,0)$ and $(0,x_2^*,0,x_4^*)$. Comparison of (26) with (30) shows that "unsymmetric" equilibria do not exist then the symmetric equilibrium is stable and that they reduce to the symmetric equilibrium then the expression in the left-hand side of (26) equals zero. Thus, each of the two branches of the equilibria given by
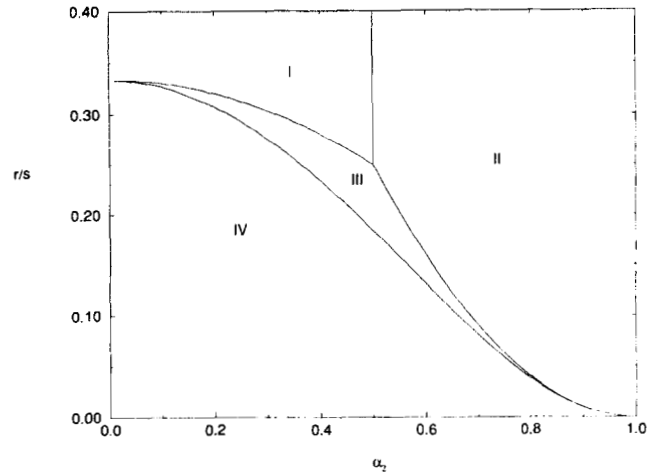

FIGURE 2.—Regions of existence and stability of different equilibria on the $(\alpha_2, r/s)$ plane for $z_0 = 0$. The roman numerals denote the regions where the following equilibria exist and are stable: I, monomorphic equilibria $(0,1,0,0)$ and $(0,0,1,0)$; II, single polymorphic equilibria $(x_1,0,x_3,0)$ and $(0,x_2,0,x_4)$; III, a pair of "unsymmetric" doubly polymorphic equilibria; IV, "symmetric" doubly polymorphic equilibrium.

(30) connects a monomorphic equilibrium with an equilibrium with one locus polymorphic bifurcating from them then they become unstable and crosses the symmetric equilibrium at the point where it becomes unstable. This pair of "unsymmetric" equilibria is stable. Figure 2 shows regions of stability of different equilibria. We see that when the symmetric equilibrium is stable it is the only stable equilibrium, while in other regions the stable equilibria (monomorphic, with one locus polymorphic, or "unsymmetric" polymorphic) occur in pairs. In the latter case results of evolution depend on the initial conditions. The main conclusion that can be drawn from this figure is that selection need not be extremely strong relative to recombination to maintain genetic variability in both loci.

DISCUSSION

Equations that describe evolution of polygenic characters under stabilizing selection are very complex and cannot be analyzed without simplifying assumptions. A standard set of such simplifications includes a weak selection approximation, equivalence of the loci with respect to their contributions to the trait value, and the location of the optimum phenotype at that of complete heterozygote. One of the common beliefs that has emerged from these simplifications is that in the absence of other factors stabilizing selection on an additive trait cannot maintain variability in more than one locus. NAGYLAKI (1989) showed, however, that if the contributions of two diallelic loci to an additive trait are sufficiently different, some forms of stabilizing selection can maintain variability in both loci. To get these results NAGYLAKI used a weak

selection approximation. This approximation is valid in the strict sense if $\Delta/r \ll 1$, where Δ is the maximum difference in fitnesses between genotypes, and r is the recombination rate (NAGYLAKI 1976). In NAGYLAKI's (1989) asymptotic results for strong selection on one or both loci, Δ is about 1, while a necessary condition for the maintenance of variability under Gaussian fitness function, $d > 1.3$ leads to $\Delta > 1 - e^{-1.3^2} = 0.8$. Hence in these situations even for nonlinked loci the inequality $\Delta/r \ll 1$ is not true. Thus, this analysis still left open the question of the dependence of the possibility of stable multilocus polymorphism on the intensity of stabilizing selection.

In this paper we have shown that if the double heterozygote has the optimum phenotype and the contributions of the loci to the trait are different, then any symmetric stabilizing selection fitness function can maintain genetic variability provided selection is sufficiently strong relative to linkage. Figure 2 shows that selection need not be extremely strong relative to recombination for the polymorphic equilibria to be stable. For example, if α_2 is about 0.5, polymorphism is stable for $r/s \leq \frac{1}{4}$. Genetic variability can be maintained for small r/s values if $z_0 \neq 0$ provided the deviation of the optimum phenotype from that of a double heterozygote is not very much. A sufficient condition for existence of locally stable polymorphic equilibrium under quadratic stabilizing selection for small r/s values is $|z_0| < (1 - \alpha_2)/2$. Decrease in difference of allelic effects (*i.e.*, increase in α_2) and increase in $|z_0|$ both decrease possibility of stable polymorphism. These results seem not to be restricted by the case of quadratic fitness; similar conclusions are valid for other stabilizing selection fitness functions. For example, one can show that for linkage sufficiently tight and "triangular" fitness function, $w(z) = 1 - s|z - z_0|$, there exists a locally stable polymorphic equilibrium, provided $|z_0| < (1 - \alpha_2)/2$. As $r/s \rightarrow 0$ this equilibrium reduces to an equilibrium in the form $(0, x_2, x_3, 0)$.

The polymorphic equilibria that were analyzed in this paper are characterized by some interesting properties. First, the mean value of the trait at equilibrium is not equal to the optimum phenotype. This means that analysis of a population at such an equilibrium would exhibit a component of apparent directional selection. Second, these equilibria have large levels of negative linkage disequilibrium which "hides" additive genetic variance. The "hidden" genetic variation will be "released" by recombination if selection is relaxed. Third, different equilibria including different polymorphic equilibria can be stable simultaneously and, hence, the outcome of evolution depends on the history. Fourth, increasing the strength of selection can result both in increase or decrease of the genotypic variance. Of special interest is a possibility of increase

in the genotypic variance as a result of increase in the intensity of selection. This can occur, for example, if the population is near a monomorphic equilibrium or an equilibrium with one locus polymorphic and this equilibrium becomes unstable when selection is strengthened. Another situation is when the population is at a stable polymorphic equilibrium and z_0 deviates from zero (see Expression 23 and 24). Our results show that global characteristics of equilibria significantly depend on relative strength of selection and linkage. For example, there is no stable polymorphic equilibrium for very weak selection, one such equilibrium for very strong selection, and several stable polymorphic equilibria for moderately strong selection.

Our overall results here that stabilizing selection does not necessarily eliminate genetic variability are consistent with some experimental results on the effects of applying strong stabilizing selection to laboratory populations. In some of these cases (FALCONER 1957; PROUT 1962), the lines subject to strong stabilizing selection did not have significant reductions in genetic variability relative to control lines, which is compatible with the results we have derived here. Note that, in contrast, explanations for the maintenance of genetic variability based on mutation selection balance would predict that strong stabilizing selection would reduce genetic variability.

LITERATURE CITED

BARTON, N., 1986 The maintenance of genetic variability through a balance between mutation and stabilizing selection. Genet. Res. **47:** 209–216.

BODMER, W. F., and J. FELSENSTEIN, 1967 Linkage and selection: theoretical analysis of the deterministic two locus random mating model. Genetics **57:** 237–265.

BULMER, M., 1973 The maintenance of the genetic variability of polygenic characters by heterozygote advantage. Genet. Res. **22:** 9–12.

BÜRGER, R., 1989 Linkage and the maintenance of heritable variation by mutation selection balance. Genetics **121:** 175–184.

FALCONER, D. S., 1957 Selection for phenotypic intermediates in Drosophila. J. Genet. **55:** 551–561.

GALE, J. S., and M. J. KEARSEY, 1968 Stable equilibria under stabilizing selection in the absence of dominance. Heredity **23:** 553–561.

GAVRILETS, S., 1993 Equilibria in an epistatic viability model under arbitrary strength of selection. J. Math. Biol. (in press).

GAVRILETS, S., and G. DE JONG, 1993 Pleiotropic models of polygenic variation, stabilizing selection and epistasis. Genetics (in press).

GIMELFARB, A., 1989 Genotypic variation for a quantitative character maintained under stabilizing selection: epistasis. Genetics **123:** 217–227.

GIMELFARB, A., 1992 Pleiotropy and multilocus polymorphism. Genetics **130:** 223–227.

GILLESPIE, J. H., and M. TURELLI, 1989 Genotype-environment interaction and the maintenance of polygenic variability. Genetics **121:** 129–138.

GUCKENHEIMER, J., and P. HOLMES, 1983 Nonlinear oscillations, dynamical systems, and bifurcations of the vector fields. Springer, New York.

HASTINGS, A., 1981 Simultaneous stability of $D = 0$ and $D \neq 0$ for multiplicative variability at two loci: an analytical study. J. Theor. Biol. **89:** 69–81.

HASTINGS, A., 1982 Unexpected behavior in two locus genetic systems: an analysis of marginal underdominance at a stable equilibrium. Genetics **102:** 129–138.

HASTINGS, A., 1985a Equilibrium properties of two-locus two-allele models. Genetics **109:** 799–812.

HASTINGS, A., 1985b Four simultaneously stable polymorphic equilibria in two-locus two-allele models. Genetics **109:** 255–261.

HASTINGS, A., 1987 Monotonic change of the mean phenotype in two-locus models. Genetics **117:** 583–585.

HASTINGS, A., 1990 Maintenance of polygenic variation through mutation-selection balance: bifurcation analysis of a biallelic model. J. Math. Biol. **28:** 329–340.

HASTINGS, A., and C. HOM, 1990 Multiple equilibria and maintenance of additive genetic variance in a model of pleiotropy. Evolution **44:** 1153–1163.

HOPPENSTEADT, F. C., 1976 A slow selection analysis of two-locus, two allele traits. Theor. Popul. Biol. **9:** 68–81.

HORN, R. A., and C. R. JOHNSON, 1985 *Matrix Analysis.* Cambridge University Press, Cambridge.

IOOSS, G., and D. D. JOSEPH, 1981 *Elementary Stability and Bifurcation Theory.* Springer, New York.

KARLIN, S., and M. FELDMAN, 1970 Linkage and selection: two-locus symmetric viability model. Theor. Popul. Biol. **1:** 39–71.

KARLIN, S., and J. McGREGOR, 1972 Application of method of small parameters to multi-niche population genetic model. Theor. Popul. Biol. **3:** 186–209.

KEARSEY, M. J., and J. S. GALE, 1968 Stabilizing selection in the absence of dominance: an additional note. Heredity **23:** 617–620.

KOJIMA, K., 1959b Stable equilibria for the optimum model. Proc. Natl. Acad. Sci. USA **45:** 989–993.

LANDE, R., 1975 The maintenance of genetic variability by mutation in a polygenic character under linked loci. Genet. Res. **26:** 221–235.

LEWONTIN, R. C., 1964 The interaction of selection and linkage. II. Optimal model. Genetics **50:** 757–782.

NAGYLAKI, T., 1976 The evolution of one- and two-locus systems. Genetics **83:** 583–600.

NAGYLAKI, T., 1989 The maintenance of genetic variability in two-locus models of stabilizing selection. Genetics **122:** 235–248.

PROUT, T., 1962 The effect of stabilizing selection on the time of development in Drosophila melanogaster. Genet. Res. **3:** 364–382.

ROBERTSON, A., 1956 The effect of selection against extreme deviants based on deviation or hemozygosis. J. Genet. **54:** 236–248.

WRIGHT, S., 1935 Evolution in populations in approximate equilibrium. J. Genet. **30:** 257–266.

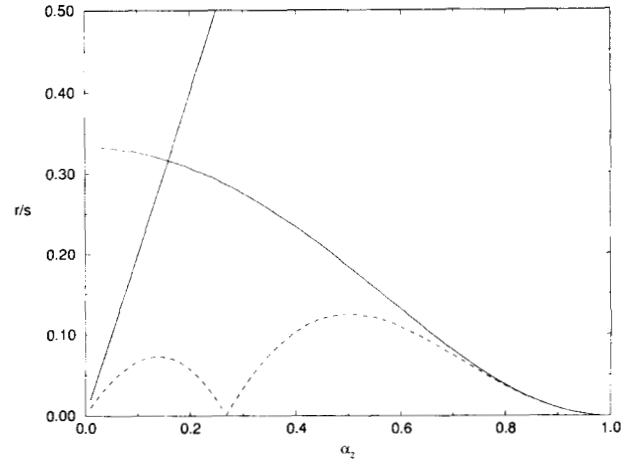WOLFRAM, S., 1988 *Mathematica: A System for Doing Mathematics by Computer.* Addison-Wesley, Reading, Mass.

FIGURE 3.—The dependence of the critical value of the ratio r/s on α_2 for $z_0 = 0$. The solid curve represents the exact dependence calculated from (26). The dashed curve represents the first order approximation calculated from (A4). The first order analysis gives feasible estimates of the gamete frequencies in the region below the straight line.

ZHIVOTOVSKY, L., and S. GAVRILETS, 1992 Quantitative variability and multilocus polymorphism under epistatic selection. Theor. Popul. Biol. **42:** 254–283.

Communicating editor: R. R. HUDSON

APPENDIX

In the main text we used regular perturbation techniques to find location of the perturbed equilibria. Substituting new perturbed equilibrium gamete frequencies $x_{i,0} + \epsilon x_{i,1}$ into the matrix that determines the stability of equilibria and calculating the corresponding eigenvalues, we can find stability conditions for the perturbed equilibria. The first order perturbation $\lambda_{,1}$ for an eigenvalue λ of the matrix $V = V_{,0} + \epsilon V_{,1}$ is

$$\lambda_{,1} = (y^T V_{,1} x)/(y^T x), \qquad \text{(A1)}$$

where x and y are a right and a left eigenvector of $V_{,0}$ that corresponds to $\lambda_{,0}$ and $(y^T x) \neq 0$ (HORN and JOHNSON 1985, Section 6.3.12).

For simplicity let us assume that $r < s \ll 1$ (*cf.* HOPPENSTEADT 1976). In this case recurrence equations (2) are approximated by the corresponding differential equations, and the stability of equilibria depends on the eigenvalues of matrix $U = \{d(\bar{w}\Delta x_i)/dx_j\}$. At equilibrium $(0, x^*_{2,0}, x^*_{3,0}, 0)$ to zero order stability matrix U computed using variables u, v and t has form

$$U_{,0} = \begin{pmatrix} \lambda_{u,0} & 0 & 0 \\ \dfrac{1+\alpha_2}{1-\alpha_2}\lambda_{v,0} & \lambda_{v,0} & -\dfrac{v_0}{4}(\lambda_{u,0}+\lambda_{t,0}) \\ 0 & 0 & \lambda_{t,0} \end{pmatrix}$$

where $\lambda_{u,0} = \lambda_{t,0} = -s\alpha_2/\bar{w}_{,0}, \lambda_{v,0} = -s(1-\alpha_2)^2(1-v_0^2)/$

$(2\bar{w}_{,0})$. The right eigenvectors of this matrix are

$$\left(\frac{1-\alpha_2}{1+\alpha_2}\frac{\lambda_{u,0}-\lambda_{v,0}}{\lambda_{v,0}}, 1, 0\right)^T, (0,1,0)^T,$$

$$\left(0, \frac{\lambda_{t,0}}{2(\lambda_{v,0}-\lambda_{t,0})}, 1\right)^T, \quad (A2)$$

while the corresponding left eigenvectors are

$$(1,0,0), \left(-\frac{1+\alpha_2}{1-\alpha_2}\frac{\lambda_{v,0}}{\lambda_{u,0}}\frac{2}{v_0}, \frac{\lambda_{u,0}-\lambda_{v,0}}{\lambda_{u,0}}\frac{2}{v_0}, 1\right), (0,0,1). \quad (A3)$$

Computing first order perturbation for the elements of matrix U, we find that the first order estimates for the eigenvalues of the stability matrix are

$$\lambda_u = \lambda_{u,0} + rw_{14}\frac{-\alpha_2-(1-\alpha_2)^2+v_0^2(1+\alpha_2^2)}{(\lambda_{v,0}-\lambda_{u,0})(2/s)}, \quad (A4a)$$

$$\lambda_v = \lambda_{v,0} + rw_{14}$$

$$\frac{\alpha_2+(1-\alpha_2)^2-v_0^2[3(1-\alpha_2)^2-\alpha_2]}{\frac{v_0^4(1-\alpha_2)^2+2v}{(\lambda_{v,0}-\lambda_{u,0})(2/s)}}, \quad (A4b)$$

$$\lambda_t = \lambda_{t,0} + rw_{14}\frac{\alpha_2 v_0^2}{(\lambda_{v,0}-\lambda_{u,0})(2/s)}. \quad (A4c)$$

Expressions (A4) can be used to find the estimates of the critical value of the recombination rate r (or the ratio r/s) under which the equilibrium becomes unstable. Figure 3 shows how $(r/s)_c$ depends on α_2 in the case when $z_0 = 0$. This figure also presents the exact dependence calculated from (26). Note that when $\lambda_{v,0} = \lambda_{u,0}$ (near α_2 value about 0.27) a right and a left eigenvector corresponding to $\lambda_{u,0}$ and to $\lambda_{v,0}$ are orthogonal and expression (A1) cannot be used. Figure 3 shows that for $\alpha_2 > 0.5$ the estimates of $(r/s)_c$ based on first order analysis are close to the exact values.