

# On the Evolution of Mutation in Changing Environments: Recombination and Phenotypic Switching

Uri Liberman,<sup>\*,†</sup> Jeremy Van Cleve<sup>‡,1</sup> and Marcus W. Feldman<sup>‡,2</sup>

<sup>\*</sup>*School of Mathematical Sciences, Tel Aviv University, Tel Aviv, Israel 69978,* <sup>†</sup>*Holon Institute of Technology, Holon, Israel 58102* and <sup>‡</sup>*Department of Biology, Stanford University, Stanford, California 94305-5020*

Manuscript received September 27, 2010  
Accepted for publication December 13, 2010

## ABSTRACT

Phenotypic switching has been observed in laboratory studies of yeast and bacteria, in which the rate of such switching appears to adjust to match the frequency of environmental changes. Among possible mechanisms of switching are epigenetic influences on gene expression and variation in levels of methylation; thus environmental and/or genetic factors may contribute to the rate of switching. Most previous analyses of the evolution of phenotypic switching have compared exponential growth rates of non-interacting populations, and recombination has been ignored. Our genetic model of the evolution of switching rates is framed in terms of a mutation-modifying gene, environments that cause periodic changes in fitness, and recombination between the mutation modifier and the gene under selection. Exact results are obtained for all recombination rates and symmetric fitnesses that strongly generalize earlier results obtained under complete linkage and strong constraints on the relation between fitness and period of switching. Our analytical and numerical results suggest a general principle that recombination reduces the stable rate of switching in symmetric and asymmetric fitness regimes and when the period of switching is random. As the recombination rate increases, it becomes less likely that there is a stable nonzero rate of switching.

**I**N large populations subject to mutation between alleles under selection that is constant over time, mutation–selection equilibria can be stable. In the neighborhood of such a stable equilibrium, if an allele at a locus that controls the rate of mutation is introduced, this allele will invade if it reduces the mutation rate (LIBERMAN and FELDMAN 1986). This is one example of the reduction principle for constant selection regimes (FELDMAN and LIBERMAN 1986). This approach, using the selectively neutral genetic modifiers of parameters such as mutation, recombination, and migration that are important features of the evolutionary process, can be viewed as an alternative to an approach using evolutionary stable strategies (ESS) (MAYNARD SMITH 1978) or to finding critical points of the mean fitness (KARLIN and MCGREGOR 1972, 1974).

It has become usual to denote the gene on which selection occurs directly as the major gene (or in the case of recombination modification, genes) and the locus that controls the parameter of interest as the modifier gene (*e.g.*, FELDMAN *et al.* 1997). If a new mutation at the modifier gene is introduced during a transient

phase of evolution, rather than near an equilibrium, the fate of the modifier mutant may be quite different: for example, an allele that causes reduction of recombination will succeed if it is introduced near stable linkage disequilibrium, but if it arises at a phase of the dynamics where the major loci are proceeding toward fixation, increase of recombination may occur and the reduction principle does not necessarily hold (MAYNARD SMITH 1980, 1988; BERGMAN and FELDMAN 1990). The evolution of modifiers of mutation, recombination, or migration rates when the regime of selection on the major gene(s) is not constant over time has seen far less mathematical or even numerical analysis than the case of constant selection. Early numerical work by CHARLESWORTH (1976) showed the failure of the reduction principle for recombination under some patterns of cyclically fluctuating abiotic selection.

Host–parasite systems may produce cyclical dynamics that have features similar to those of cyclically fluctuating abiotic selection. The evolution of host recombination in a host–parasite cyclical system has been addressed by HAMILTON (1980) and most recently by GANDON and OTTO (2007). Hamilton used a mean fitness argument to demonstrate an advantage to hosts with higher recombination, and NEE (1989) used a similar approach in finding that mutation rates should increase in both host and parasite. GANDON and OTTO (2007) showed that alleles at a recombination-modifying locus that increased recombination could succeed

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.110.123620/DC1>.

<sup>1</sup>*Present address:* Santa Fe Institute, 1399 Hyde Park Rd., Santa Fe, NM 87501.

<sup>2</sup>*Corresponding author:* Department of Biology, Stanford University, 385 Serra Mall, Stanford, CA 94305-5020. E-mail: [mfeldman@stanford.edu](mailto:mfeldman@stanford.edu)

in such host–parasite models. Mutation-increasing alleles may be favored in similar models, as shown by HARAGUCHI and SASAKI (1996) and M'GONIGLE *et al.* (2009). The latter authors also showed that under host–parasite cycling, increasing recombination between the mutation modifier and the gene under selection in the host decreased the stable mutation rate.

With increasing empirical interest in epigenetics, there has been a flurry of activity surrounding “stochastic switching,” a phenomenon observed in some studies of *Saccharomyces cerevisiae*, *Escherichia coli*, or *Bacillus subtilis*, where individual cells switch cyclically between different inheritable phenotypes (THATTAI and VAN OUDENAARDEN 2004; KUSSELL and LEIBLER 2005; ACAR *et al.* 2008). For example, experiments by BALABAN *et al.* (2004) show that switching between phenotypes may differ between different genetic strains of *E. coli*. The same group analyzed a mathematical model of this system and showed that the optimal rate of phenotypic switching was strongly dependent on the frequency of environmental changes but only weakly dependent on the strength of selection in any single environment (KUSSELL *et al.* 2005). Earlier theoretical treatments by ISHII *et al.* (1989) and LACHMANN and JABLONKA (1996) also found that optimal switching rates depended on the distribution of environmental periodicities.

Phenotypic switching may be an example of an epigenetic effect, for example based on methylation (LIM and VAN OUDENAARDEN 2007). There is some discussion as to the fraction of such epigenetic effects that are transgenerational (*e.g.*, YOUNGSON and WHITELAW 2008) and, to the extent that they are, what their evolutionary effects might be (BONDURIANSKY and DAY 2009). Most theoretical treatments of phenotypic switching have assumed that the organism is asexual and that the numbers of individuals grow exponentially (*e.g.*, KUSSELL *et al.* 2005, p. 1809; GAÁL *et al.* 2010). These studies usually do not specifically include genetic contributions to the rate of switching; exceptions are the analyses by ISHII *et al.* (1989) and SALATHÉ *et al.* (2009), both of which took fitness to be the phenotype that switches and allowed the rate of switching to be under the genetic control of a mutation-modifying locus. If switching is epigenetic and heritable, it is reasonable to propose that it is at least partially under genetic control. Further, if phenotypic switching involves epigenetic regulation of gene expression, and if this epigenetic phenomenon occurs in sexual species (*e.g.*, RAKYAN *et al.* 2003; HENDERSON and JACOBSEN 2007), it is also reasonable to investigate the importance of recombination between genes contributing to the phenotype and those contributing to the rate of switching. As pointed out by LYNCH (2007, p. 89, Table 4.1) the importance of recombination in the evolution of prokaryotes has been substantially underestimated. Inclusion of the mutation modifier and recombination carries the evolution of

phenotypic switching into the corpus of evolutionary population genetics.

One of the earliest studies of this problem in the framework of theoretical population genetics was by ISHII *et al.* (1989), who studied a haploid locus with two alleles  $A$  and  $a$ , whose fitnesses were  $1 + s(t)$  and  $1 - s(t)$ , respectively, at generation  $t$  with  $s(t)$  allowed to fluctuate through time with average zero. A second locus with alleles  $B$  and  $b$  controlled the (bidirectional) mutation rate between  $A$  and  $a$ , and the recombination rate between the two genes was  $r$ . Selection changed cyclically. In the case of complete linkage ( $r = 0$ ), they found that the ESS mutation rate maximized the long-term geometric mean population fitness. For  $r > 0$  their analysis depended on the symmetric selection coefficient,  $s$ , being  $\ll 1/n$ , where  $2n$  is the period of the selection cycle. The recombination  $r$  also appeared through the size of the product  $m$ . Their numerical analysis suggested that in this symmetric case, no matter what the value of  $r$ , if selection was strong enough ( $s$  large enough), the value  $1/n$  for the mutation rate could not be invaded. The analysis by LACHMANN and JABLONKA (1996) did not involve a modifier (and hence recombination was irrelevant) but found, similarly to ISHII *et al.* (1989), that in the symmetric selection case the (fitness) optimum mutation rate was  $\sim 1/n$ . They also claimed that this result held in asymmetric fitness regimes. An important qualitative interpretation of this result is that if the mutation rate is initially low enough, a modifier allele that increases the mutation rate can invade. Thus, violations of the Feldman–Liberman reduction principle (FELDMAN and LIBERMAN 1986) for constant environments are to be expected in cyclically varying environments.

A numerical treatment by SALATHÉ *et al.* (2009) of the symmetric case confirmed the evolutionary stability of a mutation rate of  $\sim 1/n$  in a deterministically cycling environment of period  $n$  generations. However, if the time in each environment is random, then even in the symmetric case the stable mutation rate can be vastly different from  $1/n$  (SALATHÉ *et al.* 2009, Figure 1). Further, if the fitnesses of  $A$  relative to  $a$  in environment 1 and  $a$  relative to  $A$  in environment 2 are not exactly the same, *i.e.*, there is no symmetry, then for a wide range of selection coefficients and initial mutation rates, a mutation-reducing modifier allele will succeed, potentially taking the switching rate to zero. GAÁL *et al.* (2010) proved a parallel set of results in the asexual exponential growth framework.

Our goal in this article is to study mutation modification as a mechanism for the control of phenotypic switching where the phenotype is fitness. We obtain complete analytic results for period  $2n = 2$  or  $2n = 4$  with symmetric fitnesses and recombination, where the stable values of the mutation rate  $\mu$  are 1.0 and 0.5, respectively. We also investigate an interesting property of the period, namely that there is a mathematical

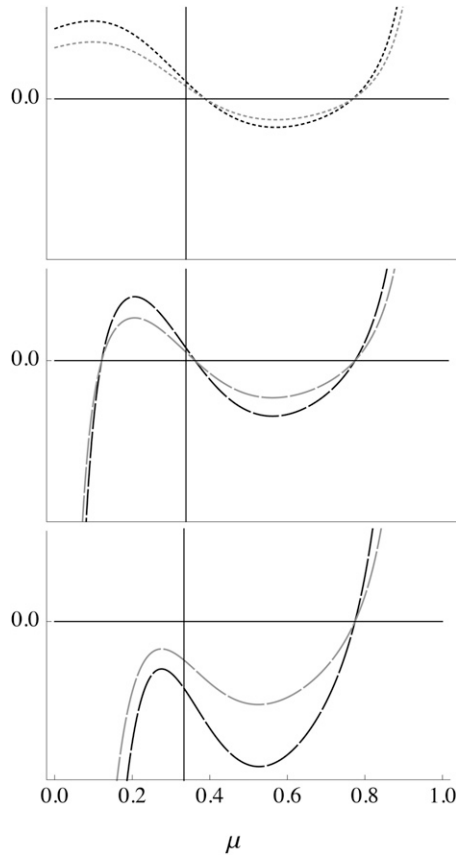


FIGURE 1.—Comparison of  $\partial\lambda_1/\partial\mu_m$  evaluated at  $\mu_m = \mu_M = \mu$  (solid curves) and  $\partial w^*/\partial\mu_M$  (shaded curves) for  $n = 3$ . In all plots,  $s_1 = 10^{-1}$  whereas  $s_3 = 10^{-1}, 10^{-0.8}$ , and  $10^{-0.6}$ , in the top, middle, and bottom plots, respectively. The vertical line is located at  $\mu = 1/n = \frac{1}{3}$ .

difference between the cases in which the selection regime changes after an odd or an even number of generations. We find that in general the presence of recombination makes it more unlikely that there is a stable nonzero mutation rate, whether the cycle period is fixed or random. As the rate of recombination increases, the extent of departure from symmetric fitnesses that permits a stable nonzero mutation rate becomes smaller.

REFERENCE MODEL: CONSTANT ENVIRONMENT

Consider a population of haploids large enough that genetic drift can be ignored. Fitness is determined by the alleles  $A$  and  $a$  at the major locus, and linked to this locus, with recombination fraction  $r$ , is a modifier locus with alleles  $M$  and  $m$  that produce mutation rates  $\mu_M$  and  $\mu_m$ , respectively. The mutation rates from  $A$  to  $a$  and  $a$  to  $A$  are the same. The four genotypes  $AM, Am, aM,$  and  $am$  have frequencies  $x_1, x_2, x_3,$  and  $x_4$ , respectively, and fitnesses  $w_1, w_2, w_3,$  and  $w_4$ , where, because the modifier locus is selectively neutral, we have  $w_1 = w_2$  and  $w_3 = w_4$ . Then the frequencies  $x'_1, x'_2, x'_3,$  and  $x'_4$  in the next generation are

$$\begin{aligned} wx'_1 &= (1 - \mu_M)w_1(x_1 - rD) + \mu_M w_3(x_3 + rD) \\ wx'_2 &= (1 - \mu_m)w_2(x_2 + rD) + \mu_m w_4(x_4 - rD) \\ wx'_3 &= (1 - \mu_M)w_3(x_3 + rD) + \mu_M w_1(x_1 - rD) \\ wx'_4 &= (1 - \mu_m)w_4(x_4 - rD) + \mu_m w_2(x_2 + rD), \end{aligned} \quad (1)$$

where  $D = x_1x_4 - x_2x_3$  is the linkage disequilibrium, and the normalizing factor  $w$  is

$$w = \sum_{i=1}^4 w_i x_i - rD(w_1 - w_2 - w_3 + w_4) = \sum_{i=1}^4 w_i x_i, \quad (2)$$

because of our fitness assumptions. We have assumed that the life cycle begins with a diploid phase during which there is Mendelian segregation with recombination followed by selection on the haploid phase after which there is mutation. Recombination, or homologous exchange, is an important step in this life cycle for a vast array of organisms, including microbes (LYNCH 2007, pp. 88–95).

In the absence of the modifier allele  $m$  (in which case recombination has no effect), the transformation (1) is linear fractional; that is, the transformation is of the form  $x'_i = (\alpha + \beta x_i)/(\gamma + \delta x_i)$ , where  $\alpha, \beta, \gamma,$  and  $\delta$  are constants, and we have used  $x_3 = (1 - x_1)$ . This allows complete determination of the global dynamics of  $(x_1, 0, x_3, 0)$ . We can summarize these dynamics as follows. When allele  $M$  is fixed, there is a unique globally stable equilibrium  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$ , where  $x_1^* = 1 - x_3^* = u^*/(1 + u^*)$  and  $u^*$  is the unique positive root of

$$Q(u) = \mu_M w_1 u^2 + (1 - \mu_M)(w_1 - w_3)u - \mu_M w_3 = 0. \quad (3)$$

We have  $x_1^* > x_3^*$  if  $(1 - 2\mu_M)(w_1 - w_3) > 0$  and  $x_1^* < x_3^*$  if  $(1 - 2\mu_M)(w_1 - w_3) < 0$ .

The evolution of mutation is determined by the stability of  $(x_1^*, 0, x_3^*, 0)$  to the introduction of allele  $m$ , that is, whether  $m$  will increase in frequency when introduced close to this equilibrium. The local stability of this equilibrium to invasion by  $Am$  and  $am$  is determined by the linear transformation

$$\begin{aligned} w^* x'_2 &= (1 - \mu_m)w_2(x_2 + rD^*) + \mu_m w_4(x_4 - rD^*) \\ w^* x'_4 &= (1 - \mu_m)w_4(x_4 - rD^*) + \mu_m w_2(x_2 + rD^*), \end{aligned} \quad (4)$$

where

$$w^* = w_1 x_1^* + w_3 x_3^*, \quad D^* = x_1^* x_4 - x_3^* x_2. \quad (5)$$

From (4), the matrix  $\mathcal{L}$  associated with this linear transformation is

$$\frac{1}{w^*} \begin{bmatrix} (1 - \mu_m)w_2 - r[(1 - \mu_m)w_2 - \mu_m w_4]x_3^* & \mu_m w_4 + r[(1 - \mu_m)w_2 - \mu_m w_4]x_1^* \\ \mu_m w_2 + r[(1 - \mu_m)w_4 - \mu_m w_2]x_3^* & (1 - \mu_m)w_4 - r[(1 - \mu_m)w_4 - \mu_m w_2]x_1^* \end{bmatrix}. \quad (6)$$

The stability of this point is determined by the eigenvalues of  $\mathcal{L}$ , which satisfy the equation  $M(z) = \det(\mathcal{L} - z\mathcal{I}) = 0$ ,

where  $\mathcal{I}$  is the  $2 \times 2$  identity matrix. The positive eigenvalue of  $\mathcal{L}$  is  $<1$  if  $M(1) > 0$  and  $M'(1) > 0$  and is  $>1$  if  $M(1) < 0$ . In the former case,  $m$  cannot invade, while in the latter case  $(x_1^*, 0, x_3^*, 0)$  is unstable and  $m$  invades. After a considerable amount of algebra we find the following.

**RESULT 1.** *The equilibrium  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$  is stable if  $\mu_m > \mu_M$  and unstable if  $\mu_m < \mu_M$ . For all recombination rates, a modifier allele that reduces the mutation rate will invade.*

We can also show that the mean fitness  $w^*$  at  $(x_1^*, 0, x_3^*, 0)$  is a decreasing function of  $\mu_M$ .

This reduction principle in constant environments was proved for selection on diploids and any number of alleles at the mutation-controlling gene by LIBERMAN and FELDMAN (1986).

CHANGING ENVIRONMENTS:  
INITIAL EQUILIBRIUM

Assume that in each generation the genotypic fitnesses change. At generation  $i$ , for  $i = 1, 2, \dots, k$ , the fitness parameters are

gamete :	$AM$	$Am$	$aM$	$am$	(7)
fitness :	$w_1^i$	$w_1^i$	$w_3^i$	$w_3^i$	

where again we assume  $M$  and  $m$  do not affect fitness.

At the beginning of generation  $i$ , for  $i = 1, 2, \dots, k$ , the population state is given by the frequency vector  $\underline{x} = (x_1, x_2, x_3, x_4)$ , and at the beginning of generation  $i + 1$  it is given by  $\underline{x}' = (x_1', x_2', x_3', x_4')$ , where  $\underline{x}' = T_i(\underline{x})$  and  $T_i$  is given by the system (1) with fitnesses (7):

$$\begin{aligned} w^i x_1' &= (1 - \mu_M)w_1^i(x_1 - rD) + \mu_M w_3^i(x_3 + rD) \\ w^i x_2' &= (1 - \mu_m)w_1^i(x_2 + rD) + \mu_m w_3^i(x_4 - rD) \\ w^i x_3' &= (1 - \mu_M)w_3^i(x_3 + rD) + \mu_M w_1^i(x_1 - rD) \\ w^i x_4' &= (1 - \mu_m)w_3^i(x_4 - rD) + \mu_m w_1^i(x_2 + rD). \end{aligned} \tag{8}$$

The normalizing factor is  $w^i = (x_1 + x_2)w_1^i + (x_3 + x_4)w_3^i$  and the linkage disequilibrium is  $D = x_1 x_4 - x_2 x_3$ .

If the population state at the beginning of the  $k$ -generation process is  $\underline{x}^o = (x_1^o, x_2^o, x_3^o, x_4^o)$ , then after generation  $k$  it is  $\underline{x}^k = (x_1^k, x_2^k, x_3^k, x_4^k)$  where  $\underline{x}^k = T(\underline{x}^o)$  and the transformation  $T$  is given by the composition of the  $k$  transformations:

$$T = T_k \circ T_{k-1} \circ \dots \circ T_1. \tag{9}$$

The combined mean fitness associated with the transformation  $T$  from  $\underline{x}^o$  to  $\underline{x}^k$  is

$$w = w(\underline{x}^k) = \prod_{i=1}^k w^i(\underline{x}^i). \tag{10}$$

To study the evolution of mutation, we first consider a population where only the  $M$  allele is present at the modifier locus. Let  $S_i$  be the transformation associated

with  $T_i$  restricted to the two gametes  $AM$  and  $aM$  only. Then  $S_i$ , for  $i = 1, 2, \dots, k$ , is given by

$$\begin{aligned} w^i x_1^{i+1} &= (1 - \mu_M)w_1^i x_1^i + \mu_M w_3^i x_3^i \\ w^i x_3^{i+1} &= (1 - \mu_M)w_3^i x_3^i + \mu_M w_1^i x_1^i, \end{aligned} \tag{11}$$

with  $w^i$  given by

$$w^i = w_1^i x_1^i + w_3^i x_3^i; \quad i = 1, 2, \dots, k. \tag{12}$$

The “total”  $k$ -generation transformation restricted to  $AM$  and  $aM$  is  $S = S_k \circ S_{k-1} \circ \dots \circ S_1$ .

In (11) we can use the ratio  $u^i = x_1^i/x_3^i$  instead of  $x_1^i$  and  $x_3^i$ , and similarly  $u^{i+1} = x_1^{i+1}/x_3^{i+1}$ . Then the transformation  $S_i$  is

$$u^{i+1} = f_i(u^i) = \frac{(1 - \mu_M)w_1^i u^i + \mu_M w_3^i}{\mu_M w_1^i u^i + (1 - \mu_M)w_3^i}; \quad i = 1, 2, \dots, k. \tag{13}$$

Thus, the total  $k$ -generation transformation on the boundary where only  $M$  is present is the composition  $f = f_k \circ f_{k-1} \circ \dots \circ f_1$ .

Note that  $f_i(\cdot)$  is a linear fractional transformation. One of the properties of such a transformation is that its composition over  $k$  generations is also a linear fractional transformation. Hence the total  $k$ -generation transformation  $f$  can be written as

$$u^k = f(u) = \frac{au^o + b}{cu^o + d}, \tag{14}$$

where, since the coefficients in (13) are positive for  $0 < \mu_M < 1$ , the coefficients  $a, b, c$ , and  $d$  are also positive. Now we can analyze the evolutionary process in terms of this  $k$ -generation transformation (14), and since it is linear fractional it converges to a unique stable equilibrium. Thus, ultimately the population converges to a  $k$ -step trajectory loop determined by the stable state of this  $k$ -step compound linear fractional transformation. In fact,  $u^*$ , the stable state of the  $k$ -step process, is the unique positive root of

$$Q(u) = cu^2 + (d - a)u - b = 0. \tag{15}$$

Using the same notation as in Result 1, this equilibrium can be expressed as  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$ , and it can be shown to be globally stable.

CHANGING ENVIRONMENTS: EXTERNAL STABILITY

To explore the dynamics of switching, and in particular to find an evolutionarily stable switching rate (if it exists), we study the external stability of the unique internally stable equilibrium  $\underline{x}^*$  to invasion by the new mutation modifying allele  $m$ . The mutation rate  $\mu_M$  produced by allele  $M$  will be evolutionarily stable if allele  $m$  cannot invade when  $\mu_m > \mu_M$  or  $\mu_m < \mu_M$ . Therefore we analyze the external stability of  $\underline{x}^*$  as a function of the difference between the new mutation rate  $\mu_m$  and the resident rate  $\mu_M$ . This will give us the direction of evolution of the switching.



A population starting from the equilibrium  $\underline{x}^*$  satisfies  $\underline{x}^* = T(\underline{x}^*) = T_k \circ T_{k-1} \circ \dots \circ T_1(\underline{x}^*)$ ; that is, after  $k$  generations of change it will end up again at  $\underline{x}^*$ . Take  $\underline{x} = \underline{x}^* + \underline{\varepsilon}$  as a “starting” population state near  $\underline{x}^*$ , where  $\underline{\varepsilon} = (\varepsilon_1, \varepsilon_2, \varepsilon_3, \varepsilon_4)$  with  $\varepsilon_i$  “small” and  $\varepsilon_1 + \varepsilon_2 + \varepsilon_3 + \varepsilon_4 = 0$  so that  $\underline{x}' = T\underline{x} = \underline{x}^* + \underline{\varepsilon}'$ . We work with the linear transformation “near”  $\underline{x}^*$ ; that is, up to nonlinear terms,

$$\underline{x}' = \underline{x}^* + \underline{\varepsilon}' = \underline{x}^* + \mathcal{L}^* \underline{\varepsilon}^T, \tag{16}$$

where  $\mathcal{L}^*$  is a matrix that depends on  $\underline{x}^*$  and may be obtained as a combination of the matrices of similar linear approximations across the  $k$  generations. The stability of equilibrium  $\underline{x}^*$  to the introduction of allele  $m$  is determined by the eigenvalues of the matrix  $\mathcal{L}^*$ .

This scenario is well known from modifier theory (FELDMAN 1972; FELDMAN *et al.* 1980; FELDMAN and LIBERMAN 1986), and the matrix  $\mathcal{L}^*$  is also known to have the structure

$$\mathcal{L}^* = \begin{matrix} & \begin{matrix} 1 & 3 & 2 & 4 \end{matrix} \\ \begin{matrix} \mathcal{L}^* \\ \mathcal{L}^* \\ \mathcal{L}^* \\ \mathcal{L}^* \end{matrix} & \begin{bmatrix} & * & * & \\ \mathcal{L}_{in} & * & * & \\ 0 & 0 & & \mathcal{L}_{ex} \\ 0 & 0 & & \end{bmatrix} \end{matrix} \begin{matrix} 1 \\ 3 \\ 2 \\ 4 \end{matrix}, \tag{17}$$

where we have swapped columns 2 and 3 and rows 2 and 3 to show the structure. The entries marked \* do not affect the eigenvalues of  $\mathcal{L}^*$ .

The eigenvalues of  $\mathcal{L}$  are therefore those of the submatrices  $\mathcal{L}_{in}$  and  $\mathcal{L}_{ex}$ , where  $\mathcal{L}_{in}$  determines the internal stability of  $\underline{x}^*$ , confined to the boundary with only  $M$  present. As  $\underline{x}^*$  is assumed to be stable there, these eigenvalues are less than one in magnitude.  $\mathcal{L}_{ex}$  is the linear approximation of evolution near  $\underline{x}^*$ , which involves only the gametes  $Am$  and  $am$  and is a combination of the matrices  $\mathcal{L}_i^{ex}$ , for  $i = 1, 2, \dots, k$ , that describe the changes in generation  $i$  when allele  $m$  is rare.

In fact, from (8) we have

$$w^i \mathcal{L}_i^{ex} = \begin{bmatrix} (1 - \mu_m)w_1^i - r[(1 - \mu_m)w_1^i - \mu_m w_3^i]x_3^i & \mu_m w_3^i + r[(1 - \mu_m)w_1^i - \mu_m w_3^i]x_1^i \\ \mu_m w_1^i + r[(1 - \mu_m)w_3^i - \mu_m w_1^i]x_3^i & (1 - \mu_m)w_3^i - r[(1 - \mu_m)w_3^i - \mu_m w_1^i]x_1^i \end{bmatrix}. \tag{18}$$

We set  $x^* = \underline{x}^1$  and describe the following generations by  $\underline{x}^2, \dots, \underline{x}^k$ . The normalizer in generation  $i$  is

$$w^i = w^i(\underline{x}^i) = w_1^i x_1^i + w_3^i x_3^i. \tag{19}$$

As each of the matrices  $\mathcal{L}_1^{ex}, \mathcal{L}_2^{ex}, \dots, \mathcal{L}_k^{ex}$  is positive,  $\mathcal{L}_{ex}$  is also positive, and, by the Perron–Frobenius theory, the largest eigenvalue of  $\mathcal{L}_{ex}$  is positive. Some properties of  $\mathcal{L}$  are documented in supporting information, File S1, Part I.

The external stability of  $\underline{x}^*$  depends on the magnitude of the positive, and largest, so-called Perron–Frobenius eigenvalue of  $\mathcal{L}_{ex}$ . If this eigenvalue is  $<1$ ,  $\underline{x}^*$  is externally stable and allele  $m$  cannot invade near

$\underline{x}^*$ , and if it is  $>1$ ,  $\underline{x}^*$  is externally unstable, and  $m$  enters the population and increases in frequency.

The characteristic polynomial of  $\mathcal{L}_{ex}$ , namely  $C(z) = \det(\mathcal{L}_{ex} - z\mathcal{I})$ , is quadratic in  $z$  with a positive  $z^2$  coefficient. Therefore the largest positive eigenvalue of  $\mathcal{L}_{ex}$  is  $<1$  when  $C(1) > 0$  and  $C'(1) > 0$ , and it is  $>1$  when  $C(1) < 0$ . It seems impossible to compute  $C(1)$  and  $C'(1)$  in general. We are, however, able to derive analytical results in some special cases that are discussed in the following sections.

CYCLICALLY FLUCTUATING ENVIRONMENTS: PERIOD 2

Suppose that the selection regime alternates between two states as specified in (20) below.

genotype	$AM$	$Am$	$aM$	$am$	
fitness regime 1	$w_1$	$w_1$	$w_3$	$w_3$	(20)
fitness regime 2	$\hat{w}_1$	$\hat{w}_1$	$\hat{w}_3$	$\hat{w}_3$	

Environments alternate, producing a cycle with period 2.

We first characterize the unique stable equilibrium  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$  on the boundary where only  $M$  is present. On this boundary the two transformations  $S_1$  and  $S_2$  of (11) are

$$S_1 \rightarrow \begin{matrix} w^1 y_1 = (1 - \mu_M)w_1 x_1 + \mu_M w_3 x_3 \\ w^1 y_3 = (1 - \mu_M)w_3 x_3 + \mu_M w_1 x_1 \end{matrix} \tag{21}$$

and

$$S_2 \rightarrow \begin{matrix} w^2 x'_1 = (1 - \mu_M)\hat{w}_1 y_1 + \mu_M \hat{w}_3 y_3 \\ w^2 x'_3 = (1 - \mu_M)\hat{w}_3 y_3 + \mu_M \hat{w}_1 y_1 \end{matrix}, \tag{22}$$

where  $x_1$  and  $x_3$  are the frequencies of  $AM$  and  $aM$ , respectively, at the beginning of generation 1,  $y_1$  and  $y_3$  at the end of generation 1, and  $x'_1$  and  $x'_3$  at the end of generation 2. The mean fitnesses are

$$w^1 = w_1 x_1 + w_3 x_3, \quad w^2 = \hat{w}_1 y_1 + \hat{w}_3 y_3. \tag{23}$$

This cycle repeats with period 2, and the combined one-cycle transformation  $S = S_2 \circ S_1$ , on the boundary where  $M$  is fixed, is

$$S \rightarrow \begin{matrix} w^1 w^2 x'_1 = [(1 - \mu_M)^2 w_1 \hat{w}_1 + \mu_M^2 w_1 \hat{w}_3] x_1 \\ \quad + \mu_M (1 - \mu_M) w_3 (\hat{w}_1 + \hat{w}_3) x_3 \\ w^1 w^2 x'_3 = [(1 - \mu_M)^2 w_3 \hat{w}_3 + \mu_M^2 w_3 \hat{w}_1] x_3 \\ \quad + \mu_M (1 - \mu_M) w_1 (\hat{w}_1 + \hat{w}_3) x_1. \end{matrix} \tag{24}$$

The normalizing factor  $w = w^1 w^2$  is the cycle mean fitness and can be expanded as

$$w = [(1 - \mu_M)^2 w_1 \hat{w}_1 + \mu_M^2 w_1 \hat{w}_3 + \mu_M (1 - \mu_M) w_1 (\hat{w}_1 + \hat{w}_3)] x_1 + [(1 - \mu_M)^2 w_3 \hat{w}_3 + \mu_M^2 w_3 \hat{w}_1 + \mu_M (1 - \mu_M) w_3 (\hat{w}_1 + \hat{w}_3)] x_3 \tag{25}$$

$$= \hat{w}_1 w_1 x_1 + \hat{w}_3 w_3 x_3 + \mu_M (\hat{w}_3 - \hat{w}_1) (w_1 x_1 - w_3 x_3). \tag{26}$$

The linear fractional transformation during the two generations,  $S$ , described in (14), is

$$u' = f(u) = \frac{[(1 - \mu_M)^2 w_1 \hat{w}_1 + \mu_M^2 w_1 \hat{w}_3] u + \mu_M (1 - \mu_M) w_3 (\hat{w}_1 + \hat{w}_3)}{\mu_M (1 - \mu_M) w_1 (\hat{w}_1 + \hat{w}_3) u + [(1 - \mu_M)^2 w_3 \hat{w}_3 + \mu_M^2 w_3 \hat{w}_1]}, \tag{27}$$

where  $u = x_1/x_3$  and  $u' = x'_1/x'_3$ .

The stable equilibrium  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$  with  $x_1^* = u^*/(1 + u^*)$  and  $x_3^* = 1/(1 + u^*)$  satisfies  $u^* = u' = f(u^*)$  and is the unique positive root of the quadratic equation  $Q(u) = 0$ , where

$$Q(u) = \mu_M (1 - \mu_M) w_1 (\hat{w}_1 + \hat{w}_3) u^2 + [(1 - \mu_M)^2 (w_3 \hat{w}_3 - w_1 \hat{w}_1) + \mu_M^2 (w_3 \hat{w}_1 - w_1 \hat{w}_3)] u - \mu_M (1 - \mu_M) w_3 (\hat{w}_1 + \hat{w}_3). \tag{28}$$

We now check the external stability properties of  $\underline{x}^*$ , that is, its stability to invasion by  $m$  when  $m$  is introduced near  $\underline{x}^*$ . Evolution of the mutation rate is determined by the external stability properties of  $\underline{x}^*$ , that is, its stability to invasion by  $m$  when  $m$  is introduced near  $\underline{x}^*$ . In File S1 we show that  $m$  will invade (*i.e.*,  $\mu_m$  invades  $\mu_M$ ) if  $M(1) < 0$ , where

$$M(1) = \frac{(\mu_m - \mu_M)(1 - r)\Delta}{x_1^*}, \tag{29}$$

and  $\Delta = \Delta(r, \mu_m)$  is shown in File S2 to be a bilinear function of  $r$  and  $\mu_m$  in  $0 \leq r \leq 1$  and  $0 \leq \mu_m \leq 1$ .

In general it is very complicated to compute  $M(1)$ , unless we assume that the fitnesses are symmetric; that is,  $\hat{w}_1 = w_3$  and  $\hat{w}_3 = w_1$ , which allows a complete analysis of  $M(1)$  and the external stability of  $\underline{x}^*$ . For general  $r$ ,  $\mu_m$ , and  $\mu_M$ , we refer to NUMERICAL ANALYSIS, below. We are able to obtain complete results when there is absolute linkage between the two loci ( $r = 0$ ) and  $\mu_m = 0$  or  $\mu_m = 1$ . When  $r = 0$ , the matrix  $\mathcal{L}_{ex} = \hat{\mathcal{L}}\mathcal{L}$ , with  $w^*$  the product of  $w^1$  and  $w^2$  at equilibrium, is given in File S1, Part II by

$$w^* \mathcal{L}_{ex} = \begin{bmatrix} (1 - \mu_m)^2 w_1 \hat{w}_1 + \mu_m^2 w_1 \hat{w}_3 & \mu_m (1 - \mu_m) w_3 (\hat{w}_1 + \hat{w}_3) \\ \mu_m (1 - \mu_m) w_1 (\hat{w}_1 + \hat{w}_3) & (1 - \mu_m)^2 w_3 \hat{w}_3 + \mu_m^2 w_3 \hat{w}_1 \end{bmatrix}. \tag{30}$$

When either  $\mu_m = 0$  or  $\mu_m = 1$ ,  $\mathcal{L}_{ex}$  is a diagonal matrix, and its eigenvalues are

$$\lambda_1^0 = \frac{w_1 \hat{w}_1}{w^*}, \quad \lambda_2^0 = \frac{w_3 \hat{w}_3}{w^*} \text{ for } \mu_m = 0, \\ \lambda_1^1 = \frac{w_1 \hat{w}_3}{w^*}, \quad \lambda_2^1 = \frac{w_3 \hat{w}_1}{w^*} \text{ for } \mu_m = 1. \tag{31}$$

The magnitude of the leading eigenvalue depends on the asymmetry of the parameters  $\hat{w}_1$  and  $\hat{w}_3$ , with respect to  $w_1$  and  $w_3$ . This asymmetry may be represented by the parameter

$$\delta = w_3 \hat{w}_3 - w_1 \hat{w}_1. \tag{32}$$

As the fitnesses are relative, and hence determined up to a multiplicative positive constant in each generation,

$\delta = 0$  implies that  $\hat{w}_3 = w_1$  and  $\hat{w}_1 = w_3$ , namely complete symmetry between the two alleles  $A$  and  $a$ , whereas  $\delta \neq 0$  implies asymmetry.

When  $\delta = 0$  (*i.e.*, the symmetric case  $\hat{w}_3 = w_1, \hat{w}_1 = w_3$  discussed in the next section), it turns out that  $\lambda_1^0$  and  $\lambda_2^0$  are  $< 1$ , whereas at least one of the eigenvalues  $\lambda_1^1$  and  $\lambda_2^1$  is  $> 1$ . Therefore when  $\mu_m = 0$ ,  $\underline{x}^*$  is externally stable for all  $0 < \mu_M \leq 1$ , and when  $\mu_m = 1$ ,  $\underline{x}^*$  is never stable when  $0 \leq \mu_M < 1$ . Thus when  $r = 0$ , a mutation-reducing allele cannot invade. The results are sensitive to the asymmetry measure  $\delta$ , as is discussed in greater detail later.

### THE SYMMETRIC CASE WITH PERIOD 2

In the symmetric case where the selection regime alternates each generation, we have  $\hat{w}_1 = w_3, \hat{w}_3 = w_1$  ( $\delta = w_3 \hat{w}_3 - w_1 \hat{w}_1 = 0$ ), and we can present a complete external stability analysis of the equilibrium  $\underline{x}^*$ . In the symmetric case, the fitness parameters fluctuate between generations such that

genotype	$AM$	$Am$	$aM$	$am$	
odd generation fitness	$w_1$	$w_1$	$w_3$	$w_3$	, (33)
even generation fitness	$w_3$	$w_3$	$w_1$	$w_1$	

where, of course, we assume  $w_1 \neq w_3$ . The unique internally stable equilibrium  $\underline{x}^* = (x_1^*, 0, x_3^*, 0)$  on the boundary where only allele  $M$  is present can be represented as  $x_1^* = u^*/(1 + u^*)$ ,  $x_3^* = 1/(1 + u^*)$ , where  $u^*$  is the unique positive root of the quadratic Equation 15, which becomes

$$\bar{Q}(u) = (1 - \mu_M) w_1 u^2 + \mu_M (w_3 - w_1) u - (1 - \mu_M) w_3 = 0. \tag{34}$$

In File S3 we use (34) to analyze the factors in (29). From (29),  $M(1) = 0$  when  $r = 1$ , in which case the larger eigenvalue is 1. When  $0 \leq r < 1$ , the sign of  $M(1)$  depends on the signs of  $(\mu_m - \mu_M)$  and the sign of  $\Delta = \Delta(r, \mu_m)$ . In fact we show in File S3 that the bilinear function  $\Delta(r, \mu_m)$  is always negative in the symmetric case. As an immediate consequence of this, together with the representation of  $M(1)$  given in (29), we secure the following result.

**RESULT 2.** *The internally stable equilibrium is externally stable when  $\mu_M > \mu_m$  and is unstable when  $\mu_M < \mu_m$  for all  $0 \leq r < 1$ . Thus with symmetric changing environments, higher mutation rates are favored.*

As in the case of constant environments, Result 2 accords with the behavior of the mean fitness at equilibrium as a function of the resident mutation rate. With symmetric changing environments we can show the following.

**RESULT 3.** *The mean fitness  $w^* = w^*(\mu_M)$  at equilibrium is a monotone increasing function of the mutation rate  $\mu_M$ .*

The proof of this result can be found in [File S4](#).

THE SYMMETRIC CASE WITH PERIOD 4

The fitnesses of the genotypes can be represented as follows:

genotype	<i>AM</i>	<i>Am</i>	<i>aM</i>	<i>am</i>	
generation 1	$w_1$	$w_1$	$w_3$	$w_3$	
generation 2	$w_1$	$w_1$	$w_3$	$w_3$	(35)
generation 3	$w_3$	$w_3$	$w_1$	$w_1$	
generation 4	$w_3$	$w_3$	$w_1$	$w_1$	

In this case, on the boundary where *M* is fixed, the four-generation recursion is

$$w\underline{x}' = T_2 \circ T_2 \circ T_1 \circ T_1(\underline{x}), \tag{36}$$

where, as in (21) and (22),

$$T_1 = \begin{bmatrix} (1 - \mu_M)w_1 & \mu_M w_3 \\ \mu_M w_1 & (1 - \mu_M)w_3 \end{bmatrix},$$

$$T_2 = \begin{bmatrix} (1 - \mu_M)w_3 & \mu_M w_1 \\ \mu_M w_3 & (1 - \mu_M)w_1 \end{bmatrix}, \tag{37}$$

and the four-generation normalization factor is then

$$w = w_1^2 w_3^2 + \mu_M (1 - \mu_M) w_1 w_3 (w_1 - w_3)^2 + \mu_M (1 - \mu_M) (w_1 + w_3)^2 (w_1 - w_3) (w_1 x_1 - w_3 x_3). \tag{38}$$

Our argument proceeds in much the same way as for the symmetric model with period 2. First we show in [File S5](#), Part I that when  $r = 0$ ,

$$(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) > 0, \tag{39}$$

where  $(x_1^*, x_3^*)$  denotes the equilibrium of the four-generation transformation. Hence, from (38)

$$w \geq w_1^2 w_3^2. \tag{40}$$

We now turn to the external stability of  $(x_1^*, 0, x_3^*, 0)$ , which is determined by the eigenvalues of  $\mathcal{L}_{\text{ex}}$ . When  $\mu_M = 0$ , we can write

$$w^* \mathcal{L}_{\text{ex}} = \begin{bmatrix} w_3 & 0 \\ 0 & w_1 \end{bmatrix}^2 \begin{bmatrix} w_1 & 0 \\ 0 & w_3 \end{bmatrix}^2 = \begin{bmatrix} w_1^2 w_3^2 & 0 \\ 0 & w_1^2 w_3^2 \end{bmatrix}, \tag{41}$$

and when  $\mu_M = 1$ ,

$$w^* \mathcal{L}_{\text{ex}} = \begin{bmatrix} 0 & w_1 \\ w_3 & 0 \end{bmatrix}^2 \begin{bmatrix} 0 & w_3 \\ w_1 & 0 \end{bmatrix}^2 = \begin{bmatrix} w_1^2 w_3^2 & 0 \\ 0 & w_1^2 w_3^2 \end{bmatrix}. \tag{42}$$

But by (40),  $w^* > w_1^2 w_3^2$ , and therefore  $\underline{x}^*$  is externally stable when either  $\mu_M = 0$  or  $\mu_M = 1$ . In other words, if

$0 < \mu_M < 1$ , then a new mutation-modifying allele cannot invade  $\underline{x}^*$  if it produces mutation rates that are too high or too low. This leaves open the possibility that there might be an intermediate value of the mutation rate  $\mu_M$  that gives rise to an equilibrium  $\underline{x}^*$  on the *M*-fixed boundary that cannot be invaded. The first indication that  $\mu_M = \frac{1}{2}$  is this value is suggested by the following.

**RESULT 4.** *In the period 4 symmetric case, if  $r = 0$ , the mean fitness  $w^* = w^*(\mu_M)$  achieves a maximum at  $\mu_M = \frac{1}{2}$ .*

The proof is in [File S5](#), Part II.

If  $\mu_M = \frac{1}{2}$ , then the single-generation transformations are very symmetric, and the equilibrium on the *M*-fixed boundary has  $x_1^* = \frac{1}{2} = x_3^*$ . To examine the invasion by  $\mu_m \neq \frac{1}{2}$  we evaluate the external stability of  $\underline{x}^* = (\frac{1}{2}, 0, \frac{1}{2}, 0)$ . The eigenvalues of the linear approximation  $\mathcal{L}_{\text{ex}}$  near  $\underline{x}^*$  are the roots of  $M(z) = 0$ , where  $M(z)$  can be expressed as

$$M(z) = (w^*z)^2 - (w^*z)(A + D) + AD - BC, \tag{43}$$

where the entries of  $\mathcal{L}_{\text{ex}}$ , *A*, *B*, *C*, *D* are all positive. As before, one eigenvalue is positive, and it can be shown that  $\det \mathcal{L}_{\text{ex}} = AD - BC = (1 - 2\mu_m)^4 w_1^4 w_3^4 > 0$ . Hence both eigenvalues are positive. The larger one is  $< 1$  if  $M(1) > 0$ , which is shown to be the case in [File S5](#), Parts III and IV. This allows us to claim the following.

**RESULT 5.** *In the period 4 symmetric case with  $0 \leq r \leq 1$ , the mutation rate  $\mu_M = \frac{1}{2}$  cannot be invaded by mutation rates either  $>$  or  $< \frac{1}{2}$ .*

For cycles of period  $m = 2n$ , when  $r = 0$ , we can show that if  $0 < \mu_M \leq 1$ , a modifier allele *m* that produces  $\mu_m = 0$  cannot invade. If *n* is even we are also able to prove that allele *m* with  $\mu_m = 1$  cannot invade. It remains to prove the latter result for odd values of *n*, and we see in [NUMERICAL ANALYSIS](#) that this could be difficult.

THE MEAN FITNESS AND EIGENVALUE CRITERIA

The analyses by [ISHII et al. \(1989\)](#) and [GAÁL et al. \(2010\)](#) formulated the dynamics of *M* and *m*, the mutation-controlling alleles, in terms of the numbers of each genotype. In particular, when  $r = 0$ , [Ishii et al.](#) concluded that the criterion for invasion and long-term increase of *m* in a population monomorphic for *M* could be obtained from the limiting geometric mean of the mean fitnesses at each generation. This allowed them to state that when  $r = 0$ , the uninvadable mutation rate “maximizes the long-term geometric average of population fitness” ([ISHII et al. 1989](#), p. 165). This harks back to early analyses on neutral modifiers of recombination and the mean fitness principle of [KARLIN and MCGREGOR \(1972, 1974\)](#), who hypothesized that uninvadable values of neutral modifiers of recombination,

mutation, and migration also maximized the mean fitness.

Our criterion for initial increase is in terms of the local stability of the equilibrium  $(x_1^*, 0, x_3^*, 0)$ , where  $x_1^*$  and  $x_3^*$  are functions of  $\mu_M$ , to invasion by  $m$ . This invasion is determined by the leading eigenvalue  $\lambda$  of the local stability matrix that gives the frequencies of  $Am$  and  $am$  near  $(x_1^*, 0, x_3^*, 0)$ , and  $\lambda$  must be a function of both  $\mu_m$  and  $\mu_M$ . As pointed out above, the relevant properties of  $\lambda$  are expressible in terms of properties of  $M(\lambda)$ , the characteristic polynomial of the local stability matrix  $\mathcal{L}_{ex}$ . The value of  $\mu_m$  that makes  $\partial\lambda/\partial\mu_m|_{\mu_m=\mu_M} = 0$  will be the stable value of the mutation rate.

We write the local stability matrix for local dynamics of  $Am, am$  near  $(x_1^*, 0, x_3^*, 0)$  as

$$\begin{bmatrix} \tilde{A} & \tilde{B} \\ \tilde{C} & \tilde{D} \end{bmatrix}, \tag{44}$$

where the entries  $\tilde{A}, \tilde{B}, \tilde{C}, \tilde{D}$  are defined by the continued operation of the linear fractional transformation corresponding to  $\mathcal{L}_{ex}$ ,  $w^*$  is a function of  $\mu_M$ , and the tilde indicates that the numerators of all entries are functions of  $\mu_m$ . We can write the characteristic polynomial  $M(\lambda)$  of matrix (44) as

$$M(\lambda) = (\lambda w^*)^2 - (\lambda w^*)(\tilde{A} + \tilde{D}) + \tilde{A}\tilde{D} - \tilde{B}\tilde{C}. \tag{45}$$

From (45) we can calculate  $\partial\lambda/\partial\mu_m$ .

For the initial equilibrium  $(x_1^*, 0, x_3^*, 0)$ , where allele  $m$  is absent, we have

$$\begin{pmatrix} x_1^* \\ x_3^* \end{pmatrix} = \begin{bmatrix} \hat{A} & \hat{B} \\ \hat{C} & \hat{D} \end{bmatrix} \begin{pmatrix} x_1^* \\ x_3^* \end{pmatrix}, \tag{46}$$

where the circumflex indicates that the numerators are functions of  $\mu_M$ . From (46), the matrix has an eigenvalue of 1, and we have

$$(w^*)^2 - w^*(\hat{A} + \hat{D}) + (\hat{A}\hat{D} - \hat{B}\hat{C}) = 0. \tag{47}$$

The stationary values of  $w^*$  with respect to  $\mu_M$  can be obtained by differentiating Equation 47 with respect to  $\mu_M$  and finding values of  $\mu_M$  such that  $\partial w^*/\partial\mu_M = 0$ . The relationship between mutation values, which maximize the mean fitness, and the uninvadable value of  $\mu$  that sets  $\partial\lambda/\partial\mu_m|_{\mu_m=\mu_M} = 0$  is given by the following.

**RESULT 6.** *Mutation rates  $\mu_M$  that are critical points of the mean fitness  $w^*$  in the case  $r = 0$  also entail that at  $\mu_m = \mu_M$ ,  $\partial\lambda/\partial\mu_m = 0$ .*

The proof is in the APPENDIX. Figure 1 plots  $\partial w^*/\partial\mu_M$  and  $\partial\lambda/\partial\mu_m$  for  $n = 3$ , and the two derivatives are seen to vanish at the same value of the mutation rate. The proof of Result 6 actually makes no use of the symmetry

of the fitnesses, so Result 6 is also true for asymmetric fitnesses.

### NUMERICAL ANALYSIS

Result 2 entails that in the symmetric case  $\delta = w_3\hat{w}_3 - w_1\hat{w}_1 = 0$ , for period 2 ( $n = 1$ ) for all values of the recombination rate, nonzero values of the resident mutation rate  $\mu_M$  will be invaded by allele  $m$  that gives rise to a higher mutation rate; *i.e.*,  $\mu_m > \mu_M$ . The mutation rate will therefore go on increasing to its maximum of 1. A similar result for period 4 ( $n = 2$ ), namely Result 5, tells us that for all  $r$ , values of  $\mu_M < \frac{1}{2}$  will be invaded by higher values  $\mu_m > \mu_M$ , but values of  $\mu_M > \frac{1}{2}$  will be invaded by lower values of  $\mu_m$ . There are no constraints on the strength of the symmetric selection as were required in the analysis by ISHII *et al.* (1989). There have been suggestions in the literature that the stability of the mutation rate  $\mu = 1/n$  for the symmetric model cycles with period  $2n$  holds for larger values of  $n$  (ISHII *et al.* 1989; LACHMANN and JABLONKA 1996). For the asymmetric model, with  $\delta \neq 0$ , however, SALATHÉ *et al.* (2009) showed that the situation was much more complicated and that if the asymmetry was large enough, zero was the stable mutation rate; mutation provided no advantage with fixed or random period cycles. Further, with stochastic environments and a rate of environmental change of  $1/n$  every generation, the stable switching rate can be up to two orders of magnitude lower than  $1/n$  (SALATHÉ *et al.* 2009, Figure 1). In addition, we noted after Result 5 above that even in the case  $r = 0$ , if  $n$  is odd there may be some anomalies.

We have therefore explored three effects numerically: the role of  $n$  and in particular whether it is odd or even, the role of asymmetry in the cycling fitness values (extending SALATHÉ *et al.* 2009), and the effect of recombination between the major locus and the mutation modifier. The equilibrium  $(x_1^*, 0, x_3^*, 0)$  is found numerically and for given parameter values, the matrix  $\mathcal{L}_{ex}$  is determined numerically together with its eigenvalues. The leading eigenvalue  $\lambda_1$  is numerically differentiated with respect to  $\mu_m$  and the derivative evaluated at  $\mu_M = \mu_m$ , where as we showed in (29) the eigenvalue is 1. The sign of  $\partial\lambda_1/\partial\mu_m|_{\mu_m=\mu_M}$  then tells us whether a larger or smaller value of  $\mu_m$  will produce  $\lambda_1 > 1$  and hence cause  $m$  to invade. Thus, points where  $\partial\lambda_1/\partial\mu$  crosses zero with a negative slope indicate values of  $\mu_M$  that cannot be invaded by any nearby value of  $\mu_m$ . These values of  $\mu_M$  can be regarded as evolutionarily stable. These results are summarized in Figure 2.

Figure 2 extends Figure 2 of SALATHÉ *et al.* (2009) by exhibiting the roles of recombination and asymmetry on the stable mutation rate. We point out first that the first row of graphs in Figure 2 represents results for  $n = 2$  (*i.e.*, period 4 case) and we see that with perfect symmetry in the cycling fitnesses, the mutation rate  $\frac{1}{2}$  is stable for all recombination rates, verifying Result 5 for



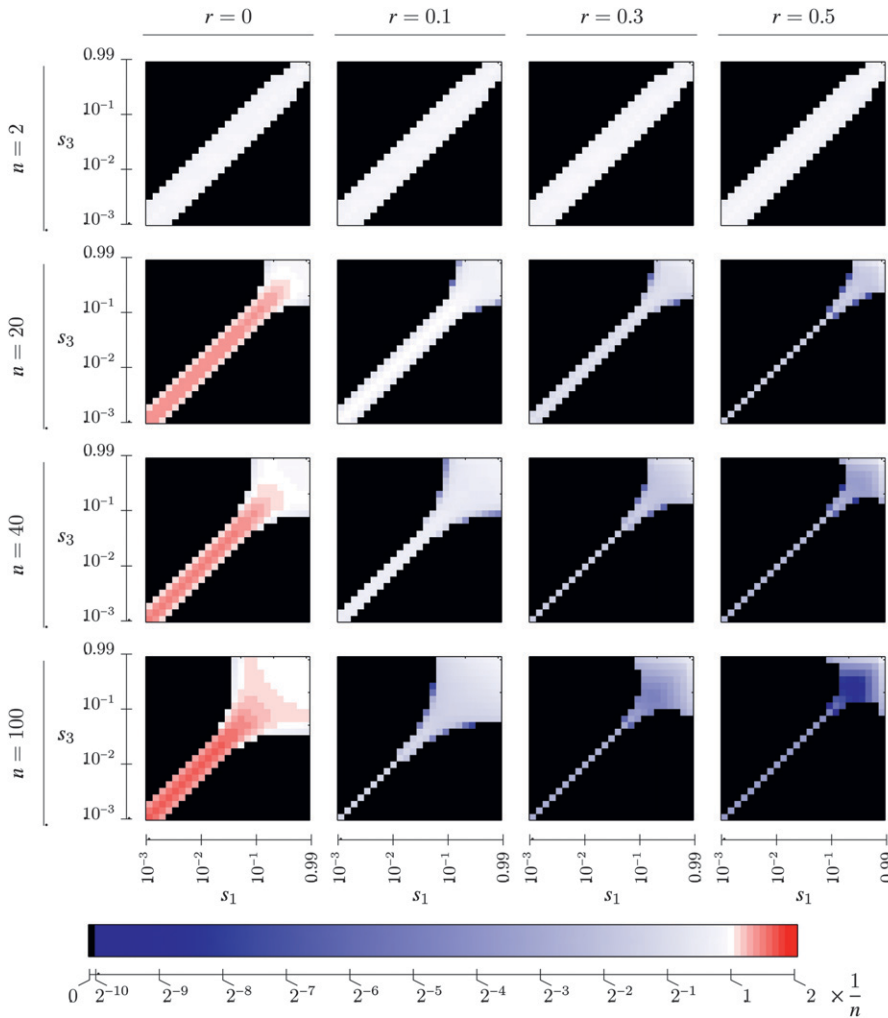


FIGURE 2.—Evolutionarily stable mutation rates as a function of the selection coefficients for the disfavored allele in each environment,  $s_1 = 1 - w_1$  and  $s_3 = 1 - w_3$ , which are plotted logarithmically. The number of generations in each environment,  $n$ , is given at the left. The recombination rate between the major locus under selection and the modifier locus is given at the top. The colors denote the stable mutation rate and are given in units of the canonical predicted rate of  $1/n$  in the colorbar at the bottom; white is a rate of  $1/n$ , shades of red are rates  $>1/n$ , and blues are rates  $<1/n$ . Black denotes evolution to zero mutation rate.

all  $r$ . In what follows, we use the notation  $w_1^{(i)} = 1 - s_1$ ,  $w_3^{(i)} = 1$  for  $n$  generations with  $w_1^{(i)} = 1$ ,  $w_3^{(i)} = 1 - s_3$  for the next  $n$  generations. Thus,  $s_1 = s_3$  corresponds to perfect symmetry in selection regimes (SALATHÉ *et al.* 2009). Note that in all graphs with  $r = 0$  and in many of the others, there is an interval of asymmetry in the selection coefficients  $s_1 = 1 - w_1$  and  $s_3 = 1 - w_3$  in which there is a stable nonzero mutation rate. As  $n$  and  $r$  increase, the stable nonzero mutation rate with  $s_1 = s_3$  decreases. For  $r = 0$  and small  $s_1$  and  $s_3$ , the extent of asymmetry that allows a stable nonzero mutation rate does not change much with  $n$ , whereas for the  $r > 0$  values shown in Figure 2 it is very sensitive to  $n$ .

To ascertain that the existence of a range of asymmetry permitting a stable nonzero mutation rate was not an artifact of the numerical procedure, for  $r = 0$  the  $n = 2$  and  $n = 3$  cases were repeated using a very accurate numerical derivative [complex step derivative (SQUIRE and TRAPP 1998)] and a finer array of  $s_3$  values for  $s_1 = 0.01$  and  $s_1 = 0.1$ . The  $n = 2$  results are in Figure 3 where there can be no doubt that this band of asymmetric fitnesses allowing a stable mutation rate at or very close to  $\frac{1}{2}$  really exists. We see that in Figure 3, A and B,  $\partial\lambda_1/\partial\mu$

is positive for  $\mu < \frac{1}{2}$  and negative for  $\mu > \frac{1}{2}$  when  $s_1 = s_3$  and that  $\mu$ -values near  $\frac{1}{2}$  are stable for a range of  $\mu$ -values that decreases as the asymmetry increases, with  $\mu = 0$  becoming stable when the asymmetry is too great. The width of the band of stability is a little  $< 1 \log_{10}$  fitness unit in both Figures 3 and 4. Figure 4 shows the same effect for  $n = 3$ . In Figure 4, A and B,  $\partial\lambda_1/\partial\mu_m = 0$  occurs close to  $\mu_m = \frac{1}{3}$ , but not exactly at  $\frac{1}{3}$ , which would be the case if  $1/n$  were the evolutionarily stable mutation rate.

Figures 5 and 6 offer another view of the stability/invasibility parameter ranges. Figure 5 sets  $r = 0$  while  $r = 0.3$  in Figure 6. In these figures, the solid areas have the leading eigenvalue  $< 1$ , while it is  $> 1$  in the open regions. In the first row of both figures we see first the stability of  $\mu = \frac{1}{2}$ , and as  $s_3$  increases the black area under the separating diagonal reflects the range of stable mutation rates near  $\frac{1}{2}$ , which disappears when  $s_1 = 0.01$  and  $s_1 = 0.03$ , leaving zero as the stable mutation rate. In general, we see that when  $s_3$  is different from  $s_1$ , an unstable equilibrium in  $\mu$  appears below the stable equilibrium. If the mutation changes in sufficiently small steps, then  $\mu$  can be trapped below the unstable equilibrium and evolve to 0. As long as the rate may take larger

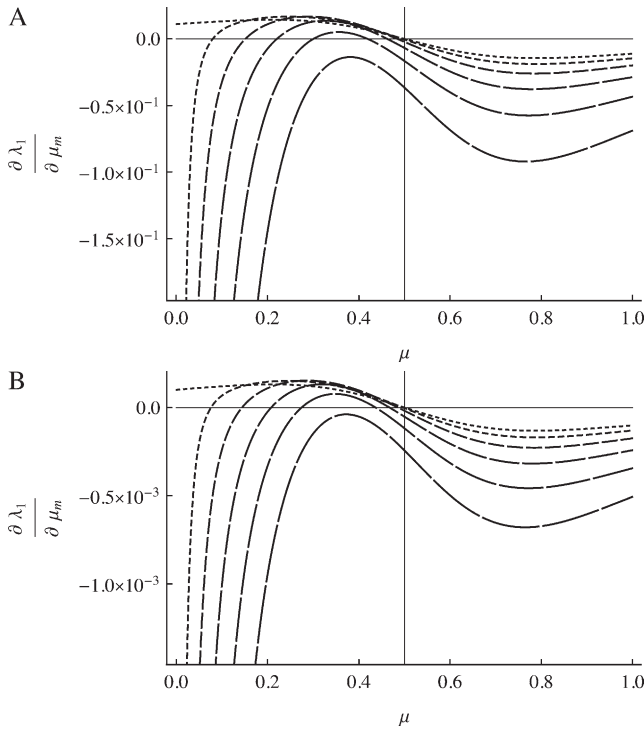


FIGURE 3.—Plot of the derivative of the leading eigenvalue,  $\lambda_1$ , with respect to  $\mu_m$  evaluated at  $\mu_M = \mu_m = \mu$  when  $n = 2$  and  $r = 0$ . (A)  $s_1 = 1 - w_1 = 10^{-1}$  and the lines from shortest dashes to longest dashes are  $s_3 = 1 - w_3 = 10^{-1}, 10^{-0.9}, 10^{-0.8}, 10^{-0.7}, 10^{-0.6}$ , and  $10^{-0.5}$ . (B)  $s_1 = 0.01$  and the lines from shortest dashes to longest dashes are  $s_3 = 10^{-2}, 10^{-1.9}, 10^{-1.8}, 10^{-1.7}, 10^{-1.6}$ , and  $10^{-1.5}$ . The vertical line is located at  $\mu = 1/n = \frac{1}{2}$ .

steps, and if the rate remains below  $\frac{1}{2}$ , it will eventually converge to the stable equilibrium. As  $s_3$  increases further, the stable and unstable equilibria annihilate one another and  $\mu$  goes to 0, if it remains below  $\frac{1}{2}$ .

With  $n$  odd, the complication alluded to after Result 5 becomes visible: the first columns of Figures 5 and 6 show the symmetric model, and the second and fourth rows are examples with  $n$  odd. With  $n = 3$  we see a stable value of the mutation rate  $< \frac{1}{2}$  and near  $\frac{1}{3}$ . In general, when  $n$  is odd, and  $r = 0$ , an unstable equilibrium value of  $\mu$  appears with  $\mu > \frac{1}{2}$ . Thus, larger values of  $\mu$  may also be invadable by even larger values, depending on the difference between the resident  $\mu_M$  and the invading  $\mu_m$ . The domains of attraction in the asymmetric cases can become very complicated; compare  $s_3 = 0.015$ ,  $n = 3$ , and  $r = 0$  with  $r = 0.3$ . Note that with  $n = 20$  and  $21$ ,  $r = 0.3$  forces stability of  $\mu = 0$  for most cases, while with  $r = 0$ ,  $s_3 = 0.015$ , and  $n = 21$ , there is a range of high mutation rates that will be invaded by even higher (although unrealistic) mutation rates. Examination of Figures 5 and 6 suggests that if both mutation rates  $\mu_M$  and  $\mu_m$  are very small, odd and even values of  $n$  give qualitatively similar results. For values of  $\mu_M$  and  $\mu_m < \frac{1}{2}$ , the patterns for  $n = 2$  and  $n = 3$  are similar for both  $r = 0$  and  $r = 0.3$ . In the unrealistic range of mutation rates  $> 0.5$ ,  $n = 2$  and  $n = 3$  show different patterns

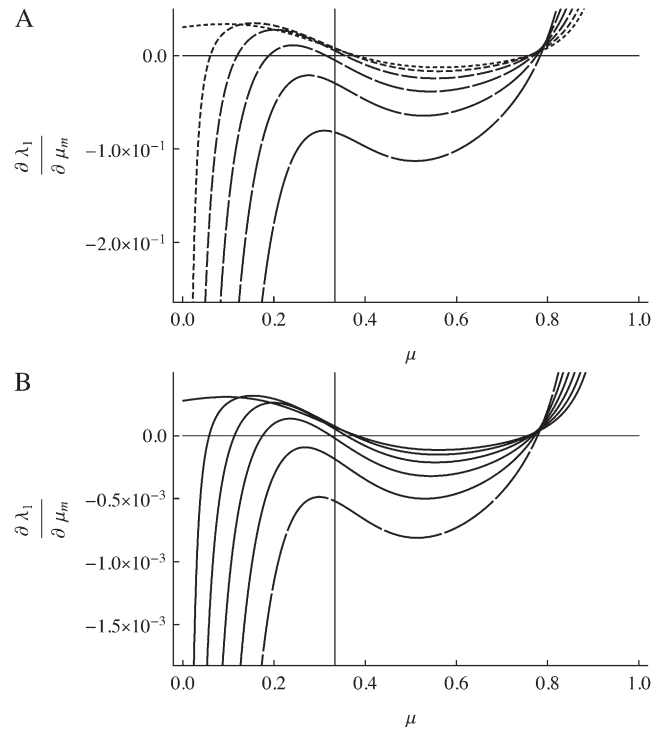


FIGURE 4.—(A and B) The same as Figure 3, except  $n = 3$ . The vertical line is located at  $\mu = 1/n = \frac{1}{3}$ .

of invasibility with  $\mu = 1$  stable for  $n = 3$  but not for  $n = 2$ .

For large values of  $s_1$  and  $s_3$  the range of asymmetry that allows a stable nonzero mutation rate increases as  $n$  increases, but for given  $n$ , it decreases as  $r$  increases. Note that for larger recombination rates and larger  $n$ , the stable mutation rates, if different from 0, are  $\ll 1/n$ . Comparing the effect of recombination when  $n$  is small with that when  $n$  is large, we have an indication from Figure 5 ( $r = 0$ ) and Figure 6 ( $r = 0.3$ ) that increasing recombination makes it more likely that the mutation rate will decrease.

SALATHÉ *et al.* (2009) showed for the symmetric selection case that if the period of the cycle was a gamma-distributed random variable, the stable switching rate dropped precipitously as the variance in the cycle period increased, compared to the case where the period was fixed at the mean of the gamma distribution. Figure 7 illustrates the interaction between recombination and variance in the cycle period. The effect of recombination on the evolutionarily stable mutation rate is striking: with  $r = 0.2$ , for example (second panel from the right in Figure 7), none of the mutation rates near  $1/n$  that are stable when the period is fixed at  $2n$  (red–pink in the leftmost panel) are stable. Moving from left to right in Figure 7, recombination is seen to strengthen the effect of randomness in the period seen by SALATHÉ *et al.* (2009, Figure 1) in shifting the stable mutation rate to substantially  $< 1/n$ . It is reasonable to expect that with asymmetries in fitness, recombination would also enhance the tendency of variance in the

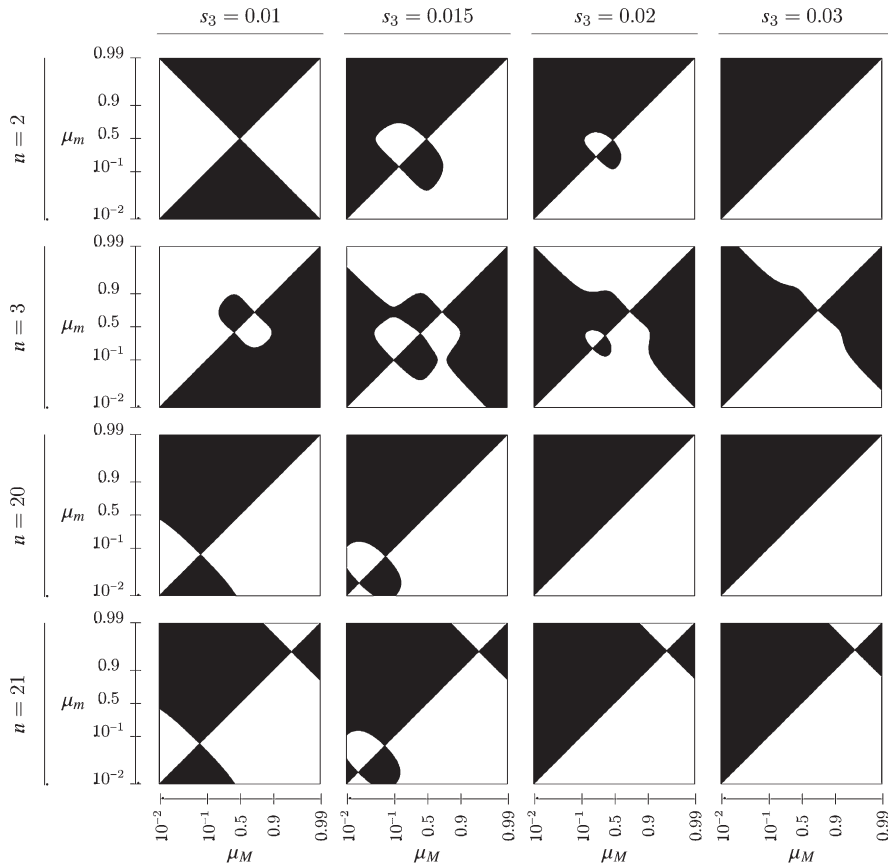


FIGURE 5.—Pairwise invasibility plots for mutation rate modifier. Combinations of resident mutation rate  $\mu_M$  and invading mutation rate  $\mu_m$  in the open regions yield a leading eigenvalue  $>1$  in absolute magnitude; the leading eigenvalue is  $<1$  in the solid regions. We assume that  $w_1 = 1 - s_1$  and  $w_3 = 1 - s_3$  and set  $s_1 = 0.01$ . Each row denotes a different value for  $n$ , the number of generations in each environment. The recombination rate is set to  $r = 0$ . Note that the  $x$ - and  $y$ -axes are logarithmically scaled from  $10^{-2}$  to  $0.5$  and from  $1 - 10^{-2}$  to  $1 - 0.5$ .

cycle period to decrease the evolutionarily stable rate of phenotypic switching.

## DISCUSSION

The contribution of epigenetic phenomena to both statistical and dynamic properties of phenotypic variation has received much recent attention. In general, the two aspects, statistical and dynamic, have been well separated. The statistical treatment by SLATKIN (2009) quantifies the contribution of epigenetic change to disease risk, where the epigenetic effect could be mutational and act on gene expression. Another recent analysis by DANCHIN and WAGNER (2010) introduces transmitted epigenetic variance as well as “culture” through transmitted social variance into calculations of heritability; addition of these effect leads them to “inclusive heritability,” which they define as all inherited components of phenotypic variance. Their framework is a special case of early work on the contributions of cultural inheritance to estimated heritability by CAVALLI-SFORZA and FELDMAN (1978) and FELDMAN and CAVALLI-SFORZA (1979) that predates the current interest in cultural inheritance as a possible cause of transgenerational epigenetics.

Such statistical treatments usually do not include recombination as a force that affects the genetic or epigenetic contributions to phenotypic variance. For ex-

ample, multilocus evolutionary simulation studies by FEINBERG and IRIZARRY (2010) of epigenetic variation were stimulated by findings of dietary modification of DNA methylation of the *Agouti* gene in mice and methylation of the *Axin*-fused allele in kinked-tail mice (COONEY *et al.* 2002; RAKYAN *et al.* 2003; WATERLAND and JIRTLE 2003). Recombination was not included in the simulations by Feinberg and Irizarry.

Studies motivated by the adjustment by microorganisms of their gene regulation to environmental change, such as those mentioned in the Introduction, ignore recombination because it is assumed to be rare in the bacteria, yeast, or cellular populations on which the models are based. As a general rule, these models take populations of cells that do not compete and compare their exponential growth dynamics under different conditions. Thus, the recent study by LEIBLER and KUSSELL (2010) focused on the historical phenotypic states of a population of nonrecombining cells, and fitness in different populations was measured as a property of these histories. Recombination would be difficult to include in such a treatment for the same reason that coalescent analysis in genetics is complicated by recombination and selection. Similarly GERLAND and HWA (2009) measure the success in populations of cells subject to mutation by the genetic load, namely the reduction below the maximum fitness possible caused by deleterious mutations in a single population. Here

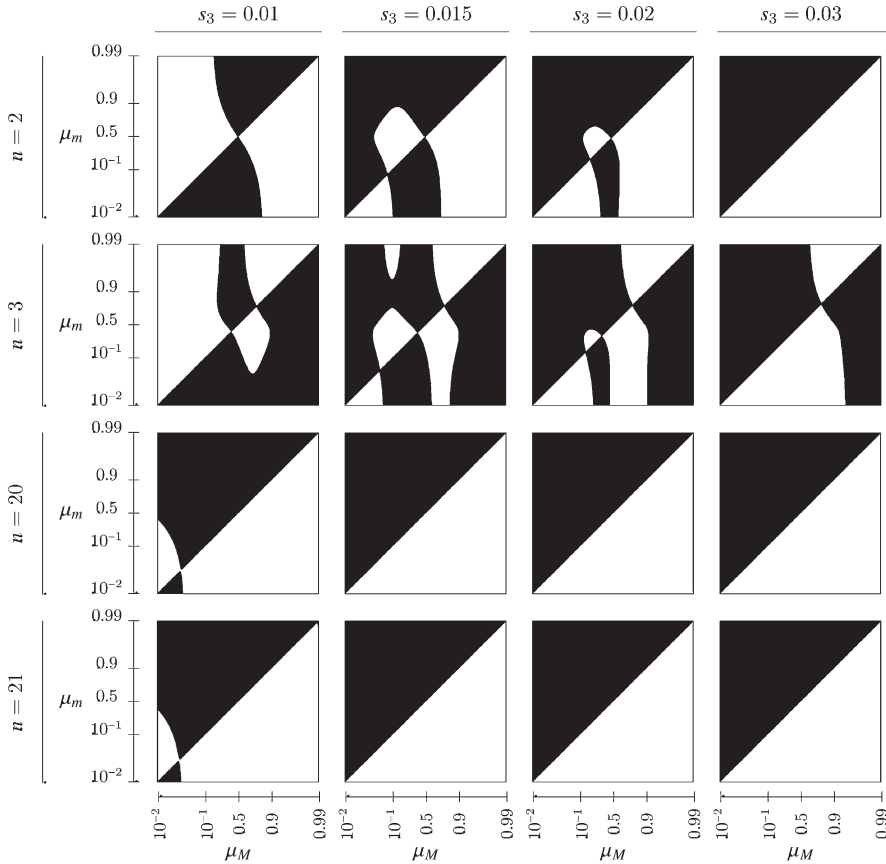


FIGURE 6.—The same as Figure 5 except the recombination rate is set to  $r = 0.3$ .

again the analysis is in terms of a single gene, and recombination does not enter into the dynamics of the different mutational states. However, LYNCH (2007, p. 89) points out that “recombination at the nucleotide level does not appear to be exceptionally low in prokaryotes when compared to that in multicellular species.”

Inclusion of recombination between the locus under selection and the locus that controls the mutation rate is important but greatly increases the difficulty of exact analysis when the selection fluctuates over time. Formally the reason for this is that at  $r = 0$  the recursion system is linear fractional, and iterates of linear fractional transformations remain linear fractional, even if the parameters are not constant over time. In particular, this guarantees that we can model the starting point of the modification process as the unique stable trajectory of the compound linear fractional system. The initial dynamics of the rare mutation-modifying allele can then be analyzed as in LIBERMAN and FELDMAN (1986). The argument makes use of the positivity of the initial increase matrix specified by  $\mathcal{L}_{\text{ex}}$  (Equations 17–19). When  $r > 0$ , the linear fractional structure is replaced by a much more complicated dynamical iteration whose properties are difficult to discern in general. With sufficiently weak selection and small values of  $|\mu_M - \mu_m|$ , however, M’GONIGLE *et al.* (2009) made considerable progress in the case where cycling was due to host–parasite interactions.

Our analysis of the symmetric cases with  $n = 1$  and  $2$ , corresponding to periods  $2$  and  $4$ , respectively, for the fitness cycle, confirms results found numerically by ISHII *et al.* (1989). They were able to study the system analytically in these cases under one of two restrictive conditions: the weak selection limit  $s \ll 1/n$  (where, in our terminology,  $s_1 = 1 - w_1$ , for example, as in Figures 1 through 7) or  $s_1$  close to  $1$  (which entails in our terminology that  $1 - w_1 \approx 0$ ). Our Results 3 and 5 and the proofs in File S1, File S2, File S3, File S4, and File S5 show analytically that  $\mu = 1$  and  $\mu = \frac{1}{2}$  are the uninvadable mutation rates for  $n = 1$  and  $n = 2$ , respectively, with no restrictions on the recombination rate or fitnesses  $w_1$  and  $w_3$ .

As pointed out by ISHII *et al.* (1989), odd values of  $n$  produce two equilibria for the mutation rate, a stable value  $< 0.5$  and an unstable one  $> 0.5$ . As  $n$  becomes larger, however, our Figures 5 and 6, with  $n = 21$  and symmetric fitnesses show that increasing  $r$  can eliminate the large unstable value of  $\mu$ ; with  $r = 0.3$ , in the first column of Figure 6, the value of  $\mu < \frac{1}{2}$  is stable from above  $\mu$ , while in Figure 5, where  $r = 0$ ,  $\mu = 1$  has a domain of stability above the unstable equilibrium.

In both symmetric cases  $n = 1$  and  $n = 2$ , we proved that the mutation rates that cannot be invaded by any other ( $\mu = 1$  for  $n = 1$  and  $\mu = \frac{1}{2}$  for  $n = 2$ ) are also the mutation rates that maximize the mean fitness  $w^*$ . Those studies (*e.g.*, ISHII *et al.* 1989; GAÁL *et al.* 2010)



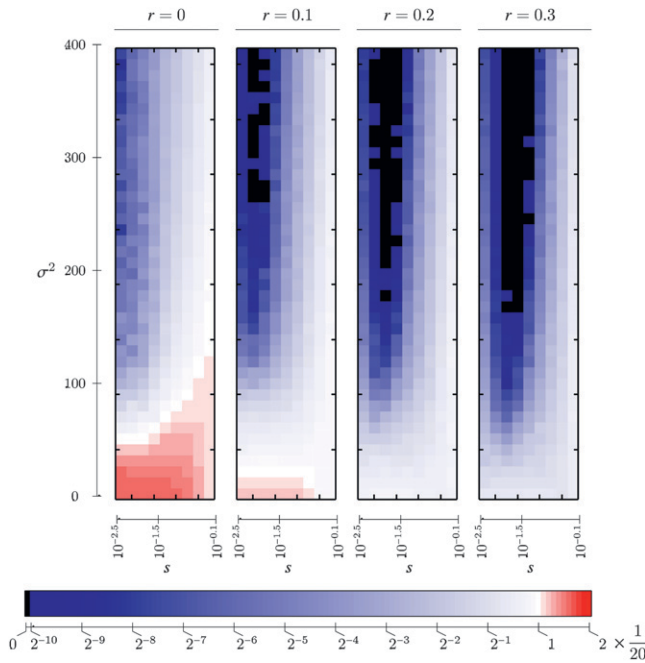


FIGURE 7.—Simulated evolutionarily stable mutation rates with  $w_1 = w_3 = 1 - s$ . The numbers of generations in each environment are drawn from a gamma distribution with mean 20 and variance  $\sigma^2$ . Each panel denotes a different value of the recombination rate given in the first row. Colors denote the stable mutation rate, as in Figure 2. Each stable rate is the average of 100 simulations; each simulation consists of iterating the recursion system (8) to determine whether a new rate  $\mu_m$  can invade an old rate  $\mu_M$ . One thousand successive new rates are sequentially assayed for whether they invade; the new rate invades and replaces the old rate if it increases by 1.001 over its initial frequency after 200,000 generations. More on the simulation methods can be found in SALATHÉ *et al.* (2009).

that argue in terms of long-term growth rates in exponentially growing populations find that this is true for any  $n$ . Our Result 5 provides an elegant resolution of the difference, with symmetric or asymmetric fitnesses and  $r = 0$ : the value of  $\mu$  where  $\partial\lambda/\partial\mu$  vanishes, namely the value that gives “eigenvalue stability,” is the same value that maximizes the logarithm of the long-term mean fitness, namely  $(w^*)^{-1} \cdot (\partial w^*/\partial\mu_M)$ . Numerical analysis confirms that  $(w^*)^{-1}$  is indeed the factor that relates these derivatives, and the proof is given in the APPENDIX.

Our analysis of symmetric selection shows some degree of robustness. From Figure 2 (and also Figures 3 and 4), there is a band of mildly asymmetric fitnesses surrounding the symmetric selection values  $s_1 = 1 - w_1 = s_3 = 1 - w_3$  in which the stable mutation rate is very close indeed to that obtained under symmetric fitnesses. For large values of both  $s_1$  and  $s_3$  this band becomes wider as  $n$  increases for all recombination values (top right of each panel in Figure 2). For weaker asymmetric selection this band narrows substantially, and the stable mutation rate becomes smaller as the recombination rate increases. Thus, as  $r$  increases, a greater range of

fitness asymmetries leads to a stable mutation rate of zero, except for  $n = 2$ . Even for  $n = 3$  in the symmetric case, however, Figure 4 shows clear distance between the stable values of  $\mu$  and the value  $\frac{1}{3}$  predicted, *e.g.*, by ISHII *et al.* (1989) and LACHMANN and JABLONKA (1996). At  $n = 100$ , in Figure 2, the stable  $\mu$  in the symmetric case is much closer to  $\frac{2}{100}$  than to  $\frac{1}{100}$ . As  $r$  increases, the stable value of  $\mu$  in the symmetric case rapidly decreases below  $1/n$ , and the band of stable nonzero mutation rates disappears. These results suggest that the optimal rate of phenotypic switching depends as much on the strength of selection in each environment as it does on the frequency of environmental changes, represented here by  $n$ ; KUSSELL *et al.* (2005) claimed that  $n$  was much more important than the magnitudes of  $s_1$  and  $s_3$  in determining the uninvadable  $\mu$ . At least for the values of  $n$  we tried, Figures 2, 5, and 6 show that  $s_1$  and  $s_3$  are indeed important.

Our earlier numerical study (SALATHÉ *et al.* 2009) showed that in asymmetric fitness landscapes ( $w_1 \neq w_3$ ), stable nonzero mutation rates required very strong selection in both environments (*i.e.*, large  $s_1$  and  $s_3$ ). Our Figures 5 and 6 show an interesting feature of the dynamical system that distinguishes odd and even values of  $n$ . The numerical analysis illustrates that there may be more than one solution to  $\partial\lambda/\partial\mu_m|_{\mu_m=\mu_M} = 0$  and that the direction of selection on  $\mu_m$  might depend delicately on the sign and magnitude of  $(\mu_M - \mu_m)$ . Why this is more likely to happen when  $n$  is odd than when  $n$  is even remains an interesting mathematical question. In both Figures 5 and 6, *i.e.*, recombination  $r = 0.0$  and  $0.3$ , for  $n = 3$ , there are three critical values of  $\mu$  when  $s_1 = 0.01$  and  $s_3 = 0.015$  and  $0.02$ ; one value is  $>0.5$  and unstable, and two values are  $<0.5$ , with the greater of these locally stable, while the smaller one is locally unstable. The same pattern is exhibited for  $n = 21$  with  $r = 0$  (Figure 5), but not for  $r = 0.3$  (Figure 6). Figures 2 and 7 suggest that increasing recombination makes it less likely that a stable nonzero mutation rate will exist with either a fixed or a random switching period (see also M’GONIGLE *et al.* 2009). This suggests that switching in sexual organisms is less likely than in asexuals, and that if it occurs by genetic regulation of gene expression, the regulators should be linked to the genes they regulate. The role of recombination becomes more important as the period becomes longer, especially in symmetric or weakly asymmetric selection regimes.

The authors thank Freddy Christiansen for his careful reading of an earlier draft and two anonymous referees for incisive and useful comments. This research was supported in part by National Institutes of Health grant GM 28016.

LITERATURE CITED

ACAR, M., J. T. METTETAL and A. VAN OUDENAARDEN, 2008 Stochastic switching as a survival strategy in fluctuating environments. *Nat. Genet.* **40**: 471–475.

- BALABAN, N. Q., J. MERRIN, R. CHAIT, L. KOWALIK and S. LEIBLER, 2004 Bacterial persistence as a phenotypic switch. *Science* **305**: 1622–1625.
- BERGMAN, A., and M. W. FELDMAN, 1990 More on selection for and against recombination. *Theor. Popul. Biol.* **38**: 68–92.
- BONDURIANSKY, R., and T. DAY, 2009 Nongenetic inheritance and its evolutionary implications. *Annu. Rev. Ecol. Syst.* **40**: 103–125.
- CAVALLI-SFORZA, L. L., and M. W. FELDMAN, 1978 The evolution of continuous variation. III. Joint transmission of genotype, phenotype and environment. *Genetics* **90**: 391–425.
- CHARLESWORTH, B., 1976 Recombination modification in a fluctuating environment. *Genetics* **83**: 181–195.
- COONEY, C. A., A. A. DAVE and G. L. WOLFF, 2002 Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J. Nutr.* **132**(Suppl. 8): 2393S–2400S.
- DANCHIN, E., and R. H. WAGNER, 2010 Inclusive heritability: combining genetic and nongenetic information to study animal culture. *Oikos* **119**: 210–218.
- FEINBERG, A. P., and R. A. IRIZARRY, 2010 Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc. Natl. Acad. Sci. USA* **107**(Suppl. 1): 1757–1764.
- FELDMAN, M. W., 1972 Selection for linkage modification: I. Random mating populations. *Theor. Popul. Biol.* **3**: 324–346.
- FELDMAN, M. W., and L. L. CAVALLI-SFORZA, 1979 Aspects of variance and covariance analysis with cultural inheritance. *Theor. Popul. Biol.* **15**: 276–307.
- FELDMAN, M. W., and U. LIBERMAN, 1986 An evolutionary reduction principle for genetic modifiers. *Proc. Natl. Acad. Sci. USA* **83**: 4824–4827.
- FELDMAN, M. W., F. B. CHRISTIANSEN and L. D. BROOKS, 1980 Evolution of recombination in a constant environment. *Proc. Natl. Acad. Sci. USA* **77**: 4838–4841.
- FELDMAN, M. W., S. P. OTTO and F. B. CHRISTIANSEN, 1997 Population genetic perspectives on the evolution of recombination. *Annu. Rev. Genet.* **30**: 261–295.
- GAÁL, B., J. W. PITCHFORD and A. J. WOOD, 2010 Exact results for the evolution of stochastic switching in variable asymmetric environments. *Genetics* **184**: 1113–1119.
- GANDON, S., and S. P. OTTO, 2007 The evolution of sex and recombination in response to abiotic or coevolutionary fluctuations in epistasis. *Genetics* **175**: 1835–1853.
- GERLAND, U., and T. HWA, 2009 Evolutionary selection between alternative modes of gene regulation. *Proc. Natl. Acad. Sci. USA* **106**: 8841–8846.
- HAMILTON, W. D., 1980 Sex versus non-sex versus parasite. *Oikos* **35**: 282–290.
- HARAGUCHI, Y., and A. SASAKI, 1996 Host-parasite arms race in mutation modifications: indefinite escalation despite a heavy load? *J. Theor. Biol.* **183**: 121–137.
- HENDERSON, I. R., and S. E. JACOBSEN, 2007 Epigenetic inheritance in plants. *Nature* **447**: 418–424.
- ISHII, K., H. MATSUDA, Y. IWASA and A. SASAKI, 1989 Evolutionary stable mutation rate in a periodically changing environment. *Genetics* **121**: 163–174.
- KARLIN, S., and J. L. MCGREGOR, 1972 The evolutionary development of modifier genes. *Proc. Natl. Acad. Sci. USA* **69**: 3611–3614.
- KARLIN, S., and J. L. MCGREGOR, 1974 Towards a theory of the evolution of modifier genes. *Theor. Popul. Biol.* **5**: 59–105.
- KUSSELL, E., and S. LEIBLER, 2005 Phenotypic diversity, population growth, and information in fluctuating environments. *Science* **309**: 2075–2078.
- KUSSELL, E., R. KISHONY and N. Q. BALABAN, 2005 Bacterial persistence: a model of survival in changing environments. *Genetics* **169**: 1807–1814.
- LACHMANN, M., and E. JABLONKA, 1996 The inheritance of phenotypes: an adaptation to fluctuating environments. *J. Theor. Biol.* **181**: 1–9.
- LEIBLER, S., and E. KUSSELL, 2010 Individual histories and selection in heterogeneous populations. *Proc. Natl. Acad. Sci. USA* **107**: 13183–13188.
- LIBERMAN, U., and M. W. FELDMAN, 1986 Modifiers of mutation rate: a general reduction principle. *Theor. Popul. Biol.* **30**: 125–142.
- LIM, H. N., and A. VAN OUDENAARDEN, 2007 A multistep epigenetic switch enables the stable inheritance of DNA methylation states. *Nat. Genet.* **39**: 269–275.
- LYNCH, M., 2007 *The Origins of Genome Architecture*. Sinauer Associates, Sunderland, MA.
- MAYNARD SMITH, J., 1978 *The Evolution of Sex*. Cambridge University Press, Cambridge, UK.
- MAYNARD SMITH, J., 1980 Selection for recombination in a polygenic model. *Genet. Res.* **35**: 269–277.
- MAYNARD SMITH, J., 1988 Selection for recombination in a polygenic model—the mechanism. *Genet. Res.* **51**: 59–63.
- M'GONIGLE, L. K., J. J. SHEN and S. P. OTTO, 2009 Mutating away from your enemies: the evolution of mutation rate in a host-parasite system. *Theor. Popul. Biol.* **75**: 301–311.
- NEE, S., 1989 Antagonistic co-evolution and evolution of genotypic randomization. *J. Theor. Biol.* **140**: 499–518.
- RARYAN, V. K., S. CHONG, M. E. CHAMP, P. C. CUTHBERT, H. D. MORGAN *et al.*, 2003 Transgenerational inheritance of epigenetic states at the murine *Axin<sup>fl</sup>* allele occurs after maternal and paternal transmission. *Proc. Natl. Acad. Sci. USA* **100**: 2538–2543.
- SALATHÉ, M., J. VAN CLEVE and M. W. FELDMAN, 2009 Evolution of stochastic switching rates in asymmetric fitness landscapes. *Genetics* **182**: 1159–1164.
- SLATKIN, M., 2009 Epigenetic inheritance and the missing heritability problem. *Genetics* **182**: 845–850.
- SQUIRE, W., and G. TRAPP, 1998 Using complex variables to estimate derivatives of real functions. *SIAM Rev.* **40**: 110–112.
- THATTAI, M., and A. VAN OUDENAARDEN, 2004 Stochastic gene expression in fluctuating environments. *Genetics* **167**: 523–530.
- WATERLAND, R. A., and R. I. JIRTLE, 2003 Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Biol. Evol.* **23**: 5293–5300.
- YOUNGSON, N. A., and E. WHITELOW, 2008 Transgenerational epigenetic effects. *Annu. Rev. Genet. Hum. Genet.* **9**: 233–257.

Communicating editor: J. WAKELEY

APPENDIX

Proof that  $\partial w^*/\partial \mu_M = 0$  if and only if  $\partial \lambda / \partial \mu_m |_{\mu_m = \mu_M} = 0$ .

From (45) we have at  $\lambda = 1$ ,

$$2 \frac{\partial \lambda}{\partial \mu_m} (w^*)^2 - \frac{\partial \lambda}{\partial \mu_m} w^* (\tilde{A} + \tilde{D}) - w^* \frac{\partial (\tilde{A} + \tilde{D})}{\partial \mu_m} + \frac{\partial (\tilde{A}\tilde{D} - \tilde{B}\tilde{C})}{\partial \mu_m} = 0, \tag{A1}$$

which we can reorganize at  $\mu_m = \mu_M$  as

$$w^* \left[ 2 \frac{\partial \lambda}{\partial \mu_m} w^* - \frac{\partial \lambda}{\partial \mu_m} (\tilde{A} + \tilde{D}) \right] \Big|_{\mu_m = \mu_M} = w^* \frac{\partial (\tilde{A} + \tilde{D})}{\partial \mu_m} \Big|_{\mu_m = \mu_M} - \frac{\partial (\tilde{A}\tilde{D} - \tilde{B}\tilde{C})}{\partial \mu_m} \Big|_{\mu_m = \mu_M}, \tag{A2}$$

or

$$\frac{\partial \lambda}{\partial \mu_m} \Big|_{\mu_m = \mu_M} = \frac{w^* (\partial (\tilde{A} + \tilde{D}) / \partial \mu_m) - \partial (\tilde{A}\tilde{D} - \tilde{B}\tilde{C}) / \partial \mu_m}{w^* [2w^* - (\tilde{A} + \tilde{D})]} \Big|_{\mu_m = \mu_M}. \tag{A3}$$

But, from Equation 47,

$$\frac{\partial w^*}{\partial \mu_M} = \frac{w^* (\partial (\hat{A} + \hat{D}) / \partial \mu_M) - \partial (\hat{A}\hat{D} - \hat{B}\hat{C}) / \partial \mu_M}{2w^* - (\hat{A} + \hat{D})}. \tag{A4}$$

Since  $\tilde{A}, \tilde{B}, \tilde{C}, \tilde{D}$  are exactly the same as  $\hat{A}, \hat{B}, \hat{C}, \hat{D}$  with  $\mu_m$  replacing  $\mu_M$ , we conclude that

$$\frac{\partial \lambda}{\partial \mu_m} \Big|_{\mu_m = \mu_M} = \frac{\partial w^* / \partial \mu_M}{w^*} = \frac{\partial \log w^*}{\partial \mu_M}. \tag{A5}$$

That is,  $\partial \lambda / \partial \mu_m |_{\mu_m = \mu_M}$  and  $\partial w^* / \partial \mu_M$  vanish together.

# GENETICS

## Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.110.123620/DC1>

### **On the Evolution of Mutation in Changing Environments: Recombination and Phenotypic Switching**

**Uri Liberman, Jeremy Van Cleve and Marcus W. Feldman**

Copyright © 2011 by the Genetics Society of America  
DOI: 10.1534/genetics.110.123620



**FILE S1**

**Part I: Some general properties of the transformation  $\mathcal{L}$**

We can gain some insight into the eigenvalues of  $\mathcal{L}$  by the following observations.

*Observation I*

$$(i) \quad \det(\mathcal{L}_i^{\text{ex}}) = \frac{(1 - 2\mu_m)(1 - r)w_1^i w_3^i}{(w^i)^2} \quad (\text{A1})$$

$$(ii) \quad \det(\mathcal{L}_{\text{ex}}) = \frac{(1 - 2\mu_m)^k (1 - r)^k \prod_{i=1}^k w_1^i w_3^i}{w^2}. \quad (\text{A2})$$

The computation of  $\det(\mathcal{L}_i^{\text{ex}})$  is straightforward and follows from the fact that  $\mathcal{L}_{\text{ex}} = \mathcal{L}_k \cdot \mathcal{L}_{k-1} \cdot \dots \cdot \mathcal{L}_1$ .

Clearly when  $\mu_m = \frac{1}{2}$ , one of the eigenvalues of  $\mathcal{L}_{\text{ex}}$  is zero. Also, if  $0 \leq r < 1$  and  $0 \leq \mu_m < \frac{1}{2}$ , the two eigenvalues are positive for all  $k$ , whereas when  $\frac{1}{2} < \mu_m \leq 1$  the two eigenvalues are positive for  $k$  even and when  $k$  is odd, one eigenvalue is positive, and the other negative.

*Observation II.*

When  $r = 1$ , the eigenvalues of each of the matrices  $\mathcal{L}_i^{\text{ex}}$  and  $\mathcal{L}_{\text{ex}}$  are zero and one.

In fact, when  $r = 1$  the matrix  $\mathcal{L}_i^{\text{ex}}$  has the form

$$\mathcal{L}_i^{\text{ex}} = \frac{1}{a_i + b_i} \begin{bmatrix} a_i & a_i \\ b_i & b_i \end{bmatrix}, \quad (\text{A3})$$

with

$$\begin{aligned} a_i &= (1 - \mu_m)w_1^i y_1^i + \mu_m w_3^i y_3^i \\ b_i &= \mu_m w_1^i y_1^i + (1 - \mu_m)w_3^i y_3^i \\ a_i + b_i &= w_1^i y_1^i + w_3^i y_3^i = w^i. \end{aligned} \quad (\text{A4})$$

Thus the two eigenvalues of  $\mathcal{L}_i^{\text{ex}}$  are zero and one. Moreover it is easily seen that

$$\mathcal{L}_i^{\text{ex}} \cdot \mathcal{L}_j^{\text{ex}} = \frac{1}{a_i + b_i} \begin{bmatrix} a_i & a_i \\ b_i & b_i \end{bmatrix} = \mathcal{L}_i^{\text{ex}}. \quad (\text{A5})$$

Hence  $\mathcal{L}_{\text{ex}} = \mathcal{L}_k^{\text{ex}} \cdot \mathcal{L}_{k-1}^{\text{ex}} \cdot \dots \cdot \mathcal{L}_1^{\text{ex}}$  also has the form (A3), and so its eigenvalues are zero and one when  $r = 1$ .

**Part II: Proof of Equation (29)**

Let  $\hat{x} = (\hat{x}_1, 0, \hat{x}_3, 0)$  be such that  $\hat{x} = T_1(x^*)$ , namely

$$\begin{aligned} w\hat{x}_1 &= (1 - \mu_M)w_1x_1^* + \mu_M w_3x_3^* \\ w\hat{x}_3 &= (1 - \mu_M)w_3x_3^* + \mu_M w_1x_1^* \end{aligned} \quad (\text{A6})$$

with

$$w = w_1x_1^* + w_3x_3^*. \quad (\text{A7})$$

Also let

$$\hat{w} = \hat{w}_1\hat{x}_1 + \hat{w}_3\hat{x}_3. \quad (\text{A8})$$

Then the external stability of  $x^*$  is determined by the largest positive eigenvalue of the matrix product  $\mathcal{L}_{\text{ex}} = \hat{\mathcal{L}} \cdot \mathcal{L}$  where

$$w\mathcal{L} = \begin{bmatrix} (1 - \mu_m)w_1 - r[(1 - \mu_m)w_1 - \mu_m w_3]x_3^* & \mu_m w_3 + r[(1 - \mu_m)w_1 - \mu_m w_3]x_1^* \\ \mu_m w_1 + r[(1 - \mu_m)w_3 - \mu_m w_1]x_3^* & (1 - \mu_m)w_3 - r[(1 - \mu_m)w_3 - \mu_m w_1]x_1^* \end{bmatrix}, \quad (\text{A9})$$

and

$$\hat{w}\hat{\mathcal{L}} = \begin{bmatrix} (1 - \mu_m)\hat{w}_1 - r[(1 - \mu_m)\hat{w}_1 - \mu_m \hat{w}_3]\hat{x}_3 & \mu_m \hat{w}_3 + r[(1 - \mu_m)\hat{w}_1 - \mu_m \hat{w}_3]\hat{x}_1 \\ \mu_m \hat{w}_1 + r[(1 - \mu_m)\hat{w}_3 - \mu_m \hat{w}_1]\hat{x}_3 & (1 - \mu_m)\hat{w}_3 - r[(1 - \mu_m)\hat{w}_3 - \mu_m \hat{w}_1]\hat{x}_1 \end{bmatrix}. \quad (\text{A10})$$

At equilibrium, we write  $w\hat{w} = w^*$ , and following (26) we have

$$w^* = \hat{w}_1w_1x_1^* + \hat{w}_3w_3x_3^* + \mu_M(\hat{w}_3 - \hat{w}_1)(w_1x_1^* - w_3x_3^*). \quad (\text{A11})$$

We can verify that

$$\det(\mathcal{L}_{\text{ex}}) = \frac{(1 - 2\mu_m)^2(1 - r)^2w_1\hat{w}_1w_3\hat{w}_3}{(w^*)^2}. \quad (\text{A12})$$

Since  $\mathcal{L}_{\text{ex}}$  is a positive matrix, it has a positive eigenvalue, and (A12) ensures that  $\det(\mathcal{L}_{\text{ex}})$  is positive when  $\mu_m \neq \frac{1}{2}$  and  $0 \leq r < 1$ . If  $\mu_m = \frac{1}{2}$  or  $r = 1$ ,  $\mathcal{L}_{\text{ex}}$  has a zero eigenvalue. Thus for all  $\mu_m \neq \frac{1}{2}$ ,  $\mathcal{L}_{\text{ex}}$  has one positive eigenvalue (the larger in magnitude) and another eigenvalue which is non-negative. The two eigenvalues are the roots of the characteristic polynomial  $C(z) = \det(\mathcal{L}_{\text{ex}} - z\mathcal{I})$ , or equivalently the roots of  $M(z) = \det(w^*\mathcal{L}_{\text{ex}} - w^*z\mathcal{I})$ .

The larger eigenvalue of  $\mathcal{L}_{\text{ex}}$  is less than one if  $M(1) > 0$  and  $M'(1) > 0$ , and greater than one when  $M(1) < 0$ .  $M(1)$  has the useful representation

$$M(1) = \frac{(\mu_m - \mu_M)(1 - r)}{x_1^*} \cdot \Delta, \quad (\text{A13})$$

where  $\Delta = \Delta(r, \mu_m) = d_{11}d_{22} - d_{12}d_{21}$  and  $\Delta(r, \mu_m)$  is a bilinear function of  $r$  and  $\mu_m$  in  $0 \leq r \leq 1, 0 \leq \mu_m \leq 1$ .

We begin by representing  $w\mathcal{L}$  and  $\hat{w}\hat{\mathcal{L}}$  as follows:

$$w\mathcal{L} = \begin{bmatrix} (1 - \mu_m)w_1 & \mu_m w_3 \\ \mu_m w_1 & (1 - \mu_m)w_3 \end{bmatrix} - r \begin{bmatrix} \alpha x_3^* & -\alpha x_1^* \\ -\beta x_3^* & \beta x_1^* \end{bmatrix} \quad (\text{A14})$$

$$\hat{w}\hat{\mathcal{L}} = \begin{bmatrix} (1 - \mu_m)\hat{w}_1 & \mu_m \hat{w}_3 \\ \mu_m \hat{w}_1 & (1 - \mu_m)\hat{w}_3 \end{bmatrix} - r \begin{bmatrix} \hat{\alpha} \hat{x}_3 & -\hat{\alpha} \hat{x}_1 \\ -\hat{\beta} \hat{x}_3 & \hat{\beta} \hat{x}_1 \end{bmatrix}, \quad (\text{A15})$$

where

$$\begin{aligned} \alpha &= w_1 - \mu_m(w_1 + w_3) & \beta &= w_3 - \mu_m(w_1 + w_3) \\ \hat{\alpha} &= \hat{w}_1 - \mu_m(\hat{w}_1 + \hat{w}_3) & \hat{\beta} &= \hat{w}_3 - \mu_m(\hat{w}_1 + \hat{w}_3). \end{aligned} \quad (\text{A16})$$

We can now compute  $w^*\mathcal{L}_{\text{ex}} = \hat{w}\hat{\mathcal{L}} \cdot w\mathcal{L}$  (using  $w^* = w\hat{w}$ ) as follows:

$$\begin{aligned} w^*\mathcal{L}_{\text{ex}} &= \begin{bmatrix} (1 - \mu_m)^2 w_1 \hat{w}_1 + \mu_m^2 w_1 \hat{w}_3 & \mu_m (1 - \mu_m) w_3 (\hat{w}_1 + \hat{w}_3) \\ \mu_m (1 - \mu_m) w_1 (\hat{w}_1 + \hat{w}_3) & (1 - \mu_m)^2 w_3 \hat{w}_3 + \mu_m^2 w_3 \hat{w}_1 \end{bmatrix} \\ &- r \begin{bmatrix} x_3^* [(1 - \mu_m)\hat{w}_1 \alpha - \mu_m \hat{w}_3 \beta] & -x_1^* [(1 - \mu_m)\hat{w}_1 \alpha - \mu_m \hat{w}_3 \beta] \\ -x_3^* [(1 - \mu_m)\hat{w}_3 \beta - \mu_m \hat{w}_1 \alpha] & x_1^* [(1 - \mu_m)\hat{w}_3 \beta - \mu_m \hat{w}_1 \alpha] \end{bmatrix} \\ &- r \begin{bmatrix} \hat{\alpha} w_1 (\hat{x}_3 - \mu_m) & -\hat{\alpha} w_3 (\hat{x}_1 - \mu_m) \\ -\hat{\beta} w_1 (\hat{x}_3 - \mu_m) & \hat{\beta} w_3 (\hat{x}_1 - \mu_m) \end{bmatrix} \\ &+ r^2 (\alpha \hat{x}_3 + \beta \hat{x}_1) \begin{bmatrix} \hat{\alpha} x_3^* & -\hat{\alpha} x_1^* \\ -\hat{\beta} x_3^* & \hat{\beta} x_1^* \end{bmatrix}. \end{aligned} \quad (\text{A17})$$

Let  $M(1) = \det(w^*\mathcal{L}_{\text{ex}} - w^*\mathcal{I})$ . Then in order to compute  $M(1)$  we multiply the first column of  $M(1)$  by  $x_1^*$ , the second column by  $x_3^*$  to get  $M(1) = \frac{1}{x_1^* x_3^*} \det(B)$  where  $B$  is the following matrix:

$$\begin{aligned} B &= \begin{bmatrix} [(1 - \mu_m)^2 w_1 \hat{w}_1 + \mu_m^2 w_1 \hat{w}_3] x_1^* - w^* x_1^* & \mu_m (1 - \mu_m) w_3 (\hat{w}_1 + \hat{w}_3) x_3^* \\ \mu_m (1 - \mu_m) w_1 (\hat{w}_1 + \hat{w}_3) x_1^* & [(1 - \mu_m)^2 w_3 \hat{w}_3 + \mu_m^2 w_3 \hat{w}_1] x_3^* - w^* x_3^* \end{bmatrix} \\ &- r \begin{bmatrix} x_1^* x_3^* [(1 - \mu_m)\hat{w}_1 \alpha - \mu_m \hat{w}_3 \beta] & -x_1^* x_3^* [(1 - \mu_m)\hat{w}_1 \alpha - \mu_m \hat{w}_3 \beta] \\ -x_1^* x_3^* [(1 - \mu_m)\hat{w}_3 \beta - \mu_m \hat{w}_1 \alpha] & x_1^* x_3^* [(1 - \mu_m)\hat{w}_3 \beta - \mu_m \hat{w}_1 \alpha] \end{bmatrix} \\ &- r \begin{bmatrix} \hat{\alpha} w_1 x_1^* (\hat{x}_3 - \mu_m) & -\hat{\alpha} w_3 x_3^* (\hat{x}_1 - \mu_m) \\ -\hat{\beta} w_1 x_1^* (\hat{x}_3 - \mu_m) & \hat{\beta} w_3 x_3^* (\hat{x}_1 - \mu_m) \end{bmatrix} \\ &+ r^2 (\alpha \hat{x}_3 + \beta \hat{x}_1) \begin{bmatrix} \hat{\alpha} x_3^* x_1^* & -\hat{\alpha} x_3^* x_1^* \\ -\hat{\beta} x_3^* x_1^* & \hat{\beta} x_3^* x_1^* \end{bmatrix}. \end{aligned} \quad (\text{A18})$$

We add the second column to the first and use the equilibrium equations for  $w^*x_1^*$  and  $w^*x_3^*$  from (25). The first column has a “constant” term, an “ $r$ ” term and an “ $r^2$ ” term. As we are adding the second column to the first, it is clear that the “ $r^2$ ” term vanishes and the “ $r$ ” term comes only from the second “ $r$ ” matrix in (A18). Using the expression (A11) for  $w^*$ , the “constant” term in the first column, first row position is

$$\begin{aligned} & [(1 - \mu_m)^2 w_1 \hat{w}_1 + \mu_m^2 w_1 \hat{w}_3] x_1^* + \mu_m (1 - \mu_m) w_3 (\hat{w}_1 + \hat{w}_3) x_3^* \\ & - [(1 - \mu_M)^2 w_1 \hat{w}_1 + \mu_M^2 w_1 \hat{w}_3] x_1^* + \mu_M (1 - \mu_M) w_3 (\hat{w}_1 + \hat{w}_3) x_3^*. \end{aligned} \quad (\text{A19})$$

Noting that

$$\begin{aligned} (1 - \mu_m)^2 - (1 - \mu_M)^2 &= (\mu_M - \mu_m)(2 - \mu_M - \mu_m), \\ \mu_m^2 - \mu_M^2 &= (\mu_m - \mu_M)(\mu_M + \mu_m), \\ \mu_m(1 - \mu_m) - \mu_M(1 - \mu_M) &= (\mu_m - \mu_M)(1 - \mu_M - \mu_m), \end{aligned} \quad (\text{A20})$$

(A19) becomes

$$(\mu_m - \mu_M) [-(2 - \mu_M - \mu_m) w_1 \hat{w}_1 x_1^* + (\mu_M + \mu_m) w_1 \hat{w}_3 x_1^* + (1 - \mu_M - \mu_m) w_3 (\hat{w}_1 + \hat{w}_3) x_3^*]. \quad (\text{A21})$$

Similarly the “constant” term in the first column and second row is

$$(\mu_m - \mu_M) [-(2 - \mu_M - \mu_m) w_3 \hat{w}_3 x_3^* + (\mu_M + \mu_m) w_3 \hat{w}_1 x_3^* + (1 - \mu_M - \mu_m) w_1 (\hat{w}_1 + \hat{w}_3) x_1^*]. \quad (\text{A22})$$

The “ $r$ ” terms are multiplied by  $(-r)$  and in the first row are

$$\hat{\alpha} x_1 x_1^* (\hat{x}_3 - \mu_m) - \hat{\alpha} w_3 x_3^* (\hat{x}_1 - \mu_m), \quad (\text{A23})$$

and in the second row

$$-\hat{\beta} w_1 x_1^* (\hat{x}_3 - \mu_m) + \hat{\beta} w_3 x_3^* (\hat{x}_1 - \mu_m). \quad (\text{A24})$$

Now using equations (A6) for  $\hat{x}_1$  and  $\hat{x}_3$ , we obtain

$$\begin{aligned} w_1 x_1^* (\hat{x}_3 - \mu_m) - w_3 x_3^* (\hat{x}_1 - \mu_m) &= w_1 x_1^* \frac{(1 - \mu_M) w_3 x_3^* + \mu_M w_1 x_1^*}{w_1 x_1^* + w_3 x_3^*} \\ &\quad - w_3 x_3^* \frac{(1 - \mu_M) w_1 x_1^* + \mu_M w_3 x_3^*}{w_1 x_1^* + w_3 x_3^*} - \mu_m (w_1 x_1^* - w_3 x_3^*) \\ &= \mu_M \frac{(w_1 x_1^*)^2 - (w_3 x_3^*)^2}{w_1 x_1^* + w_3 x_3^*} - \mu_m (w_1 x_1^* - w_3 x_3^*). \end{aligned} \quad (\text{A25})$$

We conclude that

$$w_1 x_1^* (\hat{x}_3 - \mu_m) - w_3 x_3^* (\hat{x}_1 - \mu_m) = (\mu_M - \mu_m) (w_1 x_1^* - w_3 x_3^*), \quad (\text{A26})$$



so (A23) becomes

$$\hat{\alpha}(\mu_M - \mu_m)(w_1x_1^* - w_3x_3^*). \quad (\text{A27})$$

Similarly (A24) reduces to

$$-\hat{\beta}(\mu_M - \mu_m)(w_1x_1^* - w_3x_3^*). \quad (\text{A28})$$

Hence we have a common factor of  $(\mu_m - \mu_M)$  in the first column, and we can write

$$M(1) = \frac{(\mu_m - \mu_M)}{x_1^*x_3^*} \det(A), \quad (\text{A29})$$

where  $\det(A) = a_{11}a_{22} - a_{12}a_{21}$ , with

$$\begin{aligned} a_{11} &= -(2 - \mu_M - \mu_m)w_1\hat{w}_1x_1^* + (\mu_M + \mu_m)w_1\hat{w}_3x_1^* \\ &\quad + (1 - \mu_M - \mu_m)w_3(\hat{w}_1 + \hat{w}_3)x_3^* - r\hat{\alpha}(w_3x_3^* - w_1x_1^*) \\ a_{12} &= \mu_m(1 - \mu_m)w_3(\hat{w}_1 + \hat{w}_3)x_3^* + rx_1^*x_3^*[(1 - \mu_m)\hat{w}_1\alpha - \mu_m\hat{w}_3\beta] \\ &\quad + r\hat{\alpha}w_3x_3^*(\hat{x}_1 - \mu_m) - r^2(\alpha\hat{x}_3 + \beta\hat{x}_1)\hat{\alpha}x_1^*x_3^* \\ a_{21} &= -(2 - \mu_M - \mu_m)w_3\hat{w}_3x_3^* + (\mu_M + \mu_m)w_3\hat{w}_1x_3^* \\ &\quad + (1 - \mu_M - \mu_m)w_1(\hat{w}_1 + \hat{w}_3)x_1^* + r\hat{\beta}(w_3x_3^* - w_1x_1^*) \\ a_{22} &= [(1 - \mu_m)^2w_3\hat{w}_3 + \mu_m^2w_3\hat{w}_1]x_3^* - w^*x_3^* - rx_1^*x_3^*[(1 - \mu_m)\hat{w}_3\beta - \mu_m\hat{w}_1\alpha] \\ &\quad - r\hat{\beta}w_3x_3^*(\hat{x}_1 - \mu_m) + r^2(\alpha\hat{x}_3 + \beta\hat{x}_1)\hat{\beta}x_1^*x_3^*. \end{aligned} \quad (\text{A30})$$

In  $\det(A)$  we add the second row to the first using (A30) to obtain

$$\begin{aligned} a_{11} + a_{21} &= -(2 - \mu_M - \mu_m)(w_1\hat{w}_1x_1^* + w_3\hat{w}_3x_3^*) + (\mu_M + \mu_m)(w_1\hat{w}_3x_1^* + w_3\hat{w}_1x_3^*) \\ &\quad + (1 - \mu_M - \mu_m)(\hat{w}_1 + \hat{w}_3)(w_1x_1^* + w_3x_3^*) + r(\hat{\beta} - \hat{\alpha})(w_3x_3^* - w_1x_1^*). \end{aligned} \quad (\text{A31})$$

The  $(\mu_M + \mu_m)$  term vanishes, the constant term is  $(\hat{w}_1 - \hat{w}_3)(w_3x_3^* - w_1x_1^*)$ , and since  $(\hat{\beta} - \hat{\alpha}) = (\hat{w}_3 - \hat{w}_1)$  we have

$$a_{11} + a_{21} = (1 - r)(\hat{w}_1 - \hat{w}_3)(w_3x_3^* - w_1x_1^*). \quad (\text{A32})$$

Observe that both  $a_{12}$  and  $a_{22}$  have a common factor of  $x_3^*$ . Thus

$$\begin{aligned} \frac{a_{12} + a_{22}}{x_3^*} &= (1 - \mu_m)^2w_3\hat{w}_3 + \mu_m^2w_3\hat{w}_1 + \mu_m(1 - \mu_m)w_3(\hat{w}_1 + \hat{w}_3) - w^* \\ &\quad + rx_1^*(\hat{w}_1\alpha - \hat{w}_3\beta) + rw_3(\hat{\alpha} - \hat{\beta})(\hat{x}_1 - \mu_m) - r^2(\alpha\hat{x}_3 + \beta\hat{x}_1)(\hat{\alpha} - \hat{\beta})x_1^*, \end{aligned} \quad (\text{A33})$$

which we rewrite as

$$\begin{aligned} \frac{a_{12} + a_{22}}{x_3^*} &= T + (r - 1)x_1^*(\hat{w}_1\alpha - \hat{w}_3\beta) + (r - 1)w_3(\hat{\alpha} - \hat{\beta})(\hat{x}_1 - \mu_m) \\ &\quad - (r^2 - 1)(\alpha\hat{x}_3 + \beta\hat{x}_1)(\hat{\alpha} - \hat{\beta})x_1^*, \end{aligned} \quad (\text{A34})$$

where  $T$  is independent of  $r$ .

Observe that when  $r = 1$ , from (A14) and (A15),  $w\mathcal{L}$  and  $\hat{w}\hat{\mathcal{L}}$  and their product  $w^*\mathcal{L}_{\text{ex}}$  have the same structure, namely

$$w\mathcal{L} = \begin{bmatrix} \rho & \rho \\ \sigma & \sigma \end{bmatrix}, \quad \hat{w}\hat{\mathcal{L}} = \begin{bmatrix} \hat{\rho} & \hat{\rho} \\ \hat{\sigma} & \hat{\sigma} \end{bmatrix}, \quad w^*\mathcal{L}_{\text{ex}} = \begin{bmatrix} \Omega^* & \Omega^* \\ \nu^* & \nu^* \end{bmatrix} \quad (\text{A35})$$

with

$$w = \rho + \sigma, \quad \hat{w} = \hat{\rho} + \hat{\sigma}, \quad w^* = \Omega^* + \nu^*.$$

Hence, when  $r = 1$ ,  $M(1) = \det(w^*\mathcal{L}_{\text{ex}} - w^*\mathcal{I})$  is in fact

$$M(1) = \begin{vmatrix} \Omega^* - w^* & \Omega \\ \nu^* & \nu^* - w^* \end{vmatrix}. \quad (\text{A36})$$

Therefore, repeating the steps we used above to compute  $M(1)$ , we have

$$M(1) = \frac{1}{x_1^*x_3^*} \begin{vmatrix} (\Omega^* - w^*)x_1^* & \Omega^*x_3^* \\ \nu^*x_1^* & (\nu^* - w^*)x_3^* \end{vmatrix}. \quad (\text{A37})$$

Since  $x_1^* + x_3^* = 1$ , adding the second column to the first gives

$$M(1) = \frac{1}{x_1^*x_3^*} \begin{vmatrix} \Omega^* - w^*x_1^* & \Omega^*x_3^* \\ \nu^* - w^*x_3^* & (\nu^* - w^*)x_3^* \end{vmatrix}. \quad (\text{A38})$$

Adding the second row to the first gives

$$M(1) = \frac{1}{x_1^*x_3^*} \begin{vmatrix} \Omega^* + \nu^* - w^* & (\Omega^* + \nu^* - w^*)x_3^* \\ \nu^* - w^*x_3^* & (\nu^* - w^*)x_3^* \end{vmatrix}. \quad (\text{A39})$$

But  $w^* = \Omega^* + \nu^*$ , so when  $r = 1$  we should have  $a_{11} + a_{21} = 0 = a_{12} + a_{22}$ . Therefore in (A34)  $T = 0$ , and we conclude that

$$a_{12} + a_{22} = (1 - r)x_3^*[(r + 1)(\alpha\hat{x}_3 + \beta\hat{x}_1)x_1^*(\hat{\alpha} - \hat{\beta}) - x_1^*(\hat{w}_1\alpha - \hat{w}_3\beta) - w_3(\hat{x}_1 - \mu_m)(\hat{\alpha} - \hat{\beta})]. \quad (\text{A40})$$

Thus  $(a_{12} + a_{22})$  has also a factor of  $(1 - r)$ . In addition, both  $(a_{12} + a_{22})$  and  $a_{22}$  have a common factor of  $x_3^*$ , so in fact

$$M(1) = \frac{(\mu_m - \mu_M)(1 - r)}{x_1^*} \cdot \Delta, \quad (\text{A41})$$

where  $\Delta = d_{11}d_{22} - d_{12}d_{21}$  and

$$\begin{aligned}
 d_{11} &= (\hat{w}_1 - \hat{w}_3)(w_3x_3^* - w_1x_1^*) \\
 d_{12} &= (r + 1)(\alpha\hat{x}_3 + \beta\hat{x}_1)x_1^*(\hat{\alpha} - \hat{\beta}) - x_1^*(\hat{w}_1\alpha - \hat{w}_3\beta) - w_3(\hat{x}_1 - \mu_m)(\hat{\alpha} - \hat{\beta}) \\
 d_{21} &= -(2 - \mu_M - \mu_m)w_3\hat{w}_3x_3^* + (\mu_M + \mu_m)w_3\hat{w}_1x_3^* + (1 - \mu_M - \mu_m)w_1(\hat{w}_1 + \hat{w}_3)x_1^* \\
 &\quad + r\hat{\beta}(w_3x_3^* - w_1x_1^*) \\
 d_{22} &= [(1 - \mu_m)^2w_3\hat{w}_3 + \mu_m^2w_3\hat{w}_1] - w^* - rx_1^*[(1 - \mu_m)\hat{w}_3\beta - \mu_m\hat{w}_1\alpha] \\
 &\quad - r\hat{\beta}w_3(\hat{x}_1 - \mu_m) + r^2(\alpha\hat{x}_3 + \beta\hat{x}_1)\hat{\beta}x_1^*.
 \end{aligned} \tag{A42}$$

This proves equation (29). Thus the larger eigenvalue of  $\mathcal{L}_{\text{ex}}$  is less than one if

$$M(1) > 0 \text{ and } M'(1) > 0, \tag{A43}$$

and greater than one if

$$M(1) < 0. \tag{A44}$$

**FILE S2: Proof that  $\Delta = \Delta(r, \mu_m)$  in (29) (and A41) is a bilinear function of  $r$  and  $\mu_m$**

$\Delta = d_{11}d_{22} - d_{12}d_{21}$ . We will show that the coefficients of  $r^2$  and of  $\mu_m^2$  in the expansion of  $\Delta$  are zero.

The coefficient of  $r^2$  in  $d_{11}d_{22}$  is

$$(\hat{w}_1 - \hat{w}_3)(w_3x_3^* - w_1x_1^*)(\alpha\hat{x}_3 + \beta\hat{x}_1)\hat{\beta}x_1^*, \quad (\text{B1})$$

and since  $\hat{\alpha} - \hat{\beta} = \hat{w}_1 - \hat{w}_3$  the coefficient of  $r^2$  in  $d_{12}d_{21}$  is

$$x_1^*(\alpha\hat{x}_3 + \beta\hat{x}_1)(\hat{w}_1 - \hat{w}_3)\hat{\beta}(w_3x_3^* - w_1x_1^*). \quad (\text{B2})$$

Hence the coefficient of  $r^2$  in  $\Delta$  is zero.

Similarly, since  $\alpha = w_1 - \mu_m(w_1 + w_3)$ ,  $\beta = w_3 - \mu_m(w_1 + w_3)$ , and  $\hat{\beta} = \hat{w}_3 - \mu_m(\hat{w}_1 + \hat{w}_3)$ , the coefficient of  $(\mu_m)^2$  in  $d_{11}d_{22}$  is

$$\begin{aligned} & (\hat{w}_1 - \hat{w}_3)(w_3x_3^* - w_1x_1^*) \times \\ & \left\{ w_3(\hat{w}_3 + \hat{w}_1) - rx_1^*(\hat{w}_1 + \hat{w}_3)(w_1 + w_3) - rw_3(\hat{w}_1 + \hat{w}_3) + r^2x_1^*(\hat{w}_1 + \hat{w}_3)(w_1 + w_3) \right\}. \end{aligned} \quad (\text{B3})$$

(B3) reduces to

$$(\hat{w}_1 - \hat{w}_3)(\hat{w}_1 + \hat{w}_3)(w_3x_3^* - w_1x_1^*)(1 - r)[w_3 - rx_1^*(w_1 + w_3)]. \quad (\text{B4})$$

The coefficient of  $(\mu_m)^2$  in  $d_{12}d_{21}$  is

$$\begin{aligned} & \left[ -(1 + r)x_1^*(w_1 + w_3)(\hat{w}_1 - \hat{w}_3) + x_1^*(\hat{w}_1 - \hat{w}_3)(w_1 + w_3) + w_3(\hat{w}_1 - \hat{w}_3) \right] \times \\ & \left[ (w_3x_3^* - w_1x_1^*)(\hat{w}_1 + \hat{w}_3) - r(\hat{w}_1 + \hat{w}_3)(w_3x_3^* - w_1x_1^*) \right], \end{aligned} \quad (\text{B5})$$

which simplifies to

$$(\hat{w}_1 - \hat{w}_3)(\hat{w}_1 + \hat{w}_3)(1 - r)(w_3x_3^* - w_1x_1^*)[w_3 - rx_1^*(w_1 + w_3)].$$

As (B4) and (B5) are equal, the coefficient of  $(\mu_m)^2$  in  $\Delta$  is indeed zero.

**FILE S3: Proof that with period 2 and symmetric fitnesses,  $\Delta(r, \mu_m) < 0$ , which gives Result 2**

We first show, using (33), that at  $\underline{x}^*$  we have

$$(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) > 0, \quad (\text{C1})$$

and from (26) the mean fitness  $w^*$  at  $\underline{x}^*$  is

$$w^* = w_1 w_3 + \mu_M (w_1 - w_3)(w_1 x_1^* - w_3 x_3^*), \quad (\text{C2})$$

and is, therefore, an increasing function of  $\mu_M$ .

To show (C1), we compute  $\bar{Q}(\frac{w_3}{w_1})$ :

$$\bar{Q}\left(\frac{w_3}{w_1}\right) = (1 - \mu_M) \frac{w_3^2}{w_1^2} w_1 + \mu_M (w_3 - w_1) \frac{w_3}{w_1} - (1 - \mu_M) w_3 \quad (\text{C3})$$

$$= \frac{w_3}{w_1} (w_3 - w_1). \quad (\text{C4})$$

As  $\bar{Q}(0) < 0$  and  $\bar{Q}(\pm\infty) > 0$ , when  $w_3 > w_1$ , we have  $\bar{Q}(\frac{w_3}{w_1}) > 0$  and  $u^* = \frac{x_1^*}{x_3^*} < \frac{w_3}{w_1}$  or  $w_3 x_3^* > w_1 x_1^*$ . If  $w_3 < w_1$ , then  $\bar{Q}(\frac{w_3}{w_1}) < 0$  and  $u^* = \frac{x_1^*}{x_3^*} > \frac{w_3}{w_1}$  or  $w_3 x_3^* < w_1 x_1^*$ . Hence  $(w_1 - w_3)$  and  $(w_1 x_1^* - w_3 x_3^*)$  have the same signs and (C1) follows. An alternative way to write the left side of (C1) is

$$(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) = (w_1^2 x_1^* + w_3^2 x_3^* - w_1 w_3), \quad (\text{C5})$$

and  $w_1^2 x_1^* + w_3^2 x_3^* > w_1 w_3$ . Again, using (C5) we get from (C2) that

$$w^* = (1 - \mu_M) w_1 w_3 + \mu_M (w_1^2 x_1^* + w_3^2 x_3^*). \quad (\text{C6})$$

As  $w_1^2 x_1^* + w_3^2 x_3^* > w_1 w_3$ , for all  $0 \leq \mu_M \leq 1$

$$w_1 w_3 \leq w^* \leq w_1^2 x_1^* + w_3^2 x_3^*. \quad (\text{C7})$$

Also,  $w^* > w_1 w_3$  when  $\mu_M \neq 0$ , and when  $\mu_M \neq 1$ ,  $w^* < w_1^2 x_1^* + w_3^2 x_3^*$ .

We now look for the conditions under which  $\underline{x}^*$  is externally stable. These depend on the magnitude of the larger positive eigenvalue of  $\mathcal{L}_{\text{ex}}$ , the product of (A9) and (A10). The determinant (A12) of this matrix in the symmetric case is given by

$$\det(\mathcal{L}_{\text{ex}}) = \frac{(1 - 2\mu_m)^2 (1 - r)^2 w_1^2 w_3^2}{(w^*)^2}. \quad (\text{C8})$$

As  $w^* \geq w_1 w_3$ , we conclude from (C8) that  $0 \leq \det(\mathcal{L}_{\text{ex}}) \leq 1$  for all possible values of  $r$ ,  $\mu_m$ ,  $\mu_M$ . But  $\mathcal{L}_{\text{ex}}$  is a positive matrix and therefore has a positive eigenvalue. Hence the other eigenvalue of  $\mathcal{L}_{\text{ex}}$  is positive, except when  $\mu_m = \frac{1}{2}$  when it is zero. Their product is less than or equal to one, and also at most one eigenvalue is larger than one. In this case it is clear that the larger eigenvalue of  $\mathcal{L}_{\text{ex}}$  is greater than one if  $M(1)$  of (A13) is negative, and it is smaller than one if  $M(1)$  is positive.

Since  $\Delta(r, \mu_m)$  is a bilinear function of  $r$  and  $\mu_m$ , the value of  $\Delta(r, \mu_m)$  is negative for  $0 \leq r \leq 1$ ,  $0 \leq \mu_m \leq 1$  if and only if the four ‘‘corner values’’

$$\Delta(0, 0), \quad \Delta(0, 1), \quad \Delta(1, 0), \quad \Delta(1, 1)$$

are all negative.

In the case  $r = 0$  we know, from (30), that the eigenvalues of  $\mathcal{L}_{\text{ex}}$ , in the symmetric case, with  $\mu_m = 0$  or  $\mu_m = 1$  are

$$\begin{aligned} \lambda_1^0 = \lambda_2^0 &= \frac{w_1 w_3}{w^*} && \text{when } \mu_m = 0, \\ \lambda_1^1 &= \frac{w_1^2}{w^*}, \quad \lambda_2^1 = \frac{w_3^2}{w^*} && \text{when } \mu_m = 1. \end{aligned}$$

When  $\mu_M > 0$ , (C6) and (C7) imply that  $w^* > w_1 w_3$  and the two eigenvalues  $\lambda_1^0$  and  $\lambda_2^0$  are less than 1. Therefore when  $\mu_m = 0$ ,  $\mu_M > 0$ , and  $r = 0$ , the equilibrium  $\underline{x}^*$  is stable. From (29),

$$M(1) = \frac{(\mu_m - \mu_M)(1 - r)}{x_1^*} \Delta(r, \mu_m).$$

Hence  $M(1)$  should be positive when  $\mu_m = 0$ ,  $\mu_M > 0$ , and  $r = 0$ . Thus  $\Delta(0, 0)$  is indeed negative.

Similarly, when  $\mu_M \neq 1$ ,  $w_1^2 x_1^* + w_3^2 x_3^* > w^*$  by (C6) and (C7). Therefore when  $r = 0$ ,  $\mu_m = 1$ , and  $0 \leq \mu_M < 1$  at least one of the eigenvalues  $\lambda_1^1$  or  $\lambda_2^1$  is larger than 1, and  $\underline{x}^*$  is unstable. In this case  $M(1)$  should be negative when  $r = 0$ ,  $\mu_m = 1$ ,  $0 \leq \mu_M < 1$ , which implies by (29) that  $\Delta(0, 1)$  is negative. The proof that  $\Delta(1, 0)$  and  $\Delta(1, 1)$  are negative is more complex.

We prove next that  $\Delta(1, 0)$  and  $\Delta(1, 1)$  are negative for all  $0 < \mu_M < 1$ .

In the symmetric case where  $\hat{w}_1 = w_3$ ,  $\hat{w}_3 = w_1$  we have  $\hat{\alpha} = \beta$ ,  $\hat{\beta} = \alpha$ , where  $\alpha =$



$w_1 - \mu_m(w_1 + w_3)$  and  $\beta = w_3 - \mu_m(w_1 + w_3)$ . Thus  $\Delta(r, \mu_m) = d_{11}d_{22} - d_{12}d_{21}$ , where

$$\begin{aligned}
 d_{11} &= (w_1 - w_3)(w_1x_1^* - w_3x_3^*) \\
 d_{12} &= (1 + r)x_1^*(\alpha\hat{x}_3 + \beta\hat{x}_1)(\beta - \alpha) - x_1^*(w_3\alpha - w_1\beta) - w_3(\hat{x}_1 - \mu_m)(\beta - \alpha) \\
 d_{21} &= -(2 - \mu_M - \mu_m)w_1w_3x_3^* + (\mu_M + \mu_m)w_3^2x_3^* + (1 - \mu_M - \mu_m)w_1(w_1 + w_3)x_1^* \\
 &\quad + r\alpha(w_3x_3^* - w_1x_1^*) \\
 d_{22} &= (1 - \mu_m)^2w_1w_3 + \mu_m^2w_3^2 - w^* - rx_1^*[(1 - \mu_m)w_1\beta - \mu_mw_3\alpha] - r\alpha w_3(\hat{x}_1 - \mu_m) \\
 &\quad + r^2(\alpha\hat{x}_3 + \beta\hat{x}_1)\alpha x_1^*.
 \end{aligned} \tag{C9}$$

We compute the  $d_{ij}$ 's of (C9) when  $r = 1$  and  $\mu_m = 0$ , using the fact that in this case  $\alpha = w_1$ ,  $\beta = w_3$ , and  $\beta - \alpha = w_3 - w_1$ , so that

$$\begin{aligned}
 d_{11} &= (w_1 - w_3)(w_1x_1^* - w_3x_3^*) \\
 d_{12} &= (w_1 - w_3)[-x_1^*(w_1\hat{x}_3 + w_3\hat{x}_1) - \mu_M(w_1x_1^* - w_3x_3^*)] \\
 d_{21} &= -w_1(w_1x_1^* + w_3x_3^*) - \mu_M(w_1 + w_3)(w_1x_1^* - w_3x_3^*) \\
 d_{22} &= -w_3[w_1x_1^*(1 - \mu_M) + \mu_Mw_3x_3^*].
 \end{aligned} \tag{C10}$$

When  $w_1 > w_3$  we know that  $(w_1x_1^* - w_3x_3^*) > 0$  by (C1); hence if  $w_1 > w_3$  we have that  $d_{11} > 0$ ,  $d_{12} < 0$ ,  $d_{21} < 0$ ,  $d_{22} < 0$ . Therefore clearly  $\Delta(1, 0) = d_{11}d_{22} - d_{12}d_{21}$  is negative. Observe that in the symmetric case we can reorder the gametes from  $AM Am aM am$  to  $aM am AM Am$  without changing the results, as the mutation rates from  $A$  to  $a$  and from  $a$  to  $A$  are the same. This means that our results should be the same if we interchange  $w_1 \leftrightarrow w_3$ ,  $x_1^* \leftrightarrow x_3^*$ , and  $\hat{x}_1 \leftrightarrow \hat{x}_3$ , in which case (C8) gives

$$M(1) = \frac{(\mu_m - \mu_M)(1 - r)}{x_3^*} \bar{\Delta}(r, \mu_m), \tag{C11}$$

where  $\bar{\Delta}(r, \mu_m) = \bar{d}_{11}\bar{d}_{22} - \bar{d}_{12}\bar{d}_{21}$  with the  $\bar{d}_{ij}$ 's obtained from the  $d_{ij}$ 's of (C9) by interchanging  $x_1^* \leftrightarrow x_3^*$ ,  $\hat{x}_1 \leftrightarrow \hat{x}_3$ , and  $w_1 \leftrightarrow w_3$ .

From (C8) and (C11) we have

$$\frac{1}{x_1^*} \Delta(r, \mu_m) = \frac{1}{x_3^*} \bar{\Delta}(r, \mu_m), \tag{C12}$$

and  $\Delta(r, \mu_m)$  and  $\bar{\Delta}(r, \mu_m)$  have the same signs. Thus  $\bar{\Delta}(1, 0) = \bar{d}_{11}\bar{d}_{22} - \bar{d}_{12}\bar{d}_{21}$ , where from (C10),

$$\begin{aligned}
 \bar{d}_{11} &= (w_3 - w_1)(w_3x_3^* - w_1x_1^*) \\
 \bar{d}_{12} &= (w_3 - w_1)[-x_3^*(w_3\hat{x}_1 + w_1\hat{x}_3) - \mu_M(w_3x_3^* - w_1x_1^*)] \\
 \bar{d}_{21} &= -w_3(w_3x_3^* + w_1x_1^*) - \mu_M(w_3 + w_1)(w_3x_3^* - w_1x_1^*) \\
 \bar{d}_{22} &= -w_1[(1 - \mu_M)w_3x_3^* + \mu_Mw_1x_1^*].
 \end{aligned} \tag{C13}$$

But if  $w_3 > w_1$  then  $(w_3x_3^* - w_1x_1^*) > 0$ , and consequently  $\bar{d}_{11} > 0$ , whereas  $\bar{d}_{12} < 0$ ,  $\bar{d}_{21} < 0$ , and  $\bar{d}_{22} < 0$ . So  $\bar{\Delta}(1, 0) = \bar{d}_{11}\bar{d}_{22} - \bar{d}_{12}\bar{d}_{21}$  is negative.

We thus proved that  $\Delta(1, 0)$  is negative for all  $0 \leq \mu_M \leq 1$  when  $w_1 \neq w_3$ .

When  $r = 1$  and  $\mu_m = 1$ , we have  $\alpha = -w_3$ ,  $\beta = -w_1$ , and  $\beta - \alpha = w_3 - w_1$ . In this case the  $d_{ij}$ 's of (C9) reduce to

$$\begin{aligned} d_{11} &= (w_3 - w_1)(w_3x_3^* - w_1x_1^*) \\ d_{12} &= (w_3 - w_1)[-2x_1^*(w_3\hat{x}_3 + w_1\hat{x}_1) + x_1^*(w_1 + w_3) + w_3\hat{x}_3] \\ d_{21} &= w_1w_3(x_1^* - x_3^*) + \mu_M(w_1 + w_3)(w_3x_3^* - w_1x_1^*) \\ w d_{22} &= -w_1w_3^2x_3^* - \mu_M(w_3x_3^* - w_1x_1^*)[2w_3^2 - w_1(w_1x_1^* + w_3x_3^*)], \end{aligned} \tag{C14}$$

where  $w = w_1x_1^* + w_3x_3^*$ .

When  $w_3 > w_1$  we have  $(w_3x_3^* - w_1x_1^*) > 0$  and also  $2w_3 > w_1(w_1x_1^* + w_3x_3^*)$ . Therefore in this case  $d_{11} > 0$  and  $d_{22} < 0$ . We will show that in this case  $d_{12}$  and  $d_{21}$  are both positive.

As  $w_3 > w_1$ ,  $d_{12}$  is positive if  $T$  is positive where

$$T = -2x_1^*(w_1\hat{x}_1 + w_3\hat{x}_3) + x_1^*(w_1 + w_3) + w_3\hat{x}_3. \tag{C15}$$

We use the equations for  $\hat{x}_1$  and  $\hat{x}_3$ , namely,

$$\begin{aligned} w\hat{x}_1 &= (1 - \mu_M)w_1x_1^* + \mu_Mw_3x_3^* \\ w\hat{x}_3 &= (1 - \mu_M)w_3x_3^* + \mu_Mw_1x_1^*, \end{aligned} \tag{C16}$$

with  $w = w_1x_1^* + w_3x_3^*$ . Using these equations and the fact that  $x_1^* + x_3^* = 1$ , we get

$$wT = (w_3x_3^* - w_1x_1^*)[w_3x_3^* + w_1x_1^*(1 - 2\mu_M) + w_3\mu_M(2x_1^* - 1)]. \tag{C17}$$

As  $(w_3x_3^* - w_1x_1^*)$  is positive,  $T$  is positive if

$$w_3x_3^* + w_1x_1^*(1 - 2\mu_M) + w_3\mu_M(2x_1^* - 1) > 0. \tag{C18}$$

(C18) is equivalent to

$$w_3x_3^* + w_1x_1^* - w_3\mu_M + 2\mu_M(w_3 - w_1)x_1^* > 0. \tag{C19}$$

As  $w_3 > w_1$ , in order that (C19) holds it is sufficient that

$$w_3x_3^* + w_1x_1^* > w_3\mu_M. \tag{C20}$$

Write  $x_1^* = \frac{u^*}{1+u^*}$ ,  $x_3^* = \frac{1}{1+u^*}$  where  $\bar{Q}(u^*) = 0$  and  $\bar{Q}(u)$  is given in (34). (C20) is thus equivalent to

$$w_3 + w_1 u^* > w_3 \mu_M (1 + u^*) \quad (\text{C21})$$

or

$$u^* (w_3 \mu_M - w_1) < w_3 (1 - \mu_M). \quad (\text{C22})$$

When  $w_3 \mu_M < w_1$ , (C22) clearly holds. When  $w_3 \mu_M > w_1$ , then (C22) is true if and only if  $u^*$  satisfies

$$u^* < \frac{w_3(1 - \mu_M)}{w_3 \mu_M - w_1} = t, \quad (\text{C23})$$

say. Because of the properties of the quadratic equation  $\bar{Q}(u) = 0$ ,  $u^* < t$  if and only if  $\bar{Q}(t) > 0$ . But from (34),

$$\bar{Q}(t) = (1 - \mu_M) w_1 \frac{w_3^2 (1 - \mu_M)^2}{(w_3 \mu_M - w_1)^2} + \mu_M (w_3 - w_1) \frac{w_3 (1 - \mu_M)}{w_3 \mu_M - w_1} - (1 - \mu_M) w_3. \quad (\text{C24})$$

As  $\mu_M < 1$ , the sign of  $\bar{Q}(t)$  coincides with the sign of

$$(1 - \mu_M)^2 w_1 w_3 + \mu_M (w_3 - w_1) (w_3 \mu_M - w_1) - (w_3 \mu_M - w_1)^2. \quad (\text{C25})$$

But (C25) is equal to

$$(1 - \mu_M) w_1 (w_3 - w_1), \quad (\text{C26})$$

which is positive since  $w_3 > w_1$  and  $0 < \mu_M < 1$ . So indeed  $d_{12} > 0$  when  $w_3 > w_1$ .

We now show that  $d_{21}$  is also positive when  $w_3 > w_1$ . Observe from (34) that  $\bar{Q}(1) = (1 - 2\mu_M)(w_1 - w_3)$ . Therefore when  $w_3 > w_1$  and  $0 < \mu_M < \frac{1}{2}$ ,  $\bar{Q}(1)$  is negative, and so, based on the properties of  $\bar{Q}(u) = 0$ , we have  $u^* = \frac{x_1^*}{x_3^*} > 1$  or  $x_1^* > x_3^*$ . Hence, if  $w_3 > w_1$  and also  $0 < \mu_M < \frac{1}{2}$  then  $w_3 x_3^* > w_1 x_1^*$  and  $x_1^* > x_3^*$  implying that  $d_{21}$  is positive. We check the case when  $\frac{1}{2} < \mu_M < 1$  and compute  $d_{21}$  using the representation  $x_1^* = \frac{u^*}{1+u^*}$ ,  $x_3^* = \frac{1}{1+u^*}$  with  $\bar{Q}(u^*) = 0$ . Indeed

$$(1 + u^*) d_{21} = w_1 w_3 (u^* - 1) + \mu_M (w_1 + w_3) (w_3 - w_1 u^*). \quad (\text{C27})$$

Equivalently

$$(1 + u^*) d_{21} = w_1 u^* [w_3 - \mu_M (w_1 + w_3)] + w_3 [\mu_M (w_1 + w_3) - w_1]. \quad (\text{C28})$$

When  $\frac{1}{2} \leq \mu_M < 1$ ,  $[\mu_M (w_1 + w_3) - w_1] > 0$  as  $w_3 > w_1$ . If  $[w_3 - \mu_M (w_1 + w_3)] \geq 0$  then clearly  $d_{21}$  is positive, and if  $[w_3 - \mu_M (w_1 + w_3)] < 0$ ,  $d_{21}$  is positive provided

$$u^* < \frac{w_3}{w_1} \cdot \frac{\mu_M(w_1 + w_3) - w_1}{\mu_M(w_1 + w_3) - w_3} = s, \quad (\text{C29})$$

say. (C29) is equivalent to  $\bar{Q}(s) > 0$ . Now

$$\begin{aligned} \bar{Q}(s) &= (1 - \mu_M)w_1 \frac{w_3^2}{w_1^2} \cdot \frac{[\mu_M(w_1 + w_3) - w_1]^2}{[\mu_M(w_1 + w_3) - w_3]^2} \\ &\quad + \mu_M(w_3 - w_1) \frac{w_3}{w_1} \cdot \frac{\mu_M(w_1 + w_3) - w_1}{\mu_M(w_1 + w_3) - w_3} - (1 - \mu_M)w_3. \end{aligned} \quad (\text{C30})$$

The sign of  $\bar{Q}(s)$  coincides with the sign of

$$\begin{aligned} (1 - \mu_M)w_3 [\mu_M(w_1 + w_3) - w_1]^2 + \mu_M(w_3 - w_1) [\mu_M(w_1 + w_3) - w_1] [\mu_M(w_1 + w_3) - w_3] \\ - (1 - \mu_M)w_1 [\mu_M(w_1 + w_3) - w_3]^2. \end{aligned} \quad (\text{C31})$$

(C31) is equal to

$$\begin{aligned} (1 - \mu_M) \left\{ w_3 [\mu_M(w_1 + w_3) - w_1]^2 - w_1 [\mu_M(w_1 + w_3) - w_3]^2 \right\} \\ + \mu_M(w_3 - w_1) [\mu_M(w_1 + w_3) - w_1] [\mu_M(w_1 + w_3) - w_3]. \end{aligned} \quad (\text{C32})$$

(C32) is equal to

$$\begin{aligned} (1 - \mu_M) [(w_3 - w_1) \mu_M^2 (w_1 + w_3)^2 - w_1 w_3 (w_3 - w_1)] \\ + \mu_M (w_3 - w_1) [\mu_M^2 (w_1 + w_3)^2 - \mu_M (w_1 + w_3)^2 + w_1 w_3]. \end{aligned} \quad (\text{C33})$$

As  $w_3 > w_1$ , (C33) is positive if

$$(1 - \mu_M) [\mu_M^2 (w_1 + w_3)^2 - w_1 w_3] + \mu_M [-\mu_M (1 - \mu_M) (w_1 + w_3)^2 + w_1 w_3] \quad (\text{C34})$$

is positive. Finally, (C34) equals

$$w_1 w_3 (2\mu_M - 1), \quad (\text{C35})$$

which is positive if  $\frac{1}{2} < \mu_M < 1$ . If  $\mu_M = \frac{1}{2}$  then  $[w_3 - \mu_M(w_1 + w_3)] = [\mu_M(w_1 + w_3) - w_1] = \frac{w_3 - w_1}{2}$ , which is positive, and in view of (C28)  $d_{21}$  is positive. It follows that  $d_{21}$  is always positive when  $w_3 > w_1$ .

We conclude that when  $w_3 > w_1$ ,  $d_{11} > 0$ ,  $d_{12} > 0$ ,  $d_{21} > 0$ , and  $d_{22} < 0$ , and in this case  $\Delta(1, 1)$  is negative.

Using the same symmetry argument we used for the case  $r = 1$  and  $\mu_m = 0$ , we can show in the same way that  $\Delta(1, 1)$  is also negative when  $w_1 > w_3$ , making it negative whenever  $w_1 \neq w_3$  for all  $0 < \mu_M < 1$ .

We conclude that  $\Delta(r, \mu_m) < 0$  for  $0 \leq r \leq 1$ ,  $0 \leq \mu_m \leq 1$ .

**FILE S4: Proof of Result 3**

Recall from (26) that  $w^*(\mu_M)$  is given by

$$w^*(\mu_M) = w_1 w_3 + \mu_M (w_1 - w_3) (w_1 x_1^* - w_3 x_3^*),$$

and as  $(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*)$  is always positive,  $w^*(\mu_M)$  is an increasing function of  $\mu_M$  if  $a^*(\mu_M)$  where

$$a^* = a^*(\mu_M) = (w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) \tag{D1}$$

is an increasing function of  $\mu_M$ .

The equilibrium frequencies  $x_1^*$  and  $x_3^*$  can be represented as  $x_1^* = \frac{u^*}{1+u^*}$ ,  $x_3^* = \frac{1}{1+u^*}$  where  $u^*$  is the unique positive solution of  $\bar{Q}(u) = 0$  and

$$\bar{Q}(u) = (1 - \mu_M)w_1 u^2 + \mu_M(w_3 - w_1)u - (1 - \mu_M)w_3, \tag{D2}$$

or equivalently if  $\bar{\bar{Q}}(u) = 0$ , where

$$\bar{\bar{Q}}(u) = w_1 u^2 + \frac{\mu_M}{1 - \mu_M} (w_3 - w_1)u - w_3. \tag{D3}$$

Now  $a^*$ , as a function of  $u^*$ , is

$$a^* = (w_1 - w_3) \left[ w_1 \frac{u^*}{1 + u^*} - w_3 \frac{1}{1 + u^*} \right] \tag{D4}$$

$$= (w_1 - w_3) \frac{w_1 u^* - w_3}{1 + u^*}. \tag{D5}$$

The derivative of  $a^*$ , with respect to  $\mu_M$ , is

$$\frac{da^*}{d\mu_M} = \frac{da^*}{du^*} \cdot \frac{du^*}{d\mu_M}, \tag{D6}$$

and from (D5) we have

$$\frac{da^*}{du^*} = \frac{(w_1 - w_3)(w_1 + w_3)}{(1 + u^*)^2}. \tag{D7}$$

The derivative  $(u^*)' = \frac{du^*}{d\mu_M}$  can be computed by implicit differentiation of the equilibrium equation  $\bar{\bar{Q}}(u^*) = 0$  giving

$$2w_1 u^* (u^*)' + \frac{\mu_M}{1 - \mu_M} (w_3 - w_1) (u^*)' + \frac{(w_3 - w_1)u^*}{(1 - \mu_M)^2} = 0. \tag{D8}$$

Hence

$$\left[ 2w_1 u^* - \frac{\mu_M}{1 - \mu_M} (w_1 - w_3) \right] (u^*)' = \frac{(w_1 - w_3)u^*}{(1 - \mu_M)^2}. \tag{D9}$$

We will show that  $[2w_1u^* - \frac{\mu_M}{1-\mu_M}(w_1 - w_3)]$  is always positive. It is clearly positive when  $w_1 < w_3$ . In the case  $w_1 > w_3$ , it is positive if  $u^* > \frac{\mu_M}{1-\mu_M} \cdot \frac{w_1-w_3}{2w_1}$ . Observe that

$$\bar{Q}\left(\frac{\mu_M}{1-\mu_M} \cdot \frac{w_1-w_3}{2w_1}\right) = w_1 \left(\frac{\mu_M}{1-\mu_M}\right)^2 \frac{(w_1-w_3)^2}{4w_1^2} - \left(\frac{\mu_M}{1-\mu_M}\right)^2 \frac{(w_1-w_3)^2}{2w_1} - w_3 \quad (\text{D10})$$

$$= -\frac{1}{4} \left(\frac{\mu_M}{1-\mu_M}\right)^2 \frac{(w_1-w_3)^2}{w_1} - w_3 < 0. \quad (\text{D11})$$

As  $\bar{Q}(0) < 0$  and  $\bar{Q}(\pm\infty) > 0$ , the unique positive root  $u^*$  of  $\bar{Q}(u) = 0$  should satisfy

$$u^* > \frac{\mu_M}{1-\mu_M} \cdot \frac{w_1-w_3}{2w_1}. \quad (\text{D12})$$

Thus  $[2w_1u^* - \frac{\mu_M}{1-\mu_M}(w_1 - w_3)]$  is positive, and in view of (D9) and (D7) both  $\frac{da^*}{du^*}$  and  $\frac{du^*}{d\mu_M}$  have the sign of  $(w_1 - w_3)$ , and consequently  $\frac{da^*}{d\mu_M}$  is positive. Thus we have proved  $w^*(\mu_M)$  is an increasing function of  $\mu_M$ .

**FILE S5: Proofs for Symmetric Case, n = 2; Result 4****Part I: Proof of (39);** if  $r = 0$ ,  $(w_1 - w_3)(w_1x_1^* - w_3x_3^*) > 0$ With  $M$  fixed and  $\underline{\tilde{x}} = (x_1, x_3)^T$  we have

$$\underline{\tilde{x}}' = T_2 \circ T_2 \circ T_1 \circ T_1 \underline{\tilde{x}}. \quad (\text{E1})$$

The total matrix  $\mathcal{T} = T_2 \circ T_2 \circ T_1 \circ T_1$  is written

$$\mathcal{T} = \begin{pmatrix} A & B \\ C & D \end{pmatrix}, \quad (\text{E2})$$

where  $T_1$  and  $T_2$  are given in (37) and

$$\begin{aligned} A &= w_1w_3 [(1 - \mu_M)^2w_3 + \mu_M^2w_1] [(1 - \mu_M)^2w_1 + \mu_M^2w_3] + \mu_M^2(1 - \mu_M)^2w_1^2(w_1 + w_3)^2 \\ B &= \mu_M(1 - \mu_M)w_3(w_1 + w_3)^2 [(1 - \mu_M)^2w_3 + \mu_M^2w_1] \\ C &= \mu_M(1 - \mu_M)w_1(w_1 + w_3)^2 [(1 - \mu_M)^2w_1 + \mu_M^2w_3] \\ D &= w_1w_3 [(1 - \mu_M)^2w_3 + \mu_M^2w_1] [(1 - \mu_M)^2w_1 + \mu_M^2w_3] + \mu_M^2(1 - \mu_M)^2w_3^2(w_1 + w_3)^2. \end{aligned} \quad (\text{E3})$$

The mean fitness  $w = (A + C)x_1 + (B + D)x_3$  is

$$\begin{aligned} w &= w_1w_3 [(1 - \mu_M)^2w_3 + \mu_M^2w_1] [(1 - \mu_M)^2w_1 + \mu_M^2w_3] + \\ &+ \mu_M^2(1 - \mu_M)^2(w_1 + w_3)^2(w_1^2x_1 + w_3^2x_3) + \\ &+ \mu_M(1 - \mu_M)(w_1 + w_3)^2 \{w_1x_1 [(1 - \mu_M)^2w_1 + \mu_M^2w_3] + w_3x_3 [(1 - \mu_M)^2w_3 + \mu_M^2w_1]\}. \end{aligned} \quad (\text{E4})$$

We can simplify  $w$  to

$$w = w_1^2w_3^2(1 - 2\mu_M)^2 + \mu_M(1 - \mu_M)(w_1 + w_3)^2 [\mu_Mw_1w_3 + (1 - \mu_M)(w_1^2x_1 + w_3^2x_3)]. \quad (\text{E5})$$

Also

$$\begin{aligned} w &= w_1^2w_3^2 - 4\mu_M(1 - \mu_M)w_1^2w_3^2 + \mu_M(1 - \mu_M)(w_1 + w_3)^2w_1w_3 \\ &+ \mu_M(1 - \mu_M)(w_1^2x_1 + w_3^2x_3 - w_1w_3)(w_1 + w_3)^2 \end{aligned} \quad (\text{E6})$$

$$\begin{aligned} &= w_1^2w_3^2 + \mu_M(1 - \mu_M)w_1w_3(w_1 - w_3)^2 \\ &+ \mu_M(1 - \mu_M)(w_1^2x_1 + w_3^2x_3 - w_1w_3)(w_1 + w_3)^2. \end{aligned} \quad (\text{E7})$$

As  $w_1^2x_1 + w_3^2x_3 - w_1w_3 = (w_1 - w_3)(w_1x_1 - w_3x_3)$  we can write

$$w = w_1^2w_3^2 + \mu_M(1 - \mu_M)(w_1 - w_3)w_1^2w_3 + \mu_M(1 - \mu_M)(w_1 - w_3)(w_1x_1 - w_3x_3)(w_1 + w_3)^2. \quad (\text{E8})$$



We will show that at equilibrium where  $\underline{x} = \underline{x}^*$ ,  $(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*)$  is always positive so that  $w \geq w_1^2 w_3^3$ . In the equilibrium equation  $Q(u) = 0$ , where  $x_1 = \frac{u}{1+u}$ ,  $x_3 = \frac{1}{1+u}$ , we have

$$\begin{aligned} Q(u) &= \mu_M(1 - \mu_M)w_1(w_1 + w_3)^2 [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] u^2 \\ &\quad + \mu_M^2(1 - \mu_M)^2(w_3 - w_1)(w_1 + w_3)^3 u \\ &\quad - \mu_M(1 - \mu_M)w_3(w_1 + w_3)^2 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] . \end{aligned} \quad (\text{E9})$$

Since  $0 < \mu_M < 1$ ,  $Q(u) = 0$  is equivalent to  $\bar{Q}(u) = 0$  where

$$\begin{aligned} \bar{Q}(u) &= w_1 [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] u^2 + \mu_M(1 - \mu_M)(w_3 - w_1)(w_3 + w_1)u \\ &\quad - w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] . \end{aligned} \quad (\text{E10})$$

But  $\bar{Q}(0) < 0$  and  $\bar{Q}(\pm\infty) > 0$ ; hence  $\bar{Q}(u) = 0$  has one solution  $u^* > 0$  and the other solution is negative. We compute

$$\begin{aligned} \bar{Q}\left(\frac{w_3}{w_1}\right) &= [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] \frac{w_3^2}{w_1} + \mu_M(1 - \mu_M)(w_3^2 - w_1^2) \frac{w_3}{w_1} \\ &\quad - w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] , \end{aligned} \quad (\text{E11})$$

or

$$\begin{aligned} w_1 \bar{Q}\left(\frac{w_3}{w_1}\right) &= w_3^2 [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] + \mu_M(1 - \mu_M)(w_3^2 - w_1^2) w_3 \\ &\quad - w_1 w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] . \end{aligned} \quad (\text{E12})$$

In fact

$$w_1 \bar{Q}\left(\frac{w_3}{w_1}\right) = w_3(w_3 - w_1)\mu_M . \quad (\text{E13})$$

Thus the sign of  $\bar{Q}\left(\frac{w_3}{w_1}\right)$  coincides with the sign of  $(w_3 - w_1)$ . Hence, if  $w_3 > w_1$ ,  $\bar{Q}\left(\frac{w_3}{w_1}\right)$  is positive and since  $\bar{Q}(0) < 0$ ,  $\bar{Q}(\infty) > 0$ , we have  $u^* = \frac{x_1^*}{x_3^*} < \frac{w_3}{w_1}$  or  $w_x x_3^* > w_1 x_1^*$ . If  $w_3 < w_1$  then  $\bar{Q}\left(\frac{w_3}{w_1}\right) < 0$  and  $u^* = \frac{x_1^*}{x_3^*} > \frac{w_3}{w_1}$  so that  $w_3 x_3^* < w_1 x_1^*$ . Therefore at equilibrium

$$(w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) > 0 . \quad (\text{E14})$$

## Part II: Proof of Result 4

The mean fitness on the boundary where  $M$  is fixed is  $w^*(\mu_M)$  with

$$w^*(\mu_M) = w_1^2 w_3^2 + \mu_M(1 - \mu_M)w_1 w_3 (w_1 - w_3)^2 + \mu_M(1 - \mu_M)(w_1 + w_3)^2 (w_1 - w_3)(w_1 x_1^* - w_3 x_3^*) . \quad (\text{E15})$$

Observe that  $\mu_M(1 - \mu_M)$  is maximized when  $\mu_M = \frac{1}{2}$  and also  $(1 - \mu_M)(w_1 - w_3)(w_1x_1^* - w_3x_3^*) = a(\mu_M)$  is positive. Therefore  $w^*(\mu_M)$  is maximized at  $\mu_M = \frac{1}{2}$  if and only if  $a(\mu_M)$  is maximized at  $\mu_M = \frac{1}{2}$ . When  $\mu_M = \frac{1}{2}$ ,  $x_1^* = x_3^* = \frac{1}{2}$  and  $a(\frac{1}{2}) = \frac{1}{4}(w_1 - w_3)^2$ . We will show that for any  $0 \leq \mu_M \leq 1$ ,  $a(\mu_M) \leq a(\frac{1}{2})$ . Recall that  $x_1^* = \frac{u}{1+u}$ ,  $x_3^* = \frac{1}{1+u}$ , where  $\bar{Q}(u) = 0$ . Hence

$$a(\mu_M) = (1 - \mu_M)(w_1 - w_3) \left( w_1 \frac{u}{1+u} - w_3 \frac{1}{1+u} \right). \quad (\text{E16})$$

Thus  $a(\mu_M) \leq a(\frac{1}{2})$  if and only if

$$(1 - \mu_M)(w_1 - w_3)(w_1u - w_3) \leq \frac{1}{4}(w_1 - w_3)^2(1 + u), \quad (\text{E17})$$

or if and only if

$$u \left[ (1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2 \right] \leq (1 - \mu_M)(w_1 - w_3)w_3 + \frac{1}{4}(w_1 - w_3)^2. \quad (\text{E18})$$

We consider two cases.

**Case 1:**  $w_1 > w_3$

In this case  $(1 - \mu_M)(w_1 - w_3)w_3 + \frac{1}{4}(w_1 - w_3)^2 > 0$ . If  $(1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2 \leq 0$  then (E18) clearly holds. If  $(1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2 > 0$  then (E18) holds if

$$u \leq \frac{(1 - \mu_M)(w_1 - w_3)w_3 + \frac{1}{4}(w_1 - w_3)^2}{(1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2} = t, \quad (\text{E19})$$

which is equivalent to  $\bar{Q}(t) > 0$  as  $\bar{Q}(0) < 0$  and  $\bar{Q}(\infty) > 0$ .

**Case 2:**  $w_1 < w_3$

In this case  $(1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2 < 0$ , and (E18) holds if

$$u \geq \frac{(1 - \mu_M)(w_1 - w_3)w_3 + \frac{1}{4}(w_1 - w_3)^2}{(1 - \mu_M)(w_1 - w_3)w_1 - \frac{1}{4}(w_1 - w_3)^2} = t, \quad (\text{E20})$$

which is equivalent to  $\bar{Q}(t) < 0$ .

We will therefore show that

$$w_1 > w_3 \rightarrow \bar{Q}(t) > 0 \quad (\text{E21})$$

$$w_1 < w_3 \rightarrow \bar{Q}(t) < 0. \quad (\text{E22})$$

This will prove that if  $w_1 \neq w_3$  then  $a(\mu_M) \leq a(\frac{1}{2})$ . If  $w_1 \neq w_3$

$$t = \frac{(1 - \mu_M)w_3 + \frac{1}{4}(w_1 - w_3)}{(1 - \mu_M)w_1 - \frac{1}{4}(w_1 - w_3)}. \quad (\text{E23})$$

Therefore  $\bar{Q}(t)$  equals

$$\begin{aligned} & w_1 [(1 - \mu_M)^2 + \mu_M^2 w_3] \left[ \frac{(1 - \mu_M)w_3 + \frac{1}{4}(w_1 - w_3)}{(1 - \mu_M)w_1 - \frac{1}{4}(w_1 - w_3)} \right]^2 \\ & + \mu_M(1 - \mu_M)(w_3^2 - w_1^2) \frac{(1 - \mu_M)w_3 + \frac{1}{4}(w_1 - w_3)}{(1 - \mu_M)w_1 - \frac{1}{4}(w_1 - w_3)} - w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1]. \end{aligned} \quad (\text{E24})$$

Hence the sign of  $\bar{Q}(t)$  coincides with the sign of

$$\begin{aligned} H &= w_1 [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] [(1 - \mu_M)w_3 + \frac{1}{4}(w_1 - w_3)]^2 \\ & + \mu_M(1 - \mu_M)(w_3^2 - w_1^2) [(1 - \mu_M)w_3 + \frac{1}{4}(w_1 - w_3)] [(1 - \mu_M)w_1 - \frac{1}{4}(w_1 - w_3)] \\ & - w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] [(1 - \mu_M)w_1 - \frac{1}{4}(w_1 - w_3)]^2. \end{aligned} \quad (\text{E25})$$

We compute  $H$ .

$$\begin{aligned} H &= w_1 [(1 - \mu_M)^2 w_1 + \mu_M^2 w_3] [(1 - \mu_M)^2 w_3^2 + \frac{1}{2}(1 - \mu_M)w_3(w_1 - w_3) + \frac{1}{16}(w_1 - w_3)^2] \\ & - w_3 [(1 - \mu_M)^2 w_3 + \mu_M^2 w_1] [(1 - \mu_M)^2 w_1^2 - \frac{1}{2}(1 - \mu_M)w_1(w_1 - w_3) + \frac{1}{16}(w_1 - w_3)^2] \\ & + \mu_M(1 - \mu_M)(w_3^2 - w_1^2) [(1 - \mu_M)^2 w_1 w_3 + \frac{1}{4}(1 - \mu_M)(w_1 - w_3)^2 - \frac{1}{16}(w_1 - w_3)^2]. \end{aligned} \quad (\text{E26})$$

$H$  equals

$$\begin{aligned} & (1 - \mu_M)^4 w_1^2 w_3^2 + \frac{1}{2}(1 - \mu_M)^3 w_1^2 w_3(w_1 - w_3) + \frac{1}{16}(1 - \mu_M)^2 w_1^2 (w_1 - w_3)^2 \\ & - (1 - \mu_M)^4 w_1^2 w_3^2 + \frac{1}{2}(1 - \mu_M)^3 w_3^2 w_1(w_1 - w_3) - \frac{1}{16}(1 - \mu_M)^2 w_3^2 (w_1 - w_3)^2 \\ & + \mu_M^2 (1 - \mu_M)^2 w_1 w_3^3 + \frac{1}{2}\mu_M^2 (1 - \mu_M)w_1 w_3^2 (w_1 - w_3) + \frac{1}{16}w_1 w_3 (w_1 - w_3)^2 \\ & - \mu_M^2 (1 - \mu_M)^2 w_3 w_1^3 + \frac{1}{2}\mu_M^2 (1 - \mu_M)w_1^2 w_3 (w_1 - w_3) - \frac{1}{16}w_1 w_3 (w_1 - w_3)^2 \\ & + \mu_M(1 - \mu_M)^3 w_1 w_3 (w_3^2 - w_1^2) + \frac{1}{4}\mu_M(1 - \mu_M)^2 (w_3^2 - w_1^2)(w_1 - w_3)^2 \\ & - \frac{1}{16}\mu_M(1 - \mu_M)(w_3^2 - w_1^2)(w_1 - w_3)^2. \end{aligned} \quad (\text{E27})$$

In fact  $H$  equals

$$\frac{1 - \mu_M}{16} (1 - 2\mu_M)^2 (w_1^2 - w_3^2)(w_1 - w_3)^2 + \frac{1 - \mu_M}{2} (1 - 2\mu_M)^2 w_1 w_3 (w_1^2 - w_3^2). \quad (\text{E28})$$

Thus

$$H = \frac{1 - \mu_M}{16} (1 - 2\mu_M)^2 (w_1 - w_3)(w_1 + w_3) [(w_1 - w_3)^2 + 8w_1 w_3]. \quad (\text{E29})$$

If  $\mu_M = \frac{1}{2}$  then  $H = 0$ ; otherwise for all  $0 < \mu_M < 1$  the sign of  $H$  coincides with the sign of  $(w_1 - w_3)$ . Hence when  $w_1 \neq w_3$ , the sign of  $\bar{Q}(T)$  coincides with the sign of  $(w_1 - w_3)$ , which proves that  $a(\mu_M) \leq a(\frac{1}{2})$  and  $w^*(\mu_M)$  has its maximum value when  $\mu_M = \frac{1}{2}$ .

### Part III: Proof of Result 5

We check the external stability of  $\underline{x}^* = (\frac{1}{2}, 0, \frac{1}{2}, 0)$  when  $r = 0$   $\mu_M = \frac{1}{2}$  with  $w^* = (\frac{w_1+w_3}{2})^4$ . The eigenvalues of the linear approximation  $\mathcal{L}_{\text{ex}}$  solve the equation

$$M(\tilde{z}) = \begin{vmatrix} \tilde{A} - w^*z & \tilde{B} \\ \tilde{C} & \tilde{D} - w^*z \end{vmatrix} = (w^*z)^2 - (w^*z)(\tilde{A} + \tilde{D}) + \tilde{A}\tilde{D} - \tilde{B}\tilde{C}, \quad (\text{E30})$$

where  $\tilde{A}, \tilde{B}, \tilde{C}, \tilde{D}$  are the values  $A, B, C, D$  with  $\mu_m$  instead of  $\mu_M$ .

As one of the eigenvalues is positive, by Perron-Frobenius and as  $\det \mathcal{L}_{\text{ex}} = \tilde{A}\tilde{D} - \tilde{B}\tilde{C} = (1 - 2\mu_m)^4 w_1^4 w_3^4$ , the two eigenvalues are positive and the larger is less than one if and only if  $M(1) > 0$ .

$$M(1) = M(1; \mu_m) = (w^*)^2 - w^* (\tilde{A} + \tilde{D}) + (\tilde{A}\tilde{D} - \tilde{B}\tilde{C}) \quad (\text{E31})$$

As  $\underline{x}^*$  is the equilibrium with  $\mu_M = \frac{1}{2}$ , one of the eigenvalues is 1 when  $\mu_m = \mu_M = \frac{1}{2}$ . Therefore

$$M(1; \frac{1}{2}) = (w^*)^2 - w^* (\hat{A} + \hat{D}) + (\hat{A}\hat{D} - \hat{B}\hat{C}) = 0, \quad (\text{E32})$$

where  $\hat{A}, \hat{B}, \hat{C}, \hat{D}$  are the values of  $\tilde{A}, \tilde{B}, \tilde{C}, \tilde{D}$  when  $\mu_m = \frac{1}{2}$ . As  $\hat{A}\hat{D} - \hat{B}\hat{C} = 0$ , we have

$$M(1; \mu_m) = M(1; \mu_m) - M(1; \frac{1}{2}) = w^* \left[ (\hat{A} + \hat{D}) - (\tilde{A} + \tilde{D}) \right] + (\tilde{A}\tilde{D} - \tilde{B}\tilde{C}). \quad (\text{E33})$$

But as  $M(1; \frac{1}{2}) = 0$ ,  $w^* = (\hat{A} + \hat{D})$  and

$$M(1; \mu_m) = w^* \left[ w^* - (\tilde{A} + \tilde{D}) \right] + (\tilde{A}\tilde{D} - \tilde{B}\tilde{C}). \quad (\text{E34})$$

Now  $(\tilde{A}\tilde{D} - \tilde{B}\tilde{C}) = (1 - 2\mu_m)^4 w_1^4 w_3^4$ .

$$\begin{aligned} \tilde{A} + \tilde{D} &= 2w_1^2 w_3^2 \left[ (1 - \mu_m)^4 + (\mu_m)^4 \right] + 2\mu_m^2 (1 - \mu_m)^2 w_1 w_3 (w_1^2 + w_3^2) \\ &\quad + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + w_3^2) (w_1 + w_3)^2 \end{aligned} \quad (\text{E35})$$

$$\begin{aligned} \hat{A} + \hat{D} &= 2w_1 w_3 \left\{ w_1 w_3 \left[ (1 - \mu_m)^4 + (\mu_m)^4 \right] + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + w_3^2) \right\} \\ &\quad + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + w_3^2) (w_1 + w_3)^2 \end{aligned} \quad (\text{E36})$$

$$\begin{aligned} \tilde{A} + \tilde{D} &= 2w_1w_3 \times \\ &\times \left\{ w_1w_3 \left[ (1 - \mu_m)^4 - 2\mu_m^2 (1 - \mu_m)^2 + (\mu_m)^4 \right] + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + 2w_1w_3 + w_3)^2 \right\} \\ &\quad + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + w_3^2) (w_1 + w_3)^2 \end{aligned} \quad (\text{E37})$$

$$\begin{aligned} \tilde{A} + \tilde{D} &= 2w_1^2w_3^2 \left[ (1 - \mu_m)^2 - (\mu_m)^2 \right]^2 + 2\mu_m^2 (1 - \mu_m)^2 (w_1 + w_3)^2 w_1w_3 \\ &\quad + \mu_m^2 (1 - \mu_m)^2 (w_1^2 + w_3^2) (w_1 + w_3)^2 \end{aligned} \quad (\text{E38})$$

$$\tilde{A} + \tilde{D} = 2w_1^2w_3^2 (1 - 2\mu_m)^2 + \mu_m^2 (1 - \mu_m)^2 (w_1 + w_3)^2 (w_1^2 + 2w_1w_3 + w_3)^2. \quad (\text{E39})$$

Thus

$$\tilde{A} + \tilde{D} = 2w_1^2w_3^2 (1 - 2\mu_m)^2 + \mu_m^2 (1 - \mu_m)^2 (w_1 + w_4)^4. \quad (\text{E40})$$

Therefore  $M(1; \mu_m) = w^* \left[ w^* - (\tilde{A} + \tilde{D}) \right] + (\tilde{A}\tilde{D} - \tilde{B}\tilde{C})$  is equal to

$$M(1; \mu_m) = (w^*)^2 - w^* \mu_m^2 (1 - \mu_m)^2 (w_1 + w_4)^4 - 2w^* w_1^2 w_3^2 (1 - 2\mu_m)^2 + (1 - 2\mu_m)^4 w_1^2 w_3^2. \quad (\text{E41})$$

Hence

$$M(1; \mu_m) = \left[ w^* - w_1^2 w_3^2 (1 - 2\mu_m)^2 \right]^2 - w^* \mu_m^2 (1 - \mu_m)^2 (w_1 + w_3)^4. \quad (\text{E42})$$

Equivalently, as  $w^* = \frac{(w_1 + w_3)^4}{16}$  we get

$$M(1; \mu_m) = (w^*)^2 \left\{ \left[ 1 - \frac{w_1^2 w_3^2}{w^*} (1 - 2\mu_m)^2 \right]^2 - 16\mu_m^2 (1 - \mu_m)^2 \right\} \quad (\text{E43})$$

using the mean inequality  $\sqrt{w_1 w_3} < \frac{w_1 + w_3}{2}$ , with equality if and only if  $w_1 = w_3$ . So as  $w_1 \neq w_3$ ,  $w_1^2 w_3^2 < \left(\frac{w_1 + w_3}{2}\right)^4 = w^*$ . Therefore

$$1 - \frac{w_1^2 w_3^2}{w^*} (1 - 2\mu_m)^2 > 1 - (1 - 2\mu_m)^2 = 4\mu_m(1 - \mu_m). \quad (\text{E44})$$

Hence for all  $0 \leq \mu_m \leq 1$  we get that  $M(1; \mu_m) > 0$ . We thus conclude that  $\underline{x}^*$  based on  $\mu_M = \frac{1}{2}$  is always externally stable when  $r = 0$ .

#### Part IV: Proof of Result 5

For general  $r$ , we can reorganize  $M(1)$  to have the sign of the product  $K \cdot L$  where

$$K = (1 - r)^2 (1 - 2\mu)^2 (w_1 - w_3)^2 \left[ (w_1 + w_3)^2 + 16w_1w_3 \right] \quad (\text{E45})$$

and

$$L = 2(w_1 + w_3)^4 - \left\{ (1 - r)^2 (1 - 2\mu)^2 \left[ (w_1 + w_3)^4 + 16w_1^2 w_3^2 \right] \right\}. \quad (\text{E46})$$

Now

$$\begin{aligned}
L &> 2(w_1 + w_3)^4 - \left\{ (w_1 + w_3)^4 + 16w_1^2w_3^2 \right\} \\
&= (w_1 + w_3)^4 - 16w_1^2w_3^2 \\
&= \left[ (w_1 + w_3)^2 - 4w_1w_3 \right] \left[ (w_1 + w_3)^2 + 4w_1w_3 \right] \\
&= (w_1 - w_3)^2 \left[ (w_1 + w_3)^2 + 4w_1w_3 \right] \\
&> 0.
\end{aligned} \tag{E47}$$

Since  $K$  is obviously positive, we conclude that  $M(1) > 0$  for all  $r \in (0, 1)$  when  $\mu_M = \frac{1}{2}$ . Thus  $\mu_M = \frac{1}{2}$  is not invadable for all  $r$ .