

# Waiting for a compensatory mutation: phase zero of the shifting-balance process

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*(Received 9 May 1995 and in revised form 12 January 1996)*

## Summary

In highly integrated genetic systems, changes in any one component may have a deleterious effect on fitness, but coordinated, or compensatory, change in these components could lead to an overall increase in fitness compared with the current state. Wright designed his shifting-balance theory to account for evolutionary change in such systems, since natural selection alone can not lead to the new optimal state. A largely untreated aspect of the shifting-balance theory, that of the limiting impact of waiting for the production of new mutations, is analysed here. It is shown that the average time to double fixation of compensatory mutations is extremely long (of the order of tens or hundreds of thousands of generations), because selection is too effective in large populations, and mutations are too rare in small populations. Further, the probability that a new mutant will arise and undergo fixation quickly is extremely small. Tight linkage can reduce the time to fixation somewhat, but only in models in which the double heterozygote does not have reduced fitness. It is argued that the only reasonable way for compensatory mutations to become fixed in a population is if the new mutants are first allowed to achieve a moderate frequency through the relaxation of selection. Under these conditions, the time required to reach fixation is reasonably low, although the probability of being fixed is still small when the initial allele frequencies are low. It is likely that the waiting time for fixation of new mutants, which is here called phase zero, is the major limiting factor for the success of the shifting-balance process.

## 1. Introduction

The notion that organisms are highly integrated functional systems has long held intuitive appeal to biologists. The existence of gene regulation and developmental interactions certainly support this view. One consequence of functional integration is that changes in one component of the system can lead to degradation of the system as a whole without simultaneous changes in other interacting components. For example, at the molecular level, an amino acid change in a regulatory protein might disrupt a regulatory pathway without a compensatory change in the promoter region of the gene being regulated (Tjian, 1995). There is some evidence that change of this type has led to correlated evolution at the nucleotide level (e.g. Stephan & Kirby, 1993). The difficulty of evolution in a system in which change in

individual components is separately deleterious but jointly advantageous, is that natural selection can never lead to the new advantageous state because this would necessitate that populations first move through deleterious states.

Sewall Wright created his famous shifting-balance theory of evolution in large part to deal with this problem (Wright, 1931, 1932). Wright's study of physiological and developmental genetics led him to conclude that gene interaction should be the norm in biological systems, and he felt that evolutionary change would be severely constrained if these interacting systems were prevented from evolving because of the limitations of deterministic selection. Populations would become stranded at a local optima or peaks in fitness, and would never be able to reach higher global optima. Wright's solution to this problem involved three phases. First, genetic drift would lead to a random increase in allele frequencies that would initially overcome the counterbalancing force of selection. Secondly, once the new interacting alleles became relatively more common than the original alleles, mass selection would push the popu-

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lation towards the new peak. Thirdly, populations that had successfully undergone the peak shift would then send migrants to other populations, thereby upgrading them in fitness. Various studies have investigated one aspect of the theory or another (Rutledge, 1970; Wright, 1977; Lande, 1985; Crow *et al.* 1992; Kondrashov, 1992; Phillips, 1993; Barton & Rouhani, 1993; Rouhani & Barton, 1993; Moore & Tonsor, 1994), but none have asked the seemingly fundamental question of where the variation necessary for starting the shifting-balance process comes from in the first place. The shifting-balance theory, therefore, requires another phase, phase zero, that consists of the generation of the mutations responsible for initiating the process.

Wright usually began his discussions of the shifting-balance theory at the migration–selection–drift equilibrium (e.g. Wright, 1977). He tended not to consider the time required to reach that equilibrium, presumably because dynamic solutions to his equations are much more difficult to come by, and, somewhat paradoxically, because Wright did not believe in strict equilibria; he felt that there would always be some genetic variation available to migrate into a population (J. F. Crow, personal communication). This is not really a paradox because Wright believed in the power of a dynamic equilibrium, such as that described by the equilibrium *distribution* of gene frequencies, more than the static equilibrium implied by all populations being fixed for a particular gene. Nonetheless, genetic variation must come from somewhere, and before the dynamic equilibrium can be achieved, the static equilibrium must be broken. In the shifting-balance theory, this means that at least one population must make the transition between peaks before the other phases can proceed, and this population can not undergo such a transition until mutations for the new advantageous alleles arise within it. Waiting for these mutations could take a long time.

The new variation for the advantageous genotype can enter the population in three ways. First, double mutation could lead to the *de novo* creation of the new gamete. This should be a very rare event (of the order of the square of the mutation rate) that would not be expected to be very important in most circumstances. Secondly, populations could reach fixation for each new allele sequentially. As discussed below, this involves the fixation of a temporarily deleterious allele, and would not be expected to be important unless population sizes are very small and/or selection is very weak. Finally, new deleterious mutations could simply segregate in a population via mutation pressure and drift until a mutation at the other locus allows both new alleles to come into contact in the same gamete. The alleles would then drift together across the valley until being driven by both drift and selection towards fixation at the new peak.

Here I will first outline two fitness models that can be used to depict multiple-peak gene interactions at

two loci. I will then present several methods for solving the two-locus drift problem inherent in the shifting-balance process. Two basic scenarios will be addressed. First, it will be assumed that a population starts at complete fixation, and the time to fixation under recurrent mutation will be studied. Secondly, the probability that a shift will occur in the presence of some initial variation, as might be expected after period of relaxed selection, will be calculated. In the end, it appears that initiating the shifting-balance process is difficult and time consuming, and is likely to be a major impediment to the applicability of the shifting-balance theory to natural populations.

## 2. The model

Two qualitatively different kinds of fitness models will be used (Table 1). Both models are based on a diploid gene interaction system with two loci, each with two alleles, but display different types of epistasis. In the first, the ‘deleterious intermediates’ model, any genotype composed of mismatched alleles at the two loci has reduced fitness,  $1-s$  (Haldane, 1931; Kondrashov, 1992). In the second, the ‘metabolic pathway’ model, the double heterozygote does not suffer from reduced fitness, as might be the case if the genes were sequentially involved in a metabolic pathway or when stabilizing selection acts on an additive character (for a general argument see Phillips, 1993). This model also allows for a range of dominance relationships within a locus with complete multipeak epistasis between loci (Table 1; Crow *et al.* 1990; Phillips, 1993).

Evolutionary change is assessed by tracking the frequencies of the four possible gametes, with  $x_1, x_2, x_3$  and  $x_4$  respectively representing the frequencies of gametes  $AB, AB', A'B$  and  $A'B'$ . Under the assumption of random mating, gamete frequency change is

Table 1. *Genotypic fitnesses*

	<i>AA</i>	<i>AA'</i>	<i>A'A'</i>
<b>Deleterious intermediates model</b>			
<i>BB</i>	$1+t$	$1-s$	$1-s$
<i>BB'</i>	$1-s$	$1-s$	$1-s$
<i>B'B'</i>	$1-s$	$1-s$	1
<b>Metabolic pathway model</b>			
Dominant			
<i>BB</i>	$1+t$	$1+t$	$1-s$
<i>BB'</i>	$1+t$	$1+t$	$1-s$
<i>B'B'</i>	$1-s$	$1-s$	1
Additive			
<i>BB</i>	$1+t$	$1+(t-s)/2$	$1-s$
<i>BB'</i>	$1+(t-s)/2$	$1+t/2$	$1-s/2$
<i>B'B'</i>	$1-s$	$1-s/2$	1
Recessive			
<i>BB</i>	$1+t$	$1-s$	$1-s$
<i>BB'</i>	$1-s$	1	1
<i>B'B'</i>	$1-s$	1	1

governed by the standard two-locus equations (Kimura & Ohta, 1971):

$$\Delta x_i = x'_i - x_i = \{x_i(w_i - \bar{w}) \pm r w_{14} D\} / \bar{w} \tag{1}$$

where the sign is positive when  $i = 1$  or  $2$  and negative when  $i = 3$  or  $4$ ,  $w_i = \sum_{j=1}^4 x_j w_{ij}$ ,  $r$  is the recombination rate,  $D = x_1 x_2 - x_3 x_4$  is the linkage disequilibrium parameter, and  $\bar{w} = \sum_{j=1}^4 x_j w_j$ .

(i) *Mutation and initial variation*

Populations begin in one of two possible states. In the first case examined, populations are assumed to be fixed for the  $A'B'$  gamete, with any variation in the population being generated by spontaneous mutation (this is the 'dynamic' case of Michalakis & Slatkin, 1996). In the second case, populations will be assumed to have some initial genetic variation, and mutation will not be included in the model ( $v = 0$ ; this is akin to the 'static' case of Michalakis & Slatkin, 1996).

Mutation is assumed to be unidirectional, with change from  $A'$  to  $A$  and  $B'$  to  $B$  occurring at rate  $v$ . Studying unidirectional mutation has the advantage that in a finite population there is no question of whether the new mutant will be fixed, but only of how long it will take. Since the focus of the paper is the fixation of new mutants within a single population, the existence of back-mutations only serves to make present results more conservative. As outlined below, however, unidirectional mutation can generate potentially misleading results if the relative magnitudes of mutation and selection are not accounted for. The life-cycle takes the order:

mutation  $\rightarrow$  random sampling of gametes  $\rightarrow$   
 recombination  $\rightarrow$  selection.

Gamete frequency change and the time to fixation are assessed using three separate methods: two-dimensional Kolmogorov backward equations, a two-locus Fisher-Wright Markov model, and simulations of finite populations.

(ii) *Diffusion approach*

The effects of both selection and drift on the probability of and time to fixation can be most precisely described for moderately large populations using the two-locus Kolmogorov backward diffusion equations first presented by Kimura (1955; Crow & Kimura, 1956), although I will be following the treatment of Takahata (1982), throughout. In principle, it is possible to construct set of diffusion equations for the two-locus case with arbitrary degrees of recombination, but this would unfortunately necessitate solving the equations in three dimensions. By analysing only the special cases of free recombination ( $r = 0.5$ ) and no recombination ( $r = 0$ ) the

solution can be reduced to a two-dimensional problem, but even here no general analytical solution exists. We are therefore forced into numerical solutions of the partial differential equations. For the case of free recombination, the equation becomes (Kimura, 1964):

$$\frac{\partial u}{\partial T} = \frac{p(1-p)}{4N_e} \frac{\partial^2 u}{\partial p^2} + \frac{q(1-q)}{4N_e} \frac{\partial^2 u}{\partial q^2} + M_{\delta p} \frac{\partial u}{\partial p} + M_{\delta q} \frac{\partial u}{\partial q}, \tag{2}$$

where  $T$  is a measure of generations,  $u$  is the probability of fixation as a function of  $p$  and  $q$ ,  $N_e$  is the effective population size,  $p = x_1 + x_2$ ,  $q = x_1 + x_3$ ,  $M_{\delta p} = v(1-p) + \Delta x_1 + \Delta x_2$ , and  $M_{\delta q} = v(1-q) + \Delta x_1 + \Delta x_3$  (mutational effects of the order of  $v^2$  are ignored here). Equation (2) neglects the effects of linkage disequilibrium, including that which might be generated directly by selection. These effects can be included in the diffusion model (Ohta & Kimura, 1969; Fu & Arnold, 1992), but the equation is much more difficult to solve. In practice, ignoring linkage disequilibrium yields results that are very similar to the exact Markov and simulation approaches described below (see also Littler, 1973), so this assumption does not appear to be too severe. As selection becomes very strong, we would expect the generation of linkage disequilibrium, and there is evidence from the simulations that the diffusion equation begins to underestimate the time to fixation for the deleterious intermediates model and to overestimate it for the metabolic pathway model (Fig. 1).

For complete linkage and a symmetrical fitness model, the frequencies of gametes  $AB'$  and  $A'B$  change in concert, which after defining a new parameter  $y = x_2 + x_3$ , yields the diffusion equation (Takahata, 1982)

$$\frac{\partial u}{\partial T} = \frac{x_1(1-x_1)}{4N_e} \frac{\partial^2 u}{\partial x_1^2} - \frac{x_1 y}{2N_e} \frac{\partial^2 u}{\partial x_1 \partial y} + \frac{y(1-y)}{4N_e} \frac{\partial^2 u}{\partial y^2} + M_{\delta x_1} \frac{\partial u}{\partial x_1} + M_{\delta y} \frac{\partial u}{\partial y}, \tag{3}$$

where

$$M_{\delta x_1} = vy + \Delta x_1 \text{ and } M_{\delta y} = v(2 - 2x_1 - 3y) + \Delta x_2 + \Delta x_3.$$

Equations (2) and (3) were solved numerically by approximating them with difference equations and solving them respectively on a square and a triangular gene frequency grid as described in Takahata (1982). For eqn (2), minimum grid size was  $40 \times 40$ . The equation was solved by successive iterations with initial conditions  $p = q = 1$  and  $u(1, 1) = 1$ , until fixation probabilities over the grid were stable to  $10^{-4}$  over successive iterations. Minimum grid size was  $(50 \times 50)/2$  over a triangle for eqn (3), with initial conditions  $x_1 = 1$ ,  $y = 0$ , and  $u(1, 0) = 1$ . Average time to fixation was determined by using the same difference method to approximate the integral

$$\bar{T} = \int_0^\infty u(0, 0) dT. \tag{4}$$

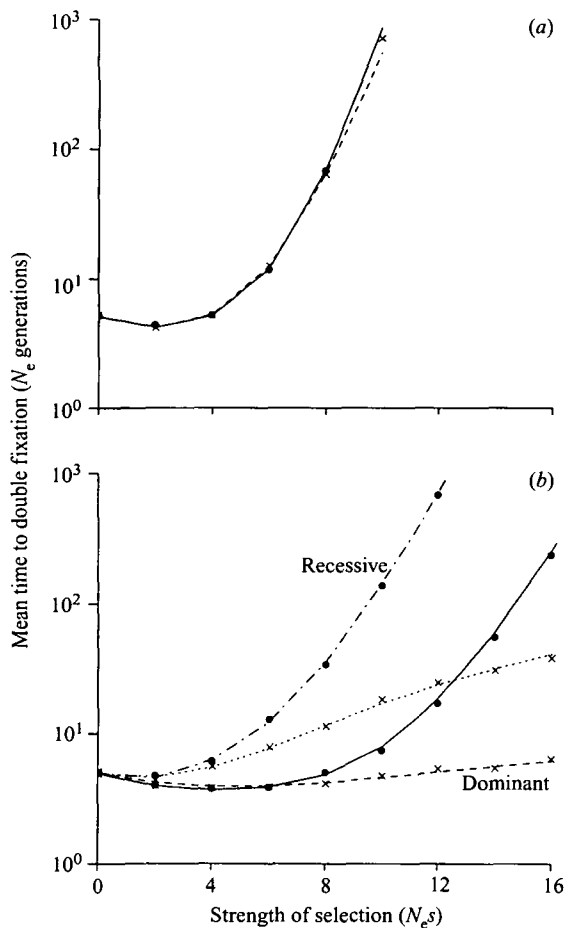


Fig. 1. Mean time to double fixation (a peak shift) as a function of the strength of selection. Lines show results from a diffusion approximation, points are from the mean of 500 simulation runs. (a) Deleterious intermediates model ( $2N_e v = 1$ ). Time to fixation increases rapidly as selection increases, although recombination rate has little influence until selection is very strong. Continuous line and circles,  $r = 0.5$ ; dashed line and crosses,  $r = 0$ . (b) Metabolic pathway model. Time to fixation again increases with selection, although dominant mutations become fixed more quickly than recessive ones. Recessive: dot-dashed line,  $r = 0.5$ ; dotted line,  $r = 0$ . Dominant: continuous line,  $r = 0.5$ ; dashed line,  $r = 0$ . Strength of selection, mutation pressure and time to fixation are all expressed relative to the effective population size. This is a neutral compensatory model in which  $N_e t = 0$ .

A similar approach using exact Fisher–Wright Markov equations is presented in the Appendix.

### (iii) Simulation approach

The accuracy of the approximations and assumptions used above were verified using Monte Carlo simulations. The simulations were semi-deterministic in that gamete frequency change generated by selection and mutation were determined using eqs (1) and (A 2). Sampling of gametes in the drift process was performed by individual sampling whenever  $N_e < 20$ ,  $x_i N_e < 3$ , or  $(1 - x_i) N_e < 3$ . In other cases, however, a pseudo-sampling method (Kimura, 1980; Kimura &

Takahata, 1983) was used to simulate drift at intermediate gamete frequencies. This involves drawing a random number once for each gametic type rather than once for each individual in the population, and provides an excellent approximation to the full sampling method as long as full sampling is used under the conditions described above (Kimura & Takahata, 1983). The accuracy of these simulations was in turn checked by a completely stochastic simulation of the entire process, but this took several orders of magnitude longer to complete. Simulation results obtained here agree almost exactly with the analytical methods described above, as well as the results obtained by a different simulation method in Michalakis & Slatkin (1996).

### 3. The waiting time for compensatory mutations

I will first present results for the time to fixation while waiting for recurrent mutations, and then analyse the probability of a peak shift when starting in a population with some initial genetic variation.

We will first envision a population that is completely fixed for alleles at two loci that put it at the top of one fitness peak. One-way mutation then introduces variation into the population, genetic drift eventually moves the population through the adaptive valley, and selection finally pushes the population to the other peak. Under recurrent one-way mutation in a finite population, it is not a question of whether a population will eventually go to fixation at the new peak, but only of how long it will take. The most obvious measure of this is the mean time to double fixation (eqn 4).

One nice attribute of using the diffusion approach to solving these problems is the realization that by multiplying both sides of eqns (2) and (3) by the effective population size, fixation time, mutation pressure and selection can all be scaled in units of  $N_e$ . A solution in this form is presented in Fig. 1. Here the mutation rate is assumed to be  $2N_e v = 1$ , which means that there is on average one new mutation entering the population every generation. (This is probably a very high mutation rate, but it is used for comparison with Kimura (1985a, b). As will be shown below, lower mutation rates lead to much longer times to fixation.) With weak selection, the average time to a peak shift is less than  $10N_e$ . As selection increases in strength, the average time to a shift increases exponentially, although the relative rate depends on both the fitness model and the recombination rate. As might be predicted, the deleterious intermediates model leads to much longer shift times than the metabolic pathway models, but surprisingly the effect of recombination is much different in the two models. In the deleterious intermediates model, recombination rate has little effect until selection is quite strong, whereas reduced recombination greatly reduces the peak shift time for the metabolic pathway models.

This is caused by a difference in the fitness of double heterozygotes in the two models. In the deleterious intermediates model, even if an  $AB$  gamete is created via mutation and that gamete can not be broken down by recombination, selection will still act against the gamete because it will mostly be combining with  $A'B'$  gametes, yielding a fitness of  $1 - s$ . Under the metabolic pathway model, however, an unrecombining  $AB$  gamete will also be primarily paired with  $A'B'$  gametes, but since in this model double heterozygotes have fitness greater than or equal to 1, they will eventually be selected for. In the latter model, then, movement to the new peak is deterministic and does not require restricted population sizes when there is tight linkage. The distinction between these two types of models only appears in the diploid case, and has not been noted before in previous treatments, which have been based exclusively on haploid models (Takahata, 1983; Kimura, 1985*a, b*; Michalakis & Slatkin, 1996).

#### (i) Effects of mutation pressure

The fact that fixation under the metabolic pathway model with restricted recombination is essentially deterministic raises the concern that we might not really be studying true peak-shift models in these cases. The problem arises when the mutation rate is large relative to the strength of selection, literally pushing the population across the adaptive valley via mutation pressure. Much the same thing can occur when migration rates overcome the effects of selection during phase three of the shifting-balance process (Crow *et al.* 1990; Barton, 1992; Kondrashov, 1992; Phillips, 1993). Indeed, the effects of dominance shown in Fig. 2*b* are the result of the same phenomena that allow more dominant gametes to invade during phase three of the shifting-balance process (Barton, 1992; Phillips, 1993).

I investigated the problem of deterministic transitions by calculating the critical mutation rate such that iterating eqn (A 2) leads to a direct traversal of the adaptive valley in the absence of drift. As was previously found in the case of migration (Crow *et al.* 1990; Barton, 1992), this rate is roughly one-tenth the strength of selection (results not shown). Under the mutation pressure present in Fig. 1, this would imply a critical selection threshold of roughly  $N_e s = 5$  – exactly the point at which the curve moves precipitously towards longer fixation times.

I consider the lower fixation times achieved when the mutation rate is high relative to the strength of selection to be an artifact of the one-way mutation model, which does not adequately reflect the peak shift problem at hand. In this light, Kimura's (1985*a, b*) conclusion that compensatory neutral mutations can evolve via drift in a reasonable amount of time when recombination is restricted must be re-evaluated, since Kimura based his conclusions on results that are essentially identical to those presented

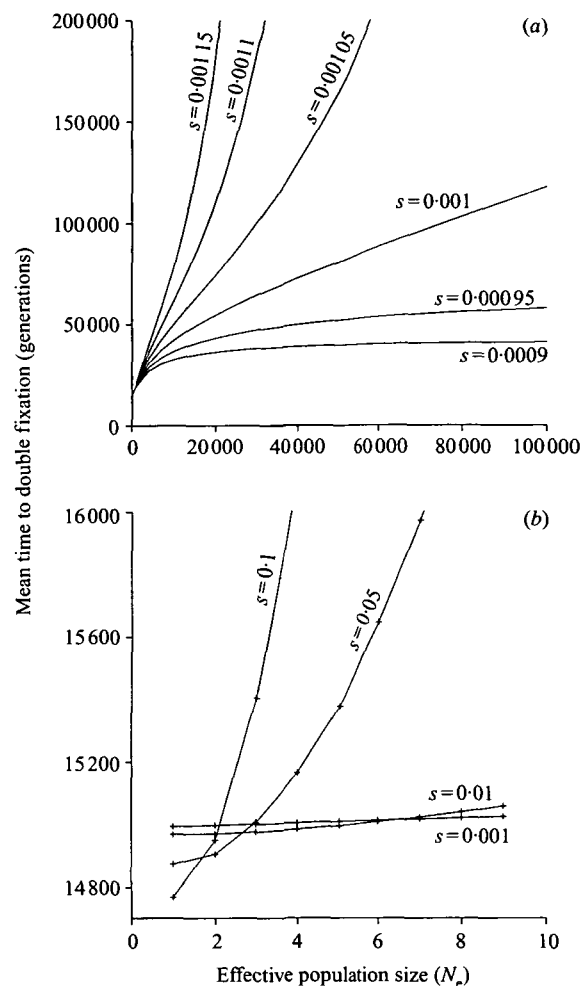


Fig. 2. Average time to double fixation for the deleterious intermediates model. The mutation rate is  $10^{-4}$ ,  $r = 0.5$ , and  $t = 0$ . (a) Large population results using the diffusion approximation. Note the large upswing in fixation times as selection exceeds the critical value,  $s = 0.001$ , for this case. (b) Small population results using the exact Markov model. Selection has little effect unless it is very strong. Time to fixation is mostly constrained by waiting for mutations to arise.

in Fig. 1 (but for a haploid model). Compensatory mutations can evolve quickly under these conditions, but drift has nothing to do with it. The root of this problem is that selection and generation time scale well with population size, but mutation rates are more naturally considered to be fixed at a particular value. Therefore, in the following results, I will fix the mutation rate, and scale the results in terms of the absolute number of individuals and the actual number of generations.

#### (ii) Fixation time and population size

Moderately large populations on average take a very long time to make the peak shift (Fig. 2). As one moves even slightly away from the critical selection rate ( $s = 0.001$  in this case), the average time to double fixation becomes extremely long (Fig. 2*a*). There is a monotonic increase in the time to fixation with

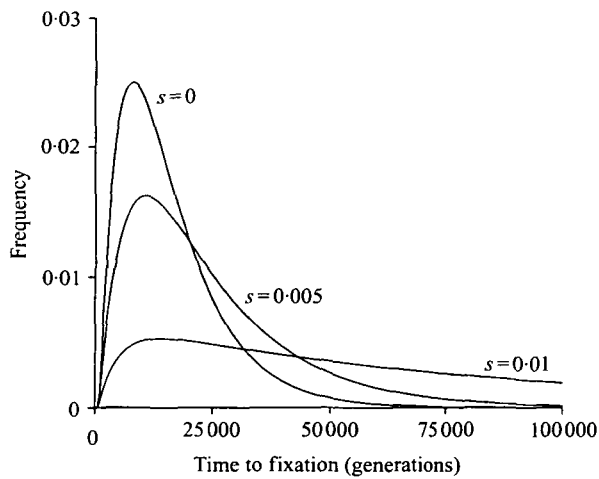


Fig. 3. Distributions of the time to double fixation for the deleterious intermediates model. The mutation rate is  $10^{-4}$ ,  $r = 0.5$  and  $t = 0$ . Note that the right tail of the distribution becomes very skewed as selection increases, but the left tail reveals that some populations will still reach fixation in a much shorter time than an average population.

population size, but even in very small populations the fixation times are very long relative to the expected demographic persistence of a population that small (Lande, 1988; Fig. 2*b*).

Very small populations tend to be mutation bound, such that only very strong selection has much of an effect on the time to fixation (Fig. 2*b*). Very small populations often undergo sequential fixation of the advantageous alleles (Markov results not shown), while larger populations ( $N_e > 20$ ) primarily undergo joint drift through the adaptive valley (simulation and diffusion results not shown). At the appropriate critical strength of selection, the time to fixation increases approximately one order of magnitude for each order of magnitude drop in the mutation rate (results not shown). For a fixed amount of selection, we would expect the time to fixation to be proportional to  $v^{-2}$  (Gillespie, 1984). Thus, with a mutation rate of  $10^{-5}$ , we would expect the fixation times to be more of the order of  $2 \times 10^5$  to  $2 \times 10^6$  generations rather than the  $2 \times 10^4$  generations shown in Fig. 2. Clearly, peak

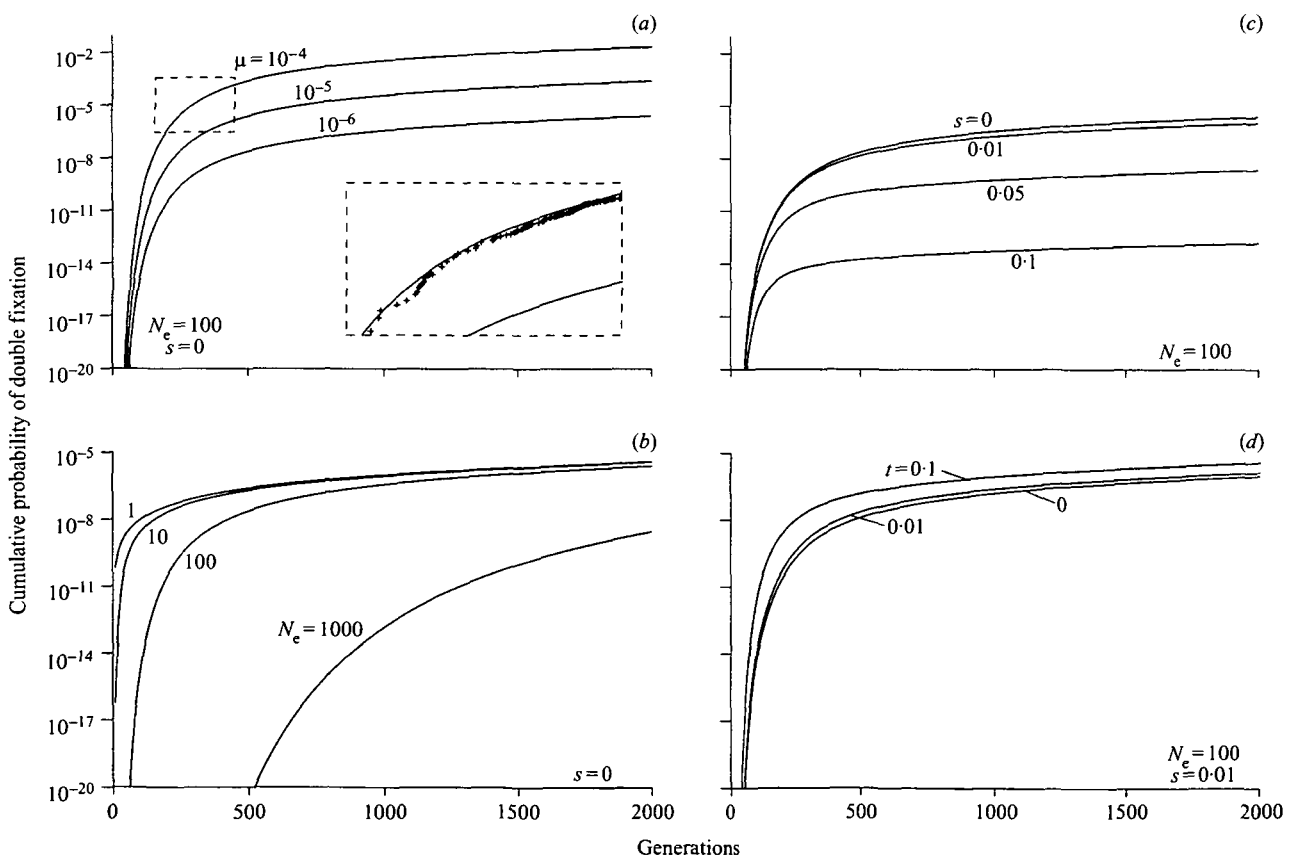


Fig. 4. Cumulative probabilities of fixation for the deleterious intermediates model. Mutation rate is  $10^{-6}$  and  $t = 0$  except where noted. Curves are the integral from time zero to the time on the  $x$ -axis of distributions such as that shown in Fig. 3, and show the probability of achieving a peak shift on or before a given time. Curves were calculated using the diffusion approximation, except where noted. (a) Effects of variation in mutation rate for a completely neutral model. Inset shows a region of the curve reconstructed from  $10^7$  simulation runs. Decreased mutation rates greatly lessen the chance that a peak shift will occur in a reasonable amount of time. (b) Effects of increasing population size in a neutral model. Rapid drift is most important for quick fixation, so the probability of fixation decreases with increasing population size. Cases for one and ten individuals were calculated using the Markov model. (c) Effects of increasing selection against intermediates. Again, since drift dominates the early part of the process, selection has little effect unless it is very strong. (d) Effects of increased selection favouring the new genotype. Increasing the height of one peak relative to the other increases the probability of undergoing a shift, but again, only when selection is very strong. In all, the probability of achieving a peak shift in a short time is very low.

shifts are not occurring very often, and the expectations is that they will be very rare and unimportant relative to demographic considerations (Lande, 1988) for most populations. It is conceivable, however, that they could still be important for a few rare populations.

(iii) Fixation time distributions

Distributions of the time to double fixation tend to have extremely long tails (Fig. 3). Thus, even as the average time to fixation grows very long when selection intensity increases, there is a certain proportion of the tail of the distribution that makes the transition relatively more quickly. Since Fig. 2 shows that quick transition times are only likely to be found in small populations, I will concentrate on the extreme left tail of the distribution. Fig. 4 shows the cumulative probability of making the peak shift within a few thousand generations. Peak shifts at this end of the distribution are definitely dominated by the waiting time to mutation, as selection has little effect unless it is very strong (Fig. 4c). Increasing the selective advantage of the new peak does increase the peak shift probability, but still does not make it large on an absolute scale (Fig. 4d). Overall, the cumulative probabilities are quite small, not exceeding  $10^{-5}$  for reasonable parameter values.

5. Peak shifts after periods of relaxed selection

Since waiting for the appropriate mutations makes peak shifts starting from a fixed equilibrium take so long, it is of interest to see how likely a peak shift is and how long one might take if there is some initial variation in the population. This might be the case

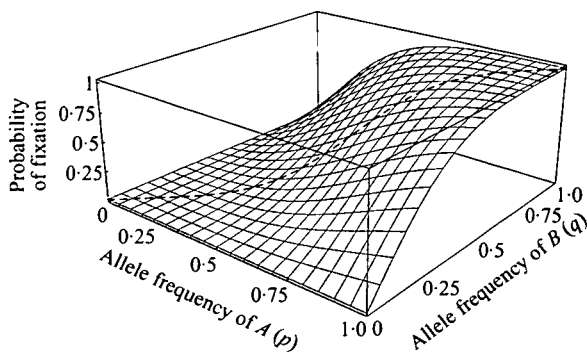


Fig. 5. Probability of double fixation with initial variation for the deleterious intermediates model. The  $x$  and  $y$  axes give the starting frequency of the alleles at the new peak under the assumption of free recombination and no linkage disequilibrium. The  $z$ -axis gives the probability of fixation based on starting at these initial allele frequencies. Probabilities are low at one side of the adaptive valley, but increase sharply when starting near the new peak. The diagonal describes the line depicting the probability of fixation as a function of the line  $p_A = p_B$ . Here,  $N_e s = 2$  and  $t = 0$ .

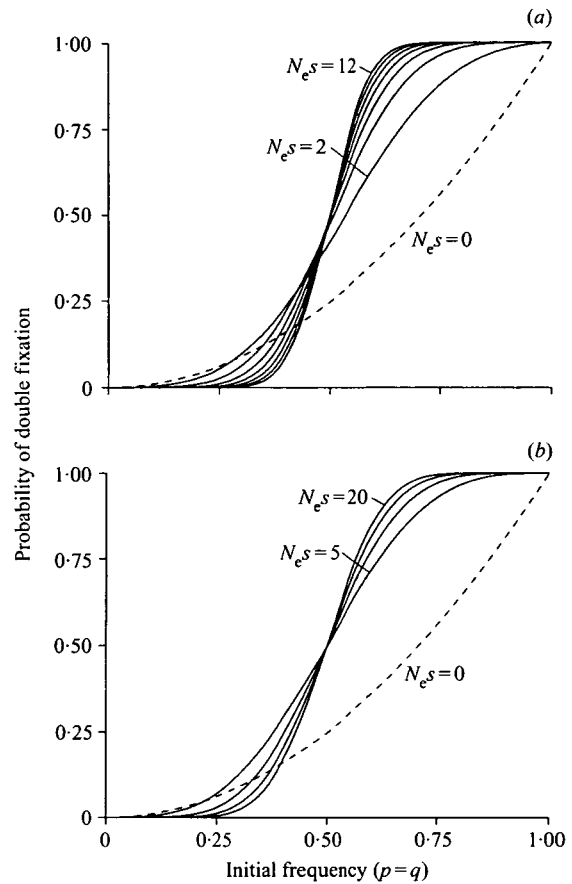


Fig. 6. Probability of double fixation with initial variation along the line  $p_A = p_B$  (see Fig. 5). (a) Deleterious intermediates model. Continuous lines show effects of increasing amounts of selection in increments of  $2N_e s$ . (b) Additive metabolic pathway model. Continuous lines show selection increments of  $5N_e s$ . In both cases, solutions are based on the diffusion approximation with  $t = 0$ .

after periods of relaxed selection or when variation is maintained via mutation–selection balance in a large population, for example. Here the primary question is whether the population will drift across the valley and reach the new peak, or become fixed at the original peak. There are again  $\binom{2N_e + 3}{3}$  different possible

states in which the population could start. One possible representation of the probability of ultimate fixation at the new peak is to plot the probability surface as a function of initial gene frequency, as is done in Fig. 5. For the models used here, these surfaces tend to be completely symmetrical, and so can be fairly well described by the height of the surface at the diagonal described by the line  $p_A = p_B$  (Fig. 5).

As might be expected, the probability of a peak shift is low when the population starts on the far side of the valley, but very high when starting near the new peak (Fig. 6). Weak selection yields a moderate chance of ultimate fixation, even when initial gene frequency is low, but as selection increases, the probability of fixation is greatly diminished when the population

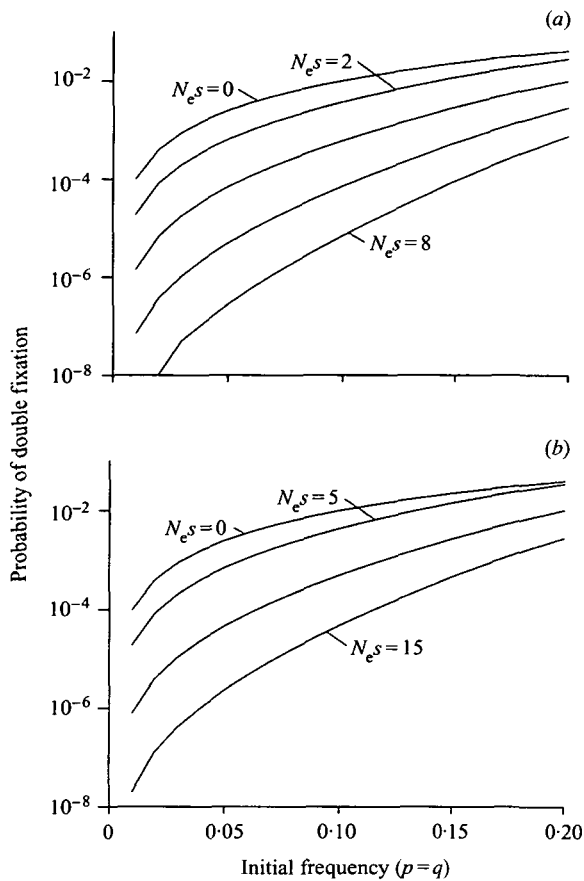


Fig. 7. The extreme left-hand tail of Fig. 6. (a) Deleterious intermediates model. Lines are in steps of  $2N_e s$ . (b) Additive metabolic pathway model. Lines are in steps of  $5N_e s$ . Note the log scale.

starts on the wrong side of the valley. This decrease is particularly pronounced when the initial frequency is low, say less than 0.1 (Fig. 7). Increasing the selective difference between the peaks ( $t$ ) shifts the minimum point of the valley towards the original peak, thereby increasing the probability of a peak shift for lower initial gene frequencies, but this does not greatly affect the probabilities at the far left of the figures (results not shown). Thus, the probability of a population undergoing a peak shift remains extremely small unless the new alleles at both loci have managed to accumulate to moderately high frequencies before selection is initiated. It should also be pointed out that the probabilities shown in Figs. 6 and 7 are the maximum values obtained when  $p_A = p_B$ . In general, the overall probability of a shift will be much lower if one or the other of the alleles is not present at an equivalently high frequency.

If the populations do ultimately manage to make the shift, they do so quite quickly, regardless of the initial frequency or strength of selection (Fig. 8). In fact, strong selection is expected to yield very fast transition times. This is because, although the probability of actually making a shift is quite low with strong selection, those populations that do make the shift must move across the valley quickly, and once

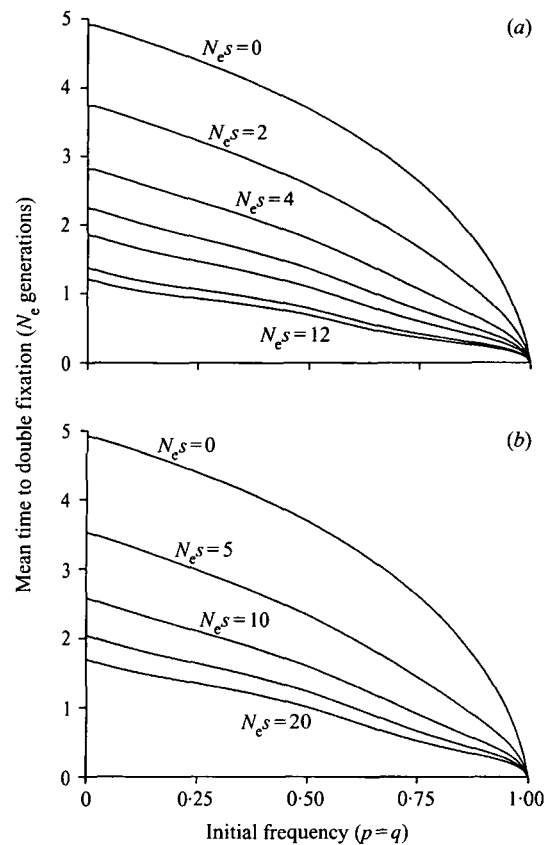


Fig. 8. Mean time to double fixation scaled in terms of  $N_e$  generations. (a) Deleterious intermediates model. (b) Additive metabolic pathway model. Notation as in Fig. 6. Peak shifts, when they occur, take place fairly rapidly.

there are rapidly fixed by selection. A similar effect of strong selection has been found in phenotypic models of peak shifts (Newman *et al.* 1985; Lande, 1986; Rouhani & Barton, 1987).

Tight linkage might be expected to aid in the shift process, but whether or not it does depends on the amount of linkage on the amount of linkage disequilibrium initially found in the population. If there is no linkage disequilibrium in the initial population, then strong linkage results in essentially the same probabilities as no linkage (Fig. 9). A population that starts out with a large negative linkage disequilibrium has few advantageous gametes present, and therefore is very unlikely to undergo a peak shift. A large positive linkage disequilibrium can increase the chance of ultimate fixation if there is reduced recombination. How much the probability is increased depends on the fitness model. In the metabolic model, tight linkage means that  $AB$  gametes are not broken up by recombination. Since these gametes are advantageous against a  $A'B'$  background, they will be directly selected for when there is positive linkage disequilibrium. The metabolic model is very similar to a haploid model in this respect. Under the deleterious intermediates model, however, tight linkage does not really benefit the  $AB$  gamete since it is deleterious against all but another  $AB$  background (Fig. 9). The



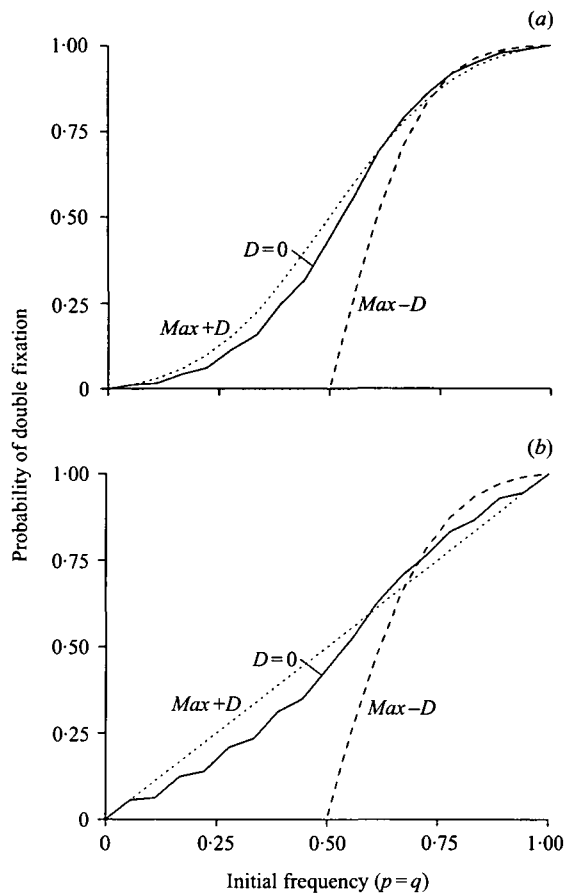


Fig. 9. Effects of limited recombination and initial linkage disequilibrium on the probability of double fixation. Each line represents an exact solution to the Markov model (eqn 8) with  $N_e = 9$ ,  $r = 0$ ,  $N_e s = 2$  and  $t = 0$ . Line for  $D = 0$  means that the population was started with no linkage disequilibrium. *Max + D* means that populations were started with the maximum amount of positive linkage disequilibrium possible for that set of allele frequencies. *Max - D* means that populations were started with the maximum amount of negative linkage disequilibrium (or the minimum achievable linkage disequilibrium) possible for that set of allele frequencies. The effect of initial positive disequilibrium is much more pronounced in the additive metabolic pathway model (b) than in the deleterious intermediates model (a). Effects in populations with free recombination are negligible.

effect of the fitness model found here parallels that found using the directional mutation approach (Fig. 1). Michalakis & Slatkin (1996) have looked at the relationship between recombination rate and selection in much more detail. They have found that if the *AB* gamete is present in the population at all, then the probability of fixation is increased by reducing the recombination rate as much as possible. In the presence of negative (repulsion) linkage disequilibrium, however, they found that there is actually an intermediate amount of recombination that optimizes the probability of fixation. Some recombination is needed to create the beneficial gametic type, but too much recombination leads to the eventual break-up of that genotype.

## 6. Discussion

The observation that base-pair changes within genes and interacting systems are often correlated at the phylogenetic level (e.g. Fitch & Markowitz, 1970; Tsukihara *et al.* 1982; Brimacombe, 1984; Stephan & Kirby, 1993) has led to the speculation that this correlated pattern might be driven by 'compensatory' evolution (Kimura, 1985*a, b*, 1990). If changes in each separate component are individually deleterious, then selection can not by itself lead to the joint evolution of the components. This is essentially the same type of situation that led Wright to propose his shifting-balance theory of evolution (Wright, 1931, 1932). The results presented here show that the very first step in this process, the generation of new mutants necessary for the coordinate evolution of the epistatic alleles, can take so long that the feasibility of the process as a whole is questionable. It is really the interaction between waiting for the new mutants and the constant elimination of these mutants via natural selection that leads to the extremely long times to fixation.

It could be argued that studying phase zero in a single population is unfair to the shifting balance theory since Wright envisioned thousands, perhaps tens of thousands, of subpopulations being subject to drift. However, any mutational variation arising at a single locus in another population would be extremely unlikely to have much of an impact on another population, since almost all migrants entering a population will be of the fixed gametic type. Migration would therefore tend to act analogously to an extremely strong back-mutation rate. Thus the times to fixation presented here, although extremely long, are actually very favourable to the shifting-balance process since any back mutation or migration would extend the process even further. Even so, the cumulative probability of achieving a peak shift in a reasonable amount of time (say before the population goes extinct or variation in migration destroys population structure) is extremely small (Fig. 4).

The low probability of achieving a peak shift must be weighed against several other factors before one can determine whether it is reasonable to expect peak shifts under this model. First, it matters whether one is interested in a particular epistatic gene combination, or whether a shift at any gene combination will do. For example, in speciation, it is not any one epistatic combination that matters, but the totality of all combinations that lead to reproductive isolation (Orr, 1995). We really do not know how general epistatic interactions of the type modelled here might be, although there are reasons to believe that they could be common (Whitlock *et al.* 1995). Secondly, the number of populations in temporary isolation also matters. If the overall population is being subdivided into thousands of demes, then each deme has a transient chance of undergoing a peak shift. Finally, if

there are repeated cycles of population subdivision and isolation, then the frequency of these cycles will determine the likelihood that one of the populations will undergo a peak shift within some finite period. The number of populations expected to undergo a peak shift within a specified time is then the product of these three factors times the cumulative probability that one of the populations will make it. This implies that some combination of gene number, population number and cycle rate must exceed 10 000 (or perhaps many orders of magnitude greater than that). Even then, the rest of the shifting balance process would have to proceed before the peak shift was fixed within the population as a whole.

Phase zero would therefore seem to present a major hurdle, if not *the* major hurdle, to initiating the shifting-balance process in a population at a fixed equilibrium. Allowing the populations to begin with at least some initial variation can greatly speed up the peak shift process, although peak shifts will still usually be unlikely when the initial frequency is low. The increase in initial frequency is probably going to have to be caused by a relaxation of selection before subdivision. In a very large population, the frequency of the favourable alleles will be determined primarily by a mutation–selection balance unless linkage is very tight (Fig. 2). The expected allele frequencies before subdivision would therefore be approximately  $v/hs$  (or  $\sqrt{v/s}$  when  $h = 0$ ; Crow & Kimura, 1970), where  $h$  is the dominance coefficient ( $h = 1$  for the deleterious intermediate model). This frequency will generally be low unless selection is weak, so on average a subpopulation derived from such a population would have a small probability of undergoing a peak shift (Fig. 7). This is only the expectation, and some populations will drift to higher frequencies, but it is still unlikely that a peak shift will occur unless the frequency of the alleles in the large population is reasonably high. This would only tend to happen if the pattern of selection were relaxed, or perhaps even reversed, prior to population subdivision.

Such a model is consistent with the verbal arguments for ‘founder-flush’ speciation via the breakup of ‘co-adapted gene complexes’ (this is actually more akin to a ‘flush-founder’ model; see Carson & Templeton, 1984; Provine, 1989). The ‘flush’ phase in this situation would need to be much longer than those usually proposed, however, as selection would need to be relaxed long enough for new mutants to rise to intermediate frequencies via drift. This could be a very long time indeed. Charlesworth & Smith (1982) have looked at this aspect of peak shifts in much more detail using computer simulations. They similarly concluded that such shifts must be preceded by many generations of relaxed selection, although peak shifts of this type would be expected to be very rare.

Ohta (1988) has also shown that compensatory mutations can arise in the case of gene duplication. Relaxed selection on the duplicate locus allows the

evolution of compensatory interactions with other such loci. Ohta’s results are thus quite consistent with the view presented here. Kimura (1985*a,b*, 1990) also felt that compensatory neutral mutations could play an important role in the coordinated, but ultimately neutral, evolution of many loci. The results of this study suggest that this is unlikely to be true. Kimura based his conclusions on models that are virtually identical to those used here and in Takahata (1983) and Michalakis & Slatkin (1996). Compensatory neutral mutations evolve in realistic amounts of time only when there is very tight linkage and selection is not too strong. Under these conditions, however, mutation pressure overwhelms selection and the drift process, and drives the new mutations to fixation (Fig. 2). Here the unrealistic assumptions of the model (unidirectional mutation) obscure the biological interpretation of the results. Compensatory neutral mutations may still evolve, but more careful models explicitly considering the issue of mutation pressure will be needed before the likelihood of this can be fully evaluated. The results presented here suggest instead that any ‘compensatory’ patterns that appear to exist at the nucleotide level are more likely to be found when changes in the individual components are not actually deleterious. The fitness consequences of such changes will need to be assessed experimentally.

Gillespie (1984) investigated a very similar model for addressing the more general question of how the spectrum of mutational variation might limit the potential response to selection. He argued that alleles that were two mutational steps from the current allele would have a difficult time becoming fixed if there were no intermediate advantageous allele one mutational step away. If we view the nucleotides within an allele as separate loci, then Gillespie’s (1984) model is nearly identical to the models with complete linkage presented above. Using the present notation and assuming a haploid model in an infinite population, Gillespie showed that the rate of fixation of the double mutant would be the product of the mutation–selection balance frequency of the one-mutation-away allele ( $v/hs$ ), the mutation rate from this point to the two-mutations-away allele ( $v$ ), and the probability of fixation of the new mutant ( $2t$ ). The average time to fixation is the inverse of this rate, or  $hs/2tv^2$ . Because of the dependence on  $v^2$ , Gillespie argued that evolution to the double mutant should be faster in large populations than small populations. This contradicts the results obtained here, in which small populations reach fixation more quickly than large populations (Fig. 2). This is because, with complete linkage, double mutants will not be broken up by recombination as they are in Fig. 2, as well as the fact that the probability of fixation for a new advantageous allele is higher in finite populations than it is in infinite populations ( $2t/[1 - \exp\{-4Nt\}]$ ; Fisher, 1930; Wright, 1931; Crow & Kimura, 1970, p. 426). When moving beyond complete linkage, the time to fixation

is a complicated function of the strength of selection and population size (Fig. 2). Nevertheless, the dependence on  $v^{-2}$  still remains, and Gillespie's (1984) argument that populations will become stuck on the 'mutational landscape' because of the long transition times between peaks is supported by the current results. The evolution of compensatory changes at more than two loci becomes even more daunting because the average time to fixation should be proportional to  $v^{-n}$ , where  $n$  is the number of loci (N. Barton, personal communication).

It is possible that the addition of other populations linked by low levels of migration to the existing model might help overcome the limitations on the shifting-balance process imposed by phase zero. For example, Barton & Rouhani (1993) have shown that peak shifts can occur at a reasonable rate in an infinite island model, regardless of the strength of selection, as long as the migration rate among populations remains around a critical value of  $Nm \approx 1$ . Linking populations via migration overcomes the slowdown in peak shifts caused by increasing the strength of selection. Nevertheless, Barton & Rouhani (1993) assume the presence of some variation in the initial population, and the time between peak shifts effectively goes to infinity when there is no initial variation (cf. Barton & Rouhani, 1993, figure 7). As mentioned above, incoming migrants would be expected to overwhelm new mutants and drive the population back towards the initial peak. However, Moore & Tonsor (1994) have simulated the entire shifting-balance process, including requiring new mutations from an initially fixed state, and found that a large proportion of runs resulted in fixation of the new genotype within 12000 generations provided that the migration rate again fell near a critical value (it should be noted that in their simulation runs the new genotype had a very large selective advantage relative to the depth of the adaptive valley). The full integration of mutation into an approach such as that presented by Barton & Rouhani (1993) awaits further work.

In conclusion, as demonstrated here and by Michalakis & Slatkin (1996), the shifting-balance process is unlikely to proceed under equilibrium conditions because mutations are unlikely to arise in small populations and selection is too effective in large populations. The time spent waiting for new mutations, phase zero, dominates the time scale for the completion of the shifting-balance process as a whole. As has been shown by Barton & Rouhani (1993) and by the results above, however, if populations start with moderate amounts of variation, the shifting-balance process can go forward. Thus, the non-equilibrium condition that must exist for the shifting-balance process to work is some relaxation of selection so that variation can enter the population. Without such fluctuations in the strength of selection, compensatory changes that involve individually deleterious effects are unlikely to evolve.

**Appendix. A Markov model**

The diffusion approach described by eqns (2) and (3) turns out to be a very general and powerful way of solving these two-locus problems. The diffusion approximation is based on the assumption that population size is not too small and selection is not too strong, such that gene frequency change is more or less continuous (Crow & Kimura, 1970). Actually, as has been frequently noted previously, the diffusion approximation performs quite well for  $N_e$  as low as 10, even in the two-locus case. Nevertheless, an exact solution to the mutation–selection–drift equations can be obtained using a Markov transition matrix formulation of gamete frequency change (e.g. Karlin & McGregor, 1968). This is only possible for fairly small population sizes, because with four gametes

there are  $\binom{2N_e + 3}{3}$  different states a diploid population of size  $N_e$  can be in. For example, when  $N_e = 10$ , there are 1771 states, and when  $N_e = 50$  there are 176851 states. The memory required to keep track of all these states (or, more precisely, the square of this number) rapidly exceeds the capacity of modern computers. A Markov approach also allows a continuous range of recombination rates to be studied exactly.

Keeping track of the four gametic types is again a three-dimensional problem, while keeping track of the transitions between states is essentially a six-dimensional problem. Letting  $\eta_1$  be the number of  $AB$  gametes,  $\eta_2$  be the number of  $AB'$  gametes,  $\eta_3$  be the number of  $A'B$  gametes, and  $\eta_4 = (2N_e - \eta_1 - \eta_2 - \eta_3)$  be the number of  $A'B'$  gametes in the previous generation, then the transition probability of moving to  $\kappa_1, \kappa_2, \kappa_3$  and  $\kappa_4$  gametes in the next generation is given by

$$\Psi_{\kappa_1 \kappa_2 \kappa_3}(\eta_1, \eta_2, \eta_3) = n! \prod_{i=1}^4 f_i (1/\eta_1, 1/\eta_2, 1/\eta_3, 1/\eta_4)^{\kappa_i} / \kappa_i! \tag{A 1}$$

for  $\kappa_1 = 0, \dots, 2N_e; \kappa_2 = 0, \dots, (2N_e - \kappa_1); \kappa_3 = 0, \dots, (2N_e - \kappa_1 - \kappa_2)$ , and

$$\left. \begin{aligned} f_1 &= x_1' + v(x_2' + x_3') + v^2 x_4' \\ f_2 &= x_2' + v(x_4' - x_2') \\ f_3 &= x_3' + v(x_4' - x_3') \\ f_4 &= x_4' [1 - v(2 + v)], \end{aligned} \right\} \tag{A 2}$$

where  $x_i'$  is defined by eqn (1) and is meant to imply the gamete frequency after selection. Equation (A 1) is somewhat awkward to work with, so the three-dimensional tensor that keeps track of the probability of being in a particular gametic state was mapped to a one-dimensional vector,  $\phi$ , by stacking all the elements of the tensor on top of one another. This allows the transition probabilities defined in eqn (A 1) to be mapped onto a standard Markov transition

matrix,  $\mathbf{M}$ . The between-generation Fisher–Wright equation is thus

$$\phi(t+1) = \mathbf{M}\phi(t). \quad (\text{A } 3)$$

Letting  $\phi_{A'B'}$  be the vector defined by the probability of being at fixation for the  $A'B'$  gamete to be 1.0, and  $\phi_{AB}$  be the vector defined by the probability of being at fixation for the  $AB$  gamete to be 1.0, then the calculations were started at  $\phi(0) = \phi_{A'B'}$  and continued until  $\|\phi(t) - \phi_{AB}\| < 10^{-4}$ . When investigating the static case without mutation, the probability of fixation could be assessed directly by solving the following equation for  $\hat{\phi}$ :

$$\phi_{AB} = \mathbf{M}\hat{\phi}. \quad (\text{A } 4)$$

The elements of  $\hat{\phi}$  then contain the probability of reaching fixation for gamete  $AB$  from the particular state gametic state associated with that element (Rutledge, 1970).

I thank J. F. Crow, C. Denniston, W. Engels, A. Kondrashov, M. Wade and M. Whitlock for helpful discussions during the early phases of this work, and N. Barton, N. Johnson and M. Whitlock for comments on the manuscript. Y. Michalakis and M. Slatkin shared unpublished results, made helpful comments on the manuscript, and provided friendly interactions where there could have been unproductive competition. This work was supported in part by National Institute of Health grant 1-F32-GM1461-01 while the author was a postdoctoral fellow at the University of Wisconsin, Madison.

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