Linkage and the Limits to Natural Selection

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ABSTRACT

The probability of fixation of a favorable mutation is reduced if selection at other loci causes inherited variation in fitness. A general method for calculating the fixation probability of an allele that can find itself in a variety of genetic backgrounds is applied to find the effect of substitutions, fluctuating polymorphisms, and deleterious mutations in a large population. With loose linkage, r, the effects depend on the additive genetic variance in relative fitness, $\mathrm{var}(W)$, and act by reducing effective population size by $(N/N_e) = 1 + \mathrm{var}(W)/2r^2$. However, tightly linked loci can have a substantial effect not predictable from N_e. Linked deleterious mutations reduce the fixation probabililty of weakly favored alleles by $\exp(-2U/R)$, where U is the total mutation rate and R is the map length in Morgans. Substitutions can cause a greater reduction: an allele with advantage $s < s_{crit} = (\pi^2/6)\log_e(S/s)[\mathrm{var}(W)/R]$ is very unlikely to be fixed. (S is the advantage of the substitution impeding fixation.) Fluctuating polymorphisms at many (n) linked loci can also have a substantial effect, reducing fixation probability by $\exp[\sqrt{2Kn}\,\mathrm{var}(W)/R]$ [$K = -1/E((u-\bar{u})^2/uv)$ depending on the frequencies (u,v) at the selected polymorphisms]. Hitchhiking due to all three kinds of selection may substantially impede adaptation that depends on weakly favored alleles.

A major objection to Darwin's theory of evolution was that natural selection, acting on slight variations, would act too slowly to account for the diverse and delicate adaptations that we see. This problem was made particularly acute by Kelvin's argument that because the earth is not yet cool, it must be young (BOWLER 1989, p. 206). The discovery of radioactivity gave both a mechanism for warming an old planet and direct estimates of its age. It is now clear from the fossil record that in the long term, most species evolve much more slowly than we know to be possible from observed responses to natural and artificial selection. However, concern over the limits to natural selection was revived by the discovery of extensive molecular variation: one of the key arguments for the neutral theory was that natural selection could not account for variation in amino acid sequence within and between species (KIMURA 1968; KING and JUKES 1969). We still do not fully understand what limits the rate of adaptation. The question is important both for understanding whether species approach this limit in nature, and for the practical task of maximizing the response to artificial selection.

HALDANE (1957) first attempted to quantify the limits to selection by showing that each substitution entailed a "substitution load"; this can be better expressed as a "lag load," defined as the net difference in mean fitness between a hypothetical population that adapted instantaneously to changing conditions, and one that adapted by means of natural selection (FELSENSTEIN 1971; MAYNARD SMITH 1976). Similar arguments were used to set limits on the number of polymorphisms that could be maintained by overdominance (LEWONTIN and HUBBY 1966; KIMURA 1968; KING and JUKES 1969), and on the number of genes that could be maintained despite deleterious mutations (HALDANE 1937). Such load arguments have not been widely accepted, because they rely on assumptions about how the effects of different loci combine, and because they involve comparison with an ideal genotype that may be vanishingly rare. Truncation selection can sustain more substitutions and polymorphism and can eliminate mutations more effectively (MILKMAN 1967; SVED *et al.* 1967; MAYNARD SMITH 1968; CROW 1970). Such counterarguments would be strengthened by evidence that natural populations do in fact show epistasis of the required form and by some evolutionary or physiological argument as to why they should. Nevertheless, they do show that the "genetic load" does not set any definite and robust limit on the extent of selection.

Recent attention has concentrated on another kind of limit, due to the interference between selection at different loci. FISHER (1930, p. 122) and MULLER (1932) pointed out that in an asexual population, advantageous mutations could only be combined if they occurred sequentially, within the same lineage; sexual reproduction can speed adaptation by combining advantageous mutations that arise in different lineages. The process was first quantified by HILL and ROBERTSON (1966), who argued that selection increases the variance in reproductive success, and so reduces the effective population size. Chance associations between

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selected loci thus reduce neutral variability, as well as reducing the probability of fixation of favourable alleles (MAYNARD SMITH and HAIGH 1974). The same "hitchhiking" effect is caused by any kind of selection—for example, against deleterious mutations (FISHER 1930; BIRKY and WALSH 1988; CHARLESWORTH *et al.* 1993; PECK 1994). Sex and recombination increase the ability of selection to act independently on different loci, and so may be favored by both group and individual selection (FELSENSTEIN 1974, 1987). The observation that DNA sequence variability is reduced in regions of low recombination—for example, in Drosophila (AQUADRO and BEGUN 1993) and on the mammalian *Y* chromosome (ELLIS *et al.* 1990)—suggests that hitchhiking may have substantial evolutionary effects.

This paper sets out a general method for calculating the probability of fixation of a favorable mutation, which can find itself in a variety of genetic backgrounds. The population is assumed to be very large ($Ns \gg 1$). The method is applied to find the effect of favorable substitutions, fluctuating polymorphisms, and deleterious mutations, but extends to cover any kind of population structure. With loose linkage, the effects can be explained by a reduction in effective population size caused by heritable variation in fitness (ROBERTSON 1961). However, tightly linked loci can have a substantial effect that is not predictable from the effective population size. Overall, the hitchhiking effect of each kind of selection may substantially impede adaptation.

GENERAL METHODS FOR FINDING FIXATION PROBABILITIES IN STRUCTURED POPULATIONS

Suppose that an allele can be found within one of several possible sites; these "sites" might represent demes within a spatially subdivided species, or alternative genetic backgrounds within a single polymorphic population. Let the probability of ultimate fixation of an allele that is present in a single copy in site i in generation t, immediately before reproduction, be $P_{i,t}$. The number of genes in each site ($2N_i$) is assumed to be large enough that different alleles are lost independently of each other. Thus, the chance that an allele in site i, and an allele in site j are both lost is $(1 - P_{i,t})(1 - P_{j,t})$; in general, the chance that a set of alleles is lost is $\prod_i (1 - P_{i,t})^{k_i}$, where there are k_i alleles to be lost in site i, and the alleles are everywhere rare ($k_i \ll 2N_i$).

The probabilities of fixation, $P_{i,t}$, can be found by iterating through one generation, in a straightforward extension of the method of branching processes. This was introduced to genetic problems by FISHER (1922) and HALDANE (1927) (see HARRIS 1963; POLLAK 1966; SCHAFFER 1970 for reviews). The allele of interest produces j offspring with probability $W_{i,j}$ when in site i. This is the distribution of the number of heterozygotes produced by a rare heterozygote (which will almost certainly mate with a homozygote for the common allele). Then

$$(1 - P_{i,t-1}) = \sum_{j=0}^{\infty} W_{i,j}(1 - P^*_{i,t})^j \qquad (1)$$

Here, $P^*_{i,t}$ is the probability that an allele in site i at time $(t - 1)$ would be fixed, given that it is passed to precisely one offspring in the next generation; $P^*_{i,t} = \Sigma_k M_{i,k} P_{k,t}$, where $M_{i,k}$ is the chance that an offspring from a parent at site i will be at site k. HARRIS (1963, Chapter II, Theorem 7.1) gives an equivalent expression in terms of generating functions. If the distribution of offspring number is Poisson with mean $(1 + s_i)$, then

$$(1 - P_{i,t-1}) = \sum_{j=0}^{\infty} \exp[-(1 + s_i)] \frac{(1 + s_i)^j}{j!} \times (1 - P^*_{i,t})^j = \exp[-(1 + s_i) P^*_{i,t}] \qquad (2)$$

The two-locus version of Equation 2 was derived by EWENS (1967). If s_i is small, and the fixation probabilities are of the same order as s_i, then this transcendental equation can be simplified by approximating $\exp(x)$ as $1 + x + x^2/2, \ldots$, and dropping terms $o(s^2)$:

$$P_{i,t-1} = (1 + s_i) P^*_{i,t} - \frac{P^{*2}_{i,t}}{2} \qquad (3)$$

(If the distribution of offspring number is not Poisson, then the second term on the right is multiplied by the variance in offspring number.) For steady selection in a single site, Equation 3 has the solution $P = 2s$ (HALDANE 1927). This is the basic expectation against which we will compare results for heterogeneous populations.

If movement between sites also changes P by a factor of order s, (*i.e.*, $M_{i,j}$ is of order s for $i \neq j$, $P^*_{1,t} - P_{i,t} = \Sigma_j M_{i,j} P_{j,t} - P_{i,t}$ is of order s^2), we can take change to occur approximately continuously in time and can approximate P^* by P:

$$-\frac{\partial P_i}{\partial t} = s_i P_i + (\sum_j M_{i,j} P_j - P_i) - \frac{P_i^2}{2} \qquad (4)$$

This extends the result derived by BARTON (1987, Equation 4b) using the diffusion approximation, to allow for variation through time.

APPLICATIONS

Interference from a selected substitution: We now apply Equation 4 to find the fixation probability of a favorable allele within a single genetically heterogeneous population. Initially, we consider the effect of a substitution at another locus, and assume that there is no epistasis (*i.e.*, that the effects on fitness multiply). It is difficult to calculate the joint fixation probability of two favorable alleles in a finite population. One must follow stochastic changes in three variables (the allele frequencies at both loci, and the linkage disequilibrium between them). Even under the diffusion approximation, the outcome depends on five parameters (the

product of population size and the selection favoring each allele, and the initial allele frequencies and linkage disequilibrium) (HILL and ROBERTSON 1966). Calculating the fixation probabilities in a very large population is much simpler. After suitable scaling, the outcome depends on only two parameters: the relative selection coefficients on the two loci, and the rate of recombination, relative to the strength of selection ($\theta = s/S$, $\rho = r/S$, defined below). Moreover, one need only consider stochastic fluctuations at one locus. This is because the fate of a favorable allele is decided while it is at very low frequency ($\approx 1/Ns$); it therefore cannot affect the fate of other rare alleles, because it is unlikely to interact with them until it becomes more common. One can thus apply branching process arguments to one locus at a time, by following the probability that a single favorable allele will be fixed, given that it is associated with one or other allele at the second locus. This argument extends to any number of loci: one need only consider the probability that each is fixed, given deterministic changes in the frequencies of all the genetic backgrounds with which it is associated.

Consider an infinite population which is segregating for two alleles (U, V) at some locus, with frequencies u, v ($u + v = 1$). We count genotypes at the haploid stage, but the analysis also applies to randomly mating diploids. The relative fitnesses of the alleles are $(1 + S):1$; $S \ll 1$. Assuming that density dependence is strong enough that population size remains constant throughout the substitution, the absolute fitnesses are $(1 + Sv):(1 - Su) + O(S^2)$. With weak selection, time can be taken as approximately continuous, so that u increases logistically through time, with $u = 1/[1 + \exp(-St)]$. Time is arbitrarily counted from the midpoint of the substitution. An allele with selective advantage s arises at another locus; for the moment, we assume that effects on fitness are additive. Its probability of fixation depends on whether it arises in the favorable background (and hence produces, initially, $1 + Sv + s$ offspring), or in the less favored background (giving $1 - Su + s$ offspring initially). Let these probabilities be P_u and P_v, respectively. The probability that an allele coupled with U recombines onto the opposite background is rv, because v is the chance that it will pair with a chromosome carrying allele V. Thus, $P^*_{u,t} = rvP_{v,t} + (1 - rv)P_{u,t}$, and similarly, $P^*_{v,t} = ruP_{u,t} + (1 - ru)P_{v,t}$. Assuming $r \ll 1$, we can apply the continuous time approximation (Equation 4) to give

$$-\frac{\partial P_u}{\partial t} = -rv(P_u - P_v) + (s + Sv)P_u - \frac{P_u^2}{2} \quad (5a)$$

$$-\frac{\partial P_v}{\partial t} = -ru(P_v - P_u) + (s - Su)P_v - \frac{P_v^2}{2} \quad (5b)$$

It is convenient to work with the average probability of fixation, $\bar{P} = (uP_u + vP_v)$, and the difference between the fixation probabilities in the two genetic backgrounds, $(P_u - P_v)$. Scaling these relative to the fixation probability in the absence of hitchhiking, $2s$, and scaling time relative to S, we define

TABLE 1

Summary of notation

P_i	The probability that a single allele in site i will ultimately be fixed; the site, i, may refer to geographic location or genetic background
$\bar{P}$	Fixation probability, averaged over all sites
s	Selective advantage of the rare allele
S	Selection on the locus that is interfering with fixation of the rare allele
θ	Scaled advantage of the rare allele; $\theta = s/S$
T	Scaled time; $T = St$
ρ	Scaled recombination; $\rho = r/S$
Π	Scaled average fixation probability; $\Pi = \bar{P}/2s$. $\Pi = 1$ with no interference
Δ	Scaled difference in fixation probability between two genetic backgrounds: $\Delta = (P_u - P_v)/2s$
R	Map length in Morgans
U	Mutation rate per haploid genome
λ	Rate of selected substitutions
Λ	Scaled rate of selected substitutions; $\Lambda = \lambda/S$

$$\Pi = \frac{(uP_u + vP_v)}{2s} \quad \Delta = \frac{(P_u - P_v)}{2s} \quad T = St,$$

$$\rho = \frac{r}{S}, \quad \theta = \frac{s}{S}, \quad u = 1/[1 + \exp(-T)]$$

$$\frac{\partial \Pi}{\partial T} = -\theta\Pi(1 - \Pi) + \theta uv\Delta^2 \quad (6a)$$

$$\frac{\partial \Delta}{\partial T} = \Delta[\rho + (2\Pi - 1)\theta + (u - v)(1 - \theta\Delta)] - \Pi \quad (6b)$$

(Notation is summarized in Table 1). For $T \gg 0$, the asymptotic solution is found by setting Π, Δ constant. As expected, this gives $\Pi = 1$: if a mutant arises long after the substitution has been completed, then its probability of fixation is unaffected and equals $2s$. Setting Δ constant for $T \gg 0$ gives a quadratic equation; when the rare allele is weakly selected ($s \ll S$, $\theta \ll 1$), $\Delta = 1/(1 + \rho)$. This is because while an allele arising with the predominant U allele has $P_u = 2s$, an allele arising in association with the deleterious V allele can only be fixed if it recombines onto the favorable background before being eliminated by selection. For small θ, $P_v = 2s\rho/(1 + \rho)$, and so $\Delta = (P_u - P_v)/2s = 1/(1 + \rho)$.

Equation 6, a and b, can be solved numerically by working back from some large time. Numerical results were obtained by using the Runge-Kutta routine NDSolve in *Mathematica* (WOLFRAM 1991), integrating over the range $T = +10$ to -40. Figure 1 shows typical patterns for the overall probability of fixation, $2s\Pi$, for $\theta = s/S = 0.001, 0.01, 0.1, 1$, while Table 2 gives the minimum fixation probability [$\min(\Pi)$], for various

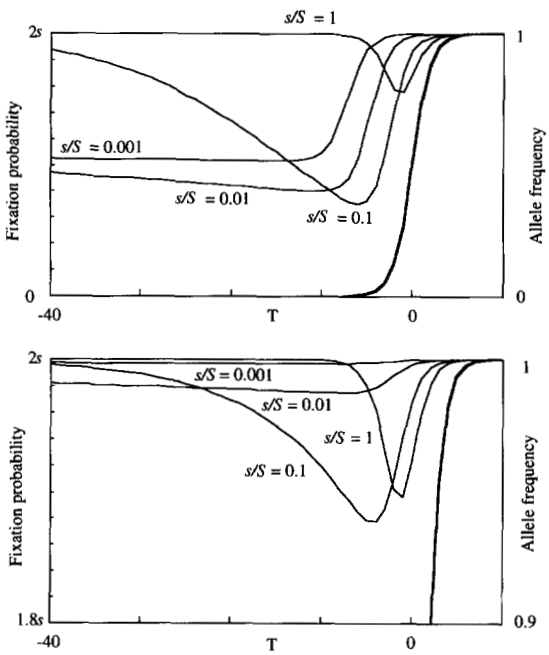

FIGURE 1.—The probability of fixation as a function of the time at which the favorable mutation occurs ($T = St$). (A) Tight linkage: $\rho = r/S = 0.1$. (B) Loose linkage: $\rho = r/S = 1$. Note that with $\rho = 1$, the vertical axis runs from $1.8s$ to $2s$. The heavy curve to the right in (A) and (B) shows the progress of the substitution [$u = 1/[1 + \exp(-T)]$].

θ and ρ. When linkage is loose (*e.g.*, $\rho = r/S = 1$), hitchhiking has little effect, the fixation probability never being reduced by >8% (at $\theta \approx 0.3$); with tight linkage ($\rho = 0.01$), it can be reduced by >95% (at $\theta = 0.03$). Unless linkage is extremely tight, the effect of a weakly selected substitution on a strongly favored allele is negligible: for example, with $\theta = 10$, and $\rho = 0.01$, fixation probability is reduced at most by 0.2% (Table 2c). In the limit of complete linkage, an allele with advantage $s < S$ (*i.e.*, $\theta < 1$) cannot be fixed if it arises in the unfavorable background, whereas if $s > S$ (*i.e.*, $\theta > 1$), $\bar{P} = 2(s - S) = 2s(1 - 1/\theta)$. If it does then fix, it will eliminate the favorable allele at the other locus; however, a little recombination will ensure that both are fixed.

The fixation probability can be substantially reduced even when selection on both loci is similar; indeed, the fixation probability is reduced to a lower value when $\theta \approx 0.1$ than when θ is extremely small. However, if the new allele is only weakly favored ($\theta \ll 1$), its fixation probability is reduced even when it arises a long time before the substitution at the second locus (for example, compare the curves for $\theta = 0.001$, $\theta = 1$ in Figure 1A, left). This is because a weakly selected allele is likely to segregate at low frequency and therefore be vulnerable, for a long period ($t \approx 1/s$, $T \approx 1/\theta$) before being fixed.

Net reduction in fixation probability: A simple measure of the net effect of hitchhiking is the total reduction in (relative) fixation probability, integrated over all possible times of origin, $\int_{-\infty}^{\infty}(1 - \Pi)\,dT$. (This is the area below the line $P = 2s$ in Figure 1.) Suppose that a favorable mutation occurs somewhere in a long time interval spanning the substitution at the second locus ($t_0 < t < t_1$, where $t_0 \ll 0$, $t_1 \gg 0$). Its probability of fixation, averaged over this period, is

$$2s\left(\int_{T_0}^{T_1} \frac{\Pi}{(T_1 - T_0)}\,dT\right)$$

$$\approx 2s\left(1 - \frac{1}{(T_1 - T_0)}\int_{-\infty}^{\infty}(1 - \Pi)\,dT\right)$$

If substitutions occur at some very low rate, $\lambda \ll S$, then the average fixation probability is reduced by $1 - \Lambda\int_{-\infty}^{\infty}(1 - \Pi)dT$, where $\Lambda = \lambda/S$. Interactions between multiple substitutions are dealt with in BARTON (1994a), where it is shown that the fixation probability declines linearly with Λ up to a threshold

$$\Lambda_{\text{crit}} = 1\Big/\left(\int_{-\infty}^{\infty}(1 - \Pi)\,dT\right)$$

above which fixation becomes very unlikely in a large population.

Figures 2 and 3 show how the net effect varies with θ and ρ. When linkage is loose ($\rho \geq 1$), the net effect is small (≤ 1.5) and is almost independent of the relative selection coefficients for $\theta < 1$ (Figure 2). It declines rapidly as the recombination rate increases (Figure 3, right). When linkage is tight ($\rho \leq 0.1$), the net effect on a weakly selected allele can become very large; it declines inversely with θ and depends only weakly on ρ. This is because the net effect depends mainly the length of time for which the new allele remains vulnerable and so is proportional to $1/\theta$.

Hitchhiking as a catastrophe: The greatest net reduction in fixation probability occurs when the new mutation is weakly selected (*i.e.*, $\theta = s/S \ll 1$). In this case, the effect is equivalent to that of a catastrophe, which suddenly reduces the number of alleles by a factor w. In APPENDIX A, an approximation for the strength w of the equivalent catastrophe is derived (Equation A5). When linkage is tight ($\rho \ll 1$), this simplifies to:

$$w = 1 - \theta^{\rho} \qquad (7)$$

This approximation can be compared with MAYNARD SMITH and HAIGH's (1974) calculation of the effect of a favorable mutation on a neutral allele which is at substantial frequency. Integrating their Equation 13 shows that if the new mutation arises with one neutral allele (V, say), the frequency of the complementary allele is reduced from u to wu, where $w = 1 - (2N)^{-\rho}$ for $2N \gg 1$. This has the same form as Equation 10, but with $1/2N$ replacing $\theta = s/S$. The difference arises because MAYNARD SMITH and HAIGH only followed the decay of the initial linkage disequilibrium produced

TABLE 2

The effect of a selected substitution on the probability of fixation of a linked allele

$\rho = r/S = 0.01$, $\theta = s/S$	min(Π)	w	$1 - \theta^\rho$	$\int_{-\infty}^{\infty}(1 - \Pi)\,dx$	$\frac{-\log(1 - \theta^\rho)}{\theta}$	$\frac{1}{(\rho + \theta)^2 - 1/4}$
0.001	0.0680	0.0669	0.0667	2698.59	2706.87	
0.00316	0.0585	0.0558	0.0559	906.98	911.84	
0.01	0.0507	0.0447	0.0450	306.46	310.09	
0.0316	0.0470	0.0336	0.0339	103.95	106.98	
0.1	0.0544	0.0236	0.0228	35.14	37.83	
0.316	0.1067	0.0176	0.0114	11.37	14.14	
1	0.5699			1.911		1.299
3.16	0.9739			0.105		0.102
10	0.9975			0.010		0.010
$\rho = r/S = 0.1$, $\theta = s/S$	min(Π)	w	$1 - \theta^\rho$	$\int_{-\infty}^{\infty}(1 - \Pi)\,dx$	$\frac{-\log(1 - \theta^\rho)}{\theta}$	$\frac{1}{(\rho + \theta)^2 - 1/4}$
0.001	0.5166	0.5126	0.4988	664.83	695.52	
0.00316	0.4576	0.4469	0.4377	251.44	261.30	
0.01	0.4005	0.3734	0.3690	95.59	99.68	
0.0316	0.3571	0.2934	0.2921	36.33	38.92	
0.1	0.3524	0.2133	0.2057	13.59	15.81	
0.316	0.4474	0.1545	0.1087	4.70	7.02	
1	0.7718			1.01		1.042
3.16	0.9755			0.098		0.096
10	0.9975			0.0099		0.010
$\rho = r/S = 1$, $\theta = s/S$	min(Π)	w	$1 - \theta^\rho$	$\int_{-\infty}^{\infty}(1 - \Pi)\,dx$	$\frac{-\log(1 - \theta^\rho)}{\theta}$	$\frac{1}{(\rho + \theta)^2 - 1/4}$
0.001	0.9986	0.9986	0.9990	1.4254	1.0005	1.330
0.00316	0.9957	0.9956	0.9968	1.3986	1.0016	1.322
0.01	0.9876	0.9865	0.9900	1.3334	1.0050	1.299
0.0316	0.9687	0.9609	0.9684	1.1849	1.0162	1.228
0.1	0.9386	0.8973	0.9000	0.9164	1.0536	1.042
0.316	0.9207	0.7526	0.6838	0.5692	1.2021	0.675
1	0.9471			0.2448		0.267
3.16	0.9857			0.0581		0.059
10	0.9980			0.0083		0.008

The effect is tabulated as a function of the ratio between the selective advantages ($\theta = s/S$). The recombination rate is $r = 0.01S$ in the first section, $0.1S$ in the second section, and S in the last section. Min(Π) gives the minimum factor by which fixation probabiltiy is reduced. The effect of a substitution on a weakly favored allele is as if that allele were suddenly reduced in numbers by a factor w; this is compared with the approximation for small θ, $1 - \theta^\rho$ (Equation 7). The net reduction in fixation probability, averaged over all times at which the mutation might occur, depends on $\int_{-\infty}^{\infty}(1 - \Pi)\,dx$; this is compared with the prediction for small θ, $-\log(1 - \theta^\rho)/\theta$ (Equation 8) and large $(\rho + \theta)$ (Equation 9).

when a new mutant arises in coupling with a particular neutral allele and neglected subsequent disequilibria produced by genetic drift. OHTA and KIMURA (1975) consider the opposite case, where a neutral mutation arises in the presence of a substitution. However, they do not find analytic solutions.

The net effect of a substitution is found by integrating Equation A1:

$$\int_{-\infty}^{\infty}(1 - \Pi)\,dT = \frac{\log(1/w)}{\theta} = \frac{-\log(1 - \theta^\rho)}{\theta} \qquad (8)$$

This simple approximation is compared with exact calculations from Equation 6 in Figures 2 and 3 (○) and in Table 2. It is in excellent agreement for $\rho \leq 1$, $\theta \leq 0.1$.

Though the numerical results indicate that the greatest effect will be of closely linked substitutions on weakly favored alleles, the cumulative effect of many loosely linked substitutions may be important. An approximation for this case is given by Equation B6 in APPENDIX B. For large $(\rho + \theta)$, this is close to

$$\int_{-\infty}^{\infty}(1 - \Pi)\,dT = \frac{1}{[(\rho + \theta)^2 - 1/4]} \qquad (9a)$$

This approximation is shown in Table 2, and by triangles in Figures 2 and 3. It is in excellent agreement whenever $\rho > 1$ or $\theta > 1$. Thus, almost the whole range of parameters is covered by the complementary approximations of Equations 8 and 9a. Note that the additive variance in relative fitness contributed by a sin-

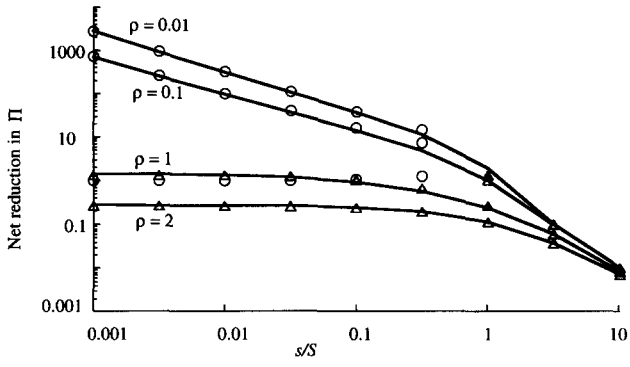

FIGURE 2.—The net effect of a substitution depends on $\int_{-\infty}^{\infty}(1 - \Pi)\,dT$ (solid curves). This is compared with the theoretical predictions for small θ, $-\log(1 - \theta^{\rho})/\theta$ (○), and for large $(\rho + \theta)$, $1/[(\rho + \theta)^2 - 1/4]$ (△), for varying linkage ($\rho = r/S = 0.01, 0.1, 1, 2$). The integral is calculated numerically over the range $T = -40$ to $+10$; the contribution for $T < -40$ is found by assuming that Π has the form $w/[w + (1 - w)\exp(T)]$.

gle substitution is $2S$; the total additive variance due to substitutions which occur at a low rate λ is $\mathrm{var}(W) = 2\lambda S$ (CROW 1970). Throughout, $\mathrm{var}(W)$ denotes the additive genetic variance in relative fitness, scaling mean fitness to $\bar{W} = 1$. In terms of the original parameters, the average fixation probability is:

$$\bar{\Pi} = 1 - \frac{\mathrm{var}(W)}{2[(r + s)^2 - S^2/4]} \quad (r + s \gg S) \quad (9b)$$

Epistasis: Suppose that the rare allele increases fitness by s_V when coupled with the V allele at the second locus, and by s_U when coupled with the U allele; let $\theta_V = s_V/S$, $\theta_U = s_U/S$. Because we assume weak selection and random mating, only the average fitness of each gamete when associated with a randomly chosen gamete need be considered. Thus, $s_V = (us_{UV} + vs_{VV})$, $s_U = (us_{UU} + vs_{UV})$. Fixation probabilities are now given by modifying Equation 5, such that the terms $(s + Sv)$, $(s - Su)$ in Equation 5, a and b, are replaced by $(s_U + Sv)$, $(s_V - Su)$. Note that the course of the substitution should

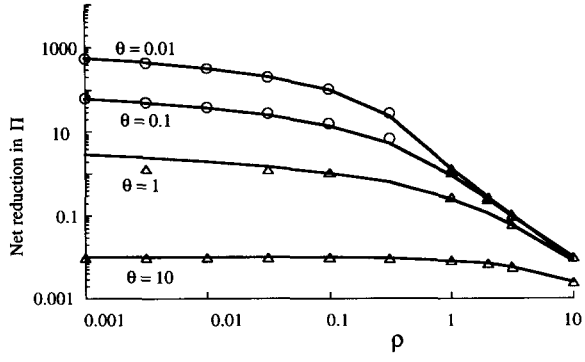

FIGURE 3.—The net effect of a substitution, $\int_{-\infty}^{\infty}(1 - \Pi)\,dT$, plotted against $\rho = r/S$ for various $\theta = s/S$ (solid curves). This integral is calculated as in Figure 2. It is compared with the theoretical predictions for small θ, $-\log(1 - \theta^{\rho})/\theta$ (○), and for large $(\rho + \theta)$, $1/[(\rho + \theta)^2 - 1/4]$ (△).

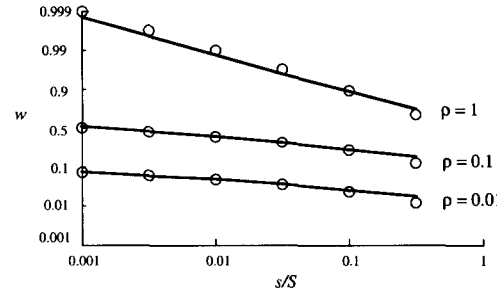

FIGURE 4.—The effect of a strongly selected substitution ($S \gg s$, $\theta = s/S \ll 1$) is equivalent to a catastrophe that reduces numbers by a factor w. This is estimated from the value at $T = St = -40$ (solid curves), and compared with the prediction for small θ, $w = 1 - \theta^{\rho}$ (○). w is plotted on a logit scale.

still be given by $u = 1/[1 + \exp(-T)]$ in these equations. This is because, although the epistatic effect of the new allele would alter the course of that substitution if it did increase to appreciable frequency, the calculations are based on following the probability of *loss* through time (Equations 1 and 2). Hence, this perturbation is irrelevant.

With dominance, s_U and s_V would change through time with changes in u, as would the selection coefficient S. The fitness of homozygotes for the rare allele can be neglected, however, provided that there is some selection on heterozygotes ($s > 0$). The fixation probability of a completely recessive allele is very small ($\approx 2s/\pi N$) for large N (CROW and KIMURA 1970, p. 427). For simplicity, only additive selection (s_U, s_V, S constant) is considered here. However, variation in the parameters through time, such as might be produced by dominance or by a changing environment, is dealt with below.

First, consider a weakly selected allele which is always favored, albeit to different degrees in different genetic backgrounds ($S > s_V > s_U > 0$). This has fixation probability $2s_V$ long before the substitution and decreases to $2s_U$ after it. Naively, one would expect the fixation probability to be twice the net selective advantage, $2(us_U + vs_V)$ (dotted curve in Figure 5A). In fact, it is lower, both because $(us_U + vs_V)$ is decreasing through time, and because of hitchhiking. These two factors reduce fixation probability during the course of the substitution, and for a time $t \approx 1/s_V$ before it (Figure 5A). If the allele does fix, it will have little effect on the second locus.

Second, consider a weakly selected allele which is only favored when associated with the new allele U ($S \gg s_U > 0 \geq s_V$). The fixation probability rises from effectively 0 to $2s_U$ (Figure 5B). With loose linkage, it becomes appreciable a time $t \approx 1/s_V$ before U increases ($\rho = 1$, 10 in Figure 5B). Hitchhiking reduces the probability toward $2us_U$ ($\rho = 0.1$, 0.01 in Figure 5B). With complete linkage, the new allele can only fix if it arises in association with U, which occurs with probabil-

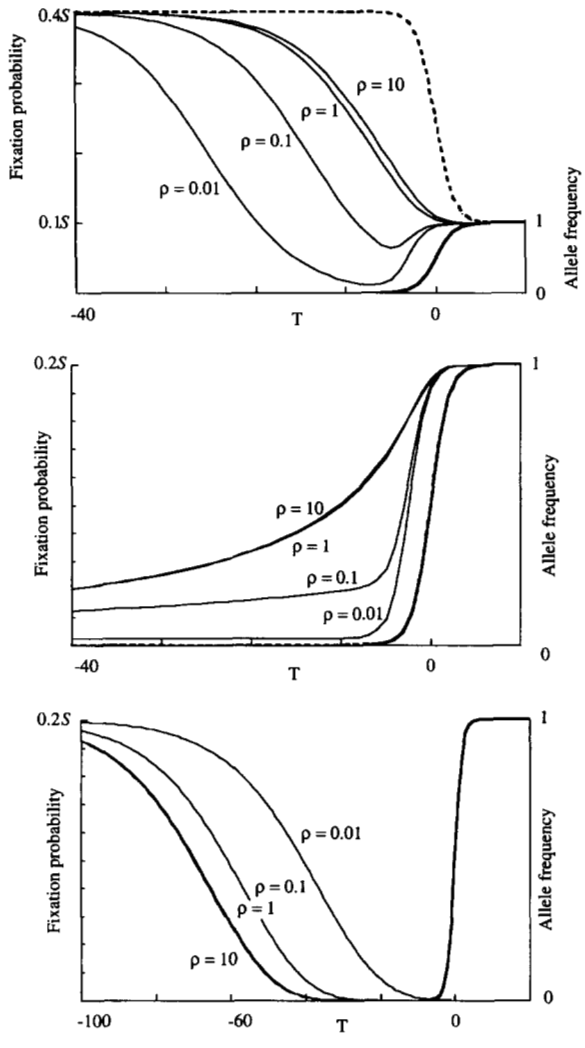

FIGURE 5.—The probability of fixation of an allele. (A) An allele that has advantage $s_V = 0.2S$ with allele V at the second locus, but only $s_U = 0.05S$ with U. The probability of fixation falls from $2s_V = 0.4S$ before the substitution to $2s_U = 0.1S$ afterward. It is lower than $2(vs_V + us_U)$ (dotted line) both because its net advantage $(vs_V + us_U)$ is decreasing and also because of hitchhiking. The curves for various $\rho = r/S$ show the reduction in fixation probability as linkage becomes tighter. The substitution (u) is shown by the heavy curve (lower right). (B) An allele that has an advantage only when coupled with allele U at the second locus ($s_V = 0$, $s_U = 0.1S$). With loose linkage ($\rho = r/S = 1, 10$), fixation probability approaches $2s_U = 0.2S$ over times $t \approx 1/s_V$ before the substitution. With tighter linkage ($\rho = 0.1, 0.01$), hitchhiking impedes fixation. As in (A), the heavy curve shows the substitution (u). (C) The reduction in fixation probability for an allele that has an advantage only when coupled with allele V at the second locus ($s_V = 0.1S$, $s_U = -0.1S$). This depends on population size; here, $NS = 100$. The prediction is that fixation probability should fall around $T = St = -S\log(8N^2 s_V s_U)/s_V = -66.5$. As in (A), the heavy curve shows the substitution (u).

ity u. The fixation probability of an allele that does arise in background U decreases from $P_U = 2(s_U + S)$ to $2s_U$. Hence, the average fixation probability is somewhat greater than $2us_U$ when $\rho = 0$.

Third, consider the converse, where the allele is only favored when associated with the original allele V ($S \gg s_V > 0 > s_U$). In a truly infinite population, it could never fix, because even if introduced long before U rises to high frequency, it would never completely replace the homologous allele and so would be eliminated once it lost its advantage. However, in a very large population, its fixation probability can be approximated as follows. If it is introduced at time $t_0 \ll 0$, it has a chance $2s_V$ of being established and of increasing to frequency $p = p_0/\{q_0 \exp[s_V(t - t_0)] + p_0\}$. p_0 varies randomly, because of stochastic fluctuations in its initial increase and has an exponential distribution with expectation $1/4Ns_V$ (N. H. BARTON, unpublished data; Equation 4 for small s). The expectation is substantially $>1/2N$ because an allele that is destined to fix is likely to rise more rapidly than expected from its selection coefficient (MAYNARD SMITH and HAIGH 1974). If the allele is to fix, the alternative allele Q must be reduced to small numbers by some time $t_1 \ll 0$. Then, $q_1 \approx (1/p_0)\exp[s_V(t_1 - t_0)]$; the chance that all $2Nq_1$ copies will be lost is $(1 - P_Q)^{2Nq_1} \approx \exp(-2Nq_1 P_Q)$, where P_Q is the probability of fixation of a single Q allele at time t_1. P_Q can be calculated numerically from Equation 6 (*cf.* Figure 5B) and must have the form $s_U C \exp(s_V t_1)$ for appropriate t_1. Integrating over the exponential distribution of p_0, the net fixation probability is:

$$\begin{aligned}\bar{P} &= 2s_v \int_0^\infty \exp(-2Nq_1 P_Q)\exp(-4Ns_v p_0)4Ns_v dp_0 \\ &= 2s_v \int_0^\infty \exp(-2Ns_U C\exp(s_v t_0)/p_0) \\ &\quad \times \exp(-4Ns_v p_0)4Ns_v dp_0 \\ &= 2s_v g[8N^2 s_v s_U C\exp(s_v t_0)] \qquad (10)\end{aligned}$$

where $g(x) = \int_0^\infty \exp(-y - x/y)\,dy = 2\sqrt{x}K_1(2\sqrt{x})$, K_1 is the modified Bessel function, and C is chosen so to splice the two approximations. The allele is unlikely to fix unless it is introduced before $t \approx -\log(8N^2|s_V s_U|)/s_V$; linkage increases the fixation probability of this ultimately disadvantageous allele (Figure 5C).

Fourth, suppose that the new allele has effect comparable with the second substituting locus, so that if it is established, it affects the subsequent evolution of that locus. The fitnesses of haplotypes (QV, PV, QU, PU) are in the ratios $(1, 1 + s_V, 1 + S, 1 + S + s_U)$. If $S > 0$, $s_V > 0$, $s_U > 0$, but $S + s_U < s_V$, then if P is fixed, it will cause the elimination of the formerly advantageous allele U at the second locus. However, this subsequent complication does not alter the calculation of the probability that P will be fixed: when the new allele (P) is so rare as to be vulnerable to random loss, it cannot affect the course of substitutions that are already established. Figure 6 shows an example with $s_V = 2S$, $s_U = 0.5S$; the dotted curve shows how the fixation probability falls from $4S$ to S as the ge-

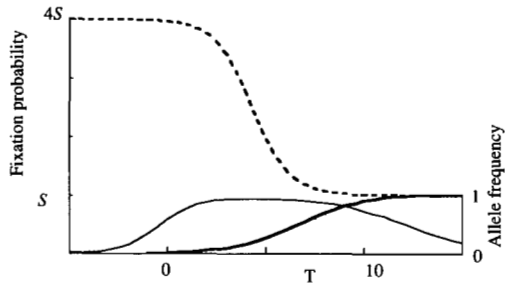

FIGURE 6.—Allele P has advantage $s_V = 2S$ when coupled with allele V at the second locus, but only $s_U = 0.5S$ when coupled with U. The two loci recombine at $r = S$. Allele U has an advantage S when coupled with Q, but a disadvantage $-0.5S$ when with P; thus, if allele P fixes, allele U will be driven out of the population unless it has already gone to fixation. The upper dotted curve shows the probability that a single copy of P will fix, as a function of the time when it is introduced, given that allele U substitutes at $T = 0$. The lower heavy curve shows the frequency of allele P if it does fix, reaching appreciable frequency after $T \approx 5$. The light curve shows the consequent elimination of U.

netic background changes from V to U. The lower curves show the outcome if allele P is established at some early time, so as to increase to appreciable frequency after $T = 5$. Allele U is eliminated (light curve), unless it fixes before P increases. In this example, because selection on the two loci is necessarily of similar strength, hitchhiking only causes a slight reduction in fixation probability. However, linkage does alter the outcome if P does fix, because epistasis builds up linkage disequilibrium. Figure 6 gives results for $r = S$; if linkage is looser, P increases to eliminate U more slowly, so that unless the population is extremely large, both alleles are likely to fix.

Finally, if allele U is only at an advantage when associated with P, and conversely, P is only at an advantage when associated with U ($s_V < 0$, $s_U > 0$, $S < 0$, but $S + s_U > s_V$), there are two stable states. Transition between them is impossible in an infinitely large population; the probability of joint fixation will decline with $\exp(-NS)$ (WRIGHT 1941). The methods presented here are not appropriate, because the fate of the alleles is not determined when they are at very low frequency, and so they will not be lost independently of each other (Equation 2). In all other cases, however, the effects of epistasis in a large population can be treated by considering stochastic fluctuations at one locus at a time.

Fluctuating selection: Balancing selection increases the level of neutral variation at closely linked loci ($r \approx \mu$) through "associative overdominance" (OHTA 1971; HUDSON *et al.* 1987). However, if the frequency of the polymorphism remains constant, the probability of fixation of favorable alleles will be unaffected. This is because at equilibrium, the favorable allele produces the same distribution of offspring, regardless of the genetic background. MARUYAMA (1970) demonstrated a similar invariance for symmetric migration among demes of constant size. Fluctuations in allele frequency have a similar effect to substitutions in reducing fixation probability: in both cases, the reduction is caused by chance associations with genetic backgrounds that are changing in abundance. Because it may be that much more additive variance in fitness is because of fluctuations in persistent polymorphisms than to substitutions of new alleles, fluctuations may cause a greater reduction in fixation probability. As before, the fixation probabilities in the two genetic backgrounds (P_U, P_V) change with the frequencies of those backgrounds (u, v). Let the marginal selection coefficient on the polymorphism be $S \cdot f(T)$, so that $\partial u/\partial T = Sf(T)uv$ (scaling time to $T = St$ as before). Integrating, $u = 1/[1 + \exp(-\int f dT)]$. Assume that $\int f dT$ remains bounded, so that the locus remains polymorphic. Stability could be ensured by either overdominance or frequency dependence; all that matters here is the actual pattern of allele frequencies, as determined by the selection coefficient, $Sf(T)$. One might consider $f(T)$ to be a stochastic variable, or a definite sequence. Equation 6 extends to give:

$$\frac{\partial \Pi}{\partial T} = -\theta\Pi(1 - \Pi) + \theta uv\Delta^2 \qquad (11a)$$

$$\frac{\partial \Delta}{\partial T} = \Delta\{\rho + (2\Pi - 1)\theta + (u - v)[f(T) - \theta\Delta]\} - f(T)\Pi \qquad (11b)$$

The average fixation probability, Π, is not directly affected by fluctuating selection at the primary locus (Equation 11a). Fluctuations generate a difference in fixation probabilities between genetic backgrounds Δ (Equation 11b), which then alters Π. Note that because the driving term in Equation 11a, $\theta uv\Delta^2$, is always positive, fixation probability must always be reduced.

Figure 7 shows typical solutions of Equation 11. Fixation probabilities differ substantially between backgrounds, the probability being lower if the new allele arises in the background that is about to decrease. However, the net probability varies very little (heavy curve). This is because when the rare allele is weakly favored, P changes slowly ($\approx\theta$; Equation 11a) In this example, $s = 0.1S$, where S is the maximum selection on the polymorphic locus. Figure 8 gives the average fixation probability as a function of recombination rate. As for a substitution, the effect is appreciable only when the new allele is selected less strongly than is the polymorphism ($\theta = s/S < 1$). Then, if linkage is tight ($\rho = r/S < 1$), the fixation probability is reduced towards a limiting value which is independent of s and r, and depends only on the pattern of allele frequencies at the polymorphic locus.

These numerical results can be understood by considering the limits of loose and tight linkage. If there is no recombination, the fixation probabilities in the dif-

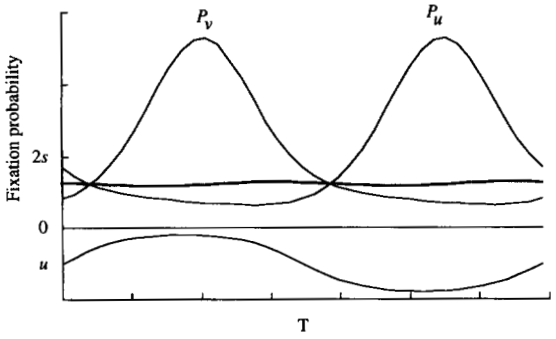

FIGURE 7.—Fluctuating selection $S \cdot \cos(\omega T)$ causes allele frequency to vary cyclically, between $u = 0.1$ and $u = 0.9$ ($\omega = 0.455$; lower part of figure). In consequence, fixation probability of an allele at a linked locus varies cyclically ($\theta = s/S = 0.1$, $\rho = r/S = 0.1$). The light curves in the upper part of the figure show P_V, P_U, relative to the value expected with no hitchhiking, $2s$. The heavy curve shows the net fixation probability, $\bar{P} = (uP_U + vP_V)$, which is reduced on average by a factor 0.613. The figure shows one cycle, spanning $t = (2\pi/\omega S) = 13.81\,S$.

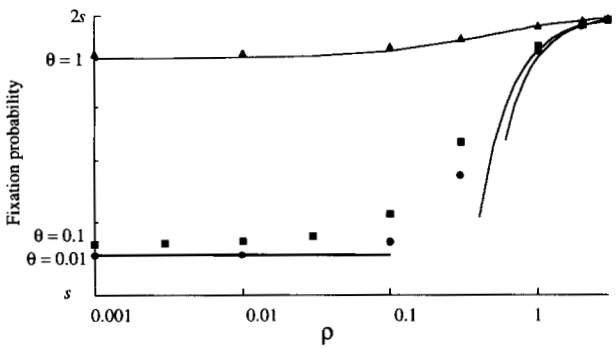

FIGURE 8.—The average reduction in fixation probability due to fluctuating selection. Allele frequency at the polymorphic locus varies between $u_{\min} = 0.1$ and $u_{\max} = 0.9$, as in Figure 7. Numerical results are plotted as a function of recombination rate ($\rho = r/S$), for alleles with advantage $\theta = 0.01$ (●), 0.1 (■) and 1 (▲). Both axes are logarithmic. The heavy line to the left shows the lower bound of $2/\{1 + I_0[\log(u_{\max}/u_{\min})]\} = 0.5519(2s)$ reached with small ρ, θ (Equation 12b; I_0 is the Bessel function). The three light curves to the right show the approximations for large $(\rho + \theta)$ (Equation 13b).

ferent backgrounds are independent of each other and are just those of an allele that multiplies at rates $s + vSf(T)$, $s - uSf(T)$, respectively. If the new allele is strongly selected ($s \gg S$), fluctuations have little effect on it. If it is weakly selected, and if fluctuations occur over a time scale $t \approx 1/S$ (as must be if the polymorphism is to persist at intermediate frequency), then the arguments of APPENDIX B lead to

$$\Delta \approx -\frac{\Pi}{uv}(u - \bar{u}) \qquad (12a)$$

$$\Pi = \frac{1}{1 + E\left(\frac{(u - \bar{u})^2}{uv}\right)}$$

$$= \frac{1}{E\left(\frac{\bar{u}^2}{u} + \frac{\bar{v}^2}{v}\right)} \quad (s, r \ll S) \qquad (12b)$$

where $E(\ )$ denotes the expectation, and $\bar{u} = E(u)$. (Note that neutral variation is reduced to a greater extent. Considering fluctuations in allele frequency within the separate genetic backgrounds show that the reduction is by a factor $(N_e/N) = 1/E[\bar{u}^2/u + \bar{v}^2/v + \mathrm{var}(u)/uv])$. The heavy line to the left of Figure 8 shows the limit of Equation 12b; the average fixation probability approaches it for $\theta \leq 0.1$, $\rho \leq 0.1$. If variations in u were extreme, $E[(u - \bar{u})^2/uv]$ would become very large, and fixation probability would tend to 0.

If linkage is loose, or if the rare allele is strongly favored, the arguments of APPENDIX A lead to

$$\Delta = \frac{f(T)}{(\rho + \theta)} \quad (\rho + \theta) \gg 1 \qquad (13a)$$

$$\Pi = 1 - \frac{E(f^2uv)}{(\rho + \theta)^2} = 1 - \frac{\mathrm{var}(W)}{2(r + s)^2} \qquad (13b)$$

Here, the average fixation probability is reduced in proportion to the additive variance in relative fitness, $E[2(Sf)^2uv]$. This is because if linkage is loose, or θ large, hitchhiking has only a transient effect, and so the ultimate fate of the selected allele is irrelevant: all that matters is the additive variance in fitness (see below). This approximation is shown by the light curves on the right of Figure 8; it is accurate for all degrees of linkage if $s \geq S$, and for $r > S$ when $s \ll S$.

Deleterious alleles: Assume that the rate of mutation to deleterious alleles is μ and that heterozygotes for such alleles have fitness reduced by S. There is no back mutation. We will assume that the mutation rate is small enough that homozygotes for the deleterious alleles are negligible, so that the equilibrium is at $u = \mu/S$. Fixation probabilities at the other locus are given by

$$-\frac{\partial P_u}{\partial t} = -rv(P_u - P_v) + (s - Sv)P_u - \frac{P_u^2}{2} \qquad (14a)$$

$$-\frac{\partial P_v}{\partial t} = -(ru + \mu)(P_v - P_u) + (s + Su)P_v - \frac{P_v^2}{2} \qquad (14b)$$

This only differs from Equation 5 in that the sign of S has been reversed and there is an additional term, $\mu(P_v - P_u)$, in Equation 14b that represents the probability μ that an allele associated with allele V will find itself associated with allele U in the next generation, as a result of mutation from V to U.

Analysis of this case is simple, because fixation proba-

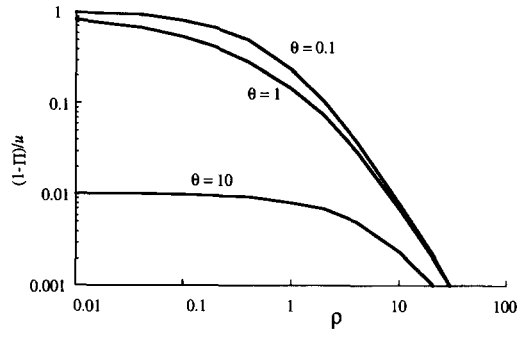

FIGURE 9.—The effect of deleterious mutations on fixation probability, relative to the maximum possible effect [*i.e.*, $(1 - \Pi)/u$], plotted against $\rho = r/S$ for $\theta = s/S = 0.1, 1, 10$. The scale $(1 - \Pi)/u$ is chosen because it is independent of u.

bilities will reach an equilibrium. As before, solution is facilitated by rescaling $T = St$, $\Pi = (uP_u + vP_v)/2s$, $\Delta = (P_u - P_v)/2s$:

$$\frac{\partial \Pi}{\partial T} = 0 = -\theta\Pi(1 - \Pi) + \theta uv\Delta^2 \quad (15a)$$

$$\frac{\partial \Delta}{\partial T} = 0 = \Delta[\rho + u + (2\Pi - 1)\theta - (u - v)(1 + \theta\Delta)] + \Pi \quad (15b)$$

In the limit of weak mutation, $u = \mu/S$ is small, and Π is close to 1:

$$\Pi = 1 - u\Delta^2 + O(u^2) \quad (16)$$

Substituting into Equation 15b and dropping terms of order u^2 leads to a quadratic equation for Δ, whose solution simplifies when linkage is very loose, or very tight:

$$\Delta = \frac{1}{2\theta}\left[-(1 + \rho + \theta) + \sqrt{(1 + \rho + \theta)^2 - 4\theta}\right] \quad (17a)$$

For $\rho = 0$:

$$\Delta = -1 \quad \Pi = 1 - u \quad (17b)$$

For $\rho \gg 1$, or for arbitrary ρ with $\theta \ll 1$:

$$\Delta \approx \frac{-1}{(1 + \rho + \theta)} \quad \Pi = 1 - \frac{u}{(1 + \rho + \theta)^2} \quad (17c)$$

The fixation probability is reduced by at most a factor $(1 - u) = (1 - \mu/S)$ when linkage is complete. Figure 9 shows the reduction in fixation probability relative to this value [*i.e.*, $(1 - \Pi)/u$], as a function of $\rho = r/S$. For loose linkage ($\rho \gg 1$), Equation 17c can be expressed in terms of the additive variance in relative fitness, $\mathrm{var}(W) = 2\mu S$:

$$\Pi = 1 - \frac{\mathrm{var}(W)}{2(S + r + s)^2} \quad (r \gg S) \quad (17d)$$

This converges to the same expression as for substitutions (Equation 9) and fluctuating selection (Equation 13b). Deleterious mutations at a single locus only cause an appreciable reduction in fixation probability when linkage is tight ($r \leq S$), *and* selection on the new mutant is weak ($s \leq S$). However, Equation 17d suggests that unlinked loci ($r = \frac{1}{2}$) can have an appreciable cumulative effect. With loose linkage ($r \approx \frac{1}{2}$), one cannot safely assume continuous time, and so the discrete version of Equation 14 should be used. At equilibrium, this gives the same equations as for weak recombination.

FISHER (1930, p. 122) gives a simple argument for the case of complete linkage ($\rho = 0$); this will be useful in the next section, where we consider multiple loci. In a large population that is in a mutation/selection balance, only the fittest class of genomes will contribute descendants to the distant future. Because the numbers of this fittest class must remain constant through time in a stable population, an individual carrying no deleterious mutations must on average leave one offspring that also carries no deleterious mutations. Because the proportion of offspring with no mutations is $(1 - \mu)$ in the present case, the absolute fitness of the fittest class must be $1/(1 - \mu)$. If a favorable allele arises in coupling with a deleterious allele, it will certainly be lost if the new genotype is still less fit than the fittest class (*i.e.*, $P_1 = 0$ if $1 + s - S < 1$, or $s < S$). If the favorable allele arises within the fittest class, its probability of fixation will depend on the absolute number of intact copies produced: $(1 + s)(1 - \mu)[1/(1 - \mu)]$. Hence, regardless of mutation rate, the fixation probability for favorable alleles that arise in the fittest class is just $P_0 = 2s$. This can be confirmed by setting $\rho = 0$ in Equation 17a. Because the favorable mutant has a chance $(1 - u)$ of arising in the fittest class, the net fixation probability is reduced by $\rho = (1 - u)$. The argument extends to any number of loci: with no recombination, mutations with a slight advantage s can only be fixed if they arise in the fittest class and have fixation probability $2s$ if they do. Because the fittest class may be extremely rare, fixation probability can be greatly reduced in an asexual population (PECK 1994). CHARLESWORTH *et al.* (1993) apply this argument to the effect of deleterious mutations on neutral variability. Here, the argument is approximate, because mutations on chromosomes carrying deleterious alleles contribute to heterozygosity, even if they cannot ultimately be fixed.

Two loci: The previous section found the effect of deleterious mutations at one locus. Because all loci within map distance $r \approx S$ will have a similar effect (Figure 9), we must consider the net influence of deleterious mutations over many loci. In principle, this can be calculated exactly, by following the probability of fixation of an allele embedded in all possible genetic backgrounds. However, this is impractical for more than a few loci, because there are so many possible genotypes. In this section, I show that the effects of

mutations at two loci are close to multiplicative. In the next section, I consider the simple case of unlinked loci of equal effect. Taken together, these analyses allow extrapolation to find the net effect of high per-genome mutation rates.

With two loci, we must follow the fixation probabilities of alleles that arise in the four possible backgrounds (P_{vv}, P_{uv}, P_{vu}, P_{uu}). The favorable allele is flanked by two loci subject to mutation at rates μ_1, μ_2 to alleles with frequencies u_1, u_2 and disadvantages S_1, S_2; recombination rates are r_1, r_2, respectively:

$$-\frac{\partial P_{uu}}{\partial t} = -r_1 v_1 (P_{uu} - P_{vu}) - r_2 v_2 (P_{uu} - P_{uv}) + (s - S_1 v_1 - S_2 v_2) P_{uu} - \frac{P_{uu}^2}{2} \quad (18a)$$

$$-\frac{\partial P_{uv}}{\partial t} = -r_1 v_1 (P_{uv} - P_{vv}) - (r_2 u_2 + \mu_2)(P_{uv} - P_{uu}) + (s - S_1 v_1 + S_2 u_2) P_{uv} - \frac{P_{uv}^2}{2} \quad (18b)$$

$$-\frac{\partial P_{vu}}{\partial t} = -(r_1 u_1 + \mu_1)(P_{vu} - P_{uu}) - r_2 v_2 (P_{vu} - P_{vv}) + (s + S_1 u_1 - S_2 v_2) P_{vu} - \frac{P_{vu}^2}{2} \quad (18c)$$

$$-\frac{\partial P_{vv}}{\partial t} = -(r_1 u_1 + \mu_1)(P_{vv} - P_{uv}) - (r_2 u_2 + \mu_2)(P_{vv} - P_{vu}) + (s + S_1 u_1 + S_2 u_2) P_{vv} - \frac{P_{vv}^2}{2} \quad (18d)$$

Linkage is assumed to be tight ($r \approx S$), so that double crossovers can be ignored. These equations can be solved numerically to give the average fixation probability, $\bar{P} = (u_1 u_2 P_{uu} + u_1 v_2 P_{uv} + v_1 u_2 P_{vu} + v_1 v_2 P_{vv})$. Because selection is weak and additive, linkage disequilibrium between the deleterious mutations is negligible. In Table 3, this is compared with the product of the effects of each locus alone. While the effects of the two loci are not precisely multiplicative, the fit is extremely close for most parameter combinations. The deviation is greatest for high mutation rates, and so Table 3 gives results for $u_1 = u_2 = 0.5$. Even when deleterious alleles are so frequent, and their effect consequently large, the deviation is only appreciable for tight linkage, and intermediate values of $\theta = s/S$. The final column of Table 3 also gives the prediction based on effective population size, an approximation that is discussed below. Agreement is comparable with the multiplicative prediction for loose linkage (when the predictions are in any case close to each other), but is much poorer for tight linkage.

Exchangeable loci: Consider a diploid organism, which has a large number of unlinked loci, each subject to a low rate of mutation. Selection acts on fecundity, such that the expected number of offspring declines geometrically with the number of deleterious mutations, as $(1 - S)^k$. The number of new mutations per haploid genome per generation then follows a Poisson distribution with mean $U = \Sigma\mu$; the number of new mutations per diploid individual is also Poisson, with mean $2U$. Selection will not generate linkage disequilibrium, and so the number of deleterious alleles (counted in diploids, before reproduction) follows a Poisson distribution with mean $2U/S$. The mean fitness of the population is $\exp(-2U)$, precisely the same value as with asexual reproduction (CROW 1970).

TABLE 3

Comparison between the effects of two flanking loci, and the effects of each locus alone

$\theta = s/S$	$\rho = r/S$	$\bar{P}_2/2s$	$(\bar{P}_2/2s)/(\bar{P}_1/2s)^2$	$\bar{P}_2\left(\frac{2}{\bar{P}_1} - \frac{1}{2s}\right)$
0.1	0	0.2500	1.00000	0.75000
	0.1	0.3689	1.00115	0.84650
	1	0.8268	1.00029	0.99199
	2	0.9296	1.00004	0.99872
1	0	0.5917	0.81219	0.79469
	0.01	0.6498	0.88480	0.86670
	0.1	0.7496	0.96503	0.95140
	1	0.9192	0.99946	0.99778
	2	0.9595	0.99997	0.99958
10	0	0.9954	1.00000	0.99999
	0.1	0.9955	0.99997	0.99999
	1	0.9962	0.99998	1.00000
	2	0.9968	0.99999	1.00000

$\bar{P}_2/2s$ gives the factor by which two equivalent loci reduce fixation probability, whilst $(\bar{P}_1/2s)^2$ gives the square of their individual effects. This is represented in the fourth column by the ratio $(\bar{P}_2/2s)/(\bar{P}_1/2s)^2$, which is usually close to 1. The final column gives the ratio between the effect of the two loci and that expected from their individual effects on effective population size (see text). The recombination rates, mutation rates, and selective effects are equal ($r_1 = r_2 = r$, $S_1 = S_2 = S$, $\mu_1 = \mu_2 = \mu$). Values are derived by numerical solution of Equation 18.

Consider an allele that increases fitness by a factor $(1 + s)$, regardless of the number of deleterious mutations present. If all loci are unlinked, its fixation probability, P_i, depends only on the number of deleterious mutations with which it is associated, i. P_i is counted in diploids, immediately before reproduction. Because the favorable allele is very rare, we need only follow the number of offspring that carry this allele. This follows a Poisson distribution, with mean W_i equal to half the expected total number of offspring.

From Equation 2:

$$(1 - P_i) = \exp(-W_i P_i^*) \quad (19a)$$

$$W_i = e^{2U}(1 + s)(1 - S)^i \quad (19b)$$

Here, W_i is the expected number of offspring of a par-

ent who carries i deleterious alleles, and also the favorable allele: only those offspring carrying the favorable allele are counted. The factor e^2U arises because we assume that the population is stable in size, so that the absolute mean fitness of the population is 1. P_i^* is the probability of fixation of a favorable allele in an individual carrying i deleterious mutations, given that that individual passes on one copy of the allele. This is the sum over the probability $\Gamma_{i,k}$ that an individual in class i passes the allele to an offspring in class k:

$$P_i^* = \sum_{k=0}^{\infty} \Gamma_{ik} P_k \qquad (20)$$

P_i^* is equivalent to P_i, except that it is measured at a different stage of the life cycle. To find $\Gamma_{i,k}$, assume that mutation occurs immediately before fertilization. The parent carries i deleterious alleles, each of which has probability ($^1/_2$) of being passed on. The offspring may also receive j_1 deleterious alleles derived by mutation in the first parent, j_2 derived by fresh mutations from the second parent, and j_3 inherited from the previous generation via the second parent. j_1 and j_2 are both Poisson distributed, with means U. j_3 is Poisson distributed with mean $(U/S) - U$, because it is drawn from the distribution among gametes after selection. Thus, $j = (j_1 + j_2 + j_3)$ is Poisson distributed with mean $(U/S) + U$. Combining these distributions,

$$\Gamma_{i,k} = \sum_{j=\max(0,k-i)}^{k} \left(\frac{[(U/S) + U]^j}{j!} e^{-[(U/S)+U]} \right) \times \left\{ \left(\frac{1}{2}\right)^i \binom{i}{k-j} \right\} \qquad (21a)$$

This simplifies to

$$\Gamma_{i,k} = e^{-[(U/S)+U]} \left(\frac{1}{2}\right)^i L_{k,i-k}\{-[(U/S) + U]\} \qquad (21b)$$

where $L_{k,i-k}[x]$ is the generalized Laguerre polynomial (Wolfram 1991). Equations 19–21 define a set of simultaneous equations which can be solved numerically by setting P_i to 0 above some large i.

Figure 10 gives results for a total mutation rate per haploid genome $U = 0.5$, with $S = 0.1$. This gives a Poisson distribution of the number of deleterious alleles (shaded bars), with mean $2U/S = 10$. The chance that an allele with advantage $s = 0.01$ will be fixed depends strongly on the genetic background in which it arises; taking the range of backgrounds, which includes 95% of the population ($i = 5$–15), the fixation probability varies by a factor 7.32 ($P_5 = 3.22s$, $P_{15} = 0.44s$). The average fixation probability is reduced by a factor $\Pi = 0.72$.

The single locus analysis suggests that mutations at unlinked loci will reduce fixation probabilities by a factor $(1 - 4\mu S)$, independent of the advantage of the new allele (Equation 17c). If effects multiply across loci, as was found with two loci (Table 3), the net reduction should be $\exp(-4US)$ for small μ. For example, with U = 0.5, $S = 0.1$, fixation probability is reduced by a factor 0.720 for $s = 0.01$, and 0.742 for $s = 0.4$; $\exp(-4US) = 0.670$. Figure 11 shows the reduction in fixation probability for $s = 0.01$, $S = 0.01$, as a function of U (○). A logarithmic scale is used, so that the multiplicative prediction $\exp(-4US)$ should appear linear (dotted line). Fixation probability does decline approximately geometrically with mutation rate for small U, but the effect is weaker than predicted for higher net mutation rates. A much better approximation is that $\Pi = 1/(1 + 4US)$ (solid curve), an approximation that is justified in the next section by an argument based on effective population size.

Peck (1994, Table 1) gives simulation results for this

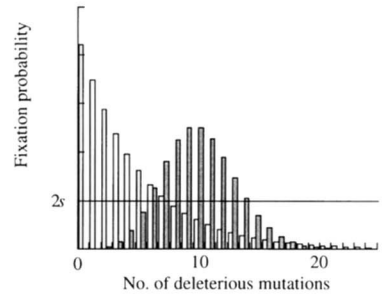

Figure 10.—The effect of deleterious mutations with effect $S = 0.1$ at a large number of unlinked loci. The net mutation rate per haploid genome is $U = 0.5$. The unshaded bars give the fixation probability of an allele with advantage $s = 0.01$, as a function of the number of deleterious alleles with which it is associated. This is measured relative to the value with no mutation, $0.0198 \approx 2s$. The shaded bars give the distribution of the number of deleterious mutations per diploid individual, which is Poisson with mean $2U/s = 10$.

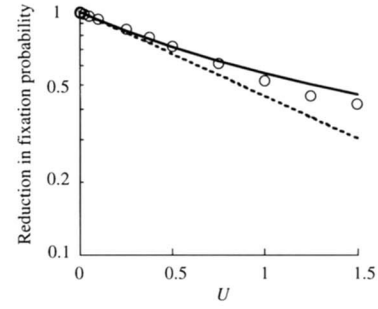

Figure 11.—The reduction in fixation probability of an allele with advantage $s = 0.01$, due to mutation at a large number of unlinked genes. Each mutation decreases fitness by a factor $(1 - S)$, with $S = 0.1$. Fixation probabilities are shown (○), calculated numerically from Equations 19–21, truncating at an upper limit of 25 deleterious mutations for $U < 1$, 35 for $U = 1$, and 50 for $U > 1$. These are compared, on a log. scale, with formulae extrapolated from Equation 17b, assuming either multiplicative effects ($\Pi = \exp(-4US)$; dotted line), or an effective population size that decreases inversely with the variance in fitness [$\Pi = N_e/N = 1/(1 + 4US)$; solid curve].

model. However, he considers somewhat lower mutation rates, and weak selection, so that the effect is weak. Simulations runs with stronger mutation and selection agree with the exact calculations from Equation 21 (J. R. PECK, personal communication).

GENERALIZATIONS

Effective population size: A general argument is that hitchhiking reduces the fixation probability simply by reducing the effective population size. On a diffusion model, the probability of fixation of a favorable allele is $2s(N_e/N)$ (CROW and KIMURA 1970, p. 426), where N_e is defined in the diffusion limit by the cumulative variance in allele frequency produced by sampling drift, $pq/2N_e = E(\delta p^2)/\delta t$ (where $\delta p = p_t + \delta t - p_t$). In the absence of natural selection, fluctuations δp are caused by random segregation at meiosis and by random, nonheritable, variation in offspring number. However, if fitness is heritable, genetic markers will become accidentally associated with genetic backgrounds of different selective value, thus causing fluctuations that persist over several generations. To apply the diffusion limit, the interval δt must be chosen to be short relative to the evolution of the favored allele, but long enough that fluctuations across different intervals are weakly correlated.

ROBERTSON (1961) argued that because associations with unlinked selected loci decay by half every generation, the cumulative perturbation is twice that in the initial generation, and the cumulative variance in allele frequency is four times that caused by noninherited variance in fitness. If, as we assume here, the noninherited variation in offspring number follows a Poisson distribution, and the population has constant size, ROBERTSON's (1961) argument gives $(N/N_e) = [1 + 2\,\mathrm{var}(W)]$, where $\mathrm{var}(W)$ is the additive genetic variance in the relative fitness of diploid individuals. Applying the same argument to linked loci gives $(N/N_e) = [1 + \mathrm{var}(W)/(2r^2)]$. The factor of $1/r^2$ appears because fluctuations in background fitness persist for $\approx r$ generations. Robertson's argument is approximate, because associations are affected by selection as well as by recombination. E. SANTIAGO and A. CABALLERO (unpublished data) give a more detailed analysis; however, this is based on a quantitative trait rather than individual loci, and so is hard to apply here. The formula $(N/N_e) = [1 + \mathrm{var}(W)/(2r^2)]$ agrees with the reduction in fixation probability of a weakly favored allele in the limit of loose linkage, for all the cases considered above: substitutions, fluctuating selection, and deleterious mutations (Equations 9b, 13b, and 17d). It is plausible that the effect of hitchhiking should be determined by the effective population size in this limit, because fluctuations due to chance associations with selected loci persist for only a short time ($r \gg s, S$), and so can be treated as equivalent to simple genetic drift in a population of smaller effective size.

Averaging over the genome: For each of the three kinds of selection considered here, we have derived approximations for the reduction in fixation probability due to a single locus, when linkage is loose or tight. To find the net effect of many loci, we must make some assumptions about how their effects combine. First, consider deleterious mutations. For unlinked deleterious alleles ($r = {}^1/_2 \gg S, s$), the argument based on effective population size predicts $\Pi = 1/[1 + 2\,\mathrm{var}(W)] = 1/(1 + 4US)$. This is shown by the solid curve in Figure 11 and is in better agreement with the exchangeable model than the multiplicative prediction (dotted line). For two loci, agreement is also good for loose linkage, but is much poorer than the multiplicative prediction for tight linkage (Table 3). This suggests that the effects of tightly linked loci combine multiplicatively, but that loosely linked loci exert their effect by increasing the genetic variance in fitness, and hence reducing effective population size. This argument is speculative; however, because the difference between the two alternative approximations is not great, it is unlikely to lead to much error. We can extrapolate to find the net effect of mutations scattered over a genetic map of length R Morgans ($R \gg 1$) by taking the product of contributions from closely linked loci (assumed to act multiplicatively), and the contribution from the majority of loci, which are effectively unlinked ($r = {}^1/_2$), and act to reduce N_e. Division of the genetic map onto several chromosomes should make little difference provided that these are not too short. The effect of deleterious mutations scattered uniformly over a genome of map length R is given by integrating Equation 17c (assuming no double crossovers):

$$\bar{\Pi} = \frac{\exp\left[-\frac{2U}{R\theta}\left(1 - \frac{1}{3\theta}\right)\right]}{(1 + 4US)} \quad (\theta \geq 1) \quad (22a)$$

$$\bar{\Pi} = \frac{\exp\left[-\frac{2U}{R}\left(1 - \frac{\theta}{3}\right)\right]}{(1 + 4US)} \quad (\theta \leq 1) \quad (22b)$$

For weakly favored alleles ($s \ll S, \theta \ll 1$), Equation 22b simplifies to $\exp(-2U/R)/(1 + 4US)$, which is the product of effects of closely linked loci (given solely by the mutation rate per unit map length, U/R), and the effects of unlinked loci [given solely by $2US = \mathrm{var}(W)$, the genetic variance in fitness caused by deleterious mutations]. This implies that as in classic load arguments, the reduction in fixation probabilities due to closely linked mutations is independent of their selective effect (provided $S \gg s$). Unlinked loci have an effect $1/(1 + 4US)$ (Equation 22b) and will have the main effect if the map is long and if most deleterious mutations have strong effects ($RS > 2$).

It is harder to extend the calculations for fluctuating

selection to many loci. When the new allele is strongly favored ($\theta \gg 1$) or when linkage is loose ($\rho \gg 1$), the fixation probability is reduced by fluctuations at a single locus by a factor $[1 - \text{var}(W)/2(r+s)^2]$ (Equation 13b), just as for deleterious mutations (Equation 17d). This similarity may be because linkage disequilibria persist for only $\approx 1/(r+s)$ generations, so that all that matters in this limit is the pattern of allele frequency change $\{\text{var}(W) = E[2(du/dt)^2/uv]\}$, rather than the ultimate fate of the alleles involved. If this is so, the analogy with deleterious mutations suggests that effects act through a reduction in N_e.

At the opposite extreme, when linkage is tight and the new allele is weakly favored ($\rho, \theta \ll 1$), the fixation probability tends to the limit $(2s)/E(\bar{u}^2/u + \bar{v}^2/v)$, where $\bar{u} = E(u)$ (Equation 12b). This formula can be derived by showing that the probability of fixation of a weakly favored allele that arises within a genetic background with average frequency $\bar{u}$ is $E(\bar{u}/u)$. Thus, it extends to multiple alleles, or multiple genetic backgrounds with frequency u_i, giving $(2s)/E(\Sigma \bar{u}_i^2/u_i)$. Now, if one considers two loci as defining four genetic backgrounds, labeled u_{00}, u_{01}, u_{10}, u_{11}, the fixation probability is given by $(2s)/E(\bar{u}_{00}^2/u_{00} + \bar{u}_{01}^2/u_{01} + \bar{u}_{10}^2/u_{10} + \bar{u}_{11}^2/u_{11})$. Provided that these loci are in linkage equilibrium, so that $u_{00} = v_1 v_2$, $u_{11} = u_1 u_2$, *etc.*, this equals $(2s)/[E(\bar{u}_1^2/u_1 + \bar{v}_1^2/v_1)E(\bar{u}_2^2/u_2 + \bar{v}_2^2/v_2)]$. Hence, effects are multiplicative in the limit of tight linkage and $s \ll S$, as for tightly linked deleterious alleles (Table 3).

To combine over loci, consider first a strongly favored allele ($s \geq S$), in which case Equation 13b applies even with tight linkage. Integrating over a long map ($R \gg 1$), and including the effects of unlinked loci:

$$\bar{\Pi} = \frac{\exp\left(-\dfrac{\text{var}(W)}{Rs}\right)}{1 + 2\,\text{var}(W)} \quad (s \geq S) \qquad (23)$$

Here, $\text{var}(W)$ is the total additive variance in relative fitness caused by fluctuations at balanced polymorphisms; it is assumed to be spread evenly over the genome. This formula suggests that weakly favored alleles [$s \leq \text{var}(W)/R$] would be very unlikely to fix. However, Equation 13b breaks down in such cases, and approaches the limiting value given by Equation 12b. Let this limit be $\Pi = (1 - K)$; it is shown by the left-hand side of Figure 8. A crude approximation is to splice Equations 12b and 13b where they cross (see Figure 8). This point is given by $K = \text{var}(W)/2n(r+s)^2$, where $\text{var}(W)/n$ is the average variance in fitness due to one of the n loci under balancing selection. It is the variance in fitness due to a single locus that matters here, because we are finding the points where two alternative approximations to the single-locus result meet. Integrating over the genome,

$$\bar{\Pi} = \frac{\exp\left(-\dfrac{\sqrt{2Kn\,\text{var}(W)}}{R}\right)}{1 + 2\,\text{var}(W)} \quad (s \ll S) \qquad (24)$$

This suggests that the cumulative effect of fluctuating selection at many polymorphic loci could be substantial if many loci are involved. However, simulations would be desirable as a check on this extrapolation from single locus results.

Finally, consider the effect of substitutions. These are qualitatively different from deleterious mutations or fluctuating selection, because successive substitutions can greatly reduce fixation probability. While many substitutions might be in progress at any one time, most of these would be at loosely linked loci, and would each have little effect. Integrating Equation 9b over the range where $(\rho + \theta) \gg 1$ leads to Equation 23, $\text{var}(W)$ now being the additive variance in fitness due to substitutions. Tightly linked loci have the dominant effect, and again, this can become very large when the new allele is weakly favored ($s \ll S$). We must therefore consider the limit where θ, ρ are both small, and the effect of a single substitution is equivalent to a catastrophe that reduces numbers by $w = 1 - \theta^\rho$ (Equation 9b).

Since it is unlikely that more than one substitution will be in progress within map distance $r \approx S$ at any one time, their effect on a rare allele can be considered as equivalent to a succession of catastrophes that reduces its numbers by factors w_1, w_2, w_3, ..., the ws depending on r and S. BARTON (1994) shows that the expected fixation probability is

$$\bar{P} = 2(s - s_{\text{crit}}) \quad \text{if} \quad s > s_{\text{crit}}$$

$$\bar{P} = 0 \quad \text{if} \quad s \leq s_{\text{crit}} \text{ where } s_{\text{crit}} = \frac{\pi^2\,\text{var}(W)}{6R\log_e(S/s)} \qquad (25)$$

This formula neglects the additional factor $1/[1 + 2\,\text{var}(W)]$ due to unlinked loci, because we expect this to be small. Note that the ratio s/S requires that the equation be solved numerically for s_{crit}; however, it only affects the threshold logarithmically. Equation 25 has the remarkable property that if the advantage of the rare allele is less than a critical value, proportional to the additive variance in relative fitness per unit map length, then it is extremely unlikely to fix in a large population. This is because each substitution reduces the frequency of the rare allele, and a succession of hitchhiking events can outweigh a slight selective advantage. Thus (barring the extremely unlikely possibility that the mutations arise in coupling), the weakly favored allele is expected to decrease in frequency. The chance that alleles with advantages below the critical value will be fixed tends to 0 as population size increases; however, it may still be much greater than the neutral value, $P = 1/2N$ (see BARTON 1994).

DISCUSSION

The analogy with spatial structure: There is a close analogy between populations that are divided into a variety of genetic backgrounds and populations that are geographically subdivided. Both genetic and spatial heterogeneity limit the power of natural selection, by reducing the chance that a favorable mutation will be fixed. The degree of interference from other selected loci depends on the random variation in fitness of an allele that finds itself in the various spatial locations or genetic backgrounds, relative to the rate of transfer between them by migration or recombination. If deme sizes and migration rates are constant, fixation probability is not affected by subdivision of the population (MARUYAMA 1970); similarly, balanced polymorphisms have no effect if they remain at equilibrium (EWENS 1967). However, if demes go extinct, and are then immediately recolonized, the fixation probability can be substantially reduced (LANDE 1985; TACHIDA and IIZUKA 1991; BARTON 1993), just as it can be reduced by substitutions at linked loci or by fluctuating polymorphisms. Variations in population structure may have different effects on alleles under different kinds of selection. For example, if extinction is frequent relative to migration, neutral variation is reduced much less than is the fixation probability of favourable alleles (BARTON 1993).

The general effect of heritable variation in fitness: Regardless of what kind of selection is acting, loosely linked loci reduce fixation probability by a factor $1/[1 + \mathrm{var}(W)/2r^2]$, where $\mathrm{var}(W)$ is the additive genetic variance in relative fitness. This is because the transient fluctuations in allele frequency caused by inherited variance in fitness act like uncorrelated "white noise," and so simply reduce the effective population size, N_e (ROBERTSON 1961). The effect of unlinked loci ($r = {}^1/_2$) on both N_e and on fixation probability depends solely on $\mathrm{var}(W)$. This may be substantial with artificial selection on a highly heritable trait: for example, if selection is based on a normally distributed trait with heritability 50% and the largest tenth is selected, the heritable variance of relative fitness is 2.24. This would reduce both fixation probability and neutral variation by a factor of 0.149, assuming no linkage. I ignore here the effect of noninherited fitness variation, which will further reduce the fixation probability. Selection that is based on the same fraction of the population, but that is spread over many independent loci, contributes much less variance in fitness. Thus, if truncation selection acts on 100 uncorrelated traits, each with heritability 50%, and if, as before, one-tenth of the population survives, $\mathrm{var}(W)$ is only 0.37. The variance in fitness is greatest, for given selective mortality, if truncation selection acts on some measure of genetic merit, as must be if load arguments are to be evaded (SVED *et al.* 1967).

It is harder to assess the heritable variance in fitness due to natural selection, even in laboratory populations. CHARLESWORTH (1987) suggests, based on the decline in viability and fertility caused by mutation accumulation, that $\mathrm{var}(W) \approx 0.005$. HOULE *et al.* (1992) use SVED's (1971) method, in which wild-type chromosomes are competed against balancers, to measure the effects of mutations on net homozygous fitness; they estimate $\mathrm{var}(W) \approx 0.013$. It is extremely hard to judge the *total* heritable variance in fitness, as opposed to the component due to deleterious mutations. CURTSINGER (1990) passed *Drosophila melanogaster X* chromosomes intact through the male line and found that the relative fitnesses of 17 different *X* chromosomes ranged from 0.63 to 1.28, with variance ≈ 0.038 [Table 3, ignoring sampling error and (strong) frequency-dependence]. K. FOWLER, C. SEMPLE, N. H. BARTON and L. PARTRIDGE (unpublished data) extend the SVED (1971) technique to find the relative fitness of wild-type chromosomes as heterozygotes against balancer chromosomes. They found that this ranged from 0.41 to 1.70 across 12 third chromosomes, with variance 0.10. These estimates include all sources of fitness variance, which may explain why they are larger than those based on mutations alone.

An upper bound is set by the total variance in relative reproductive success (the "opportunity for selection"). CLUTTON-BROCK (1988, Figure 29.1 and 29.2) reviews such measures across a variety of species, and concludes that the variance of relative fitness has a median of ~1.5 for both sexes. In principle, the heritable variance could be estimated from the lifetime reproductive success of individuals of known relationship: for example, family sizes are correlated across generations in humans (HUESTIS and MAXWELL 1932). Though such correlations may be largely due to maternal effects and cultural inheritance, they amplify random drift in much the same way as unlinked genetic variation. Fitness *components* more closely associated with fitness tend to have lower heritabilities, but only because they have large environmental variances. When standardized relative to the mean, their additive genetic variance is found to be no lower than for other metric traits (HOULE 1992), suggesting that the variance of fitness itself may be high.

These empirical estimates do not place strong bounds on the variance in fitness: if mildly deleterious mutations are the main source, $\mathrm{var}(W)$ might be negligible (≈ 0.01), whereas if a substantial fraction of the overall variance in reproductive success is heritable (for example, because of a fluctuating environment), it could cause a substantial reduction in N_e and hence in fixation probability. A general argument is that if sex is to be maintained despite its costs, then the heritable variance in fitness must be high. For example, coevolution between hosts and parasites must produce a substantial variance in fitness to produce a strong advan-

tage to modifiers that increase sex and recombination (HAMILTON *et al.* 1990). Similarly, substantial additive genetic variance must exist if it is to be worthwhile for females to choose males that carry "good genes" (CHARLESWORTH 1987).

Linked loci: *Deleterious mutations:* Linked loci further reduce the probability of fixation, below the ratio $1/[1 + 2\,\mathrm{var}(W)]$ expected from the heritable variance in fitness. Linked deleterious mutations reduce fixation probability by a factor that depends on the net mutation rate per unit map length, regardless of the selective effect: $\exp(-2U/R)$ ($s \ll S$; Equation 22b). For Drosophila, the experiments of HOULE *et al.* (1992) suggest $U > 0.25$; the map length is $R = 1.53$ Morgans (averaged over the sexes, and allowing for sex linkage) (FINCHAM 1983, p. 114). Hence, linked deleterious mutations should reduce fixation probability by a factor of at least $\exp(-2U/R) = 0.72$. More generally, classical load arguments that assume multiplicative fitnesses require $U \lesssim 1$; because most sexually reproducing higher organisms have more recombination than Drosophila, this implies a minor effect of linked deleterious mutations. CHARLESWORTH *et al.* (1993) comes to a similar conclusion for the effect of deleterious mutations on neutral variability. However, KONDRASHOV (1988) argues that if deleterious mutations have synergistic effects, a much higher mutation rate could be sustained ($U \approx 10$, say). If a substantial fraction of noncoding DNA is functional, if the number of coding genes is larger than in Drosophila (as in mammals), if many deleterious mutations are due to small insertions, deletions, *etc.* rather than base substitutions, and if the Drosophila experiments miss minor mutations, then the effect might be significant. Even if this is not usually so, linked deleterious mutations may substantially impede adaptation when recombination is restricted—for example, in predominantly selfing plants, or in regions of reduced crossing-over (*cf.* AQUADRO and BEGUN 1993; CHARLESWORTH *et al.* 1993; PECK 1994).

Fluctuating selection: It is hard to combine the effects of fluctuating selection across loci: the approximation of Equation 24 needs to be checked against simulations. However, it does suggest a substantial effect if additive genetic variance in fitness is due to fluctuations at many loci. Extreme fluctuations (for example, generated by coevolution between host and pathogen) (HAMILTON *et al.* 1990) would be most effective, but any polymorphism would be expected to fluctuate to some extent. If most amino-acid polymorphism is maintained by even moderate selection, then fixation probability could be very greatly reduced. For example, suppose that selection varies sinusoidally with amplitude $S \approx 10^{-2}$ at 10^4 loci (~10% of genes in man or mouse) (ANTEQUARA and BIRD 1994), causing allele frequencies to vary between 10 and 90%. The additive variance in fitness then averages $\mathrm{var}(W) = 0.39$. This pattern gives a maximum reduction for one locus with complete linkage of $\Pi = 1 - K = 0.448$; Equation 24 then predicts that averaged over the genome, fixation probability is greatly reduced, by a factor 0.0027. If selection were only $S \approx 10^{-3}$ on each locus (*cf.* LANGLEY *et al.* 1981), then the variance in fitness would only be 0.0039, and fixation probability would be reduced by a factor of only 0.551.

Enzyme heterozygosity is greater close to the highly polymorphic H-2 region in mice (NADEAU *et al.* 1983), as is synonymous DNA sequence variation near the F/S polymorphism at the *Adh* locus of *D. melanogaster* (HUDSON *et al.* 1987). Thus, balancing selection can have a significant influence on linked loci. As argued above, an increase in neutral variability is not incompatible with a decrease in the probability of fixation of favorable alleles. The latter is not affected by a *stable* polymorphism, even though neutral variability is increased. Conversely, fluctuations always reduce fixation probability (Equation 11a). However, their effect on neutral variability depends on the degree of linkage, relative to the time scale of fluctuations. Tightly linked loci will show higher variability, whereas loosely linked loci have less neutral diversity (*cf.* NADEAU and COLLINS 1983; SVED 1983).

Substitutions: The cumulative effect of substitutions differs qualitatively from that of deleterious mutations or fluctuating polymorphisms. The latter reduce fixation probability by a ratio which approaches some constant for weakly selected alleles ($P = 2s\Pi$, with $\Pi \approx 1$ as $s/S \to 0$). In contrast, successive substitutions make it very unlikely that alleles with selective advantage below some threshold will be fixed. The critical advantage is proportional to the heritable variance in fitness due to substitutions, per unit map length (Equation 25). It is hard to know what this value might be. If one accepts HALDANE's (1957) arguments on the substitution load, then the number of substitutions per generation must be low ($\lambda < 1/30$, say). Assuming that most amino acid substitutions are adaptive gives a similar value for higher organisms (GILLESPIE 1992, Table 1.4). If each new allele had (on average) $S = 0.05$, then the variance in fitness would be $2\lambda S = 0.0033$. Spread over 10 M, this gives the threshold $s_{\mathrm{crit}} = 0.85 \times 10^{-4}$ (Equation 25).

The role of weakly selected alleles in evolution: Several questions must be distinguished. One might ask whether weakly selected alleles occur; whether, when they occur, they are established because of their selective advantage, or merely by chance; whether they are unique, or recur; whether a large fraction of the genetic differences between species are caused by selection on weakly selected variants; and whether a significant fraction of *adaptive* differences are due to variants with little effect on fitness. The last question is the most important, because it concerns the genetic basis of adaptation. If mean fitness increases as a result of selection between alleles with very small effects, then hitchhiking could limit the rate of advance of a large population.

It is not obvious that any very weakly selected alleles should occur: one might imagine that a discrete change from one amino acid to another would necessarily cause an appreciable change in fitness. However, in metabolic pathways that involve many enzymes, the average influence of one gene on the flux must be small (KACSER and BURNS 1981). HARTL *et al.* (1985) have extended this argument to show that selection may cause average activity to increase to a level where further changes have extremely small effects. The recent discovery that most genes can be deleted without detectable effect on phenotype (TAUTZ 1992) is suggestive, but of course, "redundant" genes may still be subject to selection that is strong on an evolutionary time scale. In *Escherichia coli* (DEAN *et al.* 1988), most randomly generated mutations had no detectable effect on fitness, implying selection of ≤ 0.004. Differences between the distribution of allele frequencies at silent and replacement sites in *E. coli* suggest selection, but only of $\approx 1\text{–}5 \times 10^{-7}$ (SAWYER *et al.* 1987; HARTL 1989). Finally, null alleles are found at a frequency of ≈ 0.0025 at enzyme loci in Drosophila. If there is a balance with mutation $\mu \approx 4 \times 10^{-6}$, selection against null heterozygotes must average $\approx 1.6 \times 10^{-3}$ (LANGLEY *et al.* 1981). One would expect amino-acid changes to have even weaker effects.

Selection does not act solely on amino acid sequence: changes between synonymous codons or in noncoding regions may be important in aggregate, but may have very small individual effects. The best example of such variation comes from the bias in usage of synonymous codons. In *E. coli*, yeast, and Drosophila, this bias is strongest in highly expressed genes; however, this pattern is not found in mammals (KIMURA 1981; BULMER 1986; SHARP and LI 1986; SHIELDS *et al.* 1988). The most likely explanation is that translation is most efficient when the most abundant tRNA is used; in large populations, selection can bias codon usage in highly expressed genes. By assuming that fitness is proportional to flux, BULMER (1991) calculated that selection of $\approx 10^{-5}$ might act to bias codon usage in a highly expressed gene such as an aminoacyl tRNA synthetase in *E. coli*. A reduced bias in GC content in coding regions subject to reduced recombination suggests that hitchhiking does impede selection for codon usage bias (CHARLESWORTH 1994). It is not clear what fraction of selection can be accounted for by variation in amino acid sequence; if noncoding regions are important then very weakly selected alleles might matter.

This example shows that mutations can cause extremely small differences in fitness and yet still be subject to selection; these differences are small enough for the mutations to be strongly influenced by hitchhiking. However, we have still to discuss whether mutations of small effect make a significant contribution to adaptation. This is not quite the same as asking whether evolution is "gradual": two species might be connected by a chain of phenotypically similar intermediates, yet successive substitutions might be established by strong selection. There are many examples of adaptations based on one or a few genes under strong selection and that have evolved far too quickly for very weak selection coefficients to have been important (BISHOP and COOK 1981; ORR and COYNE 1992). Artificial selection has produced complex adaptations to domestication over hundreds of generations; and new species (notably, man) have adapted in as little as $\approx 10^5$ generations. When sister taxa hybridize, the shape of the clines that separate them can be used to estimate the number and effects of the genes responsible for reproductive isolation. For example, isolation between the toads *Bombina bombina* and *B. variegata* is based on ≈ 50 genes, each under selection of $\approx 10^{-2}$. There may well be many genetic differences with much smaller effects, but these do not contribute a significant fraction of the isolation; moreover, selection of less than $\approx 10^{-5}$ would not have been effective during the 3–4 million years for which these taxa have been diverging (SZYMURA and BARTON 1991).

The argument here assumes that adaptation is based on substitution; selection can cause a rapid change in the mean of a polygenic character, even when selection on each polygene is infinitesimally small, because small changes at many loci accumulate. However, my emphasis is on long-term evolution, which is likely to be based on substitutions, rather than shifts in polymorphic frequencies.

These arguments suggest that the differences between recent species have been established by moderately strong selection, and so cannot have been much impeded by hitchhiking. However, the basic molecular machinery has evolved over a much longer period, in very large populations, and in organisms with little recombination. Moreover, many molecular functions do not involve either the genetic code or recognition of precise binding sites, and so may be under intrinsically weak selection. Thus, hitchhiking may well have been important in limiting the rate of molecular adaptation. For example, BULMER (1991) has shown that though selection for biased codon usage in *E. coli*. is weak ($\approx 10^{-5}$), it should have produced a much stronger bias than is actually observed. Similarly, enzyme heterozygosity is much lower than expected in many species (KIMURA 1983; GILLESPIE 1992). Such discrepancies could be accounted for either by severe reductions in numbers or by hitchhiking (MAYNARD SMITH and HAIGH 1974). J. MAYNARD SMITH (personal communication) has argued that it is most unlikely that *E. coli* has been subject to sufficiently strong bottlenecks, favoring hitchhiking. However, he has also pointed out that different genotypes show very high sequence divergence, which implies polymorphisms that are too old to be compatible with either a bottleneck or hitchhiking explanation.

Hitchhiking may also be important in rapidly evolv-

ing populations, in which the variance in fitness due to substitutions becomes large: it is at just such times that a population would most benefit from favorable alleles. Substitutions under even strong selection may then be impeded, setting an upper limit on the rate of advance. The obvious example is of a population under artificial selection (HILL and ROBERTSON 1966; E. SANTIAGO and A. CABALLERO 1994), but natural populations that live in (or move into) unpredictable environments might also have a high genetic variance in fitness. Indeed, many explanations of the evolution of sex and recombination require that this state be common (HAMILTON *et al.* 1990). However, it is clear from this discussion that we need a great deal more information on the genetic basis of adaptation before we can know the extent to which linkage limits the power of natural selection.

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APPENDIX A: APPROXIMATIONS FOR A SLIGHTLY FAVORABLE ALLELE ($\theta \ll 1$)

When θ is small, the average fixation probability gradually decreases during the period before a substitution, over a time scale $T \approx 1/\theta$, and then returns rapidly toward $2s$ (*e.g.*, $\theta = s/S = 0.001$ in Figure 1A). This return occurs even while the substituting allele is rare, so that a substitution has greatest effect when in its early stages ($u < 0.01$ for $\theta = 0.001$, say). This is because while P_v is decreasing toward $2s\rho/(1 + \rho)$, P_u is still extremely large, and is decreasing from $2s(1 - \rho)/\theta$ for $\theta \ll 1$. The greatly increased probability of fixation of the new allele when coupled with U counterbalances the reduced probability when coupled with *V*, provided $u > \theta$. This pattern can be used to derive an approximate solution for small θ.

Equation 6a shows that the average fixation probability Π is reduced by the term $\theta uv\Delta^2$. This term is negligible for early times, so that $\partial\Pi/\partial T = -\theta\Pi(1 - \Pi)$. Thus:

$$\Pi \approx \frac{w}{w + (1 - w)\exp(\theta T)} \quad \text{for} \quad T \ll 0 \quad \text{(A1)}$$

This has the same form as the fixation probability before a catastrophe at $T = 0$, which reduces the number of alleles by a factor w (BARTON 1994). Numerical calculations show that Π does indeed converge to this form for small θ. During the brief period when $\theta uv\Delta^2$ is significant, the term $-\theta\Pi(1 - \Pi)$ can be ignored, so that the net change in Π from w before $T = 0$ to 1 afterward is

$$\Pi(0_+) - \Pi(0_-) = 1 - w = \int_{-\infty}^{\infty} \theta uv\Delta^2 dT \quad \text{(A2)}$$

To find an approximation for Δ, we splice together approximations for two regions: to the left, where $u \ll 1$, $\Delta \approx 1/\theta$, and to the right, where u, $\Delta \approx 1$, and where Π can be approximated by 1 (see Figure 1A).

For $u \ll 1$, $\Delta \gg 1$:

$$\frac{\partial\Delta}{\partial T} = -\Delta(1 - \rho) + \theta\Delta^2 \quad \therefore$$

$$\Delta = \frac{(1 - \rho)}{\theta(1 + A\exp[T(1 - \rho)])} \quad \text{(A3a)}$$

For u, Δ, $\Pi \approx 1$, $\rho < 1$:

$$\frac{\partial \Delta}{\partial T} = \Delta(\rho + u - v) - 1 \quad \therefore$$

$$\Delta(T) = \frac{1}{u(T)v(T)} \int_T^\infty u(\tau) \times v(\tau) \exp[\rho(T-\tau)]\, d\tau \quad \text{(A3b)}$$

These equations must be spliced together in the region where $A \exp[T(1-\rho)] \gg 1$ and $uv \approx \exp(T)$. Then, both solutions are proportional to $\exp[-T(1-\rho)]$:

$$\Delta \approx \frac{(1-\rho)\exp[-T(1-\rho)]}{A\theta}$$

$$\approx \frac{1}{u(T)v(T)} \int_T^\infty u(\tau)v(\tau) \exp[\rho(T-\tau)]\, d\tau$$

$$\approx \exp[-T(1-\rho)] \int_{-\infty}^\infty u(\tau)v(\tau) \exp(-\rho\tau)\, d\tau$$

$$\approx \exp[-T(1-\rho)] \int_{-\infty}^\infty \frac{\exp[\tau(1-\rho)]}{[1+\exp(\tau)]^2}\, d\tau \quad \text{(A4)}$$

Since

$$\int_{-\infty}^\infty \frac{\exp[T(x+1)]}{[1+\exp(T)]^2}\, dT = \frac{\pi x}{\sin(\pi x)},$$

$$A = \frac{(1-\rho)\sin(\pi\rho)}{\theta\pi\rho},$$

The dominant contribution to the integral of $\theta uv\Delta^2$ in Equation A2 is in the region where Equation A3a is valid. Hence

$$(1-w) = \left(\frac{\theta}{1-\rho}\right)^{\rho/(1-\rho)} \left(\frac{\pi\rho}{\sin(\pi\rho)}\right)^{1/(1-\rho)} \times \left(\frac{\pi\rho/(1-\rho)}{\sin[\pi\rho/(1-\rho)]}\right) \quad \text{(A5)}$$

The limit of Equation A5 when ρ is small is much simpler and is given by Equation 7.

With fluctuating selection, Π is approximately constant when θ is small (see Figure 7). Hence

$$\Pi(1-\Pi) = E(uv\Delta^2) \quad \text{(A6)}$$

From Equation 11, dropping the term $\theta\Delta(u-v)$ gives:

$$\frac{\partial \Delta}{\partial T} = \Delta[\rho + (2\Pi - 1)\theta + f(u-v)] - f\Pi \quad \text{(A7)}$$

Integrating, and using $du/dT = fuv$:

$$\Delta(T) = \frac{\Pi}{u(T)v(T)} \int_T^\infty \left(\frac{du}{d\tau}\right) \times \exp\{[\rho + (2\Pi - 1)\theta](T-\tau)\}\, d\tau \quad \text{(A8)}$$

This can be substituted into Equation A6 to give an equation for Π, which depends on the integral of (du/dt), averaged over a time scale $\rho + (2\Pi - 1)\theta$. If $[\rho + (2\Pi - 1)\theta$ is large relative to the timescale of fluctuations in u, Equation A8 simplifies to Equation 12a; Π is then approximated by Equation 12a, an expression independent of ρ and θ.

APPENDIX B: APPROXIMATIONS FOR LOOSE LINKAGE OR FOR A STRONGLY FAVORED ALLELE ($\rho + \theta \gg 1$)

Assume that the fixation probability is not much reduced, so that we can write $\Pi = 1 - \epsilon$, and ignore terms of $O(\epsilon)$. Then, from Equation 6

$$\frac{\partial \epsilon}{\partial T} \approx \theta\epsilon - \theta uv\Delta^2 \quad \text{(B1a)}$$

$$\frac{\partial \Delta}{\partial T} = \Delta[\rho + \theta + (u-v)(1-\theta\Delta)] - 1 \quad \text{(B1b)}$$

Equation B1a can be integrated explicitly, to give ϵ in terms of $\theta uv\Delta^2$:

$$\epsilon(T) = \int_T^\infty e^{-\theta(\tau - T)}\, \theta u(\tau)v(\tau)\Delta^2 d\tau \quad \text{(B2)}$$

The net effect can be found by integrating Equation B2 again, and reversing the order of integration with respect to T, τ:

$$\int_{-\infty}^\infty (1-\Pi)\, dT = \int_{-\infty}^\infty \epsilon dT = \int_{-\infty}^\infty uv\Delta^2 dT \quad \text{(B3)}$$

If we approximate the term $(1 - \theta\Delta)$ in Equation B1b by 1, on the grounds that Δ will be small with loose linkage or large θ, Equation B1b can be solved to give $\Delta(T)$ explicitly, in the same way as for Equation A3b:

$$\Delta(T) = \frac{1}{u(T)v(T)} \int_T^\infty u(\tau) \times v(\tau) \exp[(\rho + \theta)(T-\tau)]\, d\tau \quad \text{(B4)}$$

Substituting into Eq. B3 gives:

$$\int_{-\infty}^\infty (1-\Pi)\, dT$$

$$= \int_{-\infty}^\infty \frac{1}{u(T)v(T)} \left(\int_T^\infty u(\tau)v(\tau) \times \exp[-(\rho+\theta)(\tau - T)]\, d\tau \right)^2 dT$$

$$= \int_{-\infty}^\infty \int_T^\infty \int_T^\infty \frac{u(\tau_1)v(\tau_1)u(\tau_2)v(\tau_2)}{u(T)v(T)} \times \exp[-(\rho+\theta)(\tau_1 + \tau_2 - 2T)]\, d\tau_1 d\tau_2 dT \quad \text{(B5)}$$

Substituting for uv, changing variables to $z = \exp(T)$, $x_i = \exp(t_i)$, and changing the order of integration leads to

$$\int_{-\infty}^{\infty} (1 - \Pi)\, dT = \left(\frac{1}{(\rho + \theta)[2(\rho + \theta) + 1]} + \frac{2(\rho + \theta)}{4(\rho + \theta)^2 - 1} \psi'(\rho + \theta) \right) \quad \text{(B6)}$$

where $\psi'(x)$ is the second differential of $\log[\Gamma(x)]$. For large $(\rho + \theta)$, this is close to the much simpler approximation of Equation 9.

With fluctuating selection, the same arguments lead from Equation 11 to the analog of Equation B4:

$$\Delta(T) = \frac{1}{u(T)\, v(T)} \int_{T}^{\infty} f(\tau) u(\tau) v(\tau) \times \exp[(\rho + \theta)(T - \tau)]\, d\tau \quad \text{(B7)}$$

If $(\rho + \theta)$ is large compared with the time scale over which c changes, Equation B7 approximates to Equation 13a.