SUPPLEMENTAL MATERIAL

Analytical results are provided here for some special cases, all of which derive from the general approaches described in the main text.

Complete linkage; intermediate states with different deleterious effects; end states with equivalent high fitness. For the case of completely linkage, it is assumed that the sites have identical cellular and population-genetic environments so that population sizes and mutation rates are equal $N = N_A = N_B$ and $u = u_A = u_B$. In this case, $s_2 \neq s_3 > 0$ and $s_4 = 0$. Using Equations 1a-d and 3a,b, both end states have equal steady-state probabilities under the sequential model,

$$\widetilde{P}_1 = \widetilde{P}_4 = \phi_{b2}\phi_{b3}/C, \tag{S1a}$$

$$\widetilde{P}_2 = \phi_{d2}\phi_{b3}/C, \tag{S1b}$$

$$\widetilde{P}_3 = \phi_{b2}\phi_{d3}/C, \tag{S1c}$$

with the ϕ terms denoting fixation probabilities (b for beneficial, d for deleterious, and the number denoting the selection coefficient), and C being equal to the sum of the four numerators.

Both sites have equal rates evolution relative to the neutral expectation. From Equations 5a,b, for the sequential domain,

$$\left(\frac{d_N}{d_S}\right)_s = \left(\frac{d_A}{d_S}\right)_s = \left(\frac{d_B}{d_S}\right)_s = N[\,(\phi_{d2} + \phi_{d3})\widetilde{P}_1 + \phi_{b2}\widetilde{P}_2 + \phi_{b3}\widetilde{P}_3\,]. \tag{S1d}$$

Stochastic tunneling operates through the low-fitness intermediate states **aB** and **Ab**, which in large populations are maintained at selection-mutation balance frequencies u/s_2 and u/s_3, resepctively. With N individuals and equal end-state fitnesses, the rate of fixation of tunneling mutations is equal to the product of these frequencies and $Nu \cdot (1/N)$, leading to

$$\left(\frac{d_N}{d_S}\right)_t = (u^2/u)[\,(1/s_2) + (1/s_3)\,] = \frac{u(s_2 + s_3)}{s_2 s_3}. \tag{S1e}$$

Allowing for the fact that effective tunneling requires large Ns, more generally,

$$\left(\frac{d_N}{d_S}\right)_t = (1 - e^{-Ns_2/2})u/s_2 + (1 - e^{-Ns_3/2})u/s_3. \tag{S1f}$$

The total relative rate of evolution is then equal to the sum of Equations S1d and S1f.

Complete linkage; intermediate states with equivalent deleterious effects; end states with elevated but different fitnesses. In this case, the selective disadvantages of the two intermediate states are $s = s_2 = s_3 > 0$, whereas one end state has advantage s_4,. Given their linkage in the same genome, both sites are again assumed to have identical mutation rates and effective population sizes. Using Equations 1a-d and 3a,b, the steady-state probabilities under the sequential model are,

$$\widetilde{P}_1 = \phi_{bs}\phi_{ds^*}/C, \tag{S2a}$$

$$\widetilde{P}_2 = \widetilde{P}_3 = \phi_{ds}\phi_{ds^*}/C, \tag{S2b}$$

$$\widetilde{P}_4 = \phi_{ds}\phi_{bs^*}/C, \tag{S2c}$$

where $s^* = s + s_4$, with the ϕ terms again denoting fixation probabilities (b for beneficial, d for deleterious), and C being equal to the sum of the four numerators.

From Equations 5a,b, for the sequential domain, both sites are again found to have equal rates of evolution relative to the neutral expectation.

$$\left(\frac{d_N}{d_S}\right)_s = N[\,\phi_{ds}\widetilde{P}_1 + (\phi_{bs} + \phi_{bs^*})\widetilde{P}_2 + \phi_{ds^*}\widetilde{P}_4\,]. \tag{S2d}$$

The rate of stochastic tunneling can be approximated by

$$\left(\frac{d_N}{d_S}\right)_t = \frac{2Nu(1 - e^{-Ns/2})}{sC'} \cdot (\phi_{bs}\phi_{ds^*}\phi_{bs_4} + \phi_{ds}\phi_{bs^*}\phi_{ds_4}), \tag{S2e}$$

where C' is the sum of the numerators in Equations S2a and S2c. As $N \to \infty$, the tunneling rate approaches $(2Nu/s)\phi_{ds_4}$, which asymptotically approaches zero, owing to the fact that the population is permanently retained in the near-pure beneficial end state.

The total relative rate of evolution is equal to the sum of Equations S2d and S2e.

Free recombination; symmetrical fitnesses ($s_2 = s_3 = s$ and $s_4 = 0$); arbitrary population sizes (N_A and N_B) and mutation rates (u_A and u_B). The system of Equations 1a-b reduce to Equations 4a,b in the main text, and the rates of nucleotide substitution scaled to the neutral expectation are given by Equations 6a,b in the main text. The latter then expand

to

$$\frac{d_A}{d_S} = \frac{N_A[\,2\beta\phi_{bA}\phi_{dA} + \phi_{dA}\phi_{bB} + \phi_{bA}\phi_{dB},]}{\beta(\phi_{bA} + \phi_{dA}) + (\phi_{bB} + \phi_{dB})}, \tag{S3a}$$

$$\frac{d_B}{d_S} = \frac{N_B[\,2\phi_{bB}\phi_{dB} + \beta(\phi_{dA}\phi_{bB} + \phi_{bA}\phi_{dB}),]}{\beta(\phi_{bA} + \phi_{dA}) + (\phi_{bB} + \phi_{dB})}, \tag{S3b}$$

where $\beta = (N_A u_A)/(N_B u_B)$, and the fixation probabilities are defined by Equations 3a,b using the appropriate population size. For $N_A s$ and $N_B s \gg 1$, these expressions reduce to Equations 10a,b in the main text.

For the special case in which mutation rates differ, but population sizes are constant, then $\phi_b = \phi_{bA} = \phi_{bB}$, $\phi_d = \phi_{dA} = \phi_{dB}$, and Equations S3a,b reduce to Equation 7 in the main text.

Free recombination; asymmetrical fitnesses ($s_2 \neq s_3$, but $s_4 = 0$); constant population sizes ($N_A = N_B = N$); and arbitrary mutation rates (u_A, u_B). The scaled evolutionary rates of both sites are identical and independent of the mutation rates,

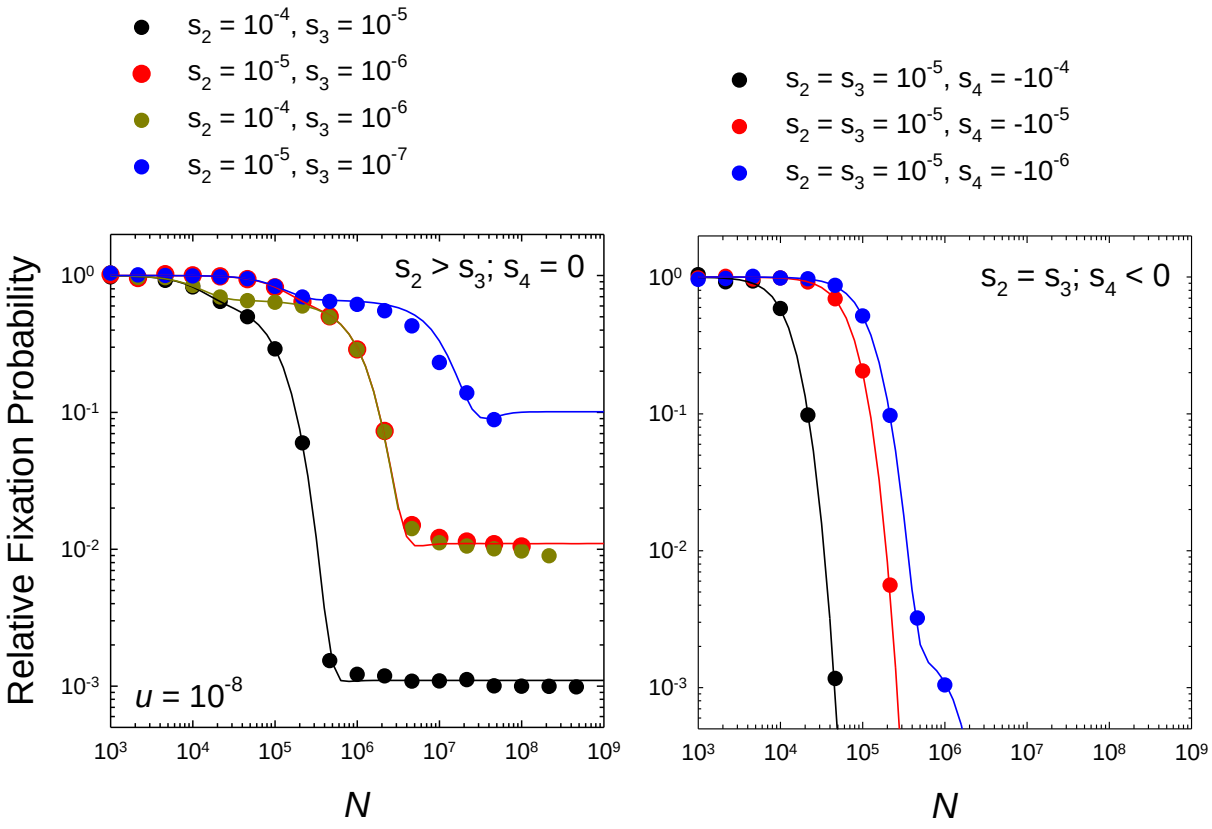
$$\frac{d_N}{d_S} = \frac{2N\phi_b(s_2)\phi_b(s_3)[\phi_d(s_2) + \phi_d(s_3)]}{C}, \tag{S4a}$$

where

$$C = \phi_b(s_2)[\phi_b(s_3) + \phi_d(s_3)] + \phi_b(s_3)[\phi_b(s_2) + \phi_d(s_2)], \tag{S4b}$$

and the fixation probabilities are defined by Equations 3a,b using fixed N and substituting the appropriate selection coefficient.

Supplemental Figure S1. Rates of substitution at the two sites scaled to the neutral expectation as a function of N, for the case of complete linkage with both sites experiencing equal mutation rates and effective population sizes. The data points are computer-simulation results, obtained over a range of N for various combinations of s_2, s_3, and s_4. The solid lines denote the predictions from theory outlined in the Supplemental Text, Equations S1d and S1f on the left, and Equations S2d and S2e on the right.



Supplemental Figure S2. Influence of the recombination rate on the scaled rate of evolution. The solid lines are the theoretical expectations in the absence of recombination (as shown in Figure 2), whereas the curved lines are the theoretical expectations (described in the text) for intermediate levels of recombination (symbol shapes denoted in the inset, for two values of s). The data points are from computer simulations with fixations recorded as transitions of one haplotype to another using a threshold frequency of 0.999.