

Evolutionary layering and the limits to cellular perfection

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Although observations from biochemistry and cell biology seemingly illustrate hundreds of examples of exquisite molecular adaptations, the fact that experimental manipulation can often result in improvements in cellular infrastructure raises the question as to what ultimately limits the level of molecular perfection achievable by natural selection. Here, it is argued that random genetic drift can impose a strong barrier to the advancement of molecular refinements by adaptive processes. Moreover, although substantial improvements in fitness may sometimes be accomplished via the emergence of novel cellular features that improve on previously established mechanisms, such advances are expected to often be transient, with overall fitness eventually returning to the level before incorporation of the genetic novelty. As a consequence of such changes, increased molecular/cellular complexity can arise by Darwinian processes, while yielding no long-term increase in adaptation and imposing increased energetic and mutational costs.

genetic load | cellular evolution | robustness | nonadaptive evolution

Although natural selection is one of the most powerful forces in the biological world, it is not all powerful. However, so ingrained is the belief in the extraordinary power of selection that when confronted by biological imperfections at the molecular and morphological levels, most investigators simply invoke pleiotropic constraints, i.e., negative functional relationships between two traits influenced by the same genes, resulting from molecular limitations, metabolic tradeoffs, etc. When made in the absence of any direct evidence, as is often the case, such adherence to the adaptationist paradigm discourages the likelihood of recognizing nonadaptive paths to the origin of organismal features (1–3).

Random genetic drift imposes a fundamental constraint on the level of perfection achievable by natural selection. As a consequence of the finite sampling of gametes and the linked nature of genomes, all populations experience stochastic fluctuations in allele frequencies that compromise the efficiency of selection. Once a high enough level of molecular perfection has been achieved so that further refinements in fitness are smaller than the prevailing force of drift, natural selection will be incapable of promoting any additional improvement (4, 5). Depending on the nature of the potential genetic variation underlying an adaptation, this drift barrier may be reached before selection is confronted with any physical, chemical, or bioenergetic constraints.

If, however, drift prevents natural selection from inexorably moving cellular features toward a state of molecular perfection, how do we account for the abundant examples of organisms using layered mechanisms for dealing with intracellular problems? For example, genome replication involves highly selective DNA polymerases, but the small fraction of initial base misincorporations are subject to correction by subsequent proofreading, and the still smaller fraction of errors that escape proofreading are generally subject to mismatch repair. The accuracy of translation also depends on a series of quality-control steps: proper loading of tRNA synthetases by their cognate amino acids, proper recognition of tRNAs by their cognate synthetases, proper codon recognition by tRNAs, and a variety of mRNA surveillance mechanisms. In many cases, proper protein folding depends on a series of chaperone

systems, and numerous mechanisms exist for the disposal of terminally misfolded proteins.

Although these layered lines of defense are clearly advantageous and in many cases essential to cell health, because the simultaneous emergence of all components of a system is implausible, several questions immediately arise. How can selection promote the establishment of additional layers of fitness-enhancing mechanisms if the established primary lines of defense are already highly refined? When such changes are assimilated into a population, does an increase in overall fitness necessarily follow? Do the preexisting mechanisms retain their full efficiency, with a permanent increase in adaptation being achieved, or does an added layer of protection inevitably result in the relaxation and eventual degeneration of earlier established mechanisms (6, 7)?

Results

Limits to Perfection. As a point of departure, we first consider the evolutionary features of an arbitrary character under a simple mutational model. A discrete array of alleles is assumed, with the upper limit to fitness being scaled to 1.0, and the selective disadvantage of allele i , $s_i = e^{-ki}$, asymptotically approaching zero with increasing i (Fig. 1). A homogeneous stepwise mutation process is assumed, such that each allele i mutates to the next highest fitness class $i + 1$ at rate u and to the next lowest class $i - 1$ at rate v . Under this model, a population is expected to evolve upwardly in the allelic series until the point is reached where the advantage of the next highest allele is so weak that selection is rendered ineffective either by the power of random genetic drift or by mutation pressure in the downward direction. This point will depend on the effective size of the population (N , assumed here to be haploid, which defines the magnitude of genetic drift $1/N$), by the granularity of the fitness effects of adjacent allelic classes (k), and by the ratio of downward to upward mutation rates (v/u).

Although the behavior of loci harboring multiple alleles can be quite complicated, two limiting cases for this stepwise-mutation model yield simple generalizations. At the small-population size extreme, $(u + v)N < 1$, there will generally be only a few alleles segregating in the population at any point in time. Such a population will then typically move between adjacent classes in a nearly stepwise fashion, and can thus be categorized reasonably well by the feature of its most recent common ancestor (8). Letting $\phi_{x,y}$ denote the probability of fixation of a single mutant allele of class y arising on a background of allele x , the approximate rate of transition of class i to $i + 1$ is $Nu\phi_{i,i+1}$ and to $i - 1$ is $Nv\phi_{i,i-1}$.

Using Kimura's (9) diffusion approximation for the fixation probabilities, noting that $s_i - s_{i+1}$ is the net selective advantage of allele $i + 1$ in an i background, and taking advantage of the fact that the ratio of fixation probabilities of advantageous and deleterious mutations with the same s is e^{2Ns} in a haploid population

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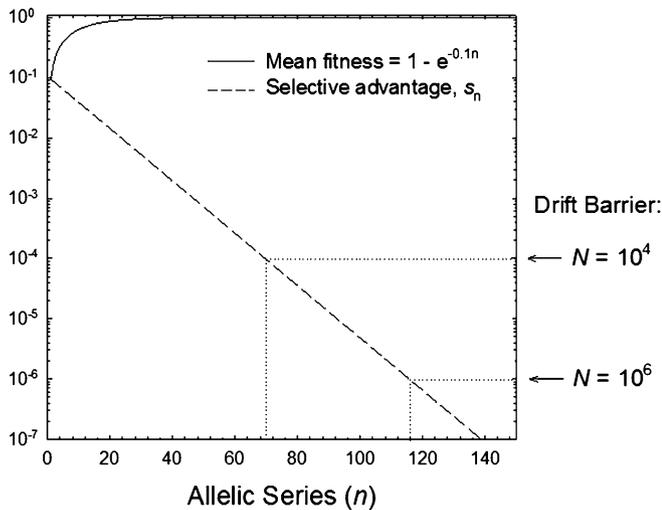


Fig. 1. Fitness function and selection coefficients for an allelic series defined by the exponential model described in the text. The drift barrier to allelic improvement is illustrated for two population sizes.

(10), a simple solution can be obtained for the equilibrium mean frequencies of each allele,

$$\hat{p}_i = \frac{(u/v)^i e^{-2N s_i}}{T}, \quad [1]$$

where T is the sum of the numerators for all \hat{p}_i . Under this model, \hat{p}_i can be interpreted as the probability that allele i is the most recent common ancestor of the full collection of alleles in the population, with adjacent allelic classes ($i - 1$ and $i + 1$) potentially segregating in between fixation periods. As shown in Fig. 2, this expression closely approximates the average long-term representation of alleles in a steady-state population with $(u + v)N < 1$, derived from the results of stochastic computer simulations.

The drift barrier to further adaptive progress is defined by the threshold allele ($i = n^*$) at the point in the array where the rate of establishment of the next best allele (the product of the upward mutation rate and the fixation probability) is approximately equal to that in the opposite direction (i.e., the rate of reversion).

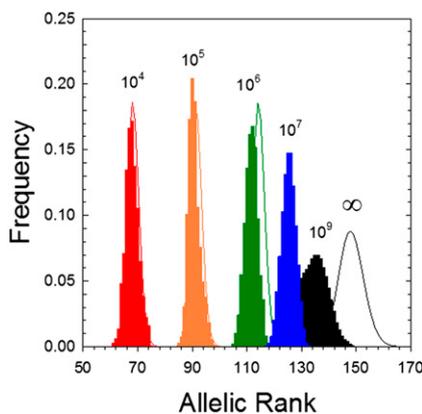


Fig. 2. Allele-frequency distributions at various effective population sizes (from 10^4 to 10^9). The histograms were derived from simulations of a Wright-Fisher population, whereas the three leftmost line graphs depict the expectations given by Eq. 1, and the results for an infinite population size were obtained from the recursion equations Eqs. 3a–3c. In all cases, the total mutation rate is $u + v = 10^{-6}$, $u/(u + v) = 0.1$, and $k = 0.1$.

Because the ratio of fixation probabilities for beneficial and deleterious alleles with equal absolute effects is e^{2Ns} , the critical selection coefficient at stochastic equilibrium is $s^* \approx [\ln(v + u)]/(2N)$ assuming $v > u$. Using the above definition for s , at stochastic selection–mutation–drift equilibrium, the population is then expected to stall near allelic position

$$n^* \approx \frac{\ln(2Nk) - \ln[\ln(v/u)]}{k}, \quad [2]$$

assuming $k \ll 1$. Allelic position n^* closely approximates the peaks of the three leftmost plots in Fig. 2.

For an effectively infinite population size, the dynamics of allele-frequency change (p to p') can be expressed as

$$p'_1 = [W_1 p_1 (1 - u) + W_2 p_2 v] / \bar{W}, \quad [3a]$$

$$p'_i = [W_{i-1} p_{i-1} u + W_i p_i (1 - u - v) + W_{i+1} p_{i+1} v] / \bar{W}, \quad [3b]$$

$$p'_{i_{\max}} = [W_{i_{\max}-1} p_{i_{\max}-1} u + W_{i_{\max}} p_{i_{\max}} (1 - v)] / \bar{W} \quad [3c]$$

where the middle equation applies to all but the first and final alleles in the series, and $\bar{W} = \sum_{i=1}^{i_{\max}} p_i W_i$. Iteration of these equations from any starting point, with i_{\max} set at an arbitrarily large value, leads to the limiting equilibrium distribution of allele frequencies.

Although a population can wander slightly above the n^* allelic state by stochastic mutation and drift, such alleles cannot be maintained by selection. Thus, for relatively small population sizes, provided there is a mutational bias in the downward direction, which seems certain in virtually all cases of molecular adaptation, a fairly tight distribution of allelic probabilities is maintained over evolutionary time, despite the fact that all allelic classes are accessible by mutation. For the extreme case in which $u = v$ (no mutational bias), alleles in states beyond the drift barrier are free to randomly evolve in both directions, and a flat distribution of \hat{p}_i is expected beyond n^* (8). However, because a random walk from n^* to some substantially higher state (n') requires on the order of $e^{\sqrt{(n'-n^*)}/u}$ generations (11), such a distribution may rarely ever be realized in nature.

The limits to adaptation can be quantified as the magnitude of fitness load resulting from the inability of selection to promote the most advantageous alleles, i.e., as the reduction of the equilibrium mean population fitness relative to the scaled maximum of 1.0,

$$L = \sum_{i=1}^{i_{\max}} s_i \hat{p}_i, \quad [4]$$

where the sum is over all alleles with nonzero equilibrium frequencies. Provided the rate of deleterious mutation exceeds the rate of adaptive mutation ($v > u$) and $(u + v)N < 1$, an equilibrium population will generally be close to the state defined by Eq. 2, and

$$L \approx \frac{\ln(v/u)}{2Nk} \quad [5]$$

This expression for the fitness load (in effect, a measure of allelic imperfection) differs from previous results that assumed the presence of just two possible alleles (e.g., ref. 12), often with mutations only producing the disadvantageous allele (e.g., ref. 13), and also from prior results with multiallelic models that have assumed either symmetrical mutation patterns (i.e., $u = v$), or Fisher's (14) geometric model in which v/u effectively increases with the degree of adaptation (8, 15, 16). Nevertheless, although

the exact expression for the drift load appears to be model dependent, a unifying feature of Eq. 5 and all prior work is an inverse relationship between the equilibrium level of molecular imperfection and the effective population size. A strong fitness gradient among alleles (large k) reduces the load because adjacent alleles are more easily discriminated by selection, and a relatively high incidence of adaptive (forward) mutations (low v/u) further reduces the load because selection is less opposed by mutation pressure.

At the other extreme, as $N \rightarrow \infty$, the population approaches the expected distribution of segregating allele frequencies under selection–mutation balance, obtainable from Eqs. 3a–3c. In this case, provided $v/u > 5$, i.e., the equilibrium load is

$$L \simeq v - u[\ln(v/u)]^2, \quad [6]$$

independent of k , although N must be enormous for this limit to be reached (Fig. 3). Eq. 6 shows that Haldane’s (13) classical result of the genetic load being equal to the deleterious mutation rate in an effectively infinite population even holds with multiple alleles, provided there are no advantageous mutations, but gives an overestimate when $u \neq 0$.

Establishment of a Secondary Line of Defense. Supposing the genotypic distribution of a primary line of defense has settled into the steady-state selection–mutation–drift equilibrium, the possibility still exists that a second line of defense with a sufficiently large initial effect on fitness can be promoted by at least transient positive selection. In a large population, such establishment will be least likely if the new mutation arises in a low background-fitness class, as such individuals are destined to be lost by selection, but colonization of a high background-fitness genotype may give a sufficiently high boost in total fitness to promote fixation of the two-layered system. To have a reasonable chance of success, the boost in fitness would need to be greater than the incremental effects of beneficial mutations for the primary line of defense. A small population transiently fixed for a relatively low quality allele may also provide a viable path to invasion of a second layer.

Consider the situation in which the mutation to a secondary layer of defense causes an improvement in fitness in a carrier equivalent

to moving up the allelic ladder by j steps, so that the absolute selective disadvantage of an individual in class i at the first layer would be reduced to $s_{i+j} = e^{-k(i+j)}$, and the probability of fixation of the new layer is given by $\phi_{i,j}$. The overall probability of establishment depends on the frequency distribution of the background trait in which the mutation for the new layer of defense arises,

$$p_{e,j} \simeq \sum_{i=1}^{i_{\max}} \hat{p}_i \phi_{i,j}. \quad [7]$$

The maximum probability of establishment arises when the second layer completely eliminates all deficiencies of the first layer, which implies a relative selective advantage equal to s_i .

An example of the sensitivity to fixation of a second layer of defense in the small population-size domain is shown in Fig. 4, where \hat{p}_i is defined by Eq. 1. With the fitness model used, virtually identical results are obtained for all population sizes in the domain satisfying $N(u + v) < 1$. The fact that the probability of fixation exceeds the neutral expectation of $1/N$ in all cases clearly demonstrates that the establishment of a second layer of defense can be promoted by selection even if the first layer is at the drift barrier. Simulations with small population sizes give slightly higher probabilities of establishment than the theoretical approximations, and those with larger population sizes yield even higher probabilities.

Consequences of Evolutionary Layering. Having been promoted by natural selection, establishment of a second layer of defense will initially lead to an increase in mean population fitness. However, this boost in fitness may also be transitory unless the presence of a second layer somehow magnifies the fitness-enhancing effects separating adjacent allelic classes (k in the above model). Consequently, depending on the nature of the molecular adaptation, the initial excursion below the drift barrier resulting from the invasion of a second layer of defense may gradually dissipate as deleterious alleles with effects too small to be opposed by selection accumulate at one or both loci, with the joint effects of the two-layered system eventually returning to the same layer of perfection as the initial one-layered system. In principle, this means that the efficiency of the first layer is unlikely to ever improve from its initial state, assuming that the equilibrium state had been reached. Likewise, assuming a similar magnitude of mutational effects for both layers (e.g., constant k), the efficiency of the secondary layer is unlikely to improve any further unless it comes at the expense of the first layer.

For the model presented above, an explicit statement can be made by considering the joint combination of alleles across both loci satisfying $n^* = n_1^* + n_2^*$, as defined in Eq. 2, with the level of perfection at each layer then being defined as $1 - e^{-kn_i^*}$. In effect, this leads to a ridge of combinations of states at the two loci defining the bivariate drift barrier $s^* = [\ln(v + u)]/(2N)$ (Fig. 5). This implies that a negative relationship is expected to evolve between the levels of perfection of the two traits in independent populations, or at various points of time within a lineage containing the two traits. With all points on the equilibrium ridge having equivalent fitness, such populations are free to drift in one direction or the other, even to the point of losing one of the traits, although directional bias may be induced by the pattern of mutation pressure.

A few examples of this behavior, generated by stochastic simulations of populations following the fixation of a newly arisen allele for the second trait, are given in Fig. 6. In all cases, the initial improvement in overall fitness resulting from the establishment of the second layer is followed by a gradual loss of perfection in each trait, until a point on the bivariate drift barrier is reached where mean fitness is essentially the same as that before invasion of the second layer.

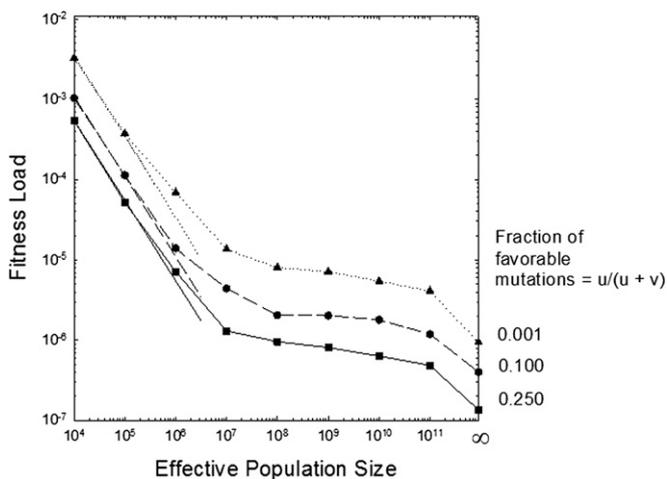


Fig. 3. Equilibrium fitness loads as a function of the effective population size. Solid, dashed, and dotted lines are for $u/v = 1/3, 1/9,$ and $1/999$, respectively. Straight lines to the left are given by Eq. 5, and data points were obtained by computer simulation of a Wright–Fisher model. The final (rightmost) data points for each curve are the infinite-population size limits obtained by use of Eqs. 3a–3c. In all cases, the total mutation rate is $u + v = 10^{-6}$, and $k = 0.1$.

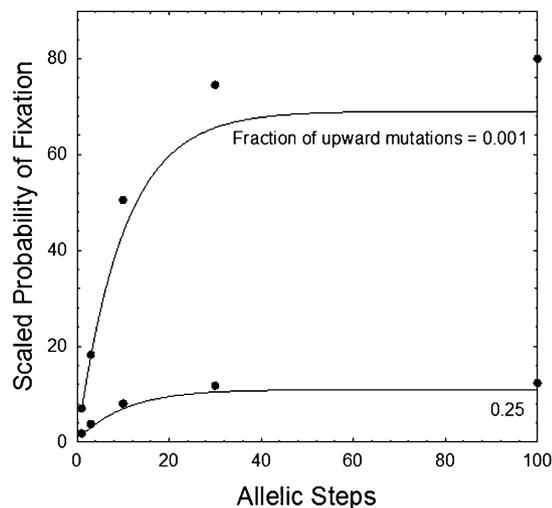


Fig. 4. Probability of establishment of a second layer of defense as a function of the initial improvement, given for the small population-size domain using Eqs. 1 and 5. In both cases, the total mutation rate is $u + v = 10^{-6}$, and $k = 0.1$. Virtually identical results are obtained for $N = 10^4$ and 10^5 . The probability of fixation is scaled to the neutral rate, being given as $Np(j)$, so a value equal to 1.0 implies simple drift to fixation without any assistance by selection. Data points were obtained from simulations of a Wright-Fisher model with complete linkage.

Discussion

Although the preceding results rely on just one of many possible mutation-selection models, they clarify the general roles that random genetic drift plays in defining the ability of natural selection to refine the performance of molecular/cellular features. In general, the level of sustainable molecular perfection is inversely proportional to the effective size of a population, with small populations experiencing the fixation of suboptimal alleles and large populations experiencing an additional segregational

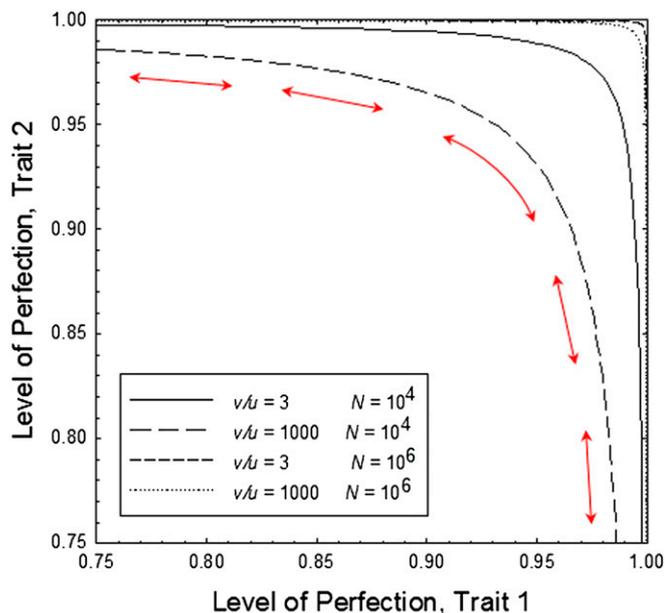


Fig. 5. Bivariate drift barrier for two ratios of rates of beneficial and deleterious mutation (u/v), and two effective population sizes (N), as defined by the model in the text, and Eq. 2. All points along each line have equal fitness, providing ridges for the drift of the bivariate system.

load from recurrent mutational introduction. In both cases, the vast majority of alleles that are likely rise to moderate frequencies are effectively neutral with respect to each other.

The concept of a drift barrier to adaptive performance is implicit in several previous theoretical studies. For example, Hartl et al. (4) suggested that enzyme activities evolve to levels at which further mutational improvements in fitness have effects smaller than $1/(4N)$, leading to the conclusion that effective neutrality is an expected outcome of natural selection. Wylie and Shakhnovich (17) provide a very similar explanation for the marginal stability of proteins, i.e., the tendency to be just one or two mutations away from misfolding (18). In effect, they argue that smaller populations will typically move down the fitness-stability function to a point that ensures larger (but still effectively neutral) effects of mutations. The idea of evolved effective neutrality is also implicit in theory on the evolution of quantitative traits under stabilizing selection in finite populations (19, 20).

Any selection coefficient between adjacent classes of $s^* \approx [\ln(v + u)]/(2N)$ defines an approximate point at which selective progress will be stalled, regardless of the relationship of s across an entire allelic series. Although the model used here assumed an exponential decline in fitness costs between consecutive allelic states, more complex allelic topographies might exhibit irregularities. For example, some adjacent classes of intermediate-state alleles might have small enough selection differentials that adaptive progress cannot be promoted by selection, even though sufficient advancement might lead to a domain where the selection gradient becomes sufficiently steep to allow further progress. Such conditions do not violate the principle of a drift barrier, but simply broaden considerations to allow for multiple possible stalling points depending on initial circumstances. For sufficiently large populations, however, such hurdles may only be transient in nature, as rare instances may arise in which multiple mutations in segregating alleles enable populations to move efficiently into a new domain (21, 22). An additional complication worth further consideration is the change in the ratio of beneficial to deleterious mutations (presumably a decline) that may arise as traits move closer and closer to the point of perfection (14), which will further inhibit progress toward molecular perfection in accordance with the preceding expression.

Despite the generality of the concepts outlined above, it appears that a drift barrier can occasionally be surmounted by the introduction of a novel feature endowing a sufficiently large improvement in a molecular pathway. However, such enhancements in fitness may often be transient, as subsequent mutations with mildly deleterious effects in one or both components cause the joint system to revert back to a point where the magnitude of mutational effects again exceeds the power of drift. There will then inevitably be a series of alternative multivariate states that are equivalent in terms of overall fitness, resulting in the evolution of negative correlations between the efficiencies of redundant pathways, as populations wander over a ridge of equivalent multitrait fitness combinations. It may be useful to consider this passive mechanism for the emergence of negative associations between functionally related traits as an alternative to the widespread notion that tradeoffs between fitness components always evolve as a direct consequence of pleiotropic constraints and/or correlated selection (23–25).

If the components of a multitiered system are completely substitutable, each component is expected to be lost with equal probability. However, mechanisms likely exist that encourage a greater probability of loss of one component of a layered system relative to another, e.g., differential metabolic costs, differential vulnerabilities to mutational breakdown, and/or the acquisition of secondary beneficial functions that ensure preservation by natural selection. One might also imagine that an overall increase in molecular refinement could be achieved if the addition of a second layer somehow altered the mutational landscape in a way that

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