

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. 54

October 2000

No. 5

Evolution, 54(5), 2000, pp. 1467–1479

COMPENSATING FOR OUR LOAD OF MUTATIONS: FREEZING THE MELTDOWN OF SMALL POPULATIONS

ART POON¹ AND SARAH P. OTTO²

Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver BC V6T 1Z4, Canada

¹*E-mail: poon@zoology.ubc.ca*

²*E-mail: otto@zoology.ubc.ca*

Abstract.—We have investigated the reduction of fitness caused by the fixation of new deleterious mutations in small populations within the framework of Fisher's geometrical model of adaptation. In Fisher's model, a population evolves in an n -dimensional character space with an adaptive optimum at the origin. The model allows us to investigate compensatory mutations, which restore fitness losses incurred by other mutations, in a context-dependent manner. We have conducted a moment analysis of the model, supplemented by the numerical results of computer simulations. The mean reduction of fitness (i.e., expected load) scaled to one is approximately $n/(n+2N_e)$, where N_e is the effective population size. The reciprocal relationship between the load and N_e implies that the fixation of deleterious mutations is unlikely to cause extinction when there is a broad scope for compensatory mutations, except in very small populations. Furthermore, the dependence of load on n implies that pleiotropy plays a large role in determining the extinction risk of small populations. Differences and similarities between our results and those of a previous study on the effects of N_e and n are explored. That the predictions of this model are qualitatively different from studies ignoring compensatory mutations implies that we must be cautious in predicting the evolutionary fate of small populations and that additional data on the nature of mutations is of critical importance.

Key words.—Compensatory mutation, conservation genetics, drift load, extinction, Fisher's model, mutational meltdown.

Received October 18, 1999. Accepted March 13, 2000.

Do small populations, in which there is a substantial probability that slightly deleterious mutations will fix, have a high risk of going extinct as a result? The majority of theoretical studies that have addressed this question affirm that many asexual and some sexual populations with low effective population size (N_e) will inevitably go extinct through mutation accumulation (Lynch and Gabriel 1990; Gabriel et al. 1993; Lynch et al. 1993, 1995a,b; Lande 1994, 1998; Butcher 1995; Schultz and Lynch 1997). According to similar work, even populations as large as our own species, given what we have measured of mutation, may be in genetic peril (Kondrashov 1995; Crow 1997; Eyre-Walker and Keightley 1999). Thus, it appears that deleterious mutation presents a genuine hazard to the conservation of species and the well-being of human populations. However, this threat may have been overestimated as an artifact of how the effect of mutation on the mean fitness of populations has been modeled. The earliest work, such as Haldane's (1937) pioneering investigations into the effect of variation in fitness in an infinite population, simplified the biology of mutations by ignoring the phenotypic origin of their effects on fitness (Hartl and Taubes 1998). Subsequent investigators have done much to improve

the biological realism within this classical framework by introducing rates of reversal, epistasis, and effect variation (Kondrashov 1994; Butcher 1995; Schultz and Lynch 1997; Lande 1998). Nevertheless, most previous investigations have assumed that mutations can be classified as either beneficial or deleterious regardless of the current phenotype.

The effect of a mutation at biochemical, physiological, morphological, and behavioral levels may, however, depend on its genomic context (Wright 1968). A mutation with a specific effect on the phenotype may be deleterious in some genomes and advantageous in others. Furthermore, interactions within and among gene products provide opportunities for one mutation to compensate for the effects of another at some level of the phenotype. Thus, new mutations may restore fitness losses incurred by previous mutations without requiring true reversals (Kimura 1990; Wagner and Gabriel 1990; Ohta 1992; Hartl and Taubes 1996; Burch and Chao 1999). These are known as compensatory, or suppressor, mutations. There is an abundance of experimental evidence (reviewed below) for mutations that conceal maladaptive mutant phenotypes at molecular, biochemical, and organismal levels. With few exceptions, we do not yet know how compensatory

mutations might influence predictions made by evolutionary models because context dependence is not a part of the classical framework. Therefore, it would be useful to determine how changing the way we model mutations affects the outcome of models addressing biological problems, such as the extinction risk of small populations. To do so requires a framework that defines a mutation by its effect on the phenotype, treating the fitness effect as a result of the phenotypic alteration. Fisher's geometrical model of adaptation (Fisher 1930), which we will describe below, is a good candidate that has been applied in a handful of studies to evolutionary models of mutation (Wagner and Gabriel 1990; Hartl and Taubes 1998; Orr 1998). One study in particular (Hartl and Taubes 1998) has derived an analytical estimate for the long-term mean fitness reduction caused by fixed mutations, which we will herein refer to as the "fixed drift load." For reasons discussed in the following section, this is an important quantity regarding the extinction risk of small populations. We have conducted a moment analysis of a model similar to Hartl and Taubes's (1998), representing the evolution of a finite population under mutation, selection, and drift. Our moment analysis provides a more comprehensive investigation of this mutational model. Furthermore, we have supplemented our analytical results with computer simulations to evaluate the precision of the analytical approximations. Differences between Hartl and Taubes's (1998) analysis and our own have substantial effects on fixed drift load predictions, which we will investigate at the conclusion of this paper.

Drift and Mutation Loads in Small Populations

Because our goal is to investigate the fitness effects of mutation on a specific kind of population, we must be able to distinguish among the different ways that mutation can impair a population. Many features of our genetic make-up are inextricably associated with a price, paid as a reduction in fitness. We refer to each of these penalties as one of many "genetic loads," owing to Muller's (1964) use of the term "load" to describe the impact of mutation on human populations (Crow 1970). The perpetual generation of deleterious mutations is sufficient to cause a genetic load. If we ignore the effects of drift, selection will deterministically establish an equilibrium frequency for deleterious mutations (Haldane 1937). This is the mutation load. In finite populations, however, genetic drift will move the frequencies of mutant alleles away from their deterministic equilibria, which can cause a further reduction of fitness known as the drift load. Theoretical work on genetic extinction has generally focused on the mutation and drift loads of finite populations.

The drift load can be apportioned into two classes. First of all, there is the load caused by temporary departures of drifting deleterious alleles from their deterministic equilibria. This effect can be substantial in moderately large populations in which genetic variation is maintained but allele frequencies fluctuate around the polymorphic equilibrium. As population size decreases, however, allele frequency distributions become more U-shaped with alleles either fixed or eliminated from the population (Wright 1937). For deleterious alleles, these fixed mutations make a second contribution to the drift load. Because the fixation probability of deleterious muta-

tions increases exponentially with decreasing population size (Crow and Kimura 1970), fixed mutations make a larger contribution to the drift load in small populations. Furthermore, fixed mutations accumulate over time, which can contribute to an incremental reduction in fitness known as the mutational meltdown process (Lynch and Gabriel 1990; Gabriel et al. 1993). Because the deterministic equilibrium frequency of deleterious mutations does not change with population size, the cumulative drift load may eventually exceed the mutation load in small populations (Lande 1995). We have chosen to investigate only the fixed drift load, not only because of its greater importance in small populations, but also because by doing so we can greatly simplify our analysis. We assume that the transient effects of segregating mutations that do not fix in the population can be ignored in the long term, which allows us to treat a population as a single genetic entity. As a result, we can model the evolution of a population by mutation, selection, and drift as a simple stochastic process.

Evidence for Compensatory Mutations

There are two types of empirical evidence that support the postulate that compensatory mutations are a common biological phenomenon. First, there is an extensive body of experimental work identifying "suppressor" mutations—an older term synonymous with compensatory mutation—that conceal the expression of a mutant gene (Hartman and Roth 1973; Jarvik and Botstein 1975). These mutations may arise within the mutant gene itself or in other genes. Compensation by additional mutations in the mutant gene (i.e., intragenic compensation) is often accomplished by restoring the original structural or functional conformation of the gene product (e.g., Hou and Schimmel 1992; Hanson et al. 1993; Kim et al. 1994; Mateu and Fersht 1999). A recent study of human hemoglobin (Kim et al. 1994) provides a good example of this process. The authors modeled the energetic folding of a mutant hemoglobin protein and identified a second base-pair mutation that best recovered normal quaternary structure. That these mutations were compensatory was then verified empirically by site-directed mutagenesis. Compensatory intragenic mutations are particularly well understood in the case of RNA structures (rRNA, Clark et al. 1984; Hancock et al. 1988; Springer et al. 1995; mRNA, Stephan and Kirby 1993; tRNA, Hou and Schimmel 1992). For example, mutations in stem structures of tRNAs can be compensated for by a matching mutation in the opposite strand (Cedergren et al. 1981; Steinberg and Cedergren 1994). Compensation by mutations in other genes (i.e., extragenic or intergenic compensation) is often mediated by the relationship between gene products. For example, a compensatory mutation in a gene involved in translation can ameliorate the phenotypic effects of a mutation by occasionally mistranslating the mutant mRNA into a normal protein product (Hartman and Roth 1973; Waterston and Brenner 1978; Murgola 1985; Yu and Spreitzer 1992; El Mezaine et al. 1998); often these compensatory mutations turn out to be point-mutations in certain tRNA genes. Alternatively, compensation can be mediated through biochemical pathways (Hartman and Roth 1973). When a mutant enzyme does not function sufficiently, the build-up of unmetabolized substrate or the lack of metabo-

lized product may have deleterious effects. Subsequent mutations that shunt flux toward an alternate pathway (Dickinson et al. 1995; Gachotte et al. 1997; Maringanti and Imlay 1999), block flux into the impaired pathway (Manning et al. 1999), or deactivate negative extragenic regulators (Matsuno and Sonenshein 1999) may compensate for this blockage.

A second source of evidence for compensatory mutation comes from studies in which direct measures of fitness compensation are made. Many classical studies find that deleterious mutations kept in laboratory populations for extended periods have decreasing fitness effects over time (Muller 1938; Haldane 1957). Furthermore, there are more recent experimental studies in which populations with reduced fitness due to the effects of a particular allele recover through compensatory effects at other loci (Elena et al. 1998; Liang et al. 1998; Lenormand et al. 1998; Burch and Chao 1999). It is worth discussing the results from Burch and Chao (1999) because they are particularly relevant to our model. Burch and Chao (1999) fixed a highly deleterious mutation in a strain of $\phi 6$ and then propagated several descendant lineages, varying the N_e among the lineages via repeated bottlenecking. The mean fitness of these bottlenecked lineages recovered over time at a rate inversely proportional to their N_e . Lineages that recovered the fitness loss in one step were inferred to have fixed a reverse mutation of the original deleterious mutation. Others, which recovered fitness in a gradual stepwise fashion, were inferred to have fixed compensatory mutations at other sites in the genome. Their experiment provides evidence that compensatory mutations are more common than a reverse mutation, and therefore only the former are seen in small populations, which have fewer genomes in which to accumulate mutations. It follows that small populations depend more heavily on compensatory mutations for recovering fitness.

Fisher's Geometrical Model of Adaptation

Fisher's (1930) geometrical model of adaptation provides a framework in which deleterious and compensatory mutations can be studied. This geometrical model was originally devised by R. A. Fisher to promote a micromutational view of adaptation, in which adaptive walks toward a fitness peak consist mostly of small steps (Leigh 1987). Fisher's model has recently made several appearances in the literature (Peck et al. 1997; Hartl and Taubes 1998; Orr 1998; Burch and Chao 1999). Its premise is that mutations can be described as changes within an n -dimensional character space in which point A represents the current state of a population and a fitness peak is represented by point O (see Fig. 1). The population's degree of adaptiveness is directly related to the distance between these two points. For convenience, we place the optimum at the origin. Thus, under selection, the mean fitness of a population at point A is a function of its Euclidean distance from the origin. A sits on the surface of a hypersphere, S_O , that is centered at O , such that the radius of S_O is equal to the distance between A and O . Moreover, every other point on the surface of S_O is equidistant from the origin and will have the same fitness value as A .

A mutation is a vector in n -space originating from point A and terminating at some other point A' . Mutations of a

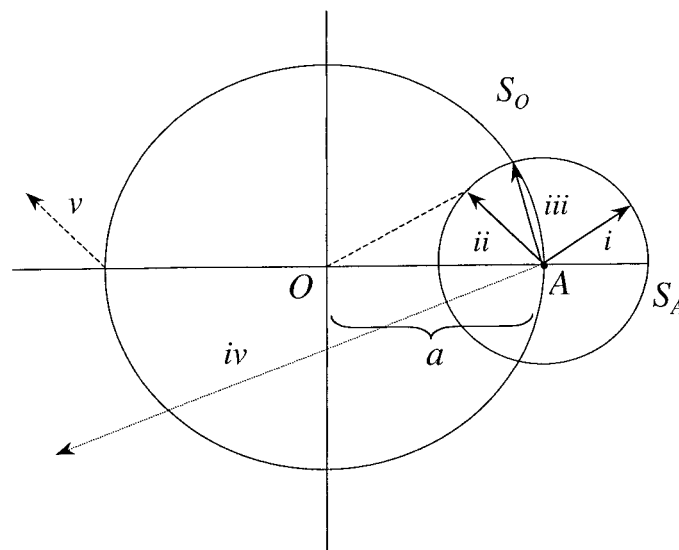


FIG. 1. Fisher's geometrical model of adaptation in two dimensions ($n = 2$). The population state is represented by point A that resides at some distance a from the optimum O , which is set at the origin for convenience. The fitness of a population is a function of a with a maximum at $a = 0$. Mutations are represented by vectors in the n -dimensional character space. (i) Mutations that increase a are deleterious. (ii) Conversely, those decreasing a are advantageous. (iii) Mutations between points on the circle S_O do not change a and are neutral. (iv) The probability that a mutation will be advantageous is a decreasing function of its length. Mutation vectors longer than the diameter of S_O cannot be advantageous. The effect of a mutation on fitness depends on its context. A mutation has an advantageous effect on one side of the optimum (ii) and a deleterious effect on the other (v).

fixed length will terminate on the surface of a hypersphere S_A centered at A ; we will have use for this notation when discussing mutation probability functions. The fitness effect of a mutation depends on where A' is relative to the surface of S_O (see Fig. 1, *i-iii*). Without this contextual information, one cannot determine the fitness effects of a particular mutation even if one knows its size and orientation in Fisher's model. Mutations between points on S_O will have a neutral effect on fitness, because there is no change in distance to the origin. A mutation that brings a population inside S_O reduces the distance to the origin and has an advantageous effect. Conversely, a mutation that moves the population outside S_O has a deleterious effect. In this model, advantageous mutations are nearly always compensatory because they are rarely direct reversals of previous mutations. It is useful to note that a correspondence exists between the size of mutations and their expected effect on fitness. Small mutations are more likely to be favorable because the local curvature of S_O becomes flatter, such that the probability of being advantageous approaches 1/2 (Fisher 1930). Although the probability that a mutation is favorable decreases with increasing mutation size (see Fig. 1, *iv*), advantageous mutations of larger effect are more likely to be incorporated into an adaptive walk because they confer a greater fitness advantage than their slightly advantageous counterparts and are therefore less likely to be lost by drift (Kimura 1983). When mutations become too large, however, this effect disappears because large mutations tend to overshoot the optimum and the mean

fitness advantage decreases. Mutations larger than the diameter of S_O are never advantageous. Unlike most classical population genetic models, the genetic context of a mutation defines its effect on fitness in Fisher's model. For example, we can visualize a vector with some fixed direction that is adaptive on one side of the origin and maladaptive on another (see Fig. 1, *ii* and *v*). Thus, in Fisher's model there are no mutations that are intrinsically and unconditionally deleterious or advantageous. It is this quality of the model that makes it useful to our purposes.

The number of orthogonal axes, n , in Fisher's model corresponds to the number of aspects by which an organism must adapt (Fisher 1930). Consequently, the model makes certain implications about the number of ways that an organism can have a suboptimal fitness. Specifically, there are 2^{n-1} more ways to have a fitness of $1 - 2\epsilon$ than $1 - \epsilon$ ($\epsilon > 0$). This becomes evident if one considers that the surface of an n -dimensional hypersphere increases at a rate proportional to the $(n - 1)$ th power of its radius. Therefore, n , to some extent, quantifies the rarity of optimal versus nearly optimal states. To better understand this, we make use of Fisher's original analogy of a microscope, except that it is now focused with n dials. The accumulation of error in the tuning of each dial results in a large number of alternate settings of the dials that could all lead to the same degree of poor focus.

METHODS

Moment Analysis of the Evolution Equation

As a population fixes mutations, it will wander in n -space at varying distances to the optimum. We use z' to denote the Euclidean distance of the population from the optimum. Throughout this analysis, we will assume that the mean environmental effect is zero. The state of a population can be represented by a probability distribution p such that $p(z', t)$ is the probability that the population state is within the interval $[z', z' + dz']$ at time t . We can describe the dynamics of this probability distribution over time with the following classic differential equation:

$$\frac{d}{dt}p(z', t) = -\mu_f p(z', t) + \mu_f \int_{-\infty}^{\infty} g(z', z)p(z, t) dz \quad (1)$$

(Kimura 1965), where z is a state variable similar to z' that is used to integrate over the entire range of previous states, μ_f is the genomic rate of fixation for new alleles, and $g(z', z)$ is the transition probability from state z to z' . The first term represents the rate that the population state leaves the interval $[z', z' + dz']$ upon mutation, and the second term integrates the cumulative probability that the population state will enter the interval $[z', z' + dz']$ from another interval $[z, z + dz]$. We assume that the occurrence of mutant alleles is rare such that the fixation probability of a mutation is unaffected by other segregating alleles. If $p(z', t)$ converges on the same distribution in the long term regardless of its initial state, then a steady state exists for this differential equation. The steady state is very useful because it will always describe the probability distribution in the long term regardless of the initial conditions. Because we are primarily interested in finding a steady state, we can ignore the details

of the approach to a steady state that allows us to redefine the time scale through a change of variables: $\tau = t\mu_f$, where τ measures time in units based on the average waiting time between fixation events. The differential equation then becomes:

$$\frac{d}{d\tau}p(z', \tau) = -p(z', \tau) + \int_{-\infty}^{\infty} g(z', z)p(z, \tau) dz, \quad (2)$$

which implies that a new mutation fixes with every time step $d\tau$. To use this equation requires that we define the transition probability density function $g(z', z)$. Because each transition represents the fixation of a new allele, $g(z', z)$ depends on the probability density function of mutations from z to z' , which we will denote as $m(z', z)$, weighted by the fixation probability $u(s)$ of the new allele, where s is the selection coefficient that depends on z' and z :

$$g(z', z) = \frac{m(z', z)u(s)}{\int_{-\infty}^{\infty} m(z', z)u(s) dz'}. \quad (3)$$

The denominator is a normalizing constant such that $\int_{-\infty}^{\infty} g(z', z) dz' = 1$. Note that the probability that a mutation moves the population from z to z' , $m(z', z)$, will depend on the number of orthogonal axes, n . For example, a mutation will have a greater chance of bringing a population closer to the origin of a plane ($n = 2$) than the origin of a three-dimensional volume ($n = 3$), because a larger proportion of mutations will lie within the hypersphere S_O . This dependence on n will be elaborated upon below, where we discuss simulation methods.

The resulting phenotypic change, once mapped to a change in the Euclidean distance from the origin, can be translated via the fitness function into a change in fitness. Because we are only concerned with changes of Euclidean distance, all calculations from this point onward will take place on the positive real line of numbers. Given a fitness function w , we can calculate the selection coefficient:

$$s = \frac{w(z') - w(z)}{w(z)}. \quad (4)$$

Note that s is a relative quantity, such that the same change in fitness causes far stronger selection when fitness is low ($w[z] \ll 1$) than when it is high ($w[z] \approx 1$). The fixation probability of a mutation in a diploid population with effective size N_e is a function of s :

$$u(s) = \frac{1 - e^{-2s}}{1 - e^{-4N_e s}} \quad (5)$$

(Crow and Kimura 1970). This function is well approximated in the neighborhood of $s = 0$ (see Fig. 2) by:

$$u(s; s < 0) = \frac{1}{2N} e^{-2N_e |s|} \quad \text{and} \quad (6a)$$

$$u(s; s > 0) = 2s + \frac{1}{2N} e^{-2N_e s}. \quad (6b)$$

To simplify the analysis, we chose a linear function to describe absolute fitness:

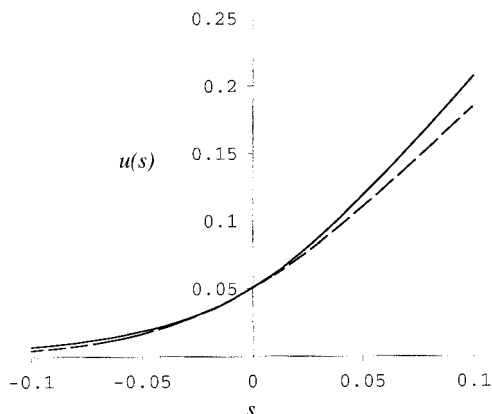


FIG. 2. Approximation of the probability of fixation $u(s)$ for a new mutation as a function of the selection coefficient s . The dashed line corresponds to the exact formula (5) (Crow and Kimura 1970) for effective population size $N = 10$. Our approximation, represented by the solid line, provides a good fit in the vicinity of $s = 0$ and overestimates the probability of fixation in the region $|s| > 0.05$.

$$w(z') = 1 - \frac{z'}{B}, \tag{7}$$

where $z' = B$ defines the point beyond which fitness is zero and where the optimal fitness was set, without loss of generality, to one. Because

$$\left| \frac{dw(z')}{dz'} \right| = \frac{1}{B}, \tag{8}$$

the intensity of selection equals the reciprocal of the upper bound.

We are interested in finding a steady state of the differential equation (2), so we set the time-derivative on the left side to zero, giving:

$$0 = -p_{eq}(z') + \int_{-\infty}^{\infty} g(z', z)p_{eq}(z) dz, \tag{9}$$

where $p_{eq}(z')$ represents the probability that the population state will be in the range $[z', z' + dz']$ at steady state. Although it is difficult to solve equation (9) for $p_{eq}(z')$ directly, we can describe the distribution, $p_{eq}(z')$, by its moments, which can be more readily analyzed. We proceed by using the method of moment generating functions (Kendall and Stuart 1963; Bürger 1991). Any density function $h(x)$ of an absolutely continuous random variable x can be transformed by integration into a moment generating function $\hat{h}(k) = \int_{-\infty}^{\infty} e^{kx}h(x) dx$, where k is a real number. The moment generating function can be expanded to yield all moments of the distribution described by $h(x)$, and it also uniquely determines $h(x)$. Applying the method of moment generating functions to $p_{eq}(z')$:

$$0 = - \int_{-\infty}^{\infty} e^{kz'} p_{eq}(z') dz' + \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{kz'} g(z', z) p_{eq}(z) dz dz', \tag{10}$$

we produce:

$$0 = -\hat{p}_{eq}(k) + \int_{-\infty}^{\infty} \hat{g}(k; z)p_{eq}(z) dz. \tag{11}$$

If we assume that the population is not too far from the optimum at steady state, we can take a Taylor series expansion of $\hat{g}(k; z)$ around $z = 0$ to produce a polynomial in z that we integrate to give the moments of p_{eq} :

$$0 = -\hat{p}_{eq}(k) + (\gamma_0(k) + \gamma_1(k)m_1 + \gamma_2(k)m_2 + \dots), \tag{12}$$

where m_i is the i_{th} moment of the distribution $p_{eq}(z)$ and γ_j is the j_{th} coefficient of the Taylor series expansion of $\hat{g}(k; z)$. Taking derivatives of equation (12) with respect to k gives us a system of equations that we limit to terms of $O(z^2)$ and greater:

$$-m_1 + (\gamma'_0|_{k=0} + \gamma'_1|_{k=0}m_1 + \gamma'_2|_{k=0}m_2) \approx 0 \quad \text{and} \tag{13a}$$

$$-m_2 + (\gamma''_0|_{k=0} + \gamma''_1|_{k=0}m_1 + \gamma''_2|_{k=0}m_2) \approx 0. \tag{13b}$$

Simulations indicate that the higher moments are small and may be safely neglected in most cases. All analytical results and approximations were compared to simulations as described in the following section. We will discuss the simulation methods next because they require a complete geometrical description of Fisher's model. Further analyses that are presented afterward require approximations that limit discussion of the model.

Simulation Methods

The evolution of a population by the fixation of new mutations is a simple Markovian stochastic process because the probability distribution depends only on the state at the previous time step (Gillespie 1992). It is relatively simple to simulate a Markov process with a Monte Carlo simulation program. We used Monte Carlo simulations of the one-dimensional and n -dimensional models to evaluate our analysis (simulation program available upon request). Uniformly distributed random numbers over the interval (0, 1) were generated with shuffling to avoid low-order correlations (*ran2* from Press 1992). These were in turn used to generate random deviates of other probability distributions. In each cycle, a mutation vector was generated with some length r drawn at random from a distribution prescribed by the number of dimensions. The mutation was then accepted or rejected according to its corresponding fixation probability. In the one-dimensional simulations, mutation lengths were drawn from an exponential distribution with rate parameter λ . We chose the exponential distribution for its mathematical simplicity (Mukai et al. 1972; Ohta 1977). Although the general consensus is that the distribution of mutant effects on fitness is probably L-shaped (Keightley 1994; Ohta 1998), there is no simple correspondence between the phenotypic and fitness effects of a mutation, and we cannot be certain which distribution is most representative. With $n = 1$, mutations were oriented toward or away from the optimum with equal probability. That is, the direction of the mutation (θ_1) was $+1$ half of the time and -1 otherwise. Although mutations can overshoot the optimum and enter negative values on the real line, we took z' to be an absolute measure of the distance from the optimum. Thus,

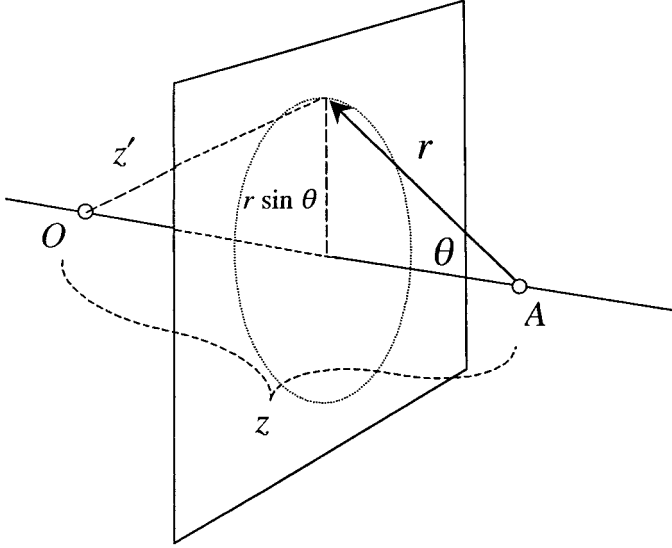


FIG. 3. The triangle scheme of mutation vectors. Any mutation can be completely described by a triangle of vectors regardless of the number of dimensions n . z represents the distance from the optimum before mutation. Each mutation is a vector of some length r and angle θ relative to z . Determining the next distance to the optimum, z' , is then a simple matter of trigonometric relations. A number of vectors share the same values r and θ , which can be quantified by the sphere with radius $r \sin \theta$ projected onto the plane orthogonal to the distance vector z . This quantity can then be translated into a probability function of θ (see text).

$$z' = |z + \theta_1 r|. \quad (14)$$

The selection coefficient (4) corresponding to a shift from z to z' then determined the probability of fixation for this mutation. If the mutation was fixed, z became z' as the population changed state and the process began another cycle.

When evolution takes place in more than one dimension, the process can be described by a triangle with one vertex at the origin, such that the two adjacent sides become radii z' and z and the remaining side becomes the mutation vector (Fig. 3; see also Hartl and Taubes 1998). Thus, regardless of how many dimensions (n) there are, the process can be summarized in two. By this scheme, any mutant vector is completely characterized by its length r and orientation θ , defined as the angle between the mutant vector and the distance vector from the optimum (Fig. 3). To determine the probability distribution of r in the n -dimensional model, we retained the exponential distribution for each axis and built up from these components using a ‘‘bottom-up’’ procedure. As a subset of all possible mutations, those of length r are also radii of a unique n -dimensional hypersphere S_A . Recall that the surface area of a hypersphere increases at a rate proportional to r^{n-1} with respect to the radius r . Using this relation, we can extrapolate from a single axis to all points on the hypersphere S_A by weighting the exponential distribution by r^{n-1} and normalizing:

$$\frac{\lambda e^{-\lambda r} r^{n-1}}{\int_0^\infty \lambda e^{-\lambda r} r^{n-1} dz'} = \frac{\lambda^n e^{-\lambda r} r^{n-1}}{\Gamma(n)}. \quad (15)$$

This is the gamma probability density function (Press 1992). For $n > 1$, equation (15) produces a bell-shaped curve with a mean mutation length equal to n/λ . We will discuss the relative merits of bottom-up and top-down approaches to modeling mutations in the discussion below. For large n and small λ , implementing equation (15) in a simulation becomes very inefficient because there is an excess of unrealistically large mutations with a near-zero probability of fixation. Consequently, we chose to truncate the distribution by the same upper bound as that defining the fitness function, B , such that only mutation lengths with a nonzero probability of fixation would be generated. Rescaling equation (15) accordingly gives us an incomplete gamma probability density function for mutation length:

$$\frac{\lambda e^{-\lambda r} r^{n-1}}{\int_0^B \lambda e^{-\lambda r} r^{n-1} dx} = \frac{\lambda^n e^{-\lambda r} r^{n-1}}{\Gamma(n) - \Gamma(n, \lambda B)}. \quad (16)$$

As n increases and λ decreases, a greater proportion of the original bell-shaped curve is truncated until only a monotonically increasing left tail remains.

Having described the distribution of mutation lengths, we turn now to a description of the distribution of θ . Increasing n expands the number of positions associated with mutation vectors of the same length r and angle θ , which in turn modifies the probability distribution of θ . To illustrate, consider that when $n = 2$ there are only two vectors associated with each value of θ ($0 \leq \theta \leq \pi$). When $n = 3$, there is a cone of vectors sharing an angle θ around radius z , tracing a circular path on a plane orthogonal to the original position vector, z (see Fig. 3). Similarly, with increasing n this path becomes a hypersphere of $n - 1$ dimensions. We can take advantage of this trend to calculate the probability distribution of θ as a function of n , apportioning the surface area of the mutant n -dimensional hypersphere S_A (see Fig. 1) into $(n - 1)$ -dimensional hyperspheres associated with values of θ . Because the radius of the hypersphere orthogonal to radius z is equal to $r \sin \theta$,

$$f(\theta) = \frac{r\pi(2r \sin \theta)^{n-2}}{\int_0^\pi r\pi(2r \sin \theta)^{n-2} d\theta} = c' \sin^{n-2}\theta, \quad (17)$$

where $0 \leq \theta \leq \pi$ (Rice 1990; Hartl and Taubes 1998) and where c' , is a normalizing constant equal to

$$c' = \frac{1}{\int_0^\pi \sin^{n-2}\theta d\theta} = \frac{\Gamma\left[\frac{n}{2}\right]}{\sqrt{\pi}\Gamma\left[\frac{1}{2}(n-1)\right]}, \quad (18)$$

where Γ denotes the gamma function (Abramowitz and Stegun 1965). Equation (17) produces a distribution similar to a Gaussian function that becomes narrower with increasing n . We used a rejection algorithm to generate pseudorandom numbers from this distribution (Press 1992). This method becomes less efficient, however, as the distribution becomes narrower, causing the simulations to take longer when n is large. For large n ($n > 10$), equation (17) is well-approxi-

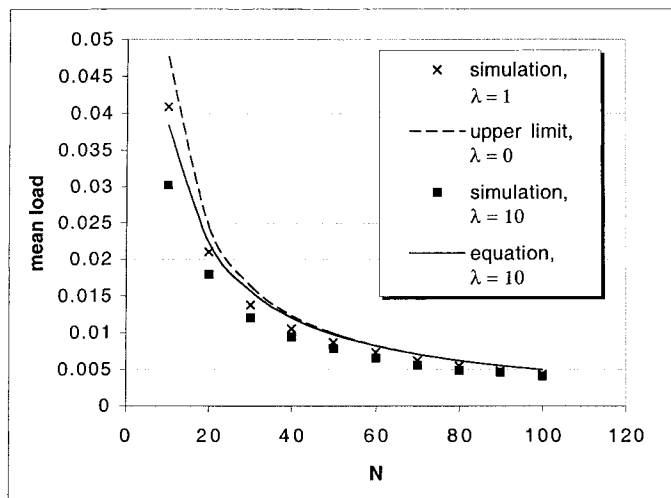


FIG. 4. The mean fixed drift load, which is equivalent to the first moment of the distribution $p_{eq}(z)$ when $B = 1$, as a function of the effective population size, N , in the one-dimensional model. Solid squares indicate the average of five simulation runs evaluated at $\lambda = 10$, each consisting of 1000 fixed mutation steps. (Throughout, the first 50 steps were ignored to minimize the influence of starting conditions.) Crosses indicate the average of five simulation runs evaluated at $\lambda = 1$, each consisting of 1000 fixed mutation steps. The solid curve represents equation (21) evaluated at $\lambda = 10$, and the dotted curve represents the upper limit approximation at $\lambda = 0$ in equation (22).

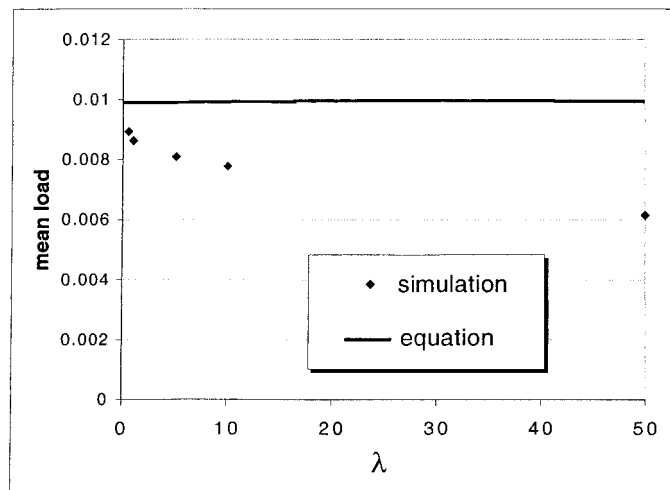


FIG. 5. The mean fixed drift load, equal to the first moment of the distribution $p_{eq}(z)$ when $B = 1$, as a function of λ in the one-dimensional model ($N = 50$). Points indicate the average of 10 simulation runs, each consisting of 1000 fixed mutation steps. Standard errors are too small to appear on the graph. Simulation results indicate that the first moment decreases monotonically with λ . The dashed line represents the second-order approximation (21).

mated by a transformed Gaussian function centered at $\pi/2$ (Fisher 1930; Hartl and Taubes 1996), which implies that most mutations point in a direction perpendicular to the direction of the optimum. We have used this approximation to generate random deviates of θ when n is large to reduce computational time. Given a mutation vector (r, θ) and the current distance from the optimum z , we can calculate the next distance z' from the optimum by the classic law of cosines (see Fig. 3):

$$z' = \sqrt{z^2 + r^2 - 2zr \cos\theta}. \tag{19}$$

Again, the mutation is fixed with probability $u(s)$, a function of the selection coefficient associated with a phenotypic change from z to z' (cf. eq. 4). This completes the derivation of the transition probability $g(z', z)$ in the n -dimensional model.

RESULTS

Evolution in One Dimension

Here we will describe the one-dimensional analysis as a means of introduction to the problem and as a segue into the general n -dimensional analysis discussed below. Recall that the transition probability $g(z', z)$ is generated from the probability density function for mutational effects on the phenotype, $m(z', z)$, weighted by the fixation probability, $u(s)$. The first step is to determine the probability density function, $m(z', z)$, based on an exponential distribution of mutation lengths. Because distance to the optimum (i.e., z, z') is an absolute quantity, every transformation from z to z' is associated with two points on the real line on either side of the

origin. Assuming exponentially distributed mutation lengths and treating advantageous and deleterious mutations separately, we have:

$$m(z', z; z' < z) = \frac{\lambda}{2} (e^{-\lambda(z-z')} + e^{-\lambda(z+z')}) \tag{20a}$$

advantageous

$$m(z', z; z' > z) = \frac{\lambda}{2} (e^{-\lambda(z'-z)} + e^{-\lambda(z'+z)}) \tag{20b}$$

deleterious.

Partitioning the denominator of equation (3) into two integrals over $z' > z$ and $z' < z$ and substituting the above equations produces a complicated solution for $g(z', z)$ in one dimension.

First-moment estimates from a second-order moment analysis of this model correspond relatively well to simulation results (see Fig. 4). The analytical solution for the first moment, which represents the mean distance from the optimum at steady state, can be approximated by the sum of two rational polynomials:

$$m_1 \approx \frac{B(2N + B\lambda)}{2N + (2N + B\lambda)^2} + \frac{B^2\lambda}{2N(2N + B\lambda)}, \tag{21}$$

where we have omitted the subscript from N_e for clarity. Equation (21) assumes that $e^{2N} \gg N^3$, and so gives a good approximation of the exact solution when $N > 10$. When compared to simulation results, we find that this second-order system makes an accurate prediction of the first moment for low values of λ (see Fig. 4). However, for large values of λ , equation (21) predicts that the first moment is nearly independent of λ , whereas simulation results show that the mean distance to the optimum decreases with λ (see Fig. 5). Consequently, equation (21) will overestimate the mean drift load

when λ is large (i.e., when the average phenotypic effect of a mutation is small).

Given that the first moment obtains a maximum value at $\lambda = 0$ in simulation results, we can approximate an upper bound estimate of the mean distance to the optimum by setting $\lambda = 0$ such that equation (21) becomes:

$$m_1 \approx \frac{B}{1 + 2N}. \tag{22}$$

By substituting equation (22) into the absolute fitness function (7), we find that the upper bound estimate of the expected drift load from fixed mutations is:

$$\bar{L} \approx \frac{1}{1 + 2N}. \tag{23}$$

Note that this upper bound estimate is independent of the selection intensity, $1/B$ (cf. eq. 8). Because equation (23) is a reciprocal function of N , the mean drift load due to fixed mutations becomes very small for $N > 10$. For reasonably sized populations ($N > 100$), the probability of fixation (5) for deleterious mutations becomes very small and most such mutations are readily compensated in this model.

Evolution in Many Dimensions

The mutation probability function $m(z', z)$ becomes very complicated when Fisher's space consists of more than one dimension. Given a population residing at a distance z from the optimum, we must consider the probability of a mutation vector's length r and direction θ hitting each point on the surface of a hypersphere with radius z' to calculate $m(z', z)$. An exact solution appears to be analytically intractable. However, simulation results show that a maximum with respect to λ exists for the first moment of the distribution $p_{eq}(z')$ at $\lambda = 0$. Therefore, we set $\lambda = 0$ to obtain an upper bound estimate of the fixed drift load for the case of exponentially distributed mutation lengths along each axis. This assumption allows a population at a distance z from the optimum to jump to any point within the n -space with equal probability. The n -space can then be thought of as a "uniform probability volume" divided into a series of concentric shells around the optimum, like an onion. It follows that the probability density function of mutations terminating on the surface of a hypersphere centered at the optimum with radius z' is:

$$m(z') = \frac{n(z')^{n-1}}{B^n}. \tag{24}$$

Here we have limited our attention to nonlethal mutations ($z' < B$). Note that equation (24) is independent of the original position z . Equation (24) is equal to the limit of (16) as λ approaches zero. To prove this, we use the following asymptotic expansion for the denominator of equation (16):

$$\Gamma(n) - \Gamma(n, \lambda B) = e^{-\lambda B} (\lambda B)^n \sum_{j=0}^{\infty} \frac{\Gamma(n)}{\Gamma(n + 1 + j)} (\lambda B)^j \tag{25}$$

(eq. 6.2.5 from Press 1992). Substituting this expansion into equation 16 and taking the limit, we have:

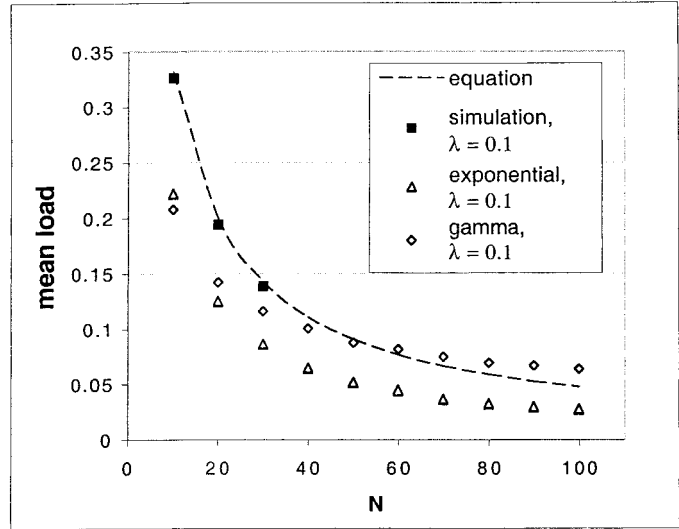


FIG. 6. The mean fixed drift load, equal to the first moment of the distribution $p_{eq}(x)$ when $B = 1$, as a function of effective population size N in the n -dimensional model ($n = 10$). The dashed curve represents the upper limit equation (27), where $\lambda = 0$. Solid squares indicate simulation results with $\lambda = 0.1$ using the original mutational model, which assumes exponential distributions of mutational effects along each axis that accumulate to produce a bell-shaped gamma distribution for total mutation length (eq. 16). We were unable to obtain simulation runs for values of N greater than 30 because mutations that fixed were extremely rare under these conditions. Open triangles represent the mean of five simulation runs consisting of 1000 fixed mutation steps each, with an exponential distribution ($\lambda = 0.1$) describing the total mutation length. Open diamonds represent the mean of five simulation runs consisting of 1000 fixed mutation steps each, with an L-shaped gamma distribution ($\lambda = 0.1, a = 0.5$) describing the total mutation length.

$$\lim_{\lambda \rightarrow 0} \frac{\lambda^n e^{-\lambda z'} (z')^{n-1}}{e^{-\lambda B} (\lambda B)^n \sum_{j=0}^{\infty} \frac{\Gamma(n)}{\Gamma(n + 1 + j)} (\lambda B)^j} = \lim_{\lambda \rightarrow 0} \frac{e^{-\lambda z'} (z')^{n-1}}{e^{-\lambda B} (B)^n \left(\frac{1}{n} + \frac{\lambda B}{(n + 1)n} + \dots \right)} = \frac{n(z')^{n-1}}{B^n}. \tag{26}$$

Our moment analysis of an n -dimensional model using this approximation produces results that are very close to those from our simulations when λ is near zero (see Figs. 6, 7). The upper limit solution for the first moment can be approximated by the sum of a simple rational polynomial and a vanishing term:

$$m_1 \approx \frac{nB}{n + 2N} - O\left(\frac{(2N)^n e^{-2N}}{\Gamma(n) - \Gamma(n, 2N)}\right). \tag{27}$$

The neglected terms are very nearly zero in the domain $N > n$. Even for $N < n$, simulations indicate that $nB/(n + 2N)$ is a good approximation for the mean distance to the optimum for low λ and provides an upper bound for m_1 as λ increases. Note that the first term reduces appropriately to equation (22) in one dimension ($n = 1$). Consequently, the mean equilibrium drift load caused by the fixation of new mutations is:

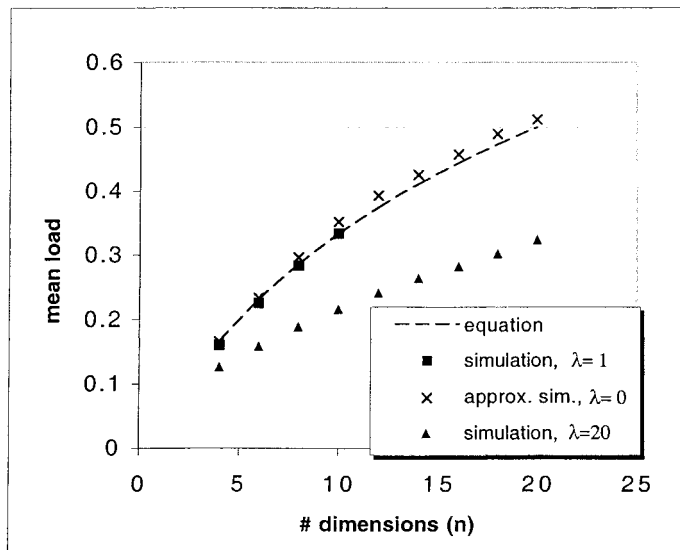


FIG. 7. The mean fixed drift load, equal to the first moment of the distribution $p_{eq}(x)$ when $B = 1$, as a function of the number of dimensions n , in the n -dimensional model ($N = 10$). The dashed curve represents the upper limit approximation (27), where $\lambda = 0$. Solid squares indicate simulation results for $\lambda = 1$. We were unable to obtain results for $n > 10$ because fixation events became extremely rare under these conditions. Crosses indicate the means of five simulation runs consisting of 500 mutation steps for a truncated distribution at $\lambda = 0$ that generated only values of r that were likely to fix. Truncating the distribution decreased computation time, but in exchange slightly overestimated the load for larger values of n . Closed triangles indicate the average of five simulation runs for $\lambda = 20$, each consisting of 1000 fixed mutation steps.

$$\bar{L} \approx \frac{n}{n + 2N}, \tag{28}$$

when λ is small, which overestimates the load when small mutations are common (higher λ). Equation (28) is again a reciprocal function of N , such that the mean drift load due to fixed mutations rapidly decreases with effective population size. Also, equation (28) is a hyperbolic function of the number of dimensions n .

Sensitivity of the Model to Its Assumptions

The features of Fisher’s geometric model of adaptation that make it mathematically useful also make it biologically oversimplified. One frequent criticism of Fisher’s model is that its spherical symmetry is too idealized to apply to real organisms. All of the orthogonal axes are standardized to fit under the same fitness function and mutational distribution. With respect to fitness, we can change the scale for each axis such that the same displacement experiences the same selection intensity. However, this would alter the mutation probability associated with a displacement along each axis. Consequently, the spherical geometry of Fisher’s model cannot perfectly capture both the fitness effects of mutations and their frequency distribution. The model also assumes that the mutational distribution is symmetrical along each axis, with an equal probability of going toward and away from the optimum. If the number of potentially compensating loci varies among traits, for example, then this assumption would not

hold. Thus, Fisher’s model is almost surely an imperfect representation of a biological system. However, models that ignore compensatory mutations are also imperfect representations. Given that neither Fisher’s model nor a model lacking compensatory mutations perfectly captures reality, modeling both is still instructive because it allows us to determine the sensitivity of evolutionary predictions to two extreme alternatives.

We have good reason to believe that the results obtained from this formulation are robust enough to changes in the assumptions to be useful for understanding the fixed drift load in small populations. There are several theoretical studies that have either examined a related problem in a roughly similar fashion (Lynch and Gabriel 1990; Hartl and Taubes 1998) or addressed a different evolutionary problem in a model analogous to Fisher’s geometric model (Robertson 1970). It is interesting that the results of all these studies bear a nontrivial resemblance to our own. Robertson (1970) studied the load caused by the drift of two alleles maintained near a polymorphic equilibrium \hat{q} by heterozygote superiority. The departure of allele frequencies from the equilibrium could be represented by the variance of Wright’s (1937) distribution, giving an expected load of:

$$\frac{\hat{q}(1 - \hat{q})}{1 + 4N\hat{q}(1 - \hat{q})} \tag{29}$$

(Robertson 1970). This model is superficially analogous to Fisher’s model for $n = 1$, in that there is an optimum located on a real line, over which selection and drift act antagonistically to determine a steady-state distribution. Note that equation (29) and our result in one-dimensional space, equation (23), are similar in their inverse dependence on population size.

A simulation study of the mutational meltdown of small populations by Lynch and Gabriel (1990), like Fisher’s model, allows for compensatory mutations. In this model, N individuals experience a Poisson distribution of mutations whose effects on fitness have a constant mean and variance. Although mutations are on average deleterious, there is a constant probability that a beneficial mutation will appear, with the restriction that individuals with a fitness greater than one are not allowed. Consequently, the model is similar to ones investigating the incorporation of unconditionally beneficial mutations (Schultz and Lynch 1997), except that beneficial mutations only appear once fitness has decayed to some extent. This differs from Fisher’s model, where the probability of a beneficial mutation continues to increase as the population moves further from the optimum and approaches 1/2. Lynch and Gabriel (1990) found that allowing for beneficial mutations caused the mean extinction time of the population to increase by orders of magnitude. The mutational meltdown is not frozen, however, unless the fraction of beneficial mutations is sufficiently high (Schultz and Lynch 1997).

More recently, Hartl and Taubes (1998) estimated the fixed drift load in their study of adaptation in Fisher’s model as:

$$\bar{L} = \frac{n + 1}{8N}. \tag{30}$$

Although we have studied the same model here, different assumptions have been made about the nature of mutations. We have evaluated the effect of each difference by running simulations under various alternative assumptions. First, Hartl and Taubes (1998) used an absolute selection coefficient $s = w(z') - w(z)$, whereas the probability of fixation for a new mutation is really a function of the relative selection coefficient $s = [w(z') - w(z)]/w(z)$ (cf. eq. 4; i.e., selective advantage, Fisher 1930). Effectively, their assumption was equivalent to relaxing selection away from the optimum. According to our simulations, changing s from an absolute to a relative measure in Hartl and Taubes's (1998) study produces the result:

$$\bar{L} \approx \frac{n}{n + 8N}. \quad (31)$$

Note that, whereas the absolute selection coefficient causes the load estimate to have a linear dependency on the number of dimensions, n , equation (28) and (31) are nonlinear functions that reach an asymptote at one as n gets large. The remaining difference between (28) and (31) results from what is assumed about selection and mutation. Hartl and Taubes (1998) used a quadratic fitness curve instead of a linear one (e.g., eq. 7). Simulations indicate that the term $8N$ in equation (31) becomes roughly $4N$ with a linear fitness function, suggesting that the fixed drift load is lowered when the fitness function curves downward away from the optimum. Furthermore, we assembled a probability distribution for mutation length, r , from component distributions in a bottom-up fashion, whereas Hartl and Taubes (1998) assumed that the total mutation length in n dimensions was uniformly distributed (see below). This difference explains the remaining discrepancy between equation (31) and (28). That both estimates of the fixed drift load are inversely related to N_e however, implies that this result from Fisher's model is fairly robust.

Comparing Hartl and Taubes (1998) and our study highlights our ignorance about how mutations affect phenotype. We assumed that the distribution for mutation length along each axis is exponential and that the total mutation length is derived from these component effects (a bottom-up derivation). However, the conventional procedure with respect to Fisher's model is to select a specific shape to the total distribution of mutational lengths, leaving the component distributions along each axis unspecified (a top-down derivation; Kimura 1983; Hartl and Taubes 1998; Orr 1998). Few data exist concerning the shape of the distribution of mutational effects on phenotype. There is a general consensus, however, that the distribution of deleterious mutant effects on fitness is L-shaped (Mackay et al. 1992; Keightley 1994; Lyman et al. 1996). For mathematical reasons, it is difficult to derive a probability density function of mutant effects on phenotype that is L-shaped using the bottom-up approach (Orr 2000). Nevertheless, the mutant effects on fitness may still have an L-shaped distribution because the phenotypic changes must first be mapped onto some fitness function. This mapping depends, however, on the current state of the population as well as on the number of dimensions of the system in Fisher's model. As the population moves further from the optimum

and as n increases, the density of mutations with little effect on fitness increases. (Recall that mutations become less likely to be oriented directly toward or away from the optimum under these conditions [Hartl and Taubes 1998].) In this case, an L-shaped distribution of fitness effects may be seen even when the distribution of phenotypic effects is bell-shaped (as in our bottom-up derivation).

In contrast, very large populations (such as the *Drosophila* populations used to generate the expectation of an L-shaped distribution of mutational effects on fitness) are expected to reside at or close to an optimum. If this is true, then the observed distribution of fitness effects should be similar in shape to the distribution of total mutation length for populations near an optimum. Consequently, it is worth investigating mutations whose total length follows an L-shaped distribution. We have run simulations using an exponential distribution and an L-shaped gamma distribution (which can be obtained from eq. 15 by setting $n = 1$ and 0.5 , respectively, with $\lambda = 0.1$ in both cases) to describe the distribution of total mutation lengths in n dimensions. In both cases, the dependency of the fixed drift load on the effective population size, N , is qualitatively similar to our original result (Fig. 6). Therefore, results from Fisher's model remain fairly robust under varying mutational models.

DISCUSSION

The Extinction Risk of Small Populations

The most important result of this model is that changing the way mutations are modeled results in qualitatively different predictions regarding the extinction risk of small populations. The inevitable extinction of small populations is a common feature of genetic models developed within the classical framework of mutations whose effects on fitness are deleterious and cannot be compensated (Lynch et al. 1993; Lande 1994). Allowing for compensatory mutations, however, we find that mean fitness does not suffer an inexorable decline, but rather reaches a steady-state level. This "freezing" of the mutational meltdown occurs because, as the population moves further from the optimum, a higher fraction of mutations increase fitness. Although it seems unlikely that all mutations can be compensated (as in Fisher's model), it is equally unlikely that compensatory mutations can be safely ignored (as in the classic framework). Whether reality lies nearer the level of compensation assumed in Fisher's model or in the classic framework is an empirical matter that has yet to be addressed. Such data are needed to resolve whether small populations are at risk of genetic meltdown and to determine the rate of this meltdown.

Previous models of the accumulation of mutations in small populations focus on unconditionally deleterious mutations with a constant fitness effect s occurring at a constant rate μ (Lynch and Gabriel 1990; Lynch et al. 1993; but see discussion above). Similar models draw unconditionally deleterious fitness effects from a continuous distribution (Lande 1994) or both deleterious and advantageous mutations at different rates (Lande 1998). In these examples, a population is unable to halt or even slow its fitness decline once it has begun, because then the influx of deleterious mutations overwhelms that of beneficial mutations. If deleterious mutations

are progressively less likely to accumulate as mean fitness decreases—as occurs when there is synergistic epistasis among deleterious mutations (Kondrashov 1994; Schultz and Lynch 1997)—the population reaches a point beyond which its mean fitness cannot decrease. Schultz and Lynch (1997) argue, however, that an unrealistically high level of synergistic epistasis is required. Interestingly, the meltdown is similarly frozen in Fisher's model, in which a population at the optimum has no beneficial mutations available to it and an increasing number as it declines in fitness. By evaluating the problem in terms of Fisher's model, we are explicitly considering the context dependence of a mutation's effect on fitness.

If there is a substantial class of mutations that are readily compensated, we expect that the drift load caused by the fixation of new mutations in small populations is unlikely to be sufficient to cause the extinction of all but the smallest populations. We have shown that the load caused by the fixation of deleterious mutations in the range $N > n$ is too small to affect a population with a modest reproductive excess. There may exist, however, a subclass of mutations that cannot be compensated. Such mutations cannot be modeled using Fisher's model and will contribute to a mutational meltdown (Lynch and Gabriel 1990), albeit at a slower rate. Of course, other risks of extinction including demographic stochasticity and ecological degradation are more than sufficient to place many small populations in peril (Lande 1988, 1993). Furthermore, small populations may be unable to adapt fast enough to a changing world, which would also place them at risk (Bürger and Lynch 1995). The pervasiveness of anthropogenic disturbance of habitats may reduce the population size of species to a point where all these forces can act in concert to cause extinction. Therefore, although our analysis lends insight into the effects of mutation on small populations when compensatory mutations are allowed, it would be unwise to base decisions regarding the conservation of endangered populations on these results.

Implications of Fisher's Model for Pleiotropy

Hartl and Taubes (1998) have discussed the relationship between the number of dimensions, n , in Fisher's adaptive space and concrete biological properties, such as the number of loci underlying a quantitative trait. It is also possible that n reflects the extent of pleiotropy in the average mutation (Orr 1998). Because our analysis substantially alters predictions of how n affects the fixed drift load, it is worth discussing the biological significance of n . To avoid confusion, we will denote the number of axes in Fisher's model as n_F and use n to denote the effective number of components that make up an organism's phenotype. Although there is an indefinitely large number of measurable characters in any organism, this does not necessarily imply that n is also large. For example, in a principal components analysis of multiple morphological measurements, most of the variation present can be accounted for by a much smaller number of axes. We can partition n into discrete sets and apply Fisher's model to each set, effectively making different assumptions about how mutation and selection act on these different trait combinations. At one extreme, letting $n_F = n$ implies that the average

mutation will nearly always have a nonzero effect on every phenotypic component, which is biologically equivalent to being completely pleiotropic. At the other extreme, n can be completely partitioned into n individual sets, each represented by a one-dimensional version of Fisher's model (i.e., $n_F = 1$). This assumes that every mutation will affect only one of the n phenotypic components at a time; there are no pleiotropic effects on any other components. The subsequent fixed drift load is predicted by the product of n and the one-dimensional result, $1/(1 + 2N)$. This load without pleiotropy, $n/(1 + 2N)$, is always greater than the load with complete pleiotropy, $n/(n + 2N)$, for $n > 1$. This implies that the pleiotropy inherent in Fisher's model substantially reduces the predicted drift load caused by new mutations, because every mutation can compensate several components at once.

Alternatively, let n represent how an organism's phenotype will respond to mutation and selection, rather than being a description of the phenotype itself (Orr 2000). Increasing n within Fisher's model (with $n_F = n$) will decrease the chance that a new mutation will counteract the effects of a previous mutation (Fisher 1930). If the organism's phenotype evolves as if it were a single character ($n = 1$), then half of the mutations that occur point in an opposite direction to the remaining half. This is equivalent to assuming that all mutations have a pleiotropic effect on every character of the organism's phenotype. When n is greater than one, then mutations will not always affect all characters simultaneously. Because the chance that maladapted characters will be little affected by a new mutation increases with n (see Fig. 3), the delay between deleterious and compensatory mutation events also increases. This explains why the fixed drift load is an increasing function of n within Fisher's model.

We have reviewed the experimental literature that provides good evidence that compensatory mutations occur, but there is a lack of empirical measures for what the overall extent of compensation might be. From Fisher's model, we can predict how n is related to measurable quantities, such as the fixed drift load and the average degree of compensation among mutations, that can be used to extrapolate estimates of n from empirical data. However, such interpretations are only as relevant as Fisher's model is an accurate representation of biology. That predictions concerning the extinction risk of small populations depend qualitatively on this issue emphasizes the importance of further research on compensatory mutation.

ACKNOWLEDGMENTS

We thank M. C. Whitlock, R. Lenski, L. Chao, R. Bürger, and G. P. Wagner for insightful discussions into the topic and the SOW group, H. A. Orr, and two anonymous reviewers for their useful comments on the manuscript. We also thank T. Lenormand, M. Maclean, and P. E. Greenwood for helpful modeling advice. This work was supported by a Natural Sciences and Engineering Research Council of Canada scholarship (NSERC PGSA-208606-1998) to AP and an NSERC grant to SPO. This paper is dedicated to the memory of Samantha Hicks.

LITERATURE CITED

- Abramowitz, M., and I. A. Stegun. 1965. Handbook of mathematical functions, with formulas, graphs, and mathematical tables. Dover Publications, New York.
- Burch, C. L., and L. Chao. 1999. Evolution by small steps and rugged landscapes in the RNA virus $\Phi 6$. *Genetics* 151:921–927.
- Bürger, R. 1991. Moments, cumulants, and polygenic dynamics. *J. Math. Biol.* 30:199–213.
- Bürger, R., and M. Lynch. 1995. Evolution and extinction in a changing environment: a quantitative-genetic analysis. *Evolution* 49(1):151–163.
- Butcher, D. 1995. Muller's ratchet, epistasis and mutation effects. *Genetics* 141:431–437.
- Cedergren, R. J., D. Sankoff, B. LaRue, and H. Grosjean. 1981. The evolving tRNA molecule. *Crit. Rev. Biochem. Mol. Biol.* 11(1):35–104.
- Clark, C. G., B. W. Tague, V. C. Ware, and S. A. Gerbi. 1984. *Xenopus laevis* 28S ribosomal RNA: a secondary structure model and its evolutionary and functional implications. *Nucleic Acids Res.* 12(15):6197–6220.
- Crow, J. F. 1970. Genetic loads and the cost of natural selection. Pp. 128–177 in K. Kojima, ed. *Mathematical topics in population genetics*. Springer, Berlin.
- . 1997. The high spontaneous mutation rate: is it a health risk? *Proc. Natl. Acad. Sci. USA* 94:8380–8386.
- Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.
- Dickinson, R. J., M. A. Sobanski, and M. J. E. Hewlins. 1995. In *Saccharomyces cerevisiae* deletion of phosphoglucose isomerase can be suppressed by increased activities of enzymes of the hexose monophosphate pathway. *Microbiology* 141(2):385–391.
- Elena, S., M. Davila, I. S. Novella, J. J. Holland, E. Domingo, and A. Moya. 1998. Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet. *Evolution* 52:309–314.
- El Mezaine, A., S. K. Lehtinen, N. Hance, L. G. Nijtmans, D. Dunbar, I. J. Holt, and H. T. Jacobs. 1998. A tRNA suppressor mutation in human mitochondria. *Nature Genet.* 18(4):350–353.
- Eyre-Walker, A., and P. D. Keightley. 1999. High genomic deleterious mutation rates in hominids. *Nature* 397:344–347.
- Fisher, R. A. 1930. *Genetical theory of natural selection*. The Clarendon Press, Oxford, U.K.
- Gabriel, W., M. Lynch, and R. Bürger. 1993. Muller's ratchet and mutational meltdowns. *Evolution* 47:1744–1757.
- Gachotte, D., C. A. Pierson, N. D. Lees, R. Barbuch, C. Koegel, and M. Bard. 1997. A yeast sterol auxotroph (*erg25*) is rescued by addition of azole antifungals and reduced levels of heme. *Proc. Natl. Acad. Sci. USA* 94:11173–11178.
- Gillespie, D. T. 1992. *Markov processes: an introduction for physical scientists*. Academic Press, Boston, MA.
- Haldane, J. B. S. 1937. The effect of variation on fitness. *Am. Nat.* 71:337–349.
- . 1957. The cost of natural selection. *J. Genet.* 55:511–524.
- Hancock, J. M., D. Tautz, and G. A. Dover. 1988. Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. *Mol. Biol. Evol.* 5:393–414.
- Hanson, D. K., D. M. Tiede, S. L. Nance, C.-H. Chang, and M. Schiffer. 1993. Site-specific and compensatory mutations imply unexpected pathways for proton delivery to the Q-B binding site of the photosynthetic reaction center. *Proc. Natl. Acad. Sci. USA* 90(19):8929–8933.
- Hartl, D. L., and C. H. Taubes. 1996. Compensatory nearly neutral mutations: selection without adaptation. *J. Theor. Biol.* 182:303–309.
- . 1998. Towards a theory of evolutionary adaptation. *Genetica* 103:525–533.
- Hartman, P. E., and J. R. Roth. 1973. Mechanisms of suppression. *Adv. Genet.* 17:1–105.
- Hou, Y. M., and P. Schimmel. 1992. Functional compensation of a recognition-defective transfer RNA by a distal base pair substitution. *Biochemistry* 31:10310–10314.
- Jarvik, J., and D. Botstein. 1975. Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. *Proc. Natl. Acad. Sci. USA* 72(7):2738–2742.
- Keightley, P. D. 1994. The distribution of mutation effects on viability in *Drosophila melanogaster*. *Genetics* 138:1315–1322.
- Kendall, M. G., and A. Stuart. 1963. *The advanced theory of statistics*. Charles Griffin & Company Ltd., London.
- Kim, H.-W., T.-J. Shen, D. P. Sun, N. T. Ho, M. Madrid, M. F. Tam, M. Zou, P. F. Cottam, and C. Ho. 1994. Restoring allostery with compensatory mutations in hemoglobin. *Proc. Natl. Acad. Sci. USA* 91:11547–11551.
- Kimura, M. 1965. A stochastic model concerning the maintenance of genetic variability in quantitative characters. *Proc. Natl. Acad. Sci. USA* 54:731–736.
- . 1983. *The neutral theory of molecular evolution*. Cambridge Univ. Press, Cambridge, U.K.
- . 1990. Some models of neutral evolution, compensatory evolution, and the shifting balance process. *Theor. Popul. Biol.* 37:150–158.
- Kondrashov, A. S. 1994. Muller's ratchet under epistatic selection. *Genetics* 136:1469–1473.
- . 1995. Contamination of the genome by very slightly deleterious mutations: why have we not died 100 times over? *J. Theor. Biol.* 175:583–594.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.
- . 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.* 142:911–927.
- . 1994. Risk of population extinction from fixation of new deleterious mutations. *Evolution* 48:1460–1469.
- . 1995. Mutation and conservation. *Conserv. Biol.* 9:782–791.
- . 1998. Risk of population extinction from fixation of deleterious and reverse mutations. *Genetica* 103:21–27.
- Leigh, E. G. 1987. *Ronald Fisher and the development of evolutionary theory. II. Influences of new variation on evolutionary process*. Oxford Univ. Press, Oxford, U.K.
- Lenormand, T., T. Guillemaud, D. Bourguet, and M. Raymond. 1998. Appearance and sweep of a gene duplication: adaptive response and potential for new functions in the mosquito *Culex pipiens*. *Evolution* 52:1705–1712.
- Liang, C., L. Rong, M. Laughrea, L. Kleiman, and M. A. Wainberg. 1998. Compensatory point mutations in the human immunodeficiency virus type 1 Gag region that are distal from deletion mutations in the dimerization initiation site can restore viral replication. *J. Virol.* 72:6629–6636.
- Lyman, R. F., F. Lawrence, S. V. Nuzhdin, and T. F. C. Mackay. 1996. Effects of single P-element insertions on bristle number and viability in *Drosophila melanogaster*. *Genetics* 143:277–292.
- Lynch, M., and W. Gabriel. 1990. Mutation load and the survival of small populations. *Evolution* 44:1725–1737.
- Lynch, M., R. Bürger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. *J. Hered.* 84:339–344.
- Lynch, M., J. Conery, and R. Bürger. 1995a. Mutation accumulation and the extinction of small populations. *Am. Nat.* 146:489–518.
- . 1995b. Mutational meltdowns in sexual populations. *Evolution* 49:1067–1080.
- Mackay, T. F. C., R. F. Lyman, and M. S. Jackson. 1992. Effects of P element insertions on quantitative traits in *Drosophila melanogaster*. *Genetics* 130:315–332.
- Manning, K., M. Al-Dhalimy, M. Finegold, and M. Grompe. 1999. *In vivo* suppressor mutations correct a murine model of hereditary tyrosinemia type I. *Proc. Acad. Natl. Sci. USA* 96(21):11928–11933.
- Maringanti, S., and J. A. Imlay. 1999. An intracellular iron chelator pleiotropically suppresses enzymatic and growth defects of superoxide dismutase-deficient *Escherichia coli*. *J. Bacteriol.* 181(12):3792–3802.
- Mateu, M. G., and A. R. Fersht. 1999. Mutually compensatory mutations during evolution of the tetramerization domain of tu-

- mor suppressor p53 lead to impaired hetero-oligomerization. *Proc. Natl. Acad. Sci. U S A* 96:3595–3599.
- Matsuno K., and A. L. Sonenshein. 1999. Role of SpoVG in asymmetric septation in *Bacillus subtilis*. *J. Bacteriol.* 181(11): 3392–3401.
- Mukai, T., S. I. Chigusa, L. E. Mettler, and J. F. Crow. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* 72:335–355.
- Muller, H. J. 1938. Reversibility in evolution considered from the standpoint of genetics. *Biol. Rev. Camb. Philos. Soc.* 14: 261–280.
- . 1964. The relation of recombination to mutational advance. *Mutat. Res.* 1:2–9.
- Murgola, E. J. 1985. tRNA, suppression, and the code. *Ann. Rev. Genet.* 19:57–80.
- Ohta, T. 1977. Extensions to the neutral mutation random drift hypothesis. Pp. 148–167 in M. Kimura, ed. *Molecular evolution and polymorphism*. National Institute of Genetics, Mishima, Japan.
- . 1992. The nearly neutral theory of molecular evolution. *Ann. Rev. Ecol. Syst.* 23:263–286.
- . 1998. Evolution by nearly-neutral mutations. *Genetica* 103: 83–90.
- Orr, H. A. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* 52: 935–949.
- . 2000. Adaptation and the cost of complexity. *Evolution in press* 54:13–20.
- Peck, J. R., G. Barreau, and S. C. Heath. 1997. Imperfect genes, Fisherian mutation and the evolution of sex. *Genetics* 145: 1171–1199.
- Press, W. H. 1992. *Numerical recipes in C: the art of scientific computing*. Cambridge Univ. Press, Cambridge, U.K.
- Rice, S. 1990. A geometric model for the evolution of development. *J. Theor. Biol.* 143:319–342.
- Robertson, A. 1970. The reduction in fitness from genetic drift at heterotic loci in small populations. *Genet. Res., Camb.* 15: 257–259.
- Schultz, S. T., and M. Lynch. 1997. Mutation and extinction: the role of variable mutational effects, synergistic epistasis, beneficial mutations, and degree of outcrossing. *Evolution* 51: 1363–1371.
- Springer, M. S., L. J. Hollar, and A. Burk. 1995. Compensatory substitutions and the evolution of the mitochondrial 12S rRNA gene in mammals. *Mol. Biol. Evol.* 12(6):1138–1150.
- Steinberg, S., and R. Cedergren. 1994. Structural compensation in atypical mitochondrial tRNAs. *Nature Struct. Biol.* 1:507–510.
- Stephan, W., and D. A. Kirby. 1993. RNA folding in *Drosophila* shows a distance effect for compensatory fitness interactions. *Genetics* 135(1):97–103.
- Wagner, G. P., and W. Gabriel. 1990. Quantitative variation in finite parthenogenetic populations: what stops muller's ratchet in the absence of recombination? *Evolution* 44:715–731.
- Waterston, R. H., and S. Brenner. 1978. A suppressor mutation in the nematode acting on specific alleles of many genes. *Nature* 275:715–719.
- Wright, S. 1937. The distribution of gene frequencies in populations. *Proc. Natl. Acad. Sci. USA* 23:307–320.
- . 1968. *Evolution and the genetics of populations*. Univ. of Chicago Press, Chicago, IL.
- Yu, W., and R. J. Spreitzer. 1992. Chloroplast heteroplasmy is stabilized by an amber-suppressor tryptophan tRNA_{CUA}. *Proc. Natl. Acad. Sci. USA* 89:3904–3907.

Corresponding Editor: H. A. Orr