Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter

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Summary

We studied the effects of population size on the inbreeding depression and genetic load caused by deleterious mutations at a single locus. Analysis shows how the inbreeding depression decreases as population size becomes smaller and/or the rate of inbreeding increases. This pattern contrasts with that for the load, which increases as population size becomes smaller but decreases as inbreeding rate goes up. The depression and load both approach asymptotic limits when the population size becomes very large or very small. Numerical results show that the transition between the small and the large population regimes is quite rapid, and occurs largely over a range of population sizes that vary by a factor of 10. The effects of drift on inbreeding depression may bias some estimates of the genomic rate of deleterious mutation. These effects could also be important in the evolution of breeding systems in hermaphroditic organisms and in the conservation of endangered populations.

1. Introduction

Several decades of experiments have shown that the vast majority of spontaneous mutations that affect fitness are deleterious (Mukai *et al.*, 1972; Simmons & Crow, 1977; Kibota & Lynch, 1996; Keightley & Caballero, 1997). The great bulk of these mutations fall into one of two classes. The first consists of lethal and sublethal mutations. These are expected to be found in appreciable frequency in large outbreeding populations, and can cause substantial inbreeding depression upon selfing or mating with close relatives. Slight inbreeding, due for instance to partial selfing or sib mating, will dramatically lower their frequency. Unless lethal mutations are totally recessive, however, their frequencies are virtually independent of population size (Nei, 1968).

The second class consists of mildly deleterious mutations that are only partially recessive. In large populations, partial self-fertilization and some forms of epistatic selection will enhance the efficacy of selection against them, resulting in lower frequencies

(purging). As a result, both inbreeding depression and the load are decreased. However, in contrast to lethals, mildly deleterious mutations can accumulate in populations of small size, where drift overwhelms the effects of selection (Kimura *et al.*, 1963). Small populations therefore suffer larger genetic loads (see for instance Lynch *et al.*, 1995). Do small populations also experience higher levels of inbreeding depression?

Here we study the effect of population size on the inbreeding depression caused by mildly deleterious alleles. Since our interest is in part motivated by plant populations in which there can be selfing, we allow for any degree of inbreeding. We show that, in small populations, drift tends to lessen the efficacy of selection which in turn decreases inbreeding depression. We also develop results for the genetic load in finite populations with inbreeding. The results have consequences for the evolution of mating systems, for estimation of the genomic mutation rate, and for conservation biology.

We begin by developing analytic approximations for inbreeding depression and the load in very large and very small populations. We then use diffusion techniques to find numerical values for these quantities in populations of intermediate size. Those results are checked against Monte Carlo simulations.

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2. Models and results

We investigate a simple model of a single diploid locus. A wild-type allele A mutates at a rate μ towards a deleterious allele a. Back-mutation from a to A occurs at a rate ν. The relative fitnesses of the AA, Aa and aa genotypes are 1, $1-hs$ and $1-s$ respectively, where s is the selection coefficient and h the dominance coefficient. We will assume that mutations are at least partly recessive ($h < 1/2$).

We measure the inbreeding depression using δ, defined as the decline in fitness in individuals produced by selfing relative to the fitness of outcrossed individuals (Charlesworth & Charlesworth, 1987). The genetic load L is the decline in fitness relative to the fitness of the optimal genotype of the population caused by the deleterious allele. For a mutation segregating at a frequency x,

$$\delta = \frac{(1+F)\,s(1-2h)\,x(1-x)}{2(1-2hsx(1-x)-sx^2)}, \tag{1}$$

and

$$L = sx((1-x)(2h+F(1-2h))+x). \tag{2}$$

Here F is the proportional excess of homozygotes caused by inbreeding, which is also equal to the correlation between uniting gametes due to non-random mating (Wright, 1969). We will use F as a measure of the inbreeding rate.

(i) *Large populations and quasi-deterministic selection*

When an infinitely large population reaches a mutation-selection equilibrium, the frequency of a deleterious allele is $\hat{x} \approx \mu/[s(h+F-hF)]$ (Ohta & Cockerham 1974). In a large but finite population of size N, the distribution of the deleterious allele frequency can be approximated by a beta distribution with mean $\hat{x}$ and variance

$$\sigma^2 \approx \mu/[s(h+F-hF)(1-4\,N_e sF(h-1)+4\,N_e sh)]$$

(see Appendix).

To find the average inbreeding depression in a large but finite population, we calculate the second-order Taylor expansion of (1) around $x = 0$ and then take its expectation over the beta distribution. Simplifying terms with the assumption that $\mu, \nu \ll hs$, the result is

$$\bar{\delta} \approx \frac{(1-2h)(1+F)\mu}{2(h+F-hF)} - \frac{(1-2h)(1+F)(1-2hs)\mu}{8(h+F-hF)^2 sN}. \tag{3}$$

The first term gives the inbreeding depression for an infinitely large population. It depends on h, F and μ, but is independent of the selective coefficients. The second term shows that inbreeding depression always declines as the population size shrinks. Examining the first derivative of $\bar{\delta}$ with respect to F shows that
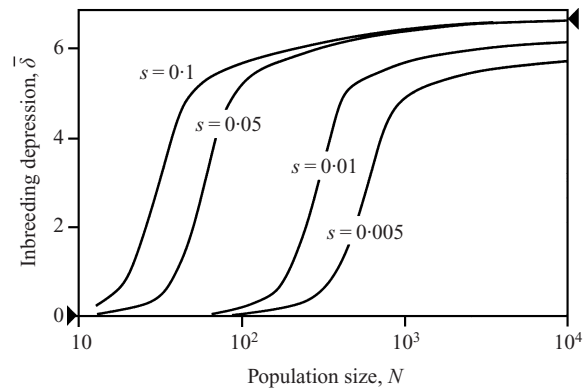

Fig. 1. Inbreeding depression ($\times 10^5$) as a function of effective population size. Curves were obtained numerically using diffusion techniques (see text). Filled triangles at the graph margins indicate the approximations for small populations (at left) and large populations (at right). The dominance coefficient is $h = 0.3$; the mutation rates are $\mu = 10^{-4}$ and $\nu = 10^{-6}$; the population is panmictic ($F = 0$).

average inbreeding depression declines as the rate of inbreeding goes up. Biologically, this occurs because inbreeding generates additional mutant homozygotes. Selection against them drives down the mutant frequency, decreasing inbreeding depression.

For the average genetic load, we take the expectation of (2) over the beta distribution, and simplify terms with the assumption that $\mu, \nu \ll hs$. That gives

$$\bar{L} \approx \frac{(2h+F-2hF)\mu}{h+F-hF} + \frac{(1-F^2)(1-2h)\mu}{4(h+F-hF)^2 sN}. \tag{4}$$

The first term on the right represents the genetic load experienced by an infinite population. This term is again independent of s (Haldane, 1937). The second term, which represents the additional load caused by drift, is always positive and is inversely proportional to N. Thus the load increases as the population size decreases. Taking the derivative of $\bar{L}$ with respect to F shows that the expected load also increases as the rate of inbreeding decreases when N is sufficiently large. As the population size tends to infinity, (3) and (4) give the classical deterministic values for $\bar{L}$ and $\bar{\delta}$ (Ohta & Cockerham, 1974).

(ii) *Small populations and strong drift*

Consider now the opposite situation in which the population is so small that drift is much stronger than selection and mutation ($s, \mu, \nu \ll 1/2N$). In this case the population will virtually always be fixed for one allele or the other; the chance of polymorphism is negligible. Then (1) shows that the expected inbreeding depression is nil: $\bar{\delta} = 0$. This result follows directly from the fact that inbreeding depression requires polymorphism.

The expected genetic load in this case is simply $\bar{L} = sx$, where x is the probability that the population is

fixed for the mutation. Following the reasoning of Bulmer (1991), $x = v\pi_a/(v\pi_a + \mu\pi_A)$, where π_a and π_A are respectively the probabilities that a single a or A mutation will spread to fixation. Those fixation probabilities are in turn simple functions of s, h, F and N (Caballero & Hill, 1992). These arguments lead to the result that

$$\bar{L} = \frac{s\mu}{\mu + v} + O(s^2). \tag{5}$$

For a panmictic population ($F = 0$), this expression is essentially the same as the one proposed without justification by Kimura *et al.* (1963); the difference between our approximation and theirs is $O(s^2)$. Equation (5) shows that in a small population, the expected load converges to its maximum value of $\bar{L} = s$ as the back-mutation rate v tends towards zero. Further, the expected load is independent of the inbreeding rate F. The intuitive explanation for this finding is that when the population is so small that drift overwhelms selection, there is little polymorphism and so selfing generates little excess homozygosity.

(iii) *Populations of intermediate size*

When the effective population size is neither so large that drift can be ignored nor so small that selection is negligible, both approximations developed above are expected to perform poorly. We therefore used two numerical approaches to obtain results for these intermediate cases.

Our first approach is based on diffusion methods. A diffusion approximation for the equilibrium allele frequency distribution $\Phi(x)$ was derived by Caballero & Hill (1992) for a population of arbitrary size N and inbreeding rate F. Using their results, we calculated the mean inbreeding depression and load by numerically integrating $L(x) \Phi(x)$ and $\delta(x) \Phi(x)$, respectively, over the interval [0, 1]. Calculations were done using the function NIntegrate of the mathematical software package *Mathematica* (Wolfram, 1996).

Secondly, we used Monte Carlo simulations for cases involving inbreeding ($F > 0$). Each simulation was started with the deleterious mutation at its deterministic equilibrium ($\hat{x}$) and run typically for 10^5 generations.

The results are shown in Figs. 1 and 2. One can distinguish three types of populations. In populations larger than about $5/hs$, our results for a large population (Equations 3 and 4) are fairly accurate. At the other extreme, when the population size is below a threshold of about $1/s$, then our small population size results (Equation 5) apply. Last, there is a intermediate range of population sizes where neither the small nor the large population size approximation
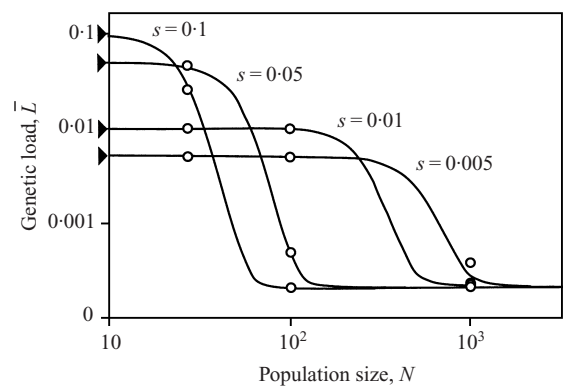

Fig. 2. Genetic load as a function of effective population size. Curves were obtained numerically using diffusion techniques (see text). Triangles at the graph margins show the limits for small populations (at left) and large populations (at right). Circles show the average load based on 500 independent replicates of Monte Carlo simulations. The mutation rates are $\mu = 10^{-4}$ and $v = 10^{-6}$; the population is panmictic ($F = 0$).

applies. In this range, the transition between the small and large population size regimes is smooth and quite rapid, spanning only about an order of magnitude in N.

3. Discussion

(i) *Factors causing inbreeding depression*

Inbreeding depression due to partially recessive mutations is thought to be a prominent evolutionary factor shaping mating systems, and can offset the intrinsic transmission advantage enjoyed by genes that increase the rate of selfing in hermaphrodites (Fisher, 1941; Charlesworth & Charlesworth, 1987). Consequently there have been numerous theoretical studies quantifying the expected amount of standing inbreeding depression in natural populations under several genetic models and mating systems (Lande & Schemske, 1985; Charlesworth *et al.*, 1991, 1992; Lande *et al.*, 1994; Muirhead & Lande, 1997). However, most models of inbreeding depression assume that population size is infinite (but see Charlesworth *et al.*, 1992).

Our analysis suggests that the inbreeding depression contributed by mildly deleterious mutations can be greatly reduced by the effects of finite population size. Information on effective population sizes is therefore of interest. *Drosophila melanogaster* is believed to have species-wide effective population size on the order of 10^6 (Kreitman, 1983; Schug *et al.*, 1997). In species such as these it is probable that deterministic expectations are correct for a large proportion of deleterious mutations. For other species, however, effective population sizes may be 10^3 or smaller (Schoen & Brown, 1991; Richman *et al.*, 1996). Moreover, the local effective population size that

governs the distribution of deleterious alleles may be substantially smaller than these estimates, which may represent species-wide numbers. In these cases, models that assume infinite population sizes are likely to give misleading predictions. Empirical studies of the relation between inbreeding depression and mating systems would be strengthened by the inclusion of demographic information along with rates of inbreeding (e.g. Van Treuren *et al.*, 1993; Ouborg & Van Treuren, 1994).

How much might the total inbreeding depression be reduced by the effects of finite population size? A substantial portion of observable inbreeding depression may be due to segregation of lethal mutations (Lande *et al.*, 1994). The expected frequency of lethal mutations is nearly independent of population size so long as they are not completely recessive (Nei, 1968). Given that empirical data suggest lethals have an average dominance level around $h = 0.02$ (Simmons & Crow, 1977), their contribution to inbreeding depression is expected to be quite insensitive to drift. Thus an important question is what fraction of the inbreeding depression is contributed by mildly deleterious mutations. Studies of *Drosophila* suggest they contribute roughly half the total (Crow, 1993). This suggests that perhaps half the inbreeding depression found in large populations of a species could be eliminated by drift in small populations. In the right circumstances, this reduction might trigger the evolution of self-fertilization in an outcrossing species.

(ii) *Consequences for the estimation of genomic mutation rates*

The genome-wide mutation rate for deleterious alleles, U, is important for many questions in evolutionary biology and conservation biology. This parameter is thought to be important, for example, to the evolution of genetic systems, the time to extinction of small population, and the management of genetic resources. Direct experimental estimation of U is, however, difficult.

An alternative is to estimate U indirectly from the inbreeding depression. This approach has been used to estimate the rate of mutation in highly selfing plants (Charlesworth *et al.*, 1990, 1994; Johnston & Schoen, 1995). More recently, Deng & Lynch (1997) and Deng (1998) extended the method to estimate the total genomic rate of deleterious mutation using data from outbred populations. Our analysis shows that such approaches will detect only a fraction of the total genome-wide mutation rate. The effective population size imposes a threshold selection coefficient. Mutations with fitness effects smaller than this threshold do not contribute to the inbreeding depression and therefore will be excluded from the estimate of the genomic mutation rate. (On the other
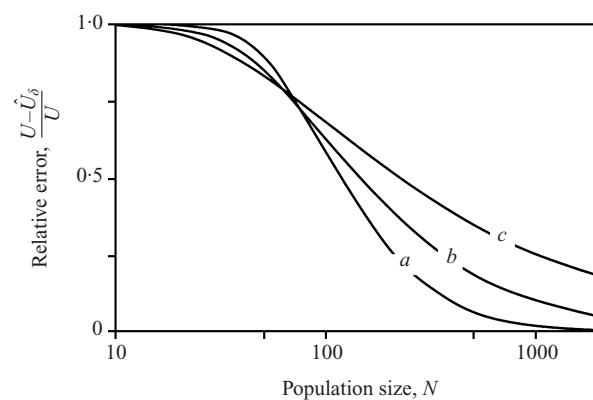

Fig. 3. Relative error in estimating the genomic mutation through inbreeding depression. The calculation assumes that fitness effects are multiplicative across loci, that only mutations with selection coefficients greater than $5/N$ are polymorphic, and that polymorphic loci are near their deterministic equilibria. For a given distribution of mutation effects $f(s)$, the relative error was calculated as $(U - \hat{U}_\delta)/U = 1 - \int_{5/N}^{1} f(s)\,ds$. (*a*) Gamma distribution $\Gamma(2, 0.025)$. (*b*) Exponential distribution. (*c*) Gamma distribution $\Gamma(0.5, 0.1)$. In all three the mean mutant effect is $s = 0.05$ and the population is panmictic ($F = 0$).

hand, if some force other than mutation maintains variation, upward bias in the estimation of U will result.) Interestingly, the average selection coefficient estimated by Deng & Lynch (1997) using inbreeding depression is $s = 0.14$, although in the present case the population sampled was presumably of the order of a million (M. Lynch, personal communication). This value is high relative to those from direct experiments, which generally report mean values of roughly $s = 0.05$ (Mukai *et al.*, 1972; Kibota & Lynch, 1996). This discrepancy could reflect a bias caused by neglecting mutations of small effect in studies based on inbreeding depression. On the other hand, Keightley & Caballero (1997) estimate an average selection coefficient of about $s = 0.4$, so it is possible that deleterious mutations indeed do have stronger effects than is typically believed.

To determine the possible error in estimates of U derived from data on inbreeding depression in outcrossed species, we assume multiplicative selection across loci. For a given distribution of selection coefficients, we can compute $\hat{U}_\delta$, the estimate for the mutation rate that one would calculate from inbreeding depression. We then calculate the relative error as $(U - \hat{U}_\delta)/U$. Fig. 3 shows results based on three distributions of selection coefficients. Choice of these distributions is suggested by recent estimates of the distribution of mutation effects in *Drosophila* (Keightley, 1994) and in *E. coli* (Santiago *et al.*, 1998). Apparently, effective population sizes of a thousand or less can produce a large error.

Despite these limitations, estimates of U based on inbreeding depression complement those based on

mutation accumulation procedures, which tend to underestimate the contributions from mutations of large effect. Furthermore, by crossing individuals between populations it should be possible to uncover the inbreeding depression (or its converse, heterosis) due to mutations of small effect fixed within some subpopulations but not others. Estimating mutation rates through inbreeding depression in a subdivided population forms the context for further research.

(iii) *Consequences of drift versus inbreeding rate*

It is well known that in infinite populations, some inbreeding enhances the efficacy of selection by increasing the frequency of homozygotes and by eliminating two copies of the deleterious allele with each selective death (Ohta & Cockerham, 1974). This 'purging effect' still occurs in finite populations, lowering both mean inbreeding depression and mean genetic load (both quantities decrease with increasing *F*). It is only when a population becomes very small and drift becomes overwhelming that the effect of inbreeding disappears.

We have shown using both numerical and analytical methods that for partly recessive mutations ($h < 1/2$) drift always results in higher mean genetic loads. This study confirms the earlier numerical results of Kimura *et al.* (1963) that were based on diffusion techniques. On the other hand, drift also decreases mean inbreeding depression. Why does drift have opposite effects on these two quantities? Drift creates variance in allele frequencies around the expectation. Load is a concave upward function of allele frequency whereas inbreeding depression is a convex function. Consequently fluctuations in allele frequency will have opposite effects on their expectations. At the limit, when drift dominates the dynamics of allelic frequencies (*N* becomes small), although the load is maximum, inbreeding depression within population also goes to zero, the population being fixed for either *A* or *a*.

Strictly speaking, rates of inbreeding are not completely independent of drift as reduced population size creates mild biparental inbreeding. But although this excess in *F* (of the order of $1/2N$) could lead to some purging, it is negligible unless the population size is extremely small, in which case drift is already overwhelming. A case where such a mild form of inbreeding could be crucial is when mutations are extremely recessive.

(iv) *Conclusion*

Recent models of mutational meltdown (Lynch *et al.*, 1995) examined the dynamics of population viability (expressed as a simple function of mean fitness) as mildly deleterious mutations accumulate in a finite population. Our study emphasizes equilibrium properties at a single locus for both mean fitness and level of inbreeding depression maintained in the population. We deliberately emphasized the effects of a single locus. Extrapolating our results to genome-wide load and inbreeding depression must be done with caution. Both epistatic interactions between loci and linkage disequilibrium caused by drift in a small population cause selective interference between loci. These effects make loci evolve non-independently and therefore single-locus results can not easily be extrapolated to the entire genome. Producing genome-wide expectations for quantities such as the mean load and inbreeding depression in finite populations will require further studies exploring such potentially intricate interactions (see, however, Schultz & Lynch (1997) for simulation results). Such expectations are badly needed to built realistic models of the evolution of self-fertilization. They would also help conservation biologists dealing with populations of decreasing size where both mean productivity and mean inbreeding depression are crucial in determining the future viability of the population.

APPENDIX. Derivation of the beta approximation for the distribution of the deleterious allele frequency at equilibrium in a large but finite population.

To derive the allele frequency distribution for a large population, we use Caballero & Hill's (1992) diffusion approximation and, following Robertson (1970), proceed by linearizing the selection term in the expression for the expected infinitesimal change of allelic frequency $E(\delta x)$. The expected infinitesimal change in allelic frequency due to mutation and selection is then

$$M(x) \equiv E(\delta x) =$$
$$-[F(1-h)+h]\,sx+(1-x)\mu-x\nu. \quad \text{(A 1)}$$

The first term of the right-hand side represents selection, followed by the effect of forward and backward mutation. Assuming binomial sampling of gametes and correcting for non-random mating ($N_e = N/(1+F)$, see Caballero & Hill, 1992), the expected squared infinitesimal change in allele frequency is found to be

$$V(x) \equiv E(\delta x^2) = \frac{x(1-x)}{2N_e}. \quad \text{(A 2)}$$

The equilibrium density for the allelic frequency is then

$$\Phi(x) = \frac{c}{V(x)} \exp\left(2\int^x \frac{M(y)}{V(y)}\,dy\right) =$$
$$cx^{4N_e\mu-1}(1-x)^{4N_e\nu+S-1}, \quad \text{(A 3)}$$

where $S = 4N_e(hs + Fs - Fhs)$ and c is a constant ensuring that

$$\int_0^1 \Phi(x)\, dx = 1$$

(see for instance, Ewens, 1979, pp. 124–125).

It follows from (A 3) that, at equilibrium in a large population with weak selection, the deleterious allele frequency follows a beta distribution with mean

$$\hat{x} \approx \mu/[s(h + F - hF)]$$

and variance

$$\sigma^2 \approx \mu/[s(h + F - hF)(1 - 4N_e sF(h-1) + 4N_e sh)].$$

These expressions for $\hat{x}$ and σ^2 assume that selection is much stronger than mutation: $\mu, \nu \ll hs$.

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