Muller's Ratchet, Epistasis and Mutation Effects

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ABSTRACT

In this study, computer simulation is used to show that despite synergistic epistasis for fitness, Muller's ratchet can lead to lethal fitness loss in a population of asexuals through the accumulation of deleterious mutations. This result contradicts previous work that indicated that epistasis will halt the ratchet. The present results show that epistasis will not halt the ratchet provided that rather than a single deleterious mutation effect, there is a distribution of deleterious mutation effects with sufficient density near zero. In addition to epistasis and mutation distribution, the ability of Muller's ratchet to lead to the extinction of an asexual population under epistasis for fitness depends strongly on the expected number of offspring that survive to reproductive age. This strong dependence is not present in the nonepistatic model and suggests that interpreting the population growth parameter as fecundity is inadequate. Because a continuous distribution of mutation effects is used in this model, an emphasis is placed on the dynamics of the mutation effect distribution rather than on the dynamics of the number of least mutation loaded individuals. This perspective suggests that current models of gene interaction are too simple to apply directly to long-term prediction for populations undergoing the ratchet.

MULLER'S ratchet (MULLER 1964; FELSENSTEIN 1974) is a process of deleterious mutation accumulation that can lead to the extinction of a population of asexuals (LYNCH and GABRIEL 1990; GABRIEL *et al.* 1993). All populations suffer decreased fitness from constantly occurring deleterious mutations (HALDANE 1937). In an infinite population, this decrease in fitness is limited by selection-mutation balance. An infinite population will attain an equilibrium distribution for fitness. A finite population undergoing constant deleterious mutation will approach the same equilibrium distribution for fitness attained by an infinite population (HAIGH 1978). However, in approaching this distribution, the number of individuals that carry no deleterious mutations will often become very low. This rarity of the fittest individuals can be critical to the persistence of a finite asexual (amictic) population. By chance, either by sampling error or mutation, these few fittest individuals may fail to contribute any mutation free progeny to the subsequent generation. All individuals of the population now have at least one deleterious mutation. Because there is no recombination between individuals, and assuming that back mutations and beneficial mutations are very rare, the population will never again have individuals as fit as were the mutation-free individuals. The maximum fitness of the population has been irreversibly lowered; the ratchet has clicked. Under some conditions, this process of lowering the maximum fitness can continue indefinitely, allowing deleterious mutations to accumulate without bound despite their damaging effects (MULLER 1964; FELSENSTEIN 1974; HAIGH 1978).

In the first formal model of Muller's ratchet (FELSENSTEIN 1974), all mutations are considered equivalent and independent in their effect on fitness. Each mutation causes a proportional decrease in the survivorship of the individual harboring the mutation. Under these assumptions, if an asexual population loses its mutation free individuals and its single mutation individuals, it will continue to accumulate mutations at a stochastically constant rate (HAIGH 1978), termed the ratchet rate. The ratchet rate is the inverse of the time in generations between successive mutations gains ("clicks") in the fittest class of individuals. This constancy of ratchet rate over the lifetime of the population is a result of the assumption that the mutations interact multiplicatively within an individual. The rate at which the ratchet clicks is not constant if synergistic epistasis among mutations is assumed. Instead, the ratchet slows as the population accumulates mutations and loses fitness (CHARLESWORTH *et al.* 1993; KONDRASHOV 1994). By the definition of synergistic epistasis (as measured on the Malthusian fitness scale), each additional mutation accepted has a greater deleterious effect than the last. Because the rate at which mutations are accepted at the population level goes down as selection against the mutations increases, synergistic epistasis can only decrease the rate of the ratchet. It has been suggested that synergistic epistasis can cause the ratchet rate to slow virtually to zero, allowing the population to persist indefinitely (KONDRASHOV 1994). However, in this study, I demonstrate that this hypothesized cessation of the ratchet under synergistic epistasis is a result of the assumption that all mutations are equivalent. Allowing mutations with a continuous distribution of effects can keep the ratchet moving despite synergistic epistasis for

fitness. Although each particular mutation has a greater impact on individual fitness than it would have in the absence of epistasis, initially less deleterious mutations become available to drive the ratchet as the initially more deleterious mutations become too damaging to accumulate. Though mutations of all effects may accumulate more slowly in the population, the overall rate of fitness loss, in contrast to the rate of the ratchet, need not decrease.

MATERIALS AND METHODS

Early models of Muller's ratchet have assumed that every mutation has the same deleterious effect s and that mutations within an individual interact multiplicatively. An individual with n mutations survives to reproduction with probability $(1 - s)^n$. It is these assumptions that are varied here. The following aspects of the model are not varied here. The population has at most K individuals. New mutations arise in an individual with frequency given by a Poisson distribution with mean μ. Mutation is assumed to affect only the probability of survival to reproduction. Reproduction occurs without recombination and with each adult having a family size with Poisson distribution of mean R. Each generation consists of reproduction, mutation, density independent selection (survivorship), and density dependent selection (reduction to K individuals independent of genotype, if more than K individuals survive), in that order. The initial population consists of K mutation-free individuals.

It is convenient to measure fitness on a natural logarithm (Malthusian) scale. This allows comparison of populations with different parameters and simplifies the discussion by making mutation effects nearly additive in the absence of epistasis. Under this scale, if the ratchet is clicking at a constant rate, then the population is also losing fitness at a constant rate. Under the multiplicative mutation interaction model, the fitness of an individual with n mutations is

$$\omega = \ln[R(1 - s)^n] = \ln(R) + [n \cdot \ln(1 - s)].$$

Each additional mutation decreases individual fitness by a constant $-\ln(1 - s)$. Under the synergistic epistatic model, the fitness of an individual with n mutations is

$$\omega = \ln(R) + \left[\frac{\ln(1 - s \cdot n(1 - \alpha))}{1 - \alpha}\right]$$

where α parameterizes epistasis. Here each additional mutation decreases individual fitness by an amount that increases with the number of mutations, the idea being that deleterious mutations interact with each other to the further detriment of the individual. This fitness function is derived from a generalization of the nonepistatic fitness function. The nonepistatic fitness function is a (discrete time) solution to the differential equation $W' = -sW$ with boundary condition $W(0) = 1$, indicating that on the arithmetic scale, fitness is lost proportionally to the current fitness (while on a Malthusian scale, fitness is lost at a constant rate). The more generalized differential equation is $W' = -sW^\alpha$, indicating that fitness is lost as a power function of the current fitness. For α less than 1, fitness will be lost at a greater than proportional rate (again on the arithmetic scale). The function ω is found by solving for W and taking the natural log. In the limit as α approaches 1, the fitness function reduces to the nonepistatic case. A broad range of strongly epistatic fitness functions is encompassed by this model, including the strongly epistatic linear (on the arithmetic fitness scale) fitness function. A Gaussian selection model is also considered, with $\omega = r - \beta n^2$. These epistatic fitness functions are used to model interactions among mutations that cause the individual harboring the mutations to have lower fitness than would be expected if the mutations act independently on fitness.

The above model was implemented as a Monte Carlo simulation on a parallel computer. To decrease run-time, an exact optimization was used that reduces the number of progeny produced only to die before reproduction. The probability of death of any individual can be partitioned into two components; the probability of death from fitness lost to all members of the population and fitness lost to only some members of the population. Because of the assumptions of the model, the most fit individual in the parental generation puts an upper bound on the fitness of all offspring (the ratchet effect). Thus, the risk of death of the fittest parent is a lower bound on the common component of risk of death in the offspring and is available before any offspring genotypes have been determined. The number of offspring that will survive the common risk of death is calculated as a binomial deviate with the common risk off death as the probability parameter and the total number of offspring as the sample size. This smaller number of surviving offspring is an upper bound on the number of offspring that need to be generated and examined for survival. Individual probability of survival is calculated from individual genotypes and then conditioned for having survived the common fitness deficit. This exact optimization is similar to the earlier fitness scaling (CHARLESWORTH *et. al.* 1993). The binomial distribution deviate algorithm used is from RIPLEY (1987). The computer programs implementing the simulations are available upon request, either in parallel C (MasPar 1992) or in sequential C.

Two continuous distributions of mutation effects were considered, the exponential distribution and the uniform distribution. The exponential distribution was chosen for several reasons; there is some evidence supporting a distribution like the exponential (MACKAY *et al.* 1992), the exponential distribution has been considered in other models (OHTA 1977; LANDE 1994), and exponential deviates are easily generated for simulation. The uniform distribution, although not intended to reflect any biological process, was chosen for ease of generation and to demonstrate that the particular form of the distribution is not crucial to the results.

For comparison to the simulations, a semianalytic approach was used to estimate the dynamics of fitness. This approximation assumes that mutations accumulate strictly independently. That is, that the probability of a mutation's fixation is a function only of its effect on fitness on the individual in which it occurs and is not a function of the population's distribution for fitness. Further, it is assumed that only mutations that occur in the fittest individual need to be considered. Given these assumptions, the rate of fitness loss can be inferred from the distribution of mutation effects, the fitness function, and the fitness of the fittest individual in the population.

Under synergistic epistasis, the actual effect of a mutation depends on its genetic background. Therefore, it is important to distinguish between the effect of a mutation as parameterized by s and the actual effect the mutation has on the individual in which it occurs. The effect of a mutation on an otherwise mutation free individual will be referred to as the mutation's inherent effect. The effect of a mutation on a particular individual, possibly with other mutations, will be referred to as the mutation's actual effect (s_a).

The contribution to the population's rate of fitness loss by a mutation of given actual effect s on individual fitness is not know and must be approximated by simulation. Assume this relationship is known and call it $\tau(s_a)$. By the assumption

of additivity, $\tau(s_a + t_a) = \tau(s_a) + \tau(t_a)$. Though not required, it is more convenient to work with the differential equation describing the fitness function than with the fitness function itself. In this case, the fitness function is described by $dW/dx = -W^{\alpha}$, where $-dx$ $(= s)$ is the mutation's inherent effect. The mutation's inherent effect will be modified by interaction with mutations already present in the individual. It is this actual effect of the mutation that will contribute to the rate of fitness loss. The mutation's actual effect on individual fitness is, by definition, $-d\omega(= s_a)$. Since $\omega = \ln(R) + \ln(W)$, $d\omega = dx \cdot -W^{\alpha-1}$. Substituting out W yields $-d\omega = dx \cdot \exp[(\omega - \ln(R))(\alpha - 1)]$. The actual effect is inflated over the inherent effect by a multiplier. The multiplier's value as a function of w depends on the fitness function. This multiplicative form allows a simple transformation of the probability distribution of inherent mutation effects, letting

$$\lambda = \exp[(\omega - \ln(R))(\alpha - 1)],\ P(s_a|\omega) = P(s_a/\lambda)/\lambda.$$

For example, for an exponential distribution of inherent mutation effects,

$$P(s_a|\omega) = \exp(s_a/\lambda\bar{s})/\lambda\bar{s}.$$

Finally, by the assumed additivity of τ, the contributions of all actual mutation effects to expected loss of best fitness can be summed together;

$$-\Delta\omega_{max}/\text{gen} = \int P(s_a|\omega_{max}) \cdot \tau(s_a) ds_a$$

$$= \int \exp(s_a/\lambda\bar{s})/\bar{s}\lambda \cdot \tau(s_a) ds_a.$$

RESULTS

Under the multiplicative model of mutation interaction, the ability of the ratchet to lead to the extinction of a population is determined both by the rate at which the ratchet clicks and the deficit in fitness per click. A class of mutations whose single effect is very slightly deleterious will result in a rapid ratchet rate, approaching the mutation rate, but with nearly negligible fitness loss per click. Mutations whose effect is more deleterious may cause much more damage per click but are subject to more effective selection and therefore a vanishingly lower click rate. The fastest rate of fitness loss is caused by mutations of intermediate, slightly deleterious, effect (FELSENSTEIN 1974; GABRIEL *et al.* 1993; LYNCH *et al.* 1993). Figure 1 shows τ, the relationship between mutation effect (s) and the rate of fitness loss per generation ($-\Delta\omega_{max}/\text{gen}$) for a few population sizes. Importantly, with the exception of the short period where the population settles into its quasi-equilibrium and the very short period where the population finally contracts to extinction, the expected rate of fitness loss per generation for a given mutation effect will be constant over the lifetime of the population.

As suggested by Figure 1, under the multiplicative model of mutation interaction, for any finite population size, there is a slightly deleterious mutation effect that will result in the fastest possible decline in fitness. Under a synergistic epistatic fitness model, however, no single mutation effect can remain slightly deleterious throughout the lifetime of a population. By definition, under synergistic epistasis, each additional mutation has larger actual effect (on the Malthusian scale) than the last. After accumulating sufficient mutations, the actual mutation effect of the next mutation becomes too large to drive the ratchet; the rate of fitness loss from the ratchet is effectively zero and the expected time to extinction is independent of the ratchet.

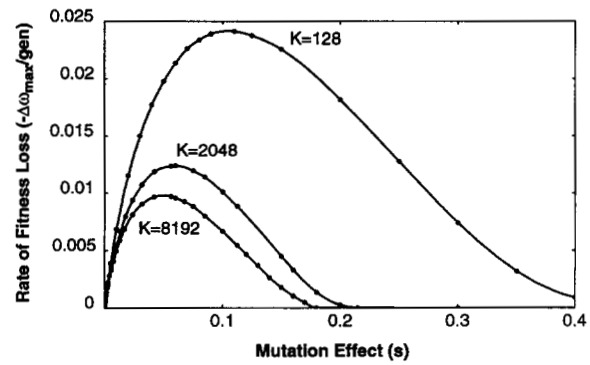

FIGURE 1.—Mean rate of decline of best individual (ω_{max}) fitness as a function of inherent mutation effect for several population sizes. In all cases $\mu = 1$. For each population size, there is an intermediate value of inherent mutation effect that maximizes the rate of fitness loss. Inherent mutation effects greater than slightly deleterious result in vanishingly low rates of fitness loss. Curves interpolated from points shown, points based on over 10,000 generations.

Figure 2 shows the actual mutation effect in the fittest individual increase with loss of maximum fitness and the corresponding rate of maximum fitness loss. There is strong epistasis,

$$\omega = \ln(R) + 2\ln(1 - \tfrac{1}{2}s \cdot n)$$

and very high initial fitness, $R = 250$. As maximum

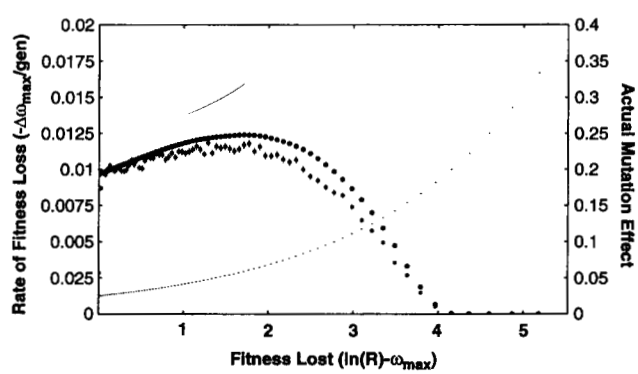

FIGURE 2.—Mean rate of decline of best individual fitness (ω_{max}), from simulations, as a function of fitness lost (medium points with error lines), derived rate of maximum fitness decline (large points), and actual mutation effect (small points, on right scale). Error lines are approximate standard error of the means. All mutations have inherent effect of $s = 0.025$. There is synergistic epistasis, $\omega(n) = \ln[R \cdot (1 - \frac{1}{2}sn)^2]$. Population parameters are ($K = 2048$, $R = 250$, $\mu = 1$). For sufficiently low maximum fitness, the population does not lose maximum fitness. Such a population will persist indefinitely.

fitness (ω_{max}) decreases, the per generation rate of maximum fitness loss ($-\Delta\omega_{max}$) ultimately decreases. Note, however, that $-\Delta\omega_{max}$ is greatest at an intermediate fitness. This is predicted from Figure 1, where $s = 0.025$ is smaller than the mutation effect that results in the greatest $-\Delta\omega_{max}$. Still, for sufficiently low ω_{max}, $-\Delta\omega_{max}$ is zero. The simulated populations attained a small but positive fitness and then persisted indefinitely. This summarizes the results from KONDRASHOV (1994). Also shown are the predicted rates of fitness loss from the semianalytic approximation. To a good approximation, the rate of fitness loss at each fitness is equal to the rate of fitness loss that would occur without epistasis but with the inflated mutation effect.

Part of the power of synergistic epistasis in stopping the ratchet comes from the assumption of a single inherent mutation effect. Because there is only one inherent effect, it is eventually inflated by epistasis beyond being able to drive the ratchet. Mutations are better modeled as arising with a distribution of inherent effects from nearly neutral to lethal. This variation in inherent effect can allow the ratchet to continue as epistasis inflates mutations' actual effects. If arbitrarily small inherent effects are included, then even at low maximum fitness there will be mutations whose actual effects allow the operation of the ratchet. A slight modification is made to the model to accommodate the continuous distribution of mutation effects. Instead of counting the number of mutations per individual, the sum of the inherent effects of the mutations is used in the fitness function. Letting each s_i be chosen from a continuous distribution, the epistatic fitness function equivalent to the fitness function used in Figure 2 is

$$\omega = \ln(R) + 2\ln\left(1 - \tfrac{1}{2}\sum_{i=1}^{n} s_i\right).$$

The ratchet no longer clicks because there are no discrete classes that share the same number of interchangeable mutations. Instead, the ratchet jumps along with step sizes that are distributed continuously, still at a particular stochastic rate. Figure 3 shows the rate of maximum fitness decrease as a function of the maximum fitness. The population and epistasis parameters are identical to those of Figure 2, but mutations here have inherent effects chosen from an exponential distribution (Figure 3A) or a uniform distribution (Figure 3B) with mean equal to the single inherent effect used in Figure 2. There is no positive maximum fitness that has a zero rate of maximum fitness loss. The ratchet will cause loss of fitness until the population is extinct. The "mutational meltdown" (GABRIEL *et al.* 1993) is illustrated by the steeply rising rate of maximum fitness loss for very low maximum fitness.

Although the rate of fitness loss never goes to zero, it does ultimately decreases as fitness decreases. The rate of fitness loss decreases as a result of the decrease in frequency of mutations whose effects are very slightly

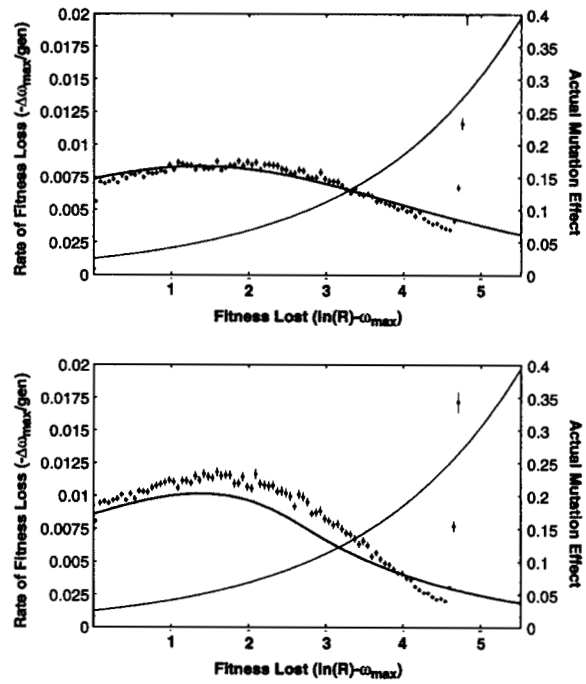

FIGURE 3.—Mean rate of decline of best individual fitness (ω_{max}), from simulations, as a function of the fitness lost (points), derived rate of maximum fitness loss (thick line), and mean actual mutation effect (thin line, on right scale). Error lines indicate approximate standard error of the means. Population parameters are the same as in Figure 2 except that inherent mutation effects occur by exponential distribution with mean of 0.025 in A and by uniform distribution with mean of 0.025 in B. Population parameters are ($K = 2048$, $R = 250$, $\mu = 1$). In both cases, the population loses maximum fitness at all maximum fitnesses. The increased rate of maximum fitness loss at very low maximum fitness is the "mutational meltdown." Such a population rapidly shrinks to extinction.

deleterious. As mutations accumulate and fitness decreases, epistasis inflates the actual effects of all subsequent mutations, skewing the distribution of mutations effects away from neutrality. The amount of this skew can be calculated by transforming the inherent mutation effect distribution by the fitness function given the fitness, yielding the distribution for the actual mutation effects. The transformation of the mean effect is shown in Figure 3. In general, the form of the distribution of mutation effects remains the same; the distribution is simply stretched by an amount determined by the fitness loss. For high fitness, few mutations are already present so the transformed mutation effect distribution is nearly identical to the original distribution. For lower fitness, more mutations are present to interact with the new mutations and the probability of incurring a mutation with greater actual effect increases. The rate of fitness loss given the fitness already lost can be predicted from the single mutation effect no-epistasis case by transforming the distribution of inherent mutation effect and finding the expected rate of fitness loss over the distribution of actual mutation effects, as shown in

the methods section above. These predictions are shown in Figure 3 and are qualitatively quite good.

The importance of this change in distribution can be seen by comparing the distribution of actual mutation effects with the rate of fitness loss function τ in the simpler nonepistatic model. Contribution of each mutation effect to the ratchet will be roughly equal to the rate of fitness loss in the nonepistatic, constant s model. At low fitness, the actual mutation effect distribution has a large proportion of its probability past the largest effect that contributes substantially to the rate of fitness loss. As fitness decreases, the proportion of actual mutation effects that contribute to fitness loss decreases.

Stronger epistasis will skew the actual mutation effect distribution even further from neutrality at low fitness. A smaller value of α will result in a larger inflation factor for a given amount of fitness lost. For any particular distribution of inherent mutation effects, sufficiently strong epistasis could, at low fitness, skew the distribution of actual effects so much that the remaining slightly deleterious mutations will not arise frequently enough to drive the ratchet at a meaningful rate.

More important than the strength of epistasis, however, is the effect of an increase in the population growth parameter, R. The population growth parameter is a measure the population's initial maximum fitness and therefore a measure of the amount of fitness that the population may lose yet still persist. An increase in R has two effects. First, it simply allows the population to persist after greater fitness loss. This effect of increasing R is seen in the no-epistasis model and is weak. The time to extinction without epistasis is approximately proportional to $\ln(R)$ (LYNCH *et al.* 1993), shown for comparison in Figure 4. This result has led to a reasoned disinterest in the interpretation of R. Second, an increase in R allows for a greater inflation of the inherent mutation effects. This can be seen by examining the function that determines the inflation factor. The largest possible multiplier of the inherent mutation effect is around $R^{1-\alpha}$. Coupled with the rapidly decreasing tail of τ, the rate of fitness loss at low fitness can decrease very rapidly with increasing R.

This second effect can be very strong and can mean the difference between quick extinction from the ratchet and immunity. By the definition of synergistic epistasis, the increase in actual effect over inherent effect of a mutation must itself increase with accumulated inherent effects. An increase in R provides for greater increase in the skew of the distribution of actual effects at low maximum fitness. Given any amount of synergistic epistasis and sufficiently large R, any particular distribution of inherent mutation effects will be skewed such that the supply of slightly deleterious mutations is on the same or lower order as the rate of back and compensatory mutations. This suggests that the presence of any epistasis can allow any asexual population to escape the ratchet through high fecundity. This sensitivity to the R parameter is shown by simulation in Figure 4 for various fitness functions. For low R, epistasis has very little effect. As R increases, the importance of epistasis increases. Under epistasis, the expected time to extinction grows rapidly and nonlinearly with $\ln(R)$. Without epistasis, the time to extinction goes up linearly with $\ln(R)$ even for high R.

DISCUSSION

Variation in mutation effect can lead to a population's extinction by the ratchet even in the presence of epistasis. Thus, the presence or even ubiquity of synergistic epistasis among mutations does not mean that the ratchet is ineffective. However, the ability of the ratchet to cause the extinction of an asexual population depends on the nature of interaction among mutations within individuals, the distribution of inherent mutation effects, and the tolerance of the population for fitness loss.

In this and previous models, a population's tolerance for fitness loss is parameterized as R, typically considered the fecundity of the model organism. Coupled with any synergistic epistasis, sufficiently high fecundity can essentially halt the ratchet (Figure 4). Some epistasis is likely and high fecundity organisms are common. However, simply producing many offspring does not warrant a large value for the R parameter. Only progeny that are expected to survive to compete with other members of the population at reproduction will contribute to R. Because the model includes only genotype-dependent and (separately) density-dependent sources of mortality, any genotype-independent and density-independent mortality must be accounted for by choosing an R lower than the expected fecundity of the modeled organism. Examples of genotype-independent and density-independent mortality include settling in the wrong environment for planktonic larvae, most kinds of predator pressure, and disease, among others. Of course, each of these factors may depend to some extent on genotype or density, but all individuals are at some (generally great) risk of failing to reproduce for reasons other than crowding and genetics. Nevertheless, fecundity puts an upper bound on R. Organisms with low inherent rates of increase, such as large vertebrates, bacteria, and Protists, are unlikely to have their rate of fitness decline via the ratchet be much affected by epistasis. Only very extreme epistatis would have any notable impact on the expected time to extinction of such a population of asexuals.

Interpreting the effect of epistasis as modeled here also requires care. Modeling epistasis as a single average interaction among mutations has problems analogous to the problems of modeling all mutations as equivalent. The average mutation effect is generally not a good indicator of how a population will behave under recurrent mutation. Semilethal mutations may greatly

influence the average mutation effect but will have very little influence on the rate of fitness loss. Slightly deleterious mutations are difficult to detect (HOULE *et al.* 1992) but dominate determination of the fate of a population undergoing the ratchet (Figure 1). Similarly, interactions among mutations are also varied and not well characterized by averaging. Subsequent mutations that happen to have strong interactions with mutations already present may have too large an actual effect to contribute to the ratchet. However, not all subsequent mutations will interact as strongly with the mutations already present. As long as there is a supply of mutations whose actual effects on the individuals in which they occur is slightly deleterious, the ratchet can continue. Epistasis is not really a genome-wide property but is the result of interactions within and between myriad metabolic pathways. Mutations accumulating in a clonal line cause complex and possibly idiosyncratic changes in the probability distribution of subsequent mutation effects. It is the dynamics of this distribution of mutation effects that determines the fate of the population. Interestingly, another model of mutation interaction, with quite different assumptions also leads to the conclusion that generalized mutation interaction will lead to the cessation of the ratchet. In that model, survivorship is assumed to be determined by underlying quantitative characters (WAGNER and GABRIEL 1990). The population starts with optimal character values. Initially, mutations cause the underlying character values to move away from their optima. However, as mutations accumulate, compensatory (beneficial) mutations become much more likely to move the characters back towards the optimum (WAGNER and GABRIEL 1990), whereas the selection against additional mutations that move the characters further from the optimum gets stronger (more deleterious). Under this model, the distribution of mutation effects change with mutation accumulation such that, as mutation accumulate, the distribution of mutation effects increasingly favors beneficial mutations and more damaging mutations, neither of which contribute to the ratchet.

Both the average-epistatic and quantitative-fitness-trait models predict that mutation accumulation will result in classes of mutations that fail to contribute to the ratchet. Undoubtedly, this is so. However, it is also likely that other models of mutation interaction (such as noninteraction) apply as well for some classes of mutations and some fitness determinants within any population. If the ratchet is halted along some fitness-contributing characters or loci but can continue via others, it will. Of course, for any given population, it is difficult to determine the distribution of mutation effects and how this distribution will change with mutation accumulation. Yet it seems very likely that within any given generation, there will be a supply of mutation effects that can drive the ratchet; the question is more how fast than if at all.

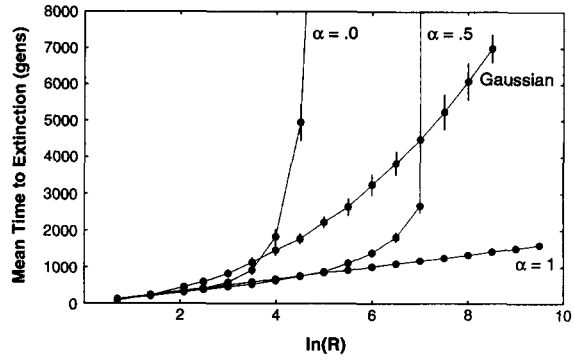

FIGURE 4. $\ln(R)$ versus mean time to extinction for simulated populations. Confidence limits are 95% ($n = 20$). For all curves, ($K = 2048$, $\mu = 1$). Each curve is labeled with its epistasis parameter (α). Curve labeled "Gaussian" has Gaussian epistasis with $\beta = 0.01$. No epistasis curve ($\alpha = 1$) shows linear relationship between $\ln(R)$ and mean time to extinction. All others show nonlinear increase of mean time to extinction with $\ln(R)$.

Both epistasis and mutation effect distribution are difficult to determine empirically and neither are well characterized in any organism (but see MUKAI 1969; WILLIS 1993). Nevertheless, in particular populations, it is possible to determine whether epistasis has stopped the ratchet. In a population where the ratchet has been halted by epistasis, the observed distribution of mutation effects would be skewed toward lethality (noted also by KONDRASHOV 1994), as shown in Figure 4. Methods of inferring mean mutation effect (SIMMONS and CROW 1977; HOULE *et al.* 1992) can be applied to putative old asexual populations. An observed mean mutation effect much higher in the asexual populations than in related sexual populations would provide good evidence for a role for synergistic epistasis in maintaining the asexual population.

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