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# Rapid fixation of deleterious alleles can be caused by Muller's ratchet

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**Summary**

Theoretical arguments are presented which suggest that each advance of Muller's ratchet in a haploid asexual population causes the fixation of a deleterious mutation at a single locus. A similar process operates in a diploid, fully asexual population under a wide range of parameter values, with respect to fixation within one of the two haploid genomes. Fixations of deleterious mutations in asexual species can thus be greatly accelerated in comparison with a freely recombining genome, if the ratchet is operating. In a diploid with segregation of a single chromosome, but no crossing over within the chromosome, the advance of the ratchet can be decoupled from fixation if mutations are sufficiently close to recessivity. A new analytical approximation for the rate of advance of the ratchet is proposed. Simulation results are presented that validate the assertions about fixation. The simulations show that none of the analytical approximations for the rate of advance of the ratchet are satisfactory when population size is large. The relevance of these results for evolutionary processes such as Y chromosome degeneration is discussed.

## 1. Introduction

The stochastic process known as Muller's ratchet has attracted a good deal of attention from theoreticians, owing to its potential importance as a factor in the evolutionary fate of asexual populations and of non-recombining portions of the genome such as the Y chromosome (Felsenstein, 1974; Maynard Smith, 1978; Charlesworth, 1996). In its classical verbal formulation (Muller, 1964; Maynard Smith, 1978), the ratchet was portrayed as a process by which the class of individuals containing the lowest number of deleterious mutations is irreversibly lost from the population by genetic drift. Consider, for example, the case of a haploid asexual population, in which mutations occur exclusively from wild-type to deleterious alleles but not in the opposite direction. If the selective effects of mutations at different loci are identical, as is usually assumed, a population can be characterized by the frequencies of genomes containing 0, 1, 2, … mutations. If the frequency of the mutational class containing the lowest number of mutations (the *least-loaded class*) is sufficiently small, it will be lost from the population after a finite number of generations. Given the assumed irreversible nature of mutation and the lack of opportunity for genetic recombination, the least-loaded class cannot be reconstituted, and will be replaced by the class with one more mutation. This class is now vulnerable to stochastic loss in the same way. There is thus a repetitive process of loss of successive least-loaded classes, in which the loss of each class can be regarded as a turn of Muller's ratchet.

As formulated in this way, there is no reference to the fate of mutant alleles at individual loci. The ratchet is, indeed, often studied theoretically in terms of a model in which the genome is assumed to have an infinite number of loci, with mutant alleles at infinitesimally low frequencies. The stochastic process can then be represented by a multinomial sampling scheme, in which the mutational classes (following mutation and selection in one generation) are randomly sampled to generate the set of mutational classes in the next generation (Haigh, 1978; Stephan *et al.*, 1993). It is thus theoretically possible for the ratchet to advance without any fixation, or even noticeable increase in gene frequencies, of mutant alleles at individual loci.

Some simulation studies of Muller's ratchet have been carried out, in which genomes with finite numbers of loci were modelled, and in which the fates of mutant alleles at individual loci were followed

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explicitly (Charlesworth *et al*., 1993; Higgs & Woodcock, 1995). The rate of fixation of deleterious mutant alleles can then be compared with the rate predicted from fixation probabilities calculated from standard diffusion equation results (Kimura, 1962). Charlesworth *et al*. (1993) studied diploid populations with either exclusively asexual reproduction, or with sexual reproduction and varying frequencies of recombination. They observed that, in randomly mating sexual populations with low rates of genetic recombination and small population sizes, fixation proceeded at much higher rates than expected from single-locus results, but that these rates declined rapidly with increasing population size. With sufficiently large population size, a non-recombining population could experience a significant rate of advance of the ratchet, in terms of successive losses of least-loaded classes and an increase in the mean number of mutations per individual, without any appreciable fixation of deleterious mutations at individual loci. This was less likely with diploid asexual populations, but some cases in which the ratchet advanced as a result of accumulation of mutations at unfixed loci were observed here too. In contrast, Higgs & Woodcock (1995) simulated asexual haploid populations and found that the advance of the ratchet exactly coincided with the fixation of deleterious alleles. Lynch and his co-workers have also equated the advance of the ratchet with fixation, but without providing detailed results on allele frequencies (Lynch & Gabriel, 1990; Gabriel *et al*., 1993).

There are thus noticeable discrepancies among the conclusions of different investigators concerning the properties of what is supposedly the same process. The question of whether the advance of Muller's ratchet can be equated with fixation events has considerable biological significance. For instance, Charlesworth (1978, 1996) argued that the degeneration of Y chromosomes and the evolution of dosage compensation of X-linked loci in the heterogametic sex may result from the operation of the ratchet, without any concomitant process of fixation of deleterious alleles at Y-linked loci. In contrast, several recent studies suggesting higher rates of molecular evolution in non-recombining genomes (compared with freely recombining genomes) have interpreted these as being due to an accelerated rate of fixation of deleterious mutations associated with the operation of Muller's ratchet (Lynch, 1996; Moran, 1996).

In order to resolve this disagreement about the nature of the ratchet, we have re-investigated all three of the cases mentioned above. We find that it is indeed the case that there is one-to-one correspondence between the fixation of deleterious alleles in the case of a haploid asexual population, but that this is not necessarily so in the diploid cases. The mechanism of fixation is more complex than is commonly assumed. Before presenting the simulation results which support this claim, we will first provide some qualitative theoretical arguments concerning the operation of the ratchet.

## 2. Qualitative theoretical considerations

### (i) *Haploid asexual population*

In the case of a haploid organism, the population can be divided into classes corresponding to genomes (haplotypes) that contain 0, 1, 2, … deleterious mutations. We denote the number of individuals carrying $i$ mutations by $n_i$. Within a class, different haplotypes may contain mutations at different loci, but the assumption of multiplicative fitnesses across loci and equal selective effects at each locus implies that these haplotypes all have the same fitness, which is equal to $(1-s)^i$ for the class with $i$ mutations. Mutation is assumed to occur according to a Poisson process, with mean $u$ per genome per generation. The population size is assumed to be fixed at $N$ breeding individuals per generation; within a given genotypic class, parents are sampled randomly for their contributions to the next generation.

The process of fixation associated with the advance of the ratchet can be understood by first considering the case in which the value of $n_0$ obtained by multiplying the corresponding deterministic equilibrium frequency by $N, \hat{n}_0$, is so large that the ratchet advances at a rate of one turn in several tens of generations (Haigh, 1978; Stephan *et al*., 1993). In addition, we assume that $Ns$ is $\gg 1$, so that a new deleterious mutation at a given locus would have a negligible chance of fixation in a freely recombing population (Kimura, 1962). In the initial phase of the process, the least-loaded class contains no mutations. As long as the least-loaded class persists in the population, the neighbouring class, whose size is $n_1$, receives new haplotypes from the zero class at an average rate of $\kappa = \langle n_0 \rangle u \exp(-u)$ per generation, where $\langle n_0 \rangle$ is the mean value of $n_0$ over the period in question. Hence, no permanent fixation of haplotypes is possible within the class with one mutation, unless $\kappa$ is so small that no new mutations enter this class in the time between fixation of a haplotype within this class and loss of the zero-mutation class. Similar principles apply to the classes with 2, 3, … etc. mutations, which all originate from classes with lower numbers of mutations. The fact that, at this stage of the process, the least-loaded class of gametes contains no mutations means that fixation of a deleterious mutation at a given locus in the population as a whole is impossible, even if fixation has occurred in all other classes.

When the zero class has been lost from the population, the least-loaded class is made up of a set of haplotypes which all have one mutation (Fig. 1). By assumption, there is no mutational flow into this class. The finite size of the least-loaded class, and the selective equivalence of all haplotypes within a mutational class, means that a purely neutral process

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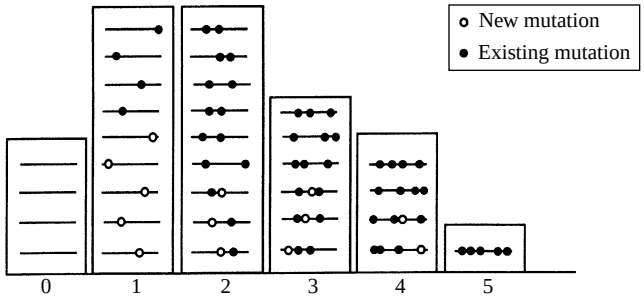
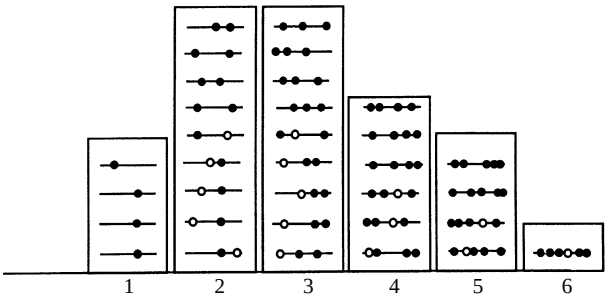
(*a*) Soon after initial stage: some mutant-free genomes present




(*b*) Zero class lost: no further input of mutations to class with one mutation




(*c*) Fixation within class with one mutation (and high frequency of this allele in classes with more than one mutation)

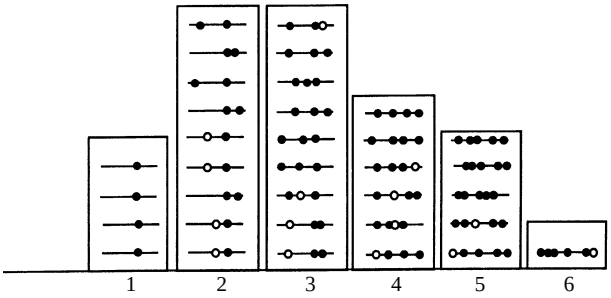



Fig. 1. Distributions of mutations in genotypes, illustrating three stages (*a–c*) during the process of fixation of mutations in the least-loaded class. Each line represents a chromosome and mutations are indicated by open (new mutations) or filled (mutations inherited from earlier generations) symbols. The column heights indicate the numbers of chromosomes with different numbers of mutations (shown on the abscissa).

of genetic drift within the new least-loaded class will cause it to become fixed for a single haplotype, if it was not previously fixed. Fixation for a haplotype corresponds to fixation of a deleterious allele at a particular locus. Because all other mutational classes in the population ultimately derive their haplotypes from the least-loaded class, the rest of the population will eventually become fixed for a deleterious allele at the locus in question, following its fixation within the new least-loaded class. Fixation in the whole population must therefore follow upon the loss of the current least-loaded class. There is no opportunity for further fixation events in the entire population until the loss of the new least-loaded class and its replacement by the neighbouring class (which now contains mutations at two loci, at least one of which is fixed in the entire population). Once this has happened, fixation can again occur at an additional locus, and so on with each further turn of the ratchet, producing a result like that observed in the simulations of Higgs & Woodcock (1995).

This establishes that, with a slow moving ratchet, there is a one-to-one correspondence between the loss of the least-loaded class and fixation of a mutation in the entire population, so that the rate at which the mean number of mutations per haploid genome increases is identical both to the rate at which least-loaded classes are lost, and to the rate of fixation of deleterious mutations within the population as a whole. If fixed loci are excluded from consideration, there thus is no increase in the mean number of deleterious alleles per haploid genome, regardless of the speed of movement of the ratchet. The cause of each fixation event is the loss of the current least-loaded class, and the fixations follow the turns of the ratchet, by many generations if $\kappa$ is large and $\langle 1/n_0 \rangle$ is small, where $\langle 1/n_0 \rangle$ is the mean of the reciprocal of the size of the least-loaded class while it persists in the

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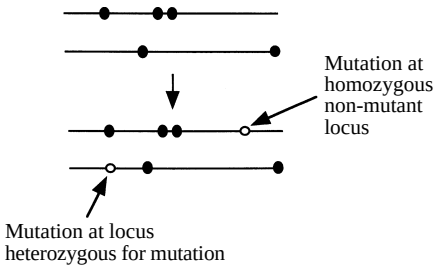


Fig. 2. Possible mutational states of loci in diploid genomes. As in Fig. 1, the lines represent chromosomes and mutations at the loci are indicated by open or filled symbols at the locations of the genes.

population. (The probability of loss of haplotypes by drift in a given generation is inversely proportional to the current size of the least-loaded class, and so the long-term rate of loss will be related to the mean of $1/n_0$.)

If the ratchet is turning rapidly (e.g. if $\hat{n}_0 < 1$), several least-loaded classes may successively be lost before fixation of a haplotype can occur in the current least-loaded class. In such cases, the argument just given must be modified. By the above argument, loss of several classes will be followed by the simultaneous fixation in the whole population of all the mutant alleles contained in the haplotype in question. The number of mutations that become fixed is equal to the number of turns of the ratchet since the last fixation event. Again, there is a one-to-one correspondence between the rate of loss of least-loaded classes and the rate of fixation of deleterious alleles.

(ii) *Diploid asexual population*

In the case of a diploid asexual population, each individual has two haploid genomes (Fig. 2). Offspring have the same genotype as their parent, plus any new mutations that may have arisen. It is reasonable to assume that the two haploid genomes of an individual acquire mutations independently, at rate $u$ for each genome, so that there is a diploid mutation rate of $U = 2u$. The fitness effect of an individual locus now requires specification of the number of mutant alleles present (0, 1 or 2). Homozygotes carrying two mutant alleles have fitness $1-s$, and heterozygotes carrying one mutant allele have fitness $1-hs$, where $h$ is the dominance coefficient.

A new mutant allele at a given locus arises in one of the two haploid genomes of the individual which originally carries it; random drift can cause the descendants of this individual to predominate in the population, but this will cause fixation of the allele only within one of the two haploid genomes that are present in each individual. If the product of population size and per locus mutation rate is sufficiently small, mutations at a given locus can be treated as unique events, so that the possibility that another mutation arises at the same locus before the first has gone to fixation can be neglected, at least in the short term, and thus the actions of selection and drift are similar to the asexual haploid case, with the selection coefficient $s$ being replaced by $hs$. If fixation is defined as spread of a new mutation through the population within one haploid set (Charlesworth *et al*., 1993), the process is completely analogous to that for the haploid case, and there will be the same correspondence between fixation and movement of the ratchet as far as the loss of the least-loaded haploid genomes is concerned.

If, however, the population size is large enough, mutations can re-occur at the same locus, and a new deleterious allele may arise in a haploid genome in an individual in which the other haploid genome already carries a mutation at the same locus. The same will be true if the ratchet has advanced sufficiently far that a substantial proportion of loci are fixed within haploid sets. When this happens, the selective equivalence of different haplotypes within the same mutational class will break down, unless deleterious alleles are sufficiently dominant. For example, among diploid carriers of two mutations, an individual whose pair of haploid genomes each contain a mutant allele at a different locus has fitness $(1-hs)^2$, whereas an individual homozygous for a deleterious allele at one locus has fitness $1-s$. These are equal when

$$h = \frac{1-\sqrt{1-s}}{s}. \tag{1}$$

For weak selection, this condition is close to $h = 0{\cdot}5$, i.e. semidominance (with $s = 0{\cdot}1$, for example, we have $h = 0{\cdot}513$, and for $s = 0{\cdot}2$ it is $0{\cdot}528$). If $h$ is less than this critical value, homozygosity for the mutant allele will impose a selective penalty within the class of individuals with two mutations, relative to individuals in the same class which are heterozygous for two mutations at different loci.

This effect means that selection will tend to resist the fixation of deleterious alleles at a locus if it has already acquired deleterious alleles by mutation in the other haploid set. This effect will be especially strong, the lower the dominance coefficient and the larger the population size. Thus, with small values of $h$, there will be only a weak relation between fixation in *both* haploid sets and loss of the least-loaded class, especially with a large population size, which increases the effectiveness of selection. Fixation of a deleterious allele at a locus within *one* of the two haploid genomes is not, of course, expected to be retarded in this way.

(iii) *Diploid, non-recombining, random-mating, sexual population*

In the case of a diploid, non-recombining, random-mating, sexual population the two haploid genomes that form an individual can segregate from each other, and combine with haploid genomes drawn at random

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from the population as a whole to form the next generation. The lack of equivalence of the fitnesses of haplotypes with the same numbers of mutations when dominance is not complete is even more obvious than in the diploid asexual case: a haplotype cannot spread to fixation within its class without causing individuals who carry it to become homozygous. If the reduction in homozygous fitness is sufficiently severe, compared with the mean fitness of heterozygous carriers of the haplotype, the probability of fixation will be decreased to an insignificant level if the size of the class is large enough. This means that losses of least-loaded classes can proceed independently of the process of fixation of deleterious alleles at individual loci, and will be accompanied by a steady rise in the mean number of mutations per individual, even if the fixed loci are disregarded. For the reasons just discussed for the asexual case, this decoupling of fixation and turns of the ratchet is most likely to be seen with low dominance coefficients and large population sizes; there will of course be intermediate sets of parameter values in which fixation is accelerated compared with the single-locus case, but does not have a one-to-one correspondence with turns of the ratchet.

## 3. Simulation methods

Stochastic simulations were done as described by Charlesworth *et al.* (1993), with a simple modification for the case of an asexual haploid population. The computer programs simulated full details of genetic transmission in multi-locus genotypes (with 1024 loci subject to deleterious mutation). This enables allele frequencies and fixation events at individual loci to be followed. The sequence of events in each generation was mutation, reproduction and selection. A population size of $N$ haploid or diploid genomes was assumed, depending on which ploidy level was investigated. Mutation was performed by assuming a rate, $u$, for an entire haploid genome, and generating haploid genomes with a Poisson distribution of mutations from wild-type to deleterious alleles. All mutations had the same selection coefficient, $s$ (and, for diploid models, the same dominance coefficient, $h$), rather than the more biologically plausible assumption that mutations at different loci can have different effects on fitness (Butcher, 1995), which is more difficult to simulate. Multiplicativity of fitness effects of different loci was assumed.

The three different genetic systems described above were modelled: haploidy with complete asexual transmission of identical genotypes from parent to offspring, except for mutation; diploidy with the same assumptions (both of these have no recombination between different genotypes); and diploidy with independent segregation of chromosomes but no within-chromosome crossing over.

The simulations were initialized with numbers of mutations per individual according to the expected distribution for an infinite population at equilibrium, and then run for 100 generations. After this stage, data were taken every 50 generations. These consisted of the numbers of mutations in the least-loaded genotype class, the numbers of fixed and non-fixed mutations, and the mean fitness of the population. Since genotypes were recorded after selection, it is important to note that comparisons of simulation results with analytical predictions should be made with predicted values of post-selection variables, such as the mean and variance of the numbers of mutations per individual. Note that for diploid asexual models, the term 'fixation' can refer either to the situation when, at a given locus, all individuals have the heterozygous genotype, or to the case when a mutant allele at a locus is present in both haploid genomes of all individuals. These are explicitly distinguished in what follows.

The rate of advance of Muller's ratchet was also found for each run. The program calculates the average number of generations between successive losses of least-loaded classes, taking into account the difference in the number of mutations between the least-loaded class and the one that has just been lost. This gives the average number of generations required for the number of mutations in the least-loaded class to increase by one. The rate of the ratchet is the reciprocal of this time. The rates calculated in this manner were very similar to rates found from the regressions of log population mean fitness on generation number.

## 4. Simulation results

### (i) *Haploid asexual populations*

Our focus was chiefly on the haploid case, as it is in this case that results appear to differ from those already obtained by simulating diploid populations (see above). We were particularly interested in testing whether the events during accumulation of mutations are as described in our interpretation of this case, outlined above. Fig. 3 illustrates the progress of the ratchet in a representative case, with successive losses of least-loaded classes preceding fixation at a locus in the least-loaded class. As described in Section 2, losses of least-loaded classes are followed after a relatively short time by fixation of a mutant allele in the class with one more mutation than the least-loaded class, and shortly after that by its fixation in the population as whole. The parameter values for this simulation were $u = 0{\cdot}35$, $s = 0{\cdot}1$ and $N = 1000$. Similar findings were obtained with a range of other parameter values.

Runs with parameters such that fixations occurred faster are, of course, more complex to interpret, because several fixations can occur in the least-loaded class before this class is lost. The genotypes at these loci then spread through the population and multiple fixations can occur in a brief time period. Despite this

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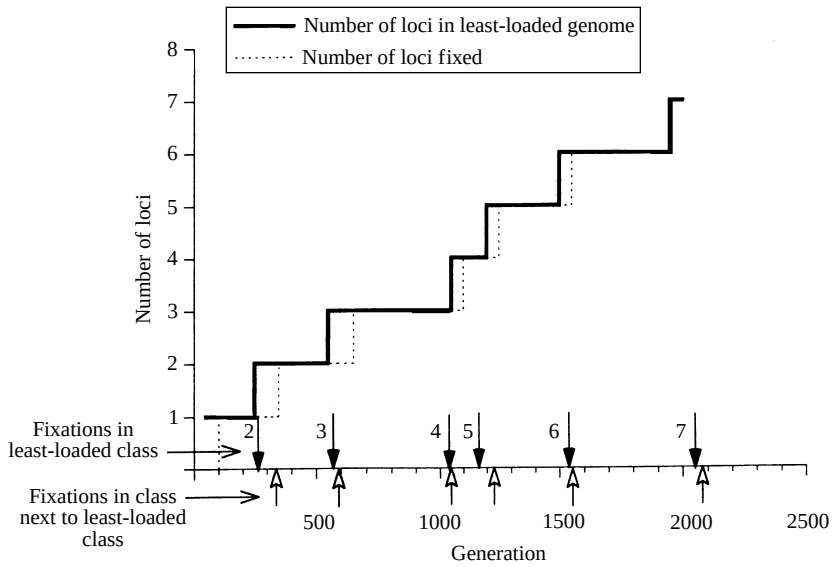


Fig. 3. Sequence of events in the first 2000 generations of a typical simulation of a haploid population of size 1000, with $u = 0{\cdot}35$, $s = 0{\cdot}1$. The numbers of loci fixed are plotted, as well as the numbers in the least-loaded genome, at intervals of 100 generations. The generation numbers at which fixations occurred are shown on the abscissa, with filled arrows for fixations in the least-loaded class of genomes, and open arrows for those in the class with one more mutation than in the least-loaded genomic class.

Table 1. *Rate of Muller's ratchet in a haploid population, and comparison with several analytical predictions*

| | | Parameters of mutation distribution | | | Mean time between losses of least-loaded classes ($T$) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $s$ | $N$ | $\hat{n}_0$ | Mean | Variance | Simulated | SA | SS | C | D |
| 0·050 | 1000 | 0·91 | 8·37 | 5·58 | $27{\cdot}3 \pm 0{\cdot}7$ | 32·4 | 35·4 | [a] | 18·7 |
| | 2000 | 1·82 | 8·24 | 5·56 | $36{\cdot}0 \pm 0{\cdot}9$ | 35·4 | 28·2 | 13·9 | 18·3 |
| 0·075 | 200 | 1·88 | 6·11 | 3·56 | $23{\cdot}9 \pm 0{\cdot}8$ | 20·3 | — | 10·5 | 17·6 |
| | 1000 | 9·40 | 5·70 | 4·01 | $66{\cdot}9 \pm 4{\cdot}2$ | 44·4 | — | 42·7 | 43·5 |
| | 2000 | 18·8 | 4·74 | 4·12 | $128 \pm 9$ | 74·4 | — | 79·9 | 67·8 |
| | 4000 | 37·6 | 4·68 | 4·20 | $3018 \pm 33$ | 135 | — | 156 | 114 |
| 0·100 | 200 | 6·04 | 4·55 | 2·93 | $43{\cdot}9 \pm 1{\cdot}1$ | 27·2 | 33·8 | 25·6 | 45·5 |
| | 1000 | 30·2 | 3·37 | 3·15 | $314 \pm 38$ | 104 | 162 | 147 | $\infty$ |
| | 2000 | 60·4 | 3·40 | 3·09 | $3263 \pm 1037$[b] | 201 | 809 | 477 | 167 |

The mutation rate, $u$ is 0·35 in each case. Most of the results are based on runs of 2000 generations, which generally accumulated enough mutations for $T$ to be estimated. Longer runs, for cases with very slow rates, are indicated in the table. Values of $T$ are shown for the present simulations, for eqn (8) and the multinomial simulations of Stephan *et al.* (1993) (SA and SS, respectively), for (A 5) of the present paper (C), and for the formula of Gabriel *et al.* (1993) (D). See text for further explanation.

The means are values for the numbers of mutations at unfixed loci.

[a] No values are shown for cases with $\hat{n}_0 \approx 1$, because the formula is not valid for such cases.

[b] Results based on 5 runs, of 10000 generations each (in one further run, no mutations had accumulated by this time).

difference, the process is essentially similar to that with a slower rate of the ratchet. As simulation results for such cases were described by Higgs & Woodcock (1995), we do not show any details here.

In addition to supporting the interpretation of the haploid ratchet process that has been outlined above, the simulations provide data on the rate of advance of the ratchet, and it is interesting to compare these with analytical predictions that have been developed. Two classes of analytical approximations have been proposed. One is based on the change in mean and higher moments of the distribution of $n_i$, treating it as a quantitative trait (Pamilo *et al.*, 1987; Gabriel *et al.*, 1993; Higgs & Woodcock, 1995; Gessler, 1995; Prügel-Bennett, 1997). The changes in mean and variance of the $n_i$ (given their current values) can be obtained by standard methods. In large populations, reasonably accurate closed forms for these can be obtained. Using the approach of Gabriel *et al.* (1993), the expected rate of change of mean (correcting for the fact that it is measured post-selection in the simulations) is

$$\langle \Delta \bar{n} \rangle \approx u(1-s) - s\langle V_n \rangle, \tag{2}$$

where the angle brackets represent means over independent replications of the process, and $\bar{n}$ and $V_n$

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are the mean and variance of the distribution of the number of mutations within a population in a given generation ($\bar{n}$ includes contributions from loci which have gone to fixation; $V_n$ is obviously affected only by segregating loci). If the ratchet settles down to a fairly constant rate, as indicated by previous work and by our simulations (see Fig. 4), the rate of change of $\bar{n}$ should approximately parallel the rate of loss of the least-loaded class. Although it is not possible to obtain a closed form for $V_n$ in the case which we are studying ($\hat{n}_0 \gg 1$), the mean of $V_n$ over replicate runs can be obtained from the simulations, and used in (2). The reciprocal of the resulting expression can be compared with the mean time between successive losses of the least-loaded class, $T$.

The other method is to use diffusion equation theory to predict the expected time to loss of the least-loaded class. This method was originally proposed by Stephan *et al.* (1993). Their eqn (8) is the appropriate formula for $T$ for the parameter sets used here (W. Stephan, personal communication). A modification of the diffusion equation approach, based on slightly simpler assumptions, is described in the Appendix. Numerical integration of (A5) yields a value for $T$.

Table 1 shows some of these comparisons between theory, based on sets of replicate runs, where averages over replicates of the means and variances (disregarding loci at which fixations had occurred) were calculated 2000 generations after initialization. In addition, the mean intervals between successive losses of the least-loaded classes were calculated for the duration of each run and averaged over runs. These are the source of the column labelled 'Simulated' for the mean time between turns of the ratchet ($T$) in Table 1 (standard errors as well as means of $T$ are shown). The columns headed 'SA' and 'SS' are obtained from eqn (8) and the multinomial sampling simulations of Stephan *et al.* (1993), respectively; the column headed 'C' is obtained from (A5) of the present paper; the approximation based on (2) is shown in the column headed 'D', using the averages of the means and variances of mutant numbers (disregarding loci which have become fixed for mutant alleles) in generation 2000, which are shown to the left of the simulation results for $T$.

Several points are noteworthy. First, the rate of the ratchet is not well predicted by the approximations when population size is large and selection is moderate or strong. Even the multinomial sampling simulation fails to predict $T$ correctly for large population sizes. The analytical approximations mostly perform even worse, especially for large $N$ (when the ratchet is moving slowly). Eqn (8) of Stephan *et al.* (1993) agrees well with the simulation results for the case of weak selection or moderate selection ($s = 0{\cdot}05$ and $0{\cdot}075$) when $N \leqslant 1000$. For larger population sizes, (A5) generally perform the best, although their predictions increasingly fall well below the simulation results as $N$ increases. Second, there is a pronounced

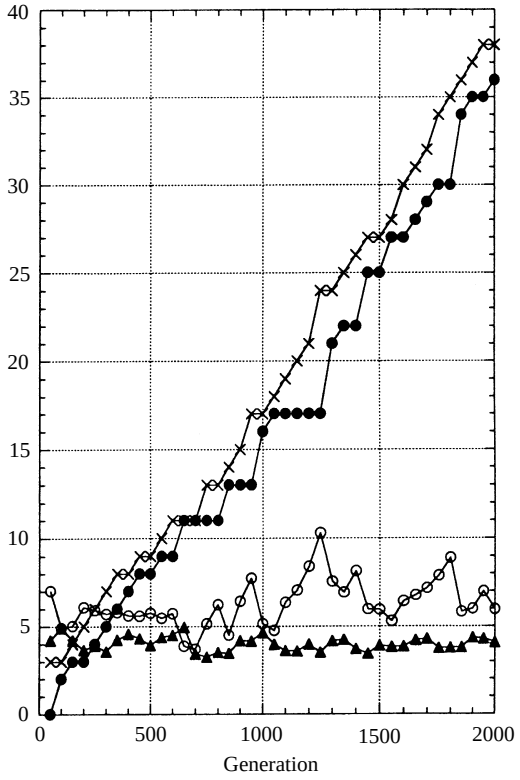


Fig. 4. Numbers of fixed mutations, numbers of mutations in the least-loaded class of genomes, and means and variances of numbers of mutations at non-fixed loci. Numbers are given per haploid genome during the first 2000 generations of a typical simulation with population of size 1000, $u = 0{\cdot}35$ and $s = 0{\cdot}075$. ●, Number fixed; ○, mean number of mutations; ▲, variance in number of mutations; ×, number in least-loaded class.

departure from a Poisson distribution for small population sizes, but the average mean and variance (discounting fixed mutations) converge as $N$ increases. Their values are in fact quite close to the deterministic equilibrium values, given by $u(1-s)/s$; e.g. for the case $u = 0{\cdot}35$ and $s = 0{\cdot}075$, the deterministic mean and variance are both equal to 4·32, compared with simulated average values of 4·68 and 4·20 with a population size of 4000. This suggests that convergence towards the deterministic equilibrium values should be observed as $N$ increases further but we were unable to investigate this because of computer speed limitations.

Examination of the results of individual simulation runs shows clearly that the mean and variance of the numbers of unfixed mutations within a population show no tendency to increase systematically, but rather fluctuate around mean values. An example is shown in Fig. 4. The increase in mean number of mutations over time is driven completely by the increased number of fixed mutations, which exactly parallels the number of mutations in the least-loaded class, as is to be expected from the results shown in Fig. 3.

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Table 2. *Results of diploid simulations at generation 2000 with no recombination but with segregation*

| | | | | Final mean numbers and standard errors | |
|---|---|---|---|---|---|
| *U* | *s* | *h* | *N* | In least-loaded class | Fixed |
| 0·1 | 0·1 | 0·05 | 50 | 77·2 ± 2·60 | 0 |
| 0·1 | 0·1 | 0·1 | 50 | 56·9 ± 1·59 | 0·545 ± 0·390 |
| 0·1 | 0·1 | 0·2 | 50 | 32·1 ± 2·80 | 1·50 ± 0·522 |
| 0·1 | 0·1 | 0·35 | 50 | 11·4 ± 1·01 | 11·1 ± 1·04 |
| 0·1 | 0·1 | 0·5 | 50 | 6·70 ± 0·684 | 6·60 ± 0·632 |
| 0·1 | 0·1 | 0·6 | 50 | 4·60 ± 0·400 | 4·50 ± 0·428 |

Ten runs were done for each set of parameters. The population size, *N*, is the diploid value.

Table 3. *Results of fully asexual diploid simulations at generation 2000*

| | | | | Final mean numbers and their standard errors | | |
|---|---|---|---|---|---|---|
| *U* | *s* | *h* | *N* | In least-loaded class | Fixed | Fixed as heterozygote |
| 0·2 | 0·2 | 0·5 | 50 | 154·1 ± 3·23 | 0 | 307 ± 5·04 |
| 0·2 | 0·2 | 0·1 | 50 | 130·6 ± 4·17 | 0·56 ± 0·294 | 259 ± 6·77 |
| 0·2 | 0·2 | 0·2 | 50 | 104·1 ± 2·66 | 1·00 ± 0·373 | 207 ± 3·73 |
| 0·2 | 0·2 | 0·35 | 50 | 71·4 ± 1·37 | 2·00 ± 0·365 | 141 ± 2·50 |
| 0·2 | 0·2 | 0·5 | 50 | 56·3 ± 1·34 | 3·20 ± 0·573 | 125 ± 3·94 |
| 0·2 | 0·2 | 0·6 | 50 | 48·4 ± 4·30 | 3·88 ± 0·398 | 119 ± 6·11 |

Ten runs were done for each set of parameters. The population size, *N*, is the diploid value.

(ii) *Diploid populations*

Our interpretation of the cause of fixations in the case of haploid populations leads to the prediction (above) that if the dominance coefficient *h* is sufficiently high in the diploid case with segregation but no recombination, the behaviour will become similar to that under haploidy, i.e. that an increase in the number of mutations in the least-loaded class will be closely followed by fixation of mutations in the population. But if *h* is sufficiently small, fixation will be retarded, and there can be a loss of least-loaded classes and an increase in mean number of mutations without fixation, as found by Charlesworth *et al.* (1993).

Table 2 shows simulation results for the case of sexual reproduction with segregation but no recombination, for a population of size 50 in which the ratchet moves quickly. The results for low *h* confirm that, in the diploid case with segregation of chromosomes, the loss of least-loaded classes under the ratchet is not paralleled by fixation events (cf. Section 2.iii). For instance, there were no fixations after 5000 generations in the case of a diploid population size of only 100, assuming a mutation rate and selection coefficient of 0·1, and with a dominance coefficient of 0·05 or 0·1. In these runs, gametes accumulated very high minimum numbers of mutations (ranging from 127 to 167), and the mean fitnesses were very low (0·10 with $h = 0{\cdot}05$, 0·034 with $h = 0{\cdot}1$). The simulation results also show that, as expected, greater dominance leads to similarity to the haploid case, i.e. with $h > 0{\cdot}5$, the numbers of mutations that have accumulated and the numbers that are fixed become identical. Increased dominance also, of course, reduces the rate of the ratchet, because selection reduces the frequency of mutations even when they are heterozygous.

A peculiarity of the cases with low dominance coefficients, which is only observed if the population is run for a long time (of the order of 100 times the population size) is that the population becomes 'crystallized' into two segregating haplotypes, within each of which deleterious alleles are fixed. This creates a situation in which there is effectively very strong heterozygote advantage, so that the two haplotypes are maintained at approximately equal frequencies. The driving force for this comes from the fact that, given sufficient time, the restricted effective population size of the least-loaded class permits deleterious alleles at two different loci to reach high frequencies in repulsion from each other; since this genotype is the ancestor of the rest of the population, it comes to consist of 10 and 01 haplotypes (where 0 and 1 represent mutant and wild-type alleles, respectively). Given the irreversible nature of mutation assumed in this model, further fixations of mutant alleles within

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each of these haplotypes will occur by the process described for the haploid case, whereas selection will be resistant to fixation of a deleterious allele at the same locus in both haplotypes. This phenomenon may be relevant to the accumulation of heterozygous deleterious mutations in small populations that has been observed by a number of *Drosophila* workers (Kidwell, 1972; Charlesworth & Charlesworth, 1985; Albornoz & Domínguez, 1994). With large population sizes, this state will be reached only exceedingly slowly for selection coefficients of the magnitude we have studied here, since the time for a deleterious allele to reach a high frequency is very long, so that this phenomenon is likely to be observable only in relatively small populations.

Table 3 shows some results for the fully asexual diploid model, in which parental genotypes are propagated exactly in the absence of mutation. In this case, fixations of mutations as heterozygotes occur much more rapidly than do fixations of mutations in both haploid sets. It will be seen that the number of loci in which mutations have been fixed as heterozygotes almost exactly parallels the mean number of mutations per individual in the least-loaded class, as would be expected if the fixation of a heterozygous mutation accompanies the loss of a least-loaded class (see Section 2.ii). The rate of fixation of mutations in both haploid sets proceeds very slowly, and is retarded by low values of the dominance coefficient, as expected (see Section 2.ii).

## 5. Discussion

The results for the case we have studied, where the deterministic prediction of the number of individuals in the least-loaded class ($\hat{n}_0$) exceeds one, are fairly clear-cut. In the haploid asexual case, losses of the least-loaded classes are followed by fixations of deleterious alleles. As has previously been shown by Higgs & Woodcock (1995) for the case of $\hat{n}_0 \leqslant 1$, the accumulation of deleterious mutant alleles in the asexual haploid case is due entirely to the fixation of alleles at individual loci, not to a steady increase in mean number of mutations at unfixed loci as has been assumed by several authors (e.g. Charlesworth, 1978, 1996). Our results appear to have elucidated the mechanism of this fixation process, as described in Section 2.i. It is the loss of the least-loaded class in a particular generation, the subsequent restriction of effective population size for the selectively equivalent haplotypes contained in the new least-loaded class, and the fact that the rest of the population will eventually trace their ancestry from this class, which jointly permit the fixation of deleterious mutations (Figs. 1, 3). With the population sizes and selection parameters assumed here ($N_e s \gg 1$), these mutations would have only an infinitesimal chance of fixation in a freely recombining genome (Kimura, 1962). Although we have assumed equal selective effects of each locus, the same principle will apply if there is variation in the selection coefficients, but clearly mutations with weaker effects will be more likely to become fixed, since haplotypes with more mutations of small effect than average will experience a selective advantage.

Fixation is thus caused by the ratchet, as classically described in terms of losses of least-loaded classes (Muller, 1964; Felsenstein, 1974; Haigh, 1978; Maynard Smith, 1978). It is not, however, legitimate to describe situations in which fixation of deleterious mutations has apparently occurred in evolution as necessarily representing examples of the ratchet, as is sometimes done (e.g. Moran, 1996; Lynch, 1996). The Hill–Robertson effect of interference between selected alleles at different loci (Hill & Robertson, 1966; Felsenstein, 1974) can cause accelerated rates of fixation of weakly selected deleterious mutations in situations where the products of selection coefficients and effective population size are so low ($N_e s \ll 1$) that no approach to the deterministic mutation–selection balance equilibrium is possible even in a freely recombining population (Li, 1987; Charlesworth *et al.*, 1993). This is a process that is conceptually distinct from the ratchet, which involves the effect of finite population size when $N_e s$ is so large that a freely recombining population would remain close to the deterministic equilibrium for a very long time, even with irreversible mutation from wild-type to deleterious alleles. In addition, the process of background selection, in which strongly selected alleles under mutation and selection restrict the effective population size experienced by weakly selected or neutral loci in a non-recombining genome, can also increase the fixation probabilities of deleterious alleles for which $N_e s$ is much smaller than for the set of strongly selected loci, but is nevertheless $\gg 1$ in the absence of background selection (Birky & Walsh, 1988; Charlesworth, 1994).

We have concentrated on the case of $\hat{n}_0 > 1$, partly because fixations in the haploid model have already been documented by Higgs & Woodcock (1995) for the case $\hat{n}_0 \leqslant 1$, but mainly because this is the parameter range which is most relevant to the problem of the evolutionary degeneration of an incipient Y chromosome or neo-Y chromosome (Charlesworth, 1996). The case of a haploid asexual genome is very similar to that of an incipient Y chromosome, which fails to recombine with its homologue in the heterogametic sex, and is thus in principle vulnerable to the operation of the ratchet. It was previously argued that the ratchet in this case does not lead to fixation of deleterious mutations at individual loci, so that the evolution of inactivation of the Y of dosage compensation in response to the increased mutational load carried by Y must involve levels of organization higher than individual genes (Charlesworth, 1978, 1996). Since the statements about fixation are now known to be wrong, this conclusion about the role of the ratchet must be modified.

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The accelerated rate of fixation of deleterious mutations that we have shown for the asexual haploid case with $\hat{n}_0 > 1$ almost certainly applies to an incipient Y chromosome. If the ratchet is operating at a significant rate, an incipient Y chromosome will therefore experience fixations of deleterious mutations with moderately large effects on fitness, given the evidence that detrimental mutations in *Drosophila* typically have heterozygous fitness effects of the order of 1–2% (Crow & Simmons, 1983; Charlesworth & Hughes, 1997). There will thus be a substantial selective premium in increasing the activity of the mutation-free X-linked loci, at the expense of their Y-linked partners that carry fixed deleterious alleles. This can cause the evolution of dosage compensation on a gene-by-gene basis, rather than at the level of whole groups of genes as was originally suggested (Charlesworth, 1978). Given the evidence for *cis*-acting factors involved in dosage compensation in *Drosophila* (Baker *et al.*, 1994), this strengthens the claim for a role of Muller's ratchet in the evolution of inactive Y chromosomes and dosage compensation, relative to the other processes that have been proposed (Charlesworth, 1996). However, our present knowledge of population sizes and mutation and selection parameters suggests that it is doubtful whether the ratchet could have operated sufficiently fast to account for the well-studied cases of degeneration of neo-Y chromosomes in *Drosophila*, although it could have been important in mammals, with their much smaller effective population sizes (Charlesworth, 1996).

Finally, the failure of the various approximations to predict the rate of movement of the ratchet when $\hat{n}_0 > 1$ in the haploid asexual case needs some comment. It appears that considerations based on the changes in the moments of the distribution give adequate approximations to the movement of the ratchet in the case when $\hat{n}_0 \ll 1$ (Gabriel *et al.*, 1993; Gessler, 1995). Gessler (1995) has argued convincingly that the movement of the ratchet when $\hat{n}_0 \ll 1$ is a quasi-deterministic process, driven by the fact that the expected frequency of the least-loaded class is always too small for it to be represented at all in the population. Agreement of the rate with a quasi-deterministic description such as (2) is thus not surprising. But this argument does not apply to the case of $\hat{n}_0 > 1$, and none of the methods proposed take into account the complexities which we have described here. It is particularly noteworthy that even the multinomial simulations of Stephan *et al.* (1993) fail to predict the movement of the ratchet when $N$ is large, which is where one might have expected the best agreement with the true results. For large $N$, the two approximations based on diffusion theory, as well as the multinomial simulations, mostly underestimate the time between turns of the ratchet (Table 1). They seem to be increasingly conservative as $N$ increases, so that it is probably safe to treat them as lower bounds to the true time between turns. Further theoretical and simulation work is needed to improve our understanding of the process.

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## Appendix

We make the simplifying assumption that the mean fitness of the population, measured relative to the fitness of the current least-loaded class, is affected mainly by fluctuations in the size of the currently least-loaded class. The frequencies of classes with 1, 2, … mutations additional to those carried by the least-loaded class are assumed to remain approximately constant until the current least-loaded class is lost from the population, and then to undergo a rapid readjustment to approach the same values as before the movement of the ratchet (Haigh, 1978; Stephan *et al.*, 1993). The simulations indicate that this procedure is valid, at least as an approximation. They also show that the expectation of the number of mutations per individual, disregarding mutations that have been lost, remains for most of the time quite close to $u/s$, the deterministic equilibrium value, if population size is sufficiently large. The simplest assumption about the mutational class frequencies is that they follow a Poisson distribution with this mean, although the simulations indicate that this assumption is not quite correct.

Denote the above measure of mean fitness by $\bar{w}$. If $f_i$ is the frequency of the class carrying $i$ more mutations than the least-loaded class, we have

$$\bar{w} = f_0 + f_1(1-s) + f_2(1-s)^2 + \ldots \quad \text{(A 1a)}$$

Let the deterministic equilibrium value for the frequency of the $i$th mutational class be $\hat{f}_i$. Under the above assumptions, the $\hat{f}_i$ are given by a Poisson distribution with mean $u/s$; in particular, the frequency of the least-loaded class is $\hat{f}_0 = \exp -u/s$. $\bar{w}$ can thus be written as

$$\begin{aligned}\bar{w} &\approx f_0 - \hat{f}_0 + \hat{f}_0 + \hat{f}_1(1-s) + \hat{f}_2(1-s)^2 + \ldots \\ &= (f_0 - \hat{f}_0) + \mathrm{e}^{-u}.\end{aligned} \quad \text{(A 1b)}$$

The deterministic change in $f_0$ due to mutation and selection is given by

$$\begin{aligned}\Delta f_0 &= \frac{f_0(\mathrm{e}^{-u} - \bar{w})}{\bar{w}} \\ &\approx -\frac{f_0(f_0 - \hat{f}_0)}{\{(f_0 - \hat{f}_0) + \mathrm{e}^{-u}\}}.\end{aligned} \quad \text{(A 2)}$$

The variance of $f_0$ due to sampling over one generation is

$$V_{\delta f_0} = \frac{f_0(1-f_0)}{N_\mathrm{e}}, \quad \text{(A 3)}$$

where $N_\mathrm{e}$ is the effective population size.

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If we assume that the distribution equilibrates rapidly after the loss of the previous least-loaded class, the time to loss of the current least-loaded class can be determined by the standard formulae for a one-dimensional diffusion process, with initial state $\hat{f_0}$. If both $f_0$ and $\hat{f_0}$ are small, the drift and diffusion coefficients (Ewens, 1979, p. 116) are approximately as follows (substituting $x$ for $f_0$ and $x_0$ for $\hat{f_0}$):

$$a(x) \approx -x(x-x_0)e^u, \tag{A 4a}$$

$$b(x) \approx \frac{x}{N_e}. \tag{A 4b}$$

Since $x = 0$ is an absorbing boundary but $x = 1$ is not, eqns (4.39) and (4.40) of Ewens (1979, p. 123) are appropriate for determining the expected time spent in an interval $x$ to $x+dx$:

$$t(x, x_0) = 2\{b(x)\,\Psi(x)\}^{-1} \int_0^x \Psi(y)\,dy \quad 0 \leqslant x < x_0, \tag{A 5a}$$

$$t(x, x_0) = 2\{b(x)\,\Psi(x)\}^{-1} \int_0^{x_0} \Psi(y)\,dy \quad x_0 \leqslant x \leqslant 1, \tag{A 5b}$$

where

$$\Psi(y) = \exp -2 \int_0^y \frac{a(z)}{b(z)} dz. \tag{A 5c}$$

In this case, (4) imply that

$$\Psi(x) \approx \exp 2N_e e^u x\left(\frac{x}{2} - x_0\right). \tag{A 6}$$

This can be substituted into (A 5a) and (A 5b). If these are then integrated over their ranges and summed, we obtain the expected time to loss of the least-loaded class. This double integral expression can be evaluated numerically, given values of the parameters. This forms the basis for the results denoted by C in Table 1.

## References


Albornoz, J. & Domínguez, A. (1994). Inversion polymorphism and accumulation of lethals in selected lines of *Drosophila melanogaster*. *Heredity* **73**, 92–97.

Baker, B. S., Gorman, M. & Marín, I. (1994). Dosage compensation in *Drosophila*. *Annual Review of Genetics* **28**, 491–522.

Birky, C. W. & Walsh, J. B. (1988). Effects of linkage on rates of molecular evolution. *Proceedings of the National Academy of Sciences of the USA* **85**, 6414–6418.

Butcher, D. (1995). Muller's ratchet, epistasis and mutation effects. *Genetics* **141**, 431–437.

Charlesworth, B. (1978). Model for evolution of Y chromosomes and dosage compensation. *Proceedings of the National Academy of Sciences of the USA* **75**, 5618–5622.

Charlesworth, B. (1994). The effect of background selection against deleterious alleles on weakly selected, linked variants. *Genetical Research* **63**, 213–228.

Charlesworth, B. (1996). The evolution of chromosomal sex determination and dosage compensation. *Current Biology* **6**, 149–162.

Charlesworth, B. & Charlesworth, D. (1985). Genetic variation in recombination in *Drosophila*. I. Responses to selection and preliminary genetic analysis. *Heredity* **54**, 71–84.

Charlesworth, B. & Hughes, K. A. (1997). The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. B. Krimbas), Cambridge: Cambridge University Press (in press).

Charlesworth, D., Morgan, M. T. & Charlesworth, B. (1993). Mutation accumulation in finite outbreeding and inbreeding populations. *Genetical Research* **61**, 39–56.

Crow, J. F. & Simmons, M. J. (1983). The mutation load in *Drosophila*. In *The Genetics and Biology of Drosophila*, Vol. 3c (ed. M. Ashburner, H. L. Carson & J. N. Thompson), pp. 1–35. London: Academic Press.

Ewens, W. J. (1979). *Mathematical Population Genetics*. Berlin: Springer-Verlag.

Felsenstein, J. (1974). The evolutionary advantage of recombination. *Genetics* **78**, 737–756.

Gabriel, W., Lynch, M. & Bürger, R. (1993). Muller's ratchet and mutational meltdowns. *Evolution* **47**, 1744–1757.

Gessler, D. G. (1995). The constraints of finite size in asexual populations and the rate of the ratchet. *Genetical Research* **66**, 241–253.

Haigh, J. (1978). The accumulation of deleterious genes in a population. *Theoretical Population Biology* **14**, 251–267.

Higgs, P. G. & Woodcock, G. (1995). The accumulation of mutations in asexual populations and the structure of genealogical trees in the presence of selection. *Journal of Mathematical Biology* **33**, 677–702.

Hill, W. G. & Robertson, A. (1996). The effect of linkage on limits to artificial selection. *Genetical Research* **8**, 269–294.

Kidwell, M. G. (1972). Genetic change of recombination value in *Drosophila melanogaster*. II. Simulated natural selection. *Genetics* **70**, 433–443.

Kimura, M. (1962). On the probability of fixation of a mutant gene in a population. *Genetics* **47**, 713–719.

Li, W.-H. (1987). Models of nearly neutral mutations with particular implications for non-random usage of synonymous codons. *Journal of Molecular Evolution* **24**, 337–345.

Lynch, M. (1996). Mutation accumulation in transfer RNAs: molecular evidence for Muller's ratchet in mitochondrial genomes. *Molecular Biology and Evolution* **13**, 209–220.

Lynch, M. & Gabriel, W. (1990). Mutation load and the survival of small populations. *Evolution* **44**, 1725–1737.

Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge: Cambridge University Press.

Moran, N. A. (1996). Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. *Proceedings of the National Academy of Sciences of the USA* **93**, 2873–2878.

Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research* **1**, 2–9.

Pamilo, P., Nei, M. & Li, W.-H. (1987). Accumulation of mutations in sexual and asexual populations. *Genetical Research* **49**, 135–146.

Prügel-Bennett, A. (1997). Modelling evolving populations. *Journal of Theoretical Biology* **185**, 81–96.

Stephan, W., Chao, L. & Smale, J. G. (1993). The advance of Muller's ratchet in a haploid asexual population: approximate solutions based on diffusion theory. *Genetical Research* **61**, 225–232.