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# Before crossing over: the advantages of eukaryotic sex in genomes lacking chiasmatic recombination

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**Summary**

Non-recombining populations should suffer from four classic population genetic disadvantages: (1) they cannot reverse Muller's Ratchet, the accumulation of deleterious mutations caused by genetic drift and mutation; (2) whenever the fix a favourable mutation they lose all unlinked favourable variants; (3) they tend to lose favourable mutations that are linked to deleterious mutations; and (4) their genetic loads can be quite high when deleterious mutations have synergistic effects. It is commonly assumed that inter-chromosomal recombination (independent assortment) can counter these phenomena, but this has been studied only for the genetic load case. In contrast, many studies have shown that recombination via crossing over can counter these phenomena. Here we first show that segregation alone can strongly decelerate Muller's Ratchet in diploids, i.e. that recombination is not the only way to do so. We then show that inter-chromosomal recombination can indeed deal with phenomena (1) to (3) above very effectively if the genome consists of a moderate number of chromosomes. Therefore, if the above advantages of genetic recombination played a large role in the initial success of eukaryotic sex, the crucial moment in the origin of sex might have been the evolution of inter-chromosomal recombination, i.e. the evolution of genome segmentation, segregation, and syngamy. Crossing over might have become established as a major recombinational device only later, eliminating the disadvantages of extensively segmented genomes.

## 1. Introduction

Recombination is considered the major advantage of eukaryotic sexual reproduction over asexuality. Recombination can indeed counter three classic population genetical processes that should affect asexual populations. The first process is the accumulation of deleterious mutations expected to occur in such populations (Muller, 1964) and the other two processes are difficulties experienced by such populations in accumulating favourable mutations (Fisher, 1930; Muller, 1932). More recently, the fact that the genetic load can be much higher in asexuals than in recombining organisms has also been stressed (Crow, 1970; Kondrashov, 1982).

'Muller's Ratchet' is the name given to the accumulation of deleterious mutations in asexual populations that occurs when, despite purifying selection, genetic drift and/or mutational pressure result in the disappearance of the least mutated genotype in a population. This is described as a 'ratchet' process because only back-mutation can reverse it. The Ratchet might be a major factor disfavouring asexuals relative to sexually reproducing organisms (Crow, 1988). Recombining populations should not suffer from the Ratchet because recombination can produce mutation-free genomes from the non-mutated genomic regions of different individuals. Studies of the Ratchet to date have focused on how its pace is affected by recombination rates, population size and strength and mode of selection, as well as mutation and outcrossing rats (Felsenstein, 1974; Haigh, 1978; Maynard Smith, 1978; Pamilo *et al.*, 1987; Bell, 1988; Charlesworth *et al.*, 1993*b*; Gabriel & Bürger, 1993; Lynch *et al.*, 1993; Stephan *et al.*, 1993; Kondrashov, 1994*a*).

In asexual populations any beneficial mutation that arises in a genome which does not carry a favourable mutation that is about to become fixed must necessarily be lost when the fixation takes place (Fisher, 1930; Muller, 1932; Barton, 1995). With recom-

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bination, however, multiple favourable mutations that arise in distinct individuals can go to fixation without excluding one another. Here we will call this interaction among favourable mutations 'Fisher–Muller interference'.

The fixation probability of a favourable mutation can be reduced by background selection acting against mutations at linked and, to a much lesser extent, at unlinked loci (Fisher, 1930; Hill & Robertson, 1966; Manning & Thompson, 1984; Birky & Walsh 1988; Charlesworth, 1994; Peck, 1994). In asexuals all loci are linked and thus selection events at any loci lower fixation probabilities at all other loci. Given the pervasiveness of deleterious mutations, favourable fixation probability should be reduced dramatically in asexual organisms but much less in recombining organisms (Barton, 1995). Here we will refer to this reduction in favourable fixation probability as 'background selection' (Charlesworth, 1994).

The above phenomena assume non-equilibrium adaptive situations and are of a stochastic nature since they occur only in finite asexual populations. In contrast, the argument from genetic load, which is at the centre of the 'mutational deterministic hypothesis' (Crow, 1970, 1983; Kondrashov, 1982, 1993), addresses an equilibrium phenomenon that also occurs in infinite populations. Theoretical studies (Kondrashov, 1982; Charlesworth, 1990) predict substantially lower loads in recombining that in non-recombining populations whenever the genomic deleterious mutation rate is large and log fitness declines faster than linearly with the number of deleterious mutations (synergistic selection). Recombination produces more extreme genotypes than those produced by mutation alone in asexual populations, and under synergistic selection this translates into a lower equilibrium number of deleterious mutations per genome and thus into a higher average fitness.

Scholars of the evolution of sex have often studied how recombination via crossing over can counter the above phenomena, but they have devoted less attention to advantages that can be conferred by other features of eukaryotic sex. The segregation of chromosomes during meiosis, for instance, is a striking feature of eukaryotic sexuality that has received relatively little attention, perhaps because of Muller's (1932) assertion that segregation is of no evolutionary value. Muller notwithstanding, several authors have shown that segregation alone can result in fitness advantages when deleterious mutations have synergistic effects (Kimura & Maruyama, 1966; Dickson & Manning, 1984; Charlesworth, 1990; Charlesworth *et al*., 1993*b*). Furthermore, Lloyd (1980), Hamilton (1980), and Weinshall (1986), as well as Barton & Post (1986), showed that segregation is advantageous in the context of fluctuating environments. Finally, the ability of segregation to allow a mutation to be fixed in double dosage at a given locus, which otherwise requires a second mutation event, has been shown to lead to advantages for sexual reproduction when favourable mutations are not dominant (Kirkpatrick & Jenkins, 1989; Wiener *et al*., 1992). However, the effect of segregation on the speed of Muller's Ratchet and the fitness consequences thereof have not been studied.

In genomes subdivided into multiple linkage groups (pairs of homologues), the independent assortment of chromosomes into gametes during meiosis, followed by the reunion of gametes (syngamy), results in inter-chromosomal recombination. In the context of the mutational deterministic hypothesis, Kondrashov (1984) and Charlesworth (1990) showed that the advantage of crossing over is greatly reduced in outcrossing organisms with moderate numbers of linkage groups. Inter-chromosomal recombination, however, has yet to receive explicit study as a substitute for chiasmatic recombination (crossing over) in decelerating the Ratchet, neutralizing the reduction in favourable fixation probability due to background selection, and avoiding Fisher–Muller interference.

Unlike chiasmatic recombination, if genomes were segmented, inter-chromosomal recombination probably began operating as soon as segregation and syngamy were evolved. It might thus have become immediately active in neutralizing the Ratchet and the reduction in favourable fixation probability due to background selection, as well as in reducing Fisher–Muller interference. Thus it is relevant to study how effectively inter-chromosomal recombination can counter these processes. Moreover, in certain protozoans, meiosis takes place without a previous replication of homologues, which makes chiasmatic recombination impossible. During this so-called one-step meiosis, chiasmatic recombination has indeed never been observed (Cleveland, 1947; Margulis & Sagan, 1986; see reviews by Raikov, 1982, 1995), but note that recombination by crossing over requires only two double-stranded DNA molecules and not four as does chiasmatic recombination (A. S. Kondrashov, personal communication). In these organisms, therefore, the advantages of sexual reproduction might be due to diploidy and/or to the ability to segregate homologues. The extant one-step meiosis might be a derived condition relative to standard eukaryotic meiosis, but this does not exclude the possibility that a similar meiotic system was ancestral to the standard meiosis (see Kondrashov, 1994*b*). Ascertaining whether inter-chromosomal recombination is advantageous will also shed light on the evolutionary maintenance of one-step meiosis.

In this paper we explore the ability of segregation and inter-chromosomal recombination to deliver the above classic advantages of sexual reproduction in the absence of chiasmatic recombination. We first present simulation results which demonstrate that segregation alone can strongly decelerate Muller's Ratchet over a wide range of parameters and we explore the fitness consequences of these decelerations. For those situa-

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tions in which segregation does not decelerate the Ratchet dramatically, we show with further simulations that a moderate number of chromosomes suffices in most cases for inter-chromosomal recombination to result in marked decelerations, provided that the population outcrosses. We then show that under such conditions, inter-chromosomal recombination can also eliminate most Fisher–Muller interference and strongly neutralize the reduction in favourable fixation probability due to background selection.

## 2. The Ratchet in segregating genomes with a single linkage group

### (i) *General considerations*

The introduction of segregation and syngamy into a ratcheting asexual diploid might decelerate the ratcheting process even if the diploid genome consists of a single pair of non-recombining homologous chromosomes. In fact, under segregation, the deleterious mutation pressure driving the ratcheting process is half of that driving the ratchet in a comparable asexual diploid. This is because, in an asexual population, the Ratchet clicks whenever the least mutated diploid genomes acquire at least one more deleterious mutation, while in a segregating population such a click requires that every single least mutated homologue in the population acquire at least one more mutation. The ratcheting unit under segregation is therefore not the whole diploid genome but rather the single homologue. A ratcheting event, however, adds two more mutations to the least mutated diploid genotype that can be produced in a segregating population, but only one if it occurs in an asexual population. Therefore, when the asexual period (the interval between clicks) is half that in the segregating population, the per-individual rate of accumulation of deleterious mutations is the same in both populations.

Another consequence of segregation is that it increases the effective population size $N_e$ of an outcrossing diploid sexual population relative to that of an asexual population with an equal number of breeding individuals. This can be easily seen in the framework provided by the Wright–Fisher model. Under this model's version of an asexual population, each new generation is produced by sampling with replacement $N$ new individuals from the $N$ reproducing asexual individuals. In a comparable segregating population that also outcrosses, the sampling is over $2N$ haploid genomes instead of over $N$ individuals, because the Wright–Fisher model assumes that gametes unite randomly. Thus the $N_e$ of an asexual population is half that of an equally numerous outcrossing segregating population. Since genetic drift is stronger in small populations, the doubling of $N_e$ due to segregation could also cause a deceleration of the Ratchet.

### (ii) *Simulation approach*

To measure the speed of the Ratchet in asexual diploids and in achiasmatic segregating populations with a single linkage group, we have used Monte Carlo simulations. The Ratchet has been studied primarily using simulations (Felsenstein, 1974; Haigh, 1978; Bell, 1988; Pamilo *et al*., 1987; Charlesworth *et al*., 1993*b*; Gabriel & Bürger, 1993; Lynch *et al*., 1993; Stephan *et al*., 1993), and only recently have approximate analytical formulae been presented by Pamilo *et al*. (1987) and Stephan *et al*. (1993). While the approximation of Pamilo *et al*. was accurate only when $N_e s \leqslant 1$, where $s$ is the deleterious effect of each mutation, Stephan *et al*.'s diffusion equation approximations apply over a larger range of $N_e s$ values. Preliminary simulations, however, showed that these approximations underestimate periods above a thousand generations. For this reason we will rely exclusively on simulation results.

To simulate the Ratchet in asexual populations, we followed Haigh's (1978) approach. We assumed a Wright–Fisher life cycle in which selection occurs before genetic drift. The number of deleterious mutations produced each generation in each individual was Poisson-distributed with mean and variance equal to the genomic deleterious mutation rate $U$, and deleterious mutations were assumed to have multiplicative cumulative fitness effects (i.e. $i$ mutations result in a fitness of $(1-s)^i$). Under this fitness scheme, one does not need to keep track of the genotypes at each locus, since the value of $(1-s)^i$ does not depend on the allelism of mutations. One can thus simply follow the numbers of individuals that carry given numbers of mutations, which speeds up runs and lowers memory requirements. We were able, therefore, to explore a wide parameter space. This approach does not allow one to study the accumulation of deleterious mutations with recessive effects, nor to keep track of fixations at single loci (Charlesworth *et al*., 1993*b*; Charlesworth & Charlesworth, 1997), but we will consider the implications of these possibilities whenever necessary. At each new generation, $N$ new individuals were produced according to the vector of multinomial probabilities defined by eqn (1) in Haigh (1978), which takes into consideration selection (i.e. the distribution of mutations in the parental population) and mutation. The most mutated genome that could arise each generation carried $U/s+3\sqrt{U/s}$ mutations, and all the residual probability of producing more mutated genomes was assigned to this most mutated class. $U/s$ is the average number and the variance of the number of mutations per individual in a large population under mutation selection balance (Haldane, 1937; Kimura & Maruyama, 1966). This variance applies approximately also to a ratcheting population (Haigh, 1978). Therefore the loss of the least mutated class through mutation was simulated exactly, but the production of genotypes more mutated

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than those in the boundary class just mentioned was not. This slight imprecision should not bias the speed with which the least mutated class is lost in the simulations, i.e. the speed of the Ratchet. The multinomial sampling procedure used to produce each new generation according to the above vector of probabilities was an implementation of Devroye's algorithm to speed up the production of multinomial samples by drawing a series of binomial samples (chapters X.4 and XI.1 in Devroye, 1986). The procedure was written and kindly provided by J. Gillespie. Our simulations produced results that were consistent with neutral predictions when $N_e s$ values were close to zero, as well as with previously published results about the Ratchet (Haigh, 1978; Pamilo, *et al*., 1987; Stephan *et al*., 1993), and with predictions from diffusion approximations (Malécot, 1952; Kimura, 1957) when such were applicable.

To estimate the period of the Ratchet in random-mating, segregating populations of $N$ reproducing diploid individuals, where each individual produces deleterious mutations with rate $U$ per generation and carries two achiasmatic homologous chromosomes, we simulated $2N$-sized Wright–Fisher populations of haploid asexual individuals each having $U/2$ as the deleterious mutation rate per generation. Such a haploid population has exactly the same population genetical behaviour as a random-mating segregating diploid population with a single linkage group, provided that mutations have multiplicative cumulative fitness effects, as assumed here, and that genetic drift occurs only after selection and before reproduction (Ewens, 1979). This last condition is the standard assumption made in Wright–Fisher models with selection (Ewens, 1979). Adding stochastic fluctuations to the genotypic proportions among zygotes before selection can increase genetic drift and might speed up the Ratchet, but here we do not attempt to study this departure from the Wright–Fisher life cycle. The approach to simulating the Ratchet process in outcrossing genomes with multiple linkage groups is presented below. The assumption of multiplicative finesses did not allow us to study dominance nor epistatic effects. For studies addressing these effects, see Charlesworth *et al*. (1993*b*) and Kondrashov (1994*a*).

### (iii) *Parameter space explored*

In evaluating the effect of segregation on the Ratchet, our central question is 'What is the advantage of a segregating population over a ratcheting but otherwise identical asexual diploid population?' To answer this question, we measured the period of the Ratchet in a variety of achiasmatic segregating populations that were otherwise identical to asexual reference populations known to ratchet with a given period. Therefore, in all the cases studied, segregation had a chance to decelerate the Ratchet and provide a fitness advantage over asexuality. We then calculated the fitness advantage of segregation and syngamy on the basis of the ratcheting periods in either population and compared this advantage with that obtained by stopping the Ratchet completely.

The population sizes examined encompassed every order of magnitude between 100 and $10^8$ individuals, and the $s$ values were 0·001, 0·005, 0·01, 0·05 and 0·1. For each combination of such $N$ and $s$ values, we determined the values of the genomic deleterious mutation rate $U$ that would let a reference asexual population ratchet with period $T_{\text{asex}}$ of 1, 10 or 100 generations (precise to $\pm 1\%$ of the desired period over 10000 ratcheting events). We then measured $T_{\text{segr}}$, the period of the Ratchet in an otherwise identical diploid achiasmatic segregating population, again over 10000 ratcheting events. Since we present the parameter space according to $T_{\text{asex}}$, $N$, and $s$, in most tables $U$ changes whenever $N$ and/or $s$ change and not just when $T_{\text{asex}}$ changes, the only exception being when we explore how the size of the segregating population affects its ratcheting speed and fitness advantage.

### (iv) *Ratcheting periods with segregation when* $N_{\text{asex}} = N_{\text{segr}}$

Table 1 shows values of $T_{\text{segr}}/T_{\text{asex}}$, the period of the Ratchet in a segregating population divided by that in the asexual reference population. The table shows that segregation lengthens the period of the Ratchet more markedly and down to smaller $s$ values when $T_{\text{asex}} \geqslant 10$ than when $T_{\text{asex}} = 1$. The lengthening of the period under segregation is more conspicuous when the population size is larger for a given $T_{\text{asex}}$. When $Ns \leqslant 1$, segregation does not decelerate the Ratchet much, since under these conditions the Ratchet is a nearly neutral process driven by mutational pressure. When $T_{\text{asex}} = 1$ and $s < 0{\cdot}01$ (i.e. regardless of $Ns$), segregation has little effect on the period of the Ratchet. In such situations, segregating populations have ratcheting periods roughly twice as long as those of corresponding reference asexuals, and therefore all populations have similar rates of accumulation of mutations (remember that a Ratchet clicks means two mutations more under segregation, but only one more under asexuality). This halving of the ratcheting speed in reaction to a halving of $U$ indicates that mutational pressure is a good predictor of the ratcheting period in these cases. While this dependence on $U$ is expected in neutral and nearly neutral situations (see e.g. $s = 0{\cdot}001$ and $N = 1000$), which are governed by mutation rates, it is surprising to find it in cases of fast ratcheting against strong selection, e.g. when $N = 10^5$, $s = 0{\cdot}01$ and $T_{\text{asex}} = 1$.

The quantity $n_0 = N\mathrm{e}^{-U/s}$ (Kimura & Maruyama, 1966) is the number of individuals with the least

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Table 1. *The Ratchet under segregation*

| | | $T_{asex}$: 1 | | | | | 10 | | | | | 100 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $N$ | $s$: | 0·1 | 0·05 | 0·01 | 0·005 | 0·001 | 0·1 | 0·05 | 0·01 | 0·005 | 0·001 | 0·1 | 0·05 | 0·01 | 0·005 | 0·001 |
| $10^2$ | | 3·1 | 2·7 | 2·3 | 2·3 | 2·1 | 8·1 | 4·4 | 2·7 | 2·4 | 2·1 | 580 | 30·3 | 3·9 | 2·9 | 2·2 |
| | $U$ | (*2·350*) | (*1·900*) | (*1·370*) | (*1·270*) | (*1·105*) | (*0·500*) | (*0·330*) | (*0·170*) | (*0·143*) | (*0·111*) | (*0·173*) | (*0·082*) | (*0·023*) | (*0·016*) | (*0·011*) |
| $10^3$ | | 3·5 | 2·9 | 2·4 | 2·3 | 2·1 | 22·4 | 6·7 | 3·1 | 2·7 | 2·3 | $> 10^4$ | $10^4$ | 7·4 | 4·4 | 2·7 |
| | $U$ | (*2·900*) | (*2·320*) | (*1·580*) | (*1·410*) | (*1·200*) | (*0·800*) | (*0·513*) | (*0·240*) | (*0·195*) | (*0·135*) | (*0·386*) | (*0·192*) | (*0·052*) | (*0·034*) | (*0·017*) |
| $10^4$ | | 3·8 | 3·1 | 2·5 | 2·4 | 2·2 | 171 | 10·4 | 3·5 | 3·0 | 2·4 | $> 10^4$ | $> 10^4$ | 21·4 | 6·5 | 3·0 |
| | $U$ | (*3·450*) | (*2·680*) | (*1·750*) | (*1·535*) | (*1·260*) | (*1·090*) | (*0·688*) | (*0·300*) | (*0·237*) | (*0·160*) | (*0·610*) | (*0·305*) | (*0·080*) | (*0·052*) | (*0·025*) |
| $10^5$ | | 4·3 | 3·3 | 2·5 | 2·4 | 2·2 | $10^4$ | 19·4 | 3·8 | 3·1 | 2·5 | $> 10^4$ | $> 10^4$ | 128 | 9·8 | 3·4 |
| | $U$ | (*3·940*) | (*3·000*) | (*1·900*) | (*1·660*) | (*1·330*) | (*1·370*) | (*0·845*) | (*0·360*) | (*0·272*) | (*0·175*) | (*0·841*) | (*0·425*) | (*0·110*) | (*0·070*) | (*0·031*) |
| $10^6$ | | 4·7 | 3·5 | 2·6 | 2·4 | 2·2 | $10^4$ | 49·5 | 4·2 | 3·3 | 2·5 | $> 10^4$ | $> 10^4$ | $10^4$ | 18·7 | 3·8 |
| | $U$ | (*4·450*) | (*3·330*) | (*2·030*) | (*1·755*) | (*1·385*) | (*1·645*) | (*1·000*) | (*0·410*) | (*0·305*) | (*0·188*) | (*1·069*) | (*0·547*) | (*0·141*) | (*0·085*) | (*0·037*) |
| $10^7$ | | 5·1 | 3·7 | 2·7 | 2·5 | 2·2 | $> 10^4$ | 359 | 4·6 | 3·6 | 2·6 | $> 10^4$ | $> 10^4$ | $> 10^4$ | 42·9 | 4·2 |
| | $U$ | (*4·950*) | (*3·625*) | (*2·150*) | (*1·850*) | (*1·440*) | (*1·900*) | (*1·145*) | (*0·460*) | (*0·335*) | (*0·205*) | (*1·298*) | (*0·664*) | (*0·169*) | (*0·101*) | (*0·041*) |
| $10^8$ | | 5·5 | 3·9 | 2·7 | 2·5 | 2·3 | $> 10^4$ | $10^4$ | 5·1 | 3·7 | 2·7 | $> 10^4$ | $> 10^4$ | $> 10^4$ | $> 10^4$ | 4·6 |
| | $U$ | (*5·440*) | (*3·850*) | (*2·250*) | (*1·190*) | (*1·475*) | (*2·170*) | (*1·300*) | (*0·500*) | (*0·370*) | (*0·220*) | (*0·540*) | (*0·784*) | (*0·193*) | (*0·116*) | (*0·046*) |

Tabulation of $T_{segr}/T_{asex}$, the period of the Ratchet in a segregating population, over that in a comparable asexual population. Periods are based on 10000 clicks of the Ratchet. The variance of each period was on the order of the square of the period. Ratios above $10^4$ are rounded to the order of magnitude. The linear relationship between $\log N$ and $U$ for a given $T_{asex}$ is also valid for $T_{asex} = 3$, 6, 30, 60, 300 and 600 generations.

mutated genotype in a large equilibrium asexual population (the so-called zero class) and has been shown to be a composite predictor of the speed of the Ratchet over certain parameter values (Haigh, 1978; Maynard Smith, 1978; Stephan *et al.*, 1993). An examination of $n_0$ values for all asexual populations in Table 1 indicates that, for a given $T_{\text{asex}}$, these values are indeed very similar when both $T_{\text{asex}} > 10$ and $s \geqslant 0{\cdot}05$. For instance, when $s = 0{\cdot}1$ and $T_{\text{asex}} = 100$, $n_0$ is 18 individuals and 20 individuals for $N = 100$ and $10^8$ individuals, respectively. These numbers become 20 and 16 individuals, respectively, for $s = 0{\cdot}05$. However, for $s = 0{\cdot}01$, $0{\cdot}005$, and $0{\cdot}001$ and the same $T_{\text{asex}}$, the corresponding values of $n_0$ become 10 and 0·4, 4 and 0·0093, and 0·0016 and $10^{-12}$ individuals, respectively. Moreover, when $T_{\text{asex}} \leqslant 10$ and $0{\cdot}001 \leqslant s \leqslant 0{\cdot}1$, $n_0$ values are always below one individual and extremely different; for example they differ by 2 and 50 orders of magnitude when $N = 100$ and $N = 10^8$ individuals, respectively. The size of $n_0$ necessary to get the same period can thus vary strongly with $N$, $s$, and $U$ and we have not attempted to discuss the effect of segregation on the Ratchet in terms of changes in $n_0$.

### (v) *Halving U versus doubling* $N_e$

Above we discussed the overall effect of segregation on the ratcheting period. Earlier we mentioned that the two consequences of segregation that can decelerate the Ratchet are the halving of the deleterious mutation rate per ratcheting unit and a doubling of $N_e$ (with outcrossing). We have attempted to separate the contributions of these two consequences of segregation to the decelerations shown in Table 1. To this end, we measured the period of the Ratchet in $N$-sized haploid populations with $U/2$ as mutation rate, (i.e. under 'segregation with no doubling of $N_e$') and in $2N$-sized haploid populations with $U$ as mutation rate (i.e. under 'segregation with no halving of $U$'). Simulations with $N$ and $U/2$ as parameters resulted in almost the same periods as with standard segregation (data not shown). Moreover, simulations with $2N$ and $U$ as parameters showed very slight deceleration, except when $s \geqslant 0{\cdot}05$ and $T_{\text{asex}} = 100$, where the period was at most 4 times longer (data not shown). These results indicate that the doubling of $N_e$ is much less effective in decelerating the Ratchet than the halving of the deleterious mutation rate per ratcheting unit.

The above results on the minor effects of doubling $N_e$ indicate that random syngamy is not necessary for attaining most of the advantages of segregation related to decelerating the Ratchet. In selfing and obligate ploidy-cycling lineages, the deleterious mutation rate relevant for the Ratchet is $U/2$, since in such lineages mutations are lost almost immediately half of the time (Charlesworth, 1990; Charlesworth *et al.*, 1993*b*; Kondrashov, 1994*c*). Such lineages, therefore, should benefit almost fully from these advantages of segregation even though they do not outcross. This is an additional advantage on top of those that result from exposing to selection deleterious mutations in double dosage when deleterious effects are not dominant (Charlesworth *et al.*, 1993*b*). Thus, the asexual ploidy cycle might be favourable not only because it lowers the genetic load (Kondrashov, 1994*c*) but also because its decelerates the Ratchet. These observations also indicate that selfing is a better 'asexual' reproductive strategy than vegetative asexuality.

### (vi) *The Ratchet and segregation: fitness consequences*

Here we examine the fitness consequences of the differences in ratcheting period between segregating and asexual populations presented in Table 1. We have chosen to describe the fitness advantage of segregation by calculating the time $t_{50\%}$ in generations for the fitness of the least mutated individuals in the reference asexual population to become 50% lower than the fitness of the least mutated individuals in a comparable segregating population. We have chosen this percentage value because 50% is the expected fitness of sexuals relative to anisogamic asexuals (Maynard Smith, 1978). It should be clear, therefore, that these comparisons of fitness only make sense in a group selection scenario and tell us nothing about whether, for example, a segregating mutant can invade a ratcheting asexual population. Often we will express these times as a multiple of that required by a non-ratcheting population to reach the twofold fitness advantage. This should serve as an indication of the efficiency of segregation relative to not ratcheting at all in leading to fitness advantages over asexuality.

The values of $t_{50\%}$ are measured in generations and were calculated from the following equation:

$$2(1-s)^{t_{50\%}/T_{\text{asex}}} = (1-s)^{2t_{50\%}/T_{\text{segr}}}, \tag{1}$$

where the formula on the left of the equal sign is twice the asexual fitness after $t_{50\%}$ generations of ratcheting with period $T_{\text{asex}}$ and the formula on the right is the fitness of a segregating population ratcheting with period $T_{\text{segr}}$ for the same length of time. Solving (1) with respect to $t_{50\%}$ gives

$$t_{50\%} = \frac{T_{\text{asex}}\, T_{\text{segr}} \log(2)}{(2T_{\text{asex}} - T_{\text{segr}}) \log(1-s)}. \tag{2}$$

In Table 2 we present $t_{50\%}$ values for all the pairwise comparisons in Table 1. The table shows that $t_{50\%}$ values are shortest when $s$ is large and $T_{\text{asex}}$ is short. This is surprising at first sight, given that in such situations segregation is less effective at decelerating the Ratchet. The table also shows that $t_{50\%}$ values are quite insensitive to $N$ when $Ns \geqslant 1$. Indeed, the effect of changing $N$ from $10^2$ to $10^8$ in the pairwise

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Table 2. *The fitness advantage of segregation*

| | | $N$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $s$ | $T_{asex}$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ | No Rachet |
| 0·1 | 1 | 20 | 16 | 15 | 13 | 12 | 11 | 11 | 7 |
| 0·05 | 1 | 54 | 45 | 40 | 35 | 32 | 30 | 28 | 14 |
| 0·1 | 10 | 89 | 73 | 67 | 66 | 66 | 66 | 66 | 66 |
| 0·05 | 10 | 251 | 195 | 168 | 151 | 141 | 136 | 135 | 135 |
| 0·01 | 1 | 258 | 220 | 196 | 174 | 159 | 150 | 141 | 69 |
| 0·1 | 100 | 660 | 658 | 658 | 658 | 658 | 658 | 658 | 658 |
| 0·01 | 10 | 1260 | 984 | 855 | 769 | 719 | 694 | 690 | 690 |
| 0·005 | 1 | 1208 | 1107 | 929 | 876 | 795 | 730 | 695 | 138 |
| 0·05 | 100 | 1450 | 1352 | 1352 | 1352 | 1352 | 1352 | 1352 | 1351 |
| 0·005 | 10 | 8272 | 5635 | 4307 | 3841 | 3490 | 3118 | 3006 | 1383 |
| 0·001 | 1 | 16448 | 10444 | 9281 | 6918 | 6979 | 6494 | 5892 | 693 |
| 0·01 | 100 | 7387 | 6898 | 6898 | 6898 | 6898 | 6898 | 6898 | 6897 |
| 0·005 | 100 | 43814 | 25576 | 19943 | 17404 | 15485 | 14507 | 13831 | 13828 |
| 0·001 | 10 | $1{\cdot}1\times10^5$ | $5{\cdot}2\times10^4$ | $4{\cdot}1\times10^4$ | $3{\cdot}7\times10^4$ | $3{\cdot}3\times10^5$ | $3{\cdot}0\times10^4$ | $2{\cdot}8\times10^4$ | 6928 |
| 0·001 | 100 | $7{\cdot}8\times10^5$ | $2{\cdot}6\times10^5$ | $2{\cdot}0\times10^5$ | $1{\cdot}7\times10^5$ | $1{\cdot}5\times10^5$ | $1{\cdot}3\times10^5$ | $1{\cdot}2\times10^5$ | 69280 |

The time $t_{50\%}$ in generations required for the asexual fitness to become 50% of that of a comparable segregating population for the parameter combinations shown in Table 1. The times on the far right are for comparisons between asexual and non-ratcheting populations. Rows are presented from top to bottom according to increasing $t_{50\%}$ values when $N$ is $10^8$ individuals (boxed column).

comparisons, while keeping the reference $T_{asex}$ constant, was at most a factor of 2 (for the case $s = 0{\cdot}001$, $T_{asex} = 100$). It is also noteworthy that for any $Ns$ where $s \geqslant 0{\cdot}005$, populations that do not ratchet at all often have $t_{50\%}$ values very similar to those of comparable populations that only segregate. In the worst case ($T_{asex} = 1$, $N = 100$, $s = 0{\cdot}005$), a non-ratcheting population is 9 times faster than a segregating population in gaining fitness over the reference asexual. On the basis of this observation, we conclude that the differences in ratcheting period between segregating and non-ratcheting populations are unlikely to be of any major consequence if what matters is to gain fitness over a ratcheting asexual population. The conclusion is strengthened by the fact that whenever fitness differences between asexual and segregating populations arise fastest in plain generations, $t_{50\%}$ values are especially close to those of non-ratcheting populations. Therefore, knowing whether or not segregation decelerates the Ratchet dramatically in a given situation is not enough to predict how fast the deceleration can translate into fitness advantages over a ratcheting asexual, except when $s$ and $T_{asex}$ are comparable across cases. Fitness differences, for instance, arise most quickly when $T_{asex}$ is short, i.e. when the Ratchet deceleration due to segregation is least conspicuous. The reason for this apparent paradox is that the ratcheting period of the asexual and the value of $s$ are the main factors that determine the speed with which the fitness advantages arise.

To summarize: (1) the fitness advantages of introducing segregation and syngamy into asexual diploid populations of various sizes arise with similar speed as long as $N$, $s$, and $T_{asex}$ are similar; (2) for a given $T_{asex}$, a segregating population increases its fitness relative to that of a comparable ratcheting asexual population more quickly when the mutations being accumulated are more strongly deleterious; (3) for a given $s$, segregation results in fitness advantages over the reference asexual population more quickly when the asexual population ratchets with a short period, despite the fact that in such cases segregation shows weaker ratchet-decelerating effects; and (4) decelerating the Ratchet beyond what segregation alone can do does not necessarily translate into a substantially improved ability to gain fitness over a ratcheting asexual population.

### (vii) *The pace of the Ratchet when $N_{segr}$ is smaller than $N_{asex}$*

Above we examined the deceleration of the Ratchet due to segregation and the resultant fitness consequences in cases where $N_{segr} = N_{asex}$. Here we examine the question of how small a segregating population can become and still retain an edge over a mutationally comparable asexual population. We measured with simulations the average period of the Ratchet in segregating populations of size $N_{segr}$ smaller than $N_{asex}$, on the basis of 10000 clicks of the Ratchet. $N_{asex}$ values were $10^4$ and $10^8$ individuals, $N_{segr}$ values ranged from $10^2$, $10^3$, to $N_{asex}$ individuals, $T_{asex}$ values were 1, 10, and 100 generations, and $s$ values were 0·1, 0·01, and 0·001.

Results for the case $N_{asex} > N_{segr}$ are shown in Table 3 in the form of $t_{50\%}$ values. The location of the boundary where segregation loses its edge is remarkably similar across columns. This means that for a given $T_{asex}$, there seems to be little influence of $s$ on the range of values of $N_{segr}$ over which segregation

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Table 3. *The fitness advantage of segregation when $N_{segr}$ is lower than $N_{asex}$*

| | $T_{asex}$: 1 | | | 10 | | | 100 | | |
|---|---|---|---|---|---|---|---|---|---|
| $N_{segr}$ | *s*: 0·1 | 0·01 | 0·001 | 0·1 | 0·01 | 0·001 | 0·1 | 0·01 | 0·001 |
| (*a*) $N_{asex} = 10^8$ | | | | | | | | | |
| $10^8$ | 11 | 272 | 5892 | 66 | 1134 | $3 \times 10^4$ | 658 | 6898 | $1 \times 10^5$ |
| $10^7$ | 14 | 331 | 8700 | 66 | 1693 | $5 \times 10^4$ | 658 | 7004 | $2 \times 10^5$ |
| $10^6$ | 19 | 422 | $2 \times 10^4$ | 76 | 3044 | $9 \times 10^4$ | 658 | $1 \times 10^4$ | $4 \times 10^5$ |
| $10^5$ | 107 | $3 \times 10^4$ | $-7 \times 10^4$ | 194 | $-2 \times 10^4$ | $-1 \times 10^5$ | 658 | $-9 \times 10^4$ | $-5 \times 10^5$ |
| $10^4$ | –33 | –555 | –7188 | –102 | –1654 | $-4 \times 10^5$ | –1803 | –4722 | $-1 \times 10^5$ |
| $10^3$ | –10 | –348 | –3844 | –29 | –684 | $-2 \times 10^5$ | –88 | –1756 | $-5 \times 10^4$ |
| $10^2$ | –5 | –157 | –2178 | –13 | –289 | –8386 | –30 | –810 | $-3 \times 10^4$ |
| No Ratchet | 7 | 69 | 693 | 66 | 690 | 6928 | 658 | 6897 | $7 \times 10^4$ |
| (*b*) $N_{asex} = 10^4$ | | | | | | | | | |
| $10^4$ | 15 | 374 | 9281 | 67 | 1619 | $4 \times 10^4$ | 658 | 7611 | $2 \times 10^5$ |
| $10^3$ | 31 | 3050 | $2 \times 10^4$ | 129 | 7036 | $-2 \times 10^5$ | 658 | $6 \times 10^4$ | $-5 \times 10^5$ |
| $10^2$ | –34 | –429 | –5493 | –81 | –1354 | $-2 \times 10^4$ | $-3 \times 10^4$ | –3752 | $-7 \times 10^4$ |
| No Ratchet | 7 | 69 | 693 | 66 | 690 | 6928 | 658 | 6897 | $7 \times 10^4$ |

Tabulation of $t_{50\%}$ values for comparisons of a segregating population of decreasing size $N_{segr}$ with an asexual one of size $N_{asex}$ which ratchets with period $T_{asex}$. Shown are results for $N_{asex}$ equal to $10^4$ (*a*) and $10^8$ individuals (*b*). *U* is constant down the columns but changes across columns such that $T_{asex}$ is as shown. Negative $t_{50\%}$ values are in favour of the asexual population, i.e. indicate that the segregating population loses fitness faster. The dotted lines separate positive and negative $t_{50\%}$ values.

remains advantageous. As $N_{segr}$ gets smaller, segregation loses its edge more quickly when $N_{segr} = 10^4$ than when $N_{asex}$ is $10^8$. This merely reflects the fact that the product of $N_{segr}$ and *s* assumes a nearly neutral value more quickly in the latter case. It is also striking that $t_{50\%}$ values are quite insensitive to the decrease of $N_{segr}$ except close to the boundary. This is due to the aforementioned fact that $t_{50\%}$ values are mainly determined by the fastest ratcheting population. Thus, a segregating population with a single linkage group can have a population size many orders of magnitude lower than a mutationally comparable ratcheting asexual population and nevertheless ratchet slower as long as the product of $N_{segr}$ and *s* is several times larger than 1·0. More importantly, such a segregating population can gain fitness over a mutationally similar asexual population almost as quickly as though it had the same population size and thus almost as quickly as if it were not ratcheting.

(viii) *Conclusions about the Ratchet and segregation in single linkage group genomes*

Our results show that chiasmatic and inter-chromosomal recombination can no longer be considered the only mechanisms for decelerating the Ratchet in eukaryotes, as segregation alone often suffices to do so. Moreover, even minor decelerations of the Ratchet caused by segregation can translate into substantial fitness gains over comparable asexual populations which ratchet. The speed with which these fitness advantages develop is similar to that obtained when the Ratchet is completely stopped, because the asexual period is the main determinant of this speed. The last two considerations are true even when the segregating population is orders of magnitude smaller than the asexual population. Thus, for many parameter configurations, diploids that already have segregation might not gain much of an advantage over asexuality from further countering the Ratchet with the help of chiasmatic recombination. The main population genetical determinant of the Ratchet-decelerating power of segregation is the halving of the deleterious mutation rate per ratcheting unit entailed by segregation, a halving that does not require outcrossing.

## 3. The Ratchet and segregation in genomes with multiple linkage groups

(i) *General considerations*

In an outcrossing segregating population with genomes subdivided into multiple pairs of independently assorting achiasmatic homologues, the ratcheting unit is still the single homologue. In such populations, the Ratchet clicks when every homologue at a given linkage group acquires at least one mutation more than were carried by the least mutated homologues at that linkage group in the previous generation. There-

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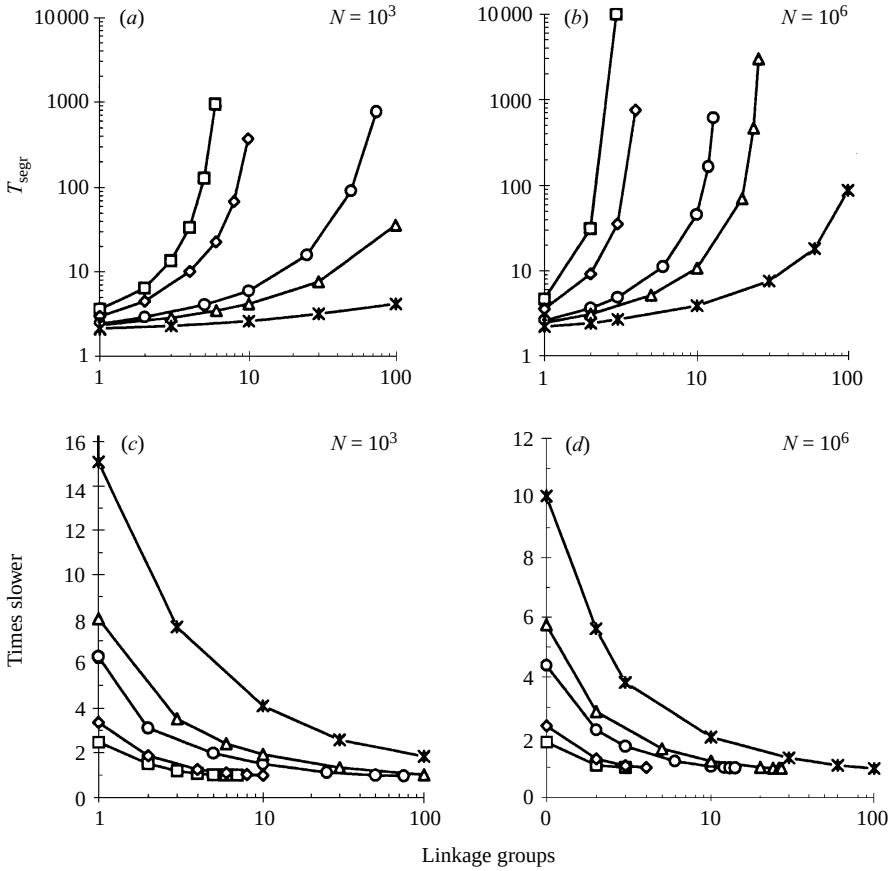


Fig. 1. The Ratchet in outcrossing segregating genomes with multiple linkage groups. The upper plots show $T_{segr}$, the genome-wide ratcheting periods for increasing numbers of linkage groups. The reference $T_{asex}$ is one generation. Squares, diamonds, circles, triangles and asterisks indicate cases where $s$ is 0·1, 0·05, 0·01, 0·005, and 0·001, respectively. The lower plots show how much slower than non-ratcheting populations, the segregating populations in the upper plots are in doubling their fitness relative to the reference asexuals.

fore, for organisms with segregation, random syngamy and a segmented genome, the Ratchet-relevant deleterious mutation rate is even smaller than with segregation alone. Thus the ratcheting period for each single linkage group of an achiasmatic segregating genome with $n$ equal-sized pairs of homologues should be markedly longer than that of a comparable asexual with $n$ times higher Ratchet-relevant mutation rate. However, the ratcheting period for the whole segregating genome is approximately $n$ times shorter than that for a single linkage group. This is because ratcheting events can occur at each of the $n$ linkage groups, so that genome-wide ratcheting events should take place on average $n$ times more frequently. We will see below, however, that the lengthening of the period due to the reduction of the mutation rate per ratcheting unit greatly outweighs the latter multiplication by $n$ except in neutral cases, where it exactly matches it.

(ii) *Simulation approach*

We have used simulations to calculate the number of equal-sized linkage groups into which the genome must be subdivided in order to produce dramatic decelerations of the Ratchet for the parameter combinations in Table 1. Our approach was the following: We first use a haploid simulation like those above to measure the ratcheting period of a single linkage group. We use $U/2n$ as the deleterious mutation rate per chromosome, where $n$ is the number of equal-sized linkage groups into which the genome is subdivided and $U$ is, as above, the deleterious mutation rate per diploid genome. Because ratcheting events can be assumed to occur $n$ times more often genome-wide than at each single linkage group, we divided the measured period by $n$. Since selection at unlinked loci lowers the effective population size $N_e$ of a population (Hill & Robertson, 1966; Felsenstein, 1974), these simulations were run with values of $N$ reduced by the factor proposed by Barton (1995). Barton found that selection of strength $s$ taking place at loci unlinked to a locus of interest, lowers the effective population size to $N_e = N/(1+4us)$, where $N$ is the number of reproducing individuals and $u$ is the deleterious mutation rate per haploid genome. Therefore the population sizes used in our haploid simulations were set equal to $2N/[1+s(U-U/n)]$, where $U-U/n$ is the deleterious mutation rate at the linkage groups not being simulated. For the parameters in Table 1, the value of $1+2Us$ is always

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Table 4. *Stopping the Ratchet when $N_{segr}$ is smaller than $N_{asex}$*

| | $T_{asex}$: 1 | | | 10 | | | 100 | | |
|---|---|---|---|---|---|---|---|---|---|
| $N_{segr}$ | $s$: 0·1 | 0·01 | 0·001 | 0·1 | 0·01 | 0·001 | 0·1 | 0·01 | 0·001 |
| (*a*) $N_{asex} = 10^8$ | | | | | | | | | |
| $10^8$ | 3 | 11 | 85 | 1 | 4 | 12 | 1 | 1 | 3 |
| $10^7$ | 3 | 13 | 105 | 1 | 4 | 15 | 1 | 2 | 4 |
| $10^6$ | 4 | 16 | 145 | 2 | 4 | 21 | 1 | 2 | 5 |
| $10^5$ | 5 | 24 | 275 | 2 | 6 | 45 | 1 | 2 | 10 |
| $10^4$ | 7 | 40 | 950 | 3 | 10 | 140 | 2 | 2 | 30 |
| $10^3$ | 11 | 140 | bt | 5 | 35 | bt | 3 | 2 | bt |
| $10^2$ | 29 | bt | bt | 16 | bt | bt | 11 | bt | bt |
| (*b*) $N_{asex} = 10^4$ | | | | | | | | | |
| $10^4$ | 4 | 32 | 800 | 1 | 6 | 100 | 1 | 2 | 15 |
| $10^3$ | 7 | 110 | bt | 2 | 20 | bt | 2 | 2 | bt |
| $10^2$ | 19 | bt | bt | 8 | bt | bt | 3 | bt | bt |

Tabulation of the number of linkage groups required to dramatically decelerate the Ratchet in the populations of Table 3.
bt, 'beyond thousand' linkage groups.

smaller than 1·6 and we saw above that the twofold difference in $N_e$ due to segregation has little effect on the period of the Ratchet (see above). Thus Barton's $N_e$ correction should have a minor effect and, in fact, repeating the simulations without the correction gives very similar results except when the correction lets $N_e s$ approach nearly neutral values. Nevertheless all results presented below were obtained using Barton's $N_e$ correction.

### (iii) *Results with multiple linkage groups*

The two upper plots in Fig. 1 show how dividing the genome into multiple linkage groups lengthens the genome-wide ratcheting period of an outcrossing segregating population, for cases with $N_{asex}$ values of $10^3$ and $10^6$ and $T_{asex} = 1$. When $Ns \leqslant 5$ and/or $s \leqslant 0{\cdot}001$, large numbers of linkage groups are required to slow down the Ratchet dramatically, while for larger $Ns$ values and $s \geqslant 0{\cdot}005$ much smaller numbers are sufficient. For cases where $T_{asex} \geqslant 10$ and $Ns \geqslant 1$, moderate numbers of linkage groups always suffice (data not shown). The lower plots in Fig. 1 show how quickly the outcrossing segregating populations in the upper plots can reach a twofold fitness advantage over otherwise identical asexuals, relative to completely non-ratcheting populations. Not surprisingly, trends corresponding to those in the two upper plots are found: small numbers of linkage groups suffice to match the speed of a non-ratcheting population, except when $Ns \leqslant 1$ and/or $s \leqslant 0{\cdot}005$, in which case only large numbers suffice. The figure shows clearly, however, that when $Ns > 1$ as few as 20 linkage groups suffice to deliver the twofold fitness advantage almost as quickly as by stopping the Ratchet. This shows again that a dramatic deceleration of the Ratchet is not required for fitness gains over a ratcheting asexual population to be obtained almost as rapidly as when the Ratchet is completely stopped.

Subdividing the genome into a few linkage groups also suffices to strongly decelerate the Ratchet when $N_{segr}$ is smaller than $N_{asex}$ (Table 4). From the figures in the Appendix, we looked up the values of $U/2n$ that would let the period for a single linkage group be longer than $n10^4 T_{asex}$ generations for various configurations of $U$, $N_{segr}$, and $s$. The table shows that often a moderate number of chromosomes suffices even when the population size of the segregating population is orders of magnitude smaller than that of the reference asexual population. However, when $s$ is 0·001 and $T_{asex} = 1{\cdot}0$ or when $Ns \leqslant 1{\cdot}0$, unrealistically large numbers of linkage groups are required. As it was noted earlier, however, also in these cases the bulk of the fitness advantages gainable over a comparable asexual population can be obtained with much smaller numbers of chromosomes (data not shown).

### (iv) *Conclusions about the Ratchet in multiple linkage group genomes*

From the above we can conclude that, when $Ns > 1$ and $T_{asex} > 10$, outcrossing and the independent assortment of homologous chromosomes during segregation suffice to stop the Ratchet almost completely when genomes have moderate numbers of linkage groups. Moreover, when $Ns > 1$ and $T_{asex} \geqslant 1$, such

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inter-chromosomally recombining populations can gain fitness over comparable ratcheting asexuals with almost the same speed as when they do not ratchet. Thus, in a contest between a segregating outcrossing population and a ratcheting asexual population with similar $U$, adding chiasmatic recombination to segregation and outcrossing is superfluous when the genome is subdivided into a moderate number of linkage groups.

## 4. Achiasmatic sexuality and the fixation of favourable mutations

### (i) *General considerations*

The advantages of segregation and outcrossing when genomes have multiple linkage groups are not limited to decelerating the Ratchet, allowing the fixation of favourable mutations in double dosage (Kirkpatrick & Jenkins, 1989; Wiener *et al*., 1992), lowering equilibrium genetic load under synergistic selection (Kimura & Maruyama, 1966; Dickson & Manning, 1984; Kondrashov, 1984; Charlesworth, 1990) and facilitating adaptation to fluctuating environments (Lloyd, 1980; Barton & Post, 1986; Weinshall, 1986, Charlesworth, 1993). Organisms that undergo these processes can indeed undergo inter-chromosomal recombination and thus enjoy to a large extent two further advantages generally attributed to chiasmatic recombination. The first of these advantages is the avoidance of Fisher–Muller interference (Fisher, 1930; Muller, 1932). In asexual populations, the fixation of a favourable mutation necessarily entails the loss of favourable mutations that had arisen in genomes not already carrying the mutation that is being fixed. In outcrossing segregating organisms with genomes subdivided into more than one linkage group, Fisher–Muller interference does not occur when the concomitant favourable mutations arise in different linkage groups. The second advantage generally attributed to chiasmatic recombination is neutralizing the reduction in the fixation probability of favourable mutations that is caused by concomitant selection against deleterious mutations at linked and unlinked loci (Fisher, 1930; Manning & Thompson, 1984; Peck, 1994; Barton, 1995). In asexual genomes all loci are linked and thus selection at any locus reduces fixation probabilities at every other locus. In genomes divided into multiple linkage groups, fixation probabilities at the locus of interest are mainly depressed by selection events at loci belonging to the same linkage group (see below).

In the following we will first discuss how well, relative to free recombination, a population with segregation, independent assortment, and outcrossing can deal with Fisher–Muller interference when the genome is divided into increasing numbers of linkage groups. Then we will compare the efficiency of inter-chromosomal recombination in neutralizing the reduction in favourable fixation probability due to background selection to the efficiency of chiasmatic recombination in the third chromosome of *Drosophila melanogaster*, a well-described chromosome that was used as a comparison.

### (ii) *Dealing with concomitant favourable mutations*

Assume that two favourable mutations arise at about the same time in a population where each genome consists of $n$ linkage groups, each of which has an identical rate of production of favourable mutations. The probability that the mutations arise on loci not located on the same linkage group (i.e. that the second mutation arises in one of the $(n-1)$ linkage groups without the first favourable mutation) equals $(n-1)/n$. Whenever this happens, the two fixation processes can take place at unlinked chromosomes and the mutations can go to fixation without excluding each other. This holds, however, only if no more than two favourable mutations are present in the population at any given time, i.e. if the mutations become fixed (or are lost) before the next two mutations arise. This probability does not predict whether the mutations will be fixed, but only whether they both can be fixed. This probability can also be calculated for cases where $k$ favourable mutations ($k \geqslant 2$) arise concomitantly in genomes with $n$ chromosomes with the help of the following formula:

$$P(k\,|\,n) = \prod_{i=1}^{k-1}\left(\frac{n-i}{n}\right), \tag{3}$$

where $P(k\,|\,n)$ is the probability that all $k$ mutations fall on distinct chromosomes.

Evaluation of (3) shows that 10 linkage groups are enough for favourable mutations arising in groups of two to fall on distinct chromosomes 90% of the time, 30 linkage groups suffice for groups of three favourable mutations to do the same, 60 for groups of four, and 100 for groups of five (but already 60 pairs of chromosomes given an 80% proportion in the last case). Thus, when concomitant mutations arise in groups of five or fewer, moderate numbers of linkage groups suffice to avoid Fisher–Muller interference 80% or more of the time.

A related but possibly more important number is $E(f\,|\,k,n)$, the average number of fixable favourable mutations when the genome has $n$ linkage groups and mutations arise in non-overlapping groups of $k$ mutations at a time. This is the average number of mutations that can go to fixation without excluding one another and equals the average number of linkage groups on which one or more of $k$ randomly placed mutations would occur. Feller (1960, chapter IX, problem 34) gives the formula for this average:

$$E[f\,|\,k,n] = \left[1-\left(1-\frac{1}{n}\right)^{k}\right]. \tag{4}$$

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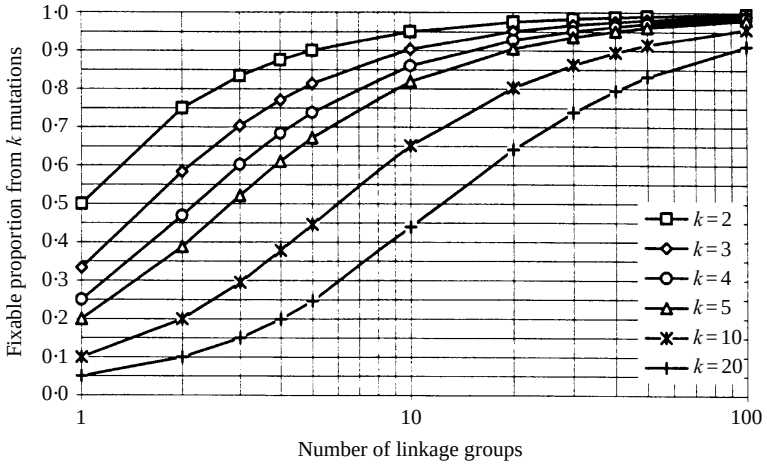


Fig. 2. Segregation and Fisher–Muller interference. In segregating outcrossing populations with genomes subdivided into multiple linkage groups, concomitant favourable mutations can go to fixation without excluding one another if they arise in distinct linkage groups. The figure shows the expected proportion of fixable favourable mutations when such mutations arise in non-overlapping groups of $k$ at a time (see text for details).

In Fig. 2, we present evaluations of (4) for various values of $k$ and $n$. The plot shows that five linkage groups suffice for an average of 90% of favourable mutations occurring in groups of two at a time to be fixable without excluding each other, 20 for groups of five, 40 for groups of ten, and 100 for groups of twenty (but note that 40 linkage groups suffice for an 80% proportion in the last case). Thus moderate numbers of linkage groups suffice for a vast majority of favourable mutations, arising concomitantly in non-overlapping groups of up to 20 mutations at a time, to go to fixation without hindering one another.

The results in the previous paragraphs indicate that in outcrossing segregating organisms with moderately subdivided genomes, Fisher–Muller interference can be neutralized almost as well as under free recombinations. The evolution of segregation, outcrossing, and a segmented genome might have sufficed to free early sexual forms from most Fisher–Muller interference.

### (iii) *Neutralizing the reduction in favourable fixation probability due to background selection*

The considerations above ignore the interference with the action of selection at a given locus due to concomitant selection against deleterious mutations at other loci (Fisher, 1930; Manning & Thompson, 1984; Charlesworth, 1994; Peck, 1994; Barton, 1995), and are therefore incomplete in describing the efficiency of inter-chromosomal recombination in facilitating the fixation of favourable mutations. Below we will compare the ability of inter-chromosomal recombination to counter the reduction in favourable fixation probability due to background selection with that of chiasmatic recombination, rather than with that of free recombination, since chiasmatic recombination can differ strongly from free recombination in this respect (Barton, 1995).

To illustrate the extent of the reduction in favourable fixation probability due to background selection in asexuals, we will calculate the fixation probability $p_{\mathrm{fix}}$ of a favourable mutation in a diploid asexual population in which deleterious mutations are produced at the rate estimated for the genome of *Drosophila melanogaster*. The potential of chiasmatic recombination to neutralize the reduction in favourable fixation probability due to background selection will be shown by calculating the $p_{\mathrm{fix}}$ in regions of the third chromosome of *D. melanogaster* with the average, highest and lowest rates of chiasmatic recombination. We will then estimate the $p_{\mathrm{fix}}$ in outcrossing segregating populations with genomes that mutate with the *D. melanogaster* deleterious rate and are subdivided into equal-sized achiasmatic linkage groups. In particular, we will determine the number of linkage groups that are necessary to match or approach the $p_{\mathrm{fix}}$ in the regions of *D. melanogaster*'s third chromosome with average, highest, and lowest rates of chiasmatic recombination. We will scale all our results by dividing $p_{\mathrm{fix}}$ by $p_{\mathrm{fixNB}}$, the fixation probability in a population free of background selection (Fisher, 1930; Charlesworth *et al.*, 1993*a*; Charlesworth, 1994). The value of $p_{\mathrm{fixNB}}$ is approximately $2s$ when $Ns \gg 1.0$ and $s \ll 1{\cdot}0$ (Haldane, 1937), but we will use exact values of $p_{\mathrm{fixNB}}$ calculated using a program kindly provided by Nick Barton that is based on eqn (2) of Barton (1995). In all cases below, we will assume that a favourable mutation that confers a fitness of $1+s$ on heterozygous carriers, arises in a population in which mutations selected against with strength $S$ in heterozygotes are produced with rate $U$ per individual per generation.

In general, when $s \leqslant S$, a favourable mutation has a substantial $p_{\mathrm{fix}}$ only if it arises in a chromosomal

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Table 5. *Favourable fixation probabilities under background selection*

| | $p_{fix}/p_{fixNB}$ (linkage groups) | | | |
|---|---|---|---|---|
| Recombination mode | *s*: 0·002 | 0·01 | 0·02 | 0·04 |
| High recombination | 0·72 (77) | 0·76 (96) | 0·80 (128) | 0·88 (110) |
| Average recombination | 0·51 (38) | 0·56 (44) | 0·65 (62) | 0·76 (46) |
| Low recombination | 0·09 (11) | 0·12 (12) | 0·19 (16) | 0·49 (20) |
| 10 linkage groups | 0·08 | 0·08 | 0·08 | 0·20 |
| 20 linkage groups | 0·29 | 0·29 | 0·29 | 0·54 |
| 40 linkage groups | 0·54 | 0·54 | 0·54 | 0·73 |
| 80 linkage groups | 0·73 | 0·73 | 0·73 | 0·85 |
| 160 linkage groups | 0·86 | 0·86 | 0·86 | 0·90 |
| $p_{fixNB}$ | 0·0040 | 0·020 | 0·039 | 0·076 |

Tabulation of the ratio $p_{fix}/p_{fixNB}$ under different recombination modes. $p_{fix}$ and $p_{fixNB}$ are the probabilities of fixation of a favourable mutation in the presence and absence of background selection, respectively. Values are presented for regions of the third chromosome of *D. melanogaster* with the highest, average, and lowest rates of chiasmatic recombination as well as for genomes with increasing numbers of linkage groups. In parentheses is the number of linkage groups required to match chiasmatic recombination.

region that carries no deleterious mutations or if it quickly recombines into such a region. In asexuals the whole genome is linked and thus only favourable mutations that arise in genomes with no deleterious mutations have substantial $p_{fix}$ values. In a large population, the proportion of such genomes is $e^{-U/S}$ and thus the asexual $p_{fix}$ is depressed by this factor on average (Kimura & Maruyama, 1966). This number, for instance, is extremely small in an asexual that produces mutations selected against with approximate strength 0·02 with a $U$ of about 1·0 – values that are close to those estimated for *D. melanogaster* (Mukai *et al.*, 1972; Crow & Simmons, 1983; Keightley, 1994).

In a population undergoing chiasmatic recombination, the probability of fixation of the favourable mutation is affected mainly by selection events as linked loci if $s \leqslant S$. Barton (1995) has derived analytical formulae to predict $p_{fix}$ values in a large population for a variety of background selection events. To illustrate the efficiency of a chiasmatically recombining chromosome, we have calculated $p_{fix}$ values for different regions of the cytogenetically well-studied third chromosome of *D. melanogaster*. To this end we integrated Barton's (1995) eqn (17a), taking into consideration the inhomogeneities in recombination intensity along the chromosome and making the same simplified assumptions about the genetic map and the deleterious mutation rate along cytological locations that were made by Hudson & Kaplan (1995). We ignored the decrease in $N_e$ due to selection events taking place at unlinked loci. This effect is expected to be identical in all populations compared here. Table 5 shows that in regions of the third chromosome that have highest recombination intensity, the value of $p_{fix}/p_{fixNB}$ is about 0·72, 0·76, and 0·80 for $s = 0{\cdot}002$, 0·01, and 0·02, respectively. The average $p_{fix}/p_{fixNB}$ over the whole chromosome (i.e. the expected value of the ratio at a randomly chosen cytological location) for the same values of $s$ is about 0·51, 0·56, and 0·65, respectively, while in regions of lowest recombination the ratio is 0·09, 0·12, and 0·19.

The probability of fixation in large achiasmatic segregating populations in which genomes have $n$ equal-sized linkage groups can be obtained by multiplying $p_{fixNB}$ by the factor $e^{-U/(2nS)}$, if $s \leqslant S$ and unlinked selection events are neglected. In a large population this factor equals the expected proportion of chromosomes at each linkage group that carry no deleterious mutations (note that this correction is independent of $s$). Table 5 lists $p_{fix}/p_{fixNB}$ ratio (i.e. $e^{-U/(2n-S)}$ for $n$ values equal to 10, 20, 40, 80, and 160 linkage groups under the assumption that $U = 1{\cdot}0$ and $S = 0{\cdot}02$, as done above. The ratios are 0·08, 0·29, 0·54, 0·73, and 0·86, respectively. The table shows that about 40 linkage groups suffice to match the values of $p_{fix}$ at a random cytological location of *D. melanogaster*'s third chromosome when $s = 0{\cdot}002$, 0·01, and 0·02, respectively. Matching the $p_{fix}$ values in regions with highest recombination requires large numbers of chromosomes, while matching those in regions with lowest recombination requires few chromosomes. Note, however, that 40 chromosomes deliver 75, 71, and 68 %, respectively, of the $p_{fix}$ values in regions with highest recombination when $s$ is 0·002, 0·01, and 0·02.

We conclude therefore that, when a mutation selected for with strength $s \leqslant 0{\cdot}02$ arises in an outcrossing segregating organism that has a moderately subdivided genome which produces deleterious mutations with $S = 0{\cdot}02$ and $U = 1{\cdot}0$ as above, the reduction in favourable fixation probability due to background selection can be neutralized almost as efficiently as in organisms with mutationally comparable chiasmatic genomes. Relatively large numbers

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of chromosomes, however, are required to match the efficiency of the high recombination regions found in the third chromosome of *D. melanogaster*.

In the case $s > S$, the favourable mutation has a substantial $p_{\text{fix}}$ only if it arises in a chromosomal region that carries fewer deleterious mutations than are necessary to balance or outweigh the fitness advantage conferred by the favourable mutation, or if the mutation quickly recombines into such a region. We calculated $p_{\text{fix}}$ values in large asexual populations with $U = 1{\cdot}0$ and $S = 0{\cdot}02$ as estimated for *D. melanogaster* with the help of Barton's program. The results show that only very large values of $s$ result in $p_{\text{fix}}$ values similar to $p_{\text{fixNB}}$ (e.g. $\log(p_{\text{fix}})$ is $-18$, $-14$, and $-11$ with $s = 0{\cdot}1$, $0{\cdot}2$, and $0{\cdot}3$, respectively). Therefore $p_{\text{fix}}$ is very reduced in large asexual populations that produce mutations with rates and deleterious effects as observed in *D. melanogaster* even when $s$ is very large. To our knowledge this phenomenon and its possible fitness consequences have not been thoroughly studied. The $p_{\text{fix}}$ values in regions of the third chromosome of *D. melanogaster* with highest, average, and lowest recombination intensity were calculated as described in the previous paragraph and are shown in Table 5 as $p_{\text{fix}}/p_{\text{fixNB}}$ ratios (we restricted our treatment of the case $s \geqslant 0{\cdot}02$ to $s = 0{\cdot}04$, given that Barton's eqn (17a) assumes that $s \ll 1{\cdot}0$ and thus might not be correct for larger values of $s$). The $p_{\text{fix}}/p_{\text{fixNB}}$ ratios for such regions were 0·88, 0·76, and 0·49, respectively, when $s$ was 0·04. To calculate $p_{\text{fix}}$ values in segregating outcrossing populations with $n$ equal-sized linkage groups, we used Barton's program again but with $U/2n$ as mutation parameter. Values are shown in Table 5. When $s = 0{\cdot}04$, $p_{\text{fix}}/p_{\text{fixNB}}$ is 0·20, 0·54, 0·73, 0·85, and 0·90 with 10, 20, 40, 80, and 160 linkage groups, respectively. Matching the ratio expected at a region with highest recombination requires 110 chromosomes but matching those at regions with average and lowest recombination requires 46 and 20 chromosomes, respectively. Note, moreover, that for a change in $p_{\text{fix}}/p_{\text{fixNB}}$ from 0·85 to 0·88, as many as 50 more chromosomes are necessary.

When $s > S$, therefore, the neutralization of the effects of background selection expected in any region of the third chromosome of *D. melanogaster* can be approached to a 90% level or higher in populations with segregation, outcrossing, and genomes subdivided into moderate numbers of chromosomes. However, matching the maximum neutralization expected in regions with the highest rates of recombination requires much larger numbers of chromosomes.

To sum up, in genomes with moderate numbers of linkage groups, the efficiency of inter-chromosomal recombination is comparable to that of chiasmatic recombination with respect to countering the reduction of the fixation probability of favourable mutations that is caused by background selection against deleterious mutations. Segregating organisms that outcross and have moderately segmented genomes which produce deleterious mutations like the *D. melanogaster* genome, can therefore be expected to be free from the reduction in favourable fixation probability due to background selection to a similar degree as mutationally comparable chiasmatic organisms whose genomes recombine like the third chromosome of *D. melanogaster*.

## 5. Why then chiasmatic recombination?

Our results imply that adding crossing over to an achiasmatic segregating *Drosophila* with 40 or 60 linkage groups should increase $p_{\text{fix}}$ values and the rate of accumulation of advantageous mutations by a small amount. However, chiasmatic recombination is known to be very reduced in very short linkage groups such as the fourth, very small chromosome of *D. melanogaster* (Hochman, 1976). It is arguable, therefore, that when chromosomes are already very small, chiasmatic recombination might be mechanically unable to take place often enough to produce a substantial effect. Thus when linkage groups are small, the portion of the reduction in favourable fixation probability due to background selection that is not neutralized by inter-chromosomal recombination could be due to events at loci that are too close for chiasmatic recombination to unlink. Chiasmatic recombination has the potential to suppress Fisher–Muller interference more efficiently when large numbers of favourable mutations arise concomitantly. It can also allow much larger numbers of loci to harbour polymorphisms whose allelic frequencies can readily respond to selection pressures arising from varying environments (Maynard Smith, 1978; Gillespie, 1992). It is of course unclear whether such advantages were important at the time of the origin of eukaryotic sexual forms and during their initial radiation.

Chiasmatic recombination, however, is certainly an alternative to inter-chromosomal recombination in the sense that it can provide all the advantages of the latter but possibly without comparable disadvantages. It is indeed likely that in cells that have to handle large numbers of chromosomes during meiosis and mitosis, chromosomal-number aberrations arise more often than in cells with fewer chromosomes. Chiasmatic recombination thus might have been the mechanism that freed organisms from such aberrations by making large chromosome numbers unnecessary. This cytological advantage might have been an important factor favouring the establishment of chiasmatic recombination as a major recombinational device.

## 6. Conclusions

We have shown that three classic advantages of eukaryotic sexuality can be provided to a large degree by inter-chromosomal recombination, a side effect of segregation in outcrossing populations with segmented

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genomes. We showed that inter-chromosomal recombination can decelerate Muller's Ratchet (Muller, 1964) dramatically and make possible the fixation of favourable mutations arising concomitantly in distinct individuals (Fisher, 1930; Muller, 1932) with an efficiency similar to that of free recombination. We also showed that it can limit the depression of the fixation probability of favourable mutations caused by concomitant selection against background deleterious mutations (Fisher, 1930; Charlesworth, 1994; Peck, 1994; Barton, 1995) with an efficiency similar to that expected for eukaryotic genomes which produce deleterious mutations at a rate similar to that estimated for the genome of *D. melanogaster* and which recombine chiasmatically like this organism's third chromosome.

Segregation and syngamy, the two most fundamental features of eukaryotic sexuality, might not have been enough to lead to the initial success of sexual forms, even though they suffice to decelerate the Ratchet strongly, as shown above, as well as to allow the fixation of favourable mutations in double dosage (Kirkpatrick & Jenkins, 1989; Wiener *et al.*, 1992), to lower equilibrium genetic loads under synergistic selection (Kimura & Maruyama, 1966; Kondrashov, 1984, 1994*c*; Charlesworth, 1990), and to give segregating populations an advantage in fluctuating environments (Lloyd, 1980; Barton & Post, 1986; Weinshall, 1986). However, segregation and random syngamy should suffice to allow inter-chromosomal recombination to take place in organisms with genomes subdivided into moderate numbers of linkage groups.

It can be argued, then, that all the classic advantages of sexual reproduction became available to early segregating eukaryotes with segmented genomes as soon as outcrossing was evolved. Chiasmatic recombination might have been evolved only later, given that it can maximize the fixation probabilities of favourable mutations, can allow populations to develop adaptations to varying environments that require fine-tuning of allelic frequencies at many loci (Maynard-Smith, 1978; Gillespie, 1992), can make possible more orderly and efficient DNA repair (Bernstein & Bernstein, 1991), and can free organisms from having to handle the large numbers of chromosomes necessary to counter the ratcheting of weakly selected mutations.

From the above we conclude that the evolution of segregation and syngamy, the two most fundamental features of eukaryotic sexuality, might have ignited the initial, possibly most crucial adaptive radiation of eukaryotic sexual forms. Outcrossing achiasmatic segregating organisms with segmented genomes did indeed not need to wait for the evolution of chiasmatic recombination before they began evolving larger genomes, occupying ecological niches that did not allow for large population sizes, etc. The study of the origin and the success of eukaryotic sex might ultimately become the study of the processes that can lead to the segmentation of eukaryotic genomes into multiple linkage groups, the study of the origin of segregation, independent assortment, and syngamy, and the study of the possibilities for ecological success that the first inter-chromosomally recombining achiasmatic organisms might have exploited. Accounts of the history of eukaryotic sex such as that given by Maynard-Smith & Szathmary (1995) in which the origin of crossing over is described as having had immense consequences (p. 156) but where inter-chromosomal recombination is totally neglected might need some revision.

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**Appendix. The period of the Ratchet as a function of $U$ and $N$ given as $s$.**

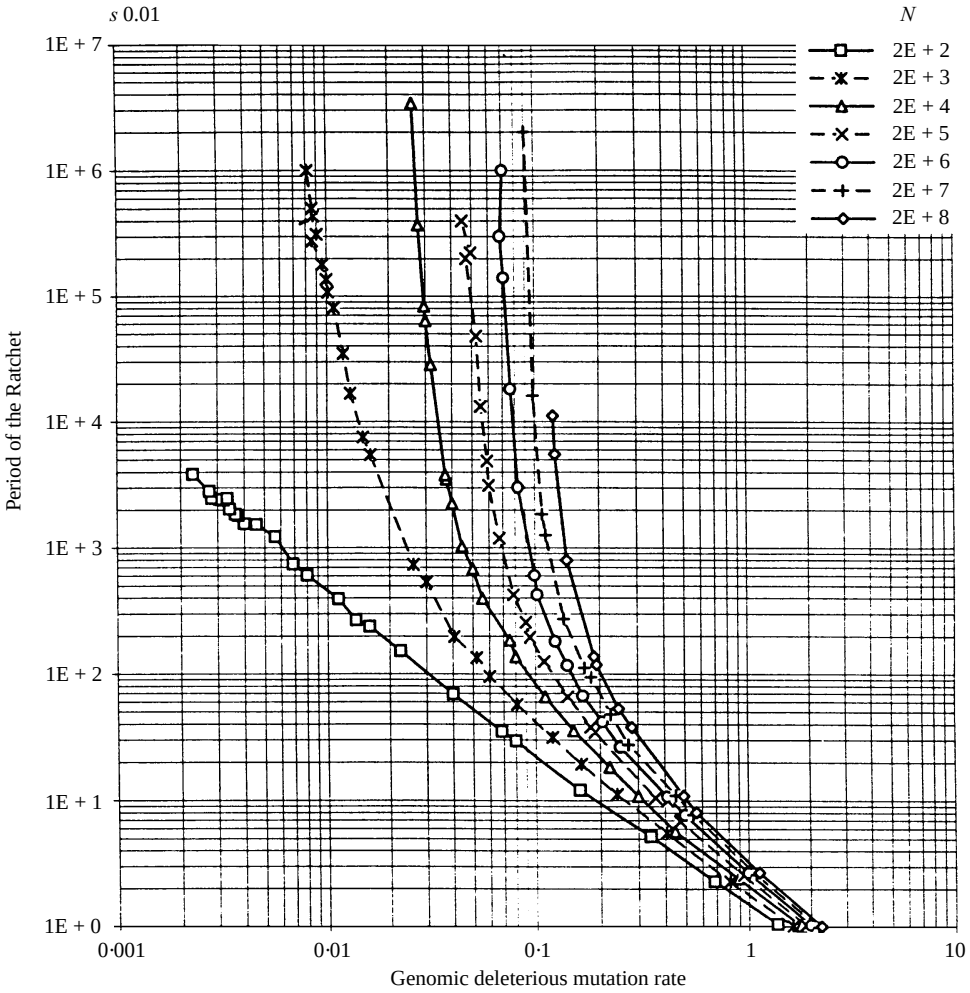


Fig. A1. $s = 0{\cdot}1$. Each period plotted is an average based on at least 100 clicks of the Ratchet for a given combination of $U$, $s$ and $N$ values. However, most periods in the proximity of 1, 10 or 100 generations are based on 10000 clicks of the Ratchet. Variances were about the square of the average period throughout the explored parameter space.

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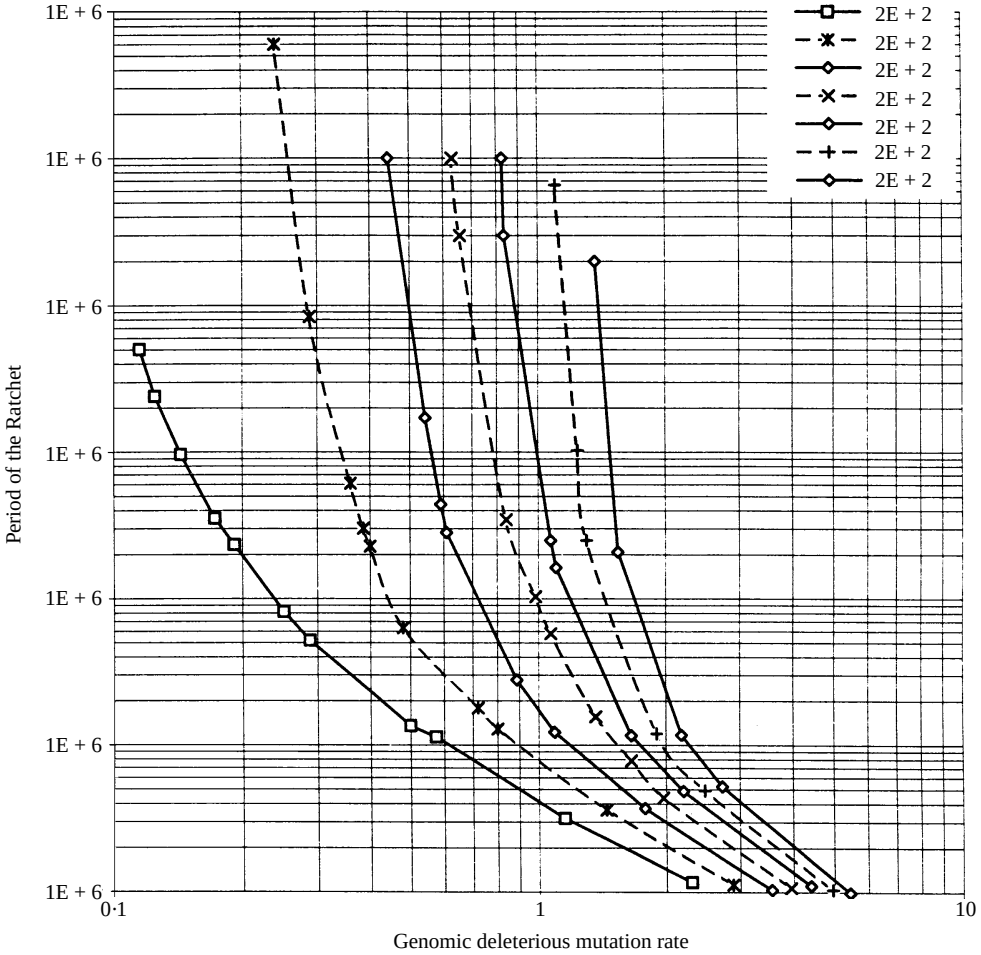


Fig. A2. $s = 0{\cdot}01$.

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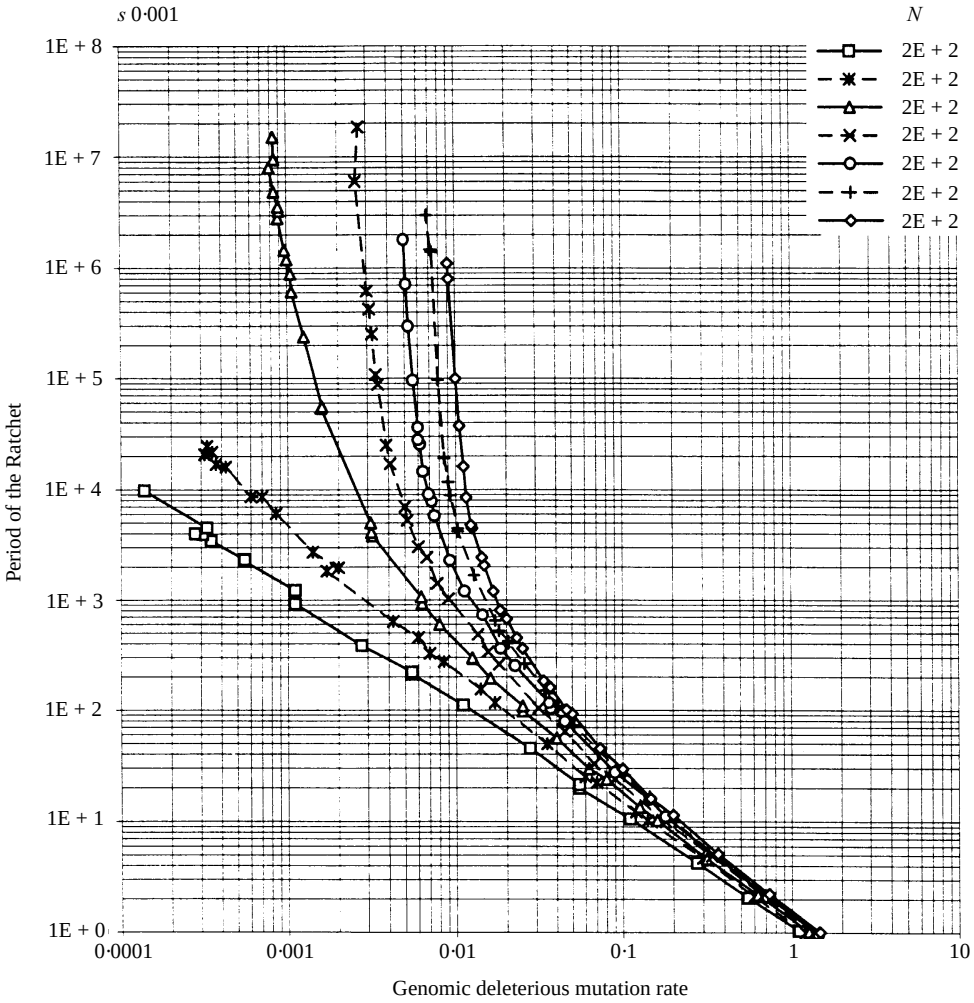


Fig. A3. $c = 0{\cdot}001$.

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