Mutation accumulation in finite outbreeding and inbreeding populations

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Summary

We have carried out an investigation of the effects of various parameters on the accumulation of deleterious mutant alleles in finite diploid populations. Two different processes contribute to mutation accumulation. In random-mating populations of very small size and with tight linkage, fixation of mutant alleles occurs at a high rate, but decreases with extremely tight linkage. With very restricted recombination, the numbers of low-frequency mutant alleles per genome in random-mating populations increase over time independently of fixation (Muller's ratchet). Increased population size affects the ratchet less than the fixation process, and the decline in population fitness is dominated by the ratchet in populations of size greater than about 100, especially with high mutation rates. The effects of differences in the selection parameters (strength of selection, dominance coefficient), of multiplicative versus synergistic selection, and of different amounts of inbreeding, are complex, but can be interpreted in terms of opposing effects of selection on individual loci and associations between loci. Stronger selection slows the accumulation of mutations, though a faster decline in mean fitness sometimes results. Increasing dominance tends to have a similar effect to greater strength of selection. High inbreeding slows the ratchet, because the increased homozygous expression of mutant alleles in inbred populations has effects similar to stronger selection, and because with inbreeding there is a higher initial frequency of the least loaded class. Fixation of deleterious mutations is accelerated in highly inbred populations. Even with inbreeding, sexual populations larger than 100 will probably rarely experience mutation accumulation to the point that their survival is endangered because neither fixation nor the ratchet has effects of the magnitude seen in asexual populations. The effects of breeding system and rate of recombination on the rate of molecular evolution by the fixation of slightly deleterious alleles are discussed.

1. Introduction

The process known as Muller's ratchet was originally understood in terms of the loss of chromosome classes from finite populations that do not have any re-combination, but are undergoing recurrent mutation to deleterious alleles (Muller, 1964; Felsenstein, 1974). In such a population, assuming multiplicative fitness interactions across loci, there will initially be a Poisson distribution of numbers of mutations per chromo-some, among the individuals present in any generation (Haigh, 1978). It is therefore possible that by chance the class of chromosomes with the lowest number of mutations is not propagated from one generation to the next, so that the 'least-loaded' chromosome type thereafter has more mutations than before. In the absence of recombination and back-mutation, it is not possible for a chromosome type with fewer mutations to arise in the population, even though such a type would have higher fitness than that of existing chromosomes. Because the process involves successive losses of the class with the lowest number of mutations, it does not cause fixation of mutations if the number of loci is large. The result is that numbers of mutations will build up over the generations, and the population mean fitness will decrease, but any particular mutant allele will remain at a low frequency in the population, being represented only in certain of the chromosomes present.

This model for deterioration of population fitness depends on restricted recombination. If much re-combination occurs, Muller's ratchet will not operate. The ratchet is also expected to operate in asexual populations (Maynard Smith, 1978), in which there is

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of course no recombination, and in highly selfing populations (Heller & Maynard Smith, 1979), as well as in situations in outcrossing sexual species where recombination is restricted, for example the differential segment of the Y chromosome (Charlesworth, 1978). Several authors have pointed out that the ratchet may lead to an irreversible decline in the mean fitness, and ultimately to extinction, of asexual populations (Manning, 1983; Maynard Smith, 1988; Lynch & Gabriel, 1990). It is important for our understanding of fitness in finite populations to know in quantitative terms how much recombination, and how much outcrossing, stop the operation of the ratchet, in populations of given size. Quantitative studies were done by Pamilo Nei & Li (1987), using simulations of population sizes of 100 diploids undergoing mutation to alleles with a dominance coefficient of 0·5. These authors also derived approximate analytical expressions for the rate of accumulation of mutations and increase in variance of numbers of mutations. Bell (1988) also studied the rate of operation of the ratchet, making some approximations whose accuracy has not been tested. Bell (1988, pp. 76–77) concluded that the product of the population size and the mean number of crossover events per bivalent must be of the order of 10^6 to prevent the ratchet's operation. Thus, Bell suggested that a small amount of recombination is sufficient to maintain population fitness in very large populations, but small populations require large recombination values to escape the operation of the ratchet.

Here, we study the speed of operation of the ratchet as a function of the diploid population size, recombination fraction, and the selfing rate, using stochastic simulations of multi-locus systems. Our aims were to study two of the unsolved problems mentioned by Maynard Smith (1988), namely how much recombination and how much outcrossing are needed to arrest the ratchet. For most of our modelling, the mutating loci were on a single chromosome. Assuming as appears reasonable that accumulation of mutations is independent for independent chromosome pairs, this is the case of most biological interest. We have therefore concentrated on runs with about 1000 loci, and a mutation rate lower than in our previous work, where the focus of interest was the level of genetic load in larger populations, due to mutations at loci throughout the genome.

This work enables us to test Bell's (1988) quantitative conclusions with respect to the frequency of recombination that will stop the ratchet in populations of different sizes, and to study the effects of the important parameters on the speed of operation of the ratchet when selection takes place in diploid populations, since most previous work (with the exception of Pamilo *et al.* 1987) has assumed haploid populations. In diploid populations, at least with outcrossing, both Muller's ratchet and fixation will be more likely to occur than in haploid populations, because of the

sheltering effect of heterozygosity, although the effects on fitness of the accumulation of mutations will also be smaller. The ratchet might also be expected to be slowed down when fitness declines faster than multiplicatively with increasing numbers of mutations carried. We have therefore also studied the effect of synergistic interactions between alleles at different loci, since this form of interaction is known to affect population mean fitness (Kimura & Maruyama, 1966; Crow, 1970).

Muller's ratchet is not the only process causing population mean fitness to decline in finite populations with low recombination. In addition to the ratchet process of loss of the genotypic class with the least mutations, fixation of mutant alleles also occurs in very small populations (Kimura, Maruyama & Crow, 1963; Felsenstein, 1974). As will be seen below, this can often be important. It is important to distinguish between these two types of process, and to identify the conditions under which each of them is expected to operate. None of the results published so far on mutation accumulation in asexual populations or with restricted recombination, either theoretical (Felsenstein, 1974; Li, 1987; Pamilo *et al.* 1987; Lynch & Gabriel, 1990) or empirical (Chao, Tran & Matthews, 1992), makes clear what contribution to the accumulation of mutations comes from each of these sources. It therefore seems that more work is required in order to understand the processes of mutation accumulation in finite populations. As will be seen, the effects of differences in the model parameters are complex, so that it is necessary to examine many different parameter combinations.

2. Methods

(i) *Construction of the model*

The populations were simulated using the stochastic multi-locus method of Fraser & Burnell (1970) and the programs used have been described previously (Charlesworth, Morgan & Charlesworth, 1992). The program for sexual reproduction assumes that individuals are hermaphrodite (i.e. that any individual can reproduce as a maternal parent or as a paternal one). In randomly choosing an individual as the paternal parent in a cross, the maternal parent was not excluded from the pool of zygotes to be sampled, so that there could be a low level of selfing in outcrossing populations of small size. The sequence of operations in each generation (mutation and reproduction, followed by selection) was the same as used by Kondrashov (1985) and by ourselves in our previous modelling work on inbreeding depression (Charlesworth *et al.* 1990, 1992). In what follows, the population size of diploid individuals will be denoted by N. The populations were assumed to be partially self-fertilizing, with a frequency of selfing (S) which could take any value between zero and one. The

number of loci was 1024. A recombination fraction of r between adjacent loci was assumed, with a binomial distribution of the numbers of recombination events, and no interference between the events at different locations. We also used a simplified version of the program to model apomictic parthenogenesis, in which entire genotypes reproduce themselves without recombination.

For the mutation process, we assumed a Poisson distribution of numbers of mutation events, with mutations occurring at loci chosen at random. The mutation rate for the whole diploid genome was denoted by U. Mutation was assumed to be unidirectional, from wild-type to mutant alleles only. This assumption is reasonable because the probability of back-mutation at any locus is likely to be much lower than forward mutation (Attwood, Schneider & Ryan, 1951). The one-way mutational process was done by changing the allelic state only for wild-type alleles, and leaving mutant alleles unchanged. When fixation for a mutant allele occurs, the mutation rate of the genome is therefore reduced. To avoid significant reduction of the mutation rate when the total number of fixations was high, loci at which fixation of mutant alleles occurred were re-set to the wild-type state, although fitness was calculated including the contribution from loci fixed for mutant alleles. As a population approaches fixation for a mutant allele, there will be a similar effect in our simulations. As will be seen, however, the proportion of the 1024 loci fixed rarely exceeded 10 % after 2000 generations, so the number of loci close to fixation (at which homozygotes will occur) in any given generation, was always very small. Even with asexual populations, and very high mutation rates, where the number of 'fixations' went as high as 383, fixation means that all individuals in the population have the same genotype at a given locus, nearly all heterozygotes, so only 19 % of alleles became immune to mutation in the worst case. The frequencies of fixed mutant alleles were very much lower than this, in all other runs, so over the time period of our runs there was only a small reduction of the mutation rate with this method.

The runs were started using distributions of numbers of mutations per individual previously calculated for populations of infinite size with free recombination, by the methods described for the multiplicative (Charlesworth *et al.* 1990) or synergistic (Charlesworth *et al.* 1991) selection models (see below). For the runs with high levels of selfing, the distributions from deterministic runs with $S = 0.99$ were used. These distributions were converted to cumulative distributions, and then (by means of random numbers) the cumulative data were used to obtain numbers of individuals with specified numbers of mutations, given the total population size to be modelled in the run. The population was then run for 2000 generations.

The state of the population was recorded every fifty generations. The variables recorded for each run

were: the numbers of heterozygous and homozygous mutations per individual in the population, the number of loci fixed for mutant alleles, the number of mutations per gamete taken over all loci, and taken over just the segregating loci, the number of mutations in the least-loaded gamete class, and the population mean fitness $\bar{w}$. For each set of parameter values, a set of 20 runs was carried out. At the end of each run, the regression of the natural logarithm of the mean fitness on generation number was recorded together with the mean values of the other variables, including correlations between the numbers of homozygous and heterozygous loci per individual. The values were stored for later calculations of the means over replicate runs, and the variances between replicates.

(ii) *Fitness functions*

In the sets of runs to be described, two fitness models were studied. Both models assume that selection takes place in the diploid stage of the life cycle, and in both models the selection and dominance parameters are assumed to be the same at all loci. In one model, fitnesses are multiplicative. In this case, the fitness of a genotype is given by the expression

$$w_{yz} = (1-s)^y (1-hs)^z, \tag{1}$$

where s is the selection coefficient against homozygotes for the mutant alleles, h is the dominance coefficient of these alleles, and y and z are the numbers of homozygous and heterozygous mutations in the genotype, respectively (Charlesworth *et al.* 1990). Our second fitness model assumes synergistic epistasis (Kimura & Maruyama, 1966; Crow 1970). For this type of model, we used a generalization of Crow's (1970) quadratic fitness model employing an 'effective number of mutations', n, which weights heterozygous mutations by the dominance coefficient (Sved & Wilton, 1989), so that $n = hz + y$. The fitness expression is then given by:

$$w_n = \exp\left[-\left(\alpha n + \frac{\beta n^2}{2}\right)\right] \tag{2}$$

(see Charlesworth *et al.* 1991).

The runs become very slow once the population mean fitness has declined to a low level (when many zygotes must be generated for each one that has high enough fitness to be permitted to survive). To speed up the runs, a scaling factor was therefore introduced, such that the threshold fitness for survival was decreased over time to such a level that approximately 10 individuals were generated for each surviving individual. We checked with several parameter sets that no differences in the results occurred as a result of this procedure, apart from the times taken for the runs.

3. Results

(i) *Effect of linkage with multiplicative fitnesses*

Table 1 and Figs 1 and 2 show the effects of changing the recombination fraction on the mean fitness, the number of loci fixed, and (for loci that had not become fixed for mutant alleles but were still segregating) the number of mutant alleles carried by the least loaded gamete class, and the mean number of mutations per gamete, for outcrossing populations with different population sizes. Only the recombination fractions that yielded some mutation accumulation are shown in the table, but the effect over the whole range of recombination values is shown in Fig. 1. The values in columns 2–6 of Table 1 are means at 2000 generations, taken over all replicate runs for each parameter set, and their standard errors. No runs were stopped before 2000 generations, even if the mean fitness had declined to biologically unrealistically low levels. The selection coefficient (s) was 0·1, and the dominance coefficient (h) was 0·2. A value of $sh = 0·02$ was estimated for detrimental mutations in *Drosophila* populations by Crow & Simmons (1983).

Initial values (from the infinite-population size case, see Section 2.1 above) for each set of runs are given in the table, at the bottom of each set of results for a given N. Comparison with these initial values enables one to tell whether a ratchet process has occurred in a set of runs, or whether the numbers of mutations are simply those to be expected in large populations with the same parameter values. It is evident from the table that, as expected, the initial values are similar to the values found after 2000 generations, for populations larger than 50 individuals with recombination rate (r) greater than 10^{-4}. In many cases, the mean numbers of mutations at segregating loci in the equilibrium populations are slightly lower than the initial values,

because low frequency mutations in the initial infinite-population distribution are liable to loss when finite population size is imposed.

The upper part of Table 1 gives results for a mutation rate U of 0·1 per diploid genome, and the lower part shows a higher mutation rate ($U = 0·5$). With all mutation rates studied, the mean fitness decreased with decreased population size. The highest recombination value shown in the table is 0·1, but the results were essentially the same for free recombination, and it can be seen in Fig. 1 that even in the smallest population ($N = 25$) the effect of increasing recombination by two orders of magnitude above 10^{-3} was slight.

Table 1 shows that the decrease in population mean fitness is caused by two factors: fixation of mutant alleles, and increase in the numbers of mutant alleles at segregating loci. With free recombination, fixation should occur at rates expected from single-locus theory. Using the method described by Charlesworth (1992) for calculating the fixation probability, the expected numbers of fixations (fixation probability $\times U \times N$) with $U = 0·1$ and the selection and dominance coefficients of Table 1, are 4·8, 0·07 and 7×10^{-6} for populations sizes of 25, 50 and 100, respectively. These are similar to our observed numbers with loose linkage (Table 1). With $U = 0·5$, the predicted values for the same population sizes are 24·3, 0·36 and 3×10^{-5}, which are also similar to our results. With a low mutation rate ($U = 0·1$), when the loci were allowed to recombine at a significant rate ($r \geqslant 0·001$), fixation of mutant alleles occurred when population size was below 100, and was frequently the main contributory factor to the decline in population mean fitness. In larger populations, fixation was rare unless the recombination frequency was extremely low. The same effect of recombination occurred with higher mutation rates, though in runs with $U = 0·5$ the number of fixations was nearly always much smaller than the number of mutations per gamete at the segregating loci.

The effect of recombination on fixation is generally not monotonic (Fig. 2). In runs with the selection parameters of Table 1, the greatest number of fixations clearly occurred with a small amount of recombination, rather than with zero recombination, with considerable increases in fixation evident between $r = 0$ and $r = 0·0001$ for $U = 0·1$, or between $r = 0·0001$ and $r = 0·001$ for $U = 0·5$ (Table 1). As will be seen below, this did not occur with a higher dominance coefficient (see Table 3). The interpretation of these effects will be given in section 4.

In addition to the effect of the recombination fraction on fixation, low recombination also increases the speed of operation of the ratchet. This can be seen in Table 1 as increases in the numbers of mutations per gamete, and in the number of mutations in the least-loaded gamete class, for segregating loci. Reduction of the recombination frequency caused a
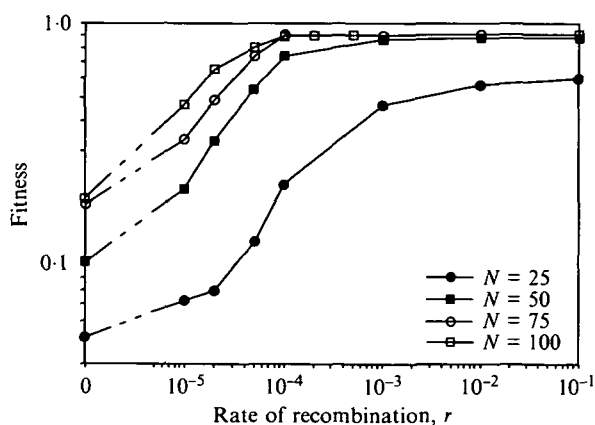

Fig. 1. Effect of reduced recombination on the mean fitness of outcrossing populations at 2000 generations after imposition of finite population sizes ranging from $N = 25$ to $N = 100$. The points plotted are averages of 20 replicate runs. The mutation rate was 0·1 per diploid genome per generation, the selection coefficient against mutant alleles was 0·1, and the dominance coefficient was 0·2.

Table 1. *The effect of restricted recombination on the decline in mean fitness in small outcrossing populations under multiplicative selection, with two mutation rates. The results presented were obtained with a dominance coefficient of* h = 0·2, *and selection coefficient* s = 0·1. *In each column, the means of each variable for 20 replicate runs are given above the standard errors*

Rate of recombination	Mean fitness	Number of fixations	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
Per-genome mutation rate $U = 0\cdot1$							
$N = 25$							
0·0	0·051	7·0	36·5	42·5	−0·962	0·99	−0·146
	0·002	1·1	2·3	2·4	−0·008	0·001	0·003
0·00001	0·073	16·9	14·1	16·5	−0·863	0·98	−0·131
	0·010	1·6	3·4	3·7	0·050	0·001	0·006
0·0001	0·220	13·4	1·1	2·5	−0·614	0·94	−0·074
	0·018	0·6	0·3	0·3	0·059	0·02	0·004
0·001	0·469	6·4	0·2	1·8	−0·326	0·84	−0·035
	0·026	0·5	0·1	0·2	0·043	0·02	0·003
0·1	0·606	3·8	0·1	1·9	−0·297	0·64	−0·019
	0·024	0·4	0·1	0·2	0·034	0·06	0·002
Initial	0·909	0	1·3	2·4	−0·003	—	—
$N = 50$							
0·0	0·104	2·5	29·8	36·1	−0·962	0·98	−0·111
	0·005	0·7	1·9	1·9	0·011	0·001	0·003
0·00001	0·211	9·2	7·2	9·9	−0·797	0·97	−0·076
	0·014	0·6	1·2	1·3	0·066	0·001	0·033
0·0001	0·742	1·7	0·5	2·3	−0·496	0·43	−0·01
	0·019	0·2	0·1	0·2	0·0496	0·06	0·002
0·001	0·876	0·1	0·1	2·2	−0·295	−0·1	−0·001
	0·011	0·1	0·1	0·1	0·035	0·04	0·001
Initial	0·909	0	1·6	2·4	0·002	—	—
$N = 100$							
0	0·191	1·6	20·8	26·1	−0·942	0·98	−0·100
	0·010	0·5	1·8	1·9	0·013	0·001	0·003
0·00001	0·468	4·2	3·3	5·7	−0·785	0·85	−0·034
	0·026	0·4	0·5	0·6	0·032	0·04	0·002
0·0001	0·897	0	0	2·2	−0·385	0·08	< −0·001
	0·007	—	—	0·1	0·034	0·03	< 0·001
Initial	0·909	0	1·5	2·4	−0·004	—	—
Per-genome mutation rate $U = 0\cdot5$							
$N = 50$							
0·0	< 0·01	10·1	212·1	221·9	−0·998	0·99	−0·520
	< 0·01	0·8	2·1	1·9	0·0004	0·001	0·007
0·00001	< 0·01	11·8	209·3	218·1	−1·000	0·99	−0·514
	< 0·01	0·07	1·7	1·6	0·0003	0·001	0·006
0·0001	< 0·01	31·9	129·3	140·1	−0·994	0·99	−0·489
	< 0·01	2·0	6·3	6·2	0·002	0·001	0·008
0·001	< 0·295	5·7	5·5	11·8	−0·569	0·71	−0·037
	< 0·018	0·6	0·4	0·3	0·032	0·04	0·003
0·01	0·558	0·3	4·2	10·6	−0·350	0·11	−0·004
	0·008	0·1	0·3	0·3	0·029	0·03	0·001
Initial	0·614	0	10·4	12·0	−0·016	—	—
$N = 100$							
0·0	< 0·01	5·5	185·8	196	−0·998	0·99	−0·443
	< 0·01	0·6	1·7	1·4	0·007	0·001	0·005
0·00001	< 0·01	5·3	177·5	188·6	−0·997	0·99	−0·435
	< 0·01	0·5	2·3	2·2	0·0006	< 0·001	0·004
0·0001	0·010	23·1	29·6	38·1	−0·945	0·98	−0·212
	0·001	1·0	1·8	2·1	0·006	0·001	0·006
0·001	0·584	0	3·9	11·1	−0·458	0·06	−0·001
	0·008	—	0·2	0·3	0·027	0·02	0·001
0·01	0·623	0	3·2	10·0	−0·276	0·04	−0·001
	0·009	—	0·2	0·3	0·017	0·01	0·001
Initial	0·615	0	9·9	12·0	0·007	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.

R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.

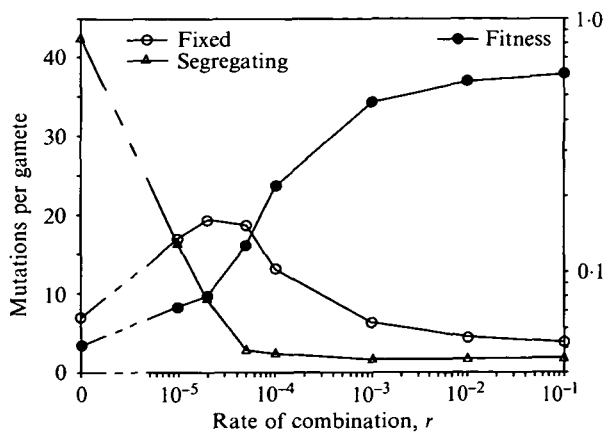‡ Regression slopes and standard errors are multiplied by 100.

Fig. 2. Effect of reduced recombination on the numbers of fixations and the mean numbers of mutations at segregating loci in outcrossing populations at 2000 generations in finite population of size $N = 25$. The points plotted are averages of 20 replicate runs. The mutation rate was 0·1 per diploid genome per generation, the selection coefficient against mutant alleles was 0·1, and the dominance coefficient was 0·2.
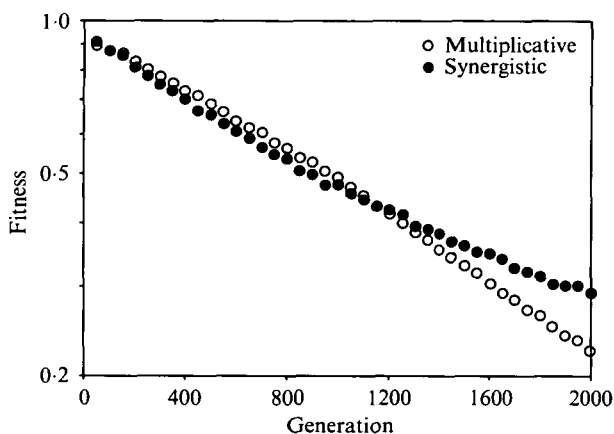

Fig. 3. Time course of decrease of the population mean fitness (averaged over 20 replicate runs) for the first 2000 generations in outcrossing populations of size $N = 100$, with multiplicative or synergistic selection. The mutation rate was 0·1 per diploid genome per generation, the recombination frequency 0·00001, and the dominance coefficient was 0·2. With the multiplicative selection model, the selection coefficient against mutant alleles was 0·1. With synergistic selection, the selection parameters were $\alpha = 0·01$ and $\beta = 0·02$.
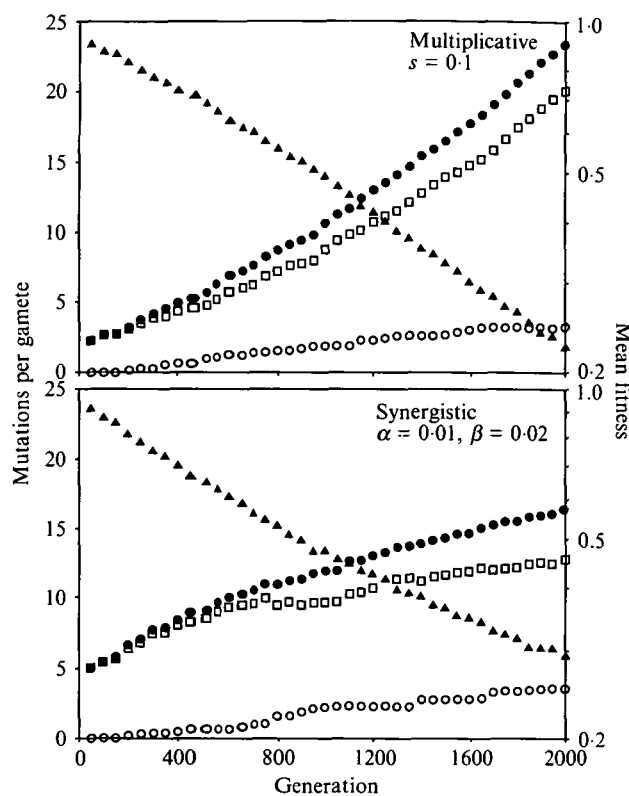

Fig. 4. Time course of decrease of mean fitness, and increase in numbers of fixed and segregating mutations in outcrossing populations of size $N = 100$, with multiplicative or synergistic selection. The filled triangles show the mean fitnesses, the filled circles the total number of mutations, and the open squares and circles are the numbers of segregating and fixed loci, respectively. The parameter values were the same as for Fig. 3.

monotonic increase in the speed of the ratchet, and also in the rate of decline of mean fitness as measured by the slope of the regression of mean fitness on generation number (Table 1, Fig. 1). With small populations, in which fixation occurred with an appreciable frequency, fixation increased at recombination frequencies at which the ratchet process of accumulation of low-frequency mutant alleles was still not apparent. Only at linkage values even tighter than required for fixation to occur did the ratchet operate at a significant rate. The effect of increased mutation was much stronger on the ratchet than on the fixation process. Thus, with high mutation rates when linkage

was tight, fixation became at most a minor, though significant, contributory factor to the decline in fitness. Even with $U = 0·5$, the ratchet operated only when linkage was extremely tight (Table 1).

The mutation rates assumed in these runs are quite high. For a chromosome carrying 1024 loci subject to mutation, as assumed here, the diploid U value of 0·1 corresponds to a mutation rate of about 5×10^{-5} per allele per generation, a value that may be reasonable given that it is assumed to include all possible detrimental mutations (Crow, 1948; Mukai et al. 1972). The value of 0·5 seems implausibly high for a single chromosome, but was included for completeness.

With multiplicative fitnesses, the decline in the natural logarithm of population mean fitness was approximately linear when the ratchet operated (Fig. 3). This parallels linear increases in the numbers of fixed and segregating mutations (Fig. 4). The slopes are given in Table 1. Similar results were probably obtained in the simulations done by previous workers. For example, Pamilo et al. (1987) state that 'accumulation rate becomes fairly steady' by 400 generations (starting from the mutant-free genotype).

Table 2. *Effects of changes in selection parameters* (*dominance coefficient*, h, *and strength of selection*, s) *on decline in mean fitness under multiplicative selection in finite outcrossing populations of* N = 50 *diploid individuals. The mean and standard error* (*below*) *after 2000 generations are reported for 20 replicates of each set of parameter values. Results presented were obtained with a per genome mutation rate of* U = 0·1

Rate of recombination	Mean fitness	Number of fixations	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
(a) s = 0·1							
h = 0·1							
0	0·159	0·9	48·9	56·9	−0·98	0·98	−0·879
	0·004	0·3	1·7	1·7	0·003	0·002	0·017
0·00001	0·181	1·9	36·1	42·7	−0·98	0·97	−0·852
	0·008	0·3	2·6	2·5	0·005	0·004	0·021
0·1	0·911	0·1	0·1	2·5	−0·22	0·05	−0·009
	0·004	0·05	0·1	0·1	0·02	0·01	0·003
Initial	0·909	0	3·6	4·7	−0·002	—	—
h = 0·2							
0	0·104	2·5	29·8	36·1	−0·96	0·98	−1·108
	0·005	0·7	1·9	1·9	0·01	0·002	0·028
0·00001	0·211	9·2	7·2	9·9	−0·80	0·97	−0·758
	0·014	0·6	1·2	1·3	0·07	0·002	0·031
0·1	0·890	0·1	0·1	2·1	−0·18	0·05	−0·005
	0·010	0·1	0·05	0·1	0·04	0·01	0·004
Initial	0·909	0	1·6	2·4	0·002	—	—
h = 0·35							
0	0·286	10·2	1·6	3·0	−0·51	0·93	−0·628
	0·022	0·8	1·2	1·3	0·07	0·01	0·049
0·00001	0·385	8·1	0·7	2·0	−0·51	0·87	−0·451
	0·034	0·6	0·2	0·3	0·06	0·04	0·038
0·1	0·889	0	0	1·5	−0·10	0·05	−0·0002
	0·006	—	—	0·1	0·03	0·02	0·003
Initial	0·908	0	0·7	1·4	−0·001	—	—
(b) s = 0·2							
h = 0·1							
0	0·099	0·2	36·6	42·5	−0·98	0·98	−1·174
	0·006	0·1	1·5	1·6	0·004	0·003	0·019
0·00001	0·286	1·0	10·8	15·2	−0·86	0·90	−0·633
	0·003	0·2	1·7	2·0	0·03	0·02	0·046
0·1	0·921	0	0	1·4	−0·11	0·03	0·001
	0·004	—	—	0·1	0·03	0·01	0·002
Initial	0·909	0	1·6	2·3	10^{-4}	—	—
h = 0·2							
0	0·239	2·7	6·9	8·8	−0·76	0·88	−0·756
	0·033	0·5	1·8	1·9	0·06	0·03	0·079
0·00001	0·588	1·9	0·4	1·5	−0·37	0·60	−0·267
	0·004	0·3	0·2	0·2	0·06	0·07	0·044
0·1	0·906	0	0	1·0	−0·05	0·02	0·003
	0·006	—	—	0·1	0·03	0·004	0·002
Initial	0·911	0	0·6	1·2	0·004	—	—
h = 0·35							
0	0·815	0·5	0·1	0·6	−0·20	0·22	−0·051
	0·030	0·2	0·1	0·1	0·04	0·06	0·018
0·00001	0·812	0·4	0·1	0·8	−0·21	0·20	−0·051
	0·027	0·1	0·05	0·1	0·04	0·06	0·022
0·1	0·905	0	0	0·6	−0·11	0·04	0·001
	0·006	—	—	0·05	0·02	0·01	0·003
Initial	0·913	0	0·3	0·6	$< 10^{-4}$	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.
R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.
‡ Regression slopes and standard errors are multiplied by 100.

(ii) *Effects of changes in the selection parameters with multiplicative fitnesses*

Table 2 shows the effects of changing the selection and dominance coefficients in outcrossing populations of size 50 with a mutation rate of 0·1. The dominance coefficient can have an important effect on the population mean fitness. With a selection coefficient of 0·1, high dominance of the mutant alleles resulted in higher mean fitness (and a correspondingly lower rate of decrease in mean fitness over time) than low dominance (comparing $h = 0.35$ and $h = 0.1$), when the loci were tightly linked, though there was no effect when the loci were loosely linked ($r = 0.1$). With complete linkage, the mean fitness at generation 2000 is significantly lower with an intermediate dominance value. Increased dominance produced greater rates of fixation of mutations at tightly linked loci (Table 2), but the accumulation of mutations by the ratchet mechanism decreased with increasing dominance, and was not significant with an h value of 0·35. With $s = 0.2$, similar effects were found, but the effect on the mean fitness at 2000 generations was a monotonic increase with increased h. We will discuss the probable reasons for the effect of the dominance coefficient below. Here, it is sufficient to note that the fixation and ratchet processes are affected in opposite ways by changes in the dominance coefficient. In considering the net effect on mean fitness, one must also remember that with greater dominance the effect on fitness of an increase in the number of mutations per gamete will be greater, so that it is not surprising that the effects on mean fitness can be complex.

Table 2 also shows that the effect of the recombination rate on the fixation and ratchet processes was generally similar, for all dominance values studied. The only important difference was that with $s = 0.1$ and $h = 0.35$ there was no decline in fixation even with extreme linkage, whereas a strong decrease in fixation was seen for $r = 0$ for more recessive alleles (see Tables 1 and 2).

The effects of changes in the selection coefficient in a random-mating population would be expected to be similar to those produced by alterations in the dominance coefficient. As might be expected intuitively, with strongly deleterious mutations (large s) the rate of accumulation of mutant alleles was slower than with smaller s values (Haigh, 1978; Pamilo *et al.* 1987; Lynch & Gabriel, 1990). This was true for both outcrossing and selfing hermaphrodite populations, but the effect was greater for the outcrossing case (Pamilo *et al.* 1987). The rate of decline in mean fitness, however, can be higher with stronger selection than with milder mutant effects, because of the greater effect of each allele on fitness (Pamilo *et al.* 1987; Lynch & Gabriel, 1990). With $U = 0.1$ and $h = 0.2$, we found that the mean fitness at 2000 generations was lower for $s = 0.02$ than 0·01 (0·459 versus 0·541), when the population size was 50, but with $N = 100$ mean

fitness was lower with $s = 0.01$. These results essentially confirm the conclusions just outlined, though it is important to realize that the selection coefficient giving the maximum deterioration in fitness depends on the values of other parameters, including the mutation rate and the population size (see also Lynch & Gabriel, 1990). Mutation to severely detrimental alleles usually resulted in higher mean fitness at 2000 generations, compared with mutations of lesser effect, but the opposite sometimes occurred with mutations of low dominance. With very strong selection against mutations ($s = 0.9$, detailed results not shown), there was no fixation with any linkage value, and the ratchet operated very slowly, if at all, and only when the mutations were quite recessive ($h = 0.1$).

(iii) *Synergistic fitness interactions*

Table 3 shows some results for the case of synergistic selection. With the selection and dominance parameters assumed, the average numbers of mutant alleles per diploid genome in large outcrossing populations with recombination was about 6. Using the result that, in outcrossing populations at equilibrium under mutation and selection, the equality $hs = U/\bar{n}$ holds (Charlesworth, 1990), we can compare the selection coefficient for the synergistic case with that in the multiplicative runs. The observed mean number of mutations corresponds to a selection coefficient per mutation of about 0·08, a value similar to that employed in the runs assuming multiplicative fitnesses with $s = 0.1$. It is therefore reasonable to make comparisons between the multiplicative and synergistic runs.

The rates of decline of mean fitness (expressed as the slopes of the natural logarithm of mean fitness) were somewhat lower than those found with the multiplicative model, for comparable recombination values and population sizes (Tables 1 and 3). The mean fitnesses at 2000 generations were correspondingly slightly higher. Since increasing the selection coefficient tended to decrease the speed of operation of the ratchet, for most recombination values (Table 2), it is not likely that the effect of synergism is caused by the slight difference in the selection coefficients, given that the synergistic runs involved effectively weaker selection, at least at the start of the runs. This is supported by the fact that there was no significant difference between the multiplicative and synergistic sets of runs in the slope of decline of fitness during the first part of the runs. It is more plausible to attribute the effect to a slowing down of the ratchet as mutations build up, in the synergistic selection case. That this happens can be seen in plots of the course of decline of mean fitness, for the two selection models (Fig. 4). There was a statistically significant decrease in the regression of the natural logarithm of mean fitness on generation number in the runs with synergism,

Table 3. *The effect of restricted recombination on the decline in mean fitness due to linkage in small outcrossing populations under synergistic selection, with a mutation rate of 0·1. The results presented were obtained with a dominance coefficient of* h = 0·2, *and selection parameters* $\alpha = 0·01$ *and* $\beta = 0·02$. *In each column, the means for 20 replicate runs are given above the standard errors*

Rate of recombination	Mean fitness	Number of fixations	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
Per-genome mutation rate $U = 0·1$							
$N = 25$							
0·0	0·065	6·7	18·9	21·9	−0·835	0·97	−0·148
	0·013	1·1	3·1	3·4	0·059	0·01	0·009
0·00001	0·142	11·4	3·8	4·9	−0·552	0·92	−0·100
	0·015	0·07	1·9	2·2	0·074	0·01	0·008
0·0001	0·400	8·4	0·3	1·3	−0·440	0·87	−0·040
	0·013	0·2	0·1	0·1	0·053	0·01	0·002
0·001	0·621	5·7	0·1	1·3	−0·207	0·61	−0·018
	0·021	0·3	0·05	0·2	0·051	0·05	0·002
0·01	0·671	5·0	0	1·4	−0·167	0·60	−0·015
	0·016	0·2	0	0·1	0·052	0·04	0·002
Initial	0·909	0	1·3	2·4	−0·003	—	—
$N = 50$							
0·0	0·172	4·7	12·4	16·8	−0·749	0·95	−0·095
	0·025	0·8	2·0	2·5	0·070	0·01	0·006
0·00001	0·396	7·8	1·1	2·4	−0·579	0·85	−0·041
	0·021	0·4	0·4	0·5	0·054	0·02	0·003
0·0001	0·708	4·3	0·3	1·9	−0·443	0·62	−0·013
	0·014	0·2	0·1	0·2	0·054	0·04	0·001
0·001	0·859	1·9	0·1	2·8	−0·361	0·30	−0·003
	0·006	0·1	0·1	0·1	0·032	0·04	< 0·001
Initial	0·943	0	3·4	4·7	−0·002	—	—
$N = 100$							
0	0·299	2·9	11·4	14·2	−0·774	0·93	−0·060
	0·024	0·8	1·9	2·2	0·063	0·01	0·005
0·00001	0·604	5·0	1·0	2·8	−0·558	0·77	−0·022
	0·019	0·2	0·3	0·3	0·049	0·04	0·002
0·0001	0·888	1·1	0·6	3·1	−0·507	0·127	−0·002
	0·004	0·13	0·1	0·1	0·005	0·046	< 0·001
0·001	0·946	0·2	0	3·2	−0·219	0·05	< −0·001
	0·006	0·1	—	0·1	0·019	0·01	< 0·001
Initial	0·943	0	3·7	4·8	0·001	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.
R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.
‡ Regression slopes and standard errors are multiplied by 100.

comparing the early and late stages of the time period investigated, but no such difference in the runs with multiplicative fitnesses. Fig. 4 also shows the increase in the mean number of mutations per gamete, and in the numbers of loci fixed for mutant alleles, and these also indicate clearly that synergism slows the ratchet.

(iv) *Effect of the outcrossing rate*

Tables 4 and 5 show the effects of high selfing rates (restricted outcrossing) on population mean fitness and mutation accumulation, in populations of various sizes. The multiplicative model with the same selection parameters as before was used. In highly selfing

populations, most individuals are homozygotes, so that the strength of selection against mutations is effectively greater than in an outbred population, where mutant alleles are usually heterozygous. One might therefore expect that one effect of selfing would be to slow down the operation of the ratchet, although the restriction of the effective amount of recombination in selfing populations would promote it (Heller & Maynard Smith, 1979). Thus restricted outbreeding should have a lesser tendency to promote the ratchet, compared with the effect of restricted recombination for the same value of U.

With a population size of 100 or greater, with free recombination and $U = 0·1$, there was at most a very small rate of decrease in fitness, even when outcrossing

Table 4. *The effect of restricted outcrossing on the decline in mean fitness in small populations under multiplicative selection. The means and standard errors (below) after 2000 generations are reported for 20 replicates of each set of parameter values. The results were obtained with a selection coefficient* s = 0·1 *and a dominance coefficient of* h = 0·2. *The initial values for each set of runs are given for the case of* S = 0·99

Selfing rate	Mean fitness	Number of fixations	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
Per-genome mutation rate $U = 0\cdot1$, unlinked loci							
N = 25							
1·0	0·263	12·3	0·05	0·69	−0·04	0·94	−0·067
	0·014	0·5	0·05	0·11	0·03	0·01	0·003
0·99	0·320	10·8	0·10	0·75	−0·08	0·91	−0·055
	0·026	0·8	0·07	0·12	0·04	0·01	0·005
0·95	0·408	8·2	0·05	0·65	−0·15	0·86	0·042
	0·021	0·5	0·05	0·11	0·04	0·02	0·003
0·9	0·521	6·0	0	0·60	−0·23	0·78	−0·032
	0·034	0·7	—	0·10	0·04	0·05	0·004
Initial	0·989	0	0·1	0·55	0	—	—
N = 50							
1·0	0·776	1·9	0·05	0·54	0·03	0·56	−0·011
	0·021	0·2	0·05	0·09	0·03	0·05	0·002
0·99	0·770	1·9	0·05	0·68	−0·03	0·48	−0·010
	0·021	0·3	0·05	0·08	0·03	0·06	0·002
0·95	0·890	0·6	0	0·63	−0·09	0·20	−0·004
	0·018	0·2	—	0·07	0·04	0·06	0·001
0·9	0·929	0·1	0	0·63	−0·14	0·10	−0·001
	0·011	0·1	—	0·07	0·03	0·04	0·001
Initial	0·989	0	0·1	0·55	0	—	—
N = 100							
1·0	0·947	0·1	0	0·48	0·04	0·07	−0·00047
	0·007	0·05	—	0·05	0·03	0·03	0·00041
0·99	0·946	0	0	0·55	−0·08	0·02	0·00002
	0·003	—	—	0·03	0·02	0·01	0·00011
Initial	0·989	0	0·02	0·55	−0·001	—	—
Per-genome mutation rate $U = 0\cdot5$, unlinked loci							
N = 100							
1·0	0·006	46·0	1·30	3·59	−0·003	0·99	−0·249
	0·001	0·8	0·26	0·23	0·02	0·002	0·006
0·99	0·024	32·6	1·30	3·79	−0·15	0·98	−0·182
	0·002	0·7	0·27	0·30	0·02	0·002	0·005
0·98	0·078	21·6	0·95	3·66	−0·26	0·97	−0·118
	0·007	0·9	0·17	0·18	0·03	0·01	0·005
0·95	0·335	7·2	0·70	3·73	−0·36	0·84	−0·039
	0·017	0·5	0·17	0·21	0·02	0·02	0·003
0·90	0·692	0·8	0·25	3·23	−0·43	0·16	−0·004
	0·015	0·2	0·10	0·12	0·02	0·04	0·001
Initial	0·948	0	0·6	2·55	0·002	—	—
Per-genome mutation rate $U = 0\cdot5$, linked loci $r = 0\cdot00001$							
N = 50							
1·0	0·004	49·8	1·26	3·71	−0·14	0·99	−0·27
	0·0004	1·1	0·36	0·36	0·02	0·0008	0·006
0·99	0·003	51·7	1·55	3·78	−0·31	0·99	−0·285
	0·0003	1·2	0·26	0·29	0·02	0·001	0·007
0·98	0·004	51·0	1·6	3·83	−0·38	0·99	−0·275
	0·01	1·3	0·32	0·32	0·03	0·001	0·007
0·95	0·002	52·7	8·05	10·69	−0·57	0·99	−0·303
	0·0003	2·0	6·16	6·35	0·04	0·0009	0·008
0·90	0·001	49·8	26·05	30·56	−0·78	9·99	−0·339
	0·0002	3·1	10·17	10·66	0·03	0·001	0·01
Initial	0·948	0	0·6	5·1	0·002	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.
R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.
‡ Regression slopes and standard errors are multiplied by 100.

Table 5. *The effect of population size on the decline in mean fitness in small selfing* (S = 1·0) *populations under multiplicative selection. The means and standard errors (below) after 2000 generations are reported for 20 replicates of each set of parameter values. The results were obtained with unlinked loci, with a selection coefficient of* s = 0·02, *and a dominance coefficient of* h = 0·2

Population size (N)	Mean fitness	Number of fixations	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
Mutation rate, $U = 0.1$							
25	0·289	60·1	0·8	1·9	0·024	0·90	−0·061
	0·009	1·5	0·2	0·2	0·028	3.3×10^{-3}	0·002
50	0·399	43·3	1·2	2·5	−0·078	0·99	−0·055
	0·011	1·3	0·2	0·2	0·027	1.1×10^{-3}	0·001
100	0·520	28·5	2·3	4·0	−0·015	0·98	−0·031
	0·010	1·0	0·4	0·4	0·025	3.0×10^{-3}	0·001
200	0·639	18·2	2·1	4·2	−0·006	0·97	−0·020
	0·011	0·8	0·5	0·4	0·020	2.5×10^{-3}	0·001
400	0·740	10·8	1·9	4·2	0·005	0·95	−0·013
	0·008	0·6	0·4	0·4	0·013	0·01	0·001
800	0·848	4·8	0·9	3·3	−0·013	0·84	−0·006
	0·008	0·4	0·2	0·2	0·009	0·003	0·001
Initial	0·950	0	0·63	2·55	0·006	—	—
Mutation rate, $U = 0.2$							
25	0·077	124·3	2·1	3·5	−0·015	0·996	−0·130
	0·0001	17·7	0·4	0·6	0·005	< 0·001	< 0·001
50	0·130	97·7	1·9	4·2	−0·032	0·993	−0·096
	0·0002	19·8	0·6	0·5	0·001	< 0·001	< 0·001
100	0·211	71·5	3·4	5·9	0·008	0·991	−0·076
	0·0002	11·3	1·3	1·4	0·002	< 0·001	< 0·001
200	0·316	50·1	4·5	7·4	0·008	0·989	−0·054
	0·0004	9·5	1·7	1·7	0·001	< 0·001	< 0·001
400	0·392	38·7	4·2	7·8	−0·014	0·989	−0·043
	0·0002	3·3	0·9	1·0	0·001	< 0·001	< 0·001
800	0·520	24·5	4·3	8·1	0·003	0·984	−0·030
	< 0·001	4·5	1·5	1·4	< 0·001	< 0·001	< 0·001
Initial	0·906	0	5·0	5·2	−0·017	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.
R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.
‡ Regression slopes and standard errors are multiplied by 100.

never occurred. With very small populations ($N = 25$), there was some decline in mean fitness with decreasing outcrossing. This was due to fixation, and the mean numbers of mutations per gamete at the segregating loci were not significantly different for the different outcrossing rates. The numbers of fixations for small N were similar to those predicted by single-locus theory (Charlesworth, 1992). For example, with the selection and dominance coefficients of Table 5, $U = 0.1$, and a population size of 25, the value is 58·8, close to the observed number. For population sizes of 50, 100 and 200, however, 31·6, 7·54 and 0·27 fixations are expected, respectively, but many more were observed. For increasing N, fixation thus fell off much less rapidly than single-locus theory predicts. The mean numbers of mutations per gamete at non-fixed loci were not greater than the expectation for the mean number after mutation, but before selection, under mutation-selection balance in an infinite popu-

lation (the approximate value of this expectation is $U(1 + 1/s)/2 = 0.55$; this is similar to the expression $U/2s$ given previously for the case of high selfing (Charlesworth *et al.* 1991), modified to give the value for the stage of the life cycle before selection). Table 4 shows that this was true even with a high mutation rate ($U = 0.5$) and a population size of 100, though in this case, as with the smaller populations when a lower mutation rate was assumed, there were many fixations.

The speed of operation of Muller's ratchet is expected to depend on the frequency of the class of genotype with no mutations (Haigh, 1978). When this frequency is small, there will be low numbers of individuals in this class, so that it is vulnerable to random loss. In the diploid sexual case, the mean number of mutations per gamete is $\bar{n} = U/2sh$, yielding the expected frequency of the zero-mutation class $p_0 = \exp\{-U/2sh\}$ (Haigh, 1978). With diploid asexual populations, the frequency of non-mutant

clones is given by $\exp\{-U/sh\}$, and for selfing, the relevant quantity to be compared is the mean number of homozygous mutant loci per individual, which is given by $U/2s$ (Charlesworth *et al.* 1991), so that $p_0 = \exp\{-U/2s\}$. Since $s \gg sh$, the frequency of the least-loaded class will be higher under selfing than for the random-mating case without recombination. For $h < \frac{1}{2}$, the same holds for asexual populations. The ratchet should also operate more slowly under selfing, because of the greater degree of expression of the mutant effects.

This interpretation predicts that the ratchet should operate in selfing populations with mutations having smaller selection coefficients. We therefore did runs with $s = 0.02$ (i.e. equal to the value of sh in the outcrossing runs of Table 1). Results for free recombination, $S = 1.0$, and a range of population sizes are shown in Table 5, for two U values. Again, with $U = 0.1$, there were many fixations for small N, but little sign of Muller's ratchet and the mean fitness declined much more slowly than for asexual populations with comparable parameter values (compare Tables 4 and 6).

Tables 4 and 5 show the results of increasing the mutation rate. For the case of $U = 0.1$ and $s = 0.02$, the above formula for selfing yields p_0 value of 0.082, a high enough frequency that, by analogy with the asexual case, the ratchet would not be expected to operate, even in a very small population. With $U = 0.2$, however, the value would be 6.74×10^{-3}, and the ratchet should operate in populations of size 100. The ratchet is indeed more apparent with $U = 0.2$, but fixation is still the dominant process in these runs. It thus appears that the ratchet is not very strongly promoted by even very high levels of inbreeding.

In all other respects, our results for inbreeding populations are consistent with those of Pamilo *et al.* (1987, p. 40), who found slower accumulation of mutations with complete selfing compared with outcrossing populations, for $N = 100$, a selection coefficient of 0.01, intermediate dominance, and free recombination. They assumed a mutation rate of $U = 0.02$ per diploid genome of 5000 loci. As mentioned above, the results presented by these authors do not distinguish between fixation and accumulation of low-frequency detrimental alleles, but both presumably occurred, as these parameters yield a very low p_0 value.

We have also done some runs to study the effect of linkage in inbreeding populations (Table 4). Our runs extend the range of recombination fractions studied down to nearly complete linkage, whereas the closest linkage studied by Pamilo *et al.* (1987) still permitted recombination between the five chromosomes. Their runs should yield slower rates of decline in mean fitness than ones in which all loci are linked, because each chromosome should degenerate independently of the others, but segregation of chromosomes would reduce the rate of decrease in fitness over the genome

as a whole by a factor approximately equal to the number of chromosomes. It is therefore important to compare the results of extremely restricted recombination with the results when segregation of chromosomes occurs.

When recombination as well as outcrossing was restricted, the ratchet (as opposed to fixation of mutations) operated only when the selfing rate was considerably lower than 1, and then increased monotonically with increased outcrossing (compare the results in Table 4 with those in Table 1 for complete outcrossing). This effect was seen only with tight linkage, and is probably due to the fact already mentioned that, with high selfing, selection on the mutant alleles operates mostly on homozygotes, and is thus effectively strongest with the greatest level of inbreeding, leading to a higher frequency of the genotypic class with the highest fitness. The finding that mutations accumulated by the ratchet only when some outcrossing occurred suggests that it is only in these populations that sufficient levels of heterozygosity exist for the mutations to be able to accumulate.

(v) *Asexual populations*

Mutation accumulation in asexual populations was thoroughly studied by Pamilo *et al.* (1987), so we have merely done a few runs with parameter values similar to those employed in our other runs. The results are summarized in Table 6. For the assumption of asexual reproduction, with no segregation of chromosomes, one is concerned with mutations on any chromosome, so the mutation rate assumed in the runs represents the whole-genome value for this case, unlike for the results for the sexual models already discussed which relate to single chromosomes. The mutation rate of $U = 0.1$ is therefore probably implausibly low for asexual populations, and a value of at least 0.5 may be appropriate (Mukai *et al.* 1972; Houle *et al.* 1992). For the case of asexual populations, it is important to note that 'fixation' means that all individuals in the population have the same genotype at a locus, but unlike the case in sexual populations these genotypes are almost exclusively heterozygous. The column for mean numbers of mutations per gamete indicates the number for loci where there is variation in the population. As found by Pamilo *et al.* (1987), accumulation is faster with asexual reproduction than with selfing (Table 6).

As with the sexual populations summarized in Fig. 3, the decline in the natural logarithm of the mean fitness of asexual populations, and the increase in the number of mutations, was linear with generation number when multiplicative fitnesses were assumed. When synergism was assumed, with the same parameters as before, the accumulation of mutations slowed over time, but there was no detectable difference in the rate of decline of mean fitness. This was presumably because the effect of a mutation is

Table 6. *Factors contributing to the decline in mean fitness in small asexually reproducing populations under multiplicative or synergistic selection. The means and standard errors (below) after 2000 generations are reported for 20 replicates of each set of parameter values. Results presented were obtained with a dominance coefficient of* h = 0·2. *For the case of multiplicative selection, the selection coefficient was 0·1, and for synergistic selection, the selection parameters were* α = 0·01, β = 0·02

Population size	Mean fitness	Number of 'fixations' (heterozygous)	Number of mutations per gamete		Within-individual correlation*	Regression of ln(mean fitness) on generation	
			Minimum	Mean		R^2	Slope‡
Multiplicative selection							
Per-genome mutation rate $U = 0·1$							
25	0·075	121·8	0·6	1·7	−0·122	0·99	−0·127
	0·004	2·4	0·2	0·2	0·059	0·001	0·003
50	0·133	94·3	0·9	2·4	−0·054	0·99	−0·098
	0·004	1·65	0·2	0·2	0·065	0·001	0·002
100	0·211	69·9	1·1	3·5	−0·143	0·99	−0·075
	0·005	1·0	0·2	0·2	0·043	0·001	0·001
200	0·322	48·3	1·2	4·0	−0·077	0·99	−0·054
	0·008	1·0	0·3	0·3	0·029	0·001	0·002
400	0·414	34·3	1·6	4·8	−0·043	0·99	−0·040
	0·012	1·3	0·3	0·3	0·026	0·002	0·001
800	0·509	22·9	1·7	5·3	−0·057	0·98	−0·030
	0·010	0·8	0·2	0·3	0·017	0·004	0·001
Initial	0·909	0	1·5	2·4	−0·004	—	—
Per-genome mutation rate $U = 0·5$							
100	< 0·001	383·4	18·6	25·0	−0·466	0·998	−0·464
	< 0·001	3·6	1·2	1·2	0·056	< 0·004	0·004
200	< 0·001	325·9	29·8	36·7	−0·403	0·998	−0·414
	< 0·001	3·5	1·2	1·3	0·031	< 0·001	0·003
400	0·001	252·6	47·8	55·8	−0·440	0·997	−0·363
	< 0·001	2·6	1·0	0·9	0·022	0·001	0·003
800	0·001	168·1	72·3	82·2	−0·300	0·998	−0·328
	< 0·001	2·8	1·8	1·5	0·025	< 0·001	0·003
Initial	0·615	0	9·9	12·0	0·007	—	—
Synergistic selection							
Per-genome mutation rate $U = 01$							
25	0·033	87·5	0·1	1·0	−0·024	0·99	−0·185
	0·002	1·2	0·1	0·2	0·019	0·002	0·005
50	0·114	68·9	0·1	1·3	−0·066	0·99	−0·117
	0·006	0·9	0·1	0·1	0·045	0·001	0·003
100	0·238	54·2	0·1	1·6	−0·055	0·99	−0·075
	0·007	0·8	0·1	0·2	0·032	0·002	0·002
200	0·352	44·2	0·4	2·2	−0·074	0·98	−0·053
	0·008	0·7	0·1	0·2	0·024	0·002	0·001
400	0·465	35·2	1·1	3·0	−0·081	0·98	−0·038
	0·008	0·7	0·2	0·2	0·017	0·003	0·001
800	0·553	27·5	1·4	4·2	−0·057	0·97	−0·028
	0·007	0·7	0·2	0·2	0·019	0·006	0·001
Initial	0·906	0	5·0	5·2	−0·017	—	—

* Within-individual correlation: correlation between number of heterozygous and number of homozygous loci per individual.

R^2: Coefficient of determination of linear regression of ln mean fitness on generation number.

‡ Regression slopes and standard errors are multiplied by 100.

4. Discussion

In understanding the effects of linkage, dominance, and strength and mode of selection on the rate of accumulation of deleterious mutations and the decline greater once several mutations are present in a genotype.

in mean fitness in finite populations, it is important to separate the two contributory processes. The first is the fixation by drift of deleterious alleles at individual loci subject to irreversible mutation from wild-type to mutant alleles. The second is the operation of Muller's ratchet *per se*, i.e. the steady increase in the frequencies of gametes carrying large numbers of mutant alleles as a result of the stochastic loss of the least-loaded class

of gametes. This can in theory occur without any noticeable concomitant fixation of deleterious alleles at individual loci (Haigh, 1978).

Our results indicate that, as might be expected, the progress of the ratchet in a random-mating population of a given size is always retarded by increased recombination among loci, and by an increase in the dominance or selection coefficient for deleterious mutations (Tables 1 and 2). The effects of these parameters on the rate of fixation of mutant alleles and mean fitness are, however, much more complex, and require some discussion.

(i) *The rate of fixation of mutant alleles in random-mating populations*

The effect of recombination on the rate of fixation of mutant alleles in random-mating populations can be understood in terms of two effects that work in opposite directions. The first is the Hill–Robertson effect (Hill & Robertson, 1966; Felsenstein, 1974), the reduction in the efficacy of selection at one locus by interference from other selected loci with which it is linked. New mutations at a given locus tend to occur in gametes carrying wild-type alleles at the majority of other loci. With tight linkage, such associations may persist for a long time, and selection against the new mutant allele will be weakened by selection in favour of wild-type alleles at the other loci. This effect therefore tends to promote a higher rate of fixation of deleterious mutant alleles with tighter linkage (Li, 1987; Birky & Walsh, 1988).

However, another effect must also occur when linkage is very tight. Because of the operation of Muller's ratchet, gametes will mostly carry many deleterious alleles, so that fixation of a mutant allele by drift will often entail the fixation of other, linked mutations carried on the chromosome that is being fixed. This homozygosity will reduce fitness considerably, and therefore linkage might be expected to work against the chance fixation of mutant alleles. The dramatic decrease in fixations in random-mating populations when linkage is complete and the dominance coefficient is low (Tables 1 and 2) appears to be caused by this effect of multiple-locus homozygosity outweighing the Hill–Robertson effect.

Two lines of evidence support this interpretation. First, there are strong negative correlations between the numbers of heterozygous and homozygous mutations per individual when linkage is very tight, which decline as recombination increases (see Tables 1 and 2 above). These reflect the correlations between the identity states of different loci due to linkage and the inbreeding effect of finite population size: identity by descent at one locus is correlated with identity by descent at linked loci (Fisher, 1949; Haldane, 1950). Since mutant alleles are generally rare at each locus, this translates into a positive correlation between loci

in the numbers of homozygous mutant alleles, as demanded by our interpretation of the retardation of fixation with very tight linkage.

The second line of evidence is provided by the increased rate of fixation of very tightly linked loci with increased dominance (Table 2). This appears counter-intuitive at first sight, because increased dominance should be equivalent to an increased strength of selection, when most mutant alleles are present in the heterozygous state. Indeed, the results in Table 2 show that the rate of fixation of mutant alleles always decreases with the selection coefficient against homozygous mutant alleles. As just discussed, however, accumulation of mutant alleles by the ratchet is expected to retard fixation when linkage is extremely tight, because of the association between loci in numbers of homozygous mutant alleles. With low dominance and very tight linkage, the ratchet successfully operates to produce a large mean number of mutant alleles per gamete, and so the effect of this association is strong. But when dominance is high, the ratchet is retarded and the correlation in homozygosity is weakened, thus permitting a higher rate of fixation with close linkage.

Felsenstein (1974) stressed the similarity between the fixation and ratchet processes, emphasizing that both depend on linkage disequilibrium and that 'both the mutational load and the rate of complete fixation of deleterious mutations should be increased for the same reason in the absence of recombination'. However, the results we have just discussed indicate that the similarity is not as complete as Felsenstein suggested. In addition, the results shown in the Tables indicate clearly that fixation is greatly slowed by increased population size, whereas the ratchet process is more strongly dependent on restricted recombination but less sensitive to population size. Furthermore, the rate of operation of the ratchet depends exponentially on U, whereas the dependence of fixation is linear. Thus high mutation rates cause the ratchet to be the dominant process, at least in situations where it can operate at all (Tables 1 and 6).

With the selection and dominance parameters of Tables 1 and 3, which are close to those suggested by the *Drosophila* data on mildly deleterious mutations affecting viability (Crow & Simmons, 1983), it is clear that with zero recombination the ratchet contributes the bulk of the accumulation of deleterious alleles in populations of 50 or more. On the other hand, increasing recombination between adjacent loci, even just to the very low level of $r = 0.00001$, with $U = 0.1$, greatly retards the ratchet, but allows fixation to proceed at an appreciable rate even with $N = 100$. A further increase in recombination to 0.0001 effectively halts both fixation and the ratchet.

(ii) *The rate of operation of the ratchet*

The speed of operation of the ratchet in relation to population size and recombination fraction was examined by Bell (1988), who concluded that the effects of these parameters could be summarised approximately for haploid populations (with a brief diploid phase followed by meiosis) by the equation $\ln r^* = -1.6 - \ln n_0$ (where n_0 is the number of the mutation-free gametes given by $\ln n_0 = \ln N - U/s$, and r^* is the recombination value, expressed as the number of crossovers per chromosome, that prevents the operation of the ratchet). This can be written as: $\ln(Nr^*) = U/s - 1.6$. Bell concluded that the ratchet should be halted by larger values of either the haploid population size, N, or of r^* than the values given by this expression.

For outbreeding diploid populations, we must replace U/s with $U/2hs$ in Bell's (1988) formulae (see 3(iv)). Then, for $s = 0.1$ and $h = 0.2$, we have $U/2hs = 12.5$ for $U = 0.5$, yielding $Nr^* \approx 54000$ or $r^* \approx 540$ for 50 diploid individuals. With such a value, we have a situation that Bell describes by stating that 'no amount of recombination will halt the ratchet'. However, inspection of the results in Table 1 for this mutation rate shows that the ratchet did not operate when the recombination fraction was 0.001 or above (equivalent to approximately 1 crossover per chromosome). Even when the runs were extended to 10000 generations, the mean number of segregating mutations per gamete remained at the same level as seen in Table 1, though there were, as expected, roughly five times as many loci fixed as at generation 2000. Similarly, in previous simulations, we found that the ratchet did not operate in diploid populations of size 400 with $U = 1.0$, $s = 0.1$ and $h = 0.2$, though Bell's formula predicts that with such a large mutation rate $Nr^* \approx 1.5 \times 10^{10}$. In general, Bell's formula suggests that the operation of the ratchet will depend on $\exp(-U/2hs)$ regardless of the recombination rate, and will thus be impossible to stop if the mutation rate is high, or hs very small. We have found many other examples of violations of these predictions, so we conclude that they are not generally valid. Only for low mutation rates ($U = 0.1$, $U/2hs = 2.5$) was agreement good. Recombination is more effective in slowing the ratchet than appeared from Bell's approximate analysis, and we find that the ratchet operates in outcrossing populations, even very small ones, only when recombination is highly restricted.

With high selfing, one would expect the effect of recombination to be slight, because heterozygotes will be rare. With complete selfing, Pamilo *et al.* (1987) indeed found little effect of differences in the amount of recombination, ranging from complete linkage on each of the 5 chromosomes assumed in the genome, to independent segregation of all loci. Since recombination affects the accumulation of mutations in outcrossing populations, the difference in the rate of accumulation between outcrossing and selfing populations is largest for populations in which there is substantial recombination (Pamilo *et al.* 1987).

(iii) *Mutation accumulation and population fitness*

The speed of operation of the ratchet and fixation processes are important in giving an understanding of when populations are likely to deteriorate in mean fitness, and possibly become vulnerable to extinction (Manning, 1983; Maynard Smith, 1988; Lynch & Gabriel, 1990). Our results suggest that species are rarely threatened by the accumulation of mutations, even when population size is very small, unless they are asexual. In random-mating populations, mutations accumulate only when recombination is severely restricted unless the mutation rate per locus is implausibly high. If there is some migration between local populations, the population size in question must be that of the species as a whole, not that of individual demes. Migration should be similar to back-mutation in that it can restore wild-type alleles, and retard the accumulation of mutations. A similar effect was shown for the case of recolonization after extinction of local asexual populations (Melzer & Koeslag, 1991).

From the results on the effect of the selection coefficient (Pamilo *et al.* 1987; Lynch & Gabriel, 1990, and the present paper), it appears that mutations of moderate effect are the most important in their consequences for the fitness of populations that meet the conditions for mutation accumulation to occur. Very slightly detrimental mutations can become fixed and can accumulate by the ratchet, but this usually has only slight effects on fitness. Very strongly detrimental mutations will not accumulate.

If the interactions between mutations at different loci are synergistic, this slows the accumulation of mutations, so that the decrease in mean fitness may be limited. This was a strong effect in sexual populations, as can be seen by comparing the values in Tables 5 and 1. In asexual populations, in which mutations accumulated to the point that mean fitness was severely decreased, synergistic selection caused little perceptible reduction in the rate of decline over time.

The effect of restricted outcrossing is more complex than previously understood (Heller & Maynard Smith, 1979). Although it is correct in theory that inbreeding permits the operation of the ratchet at greater speeds than for outcrossing populations of the same size, this comparison implicitly assumes the same strength of selection. But in real populations, increased selfing leads to increased homozygous expression of mutations, and is thus similar to an increased strength of selection, which slows down the ratchet's operation, in comparison with small outcrossing populations. Furthermore, for similar rates of mutation and similar distributions of mutant effects, the fact that selection is between different homozygous genotypes means

that the size of the least-loaded class is greater in inbred populations. Thus selfing populations will accumulate only mildly detrimental mutations. Inbreeding should not therefore endanger population fitness unless the population size is so small, and mutation rate so high, that many fixations occur. Mutation accumulation has different consequences in inbreeding populations than in outcrossing ones. In small inbred populations, mutation accumulation occurs chiefly by fixation of mutations. The classic ratchet is relatively ineffective, even with high mutation rates, though small amounts of outcrossing retard the fixation process (Table 4). In contrast, accumulation in outcrossing populations with restricted recombination is expected to result in increase in the number of mutations, but different individuals will carry different mutations, so that mutations will not reach high frequency unless population size is very small.

The acceleration of the rate of fixation by close linkage also suggests that the importance of fixation, as opposed to a classical Muller's ratchet process, should be reexamined in relation to the situation in Y chromosomes, for which it has been suggested that the ratchet may lead to loss of functional alleles (Charlesworth, 1978). It seems unlikely that rapid fixation will occur, except in very small populations, but this needs to be checked by simulation, because sheltering of the detrimental effects of mutations, due to heterozygosity with X chromosomes, together with a lower effective population size for the Y, should facilitate fixation (Nei, 1970).

In summary, our theoretical results suggest that mutations accumulate to the point that population fitness is greatly decreased only in small asexual populations or very small sexual populations with highly restricted recombination or outcrossing. Even with a population size as small as 50, with a single chromosome with 1024 loci and an average of about one crossover per generation ($r = 0.001$), the observed decline in mean fitness in 2000 generations was 3·6% with random mating, compared with the infinite population value. This would yield a decline of 18% for 5 chromosomes, assuming independent accumulation of mutations on different chromosomes. The decline in fitness in selfing populations was larger, and would be higher still with several chromosomes as can be seen from the results in Table 4 with high mutation rates. However, it was always considerably less than for asexual populations with the same parameter values (Tables 4 and 6). The rate of decline for an asexual population with multiplicative fitnesses and size 100 was slightly larger than that for a completely selfing population of size 25, and the rate for selfing populations of size 100 was 64 times lower than that for asexual populations of 800 individuals, assuming the same selection coefficient (0·1) for both cases. Even with a selection coefficient of 0·02 for the selfing case, when mutations accumulate rapidly, the rates of

fitness decline were about the same for selfing populations of size 100 as for asexual populations of size 800. It is therefore not clear that extinction due to this cause is inevitable for selfing populations (cf. Lynch and Gabriel, 1990) unless they are very small. There is some empirical evidence that asexual populations tend to be of recent evolutionary origin, compared with other closely related taxa (Maynard Smith, 1978; Avise *et al.* 1991; Quattro, Avise & Vrijenhoek, 1992), suggesting that they do not persist for long time periods. It would be interesting to make similar studies of selfing plant taxa, though if rates of fitness decline are slower for selfers than for asexuals, the patterns might not be as clear.

(iv) *Implications for molecular evolution*

The results presented here have some possible implications for molecular evolution, which may involve the substitution of slightly deleterious alleles, particularly for nucleotide substitutions that result in amino-acid replacements (Ohta, 1976). Although we have not included many runs with very low values of Ns, apart from those for inbreeding populations, fixation was sometimes quite rapid. This occurred in sexual populations when linkage was tight, as can be seen in several examples in Tables 1, 2 and 3, and with unlinked loci in inbreeding populations (Tables 4 and 5), especially with weak selection. It would presumably also occur in haploid asexual populations though, as mentioned above, 'fixation' in diploid asexual populations means that all individuals in a population have the same heterozygous genotype. In the case of random-mating populations with tight linkage, the variances of the numbers of fixations were considerably higher than the means, e.g. in Table 1 with $U = 0.1$ and $N = 100$, the ratio of variance to mean was 5 when there was no recombination. In other words, there was a clearly non-Poisson distribution of number of fixations, as has been found in DNA sequence data for replacement substitutions (reviewed by Gillespie, 1991). With weaker selection, fixation would be expected to occur in larger populations, and simulations to study this are planned.

If these preliminary findings are confirmed with weaker selection and larger population sizes, some interesting conclusions follow. In the first place, rates of molecular evolution will be influenced by the recombination rates and breeding systems of populations. At least for haploid genomes, species with asexual reproduction will be expected to evolve faster with respect to slightly deleterious mutations than if recombination occurs freely. The same conclusion was reached by Birky & Walsh (1988), but these authors modelled a single mutable locus in the presence of a completely linked 'background' block of genes under selection, rather than studying the effects in a multi-locus system. Silent, presumably selectively disadvantageous, alleles have been fixed in the clonal

female-transmitted (*monacha*) genome of *Poeciliopsis monacha* hybridogens, but not in genomes of the sexual maternal ancestor *P. monacha* (Vrijenhoek, Angus & Schultz, 1977; Leslie & Vrijenhoek, 1979; Vrijenhoek, 1979; Spinella & Vrijenhoek, 1982). Fixation could also be caused by hitch-hiking of clonal genomes when advantageous alleles are substituted. Fixation of slightly deleterious mutations should also be accelerated for highly inbreeding populations, and in regions of the genome with restricted recombination in sexual species. This is a distinct phenomenon from the observed reduction in genetic variability for neutral variants in regions of restricted recombination, which has also been ascribed to hitch-hiking (Begun & Aquadro, 1992). Fixation times of rare neutral alleles are reduced by linkage to loci subject to deleterious mutation, especially in inbreeding populations (D. Charlesworth *et al.* 1992), and this reduces the average heterozygosity for neutral substitutions (B. Charlesworth *et al.* 1992).

If fixation is accelerated in inbreeding and asexual populations, the molecular clock cannot be assumed to run at a steady rate in related species which differ in rate of recombination or breeding system, and this will present problems for phylogenetic analyses based on sequence comparisons. This effect would be expected to be greatest for replacement rather than silent substitutions, since the latter are more likely to be neutral, and hence unaffected by recombination rate or breeding system (Birky & Walsh, 1988). The ratio of replacement to silent substitutions is thus expected to be higher in highly inbreeding species compared with outbreeders, and in portions of the genome with reduced rates of recombination. This may provide a means of testing the deleterious allele theory of molecular evolution. Second, the fact that the fixation of deleterious mutations in sections of the genome with reduced recombination may proceed in a non-Poisson fashion in random-mating populations may also provide a tool for testing the deleterious allele theory. The expectation is again that the effect should be more marked for replacement than for silent substitutions.

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