J. evol. Biol. 1: 67–82 (1988)

# **Recombination and the immortality of the germ line**

Graham Bell

Biology Department, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada H3A 1B1

*Key words:* Recombination; Muller's Ratcher; DNA replication; DNA repair; evolution of sex; mutation.

### Abstract

Asexual populations irreversibly accumulate mildly deleterious mutations through the occasional stochastic loss of their least-loaded line, a process known as "Muller's Ratchet". This paper explores the dynamics of this process, and the role of recombination in halting the Ratchet. Simulation studies show that an optimal class comprising  $n_o$  individuals is lost in about  $10n_o$  generations, implying that adaptedness may deteriorate rather rapidly in geological time. Asexual organisms will persist only if they are very numerous, or if they have very small genomes, or if there is extensive negative interaction among nonallelic mutations. Otherwise, long-term persistence requires that unloaded genomes be continually generated by recombination. An approximate expression for the rate of recombination is necessary in populations of fewer than about  $10^{10}$  individuals. A further complication is introduced by mutations in sequences which specify proofreading enzymes. Since these will reduce the fidelity of their own replication, a process of positive feedback leading to an ever-accelerating loss of function is conceivable.

One of the most remarkable properties of life is simply its persistence through geological time. All living creatures are separated from their prokaryote ancestors by thousands of millions of generations, and yet retain functional genomes. To have preserved functional integrity over such vast periods of time points to an extremely effective system for the repair of the genetic material. This paper will discuss the nature of this repair system, and in particular the role of recombination in preventing the deterioration of the germ line over very long periods of time.

Bernstein et al. (1985) have suggested that the repair of DNA is the primary function of recombination. DNA may be damaged in a number of ways, for example by cross-linkage or the insertion of modified bases. If only a single strand is damaged, the original sequence can be restored by excision-repair enzymes which use the undamaged strand as a template. Damage to both strands cannot be corrected unless an alternative template, in the form of an homologous chromosome, is available. In that case, recombination can patch the damaged chromosome using its homologue as a template. This may well be an important function of recombination, but it is practicable only when the damage can be unequivocally recognized as such. It will not usually work for mutational change in which one functional base or sequence is substituted for another, since the original and the mutant states cannot be distinguished. If sequences are labelled (for example by methylation) before replication, then errors made during replication can later be corrected; this does not involve recombination.

Since they often cannot be detected by error-repair mechanisms, deleterious mutations will tend to accumulate in the population. They will not accumulate indefinitely, because heavily-loaded individuals will tend to be removed by selection. When mutation and selection are balanced, the frequency distribution of mutations per genome represents an equilibrium and no further deterioration will occur. However, this argument holds strictly only for an infinite population. In a finite population, the absolute number of individuals bearing no mutations may be small. Since it will fluctuate from generation to generation because of sampling error, this class will sooner or later disappear, leaving the class of individuals bearing a single mutation as being now the best-adapted. The process is essentially irreversible, since the singly-loaded class will itself disappear long before the unloaded class can be reconstituted from it by back-mutation. A finite asexual population therefore gradually but inexorably accumulates an increasing load of mutations, until at last it becomes extinct. This is the process first noticed by Muller (1964), and usually referred to as the Ratchet. Sexual populations do not suffer in the same way, because the unloaded class can be reconstituted by recombination between loaded gametes. In this way, recombination can function as an indirect mechanism of repair, operating not on the primary structure of the DNA but on the phenotypic consequences of mutation.

Although Muller's Ratchet is well known to theoreticians (eg. Maynard Smith, 1978; Felsenstein, 1974), very little detailed numerical work has been reported. In this paper I shall attempt to describe how rapidly the Ratchet turns; in what circumstances it represents a major threat to asexual organisms; and how much recombina-

tion is needed to prevent genetic deterioration. I shall be concerned only with outcrossed sexuality; for different opinions about the effect of close inbreeding, see Shields (1982) and Heller and Maynard Smith (1979).

### The rate of deterioration of an asexual population

In an infinite asexual population the frequency of individuals bearing a given number of mutations follows a Poisson distribution at equilibrium (Haigh, 1978). In a finite population this distribution will be realized only approximately, the size of each class fluctuating stochastically from generation to generation. The rate at which the Ratchet turns depends primarily on the number of individuals in the least-loaded class; if mutations at different loci have multiplicatively independent effects on fitness, this has the expected value

$$n_0 = N \exp(-U/s),$$

where <u>N</u> is the population size, <u>s</u> the selection coefficient and <u>U</u> the Poisson mean number of mutations per genome. For mutations of small effect and correspondingly large frequency, the optimal class will usually consist of a single individual even in large populations. I shall initially follow Haigh's suggestion of treating the optimal class as if it were an isolated population, assuming the rest of the distribution to behave deterministically, which will be reasonable provided that the optimal class is only a very small fraction of the total population. We can then ask how long it will take for an optimal class initially comprising a single individual to fluctuate to extinction. This will depend on the probability distributions of birth and death of its members.

The probability of death is simply unity if all parents die at the end of each generation, being replaced by their offspring. The probability of birth is equal to the reciprocal of the population mean fitness, since the number of offspring in each class at the beginning of each generation must be divided by the total number of offspring produced by the population in order to maintain a constant population size. The mean fitness is  $e^{-U}$  (see, for example, Haigh 1978); but since only a fraction  $e^{-U}$  will not experience a mutation, the mean birth rate of unloaded individuals is  $e^{U} \cdot e^{-U} = 1$ . If we assume that the distribution of the number of offspring per female is Poisson, the probability that a class which initially comprises a single individual will become extinct within t generations is given by:

$$P(0,1,t+1) = \exp[P(0,1,t) - 1],$$

which must be solved numerically. The mean time to extinction of such a population depends on the upper limit N placed on the number of individuals in the population. With N = 1 the population cannot increase at all, and becomes extinct, on average, within two or three generations. This time increases to about 10 generations for N = 50, but then remains about the same for N up to 1000. This is largely because the distribution of times to extinction is skewed, with most populations becoming extinct very quickly but a few lingering on for much longer periods of time. A useful benchmark in thinking about the Ratchet is that, when selection and density-regulation are ignored, a cohort founded by a single individual is likely to disappear within about 10 generations.

More generally, an optimal class of any initial size will become extinct within <u>t</u> generations with probability  $[P(0,1,t)]^{n_0}$  approximately, since all lineages are nearly independent when the class comprises a small fraction of the total population. It will become extinct with probability Q within the time

$$\mathbf{t}(0,\mathbf{n}_{\mathrm{o}},\mathbf{Q})=\mathbf{n}_{\mathrm{o}}\cdot\mathbf{t}(0,1,\mathbf{Q})$$

approximately for moderate or large t and small  $n_o$ , suggesting that the mean time to extinction is simply proportional to the equilibrium number of unloaded individuals.

In these simple approximations, the birth-rate of unloaded individuals is assumed to be independent of the number present. In fact, the birth-rate must fall as the number of unloaded individuals increases, since population size is fixed. To represent this effect, image that the frequency of unloaded individuals is perturbed from its equilibrium value  $\hat{p}_o$  to some arbitrary value  $p_o$ . Although the overall frequency of loaded individuals must change in consequence, I assume that the frequency of any particular loaded class among the loaded individuals remains the same, so that the distribution is identical to the deterministic equilibrium distribution except for the altered frequency of unloaded individuals. Thus, if the frequency of the k-th class  $(k \ge 1)$  in the equilibrium population is  $\hat{p}_k$ , its frequency among loaded individuals is  $\hat{p}_k/(1-\hat{p}_o)$ .

$$\frac{\sum_{k=1}^{\infty} \frac{\hat{p}_{k}}{(1-\hat{p}_{o})}}{k = l (1-\hat{p}_{o})} (1-s)^{k} = (\hat{w} - \hat{p}_{o})/(1-\hat{p}_{o})$$

where  $\hat{w} = e^{-U}$  is the mean fitness of the population at equilibrium. The total number of offspring produced by loaded individuals is then  $N(1-p_o)(\hat{w}-\hat{p}_o)/(1-\hat{p}_o)$ , and adding the contribution made by unloaded individuals gives the total offspring production of the perturbed population as  $Np_o + N(1-p_o)(\hat{w}-\hat{p}_o)(1-\hat{p}_o)$ . In every generation this number is renormalized to N, and the mean number of surviving unloaded offspring born to an unloaded individual is therefore

$$b_o = \hat{w}(1-\hat{p}o)/[\hat{w}(1-p_o) + (p_o-\hat{p}_o)].$$

As expected,  $b_o$  is a decreasing function of  $p_o$ , since  $\hat{w} < 1$ . We can now use this result to simulate the behaviour of the optimal class as an isolated population whose numbers are regulated through the negative response of the birth-rate to increase in density. The expected change in the number of individuals per generation will be

$$\Delta n_{\rm o} = n_{\rm o}(b_{\rm o}-1),$$

which if  $\hat{w} >> p_o$  is approximately equivalent to

$$dn_o/dt = cn_o(1 - n_o/\hat{n}_o),$$

where  $c = (1-\hat{w})\hat{p}_o/\hat{w}$ . This form is directly analogous to the familiar "logistic equation" of population dynamics. The parameter c measures the strength of the restoring force which tends to move the population back towards  $\hat{n}_o$  after perturba-



**Fig. 1.** The mean time to extinction as a function of the equilibrium population size in isolated logistic populations. An initial population is set up of N individuals whose mean birth-rate is B = 1. The number of offspring produced by each individual is randomly chosen from a Poisson distribution with mean B, these offspring replacing their parent in the population; this procedure is repeated for all N individuals, creating a new population of N' individuals. This reproduces in a similar manner, except that the new birth-rate B' is a function of N', as described in the text. The iteration continues until N' = 0, when the number of generations which have passed is recorded. Each plotted point is the mean of ten replicates: the least-squares regression line shown is y = 1.055x + 0.982.

tion; since c is small given  $\hat{w}>>p_o, \hat{n}_o$  will be approached asymptotically from above or below. The density-independent arguments discussed previously should continue to provide good approximations, since convergence will be slow unless the population is far from equilibrium.

The results of numerical simulation are shown in Fig. 1. The relationship between the mean time to extinction  $t_E$  and the equilibrium population number  $\hat{n}_o$  is found empirically to be

$$\log_{10} t_{\rm E} = 0.982 + 1.055 \log_{10} \hat{n}_{\rm o},$$

20

or roughly  $t_E = 10\hat{n}_o$ , in good agreement with the simpler models.

Once the optimal class has become extinct, the frequency distribution of load shifts backwards until a new equilibrium is approached. This process is governed by the relative strengths of mutation and selection, rather than by population size. According to Haigh (1978), the new optimal class, with one more mutation than the previously optimal class, approaches a value of 1.6 n<sub>o</sub> individuals in approximately  $(\log s - \log U)/\log (1-s)$  generations. We can therefore distinguish two phases in the replacement of an optimal class by the next least-loaded class. During the "establishment" phase the whole frequency distribution shifts backwards in a nearly determi-

LOG10 GENERATION FOR ONE TURN OF RATCHET U/s =10 15 =15 /s U/s=20 10 5 0 ō 5 10 15 20 LOG 10 POPULATION NUMBER

Fig. 2. The time required for the Ratchet to make one turn, in populations of different size. Based on the arguments in the text, and on Mukai's estimates of U and s.

nistic fashion, until the newly optimal class comprises roughly  $\hat{n}_0$  individuals. During the "extinction" phase, this newly optimal class fluctuates stochastically to zero. The sum of these two phases represents the time taken for the Ratchet to complete one turn. I have plotted this time as a function of population number in Fig. 2, for a range of values of U/s consistent with Mukai's observations of viability modifiers on the second chromosome of Drosophila melanogaster (Mukai, 1964; Mukai et al., 1972). In small populations the process is dominated by the establishment phase, and the Ratchet turns very quickly. In large populations the establishment phase is negligible, but the extinction phase may be very prolonged. By using Mukai's estimates, we can get some idea of how quickly the Ratchet will erode adaptation. He found, roughly speaking, that U/s  $\approx$  15 for mutations with a homozygous effect of 0.025 and an average dominance of 40%. In a population as large as 10<sup>10</sup> individuals, the Ratchet will turn once in about 10<sup>4.5</sup> generations. A hundred turns will reduce viability to less than 10% of its initial value in a haplont, or less than 40% in a diplont – either figure would guarantee the demise of a protist reproducing by binary fission, and would be likely to cause the extinction of a multiparous organism. This would happen within 10<sup>6.5</sup> generations, or between 10<sup>4.5</sup> years (for a protist with many generations per year) and  $10^{6.5}$  years (for an annual plant or animal). A population of  $10^{10}$ individuals would thus become extinct in the geological short term.

Many prokaryotes and eukaryotic protists maintain enormous populations, and Fig. 2 makes it clear that they will be largely immune from the effects of the Ratchet. However, very few organisms of more than about 1 g in mass will have populations of as many as 10<sup>10</sup> individuals, and such relatively large organisms will suffer severely. To put this another way, the evolution of large multicellular creatures appears to require sexuality. This interpretation is consistent with the general tendency for asexuality to predominate among unicellular organisms – many large taxa, such as euglenoid algae, have no known meiotic process – while sexual reproduction becomes more common among relatively large forms (Bell, 1982). Even within a single genus of unicells, sexuality appears to be more frequent among larger species (Bell, 1985).

Organisms with small genomes will ratchet slowly because of the low overall rate of mutation. The mitochondria of human cells are essentially asexual organisms living in closed populations of about  $10^3$  individuals, so the arguments leading to Fig. 2 appear to suggest that the mitochondrial genome should degenerate very rapidly. However, this genome is only about 1% as large as the nuclear genome, so that U/s will be about 0.1–0.2 rather than 10–20. Under these circumstances the arguments I have developed above will not apply, since the optimal class will constitute a majority of the population, but it is clear that the Ratchet will turn only very slowly. When there are very few mitochondria, each with a relatively large genome, as in yeast and *Chlamydomonas*, the mitochondrial genomes appear to be capable of recombinaton.

I have assumed so far that mutations at different loci have independent effects on fitness. Interactions between loci which make the fitness of the double mutant less than  $(1-s)^2$  will reduce the power of the Ratchet, since selection will be more effec-

tive in eliminating heavily-loaded genomes. We can represent the fitness of an individual with <u>i</u> mutations as

$$\mathbf{w}_{i} = (1 - \mathbf{s}i^{\mathrm{E}})^{i},$$

where E expresses the epistatic interaction between loci: if E = 0 the loci have independent effects on fitness, while if E > 0 the fitness of a multiple mutant is less than the product of the fitnesses of the single mutants. The way in which E affects the deterministic distribution of load at equilibrium is shown in Fig. 3. As E increases,



Fig. 3. The effect of epistasis on the mean load and on the frequency of the unloaded class at equilibrium. The values plotted here were obtained by numerical iteration with U = 0.14 and s = 0.01, hence U/s = 14.

the mean load falls and the frequency of the unloaded class increases. Even moderate degrees of epistasis produce marked increases in  $\hat{n}^{\circ}$ . For instance, as E increases from 0 to 0.1 with U/s = 14,  $\hat{n}_{\circ}$  increases by nearly an order of magnitude, from  $10^{-6}$ to  $10^{-5}$ . The Ratchet may therefore accumulate mutations at functionally related loci only very slowly; the effect of gene interaction on load has been studied previously by King (1971). The converse is also true: if the fitness of the double mutant is more than  $(1-s)^2$ , the Ratchet will turn more quickly than expected.

## The role of recombination in halting the Ratchet

Since the optimal class becomes extinct after about 10  $\hat{n}_o$  generations in an asexual population, it will be reconstituted in a sexual population if on average 0.1 unloaded genomes are produced per generation by recombination. This applies to a population of any size: a very low rate of recombination in a large population can be as effective as a very high rate in a small population. To discover how much recombination is needed in order to supply one unloaded genome every ten generations, suppose that the genome consists of a single large chromosome along which any number of crossovers may occur. We wish to calculate how much crossing-over is just sufficient to create an additional 0.1 unloaded gametes per generation.

At equilibrium, the rate of formation of unloaded gametes from loaded gametes must be equal to the rate of loss of unloaded gametes from the unloaded class. When an unloaded gamete fuses with a gamete bearing i mutations (i > 0), and j crossovers occur, let the frequency of unloaded genomes among the gametophytes produced by meiosis be X<sub>ij</sub>. Since there are four meiotic products, the number of unloaded genomes produced is 4X<sub>ij</sub>, and the number of loaded genomes produced is likewise  $4(1-X_{ii})$ . Since only two gametophytes are expected to survive if the population is to remain stationary in numbers, the number of loaded genomes produced after random elimination of two of the four meiotic products is  $2(1-X_{ij})$ . Since one loaded gamete was present initially, the extra number of loaded genomes produced is  $2(1-X_{ij})-1 = 1-2X_{ij}$ . We have to cumulate this number over the range of crossovers permitted and weight the result by the frequency of gametes bearing i mutations. If there is no recombination, then  $X_{io} = \frac{1}{2}$ , and  $1-2X_{io} = 0$ ; we need consider only those zygotes in which the partner of the unloaded gamete bears i > 0 mutations, and in which j > 0 crossovers occur. The total number of loaded genomes created from the unloaded class of  $\hat{n}_o$  is thus

$$\hat{\mathbf{n}}_{o} \sum_{i=1}^{\Sigma} [\mathbf{P}_{i} \sum_{j=1}^{\Sigma} (1-2X_{ij})\mathbf{Pr}(j)],$$

where Pr(j) specifies the probability that j cross-overs occur. This number must exceed some critical value, say K, if the Ratchet is to be halted; we know that  $K \sim 0.1$ .

If there is only one crossover, it is almost certain to combine a sequence with no mutations, from one of the two chromatids produced by the unloaded gamete, with a mutated sequence from a loaded gamete bearing several or many mutations. The other unloaded chromatid is transmitted intact, so  $(1-2X_{ij}) = \frac{1}{2}$ . A second crossover is equally likely to involve either chromatid from the unloaded gamete, either the one which has already undergone crossing-over, or the uncontaminated one. The frequency of unloaded gametophytes thus drops to  $\frac{1}{8}$ , and  $(1-2X_{ij}) = \frac{3}{4}$ . In general, if there are j crossovers, the frequency of unloaded gametophytes produced by meiosis will be nearly  $2^{-(j+1)}$  when the number of mutations borne by the loaded gamete is

fairly large. In that case, the number of unloaded genomes produced per generation is approximately

$$\hat{\mathbf{n}}_{o} \sum_{i=1}^{\Sigma} [\mathbf{p}_{i} \sum_{j=1}^{\Sigma} (1-2^{-j}) \Pr(j)].$$

The real number is somewhat greater – if i = 1, for example,  $X_{ij} = \frac{1}{2}$  irrespective of j – but the approximation will be a good one so long as U/s is large, so that lightly-loaded gametes are relatively scarce. If we suppose that there is a fixed small probability that a cross-over will form in each of the small segments into which the chromosome may be divided, the number of chiasmata per bivalent will follow a Poisson distribution. This is a biologically reasonable model, though in practice interference between chiasmata will often cause deviations from Poisson expectation. The parameter of the Poisson distribution is r, the mean number of cross-overs per chromosome. Summing the series in the expression above shows that the critical value of recombination which just prevents the Ratchet from turning is

$$r^* = -2 \log_e [1 - K/\hat{n}_o(1 - \hat{p}_o)] \sim -2 \log_e (1 - K/\hat{n}_o),$$

assuming that the deterministic frequency of the optimal class  $\hat{p}_o$  is the same in populations with and without recombination, which will be nearly true if the population is reasonably large. The model of chiasma distribution has little effect on this conclusion. If there is no variance in chiasma frequency, exactly r crossovers being formed on each chromosome, the critical value of r is

$$r^* \sim -\log_2(1-K/\hat{n}_0) = -1.44 \log_e(1-K/\hat{n}_0).$$

Greater variance than Poisson is created by a geometric distribution with parameter r', the mean number of cross-overs being r = r'/(1-r') and the critical value

$$r^* \sim 2(k/\hat{n}_o)/(1-k/\hat{n}_o),$$

which approximates the Poisson solution for  $\hat{n}_0 > 1$ .

The Poisson solution shows that no amount of recombination will halt the Ratchet if  $\hat{n}_o < K$ . Clearly, if the number of unloaded genomes K supplied by recombination exceeded the number present  $\hat{n}_o$  the population would not be in equilibrium. Consequently, populations with high mutation rates or small size will deteriorate whether or not recombination occurs. If the *Drosophila* estimate of U/s ~ 15 is representative, eukaryote species with fewer than about N = 10<sup>5</sup> members will be short-lived in evolutionary time even when they are obligately sexual. Among more abundant organisms with  $\hat{n}_o > 1$ ,  $(1-K/\hat{n}_o)$  can be approximated as  $\exp(-K/\hat{n}_o)$ , and with K ~ 0.1 there is a very simple relationship between  $\hat{n}_o$  and the critical value of recombination:

$$\log r^* \sim -1.6 - \log \hat{n}_o.$$

One way of appreciating the implications of this result is to say that, ignoring any correlation between mutation rate and population size, the product of the recombination rate (as mean number of cross-overs per genome) and population size will be a constant, with a numerical value of about  $10^6$ . Very low rates of recombination will be adequate to halt the Ratchet in large populations; for  $N = 10^{10}$ ,  $r = 10^{-4}$  is sufficient, so sexual episodes need be only infrequent in small heterogonic metazoans, such as *Volvox*, rotifers or cladocerans. In protists or prokaryotes with populations of  $10^{15}$  or so individuals, the critical rate of recombination is too low to be detectable except in very large experiments. Conversely, small populations require high rates of recombination, and more or less obligate sexuality may be a precondition for the long-term survival of species with fewer than about  $10^7$  members.

## The Ratchet and genome repair

The rate of mutation is determined by the precision with which the replication and proofreading of DNA is performed. I have so far assumed this to be a given. But the enzymes responsible for these tasks, which I shall collectively call "repair", must themselves be encoded in DNA, and the DNA of the repair system is itself liable to mutate. This leads to a difficulty. Any loss of precision by the repair system will of course increase the rate at which the genome deteriorates; but in particular the repair system will be less able to specify itself correctly. Mutations in the repair system therefore create positive feedback which will result in errors accumulating at an ever-increasing rate both in the repair system itself and in the rest of the genome. This process is similar to (and was suggested by) the "error-catastrophe" theory of somatic aging put forward by Orgel (1963), but applies to the fate of a lineage rather than an individual.

To see how a runaway process of deterioration might operate, imagine a lineage consisting of a single organism which gives birth to a single offspring before dying. There are <u>R</u> mutable sites coding for the repair system, some fraction <u>F</u> of which are mis-specified so as to decrease the precision of repair. The initial probability of misspecification in an uncorrupted genome is  $q_0$  per site per replication, and this probability is increased by a factor (1+k) by each additional error in the repair system. The number of mis-specified sites at any given time will be the number mis-specified in the previous generation, plus the product of those previously specified correctly and the prevailing error rate per site:

$$\mathbf{RF}_{t+1} = \mathbf{RF}_t + (\mathbf{R} - \mathbf{RF}_t)\mathbf{q}_t,$$

where  $q_t = q_o (1+k)^{RF}_{t}$ . The increase in the number of mis-specified sites per generation is then

$$\Delta F_t = (1 - F_t) q_o (1 + k)^{RF} t,$$





**Fig. 4.** The bivariate distribution of load in the vegetative and repair genomes at equilibrium. The contours are frequencies after 500 generations in a haplont with 4 repair loci and 15 vegetative loci. The broken lines are regressions, and represent the mean vegetative load for a given repair load ("veg") and the mean repair load for a given vegetative load ("rep"). Among individuals whose repair genome was uncorrupted, the mean number of mutations arising per generation in the vegetative genome was 0.05, with Poisson variance; the corresponding rate for the repair genome was  $(4/15) \times 0.05 = 0.0133$ . An individual whose repair genome bore  $\underline{r}$  mutations had a mutation rate in both the vegetative and repair genomes  $(1+k)^r$  times as great as that of an individual whose repair genome was 0.01.

or approximately

$$dF/dt = q_o \exp(kRF)$$

if F and k are reasonably small. The time required to pass from a small initial error frequency  $F_0$  to a final value of  $F_t$  can then be obtained by integration:

$$\mathbf{t} = (1 - e^{-\mathbf{k}\mathbf{R}\mathbf{F}})/\mathbf{k}\mathbf{R}\mathbf{q}_{o}.$$

Assuming KRF to be small, t will be of order  $1/q_o$ . Even with an uncorrupted repair system the probability of error is unlikely to be less than  $10^{-6}-10^{-7}$ , so that catastrophe will ensue after about  $10^6-10^7$  generations.

In a population with many individuals the runaway accumulation of mutations may be opposed by selection. To think about the dynamics of such populations, we can distinguish between two types of element in the genome. One type includes genes which specify metabolic or structural proteins, which I shall call collectively the vegatative genome. The second type includes loci responsible for DNA replication and proofreading, which I shall call the repair genome. Because the positive feedback within the repair genome suggests the possibility of a runaway increase in load, we might first ask whether there is any stable distribution of load, either in the vegetative or in the repair genomes, when the accumulation of mutations in the vegetative genome is opposed by selection against heavily-loaded individuals in an infinite asexual population. I have simulated a population in which the vegetative and the repair genomes each comprise a fixed number of loci, the number of mutations occurring in the genome of any given individual being a Poisson-distributed random variable whose mean is a function of the number of mutations currently borne in the repair genome. Fig. 4 shows that the population approaches a stable bivariate distribution of loads in the repair and vegetative genomes after a few hundred generations, the vegetative load among individuals with any given repair load being Poisson-distributed. This is not an artefact of the very small genomes used in the simulation; slightly more approximate models of much larger genomes have essentially identical results.

In asexual populations, a runaway increase in mutation rates is prevented by the linkage between the repair and vegetative genomes. Mutations in the repair system are in themselves selectively neutral, but are linked to the mutations in the vegetative genome which they elicit. There is therefore a positive genetic correlation between the repair load and the vegetative load, and selection against mutations in the repair genome will occur as an indirect response to selection against loss of vegetative function. It is this indirect response which maintains the stable bivariate distribution of vegetative and repair loads. In sexual populations, the effect of recombination will be to reduce the correlation between the mutations in the repair genome and the mutations the mutational load, in both repair and vegetative genomes, will be greater in sexual than in asexual populations.

This rather paradoxical conclusion implies that recombination may act to increase rather than to reduce mutational load. It leads to the prediction that mutation rates will be greater in sexual than in asexual organisms, but there appears to be no good evidence on this point (see Lynch, 1984). However, it does not imply any irreversible accumulation of load. In finite populations, one can imagine that the genome is subject simultaneously to two Ratchets, a repair Ratchet and a vegetative Ratchet. The optimal class will be smaller in a sexual than in an asexual population, as the result of recombination between the vegetative and repair genomes. However, recombination within the repair genome can still recreate unloaded repair genomes in a sexual population, while the asexual population will be unable to recover the optimal repair





**Fig. 5.** The time taken for the repair Ratchet to complete 100 turns. The contours represent this time for different values of k, given that each mutation in the repair genome increases the rate of mutation by a factor (1+k), as a fraction of the time taken when k=0.

genome once it has been lost. There will therefore be an irreversible tendency for mutation rates to increase, and moreover to increase at an increasing rate, in asexual populations. Suppose that each turn of the repair Ratchet increases the mutation rate by a factor (1+k); if there is no repair Ratchet then  $k \approx 0$ . The time taken for one turn of the Ratchet, relative to the time taken when there is no repair Ratchet, will be  $10\hat{n}_o/10\hat{n}_o$ , where  $\hat{n}_o = N \exp[-(1+k)^i U/s]$ , the equilibrium size of the optimal class when i mutations have accumulated. The corresponding factor for 100 turns is thus

$$\frac{\exp(U/s)}{100} \frac{\sum_{i=1}^{100} \exp[-(1+k)^{i} (U/s)]}{\sum_{i=1}^{100} \exp[-(1+k)^{i} (U/s)]}$$

which varies between zero (when k is very large) and unity (when k is zero). This quantity is mapped as a function of k and U/s in Fig. 5. This shows that the autocatalytic breakdown of repair systems is of little consequence when both k and U/s are small, which is likely to be the case if the repair genome is small relative to the vegetative genome, and has a correspondingly low mutation rate.

# Discussion

Outcrossed sexuality has the effect of combining mutations which have arisen independently into the same line of descent. Since the time of Weisman (1889), theoreticians have emphasized the importance of combining favourable mutations in elevating the rate of adaptive evolution (Fisher, 1930; Muller, 1932; Crow and Kimura, 1965 and 1969; Maynard Smith, 1968 and 1971). However, since mildly deleterious mutations are much more frequent than favourable mutations, a more important consequence of recombination may be its effect in slowing down the rate of maladaptive deterioration. The calculations I have presented above show that the Ratchet can be a potent evolutionary force in some circumstances. In particular, the lifespan of most obligately asexual multicellular organisms, with populations of fewer than  $10^{10}$  individuals, may be geologically brief. I would speculate that this may be why multicellularity has evolved only recently, the first two billion years of the history of life being taken up exclusively by unicells and simple filaments. Multicellularity does not seem very unlikely to arise – it requires only three genes in Volvox (Kirk, 1986) – but large and therefore rare creatures may be unable to survive for long without the efficient meiotic mechanism of recombination invented by eukaryotes. There is little direct information about the longevity of lineages of large asexual metazoans, but it has been repeatedly suggested that most parthenogenetic vertebrates date back only to the last glacial period (see Bell, 1982).

Many broad comparative patterns (reviewed by Bell, 1982) are consistent with the operation of the Ratchet. One which has already been mentioned is the prevalence of asexual reproduction among small organisms with enormous populations. Another is the very sporadic distribution of obligate asexuality among larger organisms, suggesting that asexual metazoans arise from sexual stocks but quickly become extinct. Asexual genera tend to have fewer species than sexual genera, either because asexuality lowers the rate of evolution or because it increases the rate of extinction. The close association between polyploidy and parthenogenesis among metazoans might conceivably reflect how polyploidy slows down at least the initial stages of deterioration under the Ratchet. These are patterns which might arise over long periods of time through competition between sexual and asexual species: because the Ratchet requires group selection - or some equivalent process, such as selection acting on a completely recessive allele causing recombination (see Felsenstein and Yokoyama, 1976) - it does not offer an attractive explanation of the many ecological correlates of sexuality, which presumably arise through strong selection acting over short periods of time.

The relatively rapid deterioration of asexual genomes, on a geological time scale, shows that some form of long-term genome repair is indispensable, at least for most metazoans and metaphytes. Recombination acts as a repair device in two quite different senses. On the one hand, it accomplishes *endogenous* repair by splicing out double-strand damage, acting directly on the primary structure of the nucleic acid. On the other hand, it performs *exogenous* repair, acting indirectly through the phenotypic consequences of mis-specified DNA sequences, by bringing deleterious mutations together in the same nucleus and thereby enabling them to be eliminated simul-

taneously. The necessity for this latter role emphasizes that the faithful replication of the genome is by no means a straightforward problem when a long-term view is taken. Even more profound difficulties may arise in the case of genes which specify enzymes for error detection and correction, whose breakdown will be autocatalytic. It would not be surprising to discover that proofreading genes have very sophisticated devices for eliminating errors in their own replication.

### Acknowledgements

This work was begun on sabbatical at the University of Sussex, where I benefitted greatly from conversations with John Maynard Smith and Hans Metz. The original manuscript was substantially improved by comments from J. Felsenstein and an anonymous referee. It was supported by an Operating Grant from the National Science and Engineering Research Council of Canada.

### References

Bell, G. 1982. The Masterpiece of Nature. Croom Helm, London; University of California Press, Berkelev.

Bell, G. 1985. The origin and early evolution of germ cells, as illustrated by the Volvocales, pp. 221–256. In H. O. Halverson and A. Monroy (eds.), The Origin and Evolution of Sex. Alan Liss, New York.

Bernstein, H., H. C. Byerly, F. A. Hopf and R. E. Michod. 1985. The evolutionary role of recombinational repair and sex. Int. Rev. Cytol. 96: 1–28.

Crow, J. F., and M. Kimura. 1965. Evolution in sexual and asexual populations. Am. Nat. 99: 439-450.

Crow, J. F., and M. Kimura. 1969. Evolution in sexual and asexual populations. Am. Nat. 103: 89-91.

Felsenstein, J. 1974. The evolutionary advantage of recombination. Genetics 78: 737–756.

Felsenstein, J., and S. Yokoyama. 1976. The evolutionary advantage of recombination. II. Individual selection for recombination. Genetics 83: 845-859.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford University Press.

Haigh, J. 1978. The accumulation of deleterious genes in a population – Muller's Ratchet. Theor. Pop. Biol. 14: 251-267.

Heller, R., and J. Maynard Smith. 1979. Does Muller's Ratchet work with selfing? Gen. Res. Cambridge 32: 289-293.

King, J. L. 1966. The gene interaction component of the genetic load. Genetics 53: 403-413.

Kirk, D. L., and J. F. Harper. 1986. Genetic, biochemical and molecular approaches to Volvox development and evolution. Int. Rev. Cytol. 99: 217–293.

Lynch, M. 1985. Spontaneous mutations for life-history characters in an obligate parthenogen. Evolution 39: 804–818.

Maynard Smith, J. 1968. Evolution in sexual and asexual populations. Am. Nat. 102: 469-473.

Maynard Smith, J. 1971. What use is sex? J. Theor. Biol. 30: 319-335.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press.

Mukai, T. 1964. The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics 50: 1–19.

Mukai, T., S. T. Chigusa, L. E. Mettler, and J. F. Crow. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics 72: 335–355.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. 1: 2-9.

Orgel, L. E. 1963. The maintenance of the accuracy of protein synthesis and its relevance to aging. Proc. natl. Acad. Sci. USA 49: 517.

Shields, W. M. 1982. Philopatry, Inbreeding and the Evolution of Sex. State University of New York Press, Albany.

Received 2 February, 1987;

accepted 5 June 1987.

Section Editor: H. Metz