Mutation Load Under Vegetative Reproduction and Cytoplasmic Inheritance

Alexey S. Kondrashov

Section of Ecology and Systematics, Cornell University, Ithaca, New York 14853

Manuscript received October 15, 1993
Accepted for publication January 12, 1994

ABSTRACT

For reasons that remain unclear, even multicellular organisms usually originate from a single cell. Here I consider the balance between deleterious mutations and selection against them in a population with obligate vegetative reproduction, when every offspring is initiated by more than one cell of a parent. The mutation load depends on the genomic deleterious mutation rate U, strictness of selection, number of cells which initiate an offspring n, and the relatedness among the initial cells. The load grows with increasing U, n and strictness of selection, and declines when an offspring is initiated by more closely related cells. If $Un \gg 1$, the load under obligate vegetative reproduction may be substantially higher than under sexual or asexual reproduction, which may account for its rarity. In nature obligate vegetative reproduction seems to be more common and long term in taxa whose cytological features ensure a relatively low load under it. The same model also describes the mutation load under two other modes of inheritance: (1) uniparental transmission of organelles and (2) reproduction by division of multinuclear cells, where each daughter cell receives many nuclei. The load declines substantially when the deleterious mutation rate per organelle genome gets lower or when the number of nuclei in a cell sometimes drops. This may explain the small sizes of organelle genomes in sexual lineages and the presence of karyonic cycles in asexual unicellular multinuclear eukaryotes.

MUCH attention has been paid to the differences between asexual and sexual reproduction and the evolution of sex [see KONDRASHOV (1993) for a review]. However, in some sense asexual and sexual reproduction are not so different, because in both cases an offspring appears from a single cell, produced either by mitosis, or by meiosis or syngamy. There is a more profound difference between asexual and sexual reproduction, on the one hand, and vegetative reproduction, when a progeny appears from more than one cell of a parental organism, on the other.

Vegetative growth of an organism can sometimes proceed for thousands of years and create huge individuals, *e.g.*, in trembling aspen (KEMPERMAN and BARNES 1976) and in the fungus *Armillaria bulbosa* (SMITH *et al.* 1992). Simple fission of an individual's body can lead to vegetative reproduction. This is possible in many groups, including mammals (formation of monozygotic twins). Vegetative reproduction by specialized means (*i.e.*, buds, gemmae, rhyzomes, sclerotia, soredia, and stolons) is also common in most groups of multicellular organisms, demonstrating that development of an offspring from many cells is possible.

However, in the vast majority of cases vegetative reproduction is only a facultative process, coexisting with asexual or, more often, sexual reproduction. In such cases the genetic material at least occasionally passes through "unicellular channels," which restores the genetic homogeneity of organisms. This certainly diminishes the impact of vegetative reproduction on population genetic processes. Therefore, here I will consider only obligate vegetative reproduction, where every organism can be a chimera (*i.e.*, consist of cells with different genotypes) from the very beginning of its life, and I will leave facultative vegetative reproduction for later studies. Obligate vegetative reproduction, although relatively rare, is known in bryophytes, ferns, flowering plants, fungi, lichens and animals (see DISCUSSION).

Recently, CROW (1988, p. 68) argued that obligate vegetative reproduction reduces genetic variability among the progeny, which leads to inefficient selection and can account for its rarity. Here I develop this idea and consider the efficiency of selection against deleterious mutations under four modes of obligate vegetative reproduction.

The same model also describes two types of inheritance when an offspring receives all its genes from a single cell that, however, carries many similar genomes. This happens with (1) uniparental inheritance of organelles, if selection acts on them independently from the nuclear genes (BIRKY *et al.* 1989) and (2) "asexual" reproduction by ordinary cell division, if a cell has many nuclei (MARGULIS *et al.* 1990).

MODEL

Consider a population of multicellular organisms with discrete generations and the following life cycle: growth of the organisms, when mutations occur–vegetative reproduction, after which all parents die–selection. Below the expression "initial cell of an organism" will mean one of the cells from which the organism was initiated, and not a cell which the organism may produce later to initiate its progeny.

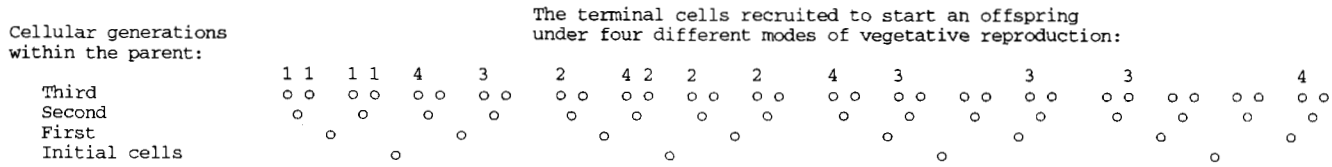

FIGURE 1.—Four modes of vegetative reproduction. The growth of a parental organism begins from its four initial cells (bottom row). After three cellular generations, 32 terminal cells of this organism are produced. Four of them must be recruited to initiate an offspring. Pattern of recruitment determines the mode of vegetative reproduction. Terminal cells that may initiate an offspring under different modes of vegetative reproduction are marked by the numbers corresponding to these modes. 1, False mode (all the recruited cells are related as closely as possible); 2, sectorial mode (the cells are recruited randomly from the descendants of the same parental initial cell); 3, random mode (the cells are recruited randomly from all terminal cells); and 4, structured mode (one cell is recruited randomly from the descendants of each parental initial cell).

During the growth period every initial cell of an organism goes through k cellular generations, to produce the "terminal" cells from which initial cells of the progeny will be recruited. The number of new deleterious mutations per initial cell per organismal generation (*i.e.*, from an initial cell of an organism to its descendent among terminal cells of the organism) has a Poisson distribution with mean U, the genomic deleterious mutation rate.

During vegetative reproduction each offspring is initiated from exactly n initial cells recruited from the terminal cells of a parental organism. Four possible modes of this recruitment will be considered [Figure 1, compare with TILNEY-BASSETT (1986) and KLEKOWSKY (1988)]. Under the false mode all initial cells of an offspring have a common ancestor shortly before vegetative reproduction. Under the sectorial mode all initial cells of an offspring are chosen randomly from all terminal cells which are descendants of one initial cell of a parent. Under the random mode all initial cells of an offspring are recruited randomly from all terminal cells. Under the structured mode one cell is recruited randomly from each group of terminal cells which originated from one parental initial cell.

If this model is applied to uniparental inheritance of organelles, n is the number of organelles transmitted to an offspring and U is the deleterious mutation rate per organelle genome per organismal generation. If the division of multinuclear cells is considered, n is the number of nuclei transmitted to a daughter cell and U is the deleterious mutation rate per nucleus per generation.

The fitness of an individual depends on the numbers of mutations in its initial cells. Efficiency of selection against mutations is measured by the mutation load (see CROW 1970) $L = 1 - \bar{W}/W_{max}$, where W_{max} is the fitness of mutation-free individuals (*i.e.*, of those which had no mutations in any of their n initial cells) and $\bar{W}$ is the mean population fitness in the mutation-selection equilibrium.

Analytical investigation of this model is described below. Besides, I have also created a simulation computer model. The population size was usually 10,000. Pseudorandom numbers were used to simulate mutation, vegetative reproduction and selection. To halt the operation of the Muller's ratchet, the random loss of all mutation-free initial cells, the number of mutations in one initial cell was artificially set to zero at the beginning of each generation, if mutation-free cells were absent. The program is written in MacFortran and is available on request.

GENERAL APPROACH

For asexual reproduction, which can be viewed as a special case of vegetative reproduction with $n = 1$, a simple way to find the mutation load was found by KIMURA and MARUYAMA (1966). They noticed that in an asexual population mutation-free progeny can appear, if reverse mutations are ignored, only to mutation-free parents with probability e^{-U}, *i.e.* when an offspring receives no new mutations. At equilibrium, selection must restore the frequency of mutation-free individuals, so that $W_{max}/\bar{W}$ must be e^{U} and $L = 1 - e^{-U}$. Of course, we have to assume that population indeed reaches mutation-selection equilibrium, which should be usually the case, unless the fitness declines unrealistically slowly when the number of mutations increases (KIMURA and MARUYAMA 1966).

This idea allows one to find immediately the mutation load under false (mode 1) and structured (mode 4) vegetative reproduction (Figure 1). False mode is genetically equivalent to apomixis, and $L = 1 - e^{-U}$ under any n, because, if we ignore mutations during the last few cellular generations, all initial cells of a progeny are identical. Under structured mode a progeny carrying no mutations in all its n initial cells appear only to the same parents with probability e^{-nU} (assuming that initial cells acquire mutations independently), and $L = 1 - e^{-nU}$.

Below I will apply the KIMURA-MARUYAMA approach to sectorial (mode 2) and random (mode 3) vegetative reproduction, which is more complicated. Here it is necessary to consider all individuals which had at least one deleterious-mutation-free cell among their n initial cells (I will call all such individuals "best"). A best individual with exactly i mutation-free initial cells ($i = 1, \ldots, n$) will be called "i-individual."

I will study the closed system of n equations connecting the numbers of i-individuals in the successive generations. This system includes a function $s(i)$, the relative fitness (either exact or average) of i-individuals. I will always assume that $s(i)$ is invariant. This is obviously

true if the fitness of an organism is completely determined by how many of its initial cells are mutation-free. Such situation is examined in the next section, where $s(i)$ is assumed known.

However, even if different i-individuals can have different fitnesses (depending on the number of mutations in their initial cells which are not mutation-free), in an equilibrium population they have some constant average fitnesses $s(i)$. Thus, consideration of the invariant $s(i)$ is still sufficient to find the load, which is a property of equilibrium populations. To do so in this situation, however, the equilibrium $s(i)$ must be estimated, which will be done later.

MUTATION LOAD UNDER GIVEN $s(i)$

Under both sectorial and random modes an offspring with any (including n) number of mutation-free initial cells can be produced by a parent which had any non-zero number of such cells among its initial cells. Still, best progeny originate only from best parents, which allows one to find the mutation load by mostly ignoring other individuals. This is essential because the whole population can be described only by the n-dimensional distribution and so makes its study difficult.

Under sectorial mode a j-parent produces an i-offspring with probability

$$a_{i,j} = \frac{j}{n}\binom{n}{i}(e^{-U})^i(1 - e^{-U})^{n-i} \qquad (1)$$

because all initial cells of an offspring are descendants of the same initial cell of a parent which was mutation-free with probability j/n, and each of them carries no mutations with probability e^{-U} (the assumption that initial cells of a progeny accumulate mutations independently is discussed below).

Similarly, under random mode

$$a_{i,j} = \binom{n}{i}\left(\frac{j}{n}e^{-U}\right)^i\left(1 - \frac{j}{n}e^{-U}\right)^{n-i} \qquad (2)$$

because an initial cell of an offspring carries no mutations with probability $(j/n)e^{-U}$.

Absolute fitnesses of i-individuals are $W_{\max}s(i)$, where $s(i)$ is a non-decreasing function and $s(n) = 1$. Then, their absolute numbers $N(i)$ in successive generations are connected by:

$$N_{t+1}(i) = W_{\max}s(i)\sum_{j=1}^{n} a_{i,j}N_t(j) \qquad (3)$$

In an equilibrium population $N_{t+1}(i) = W_{\max}\lambda N_t(i)$, where λ is the leading eigenvalue of matrix $\{(i)a_{i,j}\}$. As with asexual reproduction, this implies $W_{\max}\lambda = \overline{W}$ and $L = 1 - \lambda$, while the leading eigenvector v gives the equilibrium relative frequencies $p(i)$ of different i-individuals. Thus, we can find L and $p(i)$ for any n, U, $s(i)$ and $a_{i,j}$.

Here, in contrast with false and structured modes, the load depends on the form of selection, because λ grows if $s(i)$ increases for some i. Therefore, when $s(i) = 1$ for all i (*i.e.* if even a single mutation-free initial cell causes maximal fitness), the load is minimal. It is determined by the leading eigenvalue of matrix $\{a_{i,j}\}$ and can be easily shown to be $1 - e^{-U}$ under both sectorial and random modes, thus coinciding with the load under false mode. In this case under random mode best individuals are represented mostly by 1-individuals which produce the same or better progeny with probability e^{-U}, which leads to a relatively low load.

In contrast, when $s(i) = 1$ for $i = n$ and $s(i) = 0$ for $i \neq n$ (*i.e.*, if only n-individuals survive), the load is maximal and, as with structured mode, equals $1 - a_{n,n} = 1 - e^{-nU}$ under both sectorial and random modes. If, more realistically, $s(i)$ approaches 1 gradually as i grows from 1 to n and $s(1)$ is not too small, the load is slightly lower than $1 - s(1)e^{-U}$ and does not depend significantly on the exact shape of $s(i)$. Under random mode the load is always higher than under sectorial mode (data not presented).

Deriving the matrix $\{a_{i,j}\}$ we assumed that the initial cells of an organism accumulated mutations independently. Strictly speaking, this is true only under structured mode, where these initial cells are unrelated. However, it remains a good approximation under sectorial and random modes. Consider two initial cells of an offspring which are descendants of the same initial cell of a parent. If one of them is mutation-free, the other one is mutation-free with probability

$$P = \sum_{g=1}^{k}\frac{2^{g-1}}{2^k - 1}e^{-gU/k} = \frac{e^{-U/k}}{2e^{-U/k} - 1}\,\frac{2^k e^{-U} - 1}{2^k - 1}.$$

Derivation of this formula is simple. Two terminal cells had the last common ancestor g generations ago with probability $2^{g-1}/(2^k - 1)$, because 2^{g-1} terminal cells had the last common ancestor with a "marked" terminal cell g generations ago, and there are totally $2^k - 1$ terminal cells originated from the same initial cells as the marked cell and different from it. In this case the second cell is mutation-free with probability $e^{-gU/k}$, provided that the marked cell is mutation-free, because the probability not to acquire any mutation during one cellular generation is $e^{-U/k}$.

Thus, $P > e^{-U}$ if $k > 1$ and, particularly, the maximal load under sectorial and random modes is slightly less than $1 - e^{-nU}$, because $a_{n,n} > e^{-nU}$. However, when k grows, the expected number of cellular generations after the last common ancestor of the two cells makes a larger fraction of k, and P rapidly tends to e^{-U}. Simulations show that even under sectorial mode the assumption of independent accumulation of mutations does not alter the results substantially if $k > 4$–5 (data not presented). Under random mode the discrepancy is even smaller, because in this case different initial cells of an offspring can be descendants of different initial cells

of a parent and, thus, accumulate mutations completely independently.

MUTATION LOAD UNDER ESTIMATED $s(i)$

In reality, the fitness of an individual probably is not solely determined by the number of mutation-free cells among its initial cells. Therefore, $s(i)$ must be interpreted as the average fitness of i-individuals in an equilibrium population. We will assume that "real" selection $S(m)$ acts on the total number of mutations in all n initial cells of an organism, m. Thus, in order to find $s(i)$, we need to know $q_i(m)$, the equilibrium distribution of m in i-individuals. Genealogy of the initial cells which start a best individual is essential for this purpose.

Under sectorial mode one of the initial cells of a parent is the common ancestor for all n initial cells of a progeny (Figure 1). If at least one of the initial cells of a progeny is mutation-free, this common ancestor also must be a mutation-free initial cell, because backward mutations are ignored. If all n initial cells which start a progeny accumulated mutations independently (see above), each cell from $n - i$ non-mutation-free initial cells of an i-progeny ($i > 0$) has b mutations ($b \geq 1$) with the probability $h(b) = e^{-U}U^b/[b!(1 - e^{-U})]$ (the cell must carry at least one mutation, because otherwise we would be dealing with an $(i + 1)$-progeny). This distribution has mean $M[h] = U/(1 - e^{-U})$ and variance $\mathrm{Var}[h] = U/(1 - e^{-U}) - U^2e^{-U}/(1 - e^{-U})^2$. Thus, $q_i(m)$ has mean $Q(i) = (n - i)M[h]$ and variance $(n - i)\mathrm{Var}[h]$, and is approximately Gaussian if $n - i$ is large. Because $M[h]$ is of the order of 1 if U is small, and of the order of U if U is large, the typical number of mutations in 1-individuals (which have the highest m among best individuals) is approximately $n \max\{U, 1\}$.

If $S(m)$ decreases slowly enough with the growth of m, $s(i)$ is close to 1 for all i, and the mutation load is close to $L = 1 - e^{-U}$. Under sectorial mode this requires $S(n \max\{U, 1\}) \approx 1$. Than even 1-individuals have almost the highest fitness. Actually, a less restrictive condition $S(nU) \approx 1$ is also sufficient to make the load close to $1 - e^{-U}$: under small U the fitnesses of 1-individuals and other best individuals with a small number of mutation-free initial cells do not matter, because such individuals are very rare (data not presented).

Let us now consider random mode. Here an initial cell which is a common ancestor for all n initial cells of a best progeny also must be mutation-free. However, now different initial cells of a progeny may be descendants of different initial cells of a parent (Figure 1). Thus, the last common ancestor for all initial cells of a progeny can exist any number $T \geq 1$ of organismal generations before the one to which this progeny belongs.

The problem of finding the distribution of T is equivalent to finding the distribution of time which passes after all individuals in an asexual population with the effective size n had the last common ancestor. To be precise, these two problems are completely identical only if the number of terminal cells, from which initial cells of a progeny are recruited, is high enough to ignore the fact that there is no replacement when a cells is recruited. However, because the number of terminal cell is $n2^k$, the lack of replacement in recruiting n cells can be ignored even with small k.

If selection is absent, the expected value of T, $E(T)$, is known to be approximately $2n$ if n is large (KIMURA and OHTA 1969; BURROWS and COCKERHAM 1974, equations 3.8–3.10; KINGMAN 1982, pp. 28–29). If n is small, this is an overestimation, and for $n = 2$, where the probability of having the last common ancestor g generations ago is $(1/2)^g$, $E(T) = 2 = 1n$. The discrepancy, however, is not too large, and I will assume that $E(T) = 2n$. Then, $q_i(m)$ has mean $Q(i) = 2n(n - i)M[h]$ and variance $2n(n - i)V[h]$. Here the mean value of m for all best individuals is about $2n^2U$.

Thus, under random mode $s(i) \approx 1$ for all i and $L \approx 1 - e^{-U}$ if, with the growth of m, $S(m)$ decreases so slowly that $S(2n^2\max\{U, 1\})$ is still close to 1. In fact, as in sectorial mode, $S(2n^2U) \approx 1$ is sufficient for $L \approx 1 - e^{-U}$. Thus, if the average number of mutations acquired by n initial cells during 1 (sectorial mode) or $2n$ (random mode) generations does not cause a significant decrease of fitness, the load is close to that under false mode ("weak selection limit").

However, if $S(m)$ declines faster, sectorial and random modes lead to different loads. Under sectorial mode $q_i(m)$ does not depend on $S(m)$, so that $s(i)$ and the load can always be calculated, provided that $S(m)$ is known. In contrast, under random mode selection increases the share of those i-individuals whose ancestors carried fewer mutations and thus decreases $Q(i)$.

To deal with this case, I assume approximately that rare mutation-free initial cells are distributed in the population independently from the others ("strong selection limit"). Then, $Q(i) = \mu(1 - i/n)$, where μ is the population average of m. I also assume that $s(i) = S(Q(i))$, which is exactly correct only if within a class of i-individuals fitness depends on m linearly. Then, μ determines $s(i)$ and λ, and at equilibrium $W_{\max}\lambda(\mu) = S(\mu) = \bar{W}$. This equation has a single root $\hat{\mu}$ if $S(m)$ rapidly tends to 0 when m grows, because both $\lambda(\mu)$ and $S(\mu)$ are decreasing functions, $\lambda(0) = W_{\max}e^{-U} < S(0) = W_{\max}$, and with any $\mu\lambda(\mu) \geq e^{-nU}$. This allows one to find $L = 1 - \lambda(\hat{\mu})$ and $p(i)$ numerically. Note that if S is a function of cm ($c > 0$), $\lambda(\mu)$ does not depend on c, while $\hat{\mu}$ does.

Figure 2 shows the mutation load under four modes of obligate vegetative reproduction. The formulas $L = 1 - e^{-U}$ and $L = 1 - e^{-nU}$ were used for false and structured modes, respectively. For sectorial mode $s(i)$ was found directly from $S(m)$ and $q_i(m)$, and the load is only slightly higher than under false mode, because under the parameters chosen $S(nU) \approx 1$. In contrast, the

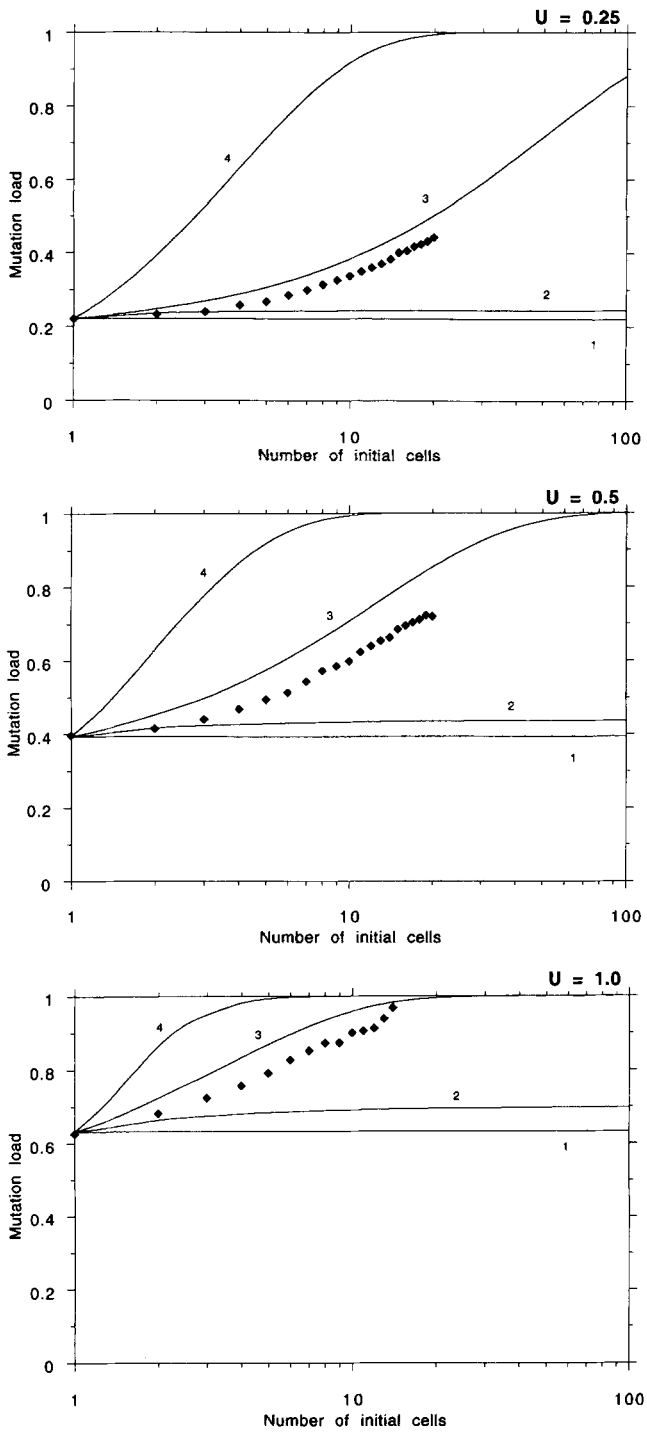

FIGURE 2.—Mutation load under false (1), sectorial (2), random (3), and structured (4) modes of vegetative reproduction with various U and n and $S(m) = \exp(-0.1(m/n) - 0.1(m/n)^2)$. Solid lines represent analytical estimates, and the results of simulations for the random mode are represented by the diamonds.

strong selection limit was used for random mode, because $S(2n^2U) \ll 1$. In addition, the results of simulations are presented for random mode. Clearly, the strong selection limit overestimates the load because in reality mutation-free cells tend to stay together and thus $Q(i) < \mu(1 - i/n)$. However, the discrepancy is not large. For three other modes, the results of simulations were very similar to analytical estimates (data not presented).

DISCUSSION

The results shown in Figure 2 demonstrate that obligate vegetative reproduction can lead to a much higher mutation load than asexual reproduction. The disadvantage of obligate vegetative reproduction grows with increasing number of initial cells n, of genomic deleterious mutation rate U, and of the stringency of selection against mutations (*i.e.*, when increase of the number of mutations in the initial cells of an organism causes more rapid decline of its fitness).

The degree of relatedness among n initial cells which start an organism is also important, and the load grows when the cells are less related (*i.e.*, from false to structured mode). Under false, sectorial, random and structured modes of vegetative reproduction (Figure 1) the expected number of organismal generations since all n initial cells had the last common ancestor, $E(T)$, equals 0, 1, $2n$ and ∞, respectively. Obligate vegetative reproduction leads to a higher load than asexual reproduction if the average number of mutations accumulated in n cells during $E(T)$ generations causes substantial decrease of fitness. This difference can be large if $Un > 1$.

Therefore, false mode does not cause any disadvantage of the obligate vegetative reproduction, sectorial mode leads only to a slight disadvantage, while random mode can be very disadvantageous, and structured mode causes the maximal disadvantage (Figure 2). This is certainly true under any conditions for false and structured modes, where the mutation load was calculated using explicit formulas. I believe that the results on sectorial and random mode are also typical, for the following reasons.

Simulations show (data not reported) that the variance of the distribution of the number of mutations per initial cell V_c is usually smaller than its mean μ/n (although the variance of m can be higher than μ, because numbers of mutations in initial cells of an organism are positively correlated). Thus, if $\mu/n > \sim 10$, the expected number of mutation-free initial cells is very small even for all organisms of a large population. Therefore, all such cells (or, more generally, all initial cells with the minimal number of mutations available) can be lost due to random drift, and this process, analogous to Muller's ratchet, goes fast (HAIGH 1978; STEPHAN *et al.* 1993). This was confirmed by simulations. Because μ is larger than the value at which $S(m)$ starts to decline rapidly, in a population protected from the ratchet $s(i) \approx 1$ for all i requires $U < \sim 10$ under sectorial mode or $2nU < \sim 10$ under random mode.

This is not restrictive for sectorial mode because with $U \gg 1$ even the minimal load is too high anyway, while with random mode this requires too small U when n is

large. Therefore, under sectorial mode the "weak selection limit" is probably applicable in most cases, which leads to a load only slightly higher than with asexual reproduction. In contrast, under random mode and substantial n the "strong selection limit" seems more realistic, which leads to a much higher load.

I have ignored intraorganismal selection among cells or intracellular selection among the organelles, which can reduce the load (KLEKOWSKI 1988). If such selection has maximal efficiency, *i.e.*, if in the organisms (cells) with at least one mutation-free initial cell (organelle) only such cells (organelles) can be recruited to start an offspring (to be transmitted to daughter cells), it can completely abolish the increase of the mutation load caused by vegetative reproduction. Currently, however, there are no data which would indicate that intraorganismal (intracellular) selection against slightly deleterious mutations is nearly that efficient. Thus, at least occasional passages of the genetic material through single-cellular channels seem necessary, either regularly during asexual or sexual reproduction, or randomly with obligate vegetative reproduction.

Implications for the evolution of vegetative reproduction: Provided that U is about 1 in multicellular organisms, as is probable (see KONDRASHOV 1988; CHARLESWORTH *et al.* 1990; HOULE *et al.* 1992; AGREN and SCHEMSKE 1993), the inefficiency of selection against deleterious mutations may be an important factor limiting the spread of obligate vegetative reproduction. Let us compare the theoretical predictions with what is known about obligate vegetative reproduction in nature.

In algae it is apparently unknown. In contrast, in many populations (and, perhaps, "species") of bryophytes, sporophytes are completely absent, and gametophytes reproduce only vegetatively by fragmentation of body and by special propagules, gemmae (see MILES and LONGTON 1990; MISHLER 1990). However, gemmae usually contain only few cells and, even more important, a gemma is always initiated by a single cell (WATSON 1967, p. 97). Thus, in this case the obligate vegetative reproduction is false. Besides, only one apical cell divides in meristems of bryophytes (PAOLILLO 1984, p. 125), so that even when an entire branch of a moss gives rise to a new organism, the vegetative reproduction is still false.

Among lower vascular plants several species of homosporic ferns in eastern United States are represented only by gametophytes and reproduce only vegetatively by gemmae, perhaps for millions of years (FARRAR 1990; RAINE *et al.* 1991). Again, gemmae consist of a small number of cells (STOKEY and ATKINSON 1958; FARRAR and WAGNER 1968) and a gemma usually develops from a single cell (STOKEY 1948; FARRAR 1974, 1990). As in bryophytes, a meristem in ferns usually has only a single dividing cell (see KLEKOWSKI 1988).

In seed plants, obligate vegetative reproduction is unknown among gymnosperms, while several known cases in angiosperms are of relatively recent origin, perhaps no older than thousands of years (see GUSTAFSSON 1946; ELLSTRAND and ROOSE 1987, Table 1). Other examples include northern populations of the birch *Betula glandulosa* (HERMANUTZ *et al.* 1989), some populations of trembling aspen *Populus tremuloides* (JELINSKI and CHELIAK 1992), the intergeneric hybrid grass *Poa labradorica* (DARBYSHIRE *et al.* 1992), and the endemic lily *Erythronium propullans* (PLEASANTS and WENDAL 1989). Perhaps in all these cases vegetative reproduction is not false, because angiosperms have multicellular meristems (see KLEKOWSKI 1988; SCHMID 1990).

Several forms of fungi are not known to produce any spores, either sexually or asexually. They are placed in the artificial taxon *Mycelia Sterilia* (see TALBOT 1971). However, some produce specialized vegetative propagules, sclerotia, that are always initiated by a single cell (BUTLER 1966, Figure 4). Probably, some representatives of *Mycelia Sterilia* can, in fact, produce spores under appropriate conditions, while some truly sterile forms may be still undiscovered. However, in fungi even vegetative reproduction by fragmentation of a mycelium is false, because hyphae are filaments of individual cells.

Many populations of lichens reproduce only vegetatively by propagules called soredia and isidia. A majority of them are probably of relatively recent origin, because the related forms with sexual reproduction also exist, usually within a smaller geographical range (BOWLER and RUNDEL 1975; TEHLER 1982). However, in some cases a whole distinct "species" of lichens apparently reproduces only vegetatively. It seems that soredia and isidia are formed by many cells; at least no single precursor cells are obvious (JAHNS 1973, Figures 92–107). Still, because lichen thalli are based on fungal mycelia which consist of branching hyphae and not of three-dimensional tissue (JAHNS 1988), adjacent hyphae in the thallus can have the same ancestral cell within this thallus. If so, formation of soredia and isidia may represent a case of sectorial vegetative reproduction. This is worth studying.

Few cases of obligate vegetative reproduction have been reported in animals, and sometimes it is not clear whether sexual or asexual reproduction is really completely absent (JACKSON *et al.* 1985). Still, only vegetative reproduction is known for North American populations of the sea anemone *Haliplanella lineata* (see HUGHES 1989, p. 183), for several populations of flatworms from family Planariidae, for many forms of freshwater oligochaetes from families Aelosomatidae and Naididae and the soil oligochaete *Enchytraeus fragmentosus* (Enchytraeidae) (BELL 1959), and for one form of polychaetes, *Zeppelina* [see BELL (1982, Ch. 3) for an excellent review]. In all these cases obligate vegetative reproduction is of obviously recent origin. Probably no unicellular channels separate a vegetatively produced offspring from its parent in animals.

Thus, the general tendency seems clear. Obligate vegetative reproduction may be common (bryophytes and, perhaps, lichens) or ancient (homosporic ferns) in the taxa where propagules develop from single cells or unicellular channels frequently appear during the growth of an individual. In contrast, if propagules are initiated by many cells and no unicellular channels appear during growth, obligate vegetative reproduction in rare and of recent origin (angiosperms and animals).

This tendency is consistent with my analysis, because the conditions favoring obligate vegetative reproduction are those which diminish the mutation load to its value under asexual reproduction, $L = 1 - e^{-U}$, effectively making $n = 1$. However, even in angiosperms and animal populations with obligate vegetative reproduction sometimes seem to exist for a considerable time. Does this mean that they still have not reached mutation-selection equilibrium? Or even in these taxa do some unknown mechanisms ensure occasional passage of the genetic material through unicellular channels during the growth of an organism? Or could intraorganismal selection be important? We do not know, and only experiments can answer these questions.

Implications for the evolution of organelle genomes: Data from sequence comparisons (BROWN *et al.* 1986) suggests that in the mammalian mitochondrial genome $U \sim 10^{-3}$ while the number of mitochondria in an egg can be much larger than 10^3. Thus, $nU \gg 1$, which can lead to a high mutation load. Therefore, bottlenecks in the number of organelles in the female germ line, before the formation of eggs, can be significantly beneficial. Furthermore, the transfer of genes from organelles to the nucleus can reduce the mutation load because a piece of DNA in an organelle genome can cause a much higher load than in the nucleus.

The following succession of events seems plausible. (1) A copy of organelle gene is randomly transferred to a nucleus, which may happen relatively frequently (MARTIN *et al.* 1993). However, this transferred copy should initially work rather poorly, if at all, because of new genetic environment. (2) Mutations which improve functioning of this copy in the nucleus are selected, because they make mutations in the corresponding organelle gene less deleterious and reduce the mutation load. (3) Mutations which impair functioning of the organelle gene accumulate, because they are not selected against any more. Thus, after a while, the organelle gene becomes non-functional. (4) Deletions in the resulting pseudogene are selected, probably because of competition between organelles for more rapid DNA replication. This completes the transfer of a gene from organelle to nuclear genome.

The current model does not allow one to consider the mutation load under biparental transmission of organelles. Presumably, however, in this case the load is lower, because mixing of organelles from different sources should cause an increase in the variance. If so, the evolution of anisogamy must be driven by other factors, different from the reduction of the mutation load. Probably, even rare paternal transmission of organelles can reduce the load in anisogamous organisms.

If a cell has organelles of several types (or organelles of the same type that always divide simultaneously), this is equivalent to the structured mode of vegetative reproduction, which causes the highest load. Thus, increased mutation load could limit the number of different symbionts in early evolution of eukaryotes.

Implications for the evolution of reproduction by multinuclear cells: Several cases of multinuclearity in apparently obligately asexual organisms are known. A primitive ameba *Pelomyxa palustris* (*Karyoblastea*) usually has thousands of nuclei per cell. In the fall and in the spring, however, the cells undergo plasmotomy, which leads to production of many mononucleate cells (see MARGULIS *et al.* 1990, p. 174). Similar karyonic cycles are possible in many other multinuclear amoebas, currently classified as *Rhyzopoda* (WILLUMSEN *et al.* 1987). At least in some cases a wide variation in the number of nuclei per cell was noticed (SERAVIN and GUDKOV 1985). Obviously, bottlnecks in the number of nuclei per cell increase the relatedness among the nuclei in a cell and thus reduce the mutation load, making the mode of nuclear transmission closer to false vegetative reproduction. In contrast, the worst mode of the nuclear transmission would be if all the nuclei always divide simultaneously, which is analogous to structured vegetative reproduction. Ploidy cycles, where a cell always has a single nucleus, but its ploidy varies with time (see RAIKOV 1982) also can reduce the mutation load, compared with permanent diploidy or polyploidy, due to a similar mechanism (A. S. KONDRASHOV, submitted for publication).

This work was inspired by discussions with J. F. CROW, assisted by advice of M. TURELLI, R. BÜRGER, J. WILLIS, C. D. HARVELL and B. NORMARK, and supported by the National Institutes of Health grant GM 36827 to M. LYNCH.

LITERATURE CITED

AGREN, J., and D. SCHEMSKE, 1993 Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. Evolution **47:** 125–135.

BELL, A. W., 1959 *Enchytraeus fragmentosus*, a new species of naturally fragmenting oligochaete worm. Science **129:** 1278.

BELL, G., 1982 *The Masterpiece of Nature*. University of California Press, Berkeley.

BIRKY, C. W., P. FUERST and T. MARUYAMA, 1989 Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics **121:** 613–627.

BOWLER, P. A., and P. W. RUNDEL, 1975 Reproductive strategies in lichens. Bot. J. Linn. Soc. **70:** 325–340.

BROWN, G. G., G. GALADETA, G. PEPE, C. SACCONE and E. J. SBISA, 1986 Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. J. Mol. Biol. **192:** 503–511.

BURROWS, P. M., and C. C. COCKERHAM, 1974 Distributions of time to fixation of neutral genes. Theor. Popul. Biol. **5:** 192–207.

BUTLER, G. M., 1966 Vegetative structures, pp. 83–112 in *The Fungi*, Vol. II, edited by G. C. AINSWORTH and A. S. SUSSMAN. Academic Press, New York.

CHARLESWORTH, B., D. CHARLESWORTH and M. T. MORGAN, 1990 Genetic loads and estimates of mutation rates in highly inbred plant populations. Nature **347:** 380–382.

CROW, J. F., 1970 Genetic loads and the cost of natural selection, pp. 128–177 in *Mathematical Topics in Population Genetics*, edited by K. KOJIMA. Springer, Heildelberg.

CROW, J. F., 1988 The importance of recombination, pp. 56–73 in *The Evolution of Sex: An Examination of Current Ideas*, edited by R. E. MICHOD and B. R. LEVIN. Sinauer, Sunderland.

DARBYSHIRE, S. J., J. CAYOUETTE and S. I. WARWICK, 1992 The intergeneric hybrid origin of *Poa labradorica* (*Poaceae*). Plant Syst. Evol. **181:** 57–76.

ELLSTRAND, N. C., and M. L. ROOSE, 1987 Paterns of genotypic diversity in clonal plant species. Am. J. Bot. **74:** 123–131.

FARRAR, D. R., 1974 Gemmiferous fern gametophytes–*Vittariaceae*. Am. J. Bot. **61:** 146–155.

FARRAR, D. R., 1990 Species and evolution in asexually reproducing independent fern gametophytes. Syst. Bot. **15:** 98–111.

FARRAR, D. R., and W. H. WAGNER, 1968 The gametophyte of *Trichomanes holopterum* Kunze. Bot. Gaz. **129:** 210–219.

GUSTAFFSON, A., 1946–1947 *Apomixis in Higher Plants*. Lunds Universitets Arsskrift, Lund.

HAIGH, J., 1978 The accumulation of deleterious genes in a population– Muller's ratchet. Theor. Popul. Biol. **14:** 251–267.

HERMANUTZ, L. A., D. J. INNES and I. M. WEIS, 1989 Clonal structure of arctic dwarf birch (*Betula glandulosa*) at its northern limit. Am. J. Bot. **76:** 755–761.

HOULE, D., D. K. HOFFMASTER, S. ASSIMACOPOULOS and B. CHARLESWORTH, 1992 The genomic mutation rate for fitness in *Drosophila melanogaster*. Nature **359:** 58–60.

HUGHES, R. N., 1989 *A Functional Biology of Clonal Animals*. Chapman & Hall, London.

JACKSON, J. B. C., L. W. BUSSAND and R. E. COOK, 1985 *Population Biology and Evolution of Clonal Organisms*. Yale University Press, New Haven, Conn.

JAHNS, H. M., 1973 Anatomy, morphology, and development, pp. 3–58 in *The Lichens*, edited by V. AHMADJIAN and M. E. HALE. Academic Press, New York.

JAHNS, H. M., 1988 The lichen thallus, pp. 95–143 in *Handbook of Lichenology*, Vol. I, edited by M. GALUN. CRC Press, Boca Raton, Fla.

JELINSKI, D. E., and W. H. CHELIAK, 1992 Genetic diversity and spatial subdivision of *Populus tremuloides* (*Salicaceae*) in a heterogeneous landscape. Am. J. Bot. **79:** 728–736.

KEMPERMAN, J. A., and B. V. BARNES, 1976 Clone size in American aspens. Can. J. Bot. **54:** 2603–2607.

KIMURA, M., and T. MARUYAMA, 1966 The mutation load with epistatic gene interactions in fitness. Genetics **54:** 1337–1351.

KIMURA, M., and T. OHTA, 1969 The average number of generations until fixation of a mutant gene in a finite population. Genetics **61:** 763–771.

KINGMAN, J. F. C., 1982 On the genealogy of large populations. J. Appl. Probab. **19A:** 27–43.

KLEKOWSKI, E. J., 1988 *Mutation, Developmental Selection, and Plant Evolution*. Columbia University Press, New York.

KONDRASHOV, A. S., 1988 Deleterious mutations and the evolution of sexual reproduction. Nature **336:** 435–440.

KONDRASHOV, A. S., 1993 Classification of hypotheses on the advantage of amphimixis. J. Hered. **84:** 372–387.

MARGULIS, L., J. O. CORLISS, M. MELKONIAN and D. J. CHAPMAN (Editors), 1990 *Handbook of Protoctista*. Jones & Bartlett, Boston.

MARTIN, W., H. BRINKMANN, C. SAVONNA and R. CERFF, 1993 Evidence for a chimeric nature of nuclear genomes: eubacterial origin of eukaryotic glyceraldehyde-3-phosphate dehydrogenase genes. Proc. Natl. Acad. Sci. USA **90:** 8692–8696.

MAYNARD SMITH, J., 1989 *Evolutionary Genetics*. Cambridge University Press, Cambridge.

MISHLER, B. D., 1990 Reproductive biology and species distinctions in the moss genus *Tortula*, as represented in Mexico. Syst. Bot. **15:** 86–97.

MILES, C. J., and R. E. LONGTON, 1990 The role of spores in reproduction in mosses. Bot. J. Linn. Soc. **104:** 149–173.

PAOLILLO, D. J., 1984 Cell and plastid cycles, pp. 117–142 in *The Experimental Biology of Bryophytes*, edited by A. F. DYER and J. G. DUCKETT. Academic Press, London.

PLEASANTS, J. M., and J. F. WENDAL, 1989 Genetic diversity in a clonal narrow endemic, *Erythronium propullans*, and in its widespread progenitor, *Erythronium albidum*. Am. J. Bot. **76:** 1136–1151.

RAIKOV, I. B., 1982 *The Protozoan Nucleus*, Springer, Berlin.

RAINE, C. A., D. R. FARRAR and E. SHEFFIELD, 1991 A new *Hymenophyllum* species in the Appalachians represented by independent gametophyte colonies. Am. Fern J. **81:** 109–118.

SCHMID, B., 1990 Some ecological and evolutionary consequences of modular organization and clonal growth in plants. Evol. Trends Plants **4:** 25–34.

SERAVIN, L. N., and A. V. GUDKOV, 1985 Similarity and difference between two marine limax amoebae, *Gruberella flavescens* and *Euhyperamoeba fallax* (*Lobosea, Gymnamoebia*). Zool. Zh. **64:** 1090–1093 (in Russian).

SMITH, M. L., J. N. BRUHN and J. B. ANDERSON, 1992 The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature **356:** 428–431.

STEPHAN, W., L. CHAO and J. G. SMALE, 1993 The advance of Muller's ratchet in a haploid asexual population: approximate solutions based on diffusion theory. Genet. Res. **61:** 225–231.

STOKEY, A. G., 1948 Reproductive structures of the gametophytes of *Hymenophyllum* and *Trichomanes*. Bot. Gaz. **109:** 363–380.

STOKEY, A. G., and L. R. ATKINSON, 1958 The gametophyte of the *Grammitidaceae*. Phytomorphology **8:** 391–403.

TALBOT, P. H. B., 1971 *Principles of Fungal Taxonomy*. Macmillan, London.

TEHLER, A., 1982 The species pair concept in lichenology. Taxon **31:** 708–717.

TILNEY-BASSETT, R. A. E., 1986 *Plant Chimeras*. Arnold, London.

WATSON, E. V., 1967 *The Structure and Life of Bryophytes*. Hutchinson University Library, London.

WILLUMSEN, N. B. S., F. SIEMENSMA and P. SUHR-JESSEN, 1987 A multinucleate amoeba, *Parachaos zoochlorellae* (Willumsen 1982) comb. nov., and a proposed division of the genus *Chaos* into the genera *Chaos* and *Parachaos* (*Gymnamoebia, Amoebidae*). Arch. Protistenk. **133:** 199–217.

Communicating editor: A. G. CLARK