

Mutation, Multilevel Selection, and the Evolution of Propagule Size during the Origin of Multicellularity

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ABSTRACT: Evolutionary transitions require the organization of genetic variation at two (or more) levels of selection so that fitness heritability may emerge at the new level. In this article, we consider the consequences for fitness variation and heritability of two of the main modes of reproduction used in multicellular organisms: vegetative reproduction and single-cell reproduction. We study a model where simple cell colonies reproduce by fragments or propagules of differing size, with mutations occurring during colony growth. Mutations are deleterious at the colony level but can be advantageous or deleterious at the cell level (“selfish” or “uniformly deleterious” mutants). Fragment size affects fitness in two ways: through a direct effect on adult group size (which in turn affects fitness) and by affecting the within- and between-group variances and opportunity for selection on mutations at the two levels. We show that the evolution of fragment size is determined primarily by its direct effects on group size except when mutations are selfish. When mutations are selfish, smaller propagule size may be selected, including single-cell reproduction, even though smaller propagule size has a direct fitness cost by virtue of producing smaller organisms, that is, smaller adult cell groups.

Keywords: levels of selection, mutation load, multicellularity, evolutionary transitions, altruism, endosymbiosis.

Ever since Darwin, we have understood that evolution by natural selection requires heritable variation in fitness. Evolutionary transitions to a new, higher level of selection require the reorganization of genetic variation at two (or more) levels so that heritable variation in fitness may emerge at the new level of organization. For example, in

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the case of the transition from single cells to multicellular organisms, single cells must, as it were, relinquish their claim to flourish and multiply in favor of the multicellular group.

Mutation

Mutation is a source both of novelty and ruin for living systems. Ultimately, all prospects for evolutionary advance rely on mutation, yet, in the short run, most mutations are deleterious. During the origin of multicellular groups, mutation enhances the scope for selection and conflict both within and between groups of cells. Our general working hypothesis is that coping with mutation in a multi-level context was a fundamental issue during evolutionary transitions.

Because of the hierarchical nature of selection during evolutionary transitions, there are at least two levels of selection at which to consider mutational effects (e.g., in the case of the origin of multicellularity, the fitness of the cell and the fitness of the cell group [emerging organism]). Deleterious mutation may detract from both the fitness of the group and the fitness of component cells. Such mutations are termed “uniformly deleterious” since they are deleterious at both levels of selection. The evidence for uniformly deleterious mutations has recently been reviewed in the case of multicellular organisms (Otto and Hastings 1998). For uniformly deleterious mutations, the occurrence of selection among cells may have the benefit of lowering the overall mutation load in the population of organisms, and this effect has been considered by several authors (Crow 1970; Whitham and Slobodchikoff 1981; Otto and Orive 1995; Otto and Hastings 1998; Michod and Roze 1999). The converse of uniformly deleterious mutations are “uniformly advantageous” mutations that benefit both the cell and the group or organism.

“Selfish mutations” benefit the cell’s replication rate but detract from group fitness. There is considerable evidence that selfish mutations threaten the individual integrity of extant organisms. In multicellular organisms, selfish mutations in somatic cells affect critical genes that regulate

cell proliferation and survival and, in turn, cause cancer. Both somatic mutation (Nowell 1976; Dennis et al. 1981; Farber 1984; Temin 1988; Blumenthal 1992; Ramel 1992; Coppes et al. 1993; Hague et al. 1993; Ionov et al. 1993; Kupryjanczyk et al. 1993; Chigira and Watanabe 1994; Miyaki et al. 1994; Nielsen et al. 1994; Shibata et al. 1994; Akopyants et al. 1995; Hoff-Olsen et al. 1995; Talbot et al. 1995; Tsiotou et al. 1995) and within-organism selection (Nowell 1976; Dennis et al. 1981; Michelson et al. 1987; Temin 1988; Gatenby 1991) are critical in the development of many human cancers. Repair, arrest of cell division, and cell suicide are all active, genetically programmed responses to selfish mutations. The widespread existence of programmed cell death (apoptosis) is testimony to the significant threat selfish mutations pose to organism fitness (Evan and Littlewood 1998). If selfish mutations (mutations leading to cell proliferation at the expense of the organism) were not a common threat to organismal fitness, adaptive responses such as programmed cell death and programmed arrest of cell division would not have evolved.

While there is considerable evidence that selfish mutations are relevant to modern metazoans, it is also likely that they are relevant to the early stages of evolutionary transitions, when existing functions of lower-level units (cells) are directed to benefit the group. For free-living cells, life-history evolution will optimally allocate the time and energy of cells to conflicting life-history functions (such as motility and replication), and loss of function mutations would be deleterious. However, in a group setting, cell behaviors and activities will (in conditions conducive to effective group selection) be adjusted away from the cell optimum toward levels that are more beneficial to the group (thereby costing the cell). Loss of function mutations in conflicting life-history components, say, for the capacity of a cell to become motile (which conflicts with cell replication), would then be selfish since the cell would benefit at the expense of the group because the cell can now spend more time replicating (so long as there are other cooperative cells in the group providing the needed function, which, in this case, is motility). For this reason, we believe that selfish mutations are of general relevance to evolutionary transitions. Indeed, one of the central results of this article is that had it not been for the occurrence of some selfish mutations, the single-cell stage of the life cycle (so prevalent in modern organisms) would not have evolved as a means to mediate conflict by ensuring high kinship among cells. The converse of selfish mutations are "altruistic mutations" that detract from cell fitness but benefit the organism. The general hypothesis explored in this article is that coping with different kinds of mutation in a multilevel context (uniformly deleterious, uniformly

advantageous, selfish, and altruistic) was a fundamental issue during evolutionary transitions.

Reproductive Mode

Multicellular organisms reproduce in several ways: by fragmentation, by aggregation, or by single-cell reproduction (involving, perhaps, a zygote, spore, or stem-cell stage). These basic reproductive modes may involve asexual and/or sexual life cycles. In this article, we consider vegetative reproduction, where the offspring originates from a group of cells taken from the adult (a propagule), and single-cell reproduction, where development starts from only a single cell. While vegetative reproduction is always asexual, single-cell reproduction can be sexual (the original cell comes from the fusion of two gametes and is called a "zygote") or asexual (in that case, the primordial cell is called a "spore"). Indeed, an alternative hypothesis for passing the life cycle through a single-cell stage is for sexual fusion with another such cell from another organism. However, there are many multicellular forms that use a single cell during asexual reproduction, such as *Volvox* and other Volvocales, cellular slime molds, myxobacteria, and many plants that reproduce through apomictically produced seeds. We explore the evolution of vegetative and single-cell reproduction here in the context of their effects on fitness and heritability at a new level of selection and individuality: the multicellular organism.

Vegetative reproduction is widespread in plants and involves different mechanisms, including fragmentation, production of specialized propagules (gemmae of bryophytes and pteridophytes), creeping stems (runners, stolons, rhizomes, etc.), and modified shoot bases (bulbs). Usually, this mode of reproduction is facultative and coexists with single-cell reproduction; however, some cases of obligate vegetative reproduction are known (see references in Kondrashov 1994). In animals, vegetative reproduction occurs mainly in invertebrates by diverse mechanisms (fragmentation, laceration, budding, fission, etc.; Hughes 1989). Some rare cases of obligate vegetative reproduction have been reported (Bell 1959; Hughes 1989; Åkesson and Rice 1992). In fungi and lichens, vegetative reproduction occurs by fragmentation and by the means of specialized organs called soredia and isidia.

Kondrashov (1994) developed a model to study the consequences of obligate vegetative reproduction on the mutation load (reduction in the average fitness of a population due to deleterious mutations) when selection occurs only at the organism level and not among cells within the organism. In his model, the development of an organism starts from a propagule made of N cells. A main result of Kondrashov's article is that the equilibrium mutation load increases as propagule size (N) increases and as the re-

latedness among the cells forming a propagule decreases (this relatedness depends on the way cells are sampled to form propagules). Intuitively, when propagule size is large or when relatedness among cells within propagules is low, the genetic variance between offspring is low, and therefore individual selection is rather inefficient at eliminating deleterious mutations. As propagule size decreases, the variance between offspring increases, and selection against mutants at the organism level becomes more efficient. In other words, mutant cells are better eliminated when they are not mixed with nonmutant cells in propagules.

Otto and Orive (1995) extended Kondrashov's model by including selection among cells within the organism. Mutations can have an effect on the rate of replication of cells during the development, and this allows a better elimination of deleterious mutations if they are also deleterious at the cell level. Otto and Orive showed that cell selection can reverse the effect of propagule size on the mutation load; when cell selection is more efficient than organism selection at eliminating mutants, the mutation load decreases as propagule size increases.

Individuality

We have been interested in the evolutionary transition from unicellular to multicellular organisms and the emergence of individuality at the level of the organism (Michod 1996, 1997*a*, 1997*b*, 1999; Michod and Roze 1997). During the first stages of the evolution of multicellularity, the fitness interests of cells were likely not aligned with the interests of the cell group, and this would have generated conflicts between the cell and organism levels (Buss 1987; Michod 1997*a*). For example, there was probably a trade-off between motility and reproduction in the life histories of the cells that formed the first metazoans (Margulis 1981) due to a limited number of microtubule organizing centers per cell (a cell could not build a mitotic spindle and have a flagellum at the same time). During unicellular life, cells probably switched between motile and reproductive stages depending on environmental conditions. In a multicellular context, a cell that would remain mitotic and never differentiate into a motile cell (or have a lower probability of differentiating) would increase in frequency within the group, reducing the fitness of the group (assuming that motility is advantageous for the group). Conflict between levels of selection may have occurred often during the evolution of higher levels of complexity (Maynard Smith and Szathmáry 1995; Keller 1999). The resolution of such conflicts involves the evolution of modifiers increasing the strength of selection at the higher level, relative to the lower level. This can be achieved by modifying the variance in fitness and/or the heritability of fitness at the two levels of selection. For example, increasing the relatedness among

group members increases the opportunity for selection between groups relative to selection within groups (by increasing the between-group variance and decreasing the within-group variance). Increasing the heritability of fitness at the higher level can have the same result; for example, we have shown previously that the early segregation of a germ line during ontogeny increases the strength of selection between organisms by increasing heritability at the organism level (if mutations are less frequent in the germ line than in the soma; Michod 1996; Michod and Roze 1997).

Multicellularity probably evolved because of advantages for cells of group living (ability to catch bigger prey, better avoidance of predation, a buffered environment within the group, etc.). However, most multicellular organisms begin their life cycle as a single cell. If group living is so advantageous, why are multicellular organisms going back to a unicellular stage at the start of each generation? A common hypothesis is that this unicellular bottleneck acts as a conflict mediator by increasing the kinship among cells in the organism and aligning the interests of cells with the interest of the organism (Bell and Koufopanou 1991; Maynard Smith and Szathmáry 1995; Grosberg and Strathmann 1998). Primitive cell groups (or cell colonies) probably reproduced by fragmentation like present colonial Choanoflagellates (Leadbeater 1983). In this article, we study the evolution of propagule size in the context of two selective factors: mutation load and adult organism size. As we discuss here, the way in which these factors select for propagule size is reminiscent of the way in which deleterious mutation selects for another aspect of the reproductive system: sexual versus asexual reproduction (Kondrashov 1988).

Model

Life Cycle

The model life cycle is represented in figure 1. Development starts from a group of N cells. Mutation occurs during development at a rate μ per cell division. These mutants have a deleterious effect on the fitness of the group (parameter β ; see below).

During the development of the organism, nonmutant cells replicate at a rate c , which is fixed at 1 in this article, without loss of generality. Mutant cells replicate at a rate bc ; if $b > 1$, mutants are selfish (they increase in frequency in the group but decrease the fitness of the group), while if $b < 1$, mutants are uniformly deleterious and are disadvantaged both at the cell and the organism levels. After development, each organism generates propagules of N cells. In this model, adult size is not fixed but depends on rates of cell division (b , c) and time available for devel-

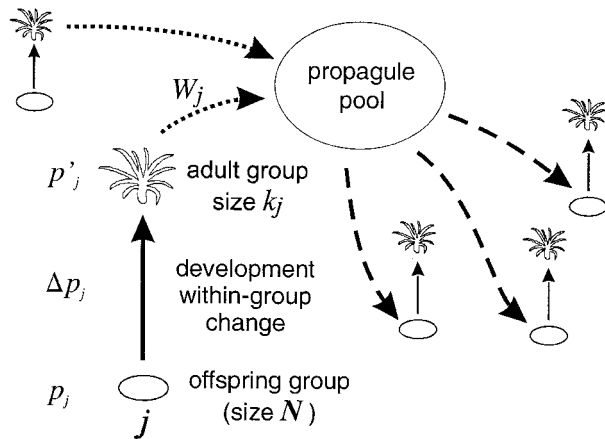


Figure 1: Model life cycle. In the case of single-cell reproduction, the offspring “group” is just one cell. The subscript j refers to the number of mutant cells in the offspring group; $j = 0, 1, 2, \dots, N$, where N is the total number of cells in the offspring group (termed propagule size; $N = 1$ for single-cell reproduction). The variables k_{mj} and k_j refer to the number of mutant cells and the total number of cells, respectively, in the adult cell group (i.e., after development) produced by an offspring group of type j . The variables p_j ($p_j = j/N$) and p'_j ($p'_j = k_{mj}/k_j$) refer to the frequency of mutant cells within the cell group before and after development, respectively, with $\Delta p_j = p'_j - p_j$ being the change in mutant frequency within the cell group during development. W_j is the fitness of group j , defined as the expected number of propagules produced by the group (dotted arrows). Two components of group fitness are considered: the size of the adult group and its functionality (parameters α and β ; see text). The propagules then form offspring groups of the next generation (dashed arrows). The basic terms and variables are given in table 1.

opment (t). The fitness of the organism is the expected number of propagules it produces; this depends both on the size of the organism and on the frequency of mutant cells in the adult, as explained in the section entitled “Selection at the Organism (Cell-Group) Level.”

A propagule of type j is a propagule containing j mutant cells. Let k_{mj} be the number of mutant cells in the adult form of an organism of type j and k_j be the total number of cells in this organism. The calculation of these k variables is given below. The variable $p_j = j/N$ is the frequency of mutants in a propagule of type j , while $p'_j = k_{mj}/k_j$ is the frequency of mutants in the adult form.

During reproduction, the N cells forming a propagule are sampled from the cells in the adult. Kondrashov (1994) considered four different modes of sampling that led to different values of the relatedness among cells in a propagule. In this article, we will consider only the “random mode,” where the N cells are sampled randomly in the adult. Let f_{ij} be the expected frequency of propagules of type i among all the propagules produced by an organism of type j as given by equation (1):

$$f_{ij} = \binom{N}{i} (p'_j)^i (1 - p'_j)^{(N-i)}. \quad (1)$$

A hypergeometric distribution (sampling without replacement) would be more appropriate, but here the binomial is a good approximation.

Let x_j be the number of propagules of type j (with j mutant cells) in the whole population (whose size is assumed to be very big) at the beginning of a generation. The dynamics of the system are given by equation (2):

$$\mathbf{x}_{t+1} = \mathbf{A}_N \mathbf{x}_t, \quad (2)$$

where \mathbf{x}_t is the vector $(x_0, x_1, x_2, \dots, x_N)$ at generation t , \mathbf{A}_N is the $(f_{ij} W_j)$ matrix, and W_j is the fitness of an organism of type j as defined below. We have not solved this system to find an expression for the equilibrium in the general case (N not fixed), but the equilibrium distribution of the different types of propagules in the population is given by the first right eigenvector of the \mathbf{A}_N matrix. Furthermore, the equilibrium rate of growth of the population is given by λ_N , the first eigenvalue of \mathbf{A}_N (Caswell 1989). This gives us a measure of the average fitness of the population at equilibrium, and therefore the equilibrium mutation load is given by $L = 1 - \bar{W}/W_{\max} = 1 - \lambda_N/W_0$, where W_0 is the fitness of an organism starting its development from a propagule without any mutant cells. Kondrashov (1994) used the same method to calculate the load in his model of obligate vegetative reproduction.

Selection at the Organism (Cell-Group) Level

Multicellularity evolved initially because of advantages for cells due to living in groups. We consider two distinct aspects of group living in the determination of group fitness: group size and group functionality. We take the fitness of the multicellular group or organism (W_j) to be the expected number of offspring propagules it produces. We assume that fitness depends on the size of the adult organism (total number of cells in group after development) and the frequency of mutant cells (mutation is assumed to detract from the functionality of the group) as given in equation (3):

$$W_j = \frac{k_j^\alpha}{N} [1 - \beta(p'_j)^\pi]. \quad (3)$$

In equation (3), the effect of adult group size (k_j) on fitness is expressed as a power function (k_j^α). If $\alpha = 0$, there is no effect of size on fitness; $\alpha = 1$ means the dependence is linear, and $\alpha < 1$ and $\alpha > 1$ mean that the increase of fitness with size is less than linear or greater

than linear, respectively. The number of propagules that an organism of a given size can produce must decrease with the size of these propagules; to represent this effect in equation (3), we divide by propagule size (N). The second term of our fitness function measures how the functionality of the group decreases with the frequency of mutants in the adult (p_j). The parameter β in equation (3) measures the deleterious effect of mutation at the group or organism level, while the parameter π controls the shape of the relationship between fitness and frequency of mutant cells. If $\pi = 1$, fitness decreases linearly with the frequency of mutants, while if $\pi < 1$ ($\pi > 1$), the decrease in fitness slows down (accelerates) as the frequency of mutant cells increases. For comparison purposes, in our previous work, we defined β as the benefit of cooperation at the group level instead of as the cost of mutation at the group level (due to the loss of cooperation). The relationship between the two definitions is simple; one is the negative of the other.

Propagule size (N) influences fitness in equation (3) in several ways. First, propagule size affects the variance within and between offspring; smaller N increases the variance between offspring and decreases the variance within offspring. Consequently, larger N decreases the opportunity for between-group (organism) selection and increases the opportunity for within-group change. Second, propagule size has direct effects on fitness through the term k_j^α/N in equation (3). All other factors being equal, smaller N increases the number of possible fragments but decreases the adult size k_j . The parameter α tunes the relative role played by these two effects. It is shown in the next section that the number of cells in the adult (adult size [k_j]) increases linearly with N ; therefore, if $\alpha > 1$, k_j^α/N increases as N increases, while if $\alpha < 1$, k_j^α/N decreases as N increases.

The case $\alpha > 1$ (in which the function k_j^α/N in eq. [3] increases as N increases) may be the most appropriate for the simple cell colonies on the brink of multicellularity. Since N is the propagule size and k_j is the size of the adult group, k_j/N is the maximum number of propagules that can be produced. Therefore, without any advantage of having a bigger size, the number of propagules produced by a group would increase linearly with group size. If we assume that for some reason having a bigger size is advantageous, and, after all, the advantages of larger size are likely the point of group formation, the number of propagules produced would increase faster than linearly with group size ($\alpha > 1$). However, it seems likely that fitness would increase with size up to an optimal size above which an increase in size would have no effect on fitness or would even decrease fitness. In our model, we assume that we are below this optimum so that fitness increases monotonously with organism size. This seems reasonable for

primitive groups in which group formation is just beginning and in which the means by which cells stay together are poorly developed.

Selection at the Within-Organism (Cell) Level

We now turn to the calculation of the k variables that express the numbers of different cell types in the adult. The parameter t measures the time available for development. We assume no back mutation, so an initial mutant cell produces 2^{bct} mutant cells in the adult (bc is the division rate of a mutant cell). An initial nonmutant cell can produce different numbers of mutant cells in the adult with different probabilities. To our knowledge, nobody has succeeded in finding an exact expression for this probability distribution, but it is possible to calculate the average of the distribution. We assume here that each nonmutant initial produces the average number of mutant and nonmutant cells, which are $[\mu 2^{bct} - 2^{ct}(1 - \mu)^{ct}]/(-1 + 2^{b-1} + \mu)$ and $2^{ct}(1 - \mu)^{ct}$, respectively. The derivation of these expressions is explained in figure 2 and formed the basis for previous work (Otto and Orive 1995; Michod 1997a; Michod and Roze 1997). Table 1 summarizes the parameters and variables of the model. Since we use the average number of mutant and nonmutant cells in our recurrence equations (eq. [2]) instead of the whole distribution, we are making an approximation. We have checked the implications of this approximation by computer simulation in which mutation during the development is stochastic (so that a given type of propagule can produce adults with different numbers of mutant cells with different probabilities) and found some minor quantitative differences (but no qualitative difference) from the results presented here for the parameter values used in this article (unpublished results available from the authors on request).

The variables k_{mj} (number of mutant cells in the adult) and k_j (total number of cells in the adult) are then given by equations (4a) and (4b):

$$k_{mj} = j2^{bct} + (N - j) \frac{\mu 2^{bct} - 2^{ct}(1 - \mu)^{ct} \mu}{-1 + 2^{b-1} + \mu}, \quad (4a)$$

$$k_j = k_{mj} + (N - j)2^{ct}(1 - \mu)^{ct}. \quad (4b)$$

Mutation Load and Reproductive Mode

We first study how the different aspects of fitness and the reproductive system (especially propagule size [N] and group size through the parameter α) interact with the mutational properties of cells to affect mutation load. A central result of our model is that mutation load is not often a predictor of the outcome of selection, and in the

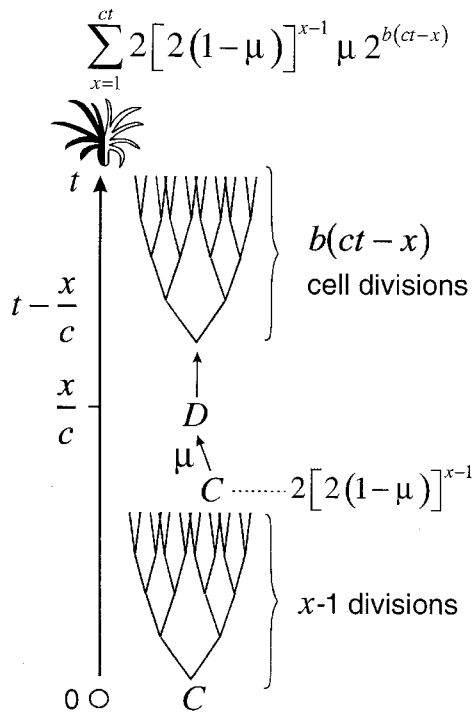


Figure 2: Calculation of the number of mutant cells in the adult stage descended from a single nonmutant cell in the initial propagule. Consider cells that have divided and not yet mutated for $x - 1$ divisions and are now in the process of cell division x . The total number of these cells is $2[2(1 - \mu)]^{x-1}$. Some of these cells (μ) will mutate for the first time, and the resulting mutants will undergo $b(ct - x)$ more cell divisions. The time taken to get x cell divisions is x/c , if c is the replication rate of nonmutant cells. The time left is $t - x/c$. The number of cell divisions the mutant will undergo is then $bc(t - x/c) = b(ct - x)$, if bc is the replication rate of mutant cells. In the case of selfish mutations, mutant cells replicate faster than nonmutant cells, so $b > 1$. In the case of uniformly deleterious mutations, mutant cells replicate slower than nonmutant cells, so $b < 1$. Mutants are neutral at the cell level if $b = 1$. The number of mutant cells at the end of development is given by the sum on top, which simplifies to the expression given in the text. The case of diploidy has been considered in a previous article (Michod 1997b).

section “Competition between Different Reproductive Modes,” we study how the joint effects of mutation load and the direct effects of N (especially on adult group size) affect the evolution of N itself.

Fitness Independent of Group Size

In figure 3, we plot mutation load (ordinate) as a function of selection at the cell level (b [abscissa]) for different propagule sizes ($N = 1, 2, 10$) when fitness is independent of group size ($\alpha = 0$) as has been assumed in previous studies (Otto and Orive 1995). In this case, the fitness function in equation (3) reduces to $[1 - \beta(p')^\pi]/N$. The

different plots of figure 3 correspond to different values of π (which controls the shape of the relationship between frequency of mutant cells and fitness). The load under single-cell reproduction ($N = 1$) is the same in the three plots; indeed, in this case, it is possible to show that the load equals $1 - p'_0$ and does not depend on how mutants are selected against at the organism level. However, when $N > 1$, the load depends on the shape of the fitness function (the curves for $N = 2$ and $N = 10$ are different in the three plots). Figure 3B and 3C shows that when $\pi \geq 1$, there is a threshold value of selection at the cell level ($\sim b = 0.98$) below (above) which mutation load increases (decreases) with decreasing N . Otto and Orive (1995) have studied a model similar to ours, where the fitness of the organism equals $1 - \beta p_j$, and mutations are uniformly deleterious (replication rate $b < 1$). They also found that when cell selection is weak (that is, b is less than but close to 1), the equilibrium mutation load increases with propagule size (N ; see also Kondrashov 1994). When cell selection is stronger (that is, for lower values of b), they found the opposite result, as have we (fig. 3B, 3C); the equilibrium mutation load is lower for larger propagule sizes. In the case of selfish mutants ($b > 1$), the load always increases as N increases (since selfish mutants are advantaged in intraorganismal selection), and the load becomes greater and greater as b increases ($b > 1$; see fig. 3B, 3C). Interestingly, when $\pi < 1$ (fig. 3A), intermediate values of N can minimize the load for some parameter values (in fig. 3A, the load is lower with $N = 2$ than with $N = 1$ and $N = 10$ when $b < 0.97$, approximately). This results holds for other parameter values.

For a specific kind of mutation, that is, for specific values of b and β , a reduction in propagule size will change the equilibrium mutation load. It can be seen in figure 3 that the load may be higher or lower under spore reproduction than under fragmentation, depending on the strength and direction of selection at the cell and organism levels. To have an idea of the overall effect of a reduction in propagule size (say from N greater than unity to N equals unity) on the load when different kinds of mutations are occurring simultaneously, we integrate the load under fragmentation and spore reproduction over a distribution of selection coefficients at the cell level. If we assume independent fitness effects at each locus and linkage equilibrium, the overall mutation load can be approximated by the sum of the mutation loads obtained by considering each locus independently (Crow and Kimura 1970).

For simplicity, we fix selection at the organism level and consider a distribution of mutational effects at the cell level. We obtained the frequency distribution of mutational effects at the cell level (b) by assuming that a proportion (ρ) of the mutations are selfish ($b > 1$; see also fig. 4). The frequencies of the different types of selfish

Table 1: Parameters and variables of the model

Symbol	Definition
N	Number of cells in a propagule
μ	Mutation rate per cell division
c	Replication rate of a nonmutant cell
bc	Replication rate of a mutant cell
t	Time available for development
α	Effect of adult size on fitness
β	Effect of mutation at the organism level
x_j	Number of propagules of type j (with j mutant cells) in the whole population
k_{mj}	Number of mutant cells in the adult stage of a propagule of type j
k_j	Total number of cells in the adult stage of a propagule of type j
p_j	Frequency of mutants in a propagule of type j
p'_j	Frequency of mutants in the adult stage of a propagule of type j
W_j	Fitness of an organism of type j
f_{ij}	Frequency of propagules of type i among the offspring of an organism of type j
p	Frequency of mutants in the total population

mutants (different values of b) are given by an exponential distribution of mean $1 + \sigma_s$ (distribution 3 on fig. 4). Among uniformly deleterious mutants ($b < 1$), a proportion θ are mildly deleterious at the cell level (b close to 1), and their frequencies follow an exponential distribution of mean $1 - \sigma_{D1}$ (distribution 2). The other uniformly deleterious mutants (in proportion $1 - \theta$) are strongly deleterious at the cell level (b close to 0), and their frequency distribution is exponential with mean σ_{D0} (distribution 1). Figure 4 shows the shape of the whole distribution.

For a given propagule size (N), we calculate the overall mutation load as $L_N = \int_{\text{distr}(b)} L(b) \text{distr}(b) db$, where $L(b)$ is the mutation load obtained for a given value of b and $\text{distr}(b)$ is the frequency of mutations whose effect at the cell level is b (shown in fig. 4). Then to see the effect of the evolution of single-cell reproduction on the mutation load, we compare L_1 and L_N (the load under single-cell reproduction and under propagule reproduction).

Figure 5 shows the effect of some parameters of the frequency distribution given in figure 4 on the sign of the difference between L_1 and L_N . In figure 5, σ_{D0} (the average of the distribution of strongly deleterious mutations at the cell level) is fixed to 0.05. Along the X-axis of figure 5, we increase σ_{D1} and σ_s , the standard deviations of the distributions of mildly deleterious and selfish mutations (see fig. 4). We set $\sigma_{D1} = 3\sigma_s$ (distribution 2 is wider than distribution 3). The Y-axis is the limit value of ρ (the proportion of selfish mutations) above which the load is lower under single-cell reproduction than under propagule reproduction ($L_1 < L_N$). The different curves are for different values of θ , which is the fraction of uniformly deleterious mutations that are mildly deleterious at the cell level (see fig. 4).

Figure 5 shows that when the fitness of the organism does not depend on its size ($\alpha = 0$), even though most

deleterious mutations at the cell level generate a higher load under single-cell reproduction than under propagule reproduction, a small proportion of selfish mutations can be sufficient to have a lower load under single-cell reproduction. This is due to the fact that mutants with higher cell replication stay at a higher equilibrium frequency in the population and therefore have more influence on the value of the mutation load.

Effect of α and Group Size on Mutation Load

In the preceding section, "Fitness Independent of Group Size," we studied the case in which the number of cells in a group (group size) had no influence on the performance of the group (that is, $\alpha = 0$) because this assumption had been made in previous studies of this problem (Kondrashov 1994; Otto and Orive 1995). However, this assumption probably does not hold for primitive cell colonies; a bigger colony can produce more fragments, and there may be other advantages of a larger size (e.g., bigger things eat smaller things). This influence of size has not been studied previously and, as we show below, affects the mutation load in important ways. We now turn to the analysis of the model when α (the parameter that measures how group size affects fitness) is >0 . Recall that $\alpha < 1$ and $\alpha > 1$ mean that the increase of fitness with size is less than linear or greater than linear, respectively.

Figure 6 shows the relation between propagule size and mutation load as a function of the parameters α , b , and β . To make this figure, we fixed N (here, $N = 5$) and determined numerically the limit between the region where the load increases with increasing N (above surface in fig. 6) and the region where the load decreases with increasing N (below surface). Concerning selfish mutants ($b > 1$), the load is always lower when N decreases because

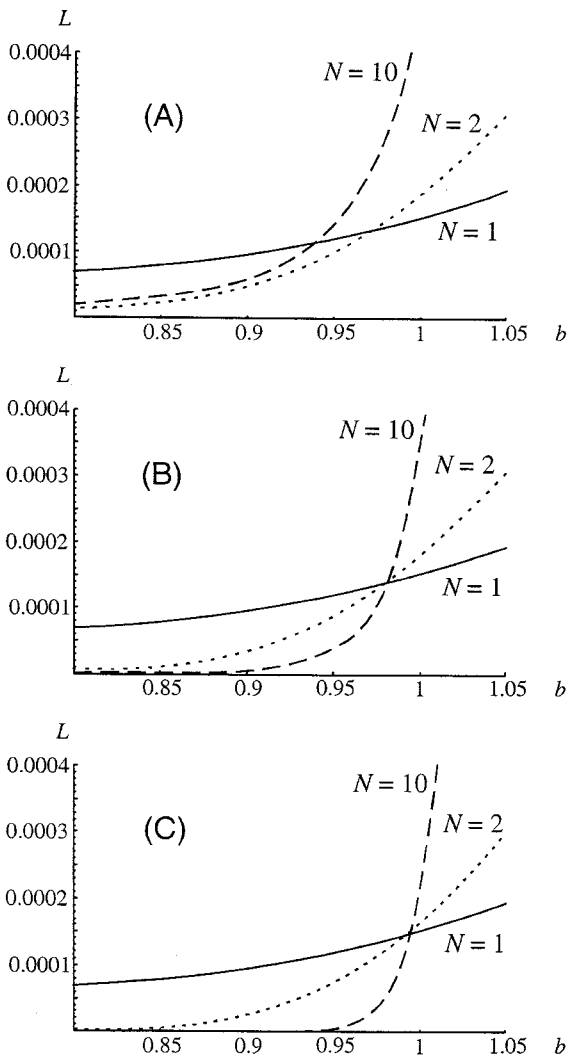


Figure 3: Mutation load as a function of mutant-cell replication rate when fitness does not depend on the size of the organism ($\alpha = 0$). The parameter b on the X-axis is the rate of replication of mutant cells. Nonmutant cells replicate at rate 1. A, $\pi = 0.5$; B, $\pi = 1$; C, $\pi = 2$. Values of the other parameters: $\mu = 10^{-5}$, $t = 15$, $\beta = 0.2$.

selfish mutants are better eliminated when the variance between organisms is higher. In the case of uniformly deleterious mutants ($b < 1$), the results are very different depending on the value of α , and the remaining discussion in this section is restricted to uniformly deleterious mutations.

When $\alpha < 1$, the figure shows that there is a limit value of b (surface of fig. 6A) below which the load is higher under single-cell reproduction than under propagule reproduction. In that case, cell selection is more efficient than organism selection at eliminating mutants. This limit value of b is close to 1 except when α approaches 1.

When $\alpha \geq 1$, all mutants generate a lower load under single-cell reproduction (fig. 6B). Therefore, when $\alpha \geq 1$, the evolution of a modifier coding for single-cell reproduction will always reduce the mutation load whatever the replication rate of mutant cells.

The importance of α in modulating the effect of propagule size on mutation load when mutations are uniformly deleterious can be understood as follows. During the growth of a cell group, uniformly deleterious mutants decrease in frequency within the group (unless there was no mutant in the initial group). Since group size is not fixed, the more mutant cells, the smaller the adult group will be (recall mutant cells replicate more slowly than non-mutant cells). This effect of cell selection on group size reduces the frequency of mutant cells in the population. However, part of this effect of cell selection in reducing the load is lost when organisms reproduce if fitness increases less than linearly with size. The more variance between groups (i.e., the smaller N), the greater this loss will be; therefore, if the mutational effects are strong at the cell level compared with the organism (group) level (β small and/or b small), larger propagules can lead to better elimination of deleterious mutants. If the mutational effects are large at the organism level compared with the cell level (b large and/or b close to unity), then the loss in effectiveness of cell selection (stemming from $\alpha < 1$) is less important and smaller propagules lead to more effective elimination of mutations (smaller propagules always enhance the opportunity for between-organism selection).

Figure 7 illustrates these points and forms the basis of equation (5), which predicts whether more organism se-

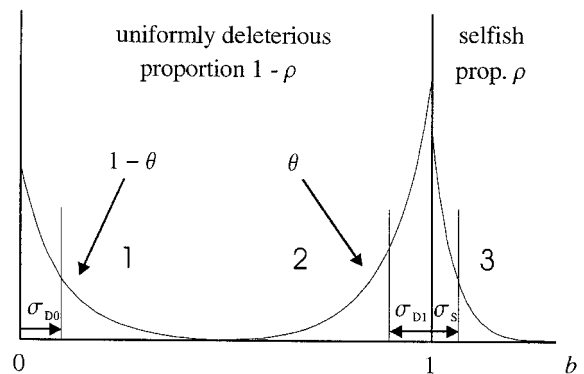


Figure 4: Hypothetical distribution of mutational effects at the cell level (b). The vertical bars represent the averages of the three exponential distributions. A proportion ρ of mutations are selfish ($b > 1$), a proportion $(1 - \rho)\theta$ are mildly deleterious at the cell level ($b < 1$, b close to 1), and a proportion $(1 - \rho)(1 - \theta)$ are strongly deleterious at the cell level (b close to 0).

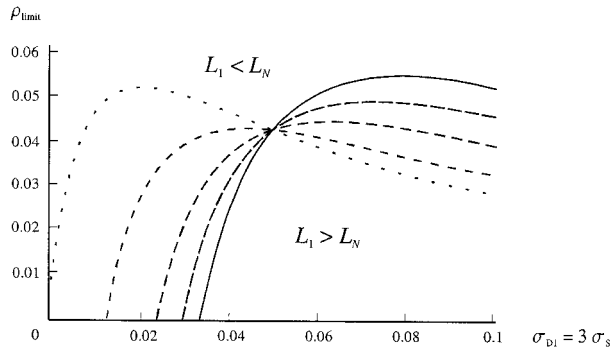


Figure 5: Effect of propagule size on mutation load as a function of the distribution of mutational effects. Effects of some parameters of the distribution of mutational effects at the cell level on the sign of the difference between the mutation load under single-cell reproduction and propagule reproduction (with $N = 10$). See text for explanation. Dotted curve, $\theta = 0.1$; solid curve, $\theta = 1$; dashed curves, $\theta = 0.25, 0.5, 0.75$. Values of the other parameters: $t = 15$, $\beta = 0.2$, $\pi = 1$, $\mu = 10^{-5}$, $\sigma_{D0} = 0.05$.

lection (selection at the cell-group level) serves to reduce mutation load. The small circles on the first line of figure 7A–7C represent propagules at the beginning of a generation. The black parts represent mutant cells. The bigger circles on the second line represent adult organisms. The different adult sizes are represented by different circle sizes on the second line (the circles are bigger on the second line because the groups have grown in size). The third line represents group fitness (the whole set of propagules generated by each organism). The size of the circles represents the fitness of the organism, and the black part represents the average frequency of mutant cells in the propagules (which is the same as the frequency of mutants in the adult of the second line). The parameters α or β tune the relation between fitness and adult size or mutant frequency, respectively; in other words, these parameters affect the transition from the second to third lines in figure 7A–7C.

Figure 7A represents the case where $\alpha < 1$ and $\beta = 0$ so that the mutations have no effect at the organism level (in that case, $W_j = k_j^\alpha/N$). Because $\alpha < 1$, the fitness of the organism increases less than linearly with its size, and therefore there are fewer differences between the sizes of the circles of the third line (fitness of the organism) than between the sizes of the circles of the second line (size of the organism). It is easy to see that the frequency of mutant cells in the whole population is higher in the third line than in the second line. Cell selection serves to decrease the frequency of mutants by within-group change and by creating size variance among groups; however, the less-than-linear effect of organism size on fitness effectively erases some of the size variance and, along with it, the

reduction in mutation load that would result should this size variance be expressed as fitness variance. More organism selection (lower N) means more size variance erased, and therefore a reduction in propagule size will increase the mutation load. When $\beta > 0$, the differences in fitness between the different types of organism are greater (the sizes of the circles of the third line are more different).

Figure 7B represents the case where β equals the limit value represented in figure 6C and 6D. In this case, the differences in fitness are the same as the differences in size between the different organisms, and therefore reproduction does not change the frequency of mutant cells in the population. Selection is context independent (or frequency independent) in that case since the number of progeny produced by an initial cell after growth and reproduction does not depend on the composition of the group but only on intrinsic properties of that cell. Therefore, N has no effect on selection in that case. Figure 7C represents the case where β is above this limit value; differences in fitness are greater than differences in size, and therefore organism selection reduces the frequency of mutants in the population. In that case, a reduction in propagule size decreases the load by increasing the effect of organism selection.

When $\alpha = 1$ (fitness increases linearly with size), the cases where $\beta = 0$ and $\beta > 0$ are represented by figure 7B and 7C, respectively. When $\alpha > 1$, differences in fitness are always greater than differences in size for any value of β (fig. 7C). Therefore, when $\alpha \geq 1$, organism selection always reduces the frequency of mutants in the population, and so in this case, the mutation load is always lower under single-cell reproduction than under propagule reproduction.

This discussion can be formalized by a covariance expression calculated from the frequency of mutants in the population at the different times of the life cycle (expressions given in fig. 7). The frequency of mutants is changed by an amount Δp^W during the development due to within-organism selection. In the case of uniformly deleterious mutants, Δp^W is negative. Then the frequency of mutants is changed by an amount Δp^B during reproduction. Recall that before organisms reproduce, replication of cells has reduced the frequency of mutants within organisms, and organisms with more mutants tend to be smaller in size. When organisms reproduce, the frequency of mutants can increase if organism fitness is not very sensitive to organism size. It is shown easily (by comparing the expressions in fig. 7A) that the mutant frequency is reduced by organism reproduction ($\Delta p^B < 0$) if

$$\frac{\text{cov}(W_j, p_j')}{\bar{W}} < \frac{\text{cov}(k_j, p_j')}{\bar{k}}, \tag{5}$$

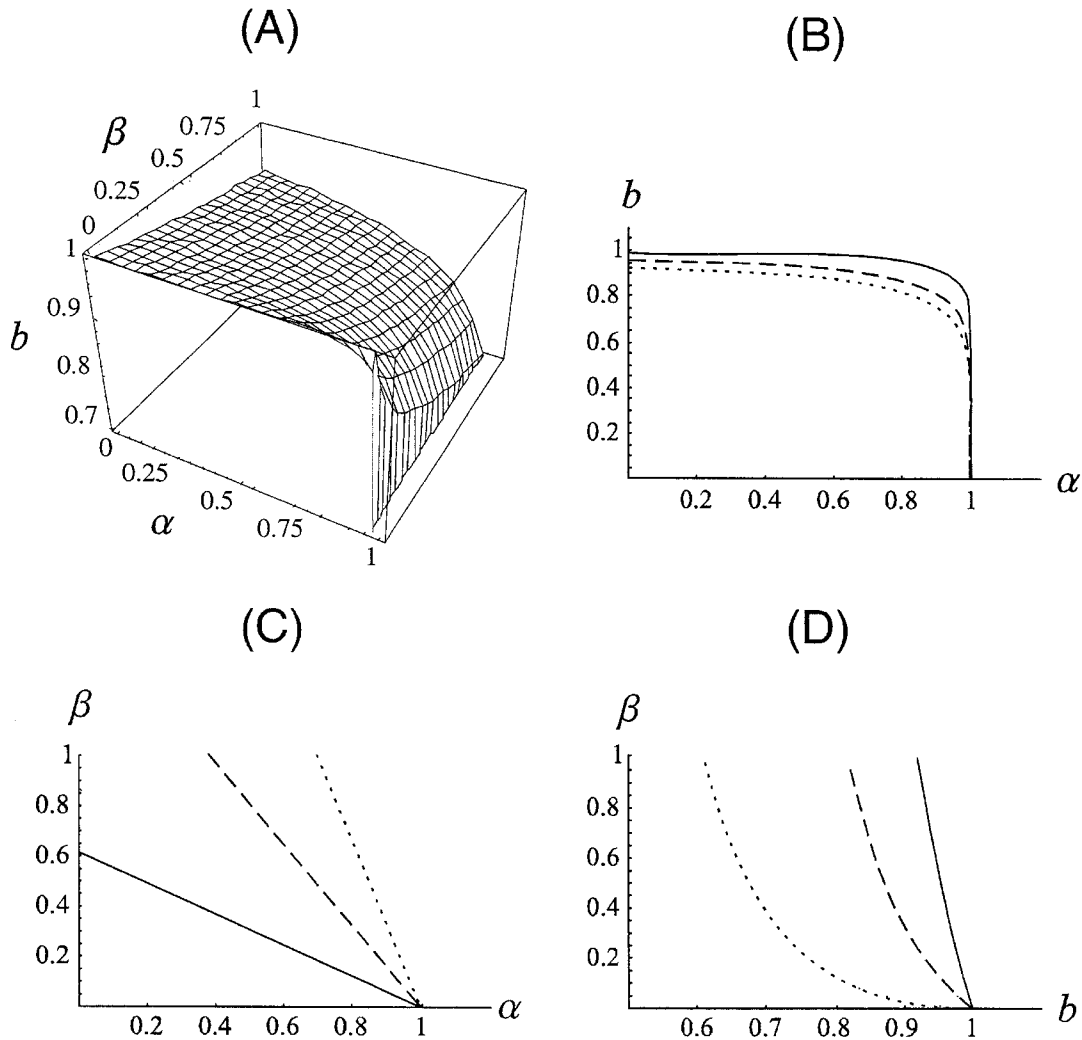


Figure 6: Mutation load and propagule size for uniformly deleterious mutations. The mutation load is lower under single-cell reproduction (than with fragmentation $N = 5$) above the surface in A and above and to the right of the curves in B, C, and D (which are slices in the three-dimensional surface). Beneath the surfaces, fragmentation has a lower mutation load. Surfaces are plotted in $\alpha/b/\beta$ space (α measures how group size influences fitness, b is the replication rate of mutant cells, and β the deleterious effect of mutation at the organism level). When $b > 1$, mutants are selfish, and the load always decreases as N decreases. When $b < 1$, mutants are deleterious at both levels (uniformly deleterious), and the effect of N on the load depends on the values of α , b , and β (explained further in fig. 7). Parameter values: B, $\beta = 0.1$ (solid), $\beta = 0.5$ (dashed), $\beta = 1$ (dotted); C, $b = 0.95$ (solid), $b = 0.9$ (dashed), $b = 0.85$ (dotted); D, $\alpha = 0.1$ (solid), $\alpha = 0.8$ (dashed), $\alpha = 0.98$ (dotted); A–D, $t = 15$, $\pi = 1$, $\mu = 10^{-5}$.

where W_j , k_j , and p_j' are the fitness, size, and frequency, respectively, of mutant cells in an organism of type j and \bar{W} and \bar{k} are the average fitness and average adult size in the population, respectively. Note that in the case of uniformly deleterious mutants, these covariances are both negative (both size and fitness decrease as the proportion of mutants in the adult increases). Equation (5) indicates that increasing between-organism selection at the expense of within-organism selection (as is done by reducing N)

reduces the frequency of mutants in the population if the covariance between frequency of mutants in the adult and fitness is more negative than the covariance between frequency of mutants in the adult and adult size. When fitness increases more than linearly with adult size ($\alpha > 1$) or when mutants are selfish, this condition is always satisfied.

The condition $\alpha > 1$ seems to be the most realistic case for primitive cell groups. Indeed, there is a linear relationship between the size of a group and the number of

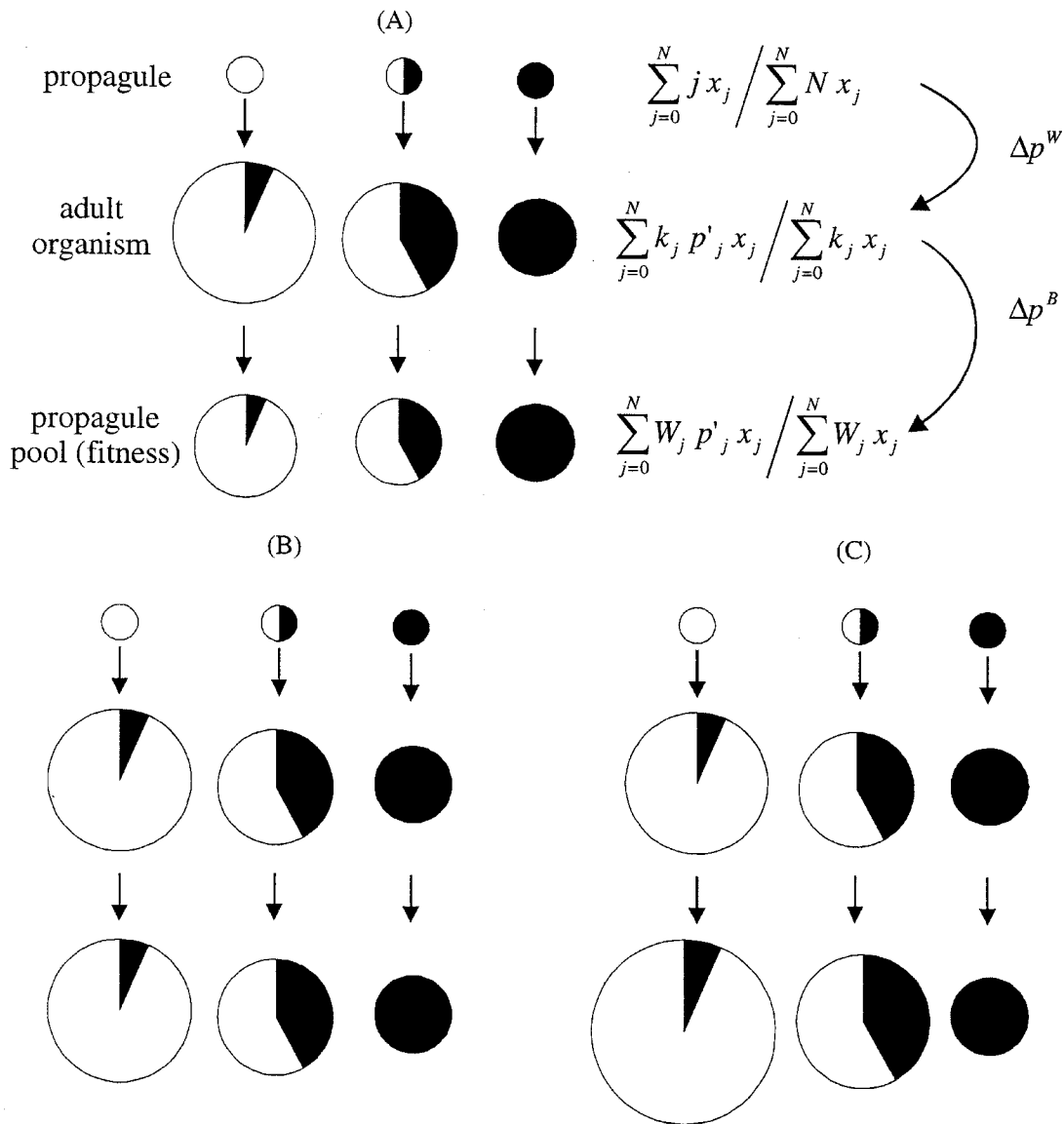


Figure 7: Multilevel selection and mutation load. Diagrams representing the change in frequency of mutant cells due to within- and between-organism selection. The three lines of each figure represent the population before growth of the organisms, after growth and before reproduction, and after reproduction. See text for explanation. Panels A–D have different interpretations depending on α . In general, the deleterious effect of mutation at the organism level increases from A to C. When $\alpha < 1$ in A, there is no effect of mutation at the organism level ($\beta = 0$); in B, the effect of mutation at the organism level equals a value β_{limit} such that the reproduction of organisms does not change the frequency of mutants in the population. In C, $\beta > \beta_{\text{limit}}$, and the reproduction of organisms reduces the frequency of mutants. When $\alpha = 1$, A is not applicable, and B and C correspond to the cases where $\beta = 0$ and $\beta > 0$, respectively. When $\alpha > 1$, A and B are not applicable, and C corresponds to the case where $\beta \geq 0$. The expressions on the right of A represent the frequency of mutant cells in the whole population at the three different stages. Variables Δp^W and Δp^B are the changes in mutant frequency due to within- and between-organism selection, respectively.

fragments that it can produce; if other advantages of size are taken into account (and such advantages probably played a role during the emergence of early cell groups), the number of fragments produced would increase more than linearly with size. Our model shows that in this case,

the evolution of single-cell reproduction (from fragmentation) would have reduced the mutation load caused by deleterious mutations at the organism level whatever the effect of mutations on cell replication rates may be. However, the fact that the load is lower under spore repro-

duction does not mean that spore reproduction would be selected in a population of fragmenters because direct effects of propagule size on fitness are likely to play a role in the evolution of propagule size. We now consider these direct effects along with the effects on mutation load.

Competition between Different Reproductive Modes

In our model, two factors can influence the evolution of N : first, the trade-off between propagule size and number of propagules produced and second, the influence of propagule size on the relative strengths of within- and between-organism selection and the concomitant effects on mutation load. We expect that the first factor should often predominate since it has immediate effects on fitness, while the effect of N on the within- and between-group variance should have consequences in the longer term. In this section, we are interested in the outcome of selection between a spore reproducer and a propagule reproducer, taking into account both factors in the face of deleterious mutations occurring within the organisms. Of course, there are many specific factors that we do not take into account here that are likely to influence the evolution of propagule size (e.g., a smaller size could allow better dispersal), but we think that our simple model still provides insights about the general issues involved in the evolution of propagule size (N).

We have seen that for a given propagule size (N) there can be $N + 1$ types of propagules depending on their composition of mutant/nonmutant cells. The variable x_j is the number of propagules of type j (containing j mutant cells) in the whole population at the beginning of a generation. The dynamics of the system are given by $\mathbf{x}_{t+1} = \mathbf{A}_N \mathbf{x}_t$ (eq. [2]). In time, the frequencies of the different types of propagules reach an equilibrium given by the first eigenvector of \mathbf{A}_N , and the population size increases at a rate given by λ_N , the first eigenvalue of \mathbf{A}_N . We use this λ_N as a measure of fitness associated with propagule size N . We determine whether a spore reproducer will invade a population of fragmenters by comparing the values of λ_1 and λ_N . Furthermore, we use this invasion criteria λ_1/λ_N as an operational measure of the fitness of spore reproduction. We assume here that propagule size is genetically encoded but that different genotypes coding for different propagule sizes are never mixed in the same organism (which would complicate the model). Therefore, the propagules produced by a given organism all have the same size.

In the following, we consider only cases where $\alpha > 1$. As discussed previously, we think that this is probably the most realistic case for primitive cell groups, and in this case, the two factors (mutation load and the direct effects of changing N) acting on propagule size are in opposition. When $\alpha > 1$, fitness increases more than linearly with the

size of the organism, and this selects for bigger propagule sizes, at least when mutation is absent. Indeed, the size of the adult (k_j) increases linearly with N ; therefore, when $\alpha > 1$, the term k_j^α/N of the fitness function (eq. [3]) increases as N increases. However, the previous section showed that when $\alpha > 1$, the equilibrium frequency of deleterious mutations is lower for smaller propagule sizes, whatever the replication rate of mutants within the organism. We want to see which of these two effects predominates in the evolution of propagule size for given values of parameters.

Figure 8 shows the result of the competition for different values of α and b . Here we assume that $\beta = 1$ (organisms entirely composed of mutant cells have zero fitness) so that selfish mutants cannot go to fixation. Figure 8 shows that there is a limit value of b below which fragmentation is advantageous and above which spore reproduction is better. Spore reproduction does better than fragmentation when b is high (mutant cells have a high advantage at the cell level). Indeed, coping with mutation is an important issue when mutants spread at a high rate within cell groups; in that case, it is advantageous to reduce the size of propagules in order to eliminate mutants more efficiently even though this has a cost in terms of diminished adult size. The parameter α measures the advantage of size, and therefore it also represents the cost of spore reproduction. There is no cost when $\alpha = 1$, and the curve shown in figure 8 falls to 0 as α goes to 1. As α increases, the cost becomes more and more important, and therefore the curve goes up.

Figure 8 shows that depending on the effect of selection at the cell level, smaller or larger propagule size may win. We now use the distribution of mutational effects pre-

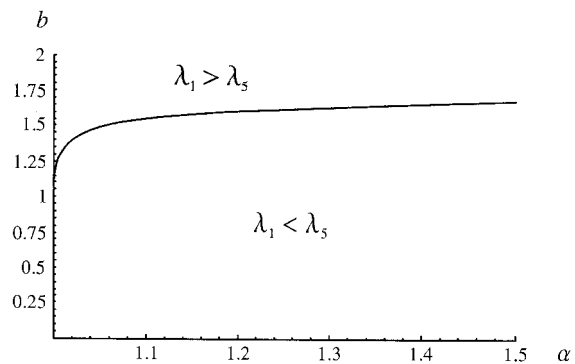


Figure 8: Competition between a fragmenter (here, $N = 5$) and a spore reproducer ($N = 1$). Parameter α is the effect of adult size on fitness, and b is the replication rate of mutants cells within the organism. The spore reproducer wins above the curve, and the fragmenter wins below. Values of the other parameters: $\mu = 10^{-5}$, $\beta = 1$, $\pi = 1$, $t = 20$.

sented in figure 4 to study the outcome of competition between fragmentation and spore reproduction when there is a distribution of effects of mutation at the cell level. We calculate the average selection coefficient for spore reproduction by integrating the selection pressure that each locus would cause on spore reproduction over the distribution (assuming no interaction and no linkage disequilibrium among loci). This approach has already been used by Barton (1995) to obtain a genome-wide measure of the selective force on a modifier of recombination. In our case, the selection pressure for spore reproduction is given by λ_i/λ_N for given effects of mutation; to obtain an average selection coefficient, we numerically integrate λ_i/λ_N over the distribution shown in figure 4.

Representative results are shown in figure 9. The X-axis of figure 9 is the average advantage of selfish mutations at the cell level (average of distribution 3 of fig. 4), and the Y-axis is the proportion of selfish mutations (ρ). Spore reproduction is more advantageous than fragmentation above the curves (the different curves are for different values of α). Figure 9 shows that the minimal proportion of selfish mutations for spore reproduction to be advantageous decreases in a steplike manner as the average advantage of selfish mutants at the cell level increases. When the advantage of selfishness is too low, spore reproduction is not advantageous, but when this advantage is higher than a threshold value, spore reproduction can be selected provided that only a small proportion of mutations are selfish. The position of the threshold depends on the values of the other parameters; in particular, the threshold moves rapidly as α increases, as shown by figure 9. The other parameters have less effect than α on the position of the threshold.

Discussion

A common view on the evolution of propagule size is that lower propagule size is advantageous because it increases kinship among cells, thereby increasing selection at the cell-group or organism level (Bell and Koufopanou 1991; Maynard Smith and Szathmary 1995). We have tested this hypothesis in the context of coping with deleterious mutations, that is, mutations that are always deleterious at the organism level and may be deleterious or advantageous at the cell level. A main result of this article is that the effect of propagule size on the mutation load depends critically on the relation between adult size and fitness. When fitness increases less than linearly with size, the effect of N on the load depends on the strength and direction of selection at the cell and organism levels. Selfish mutations are always better eliminated for smaller propagule sizes, but uniformly deleterious mutations are usually better eliminated for larger propagule sizes unless their effect

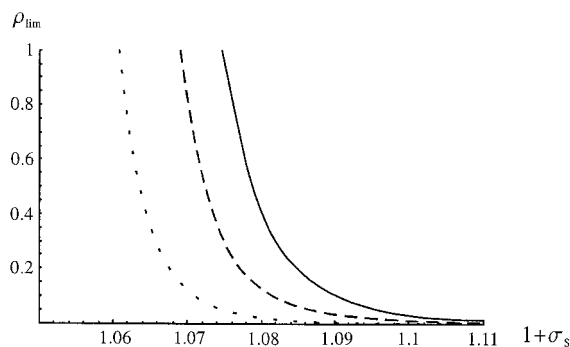


Figure 9: Competition between a fragmenter ($N = 5$) and a spore reproducer. The X-axis is the average effect of selfish mutations at the cell level, and the Y-axis is the proportion of selfish mutations. Spore reproduction is selected above the curves, and fragmentation is selected below the curves. Dotted line, $\alpha = 1.01$; dashed line, $\alpha = 1.05$; solid line, $\alpha = 1.1$. Values of the other parameters: $\mu = 10^{-5}$, $\beta = 1$, $\pi = 1$, $t = 20$, $\sigma_{D0} = \sigma_{D1} = 0.05$.

at the cell level is very small. We showed that when these different types of mutations are present simultaneously, the effect of selfish mutations predominates; the load is lower for smaller propagule sizes even when the proportion of selfish mutations is small. This is due to the fact that the mutation/selection equilibrium is much higher for selfish than for uniformly deleterious mutants.

When fitness increases more than linearly with size, the mutation load is always lower for smaller propagule sizes for any effect of mutation at the cell level. However, in this case, the effect of size on fitness leads to a direct-selection pressure favoring reproduction through large propagules. This tendency can be reversed if some mutations generate selfish cells and if the rate of proliferation of these cells is high enough so that the benefits of a more efficient selection against those mutants compensate for the cost of having a smaller size. We should remind the reader that our representation of the effect of size on fitness has limitations; fitness probably increases with size up to a certain point. With such an intermediate-optimum fitness function, the advantage of having a bigger size would be less pronounced than in our model; therefore, we can consider our competition model as a worst case for the evolution of spore reproduction. Still, our model should provide a good approximation to the early evolution of groups in which the mechanisms promoting group formation are poorly developed and most groups are below the optimal size. As we discussed previously, we think that the case where fitness increases more than linearly with size is realistic for simple cell groups reproducing by fragmentation because bigger groups can generate more fragments, and there may be other advantages of larger group

size. However, one can also envision cases where fitness would increase less than linearly with size, especially if there is a kind of cell specialization in the group (even if this specialization is just the difference between “inside” and “outside” cells in a sphere or other volume as was pointed out by one of our reviewers). Data on the effect of size on fitness in simple multicellular organisms are crucially needed to determine what would be the most realistic fitness function, and we are planning to do experiments using *Volvox* in order to estimate α .

As shown by Kondrashov (1994), the effect of vegetative reproduction on the load depends both on propagule size and on the relatedness among cells within propagules. He considered four different modes of sampling cells in the adult to form propagules that led to different values of the relatedness among those cells. Here we used only the “random mode”; the cells forming a propagule are sampled randomly in the adult. Another sampling mode considered by Kondrashov is the “false mode,” where the cells forming a propagule are chosen as closely related as possible. In this case, he assumes that no mutation occurs during the cell divisions separating the recruited cells from their common ancestor so that all the cells of a propagule are identical. He concludes that this case is genetically equivalent to $N = 1$ and that propagule size has no effect on the load. However, Kondrashov’s assumption is unrealistic when the initial number of cells in the offspring is large, as in many organisms reproducing by fragmentation or fission. In that case, the production of mosaic descendants must be frequent. The mode of propagule formation in real organisms must lie somewhere between the false mode and the random mode, with the exact position on this continuum depending on the developmental pattern of the organism (cell mobility in particular). If cells do not migrate much during development, propagule size should have less effect on the load than predicted by our model because, in that case, the cells of a propagule will be genetically identical most of the time, unless propagule size is fairly large. However, our qualitative results still hold.

We have assumed that the growth of organisms was undetermined in the sense that organism size depended only on propagule size and the replication rates of their component cells. Therefore, different types of propagules generate adults of different sizes. How would our results be affected if adult size was fixed as it is in many complex multicellular organisms? Two cases may be distinguished: First, the timing of reproduction is fixed and not affected by the rate of growth. Second, organisms reproduce as soon as they reach the fixed adult size, and therefore organisms growing faster reproduce earlier. The first case, in which adult size and the timing of reproduction is fixed for all organisms, is similar to our case above in which $\alpha = 0$. The second case, in which organisms growing fas-

ter replicate earlier, is similar to our case in which $\alpha = 1$. We now explain why we think this.

In the first case, we imagine that all the organisms in the population reproduce at the same time (determined, e.g., by environmental conditions such as temperature or photoperiod). The organisms grow until they reach the fixed adult size and stay at that size until the time of reproduction; therefore, in that case, there is no advantage as a result of growing faster. Fixed adult size would have the effect of reducing the load of selfish mutations since the advantage of selfish mutants at the cell level is reduced (because their faster growth rate would not result in a larger adult size or quicker reproduction). The load is lower for selfish mutations, but the effect of N on reducing the load for selfish mutants remains the same (since selfish mutants are always eliminated more efficiently when the variance between organisms is high); that is, the load decreases as N decreases. Matters change, however, in the case of uniformly deleterious mutants. Uniformly deleterious mutants are advantaged by a fixed adult size since organisms containing a high number of mutant cells reach the same adult size in the end. The way in which this advantage affects mutation frequency and load when N changes depends on the partitioning of variance; the more variance between organisms (and the less variance within), the less cell selection will be effective against uniformly deleterious mutants. However, organism selection is more effective for greater variances between organisms. Therefore, the effect of N on the load will depend on the relative strengths of cell and organism selection, like in our model when $\alpha = 0$ (no effect of size).

In the second case, we assume that organisms reproduce as soon as they reach the fixed adult size, and therefore faster growth results in earlier reproduction. Here all differences in cell replication rates are translated into fitness differences at the group level in terms of rate of reproduction of the group. If mutations have no effect on the functionality of the group ($\beta = 0$), the reproduction of organisms will not change the frequencies of the different types of cells in the population, and selection is frequency independent; the number of offspring cells that a given cell will produce at any given time in the future does not depend on the other genotypes in the group. Therefore, the mutation load does not depend on propagule size. In our model, this corresponds to the case represented by figure 7B. If $\beta > 0$, selection becomes frequency dependent, and the mutants cells are better eliminated when the variance between organisms is greater, that is, when N is smaller. Therefore, the effect of N on the load is the same as in our model when $\alpha = 1$; it has no effect if $\beta = 0$, while if $\beta > 0$, a reduction in propagule size will always reduce the load.

One can also note that in variable environments where

resources can be depleted, faster growth may be advantageous if there is a risk of not reaching adult size because resources are lacking. This would give an extra advantage to faster cell replication. In this article, we have not taken environmental variability into account; this would necessitate more modeling work.

As noted by Kondrashov (1994), similar questions arise in the case of uniparental transmission of organelles or endosymbionts, where groups of organelles (instead of groups of cells) are transmitted from one generation to the next. The total number of organelles (or endosymbionts) in a host is probably fixed, and the time at which the host reproduces probably does not depend on the rate of proliferation of organelles or endosymbionts, which makes this system similar to the case of organisms with fixed adult size, where the timing of reproduction does not depend on the rate of growth of the organism (case 1 of the previous paragraph). Here again, we expect that selfish mutations (advantageous for the organelle but deleterious for the host) should be better eliminated when the number of organelles transmitted to the offspring is small, while mutations deleterious both for the organelle and the host could be better eliminated for smaller or larger numbers of organelles transmitted depending on the relative strengths of selection at the two levels. However, the numbers of transmitted organelles also affects the importance of genetic drift, which we do not consider in this article. Stochastic population genetics models covering the case of two levels of selection are needed to investigate these questions. Simulation studies of such host-endosymbionts systems have already been performed (Rispe and Moran 2000).

The way in which the direct (adult size) and indirect (mutation load) consequences of changes in propagule size select for smaller size (single-cell reproduction) is reminiscent of the way in which deleterious mutation selects for another aspect of the reproductive system: sexual versus asexual reproduction (Kondrashov 1988; Bell and Koufopanou 1991). Indeed, Bell and Koufopanou (1991) argued that the single-cell spore stage evolved to reduce mutation load (what they termed "exogenous repair") during both the sexual and asexual life cycles by increasing the variance in mutation load among offspring. Decreased propagule size, like sex, increases the fitness variance between organisms, and this serves, in many cases, to eliminate deleterious mutations more effectively (we discuss some counterexamples above). Mutation load is usually (but not always) lower when propagule size is smaller, as can be the case with sexual reproduction (when compared to asexual reproduction) if mutations are synergistic. As with sex, there are direct fitness costs of smaller propagule size; most important, smaller propagules produce smaller organisms (all other things being equal), and smaller or-

ganisms are usually less fit than larger ones. Consequently, there is a direct fitness cost of decreasing mutation load by smaller propagule size, as there are direct fitness costs of reducing the mutation load through sexual reproduction (the cost of males). We have studied here how evolution will sort out these conflicting effects of propagule size on fitness and have found that selfish mutations, mutations that harm the organism but allow cells to replicate faster, have a dominant role to play even if they occur relatively infrequently.

Acknowledgments

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