

Some Aspects of Reproductive Mode and the Origin of Multicellularity

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Evolutionary transitions require the organization of genetic variation at two (or more) levels of selection so that fitness heritability may emerge at the new higher level. For example, in 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. In this paper we consider the consequences on fitness variation and heritability of two main modes of reproduction used in multicellular organisms: vegetative reproduction, where the offspring originates from a group of cells of the adult (a propagule), and single-cell reproduction, where development starts from only one cell. Most modern organisms pass through a single-cell stage during their life-cycle, a possible explanation being that the single-cell stage increases the effectiveness of organism selection relative to cell selection, by increasing the kinship among cells within the organism. To study this hypothesis we consider simple cell colonies reproducing 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 (termed “selfish” or “uniformly deleterious” mutants, respectively). In our model fragment size affects fitness in two ways, through a direct effect on 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 groups. Using continuous distributions of mutational effects, we show that selfish mutants have an important effect on mutational load and selection on propagule size, even when selfish mutations are relatively infrequent. We then consider the role of deleterious mutation in the evolution of the germ line. Two possible ways to mediate conflict in the germ line are considered: reduction in development time (of the germ line relative to the soma) and lowered mutation rate in the germ line. The evolution of shorter development time in the germ line depends critically on whether and how the number of gametes influences fitness. If there is a direct effect of the number of gametes on fitness, it will be difficult for shorter development times in the germ line to evolve. We conclude that a lowered mutation rate in the germ line relative to the soma provides the most robust rationale for the origin of the germ line.

Keywords: Levels of selection, germ line, mutation, mutation load, altruism, group selection, evolutionary transitions

1. Introduction

Evolution by natural selection requires heritable variation in fitness. Evolutionary transitions require the organization of genetic variation at two (or more) levels of selection so that fitness heritability may emerge at the new higher level. For example, in 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. Multicellular organisms use a variety of basic reproductive modes such as fragmentation, aggregation, and stem-cell or spore reproduction involving both asexual and sexual life styles. In this paper we consider two main modes: vegetative reproduction, where the offspring originates from a group of cells of the adult (a propagule), and single-cell reproduction, where development starts from only one cell. Single-cell reproduction may involve a germ line (second part of paper) or not (first part of paper). 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 “zygote”), or asexual (in

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that case the primordial cell is called “spore”). Aspects of the reproductive system, such as propagule size (in case of fragmentation) or a germ line (in case of sexual or spore reproduction), have fundamental effects on genetic variability and heritability at the two levels of selection. Here we explore the evolution of these aspects of the reproductive system in the context of their effects on fitness and heritability at a new level of selection and individuality of the multicellular organism.

2. Evolution of propagule size

2.1. Overview

Multicellularity probably arose because of the advantages for cells of group living (ability to catch bigger prey, avoidance of predation, buffered environment within the group...). However, most multicellular organisms begin their life-cycle as a single cell. If group living is so advantageous, why 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 (Maynard Smith and Szathmary, 1995). The first multicellular metazoans were probably similar to present colonies of choanoflagellates, which reproduce by fragmentation by producing propagules (Leadbeater, 1983). We have studied the evolution of propagule size in the context of two selective factors: mutation load and adult organism size. As we will see, the way in which these factors select for propagule size are reminiscent of the way in which deleterious mutation selects for another aspect of the reproductive system, sexual versus asexual reproduction. Decreased propagule size, like sex, increases the fitness variance between organisms and this serves, in many cases, to more effectively eliminate deleterious mutations (we discuss some counter examples below). Mutation load is usually (but not always) lower when propagule size is smaller. However, there are direct fitness costs of smaller propagule size, most importantly, smaller propagules produce smaller organisms (all other things being equal), and smaller organisms are usually less fit than larger ones. Consequently, there is a direct fitness cost of decreasing mutation load. We study

how evolution will sort out these conflicting effects of propagule size and find that selfish mutations, mutations that harm the organism but allow cells to replicate faster, have a dominant role to play.

2.2. Life cycle

Development starts from a group of N cells, N is fixed in our model for simplicity. 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 below). During the development of the organism (lasting t time units), nonmutant cells replicate at a rate c , which will be fixed to 1 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 disadvantageous at both 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 development (t).

The fitness of the adult group j , W_j , is the absolute number of offspring it produces. The subscript j refers to the number of mutant cells in the offspring group; $j = 0, 1, 2, \dots, N$. Since N is the propagule size, the total number of propagules that an organism of size k_j can produce is k_j/N . This number is modified in two ways depending on the size and functionality of the adult group. The functionality of the group is assumed to decrease linearly with the frequency of mutants in the adult, p'_j , the parameter β measuring the deleterious effect of mutation at the organism level. The frequency of mutants in the adult can be calculated from the mutation rate, μ , development time, t , and cell replication rates, unity and b , as described elsewhere (Michod, 1996, 1997; Michod and Roze, 1999). Multiplicative models and other nonlinear formulations can be considered in the same framework, but are not studied here. The effect of adult group size on fitness is expressed as a power function, k_j^x , although, again, other functions may be more appropriate for special situations. For example, there may be an intermediate optimum organism size and a Gaussian function could be included instead. In any case, we take fitness to be the product of these three factors:

$$W_j = (k_j / N)k_j^\alpha (1 - \beta p'_j)$$

Grouping the k_j terms we obtain:

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

where $\alpha = 1 + X$. The effect of adult size on fitness is determined by the parameter α : for example, $\alpha = 0$ means there is no effect of size on fitness, $\alpha = 1$ means the dependence is linear, $\alpha < 1$ or $\alpha > 1$ means that the increase of fitness with size is less than linear, or greater than linear, respectively. In case of selfish mutants, mutant cells replicate faster or survive better than cooperating cells and therefore produce a larger but less functional adult. In case of uniformly deleterious mutations, both components of fitness are diminished. Organism size is assumed to be indeterminate and to depend on the time available for development as well as rate at which cells divide.

Propagule size, N , influences fitness in two ways. First, propagule size affects the genetic variance within and between offspring groups: smaller N increases the between group variance relative to the within group variance. Consequently, larger N decreases the opportunity for between group (organism) selection relative to the opportunity for within group change. The opportunity for selection at the two levels affects mutation load as we discuss below. Second, propagule size has direct effects on fitness through the term k_j^α / N in (1). All other factors being equal, smaller N increases the number of possible fragments, but decreases the resulting adult size, k_j . It can be shown that adult size, k_j , increases linearly with N , therefore, if $\alpha > 1$, the first component of fitness, k_j^α / N , in (1) increases as N increases, while, if $\alpha < 1$, this component decreases as N increases. The parameter α tunes the relative role played by the between and within group variances and the direct effects of propagule size.

2.3. Mutation load

2.3.1. Fitness independent of group size

In Figure 1 we plot mutation load (ordinate) as a function of selection at the cell level (abscissa) for different propagule sizes ($N = 1, 2, 10$) when fitness

is independent of group size ($\alpha = 0$). In this case, our fitness function reduces to $1 - \beta p'_j / N$. Figure 1 shows that there is a threshold value of selection at the cell level (approx. $b = 0.98$) 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 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 we have (Fig. 1), that the equilibrium mutation load is lower for larger propagule sizes. In case of selfish mutants ($b > 1$), the load always increases as N increases (since selfish mutants are advantaged in intra-organismal selection), and the load becomes greater and greater as b increases (see Fig. 1, $b > 1$).

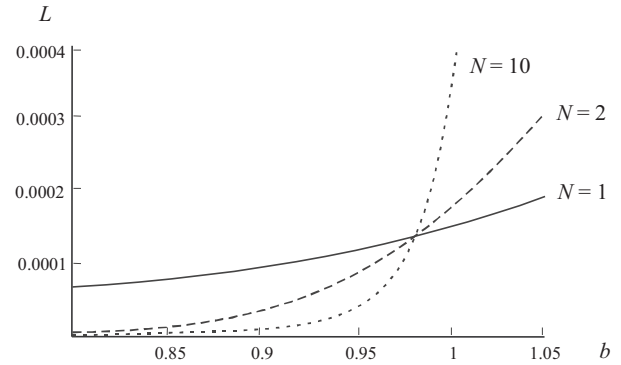


FIG. 1. Mutation load as a function of mutant cell replication rate, when fitness does not depend on the size of the organism ($\alpha = 0$). The parameter b on the x -axis is the rate of replication of mutant cells. Nonmutant cells replicate at rate 1. Values of the other parameters: development time $t = 15$, affect of mutation on group fitness $\beta = 0.2$, mutation rate $\mu = 10^{-5}$

2.3.2. Distribution of mutational effects

For a specific kind of mutation, that is for specific values of b and β , a reduction in propagule size will lead to a certain change in the equilibrium mutation load. It can be seen in Figure 1 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

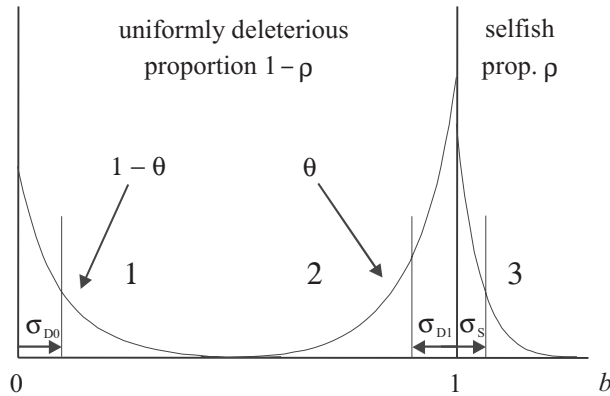


FIG. 2. Hypothetical distribution of mutational effects at the cell level (b). The vertical bars represent the averages of the 3 exponential distributions. A proportion ρ of mutations are selfish ($b > 1$), a proportion $(1 - \rho)\theta$ are mildly deleterious at the cell level ($b < 1$, b close to 1), and a proportion $(1 - \rho)(1 - \theta)$ are strongly deleterious at the cell level (b close to 0)

propagule size (say from N to 1) 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. The frequency distribution of mutational effects at the cell level (b) is determined as follows (see also Fig. 2). We assume that a proportion ρ of the mutations are selfish ($b > 1$). The frequencies of the different types of selfish mutants (different values of b) are given by an exponential distribution of mean $1 + \sigma_s$ (distribution 3 in Fig. 2). 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 in Fig. 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 in Fig. 1). Figure 2 shows the shape of the whole distribution. Data about distributions of mutational effects at the cell level are scarce; however, available data seem to support the kind of distribution assumed here (Thaker and Kankel, 1992).

For a fixed propagule size (N), we calculate the overall mutation load as

$$L^N = \int_{distr(b)} L(b) distr(b) db$$

where $L(b)$ is the mutation load obtained for a specific value of b , and $distr(b)$ is the frequency of mutations whose effect at the cell level is b (shown in Fig. 2). Then we can compare the overall load under fragmentation and spore reproduction, for given parameters of the distribution of mutational effects.

As already mentioned, for selfish mutations, mutation load always decreases with decreasing propagule size, N ; however, for uniformly deleterious mutations, mutation load decreases with decreasing N when mutations are slightly deleterious at the cell level, but mutation load increases with decreasing N when mutations are strongly deleterious. When both selfish and uniformly deleterious mutations occur simultaneously (that is when there is a distribution of mutational effects at the two levels of selection), there is a proportion of selfish mutants, ρ_{limit} , above (below) which mutation load decreases (increases) with decreasing N . We have studied this limit proportion of selfish mutants elsewhere (also see Fig. 6 below) and have shown 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 (Roze and Michod, 2000). 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.

2.3.3. Effect of organism size

In the preceding section we assumed that the number of cells in a group had no influence on the performance of the group. However, this assumption probably does not hold for primitive cell colonies: bigger colonies produce more fragments, in addition there are likely other advantages of larger size (bigger things eat smaller things, for example). Including organism (group) size has important effects on the mutation load and propagule size. We now turn to the analysis of the model when α [the parameter which measures how group size affects fitness; see (1)] is

greater than zero. Recall that $\alpha < 1$ or $\alpha > 1$ means that the increase of fitness with size is less than linear, or greater than linear, respectively. Figure 3 shows how the relation between propagule size and mutation load depends on the parameters α , b and β . The surface in Figure 3a divides the parameter space into two regions with respect to whether single-cell reproduction decreases (above the surface) or increases (below) the mutation load relative to propagule reproduction ($N = 5$). When mutations are selfish, $b > 1$, single-cell reproduction always lowers the mutation load. When α is less than 1, Figure 3 shows that there is a limit value of b (the surface in Figure 3a) 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 (Fig. 3b).

When α is greater than or equal to 1, all mutants generate a lower load under single-cell reproduction (Fig. 3b). Therefore when α is greater or equal to 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 the effect of propagule size on the load due to uniformly deleterious mutations 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 are present in the group, the smaller the group will be. This serves to reduce the frequency of mutant cells in the population. However, part of this effect of cell selection is lost when organisms reproduce, if fitness increases less than linearly with size. The more variance between groups (i.e., the smaller N), the

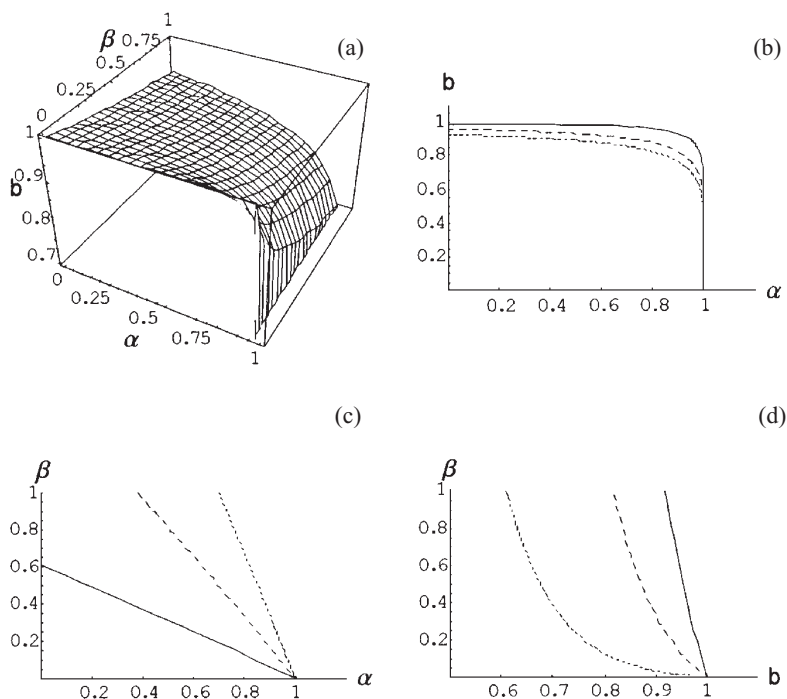


FIG. 3. Regions where the mutation load increases and decreases when the propagule size decreases, in the $\alpha/b/\beta$ space (α measures how group size influences fitness, b is the replication rate of mutant cells and β the deleterious effect of mutation at the organism level). When $b > 1$, mutants are selfish and the load always decreases as N decreases. When $b < 1$, mutants are deleterious at both levels and the effect of N on the load depends on the values of α , b and β . The load increases as N decreases below the surface in (a) and below the curves in (b), (c) and (d) (which are slices in the 3 dimensional surface). 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). In all graphs $t=15$ and $\mu = 10^{-5}$.

greater this loss will be, and therefore if the strength of organism selection is weak compared with cell selection, larger propagules can lead to better elimination of deleterious mutants. Figure 4 illustrates this point.

The small circles on the first line of each panel in Figure 4 represent propagules at the beginning of a generation. They are all equal in size to N , since N does not vary, however, the mutant composition of these propagules may differ as represented by the black area. The propagules grow into adult organisms of different sizes represented by the bigger circles of different size and mutant composition in the second line. The adult organisms represented in the second line of each panel may have a different mutant composition than the propagules they derive from because mutant cells replicate at a different rate. The third line represents the whole set of propagules produced by the adult organisms in line

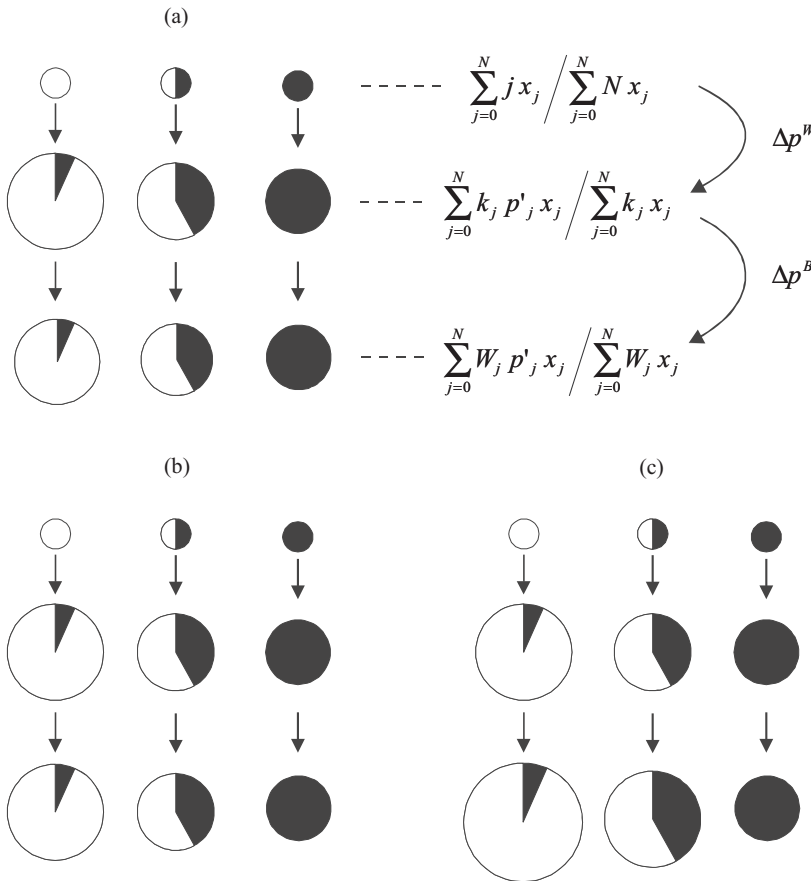


FIG. 4. Diagrams representing the change in frequency of mutant cells due to within and between organism selection. The three lines of each figure represent the population before growth of the organisms, after growth and before reproduction, and after reproduction. The expressions on the right of figure (a) represent the frequency of mutant cells in the whole population, at the three different stages. Δp^W and Δp^B are the change in mutant frequency due to within and between organism selection, respectively. The differences between panels (a), (b) and (c) are discussed in the text

two. In other words, the size of the circles represents the fitness of the organism, and the black area represents the average frequency of mutant cells in the propagules (which must be the same as the frequency of mutants in the adult of the second line).

Figure 4a represents the case where α is less than one and β equals zero (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 less 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: organism selection increases the frequency of mutant cells, and therefore a reduction in propagule size will increase

the mutation load (by increasing the effect of organism selection). When β is greater than zero, 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 4b represents the case where β equals the limit value represented in Figure 3c and Figure 3d. 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 4c represents the case where β is above this limit value: differences in fitness are more important 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 α equals one (fitness increases linearly with size), the case where β equals zero is represented by Figure 4b, and the case where β is higher than zero by Figure 4c. When α is higher than one, differences in fitness are always greater than differences in size, for any value of β (Fig. 4c). Therefore when α is greater or equal to one, 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 Figure 4). The frequency of mutants is changed by an amount Δp^W during the development, due to within-organism selection. In case of uniformly deleterious mutants, Δp^W is negative. Then the frequency of mutants is changed by an amount Δp^B during reproduction. It is shown easily that the mutant frequency is reduced by organism selection ($\Delta p^B < 0$) if:

$$\frac{\text{Cov}(W_j, p'_j)}{\bar{W}} < \frac{\text{Cov}(k_j, p'_j)}{\bar{k}} \quad (2)$$

where W_j , k_j and p'_j are the fitness, size and frequency of mutant cells in an organism of type j , and \bar{W} and \bar{k} the average fitness and average adult size in the population, respectively. Note that in 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 2 shows that organism selection 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 case when $\alpha > 1$ seems to be the most realistic case for our primitive cell groups. Indeed there is a linear relationship between the size of a group and the number of 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. However, the fact that the load is lower under spore reproduction does not mean that spore reproduction would be selected in a population of fragmenters, as other factors are likely to play a role in the evolution of propagule size. This is what we discuss in the next part.

2.3.4. Competition between different reproductive modes

In our model two factors can influence the evolution of N : (i) the direct relationship of propagule size on fitness (discussed further below), and (ii) the influence of propagule size on the relative strengths of within and between organism selection against mutants. In this section, we are interested in studying the outcome of selection between a spore reproducer and a propagule reproducer when both of these factors are taken into account. Several factors that we do not take into account here are likely to influence the evolution of propagule size (for example there is the important effect of genetic drift that we are ignoring, in addition a smaller size could allow a better dispersal), nevertheless we think that our simple model provides some general insights about the major effects.

As explained previously (1), the fitness of an organism is given by

$$W_j = \frac{k_j^\alpha}{N} (1 - \beta p'_j),$$

where k_j is the size of the adult, and p'_j the frequency of mutants in the adult. The size of the adult may be further decomposed into propagule size, N as follows, $k_j = N(p_j k_m + (1 - p_j) k_{nm})$, where p_j is the frequency of mutants in the initial propagule ($p_j = j/N$), and k_m and k_{nm} the number of cells produced by a single initial mutant, and nonmutant cell, respectively (therefore $p'_j = k_m / (k_m + k_{nm})$). The variables k_m and k_{nm} depend on the parameters b , t , and μ (Roze and Michod, 2000). Using this decomposition of group size, we can write fitness as $W_j = N^{\alpha-1} (p_j k_m + (1 - p_j) k_{nm}) (1 - \beta p'_j)$. Writing fitness in this way shows that propagule size, N , has a direct effect on fitness: when α is less than one, $N^{\alpha-1}$ decreases as N increases, while when α is greater than one, $N^{\alpha-1}$ increases as N increases. Therefore, when no mutations occur ($p_j = p'_j = 0$ for all j), spore selection is selected when α is less than one, while fragmentation is selected when α is greater than one. This is what we mean by direct selection on propagule size. When mutations occur, there is an indirect selective pressure on N , besides the direct selective pressure just discussed. Indeed, N affects the efficiency of selection against mutants, as discussed in the previous section. The direct and indirect selective pressures on N can go in opposite directions, and in that case it is not clear what is the

outcome of selection. In the following, we show that the direct effect predominates when mutants are uniformly deleterious, while the indirect effect can predominate when mutants are selfish.

We have seen that for a given propagule size, N , there can be $N + 1$ types of propagules depending on their composition in 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. As shown elsewhere, the dynamics of the system are given by a matrix equation $x_{t+1} = A_N x_t$ (Michod and Roze, 1999; Roze and Michod, 2000). After a long enough time, the frequencies of the different types of propagules reach an equilibrium given by the first eigenvector of A_N , and the population size increases at a rate given by λ_N , the first eigenvalue of A_N . We use this λ_N as a measure of fitness associated with propagule size N . We can determine whether a spore reproducer can invade a population of fragmenters by comparing the values of λ_1 and λ_N . Furthermore, we can use λ_1/λ_N as a measure of the selection coefficient for spore reproduction.

Figure 5 shows the result of the competition for different values of α and b . Here we assume for simplicity that $\beta = 1$ (organisms entirely composed of mutant cells have no fitness), so that selfish mutants can never go to fixation. The story is more compli-

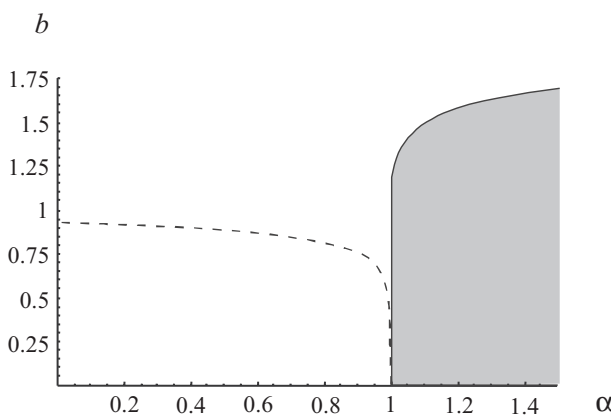


FIG. 5. Competition between a fragmenter (here $N = 5$) and a spore reproducer ($N = 1$). α is the effect of adult size on fitness, b the replication rate of mutants cells within the organism. The fragmenter wins in the shaded area, and the spore reproducer everywhere else. The mutation load is lower under fragmentation under the dashed curve, and lower under spore reproduction everywhere else. Parameter values: $\mu = 10^{-4}$, $\beta = 1$, $t = 15$. Note that the dashed curve here is the dotted curve given in the top right panel in Figure 3

cated for intermediate values of β , as discussed elsewhere (Roze and Michod, 2000). The region where the mutation load is lower under fragmentation is the area below the dashed curve of Figure 5, everywhere else (above and to the right of the dashed line) the load is lower under spore reproduction. When α is greater than one, the mutation load is always lower under spore reproduction, for any effect of mutation at the cell level. Indeed when α is greater than one, organism selection always reduces the frequency of mutants in the population, and therefore a smaller N reduces the load, by increasing the strength of organism selection. The effect of cell selection does not depend on N , because the replication rate of a cell does not depend on the frequency of mutants in the organism.

Figure 5 shows that the region where the fragmenter wins (shaded area) does not correspond to the region where the load is lower under fragmentation (below the dashed curve). This is due to the direct effect of N on fitness: when α is less than one, the term $N^{\alpha-1}$ of the fitness function decreases as N increases, while when α is greater than one, $N^{\alpha-1}$ increases as N increases. Figure 5 shows that, in case of uniformly deleterious mutants ($b < 1$), this direct effect predominates: spore reproduction wins when α is less than one, and fragmentation when α is greater than one.

Things are different, however, in case of selfish mutants ($b > 1$). Figure 5 shows that when α is greater than one, spore reproduction does better than fragmentation when b is high. 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 spore reproduction is costly when α is greater than one. There is no cost when α equals one, and the curve which limits the shaded area in Figure 5 falls rapidly to zero as α goes to 1. As α increases, the cost becomes more and more important, and therefore the curve goes up. Interestingly, the effect of size seems to plateau out quickly.

What is the outcome of selection, when both selfish and uniformly deleterious mutations occur at the same time? We now use the distribution of mutational effects presented in Figure 2 to study the outcome of competition between fragmentation and spore reproduction when there is a distribution of

mutational effects at the cell level like in Figure 2. 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 been used by Barton to obtain a genome-wide measure of the selective force on a modifier of recombination (Barton, 1995). In our case, the selection pressure for spore reproduction is given by λ_1/λ_N , for given effects of mutation; to obtain an average selection coefficient we integrate λ_1/λ_N over the distribution shown in Figure 2.

Some results are shown in Figure 6 taken from a fuller exploration given elsewhere (Roze and Michod, 2000). Here α is greater than one, and therefore propagule reproduction is selected when mutants are uniformly deleterious, while selfish mutants select for spore reproduction, when b is above a limit value (Fig. 5). The x -axis of Figure 6 is the average advantage of selfish mutations at the cell level (average of the distribution 3 of Figure 2), 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 $\alpha > 1$). Figure 6 shows that the minimal proportion of selfish mutations for spore reproduction to be advantageous decreases in a step-like 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 in Figure 6. These results are robust to shifts in the distribution of mutational effects (for example, when gamma distributions are used instead of exponential distributions).

2.3.5. Discussion of the evolution of propagule size

A main result of this section 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

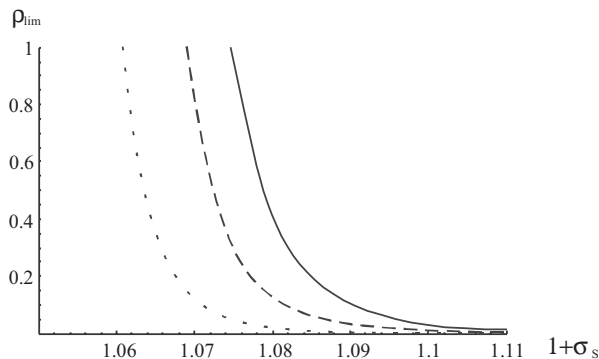


FIG. 6. Competition between a fragmenter ($N = 5$) and a spore reproducer. The x -axis is the average effect of selfish mutations at the cell level, the y -axis the proportion of selfish mutations. Spore reproduction is selected above the curves, fragmentation below the curves. Dotted line: $\alpha = 1.01$, dashed line: $\alpha = 1.05$, solid line: $\alpha = 1.1$. Values of the other parameters: $\mu = 10^{-5}$, $\beta = 1$, $t = 20$

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 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 (which seems to be the most realistic case for simple cell groups reproducing by fragmentation), 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 note here that our representation of the effect of size on fitness is not very realistic: fitness probably increases with size, up to a certain point. With such a 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.

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, leading 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. An other 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 initial cells of a progeny are identical. He concludes that this case is genetically equivalent to $N = 1$, and that propagule size has no effect on the load. However, this assumption is unrealistic when the initial number of cells in the offspring is big, 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, 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 the development, propagule size should have less effect on the load than predicted by our model. However, our qualitative results still hold.

Another aspect worth discussing concerns the stochastic nature of the mutation process. This stochasticity is absent in our model, since we assume that all the nonmutant cells present in a propagule produce an average number of mutants during the development of the organism. Such a model ignores the variability between organisms due to the fact that mutations may occur at different times in different organisms. Models taking stochasticity into account are studied elsewhere (Roze and Michod, 2000), confirming our qualitative results about the effect of N on the mutation load.

We have assumed that the growth of organisms was undetermined meaning that organism size depended 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: (i) the timing of reproduction is fixed (say by environmental conditions such as temperature or photoperiod) and not affected by the rate of growth, and (ii) 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 faster replicate earlier, is similar to our case in which $\alpha = 1$. We now explain why we believe this is so.

In the first case, where the timing of reproduction is fixed, there is no advantage 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 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).

The second case, where faster growth results in earlier reproduction, is similar to our model when $\alpha = 1$, since in that case selection is context-independent when mutations are neutral at the organism level. Therefore a reduction in propagule size will always reduce the load, provided that mutations are deleterious at the organism level.

3. Evolution of the germ line

3.1. Overview

Three different types of hypotheses have been proposed to explain the evolution of germ-soma differentiation:

(i) Division of labor (Bell, 1985; Koufopanou, 1994): the organism has a greater fitness when some cells specialize at reproduction, and the other cells into other functions, because each task can be performed more efficiently. This corresponds to Adam Smith's theory on the benefits of division of labor in human societies, applied to multicellular organisms.

(ii) Conflict mediation (Buss, 1987; Michod, 1996): the evolution of early organisms is threatened by the spread of cancer-like cells, especially as organisms get bigger. The segregation of a germ line early in the development, with a lower number of cell divisions and/or a lower mutation rate than in the soma, decreases the proportion of mutant cells in the gametes and the heritability of fitness at the cell group, or organism, level.

(iii) Fresh epigenetic start (Jablonka and Lamb, 1995): as development gets more complex, it becomes difficult for a cell to de-differentiate and start a new organism. At some point it became necessary to set aside a group of undifferentiated cells at the beginning of the development, to produce the next generation of organisms.

A corollary of the first two hypotheses is that having a germ line becomes more and more advantageous as organisms increase in size. Indeed division of labor is more fruitful in a big group than in a small one (first hypothesis), and the frequency of mutants occurring during the development increases with development time (second hypothesis). Therefore, one might think that a consequence of both these hypotheses is that big organisms should have a germ line, while small ones should not. However, this deduction is not valid across taxa, because organisms from different taxa are subject to different sets of constraints. For example, we expect that organisms from different taxa should differ in their susceptibility to selfish mutants: in plants, for example, selfish mutants do not have much opportunity to spread within the organism, due to the mechanical constraint of the rigid cell wall, while in animals, cell mobility increases the risk of proliferation of selfish cells. Therefore, having a germ line could be more

advantageous for a small animal than for a large plant. So we do not think that comparisons made by Pál and Szathmáry between loosely related taxonomic groups provide a powerful test for these hypotheses (Pál and Szathmáry, 2000). Comparisons between closely related species, however, are more useful, because these species may be subject to similar constraints. The *Volvocales*, for example, illustrate the association of larger size with earlier germ-soma differentiation, in agreement with the division of labor and conflict mediation hypotheses (but, of course, a correlation is not a proof of a causal relationship).

The epigenetic hypothesis implies that an early segregation of the germ line should be more advantageous as cell differentiation becomes more complex. However, one may argue that the complexity of differentiation increases in parallel with the size of organisms, since the benefits of differentiation should be greater for larger cell groups. Therefore, in that case too, one could expect a correlation between bigger size and earlier germ-soma differentiation.

3.2. Modifier model

We developed a model to study the evolution of germ-soma differentiation (Michod, 1996; Michod and Roze, 1997). In this model, we start from a population of organisms (cell groups) without a germ line (after development, all the cells of an organism can become gametes). Mutations occur during the development and are maintained in the population at a mutation-selection equilibrium. These mutations are deleterious at the organism level, but can be advantageous or deleterious at the cell level ("selfish" and "uniformly deleterious" mutations, respectively), depending on the value of the parameter b discussed above. For simplicity we use a one locus – two alleles model to represent the mutation process, the two alleles being called C and D ("cooperative" for the nonmutant, and "deleterious" for the mutant). Then we study the evolution at a modifier locus, coding for early germ-soma differentiation. The two alleles at this locus are M (germ-soma differentiation) and m (no differentiation). We assume that the different events of the life-cycle take place in the following order: fecundation – meiosis – development – gamete production (haploid life-cycle). Development starts from a single cell, whose genotype

can be *CM*, *Cm*, *DM*, or *Dm* (labeled 1, 2, 3 and 4, respectively). The original papers should be referred for more details about the model (Michod, 1996b).

We shall discuss here only the case where recombination between the two loci is absent (equivalent to asexual reproduction), which is the easiest case to interpret. In that case, if the population is polymorphic at the *C/D* locus before allele *M* appears, the condition for *M* to increase in frequency (and go to fixation) once it appeared by mutation is:

$$f_1 W_1 > f_2 W_2 \quad (3)$$

where W_i is the fitness (number of gametes produced), and f_i the proportion of *C* cells among the gametes of an organism starting its development from a cell of genotype *i*. Therefore, the modifier is selected if it increases the total number of “good” gametes (carrying the *C* allele) produced by the organism.

One may think that the mutation rate could be lower in the germ line, because DNA is less exposed to the damaging products of the metabolism, like oxygen radicals. A modifier coding for a germ line where the mutation rate is lower would always be selected, all other things being equal, as we have studied previously. The frequency of mutants could also be lower in the germ line if the number of cell divisions is smaller; indeed the proportion of selfish mutants in a cell lineage increases to one as the number of cell divisions increases, while the proportion of uniformly deleterious mutants increases to reach a mutation–selection equilibrium. In our previous model, we studied the evolution of a germ line modifier, when the number of cell divisions in the germ line is smaller than the number of cell divisions in an organism without germ line. To obtain an expression for the fitness of an organism with germ-soma differentiation (originating from a *CM* spore), we assumed that somatic cells produce some resource that is distributed equally among the gametes. The number of gametes produced is the size of the germ line after development, which we call K_1 . The total amount of resource produced by the soma is given by $(k_1 - K_1)(1 + \beta f_1^S)$, where $k_1 - K_1$ is the number of somatic cells after development (k_1 is the total number of cells in the adult, and K_1 the number of germ cells), f_1^S the proportion of cooperative cells in the soma, and β a parameter measuring the advantage of cooperation for the organism (the more coop-

eration among somatic cells, the more resource produced). The amount of resource received by each gamete is the total amount of resource produced, divided by the number of gametes, which is $(k_1 - K_1)(1 + \beta f_1^S) / K_1$. We then write fitness as the number of gametes produced times the amount of resource per gamete:

$$W_1 = K_1 \frac{(k_1 - K_1)(1 + \beta f_1^S)}{K_1} = (k_1 - K_1)(1 + \beta f_1^S). \quad (4)$$

Similarly, the fitness of an organism without germ-soma differentiation (originating from a *Cm* spore), is written $W_2 = k_2(1 + \beta f_2)$, where k_2 is the total number of cells in the adult, and f_2 the proportion of cooperative cells in the adult. We assume that organisms originating from a *CM* and a *Cm* spore have the same adult size ($k_1 = k_2$). The frequency of cooperative cells in the soma of a *CM* organism is about the same as in an adult *Cm* organism ($f_1^S \approx f_2$), and therefore a *CM* organism has a lower fitness than a *Cm* organism ($W_1 < W_2$). In other words, having a germ line is costly in terms of fitness. Nevertheless, we found that the *M* allele can be selected, because it increases the proportion of cooperative cells among the gametes ($f_1 > f_2$, where f_1 is the proportion of *C* cells in the germ line of a *CM* organism).

4. Discussion of the evolution of the germ line

We have used (4) in our previous studies of the evolution of the germ line to study the conditions under which conflict mediation leads to the evolution of a germ line with a lower number of cell divisions or lower mutation rate than the somatic line (Michod, 1996). Our previous results concerning a reduction in development time in the germ line are limited in that (4) corresponds to a rather ideal case where fitness depends only on the amount of resource produced by the soma and not upon the number of cells in the germ line (this is due to the fact that the K_1 terms cancel out in (4)). Therefore our model assumed that distributing a given quantity of resource between a high number of gametes or between a few gametes does not affect fitness (in the first case lots of gametes of low quality are produced, while in the second case a few gametes of high quality are produced, and fitness is the same in both cases). It would be interesting and important to study this model when this assumption is relaxed. When this is

done, there will be direct costs on the number of gametes produced, W_1 , of lowering the development time of the germ line. We think these direct costs (lower W_1) will likely swamp out the beneficial effects of increased heritability (higher f_1), and make condition (3) harder to satisfy, in turn making it difficult to evolve greater individuality by shortening the development time of the germ line.

The evolution of enhanced heritability and individuality at the organism level through decreased mutation rates in the germ line does not suffer from this complication that plagues shorter development times. By decreasing the mutation rate, heritability (f_1) increases with no direct costs in terms of the number of gametes produced (W_1) in (3). Therefore, we think that the likelihood of lower mutation and DNA damage rates (by sequestering the germ line away from the energetic and metabolic demands of the soma) provides a quite general and sound basis for the evolution of the germ line. In a way, this interpretation of the function of the germ line harkens back to the division of labor hypothesis in that the germ line specializes in high quality genetic information with low numbers of mutations and the soma specializes on energetic and metabolic function.

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