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Life-history evolution and the origin of multicellularity

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Abstract

The fitness of an evolutionary individual can be understood in terms of its two basic components: survival and reproduction. As embodied in current theory, trade-offs between these fitness components drive the evolution of life-history traits in extant multicellular organisms. Here, we argue that the evolution of germ–soma specialization and the emergence of individuality at a new higher level during the transition from unicellular to multicellular organisms are also consequences of trade-offs between the two components of fitness—survival and reproduction. The models presented here explore fitness trade-offs at both the cell and group levels during the unicellular–multicellular transition. When the two components of fitness negatively covary at the lower level there is an enhanced fitness at the group level equal to the covariance of components at the lower level. We show that the group fitness trade-offs are initially determined by the cell level trade-offs. However, as the transition proceeds to multicellularity, the group level trade-offs depart from the cell level ones, because certain fitness advantages of cell specialization may be realized only by the group. The curvature of the trade-off between fitness components is a basic issue in life-history theory and we predict that this curvature is concave in single-celled organisms but becomes increasingly convex as group size increases in multicellular organisms. We argue that the increasingly convex curvature of the trade-off function is driven by the initial cost of reproduction to survival which increases as group size increases. To illustrate the principles and conclusions of the model, we consider aspects of the biology of the volvocine green algae, which contain both unicellular and multicellular members.

Keywords: Cost of reproduction; Germ–soma differentiation; Fitness; Body size; Evolutionary transitions; Life-history evolution; Volvox

1. Introduction

Fitness can be understood in terms of its two basic components: survival (viability) and reproduction (fecundity). Investment in one component often detracts from the other, leading to trade-offs in fitness components. A wide body of work shows that fitness trade-offs underlie the evolution of diverse life-history traits in extant organisms (Stearns, 1992; Charlesworth, 1980). We show here that trade-offs between survival and reproduction have special significance during evolutionary transitions; in particular, they may drive the evolution of individuality during the transition from unicellular to multicellular organisms.

The emergence of individuality during the unicellular–multicellular transition is based on the evolution of cells that differentiate and specialize in reproductive and survival-enhancing vegetative functions. In unicellular individuals, the same cell must contribute to both fitness components, these contributions typically being separated in time. In multicellular groups, cells may specialize during development in either component, leading to the differentiation and specialization in reproductive (germ) and

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vegetative survival-enhancing functions (soma)—what we term germ–soma or "G–S" specialization. As cells specialize in these different but essential fitness components, they relinquish their autonomy in favor of the group and, as a result, fitness and individuality are transferred from the cell level to the group level. We argue here that the evolution of G–S separation and the emergence of individuality at the new higher level are consequences of fitness trade-offs among life-history components—in short, that life-history evolution is a fundamental factor in evolutionary transitions. We first present an overview of the volvocine green algae, which are the organisms we had in mind when constructing the models. Although we discuss the models with regard to the volvocine algae, we have kept the assumptions of the models general so that they will apply to other groups.

2. The volvocine green algae

The evolution of multicellular organisms from unicellular and colonial ancestors is the premier example of the integration of lower level units into a new, higher level individual. Unfortunately, for the major multicellular lineages, the factors underlying their origin lie hidden deep in their evolutionary past, obscured by hundreds of millions of years of evolution. In contrast, according to one estimate (Rausch et al., 1989), the colonial volvocine algae (Fig. 1) diverged from a unicellular ancestor just 35 million years ago, providing a unique window into this major transition.

Volvocine algae are flagellated photosynthetic organisms that range from unicellular (i.e. *Chlamydomonas*) and multicellular forms with no cell differentiation (e.g., *Gonium* and *Eudorina*; 8–32 cells) or incomplete G–S differentiation (*Pleodorina*; 64–128 cells) to multicellular forms with complete G–S separation (i.e. *Volvox*; 500–50,000 cells) (Kirk, 1998). In multicellular volvocine colonies the number of cells is determined by the number of cleavage divisions that take place during their initial formation, and cell number is not augmented by additional cell divisions (Kirk, 1997). In colonies without G–S separation (i.e., *Gonium*, *Eudorina*), each cell gives rise to a daughter colony. The life cycle corresponds to one of discrete generations as the parent colony dies as soon as the daughter colonies hatch.

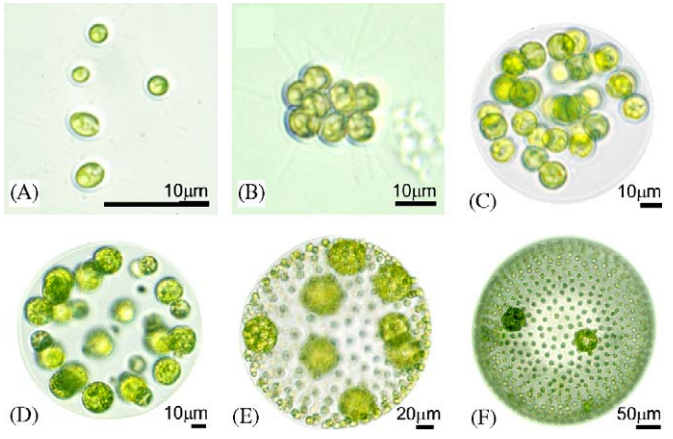

Fig. 1. Subset of volvocine species which shows an increase in complexity, cell number, volume of extracellular matrix, division of labor between somatic and reproductive cells, and proportion of somatic cells. A: *Chlamydomonas reinhardtii*; B: *Gonium pectorale*; C: *Eudorina elegans*; D: *Pleodorina californica*; E: *Volvox carteri*; F: *Volvox aureus*. Where two cell types are present (D, E and F), the smaller cells are the vegetative sterile somatic cells, whereas the larger cells are the reproductive germ cells. Picture credit: C. Solari.

It is believed that all multicellular volvocine algae have evolved from a common ancestor similar to the extant *Chlamydomonas reinhardtii* (Coleman, 1999; Larson et al., 1992). Within this closely-related monophyletic group (Buchheim et al., 1994; Coleman, 1999; Larson et al., 1992; Nozaki et al., 2000, 2002, 2003; Nozaki, 2003), significant evolutionary transitions have occurred repeatedly within a relatively short period of time (possibly as short as 35 million years (Rausch et al., 1989), as already mentioned), suggesting strong selective pressures driving the evolution of multicellularity and G–S specialization.

Although several model systems have been used to investigate the origins of multicellularity, including choanoflagellates (King and Carroll, 2001), cellular slime molds (Strassmann et al., 2000; Foster et al., 2002; Queller et al., 2003) and myxobacteria (Velicer et al., 2000; Shimkets, 1990), volvocine algae exhibit a number of features that make them especially suitable for our work (see the Volvocales Information Project at www.unbf.ca/vip). Like most familiar multicellular forms, and unlike other model experimental systems such as slime molds or myxobacteria, multicellular volvocine algae develop from a single cell, so the cells in the group are related. They can easily be obtained from nature (where uni- and multicellular forms coexist) and maintained in the lab under realistic conditions that allow for an eco-physiological framework. Many aspects of their biology have been studied (Kirk, 1998) (cytology, biochemistry, development, genetics, physiology, natural history, ecology and life-history). The 'social' genes necessary for group living and fitness reorganization have been identified in *V. carteri* (Kirk et al., 1999; Miller and Kirk, 1999), indicating that the underlying genetics of cellular differentiation and G–S specialization is likely simple and may not involve many genetic steps (Kirk, 1997, 1998).

3. Overview of models

The models studied below focus on the trade-offs between survival and reproduction and on how these trade-offs change as group size increases and cells specialize in reproductive and vegetative functions. The models are based on three general assumptions: (i) there are both advantages and disadvantages associated with increasing group size, (ii) generations are discrete, so that fitness is the product of viability and fecundity, and (iii) variation in

fitness exists primarily at the group or colony level; within-group variation is assumed negligible.

Larger group size may be beneficial for survival (for example, in terms of predation avoidance, ability to catch bigger prey, or a buffered environment within a group), as well as for reproduction (for example, in terms of a higher number or quality of offspring). Reduced predation is likely to be especially important in the volvocine algae (Morgan, 1980; Pentecost, 1983; Porter, 1977; Reynolds, 1984; Shikano et al., 1990). However, we do not explicitly model or discuss further this assumed advantage of larger groups.

Increasing group size may also detract from fitness, because of the increasing need for local resources, less effective movement within the environment, and longer generation time. In volvocine algae, these disadvantages of larger size are the result of (i) the 'flagellation constraint' which impedes motility in dividing cells (Koufopanou, 1994) and (ii) the 'enlargement constraint' which refers to the transport and hydrodynamic problems associated with the metabolism and translocation of an increasingly larger colony (Solari et al., 2005a, b). Bell (1985) has also discussed with respect to the volvocines the effect of increased colony size on the increased generation time and the resources needed.

The flagellation constraint impedes motility, and thus viability, during cell division (Koufopanou, 1994), and is a consequence of the coherent glycoprotein cell wall that does not allow the flagellar basal bodies to move laterally and take the expected position of centrioles in cell division while still attached to the flagella (as they do in naked green flagellates). This constraint sets an upper limit of five for the number of times a cell can divide while still maintaining an active flagellum, and thus becomes critical at about the 32-cell stage.

The enlargement constraint stems from the particular way in which volvocine algae reproduce. Because post-embryonic cell divisions are not possible (although the young colonies do increase in size after their release from the mother colony through an increase in cell size and volume of extracellular matrix), the embryo contains all the cells present in the adult. Consequently, the larger the number of cells in the colony, the larger the embryo that develops and must be supported by the swimming mother colony. And, the larger the colony, the larger the investment needed for there to be any reproduction at all. This initial cost of reproduction is especially acute in species in which cells do not double in size and then undergo binary fission, but grow about $N = 2^d$ fold in size and then undergo a rapid synchronous series of d divisions (under the mother cell wall). This type of cell division, which is considered the ancestral developmental program in this lineage (Desnitski, 1995), is known as "palintomy" and is thought to have predisposed these algae to multicellularity (Kirk, 1998). It occurs in the smaller species (including *Chlamydomonas*, *Gonium*, *Eudorina* and *Pleodorina*) and in some of the G–S specialized *Volvox* species (e.g., *V. carteri*) (Fig. 1).

The assumption of selection at the group level (assumption (iii)) is likely to hold in volvocine algae because of their mode of reproduction and colony formation, in which all cells in the group are derived clonally from a single cell after a specific number of cell divisions, d ($d = 3$ for *Gonium*, $d = 5$ for *Eudorina*, $d = 6-7$ for *Pleodorina* and $d = 8-16$ for species of *Volvox*). We have previously studied the conditions under which multilevel selection may select for systems of conflict mediation that enhance selection at the group level (Michod, 1996, 1997, 1999; Michod and Roze, 1997, 1999; Michod et al., 2003). Another factor favoring selection at the group level is "parental control" of the cell phenotype, in which the behavioral phenotype (i.e., the cell fate) is determined during development by the "mother" cell. This is the case in *V. carteri*, as the cell fate (somatic or germ) is established early in development through a series of asymmetric cell divisions of the anterior blastomeres (for discussion see Michod et al. (2003)). It is well known that it is easier for cooperation to be maintained in a group under parental control than under offspring control (in which the phenotype is determined by the genotype of the cell), because the sacrifice of cooperation is spread over the different genotypes present in the cell group (see, for example, Michod (1982)).

Here we consider a group of cells and seek to understand the selective pressures that mold the allocation of energy and resources at the cell level to the two fitness components of the group, survival and reproduction. We present two models. In the fitness isocline model we consider whether, as groups increase in size, when a single new cell is added, it could increase the fitness of the group by changing its reproductive effort from what the existing cells in the group have been doing. In the full optimization model we consider whether a small change in behavior of one or several cells could increase the fitness of the group. The optimization model is clearly more general, but we begin with the fitness isocline model for heuristic reasons. Throughout, we seek qualitative results that are independent of the specific functions involved (so long as these functions meet the general assumptions stated: differentiability, concavity, convexity, etc.).

4. Fitness isocline model

Consider a group of $N-1$ cells with a group viability V_{N-1} and fecundity B_{N-1}. We would like to predict the allocation of energy and resources to reproduction, e, and survival-enhancing vegetative functions, $1-e$, for the Nth cell, resulting in $b(e)$ and $v(1-e)$ contributions to fecundity and viability, respectively. The variable e is the familiar reproductive effort variable of life-history theory (Stearns, 1992; Charlesworth, 1980). Since both b and v are assumed to be monotonic functions of e, we follow precedence in this area and generally work in terms of b and v directly (instead of in terms of e). We assume a simple additive model of fitness at the group level (termed "group selection

I'' by Damuth and Heisler (1988)) so that the fitness components of the group are the sum of the contributions of the cells, or considering the additional Nth cell, the fitness of the group is $W=(V+v)(B+b)$, where $V=V_{N-1}$ and $B=B_{N-1}$. We suppress the group size subscript here and in what follows for notational simplicity. Additivity of cell contributions to viability and fecundity might apply, for example, to the simpler forms of volvocine algae considered in Fig. 1, in which cells stay together after cell division.

The new fitness of the group with the additional cell is then given by

$$W = bv + bV + vB + BV. \tag{1}$$

We would like to maximize the fitness, W_i, contributed by the new cell given by

$$W_i = bv + bV + vB. \tag{2}$$

For fixed W (fixed V and B), the fitness contributed by the new cell, W_i, is a function of two variables, b and v. We can plot isoclines for W_i by using Eqs. (3) and (4) to plot v as a function of b as done in Fig. 2:

$$v = \frac{W_i - bV}{b+B}, \tag{3}$$

$$v'(b) = -\frac{W_i + BV}{(b+B)^2} < 0 \quad \text{and}$$

$$v''(b) = 2\frac{W_i + BV}{(b+B)^3} > 0. \tag{4}$$

We note a few points about Fig. 2 that will be useful below. The isoclines are convex functions (first derivative increasing) which do not overlap and, for increasing fitness return, W_i, they occur increasingly farther from the origin. For any particular W_i, W_i/B and W_i/V are the maximal fitness that could be attained at the group level for viability and fecundity, respectively. Using Eq. (4), the slopes of tangents to the isocline at these points are indicated in Fig. 2.

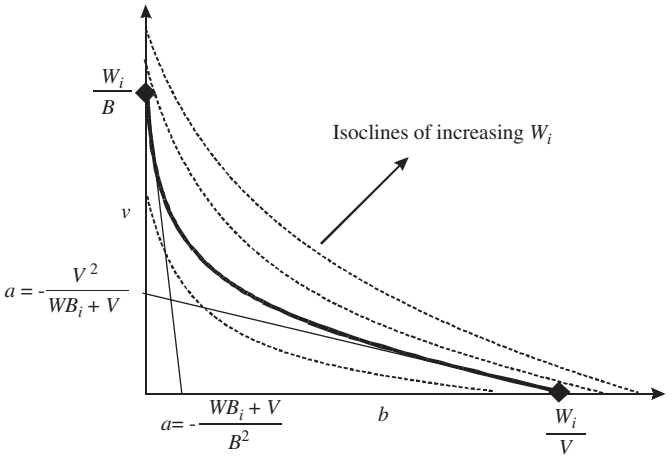

Fig. 2. Fitness isoclines for the contribution of the new cell to viability, v, and fecundity, b, at the group level. Four isoclines are shown, the heavy solid line is the isocline of interest, the others are dashed. Tangents to the isocline are shown at the maximal contributions possible: W_i/V for reproduction and W_i/B for viability.

In addition to the fitness relations at the group level graphed by the isoclines in Fig. 2, we assume there is an intrinsic relation that links b and v within the cell because of cell physiology and/or other constraints. We refer to this intrinsic relation as the "trade-off function", as it embodies life-history trade-offs between the two fitness components at the cell level. During the origin of multicellularity, we expect these trade-offs to depend upon the size of the group that the cells must create (investigated below), as well as a host of other factors; but, for the moment, we consider the implications of the simple linear relation

$$v = v_{\max} - \alpha b. \tag{5}$$

As illustrated in Fig. 3A with a linear intrinsic function, the cell will likely invest in both reproductive and viability-related functions. Indeed, a simple inductive argument given in Appendix A shows that for a linear intrinsic function (Eq. (5)), cell groups have no incentive to specialize. No matter how large the group is, provided that the $N-1$ first cells exert intermediate reproductive effort at $b = b_{\max}/2$ and $v = v_{\max}/2$, it is optimal that the Nth cell exerts the same effort (this yields the best unspecialized group; some specialized groups may achieve the same level of fitness, but not a higher one). In the case of a linear trade-off, the ratio of viability to fecundity at the group level is determined directly by the trade-offs at the cell level (as represented by α) and is given by

$$V = \alpha B. \tag{6}$$

Since α governs the basic relationship between survival and reproduction at the cell level, it imposes severe constraints on fitness components V and B at the group level (Eq. (6)). Indeed, as there is yet nothing else in the model that might change the relationship between viability and fecundity at the group level, we may expect that Eq. (6) will hold as the group increases in size, so long as we assume the linear constraint at the cell level (Eq. (5)). Below we consider a cost of reproduction to survival that increases as the size of the group produced by the cell increases. This cost changes the basic relationship between survival and reproduction at the group level from that given in Eq. (6), because certain fitness advantages of cell specialization may be realized by the group, but not the cell.

From the graphs in Fig. 3 (B and C), we may anticipate a central result of the model. Note that as the intrinsic curve becomes convex (meaning its derivative increases with b), the cell will specialize in viability (panel B) or fecundity (panel C) functions to attain the maximum fitness gains allowed at the group level. Such specialization in viability or fecundity functions is tantamount to the evolution of soma (panel B) and germ (panel C). In what follows, we approximate a convex intrinsic function in a piecewise linear fashion.

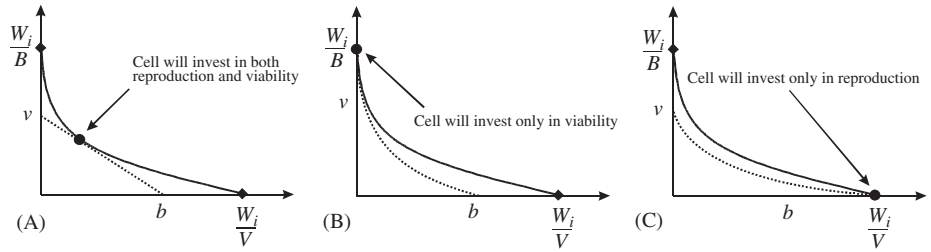

Fig. 3. Optimal investment strategy determined by the intrinsic functions and the fitness isocline. For a linear intrinsic curve (A), the new cell will perform a mix of viability and fecundity functions. For convex intrinsic curves, the new cell will more likely specialize, for example, in survival (B) or reproduction (C).

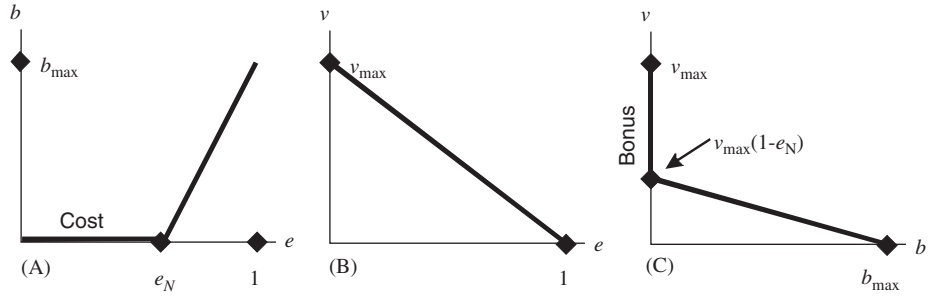

Fig. 4. Initial cost of reproduction. The piecewise convex curve in panel (C) is formed out of the functions in panels (A) and (B) and approximates the convex curves in Fig. 3. In panel (A) the reproductive effort e_N is the initial (or fixed) cost of reproduction. In panel (C) the quantity $v_{max} - v_{max}(1 - e_N)$ is the bonus to viability of soma specialization. This bonus is realized only in groups. The negative of the bonus may be referred to as the initial cost of reproduction to survival. See text for further explanation.

An initial investment is often necessary to get any reproduction. For example, growing the embryo inside the mother colony in the case of the volvocine algae takes time, energy and resources away from other functions (or a mating display, producing a flower, etc.). These initial reproductive costs tend to create a convex relationship between reproductive effort, e, and fecundity, b(e), as depicted in Fig. 4A in a piecewise linear way. We assume that this initial cost of reproduction detracts from survival and so we term it "initial cost of reproduction to survival" or sometimes just "initial cost of reproduction". This initial cost will depend on the group size N which the cell must produce, e_N. Combining this initial cost of reproduction (Fig. 4A) with a linear intrinsic function for viability (Fig. 4B) and using the construction given in Fig. 4, we obtain the piecewise linear intrinsic function relating v and b given in Eq. (7) and plotted in Fig. 4C. By varying the initial cost of reproduction, the piecewise linear curve in Eq. (7) (Fig. 4B) can approximate the convex curves graphed in Fig. 3 (panels B and C).

$$v = v_{max}(1 - e_N)\left(1 - \frac{b}{b_{max}}\right), \quad v \leqslant v_{max}(1 - e_N),$$

$$v = v_{max}(1 - e), \quad v > v_{max}(1 - e_N). \tag{7}$$

In Fig. 4, the initial cost of reproduction to survival is the vertical portion of the intrinsic curve running along the v-axis from $v_{max}(1 - e_N)$ up to v_{max}. The modulus of this initial cost also equals the benefit to viability of soma specialization stemming from not having to pay the initial cost of reproduction. For the volvocine green algae with palintomic development (Fig. 1), the initial cost of reproduction, e_N in Fig. 4, is directly related to the group size N which the reproductive cell must produce, and thus to the cell size the reproductive cell must attain before initiating the rapid series of embryonic divisions to create the daughter colony.

We note three points about this benefit of soma specialization, the "bonus" diagrammed in Fig. 4C. First, this bonus is only obtainable through group living and is only expressed at the group level, it is not an option open to solitary cells. Second, it changes the basic relationship that governs the fitness components at the cell level into a new relationship between viability and reproduction at the group level. Third, the benefit will likely change with the size and organization of the group. For example, if there are already many somatic cells in the group, the benefit of a new somatic cell may be small.

If we assume that at the colony size at which the initial cost of reproduction becomes operative, the ratio of survival to reproduction at the group level is α (as the linear constraint predicts), $V = \alpha B$, then a straightforward analysis is possible. In this case, we must have $Bv_{max} > Vb_{max}$, so germ specialization never pays as a first step from undifferentiated cells. Assuming $V = \alpha B$, a critical value for the initial cost of reproduction, e_N, can be derived to determine whether the new cell will specialize in somatic functions or remain undifferentiated. This critical cost is obtained by investigating the conditions when $Bv_{max} > W_i^*$, where W_i^* is the maximal value of added

fitness obtained for optimal intermediate allocation to reproduction, b^*, and survival, v^*. The critical value of the initial cost of reproduction is given by

$$e_N^{crit} = \frac{b_{\max}}{4B + b_{\max}}. \quad (8)$$

If $e_N > e_N^{crit}$, soma specialization pays. Otherwise, the cell continues to allocate resources to both survival and reproduction.

By inspecting Eq. (8), we can see that the larger the group fecundity (B) is, the smaller the initial cost of reproduction may be for soma specialization to evolve. In other words, in colonies with larger fecundity (and, all things being equal, this means larger colonies) it is easier for a specialized and sterile soma to evolve. This may be explained as follows. The difference between the added fitness brought about by a cell specializing in soma and the added fitness brought about by a cell having the same fecundity, $b = b_{\max}/2$, as the first $N-1$ cells is $(v_{\max} - v_0)B - v_0 b_{\max}/4$. The first term is the advantage of specialization linked to the initial cost of reproduction. The second term is the loss linked to the fact that specialization disrupts the balance between viability and fecundity (see Section 5.4.1 for more discussion). While this loss is independent of colony size, the advantage increases with colony fecundity. Therefore, it is more likely for the advantage of specialization to exceed the loss due to specialization for larger and already more fecund colonies.

The significance of the loss due to specialization is a result of the assumption that only one cell changes. In particular, if the cell reduces its reproductive effort, there must be a loss to fecundity that must be overcome for this specialization to pay in the overall group fitness. In the optimization model considered next, we allow two (or more) cells to simultaneously change their allocation strategy. If one cell increases and another cell decreases their respective reproductive efforts by the same amount, the total fecundity will remain the same (hence, there is no fecundity loss to the group), but gains in viability are possible under convex curvature.

5. Optimization model

5.1. Overview

We now apply optimization theory to the cell group, so as to consider all the cells simultaneously and study strategies in which cells jointly increase or decrease their reproductive effort so as to maximize the fitness of the group. In the fitness isocline model, we considered how a single new cell could maximize its fitness contribution to the group. In the optimization model we test whether small deviations by two or more cells could increase the fitness of the group. The stability conditions of the optimization model include, and are more general than, the stability conditions of the fitness isocline model.

5.2. The model

Consider groups of N cells, with cells indexed $i = 1, 2, \ldots, N$. Let $e_1, e_2, \ldots, e_N$ be the reproductive effort for each cell, and let $b_1, b_2, \ldots, b_N$ be the resulting contribution to the fecundity of the group. As we did above, we assume the contribution to fecundity is an increasing function of reproductive effort; therefore, we can work in terms of fecundity, instead of reproductive effort. Let $v_1, v_2, \ldots, v_N$ be the vegetative, viability-enhancing capabilities of each cell. As more effort is put into reproduction, less is available for vegetative functions, resulting in a trade-off between the contributions to the fitness components of the group. We assume that if $b = b_{\max}$ then $v = 0$, and if $b = 0$ then $v = v_{\max}$. As above, for simplicity, we assume that the viability and fecundity of the group, V and B, respectively, are simple additive functions of the cell properties given by

$$B = \sum_{i=1}^{N} b_i \quad \text{and} \quad V = \sum_{i=1}^{N} v_i. \quad (9)$$

Note that while in the fitness isocline model V and B denoted the contribution to viability and fecundity of the first $N-1$ cells, here they denote the viability and fecundity of the whole colony. While it seems biologically reasonable to assume additivity of the contributions to fecundity of the group, additivity of the contributions to viability is more questionable. We have in mind a trait-like flagellar motility (or mixing) as a proxy for viability and assume there is a simple linear relationship between the effort or time a cell invests in flagellar action and the overall motility of the group. While this assumption may hold over a limited range, it would likely fail as the group gets larger and more integrated. We show in Appendix B.3 that we may dispense with the additivity assumption as it applies to viability so long as we maintain it for fecundity and still reach the same qualitative conclusions.

For our purposes, it is not necessary to normalize fitness, since the analysis of optimal behavior in the optimization model or in the fitness isocline model would not change. Normalizing fitness means multiplying the fitness we have by a coefficient which depends only on the size of the colony. When we ask, for a colony of a particular size, should cells specialize or remain generalists, normalization would not change the answer, because the maxima for the normalized or not-normalized fitness functions would be the same.

We assume that group fitness, W, is the product of viability and fecundity. This is appropriate for a life cycle involving discrete generations as is the case with the volvocine green algae.

$$W = VB. \quad (10)$$

Although the multiplicative decomposition of fitness into viability and fecundity assumed in Eq. (10) applies when generations are discrete, most of the qualitative points

made in the following sections would still hold were fitness a more general function $W(V,B)$ which was nonnegative, zero if and only if $V=0$ or $B=0$, and strictly increasing in both arguments whenever V and B are both positive. In particular, the fundamental point that cell specialization allows the group to increase fitness under conditions of convexity holds for this more general fitness function (because specialization can retain the same group fecundity while increasing viability).

As already mentioned, the additivity assumed in Eq. (9) is an example of group selection of type I as discussed by Damuth and Heisler (1988). However, there are interesting implications of combining the fitness components at the group level after first summing the cell contributions (as assumed in Eqs. (9) and (10)). Most important (and critical to our analysis below) is the fact that, if one cell has a high fecundity (and hence a low viability, so that it would have a low fitness by itself), this may be compensated for if another cell has a high viability (and hence low fecundity). Consequently, even though each of these cells by itself would have a low fitness, together they can bring a high fitness to the group (especially under conditions of convexity of the trade-off). This kind of joint effect is a first step towards integration of the group, and would not be possible if we used as group fitness the average cell fitness, $(1/N)\sum_{i=1}^{N} v_i b_i$.

More formally, the normalized fitness, VB/N^2, is greater than the average cell fitness by the negative of the covariance between the two fitness components. Since in our case the covariance is negative, the normalized fitness associated with Eq. (10), VB/N^2, is greater than the average cell fitness, $(1/N)\sum_{i=1}^{N} v_i b_i$, by the magnitude of the covariance between fitness components. This covariance effect at the group level appears to be quite general. Its contribution to a property like fitness depends on the property being a multiplicative function (or some other function requiring a strong balance) of two components (e.g., viability and fecundity) which themselves covary so that higher values of one component bring lower values of the other (the trade-off principle). Of course, if there is no variance in these components among the lower level units (cells) then there is no covariance and no effect at the group level. What factors might produce variance among the lower level units? We can think of two factors: noise, and the curvature of the trade-off function being convex.

5.3. Implications of different curvatures of the trade-off function

When investigating the implications of the different possible curvatures of the trade-off function, $v(b)$, we will repeatedly make use of the definitions of convex and concave functions. For a strictly convex (concave) function $v(b)$, if we take a particular point, say b^*, and two points equidistant below and above b^*, say b^- and b^+, respectively, then $v(b^-)+v(b^+)>(<)\,2\,v(b^*)$. If b is fecundity and $v(b)$ viability, then convexity of v implies that there is an advantage to specializing in the two components of fitness, while concavity implies there are diminishing returns on an investment in either component. We first assume that there is no initial cost of reproduction.

5.3.1. Concave trade-off

If the function $v(b)$ is strictly concave, then the cell group should remain unspecialized. More precisely, all cells should exert the effort b^* that maximizes the product $bv(b)$. The key to this result is to observe that if two cells, i and j, have different reproductive efforts, $b_i \neq b_j$, then they could both change their fecundities to $(b_i+b_j)/2$. This change in reproductive effort would not change the overall fecundity of the group but would (by definition of concavity) increase group viability, and hence increase fitness. Indeed, the change in group viability would be $\Delta V = 2v((b_i+b_j)/2) - v(b_i) - v(b_j)$, which is positive because $v(b)$ is a strictly concave function. This shows that all cells should exert the same effort. If this common effort is b then the viability is $V=Nv(b)$, the fecundity is $B=Nb$ and the fitness is $W=N^2bv(b)$. Thus, independently of the number of cells in the colony, the optimal value of the cell fecundity common to all cells is the one that maximizes the product $bv(b)$. The result that the optimal fecundity for each cell does not depend on the number of cells is likely not robust and depends crucially on the assumptions that viability is additive and that the trade-off function $v(b)$ does not depend on the size of the colony. In contrast, the result that a concave trade-off function selects against specialization is robust, as we show in Appendix B.

5.3.2. Linear trade-off

If the function $v(b)$ is linear ($v(b)=v_{\max}-\alpha b$, as in Eq. (5)) then the group viability only depends on the group fecundity (and not on the particular values of the component cell fecundities). Indeed, we have $V=\sum_i v_i = \sum_i (v_{\max}-\alpha b_i) = Nv_{\max}-\alpha B$.

Thus, any values of the fecundities $b_1,\ldots,b_N$ leading to the same global fecundity B yield the same fitness $W=(Nv_{\max}-\alpha B)B$. The possible values of B range from 0 to $Nv_{\max}/\alpha$. The maximum fitness is obtained for $B=Nb_{\max}/2$ (hence $V=Nv_{\max}/2$) and is equal to $W^*=N^2b_{\max}v_{\max}/4$. Any arrangement of the fecundities $b_1,\ldots,b_N$ such that $\sum_i b_i = Nb_{\max}/2$ is optimal and these are the only optimal choices of the fecundities. In particular, assuming that the $N-1$ first cells have a fecundity $b=b_{\max}/2$, then it is optimal for the Nth cell to exert the same effort, which yields the first result of the fitness isocline model. Formally, the group of cells behaves as if there was just one cell. There is no incentive to specialize and so no individuality at this stage.

5.3.3. Convex trade-off

If the function $v(b)$ is strictly convex, then the vast majority of cells will specialize (some in soma, some in germ). It may be that, at most, one cell remains unspecialized if, for example, there is an odd number of

cells in the group. If there is an even number of cells, then half should specialize in germ and half in soma. Indeed, this yields a fitness of $W = \frac{1}{4}N^2 v_{\max} b_{\max}$, which is the same fitness obtained in the more favorable linear case considered above with the same values of $b_{\max}$ and $v_{\max}$. The linear trade-off is more "favorable" in the sense that, for any value of the contribution to fecundity b, the contribution to viability $v(b)$ is higher or equal in the linear case than in the convex case. As a result, for a convex trade-off, complete specialization in equal proportions must be optimal since it attains this highest possible fitness. If there is an odd number of cells, it may be that one cell remains unspecialized (for instance, when there is just one cell), but at most one cell may remain unspecialized. Indeed, assume that two cells i and j have an intermediate fecundity; without loss of generality assume $b_i \leqslant b_j$. Let δ be positive and smaller than both b_i and $b_{\max} - b_j$. If cell i decreases its fecundity while cell j increases its fecundity by the same amount δ, then the global fecundity of the colony does not change. However, the viability increases, hence the fitness increases. Indeed, the change in viability is $\Delta V = v(b_i - \delta) + v(b_j + \delta) - (v(b_i) + v(b_j))$, which is positive due to the strict convexity of the function $v(b)$, as shown in Appendix B.1.

5.3.4. Neither convex nor concave

It might be that the function $v(b)$ is neither concave nor convex. In that case, in the absence of additional information, whether specialization pays cannot be decided. Some partial results may be obtained though. For instance, assuming that $v(b)$ is differentiable, if at a fitness maximum a cell i has an intermediate fecundity b_i, then we must have $\partial v/\partial b(b_i) = -V/B$ (taking the derivative of fitness with respect to fecundity of cell i and setting it to zero using Eqs. (10) and (9)). Also, if $v(b)$ is twice differentiable, then at a fitness maximum at most one cell may have an intermediate fecundity b' such that the trade-off function is locally strictly convex at b' (that is, $\partial^2 v/\partial^2 b(b') > 0$). This generalizes the above result on convex trade-offs. The proof of this result (omitted for brevity) consists in differentiating the fitness function and investigating the standard first- and second-order optimality conditions.

5.4. Initial cost of reproduction

We now investigate the effect of an initial cost of reproduction. Formally, letting $v_{\max}$ be the contribution to viability of a completely specialized somatic cell ($b = 0$) and letting v_0 be the limit of $v(b)$ when the fecundity $b > 0$ tends to zero, we assume that $v_{\max}$ is greater than v_0 and study how this modifies the results of the preceding section. Recall that $v_{\max} - v_0$ is the bonus discussed in Fig. 4. Intuitively, an initial cost of reproduction makes the trade-off function more "convex-like", and thus tends to select for specialization.

5.4.1. Convex or linear trade-off

If the trade-off function $v(b)$ is strictly convex, then adding an initial cost of reproduction only reinforces the conclusion that specialization should be favored. More interesting are the cases of a linear trade-off (considered now) or a concave trade-off (considered below). Recall that in the linear case, without an initial cost of reproduction, the maximal fitness may be obtained with or without specialization. Thus, in the previous case, the colony is indifferent to specialization or no specialization. An initial cost of reproduction, however small, tilts the balance in favor of specialization. Indeed, assume that for $b > 0$, $v(b) = v_0 - \alpha b$ with $v_0 = \alpha b_{\max} < v_{\max}$. If no cell specializes, then, as discussed in the preceding section, the best fitness that the colony can obtain is $W^* = N^2 b_{\max} v_0/4$. This will be the case when the group fecundity is $B^* = N b_{\max}/2$, and the group viability is $V^* = N v_0/2$. We now apply the argument above concerning a pair of cells and whether they might jointly specialize by considering a colony in which $N-2$ cells have fecundity $b = b_{\max}/2$, and in which the two other cells are specialized, one in soma and one in germ. The overall fecundity of the colony is still B^*, but the viability is now $V = (N-2)v_0/2 + v_{\max} = N(v_0/2) + v_{\max} - v_0 > V^*$; hence, the fitness is greater than W^*. Therefore, if some of the cells, specialize, the colony can obtain a greater fitness than if all cells are generalists. If there is an even number of cells then, as in the case of a convex trade-off and no initial cost of reproduction, half of the cells should specialize in soma and half in germ.

Note that, with a linear trade-off, specialization occurs as soon as there is any initial cost of reproduction. This contrasts with the fitness isocline model, in which specialization requires that the initial cost of reproduction be greater than a critical value (Eq. (8)). To understand this difference, note that in the fitness isocline model we assumed that the behavior of the $N-1$ first cells was fixed. Thus, if the Nth cell specializes in soma, this yields a benefit to viability (the bonus to specialization linked to the initial cost of reproduction), but disrupts the balance between fecundity and viability. For specialization to be optimal in the fitness isocline model, the benefits must outweigh the costs. In the more general optimization model considered here, if a cell specializes in soma, the other cells may increase their reproductive effort in order to compensate for the corresponding loss of fecundity. Thus, the group may obtain the benefits of specialization without having to pay for a disruption in the balance between fecundity and viability.

5.4.2. Concave trade-off

We assume now that the trade-off function $v(b)$ is strictly concave ($0 < b \leqslant b_{\max}$), but that there is an initial cost of reproduction, so that $v_{\max} > v_0$ (Fig. 5). As in the preceding section, if two cells i and j which are not specialized in soma have a different fecundity, e.g. $b_i > b_j > 0$, then by changing their fecundity to $(b_i + b_j)/2$, they would retain the same overall contribution to group fecundity while increasing

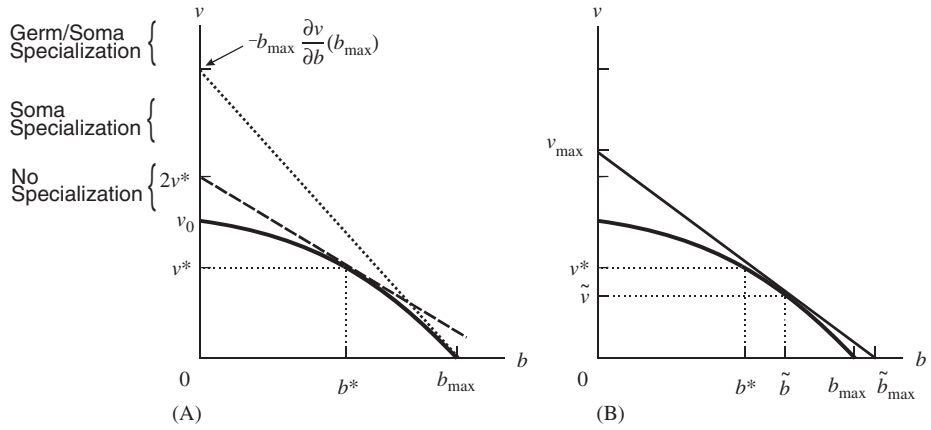

Fig. 5. Evolution of soma and germ with concave trade-off and cost of reproduction. The kinds of colonies predicted by the model are given along the ordinate in panel (A) for different regions of v_{max}. The quantities b^* and v^* are the fecundity and viability, respectively, of the cells in the optimal unspecialized colony. The solid concave curve (identical in both panels) is the trade-off between viability and fecundity. Three tangents relevant to the analysis are drawn. In panel (A), the dotted and dashed lines are the tangents to the trade-off curve at $b = b_{max}$ and $b = b^*$, respectively. In panel (B), the dashed line is the tangent to the curve at $b = \tilde{b}$. The significance of these points is discussed in the text and Appendix B.2. The quantity $2v^* - v_0$ is the detriment to soma specialization due to the concavity of the trade-off. If $v_{max} > 2v^*$, then specialization in soma pays. See text for further explanation.

their contribution to group viability. It follows that, at a fitness maximum, all cells which are not specialized in soma must have the same fecundity. An immediate consequence is that specialization in germ without specialization in soma cannot pay as a first step. The question is whether specialization of some cells in soma allows for an increase in fitness.

Assuming that the function $v(b)$ is twice differentiable, an analysis given in Appendix B.2 gives the conditions under which soma specialization will evolve, and, if soma evolves, whether germ specialization will also evolve. We now summarize those conditions referring to Fig. 5. Let b^* denote the fecundity of the cells in the best unspecialized colony (i.e. b^* is the fecundity which maximizes the product $bv(b)$). If Eq. (11) holds, specialization does not pay (the best colony is the one in which all cells have intermediate fecundity b^*).

$$v_{max} \leqslant 2v^*. \tag{11}$$

Since b^* maximizes $bv(b)$, we have $v^* + b^*(\partial v/\partial b)(b^*) = 0$. It follows that Eq. (11) is equivalent to

$$v_{max} \leqslant v^* - b^*\frac{\partial v}{\partial b}(b^*). \tag{12}$$

Eq. (12) means that the tangent to the trade-off curve at $b = b^*$ crosses the line $b = 0$ below v_{max}. On the other hand, if the reverse of Eq. (12) (given in Eq. (13)) holds, then some cells should specialize in soma.

$$v^* - b^*\frac{\partial v}{\partial b}(b^*) = 2v^* < v_{max}. \tag{13}$$

Furthermore, if, in addition to satisfying Eq. (13), $v_{max} < -b^*(\partial v/\partial b)(b^*)$, then the reproductive cells should have an intermediate fecundity; more precisely, they should have fecundity $\tilde{b}$ such that the tangent to the trade-off curve at $b = \tilde{b}$ crosses the line $b = 0$ precisely at v_{max} (Fig. 5 panel (B)) (that is $v_{max} = \tilde{v} - \tilde{b}(\partial v/\partial b)(\tilde{b})$, see Appendix B.2). The proportion of somatic cells should be such that the mean viability is $v_{max}/2$. The mean fecundity is then $\tilde{b}_{max}/2$, $\tilde{b}_{max} = -v_{max}/[(\partial v/\partial b)(\tilde{b})]$ and the fitness $W = N^2 v_{max}\tilde{b}_{max}/4$, which is the highest obtainable fitness for a colony facing a linear trade-off $v = v_{max} - \alpha b$ with $\alpha = -\partial v/\partial b(\tilde{b})$.

Finally, if

$$v_{max} \geqslant -b_{max}\frac{\partial v}{\partial b}(b_{max}), \tag{14}$$

then half of the cells should specialize in soma ($b = 0$) and the other half in germ ($b = b_{max}$). This yields the fitness $W = N^2 v_{max} b_{max}/4$.

The above analysis shows that there are two threshold values for v_{max} (in the region $v_{max} > v_0$ (see Fig. 5)). The first threshold, $v_{max} = 2v^* = v^* - b^*(\partial v/\partial b)(b^*)$, concerns specialization in soma. The second threshold, $v_{max} = -b_{max}(\partial v/\partial b)(b_{max})$, concerns specialization in germ. In the case of a linear trade-off, both thresholds are equal to v_0. Thus, while $v_{max} - v_0$ is the bonus of soma specialization linked to the cost of reproduction, we may see $2v^* - v_0$ as the detriment of soma specialization linked to the concavity of the trade-off. Eq. (11) (or, equivalently, Eq. (12)) expresses the condition that the bonus of soma specialization is smaller than its detriment.

As detailed in Appendix B.2, if we take into account the fact that the proportion of somatic cells must be a multiple of $1/N$, then the condition for soma specialization is slightly more demanding than Eq. (13) and is more easily satisfied if the number of cells in the colony is large. This effect of colony size in facilitating the evolution of soma specialization may be interpreted as follows: there is a tension between being efficient (that is, having high ratios of viability/(resources allocated to viability) and fecundity/(resources allocated to fecundity)) and keeping a balance

between viability and fecundity. If a cell specializes in soma, then in order to keep a balance between fecundity and viability, the other cells must increase their fecundity. If there are only a few other cells in the colony, then they will have to increase their fecundity a lot, possibly moving to inefficient functioning points. This is likely to be the case when there are decreasing returns on efforts (that is, when the fecundity/(resources to fecundity) ratio decreases as the resources alloted to fecundity increase, and similarly for viability), which corresponds to a concave trade-off. In contrast, in a large colony where there are more cells available, a balance between viability and fecundity can more easily be maintained since the increase in reproductive effort by the rest of the colony can be divided among more cells, which would then not greatly effect each cell's efficiency. Thus, it is possible to reap the advantage of soma specialization linked to the cost of reproduction while maintaining both a balance between viability and fecundity and an efficient functioning of all cells.

6. Discussion

We have concluded that during the origin of multicellularity convex trade-offs between survival and reproduction, such as those created by a significant initial cost of reproduction to survival, select for specialization in the two fitness components as colonies increase in size. As a result of this specialization, the individuality of the cell group is enhanced. The conclusion about the role of convexity in specialization is very general; in particular, we have shown it holds for more general fitness functions than multiplicative (Eq. (10)) and for non-additive viabilities. The main point is that when the trade-off is convex, specialization allows for the increase of one component of fitness (we focused on viability) without any decrease in the other component (fecundity).

The conclusion that convexity favors specialization resembles the standard results of life-history theory, which state that convex fitness trade-offs select for specialization in reproductive function as organisms increase in age. That is, convex fitness trade-offs select for semelparity or "big bang" reproduction in which there is no reproduction until the last stage of the life cycle (Schaffer, 1974; Charlesworth and Leon, 1976). Big bang reproduction is analogous to cell specialization in the sense that age classes specialize in either no reproduction or complete reproduction (for the last class).

However, the life-history problem of optimization of reproductive effort over the lifespan of an individual is different in important ways from the problem of optimization of the reproductive effort of cells in a group. There is, most fundamentally, the very question we wish to answer: is the individual the cell or the cell group? While we do assume selection at the group level, without cell specialization there is no property that would make the group indivisible and hence a true individual. In answering this question, we are not concerned with how the reproductive effort at the group level changes, but rather with whether there is specialization at the lower level among cells. Indeed, in our argument for cell specialization in the optimization model, the average reproductive effort at the group level does not change at all. However, how this effort is distributed among cells can be critical for the group in terms of its viability and individuality.

Consider, for example, the following question in life-history theory which may seem similar to the one studied here. How will the trade-off between viability and fecundity evolve as an external parameter, such as the quality of the environment, varies (Kisdi et al., 1998)? Here we also investigate the evolution of the trade-off between viability and fecundity as another parameter, the size of the group, varies. However, the question we investigate is not how the investment in fecundity will evolve as this additional parameter varies, as in Kisdi et al. (1998), but whether some cells will specialize (again, the overall investment in fecundity staying more or less the same).

On a qualitative level, what we have studied is how the relative changes in viability and fecundity linked to cell specialization evolve as colony size increases. Due to the assumed multiplicative nature of fitness (Eqs. (1) and (10)), $W = VB$, what matters when a cell changes its reproductive effort are the relative changes in viability and fecundity for the group. Formally, if a cell specializes in soma, leading to a decrease in group fecundity of δB and an increase in group viability of δV, then the change in group fitness is $\Delta W = -V\delta B + B\delta V - \delta B\delta V$. If we neglect the last term (which is a second-order term), then we see that the condition for fitness to increase is that the relative increase in viability $\delta V/V$ be greater than the relative decrease in fecundity $-\delta B/B$. This is more likely to be the case if viability is low, as will occur if colonies increase in size without specializing in somatic functions.

What is the fecundity viability trade-off curve like in single-celled organisms? The multiplicative nature of fitness requires that single-cell organisms be generalists and have intermediate efforts at both reproduction and viability, regardless of the curvature of the trade-off curve. Nevertheless, the curvature of the trade-off determines whether the unicellular habit will be stable to two- (or greater) cell groups. This will be the case when the trade-off curve is strictly concave. Since, in nature, smaller groups are not specialized (Fig. 1), our model suggests that the trade-off is concave rather than convex in single-celled species. Furthermore, a concave trade-off seems more natural for small groups, as it expresses a law of decreasing return on efforts.

The curvature of the function describing the relationship between the two main fitness components' reproduction and survival is a basic issue in life-history theory (Benkman, 1993; Michod, 1978; Schaffer, 1974; Benson and Stephens, 1996; Blows et al., 2004; Carriere and Roff, 1995; Kisdi, 2001; Reznick, 1985; Roff, 2002; Rueffler et al., 2004; Sato, 2002; Strohm and Linsenmair, 2000; Takada and Nakajima, 1996; Levins, 1968; Stearns, 1992). Despite

the central relevance of this issue to life-history theory, a recent review (Rueffler et al., 2004) of the data concerning the curvature of the trade-off curve states: "Unfortunately, there is no study known to us which has revealed the details of this curvature for any life-history trade-off in a specific organism. However, these curvatures are central in life-history theory which indicates a major gap between theory and empirical knowledge...".

Our analysis predicts that a large initial cost of reproduction to survival is sufficient to select for G–S specialization. Measuring this initial survival cost of reproduction is empirically more practical than measuring the complete curvature of the trade-off curve between survival and reproduction, the latter having been studied in a variety of organisms with no clear results (if the above quotation is accepted). We return now to the volvocine green algae (Fig. 1) and consider this central prediction of our model.

As already discussed when introducing the volvocine algae (Fig. 1), the investment of the parent colony in reproductive cell growth illustrates an initial cost of reproduction to survival, which increases with organism size. Besides using more resources, a larger embryo increases the volume, mass and drag of the mother colony, as has been quantified in Fig. 4 of Solari et al. (2005b). Solari et al. (2005b) show that these initial survival costs increase with colony size, requiring more swimming force as well as more flagellar mixing (for nutrient acquisition and removal of waste) per embryo.

We believe the need to pay this initial cost of reproduction to survival accounts for the observed increase in the somatic/reproductive (S/R) cell ratio as colony size increases in the volvocine algae (see Table 3 of Solari et al., 2005b). We think that the evolution of soma (as well as the evolution of increased S/R ratios) provides the benefits that compensate for the increasing initial costs of reproduction in colonies of increasing size. There are also direct costs of germ and soma specialization which must be overcome by these benefits, as germ specialization reduces the number of cells available for vegetative functions and soma specialization reduces the number of reproducing cells.

The benefits of soma specialization include: (i) colony motility while reproducing (overcoming the flagellation constraint discussed in Section 3), (ii) motility while large (overcoming the enlargement constraint discussed in Section 3), (iii) increased resource uptake due to the 'source-sink' effect (in which somatic cells transfer resources to germ cells which act as a sink) (Bell, 1985; Koufopanou and Bell, 1993; Solari et al., 2005a), and (iv) enhanced uptake of resources and removal of waste by flagellar beating (Niklas, 1994, 2000; Solari et al., 2005a).

In addition, soma specialization reduces the detriment to viability of germ specialization. Once larger colonies invest in a high proportion of somatic cells, non-somatic cells can focus on reproduction rather than contribute to vegetative functions which are sufficiently dealt with by somatic cells. When soma separation is complete, germ specialization can provide additional benefits, such as decreased generation time, increased productivity by specialization at photosynthesis, and hydrodynamic advantages stemming from the location of germ. Since specialized germ cells are non-flagellated and do not contribute to motility, they are located in the interior of the colony, making the colony spheroid smaller and lowering drag (Solari et al., 2005b).

Single gene mutations in life-history traits can be a powerful approach to understanding the cost of reproduction and trade-offs between life history traits, both long standing topics of considerable interest (Reznick, 1985; Roff, 2000, 2002). Various *V. carteri* developmental mutants are known (Kirk, 1998), which differ in the basic factors hypothesized in our models for the origin of multicellularity: group size, S/R ratio, type and timing of G–S specialization, and motility; yet they differ in just one or a few genes. These mutants include lag^- (germ cells perform motility functions before reproducing; these mutant colonies are similar to *Volvox* species such as *V. aureus* and *V. rouselletti*), $regA^-$ (somatic cells regenerate to become reproductive), and $glsA^-/regA^-$ (all cells perform vegetative functions first and then become reproductive; this mutant is similar to *Eudorina*; see Fig. 1).

These mutants are especially useful for studying fitness decomposition at the cell and group levels, because a certain known number of cells (or amount of tissue) have changed their reproductive effort. We can measure the consequences of this change at the colony level, and in this way estimate the contribution to the group fitness of the changed effort at the cell level as is required by our model in Fig. 4. In the $regA^-$ mutants, ~235 cells have changed their phenotype from somatic to unspecialized; in lag^-~9 cells have changed their phenotype from germ to unspecialized; and in $glsA^-$ $regA^-$ there are ~561 unspecialized cells — similar to a *Eudorina* colony, but larger.

As a result of these changes in reproductive effort at the cell level, the size, productivity and motility of the group change (Solari et al., 2005b; Solari, 2005). For example, in colonies with the $regA^-$ mutation, as once-specialized somatic cells ($b = 0$ in Fig. 4) begin exerting reproductive effort ($b > 0$), there is not only a large decrease in colony motility, but also a large decrease in the motility contributed by a single changed cell. Specifically, the average force exerted for group motility by a single motile cell is about half in the $regA^-$ mutant and a quarter in the $glsA^-$ $regA^-$ mutant compared to the wild type (Solari et al., 2005b). The initial cost of reproduction to survival that underlies the convex nature of the fitness trade-offs (Fig. 4) is real and directly measurable in these organisms, and attributable to a change in the effort exerted by single cells within the cell group as required by the models considered above.

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Appendix A

We use Eq. (5) and build the group one cell at a time, assuming that after a cell is added it does not change its strategy. For the first cell, $V = B = 0$ and the isoclines are simply $v = W_i/b$. Maximizing W_i (Eq. (3)) subject to Eq. (5) gives the optimum strategy for the cell defined by

$$b^* = \frac{v_{\max}}{2\alpha} = \frac{b_{\max}}{2} \quad \text{and} \quad v^* = \frac{v_{\max}}{2}. \tag{A.1}$$

The additional fitness (Eq. (3)) is now given by

$$W_i^* = \frac{b_{\max} v_{\max}}{4}. \tag{A.2}$$

We now add the second cell. The isocline (Eq. (2)) now has $V_1 = v_{\max}/2$ and $B_1 = b_{\max}/2$. Note that $V_1 = \alpha B_1$. For the second cell, maximizing the added fitness (Eq. (3)) subject to the linear constraint (Eq. (5)) gives

$$b^* = \frac{b_{\max}}{2} \quad \text{and} \quad v^* = \frac{v_{\max}}{2}, \tag{A.3}$$

with added fitness given by

$$W_i^* = \tfrac{3}{4} V_1 B_1. \tag{A.4}$$

The new 2-cell group has $V_2 = 2v_{\max}/2 = v_{\max}$ and $B_2 = 2b_{\max}/2 = b_{\max}$ and Eq. (A.4) holds since, of course, $v_{\max} = \alpha b_{\max}$. If we now consider the $N+1$ cell and maximize W_i subject to the linear constraint (Eq. (5)), and that for the N cell group $V_N = \alpha B_N$ (Eq. (A.2)), we find again Eq. (A.1) and $B_{N+1} = B_N + b_{\max}/2 = (N+1)b_{\max}/2$ and $V_{N+1} = V_N + v_{\max}/2 = (N+1)v_{\max}/2$. The optimal value of intermediate reproductive effort can be obtained by maximizing W_i subject to the linear intrinsic constraint. For use in the text in deriving Eq. (8), we assume $V = \alpha B$. When there is a cost of reproduction, using the piecewise linear curve defined in Fig. 4, we obtain the optimal intermediate values to be

$$b^* = \frac{b_{\max}}{2} \quad \text{and} \quad v^* = \frac{(1-e_N)v_{\max}}{2}. \tag{A.5}$$

Using the values in Eq. (A.5), we obtain as the maximal added fitness for intermediate strategies

$$W_i^* = \frac{(4B + b_{\max})(1-e_N)v_{\max}}{4}. \tag{A.6}$$

Appendix B

B.1. Proof of a result on convex trade-offs

Assume that $v(b)$ is strictly convex. Let $b_i < b_j$ be intermediate fecundities and let $\delta > 0$ be smaller than b_i and $b_{\max} - b_j$. From the mean-value theorem, it follows that there exist fecundities b' in $[b_i - \delta, b_i]$ and b'' in $[b_j, b_j + \delta]$ such that

$$v(b_i) = v(b_i - \delta) + \delta \frac{\partial v}{\partial b}(b') \quad \text{and}$$

$$v(b_j + \delta) = v(b_j) + \delta \frac{\partial v}{\partial b}(b'').$$

It follows that

$$v(b_i - \delta) + v(b_j + \delta) - (v(b_i) + v(b_j)) = \delta \left[\frac{\partial v}{\partial b}(b'') - \frac{\partial v}{\partial b}(b') \right]. \tag{B.1}$$

Since the function v is strictly convex, its first derivative is strictly increasing. Therefore, since $b'' > b'$, Eq. (B.1) is positive, as claimed in Section 5.3.3. The result still holds if the function v is not differentiable (proof omitted).

B.2. Proof of results on concave trade-offs with an initial cost of reproduction

We compute here the optimal behavior of a colony facing a strictly concave trade-off with an initial cost of reproduction. We first assume, as an approximation, that the proportion of somatic cells can take any value. We then discuss how taking into account the fact that the proportion of somatic cells must be a multiple of $1/N$ changes the results. Throughout, we assume that the contributions to viability are additive (the case of non-additive viabilities is treated in Appendix B.3). We also assume for simplicity that the function $v(b)$ is twice differentiable, so that the fitness function is twice differentiable. This allows us to use the standard first- and second-order optimality conditions of optimization theory (see below). However, this assumption is not necessary: as discussed at the end of this section, a graphical analysis shows that the same conclusions may be reached if the function v is not differentiable.

The fitness of a colony with a proportion p of somatic cells and fecundity b for all other cells is

$$W(p, b) = N^2[p v_{\max} + (1-p)v(b)] \cdot (1-p)b. \tag{B.2}$$

As explained in Section 5.4.2, in the best colony, all non-somatic cells have the same fecundity. Thus, to find the best colony, we only need to find the values of p and b that maximize Eq. (B.2). Let $\tilde{p}$ and $\tilde{b}$ be such optimal values and let $\tilde{v} = v(\tilde{b})$. Specialization of some cells in soma is optimal if $\tilde{p} > 0$. Specialization of the non-somatic cells in germ is optimal if $\tilde{b} = b_{\max}$. Recall that b^* denotes the optimal value of the fecundity for a non-specialized colony. If specialization in soma does not pay ($\tilde{p} = 0$), then $\tilde{b} = b^*$; but, if specialization in soma pays, then we expect (and we will prove) that the optimal fecundity of the non-somatic cells $\tilde{b}$ is greater than b^*.

Since the values $\tilde{p}$ and $\tilde{b}$ are optimal, we have

$$\frac{\partial W}{\partial b}(\tilde{p}, \tilde{b}) = 0 \tag{B.3}$$

if $\tilde{b} < b_{\max}$,

$$\frac{\partial W}{\partial b}(\tilde{p}, \tilde{b}) \geqslant 0 \tag{B.4}$$

if $\tilde{b} = b_{\max}$, and finally, if $\tilde{p} > 0$,

$$\frac{\partial W}{\partial p}(\tilde{p}, \tilde{b}) = 0. \tag{B.5}$$

To use these conditions, we need to compute the partial derivatives of W. We get

$$\frac{\partial W}{\partial p}(p, b) = N^2 b[(1-2p)v_{\max} - 2(1-p)v(b)], \tag{B.6}$$

$$\frac{\partial W}{\partial b}(p, b) = N^2(1-p)\left[(1-p)\left[b\frac{\partial v}{\partial b}(b) + v(b)\right] + pv_{\max}\right]. \tag{B.7}$$

From Eqs. (B.5) and (B.6) we obtain

$$\tilde{p} = \frac{v_{\max} - 2\tilde{v}}{2[v_{\max} - \tilde{v}]} \tag{B.8}$$

and

$$\tilde{p}v_{\max} = (1-\tilde{p})(v_{\max} - 2\tilde{v}). \tag{B.9}$$

Furthermore, plugging Eq. (B.9) into Eq. (B.7) we get

$$\frac{\partial W}{\partial b}(\tilde{p}, \tilde{b}) = N^2(1-\tilde{p})^2\left[\tilde{b}\frac{\partial v}{\partial b}(\tilde{b}) + (v_{\max} - \tilde{v})\right]. \tag{B.10}$$

Therefore, Eqs. (B.3) and (B.4) imply that

$$v_{\max} \geqslant \tilde{v} - \tilde{b}\frac{\partial v}{\partial b}(\tilde{b}), \tag{B.11}$$

with equality if $\tilde{b} < b_{\max}$.

We now distinguish three cases which correspond, respectively, to no specialization, specialization in soma but not in germ, and specialization in soma and in germ. Let $v^* = v(b^*)$.

Case 1: $v_{\max} \leqslant 2v^*$. It follows from Eq. (B.8) that for specialization to be optimal, i.e. $\tilde{p} > 0$, we must have

$$v_{\max} > 2\tilde{v}. \tag{B.12}$$

Since $v_{\max} \leqslant 2v^*$, this implies $v^* > \tilde{v}$, hence $\tilde{b} > b^*$.

Since the function v is concave, it follows that

$$\tilde{v} - \tilde{b}\frac{\partial v}{\partial b}(\tilde{b}) > v^* - b^*\frac{\partial v}{\partial b}(b^*).$$

Together with Eq. (B.11), this implies that

$$v_{\max} > v^* - b^*\frac{\partial v}{\partial b}(b^*) = 2v^*$$

(the latter equality is proved in Section 5.4.2). This contradicts the assumption $v_{\max} \leqslant 2v^*$. It follows that if $v_{\max} \leqslant 2v^*$, then specialization is not optimal.

Case 2: $2v^* < v_{\max} < -b_{\max}(\partial v/\partial b)(b_{\max})$. Applying Eq. (B.6) at $p = 0$ and $b = b^*$ we obtain

$$\frac{\partial W}{\partial p}(0, b^*) = N^2 b^*(v_{\max} - 2v^*) > 0. \tag{B.13}$$

Thus a colony with some somatic cells and fecundity b^* for the non-somatic cells would have a higher fitness than the colony in which all cells have fecundity b^* (i.e. the best unspecialized colony). This implies that at a fitness maximum, the proportion of somatic cells is positive: $\tilde{p} > 0$. Furthermore, since

$$v_{\max} < -b_{\max}\frac{\partial v}{\partial b}(b_{\max}) = v(b_{\max}) - b_{\max}\frac{\partial v}{\partial b}(b_{\max})$$

it follows that if $\tilde{b} = b_{\max}$, then Eq. (B.10) is negative, contradicting Eq. (B.4). Therefore, $\tilde{b} < b_{\max}$, i.e. the non-somatic cells should be generalists. This implies that Eq. (B.11) holds with equality. That is,

$$v_{\max} = \tilde{v} - \tilde{b}\frac{\partial v}{\partial b}(\tilde{b}). \tag{B.14}$$

Since the equation of the tangent to the trade-off curve at $\tilde{b}$ is

$$v = \tilde{v} + (b - \tilde{b})\frac{\partial v}{\partial b}(\tilde{b}),$$

Eq. (B.14) means that this tangent crosses the line $b = 0$ exactly at $v = v_{\max}$, as depicted in Fig. 5. This implies that $\tilde{v} > v^*$.

Using Eq. (B.8), we compute the viability, fecundity and fitness of the colony to obtain $V = Nv_{\max}/2$, $B = Nv_{\max}/2\alpha$, and $W = N^2 v_{\max}^2/4\alpha$, with $\alpha = -(\partial v/\partial b)(\tilde{b})$.

Case 3: $v_{\max} \geqslant -b_{\max}(\partial v/\partial b)(b_{\max})$. As in case 2, specialization in soma pays: $\tilde{p} > 0$, but now $\tilde{b} = b_{\max}$ (otherwise Eq. (B.10) would be positive, contradicting Eq. (B.3)). That is, the non-somatic cells should be germ (and not generalist). The above formulae for p, V, B and the fitness W still hold. In particular, Eq. (B.8) gives $p = \frac{1}{2}$; thus, half of the cells should specialize in germ and half in soma.

Note that our results do not require that the function $v(b)$ stays the same. In particular, the trade-off function could change as the number of cells increases ($v = v(b, N)$), in which case the optimal fecundity of the cells in an unspecialized colony may depend on the size of the colony. In addition, it is not necessary that the function v be concave (it could be, e.g., neither convex nor concave). A graphical analysis (included as supplementary material in the online version of this article) shows that provided that there exists a fecundity $\tilde{b}$ which is greater than the mean fecundity in the best unspecialized colony and such that the line joining the points $(0, v_{\max})$ and $(\tilde{b}, v(\tilde{b}))$ is above the graph of v, then specialization will be favored.

Taking into account the fact that the proportion of somatic cells is a multiple of $1/N$: We now discuss how the results change if we take into account the fact that the proportion of somatic cells cannot vary continuously but must be a multiple of $1/N$. Consider a colony in which a cell specializes in soma and the other cells increase their fecundities from b^* to $(N/(N-1))b^*$. This colony would have the same fecundity $B^* = Nb^*$ as the best unspecialized

colony, but its viability would be

$$V = v_{\max} + (N-1)v\left(\frac{Nb^*}{N-1}\right). \quad \text{(B.15)}$$

Thus, a sufficient condition for specialization to be favored is that $V > V^* = Nv^*$, or equivalently

$$v_{\max} > Nv^* - (N-1)v\left(\frac{Nb^*}{N-1}\right). \quad \text{(B.16)}$$

Noting that $Nb^*/(N-1) = b^*(1+1/(N-1))$ and approximating the right-hand side by a Taylor expansion including up to second-order terms, Eq. (B.16) becomes

$$v_{\max} > v^* - b^*\frac{\partial v}{\partial b}(b^*) - \frac{(b^*)^2}{2(N-1)}\frac{\partial^2 v}{\partial b^2}(b^*). \quad \text{(B.17)}$$

This is exactly Eq. (13) when the trade-off is linear, but is more demanding for a concave trade-off as the second derivative of v is then negative. Furthermore, for a concave trade-off, Eq. (B.17) reduces to Eq. (13) in the limit of a very large number of cells, but may be significantly more demanding when N is small.

Intuitively, if N is small and $v_{\max}$ is only slightly greater than $v^* - b^*(\partial v/\partial b)(b^*) = 2v^*$, then if a cell specializes in soma, the resulting proportion of somatic cells, $1/N$, might be much higher than the optimal proportion of somatic cells given in Eq. (B.8) and specialization in soma need not be favored even though Eq. (13) is satisfied.

B.3. Non-additive viabilities

Up to now, we assumed for simplicity that the cells contributions to the viability of the group were additive. We show here that we may dispense with this assumption. The assumptions we keep are that the fecundities of the cells are additive, $B = \sum_i b_i$, and (for some results) that the viability V of the group is a symmetric function of the fecundities; that is, the cells are interchangeable in the sense that if cell i and cell j exchange their fecundities, then the viability of the group does not change. We first consider trade-offs with no initial cost of reproduction and show that a convex (concave) trade-off selects for (against) specialization.

Convex trade-off: Assume that the function V is strictly convex. Then in an optimal group, at most one cell may have an intermediate fecundity. Indeed, assume by contradiction that two cells, say cells 1 and 2, have an intermediate fecundity. If cell 1 increases its fecundity by some small quantity x and cell 2 simultaneously decreases its fecundity by the same quantity, then the fecundity of the group does not change but the viability becomes

$$f(x) = V(b_1 + x, b_2 - x, b_3, \ldots, b_N).$$

It follows from the strict convexity of V that the function f is strictly convex. Therefore, for $x > 0$, $f(x) + f(-x) > 2f(0)$. It follows that at least one of the quantities $f(x)$ and $f(-x)$ is strictly greater than $f(0)$. Without loss of generality, assume $f(x) > f(0)$. This means that, while the fecundity of a colony with fecundities $b_1 + x, b_2 - x, b_3, \ldots, b_N$ is the same as the fecundity of the initial colony, its viability, hence its fitness, is higher. Therefore, the initial colony was not optimal.

Linear trade-off: In this case, assuming that V is symmetric in the fecundities, then viabilities are additive and we are back to the model of Section 5.3.2. Indeed, if the trade-off is linear then there exist constants $V_{\max}$, $\alpha_1, \ldots, \alpha_N$ such that

$$V(b_1, \ldots, b_n) = V_{\max} - \left(\sum_i \alpha_i b_i\right).$$

If V is symmetric, then the constants α_i are all equal. Letting α be the common value of the α_i and $v_{\max} = V_{\max}/N$, we get

$$V(b_1, \ldots, b_n) = \sum_i (v_{\max} - \alpha b_i)$$

as in the case of additive viabilities.

Concave trade-off: If the viability V is strictly concave and symmetric, then in an optimal colony, all cells have the same fecundity. Indeed, consider a colony with fecundities $b_1, \ldots, b_N$ and assume that two cells, say cells 1 and 2, have different fecundities. Let

$$g(x) = V\left(\frac{b_1 + b_2}{2} + x, \frac{b_1 + b_2}{2} - x, b_3, \ldots, b_N\right)$$

so that $V(b_1, \ldots, b_N) = g([b_1 - b_2]/2)$. If V is strictly concave, then so is g, so that for $x = [b_1 - b_2]/2 \neq 0$

$$g(x) + g(-x) < 2g(0).$$

Furthermore, if V is symmetric, then

$$g(-x) = V(b_2, b_1, b_3, \ldots, b_N) = V(b_1, b_2, b_3, \ldots, b_N) = g(x)$$

so that $g(x) < g(0)$. It follows that a colony with fecundities $(b_1 + b_2)/2, (b_1 + b_2)/2, b_3, \ldots, b_N$ would have the same group fecundity but a higher group viability than a colony with fecundities $b_1, b_2, b_3, \ldots, b_N$.

Initial cost of reproduction: We now consider the effect of an initial cost of reproduction. By an initial cost of reproduction, we mean that if cell i specializes in soma, the viability is substantially higher than if it provides a little fecundity. Formally,

$$V(b_1, \ldots, b_{i-1}, 0, b_{i+1}, \ldots, b_N) > V(b_1, \ldots, b_{i-1}, 0^+, b_{i+1}, \ldots, b_N)$$

with

$$V(b_1, \ldots, b_{i-1}, 0^+, b_{i+1}, \ldots, b_N) = \lim_{\varepsilon \to 0, \varepsilon > 0} V(b_1, \ldots, b_{i-1}, \varepsilon, b_{i+1}, \ldots, b_N).$$

When the fecundities are additive, then the difference

$$V(b_1, \ldots, b_{i-1}, 0, b_{i+1}, \ldots, b_N) - V(b_1, \ldots, b_{i-1}, 0^+, b_{i+1}, \ldots, b_N)$$

is simply the difference between $v_{\max}$ and v_0 (see Fig. 5).

We focus on the case of a concave trade-off (the case of a convex or linear trade-off is easily dealt with as in Section 5.4.1.). Recall the above argument showing that if the function V is strictly concave and symmetric and if there is no initial cost of reproduction, then all cells should have the same fecundity. The same argument shows that, when there is a cost of reproduction, all non-somatic cells should have the same fecundity.

Let b^* denote the fecundity of the cells in the best unspecialized colony and $B^* = Nb^*$. If, starting from the best unspecialized colony, one cell specializes in soma and the other cells increase their fecundities to $b' = Nb^*/(N-1)$, then the global fecundity does not change but the viability goes from $V(b^*, \ldots, b^*)$ to $V(0, b', \ldots, b')$. Thus, a sufficient condition for fitness to increase is that

$$V(0, b', \ldots, b') > V(b^*, \ldots, b^*) \quad \text{with } b' = \frac{Nb^*}{N-1}. \tag{B.18}$$

In the case of a linear (and symmetric) trade-off, the quantity

$$V(0^+, b', \ldots, b') - V(b^*, \ldots, b^*) \tag{B.19}$$

is zero. Thus we may see this quantity as a detriment to soma specialization due to the concavity of the trade-off. Eq. (B.18) expresses that if the bonus to soma specialization

$$V(0, b', \ldots, b') - V(0^+, b', \ldots, b')$$

is greater than the detriment in Eq. (B.19), then specialization in soma is favored.

Another perspective is as follows: let $V(p, b)$ and $W(p, b)$ denote, respectively, the viability and fitness of a colony with a proportion p of somatic cells and fecundity b for the non-somatic cells. Assume for simplicity that p can vary continuously and let

$$f(p) = V(p, b^*/(1-p))$$

denote the viability of a colony with a proportion p of somatic cells and fecundity $b^*/(1-p)$ for the other cells, so that the global fecundity equals B^*. A sufficient condition for specialization is that

$$\frac{\partial f}{\partial p}(0) > 0. \tag{B.20}$$

This expresses the fact that by having some cells specialized in soma, a colony can retain the same fecundity as the best unspecialized colony but increase its viability.

Simple computations show that Eq. (B.20) is equivalent to

$$\frac{1}{V}\frac{\partial V}{\partial p}(0, b^*) > 1 \tag{B.21}$$

which means that the relative increase in viability provided by specialization in soma should be greater than a certain threshold (when the viabilities are additive, Eq. (B.21) boils down to $(v_{\max} - v^*)/v^* > 1$ or equivalently $v_{\max} > 2v^*$). An effect of an increasing initial cost of reproduction is that the relative increase in viability provided by specialization in soma increases with the size of the colony (In volvocine algae, this is essentially because, in the absence of somatic cells, the viability gets lower and lower, hence $1/V$ increases). Thus, the higher the initial cost of reproduction, the more likely it is that Eq. (B.21) will be satisfied, hence specialization favored.

Appendix C. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtbi.2005.08.043.

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