A General Allometric and Life-History Model for Cellular Differentiation in the Transition to Multicellularity

Cristian A. Solari,[1,*] John O. Kessler,[2] and Raymond E. Goldstein[3]

1. Consejo Nacional de Investigaciones Científicas y Técnicas Researcher, Laboratorio de Biología Comparada de Protistas, Departamento de Biodiversidad y Biología Experimental (Facultad de Ciencias Exactas y Naturales), Universidad de Buenos Aires, Buenos Aires, Argentina C1428EHA; 2. Department of Physics, University of Arizona, Tucson, Arizona 85721; 3. Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, Wilberforce Road, University of Cambridge, Cambridge CB3 0WA, United Kingdom

Submitted February 14, 2012; Accepted October 8, 2012; Electronically published January 25, 2013

abstract: The transition from unicellular, to colonial, to larger multicellular organisms has benefits, costs, and requirements. Here we present a model inspired by the volvocine green algae that explains the dynamics involved in the unicellular-multicellular transition using life-history theory and allometry. We model the two fitness components (fecundity and viability) and compare the fitness of hypothetical colonies of different sizes with varying degrees of cellular differentiation to understand the general principles that underlie the evolution of multicellularity. We argue that germ-soma separation may have evolved to counteract the increasing costs and requirements of larger multicellular colonies. The model shows that the cost of investing in soma decreases with size. For lineages such as the Volvocales, as reproduction costs increase with size for undifferentiated colonies, soma specialization benefits the colony indirectly by decreasing such costs and directly by helping reproductive cells acquire resources for their metabolic needs. Germ specialization is favored once soma evolves and takes care of vegetative functions. To illustrate the model, we use some allometric relationships measured in Volvocales. Our analysis shows that the cost of reproducing an increasingly larger group has likely played an important role in the transition to multicellularity and cellular differentiation.

Keywords: body size, cost of reproduction, germ-soma differentiation, life-history evolution, multicellularity, Volvocales.

Introduction

It is generally assumed that various selective pressures, such as predation or the need for increased motility, push unicellular organisms to increase in size. However, general constraints set an upper limit to cell size, such as the decrease in the surface to volume ratio, which reduces the exchange of nutrients and wastes for cells with approximately spheroidal convex shape. Given these constraints, the aggregation of mitotic products held together by a cohesive extracellular material might have enabled certain organisms to increase in size by increasing cell number (instead of cell size). In some cases, natural selection has favored this strategy, as illustrated by the multiple independent origins of colonial and multicellular organisms in, for example, algae (Niklas 1994, 2000; Graham and Wilcox 2000). Large size can be beneficial for both viability (e.g., in terms of predation avoidance, higher motility to reach resources; e.g., Porter 1977; Morgan 1980; Sommer and Gliwicz 1986) and fecundity (e.g., higher number or quality of offspring), the two basic fitness components. Nevertheless, a large size can also become costly, in terms of both viability (e.g., increased need for local resources) and fecundity (e.g., increased generation time). As size increases, such costs can reach a point where the fitness of the emerging multicellular individual might be negatively affected. Consequently, to maintain levels of fitness that allow for further increase in size, the benefits have to be increased and/or the costs have to be reduced. Here we propose that cellular specialization in the emergent multicellular groups may have evolved as a means to deal with the costs associated with the production of large multicellular colonies and their metabolic requirements.

In the first group of cells that formed the simplest colonies, all cells retained both vegetative and reproductive functions and remained undifferentiated. From these colonial organisms, natural selection generated more complex forms with cellular differentiation, with cells specialized in vegetative (i.e., soma) and reproductive (i.e., germ) functions. Germ-soma separation helps to create the emergence of a higher level of individuality because both cell lines depend on each other for the success of the whole organism. As cells specialize in the different fitness components (i.e., fecundity and viability), they relinquish their autonomy in favor of the group, and as a result, fitness and individuality are transferred from the cell to the group level.

* Corresponding author; e-mail: casolari@bg.fcen.uba.ar.

Am. Nat. 2013. Vol. 181, pp. 369–380. 0003-0147/2013/18103-53660$15.00.
DOI: 10.1086/669151

It is well known that the transition from unicellular to multicellular organisms and cellular differentiation has happened independently multiple times and has even been labeled a "major minor transition" in a recent review on the subject (Grosberg and Strathmann 2007; this work cites most of the relevant literature). Recently, several valuable models have contributed to the understanding of the origin of multicellularity and cellular differentiation (Michod et al. 2006; Willensdorfer 2009; Gavrilets 2010). Michod et al. (2006) argue that the trade-off between fecundity and survival drives the transition to multicellularity. They explore in detail the curvature of this trade-off, predicting that it becomes increasingly convex as group size increases in multicellular organisms. Willensdorfer (2009) uses a quantitative trait that affects fitness via a benefit function that is determined by the number and kind of cells. In his model, he points out that many benefit functions allow the evolution of multicellularity with cellular differentiation and that these functions can have convex, concave, or linear forms. Gavrilets (2010) agrees with Grosberg and Strathmann (2007); he used a genetics approach in which one major gene controls for survival functions and the other for fecundity functions, finding that complete germ-soma differentiation can be achieved relatively easily and quickly via the evolution of developmental plasticity.

Here we present a model using life-history theory and allometry inspired by the volvocine green algae. Its purpose is to provide further insight into the dynamics of cellular differentiation as size increases. We model the two fitness components (fecundity and viability) and compare the fitness of hypothetical colonies of different sizes with varying degrees of cellular differentiation to try to understand the general principles underlying the evolution of multicellularity.

Volvocales are an ideal model system for studying the transition from unicellular to multicellular organisms because they are composed of an assemblage of lineages featuring varying degrees of complexity in terms of colony size, colony structure, and cell specialization (e.g., Koufopanou 1994; Kirk 1998; Solari et al. 2006*b*; Herron and Michod 2008). These aquatic flagellated organisms range from unicellular species, such as *Chlamydomonas*, to colonies composed of four to 64 cells with no cellular differentiation (e.g., *Gonium* and *Eudorina*), to multicellular individuals comprising 1,000–50,000 cells with complete germ-soma separation (e.g., *Volvox*; fig. 1). In this lineage, the transition to cellular specialization has occurred multiple times (e.g., Nozaki et al. 2006; Herron and Michod 2008). *Volvox* species with germ-soma specialization have evolved several times independently from quite different ancestors. In short, Volvocales comprise a group of closely related lineages with different degrees of cell specialization

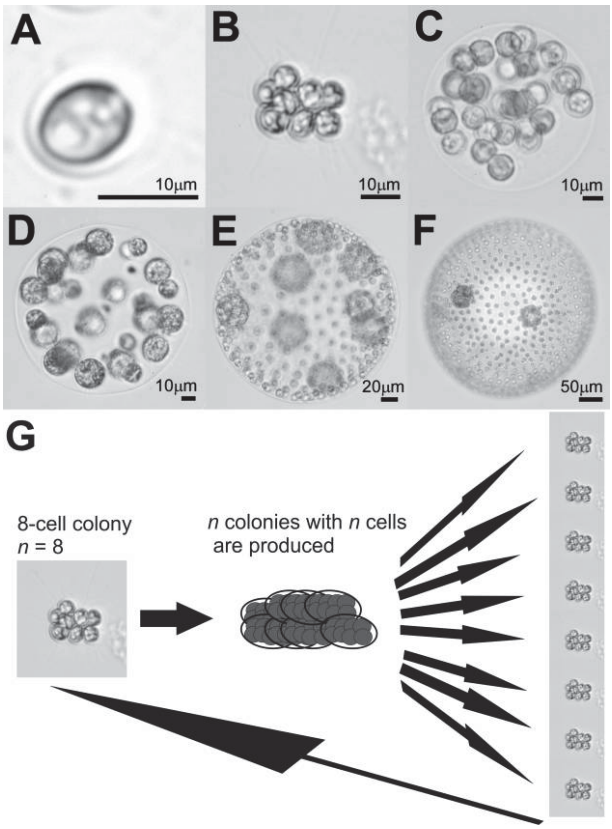

Figure 1: Subset of Volvocales showing differences in cell number, size, and degree of specialization. In the species where two cell types can be identified, the smaller cells are sterile and somatic, and the larger ones are reproductive. *A*, *Chlamydomonas reinhardtii*. *B*, *Gonium pectorale*. *C*, *Eudorina elegans*. *D*, *Pleodorina californica*. *E*, *Volvox carteri*. *F*, *Volvox aureus*. *G*, Autocolony process for an eight-cell colony. Photograph from *G. pectorale*. The process can be performed with continuous growth before the division phase, as in most Volvocales, or by binary fission with growth between divisions.

that seem to represent alternative stable states (Larson et al. 1992).

The trade-offs between fecundity, viability, and size have recently been studied in detail in Volvocales (Short et al. 2006; Solari et al. 2006*a*, 2006*b*). In these organisms, the constraints and opportunities of flagellar motility may have been the major driving force in the transition to multicellularity and germ-soma separation as colony size increased. Some of the allometric relationships that have been derived are used to illustrate the model presented here.

The Model

Fecundity

For the model, we assume the simplest scenario. Conflict mediation theory is not necessary for modeling this tran-

sition because cells in these emerging colonial organisms are mitotic products and genetically identical. Thus, within-group variation is negligible; variation in fitness exists primarily at the group/colony level. The new emerging colonial organism reproduces asexually and has a discrete generation time. We do not take into account the structural costs associated with the extracellular material that is needed to hold the colony together. An organism can have many cell types; we model a colonial organism that first invests in only one somatic cell type. We also assume that cell number in the organism is fixed throughout its development, that the intrinsic growth rate of a unicell is the maximum rate attainable by cells that form groups, and that initial cell size is the same for both somatic and reproductive cells.

Several aspects of the life history of Volvocales fit the assumptions of the model. In Volvocales, population growth is achieved via asexual reproduction; they go through the sexual phase to produce resistant spores only when conditions for survival are not met. Volvocales have a discrete generation time; when new colonies hatch, the mother colony disintegrates through apoptosis. Germsoma differentiated *Volvox* colonies have only two cell types. Because the number of cells in Volvocales is determined by the number of cleavage divisions that take place during embryonic development, cell number is not augmented by accretionary cell divisions after juveniles hatch (Kirk 1997).

In the hypothetical primordial colonies, which are composed of a number of undifferentiated cells n held together by some cohesive material, we assume an autocolony process in which each cell in the new colony grows to produce the next generation of colonies with the same cell number n (fig. 1*G*; Kirk 1998). The process can go through a continuous growth phase and a subsequent division phase, as in most volvocine algae (palintomy; Kirk 1998), or via binary fission, as in most organisms. There are other colonial/multicellular organisms with different forms of reproduction and growth, such as filamentous algae or fungi, which can reproduce via fragmentation or budding (Grosberg and Strathmann 2007). We will first examine the model with the autocolony assumption and illustrate it with the Volvocales. Then, to simulate the other life cycles, we will explore relaxing this assumption.

If we use a standard exponential growth model for the growth of the reproductive cells, or what we can call embryos, or the developing daughter colonies, then the size m (i.e., mass or volume) to which each cell/embryo within the mother colony grows obeys

$$m = m_0 e^{rt}, \tag{1}$$

where m_0 is the initial size for the reproductive cells in newly produced colonies, r is the intrinsic growth rate for such cells/embryos, and t is time. As the number of cells n in the colony increases, the size m and the number of divisions d it performs to produce a daughter colony of the same type increase. As stated, the cell/embryo can reach size m through continuous growth before the division phase as in Volvocales or through binary fission. The size m that reproductive cells/embryos reach can also be defined by the initial cell size m_0 in newly produced colonies times the number of cells n in the mother colony, $m = nm_0$, because each cell/embryo grows by a factor of n to produce daughter colonies of the same type. If we solve for cell number, $n = m/m_0$, and insert this relationship into equation (1),

$$\frac{m}{m_0} = e^{rt} \rightarrow n = e^{rt}; \tag{2}$$

$$\text{if we solve for time, } t = \frac{\ln(n)}{r}.$$

For the sake of simplicity, if we assume that cell division d is instantaneous and does not contribute to generation time T, then $t = T$. Equation (2) clearly shows that generation time T increases if the number of cells n in the colony increases and decreases if the cell growth rate r increases.

Because we assume that colonies have discrete generation time, the per generation fecundity *Ro* (Stearns 1992) of the group of cells or colony is equal to the number of cells in the colony because all cells produce daughter colonies of the same type (i.e., autocolony):

$$Ro = n = 2^d. \tag{3a}$$

For example, in an eight-cell colony ($n = 8$), each cell/embryo grows by a factor of n (continuously before the division phase or between divisions; fig. 1*G*), undergoing three divisions ($d = 3$, $n = 2^d$) to develop a daughter colony with eight cells with initial size m_0.

By assuming a discrete generation time, *Ro* can also be written in a simple way as a function of the fecundity rate λ (Stearns 1992):

$$Ro = \lambda^T, \tag{3b}$$

where T is generation time. Because $n = e^{rT}$ (eq. [2]) and $Ro = n$ (eq. [3]), then

$$Ro = n = e^{rT} = \lambda^T,$$

$$\text{or } \lambda = e^r. \tag{4a}$$

Our autocolony assumption and simplified modeling (eq. [4a]) allows us to reach the first conclusion. If the intrinsic cell growth rate r is constant and not size dependent, the fecundity rate for colonies composed of undifferentiated cells is the same regardless of size (i.e., the size term n

cancels out). In short, in an ideal world with no size constraints or benefits, size does not matter; organisms of different sizes have the same fitness (i.e., fecundity rate).

Let us now investigate what happens if these colonies invest in soma and a proportion s of cells in the colony become sterile (do not reproduce) and perform only vegetative functions, therefore not contributing to the next generation of colonies. In this case, fecundity depends only on the cells that reproduce,

$$Ro = n(1 - s). \tag{4b}$$

If $s = 0$, then $Ro = n$, and if $s = 1$, all cells are somatic and sterile, so $Ro = 0$. Thus, increasing the number of sterile somatic cells decreases Ro and subsequently the fecundity rate λ. Therefore, as in equation (4a), because $Ro = n(1 - s)$ (eq. [4b]), then

$$Ro = n(1 - s) = e^{rT}(1 - s) = \lambda^T,$$

$$\text{or } \lambda = e^r(1 - s)^{1/T}. \tag{4c}$$

Because $1/T = r/\ln(n)$ (the inverse of eq. [2]) and $\ln(n) = d\ln 2$ (applying the natural logarithm to eq. [3a]), then $1/T = r/d\ln 2$. By inserting this relationship into equation (4c), we obtain

$$\lambda = e^r(1 - s)^{1/T} \rightarrow \lambda = e^r\left[(1 - s)^{r/\ln 2}\right]^{1/d}. \tag{4d}$$

Equation (4d) shows, as we already knew, that investing in soma (s) decreases the fecundity rate (λ), but it also shows that the negative effect of soma decreases and dilutes as colony size increases. An increase in colony size (n; thus, the number of divisions d; $d = \log_2(n)$) decreases the exponent of the proportion $(1 - s)$, thus increasing the fecundity rate. Figure 2 plots λ as a function of the number of divisions d a reproductive cell/embryo undergoes to produce a colony of the same type. This figure shows that the cost of investing in a proportion of somatic cells decreases with size. In short, regardless of the costs and benefits that size might have on fitness, larger size gives a direct scaling benefit to cellular differentiation by decreasing the effect of its cost on the fecundity rate.

Of course, we know that the growth rate r of reproductive cells, on which the fecundity rate greatly depends (eq. [4d]), is not constant but dependent on the supply and demand of resources, which in turn depend on size and cellular differentiation. We will now explore how size and cellular differentiation may change the growth rate.

The resources needed for a colony to produce the next generation depend on the total number of cells in the colony and the ratio of somatic to reproductive cells. For example, if there is no cellular differentiation, a 128-cell colony grows enough to produce 128 daughter colonies with 128 cells each (total production $= n^2 = 16{,}384$ cells). But, assuming that somatic cells do not grow or reproduce,

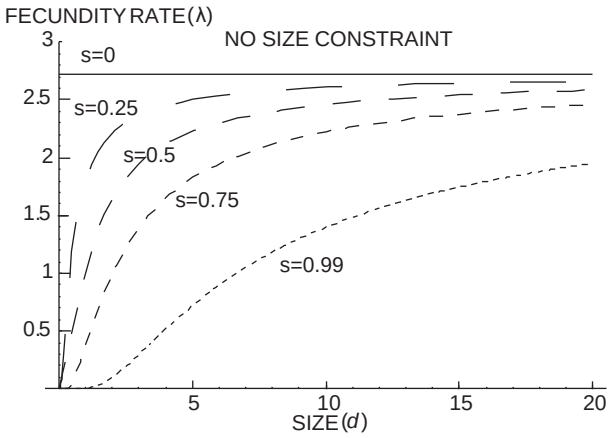

Figure 2: Fecundity rate of colonies with no size dependency (the cell growth rate $r = 1$ remains constant as size increases) as a function of size d ($\log_2 n$) for different proportions of somatic cells s.

if the same 128-cell colony sequesters three-fourths of its cells for somatic functions ($s = 0.75$), then only 32 reproductive cells ($1 - s = 0.25$) produce 32 daughter colonies with 128 cells each (total production $= n^2(1 - s) = 4{,}096$ cells). The cost of reproduction of the soma-differentiated colony is lower, in this case, 4 times lower than the cost of reproduction of the undifferentiated colony. Thus, we model the cost of reproduction C (i.e., the resources needed to produce the next generation) as proportional to the total number of cells a colony has to produce, the number of reproductive cells times the amount of cells in the colony, $C \sim n \times n(1 - s) = n^2(1 - s)$. C increases exponentially with size for undifferentiated colonies ($C \sim n^2$), but this cost can be eased and shifted to a larger size by the increase in proportion of sterile somatic cells (fig. 3*A*).

On the other hand, the vegetative functions B needed to acquire resources to grow and reproduce are performed by the sterile somatic cells ns that lose reproductive functions and the undifferentiated reproductive cells that retain those functions $n(1 - s)(1 - g)$, $B = ns + n(1 - s)(1 - g)$. For the sake of simplicity, we assume an additive equal contribution of both cell types. Parameter g goes from no specialization ($g = 0$), meaning that reproductive cells retain full vegetative functions, to full specialization in reproductive functions ($g = 1$), meaning that reproductive cells lose all vegetative functions. With no germ specialization ($g = 0$), $B = n$, since both cell types contribute to vegetative functions in the same way. Figure 3*B* shows that the cost of germ specialization to the vegetative contribution B can be compensated by increasing the proportion of somatic cells s. In short, soma specialization can have an indirect benefit to the colony by decreasing the repro-

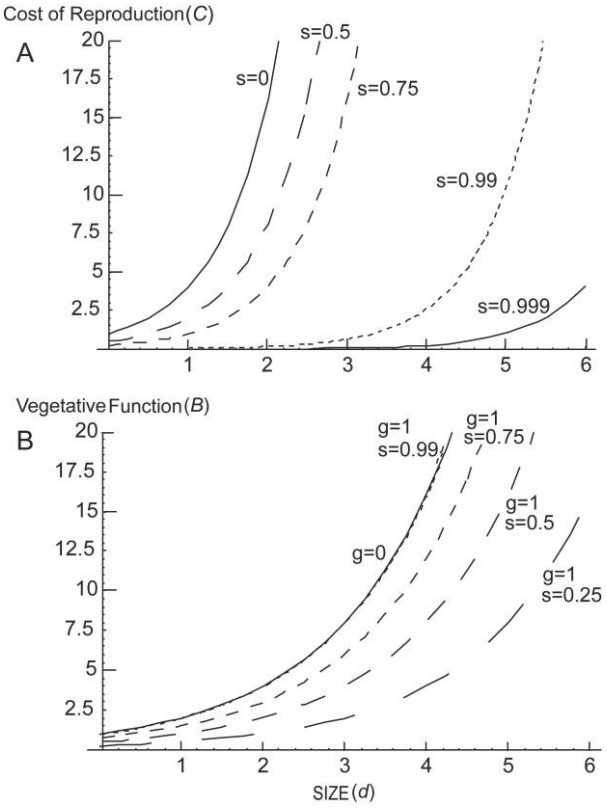

Figure 3: Demand C and supply B of resources as a function of size d ($\log_2 n$) for different proportions of somatic cells s. *A*, Plot of C versus d. The cost of reproduction C increases exponentially with size in undifferentiated colonies. The slope of C can be lowered by increasing the proportion of somatic cells s. *B*, Plot of B versus d. When there is no germ specialization, $B = n$ because undifferentiated and somatic cells contribute equally to the vegetative functions (supply of resources). With germ specialization ($g = 1$), $B = ns$; when $s = 0$, $B = 0$, but its slope increases as s increases until almost reaching the supply level with no germ specialization.

duction costs (the demand for resources C) and a direct benefit by helping the reproductive cells to acquire resources for their metabolic needs (the supply of resources B).

We use the ratio between the supply B and demand C of resources (BC_r) as the factor that may limit the intrinsic growth rate r of reproductive cells/embryos as colonies increase in size:

$$BC_r = \frac{b[ns + n(1 - s)(1 - g)]^{\beta}}{c[n^2(1 - s)]^{\alpha}} = \frac{bB^{\beta}}{cC^{\alpha}}, \quad (5)$$

where b and c are the normalization constants that reflect, respectively, the acquisition (the inflow of nutrients) and the consumption (metabolic rate) of resources of the unicellular organism that is basal to the multicellular lineage, and α and β are the scaling exponents for the metabolic demand C and the supply of resources B, respectively. The ratio between the two normalization constants (b/c) gives an indication of the size threshold at which supply might not meet demand. As this ratio increases, the threshold at which supply does not meet demand moves to a larger size (cell number). The scaling exponents reflect the supply and demand dynamics of the multicellular group as size increases, which can depend on several factors (e.g., geometry); we will later explore the changes in the scaling exponents when comparing the fitness functions.

Taking into account equation (5), we now model the intrinsic growth rate r of reproductive cells as a function of colony size and germ specialization:

$$\text{if } BC_r \geq 1,\ r = (1 + u_g g)r_0,$$
$$\text{if } BC_r < 1,\ r = (1 + u_g g)r_0 BC_r, \quad (6)$$

where r_0 is the growth rate of a cell with no size constraints on its metabolic rate, such as a unicellular organism, and u_g is the benefit of germ specialization on the growth rate. Equation (6) is a stepwise function because we assume r_0 to be the maximum possible rate for an undifferentiated cell. If $BC_r \geq 1$, supply meets the demand of resources, so cells grow at their maximum possible rate. If $BC_r < 1$, the supply of resources does not meet the demand and limits r. The germ specialization benefit ($1 + u_g g$), which transfers resources from vegetative to reproductive functions, is an intrinsic benefit of reproductive cells, therefore independent of colony size n. Figure 4*A* shows how the investment in soma shifts the size constraint on the growth rate to a larger size. Figure 4*B* shows how germ specialization increases the growth rate regardless of soma specialization. Figure 4*C* shows how an increase in the ratio between the supply and demand normalization constants ($b/c = 8$ instead of 1) also shifts the size constraint on the growth rate to a larger size.

We now insert equation (6) into equation (4d) to evaluate the effects of size and cellular specialization on the fecundity rate (fig. 5). Fecundity rate curves of hypothetical colonies form peaks that shift to larger size as the proportion of somatic cells s increases. For a fixed proportion of somatic cells s, there is a colony size that optimizes the fecundity rate, with this optimal size increasing as s increases. As colony size increases, depending on the proportion of somatic cells s, the germ specialization benefit ($1 + u_g g$), and the threshold size b/c, colonies with specialized germ cells ($g = 1$; fig. 5*B*, 5*D*) might have higher fecundity rates than colonies with nonspecialized reproductive cells (fig. 5*A*, 5*C*). In general, for a fixed proportion of somatic cells, with a low proportion of soma, germ-

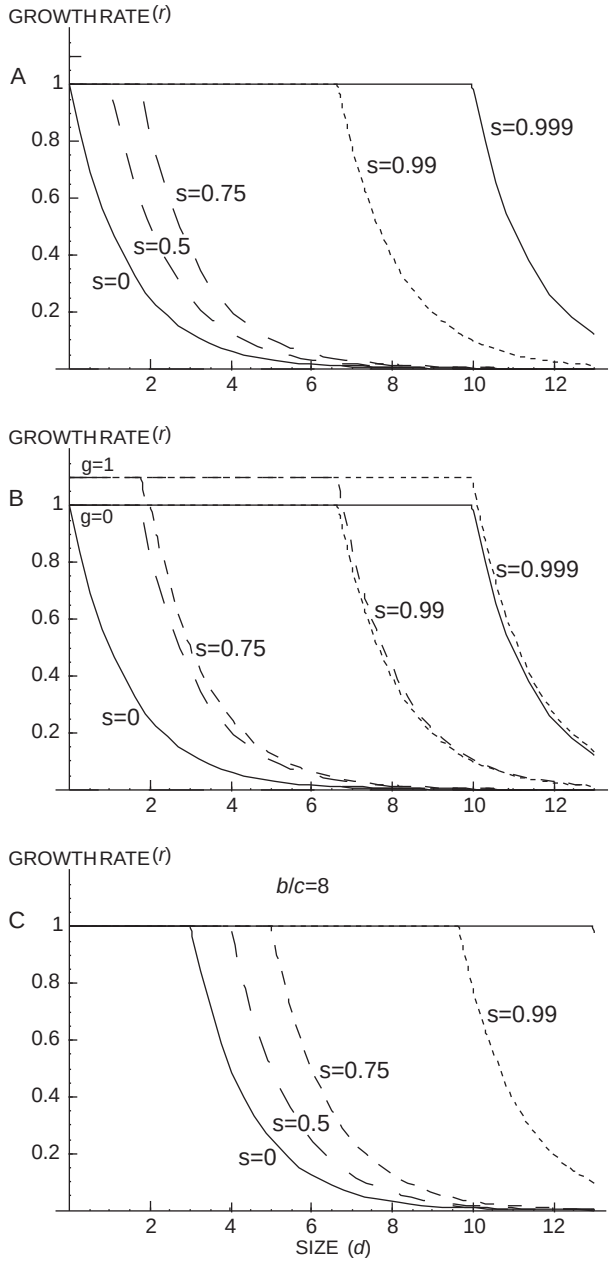

Figure 4: Reproductive cell growth rate r as a function of size d ($\log_2 n$) for different proportions of somatic cells s (the unicellular growth rate $r_0 = 1$, scaling exponents $\alpha = \beta = 1$; eq. [6]). *A*, Plot of r versus d without germ specialization ($g = 0$) and no difference between the supply and demand on the unicell ($b/c = 1$). As the proportion of somatic cells s increases, r curves shift to larger size. *B*, Plot of r versus d with ($g = 1$, $u_g = 0.1$) and without ($g = 0$) germ specialization. Germ specialization allows an overall increase in the growth rate regardless of d and s. *C*, Plot of r versus d with the ratio between the supply and demand constants $b/c = 8$ (in the unicell, supply capabilities are 8 times higher than demand capabilities). This also allows the size constraint to shift to larger size.

specialized colonies have lower fecundity rates than colonies with undifferentiated reproductive cells because there is a low proportion of cells supplying resources; for a high proportion of soma, large colonies with specialized germ cells might have higher fecundity rates than their undifferentiated counterparts. As b/c increases, curves are shifted to a larger size, increasing fecundity rates levels (fig. 5*C*, 5*D*).

We now investigate what happens in the Volvocales with the supply and demand dynamics as size increases and how it affects the growth rates of colonies. We know that flagella are used for self-propulsion, but collective flagellar beating may also serve to enhance the molecular transport of nutrients (Short et al. 2006; Solari et al. 2006*a*). Previously calculated thresholds and scaling relationships in the Volvocales can be used for nutrient uptake (B) to calculate cell growth rates (r) for the fecundity (λ) in the model.

Volvocales show a diffusive bottleneck as colonies increase in size (Short et al. 2006). When the demand for essential molecules exceeds the diffusive current, metabolism is constrained. This bottleneck can be circumvented by the increased advection generated by the flagellated cells arrayed at the surface of the colony. The mixing of the medium maintains a high nutrient concentration around the colonies; this helps the nutrient uptake influx keep up with increased metabolic demands as size increases. Short et al. (2006) showed that the absorption rate of nutrients in organisms with a spherical design such as the Volvocales is $I_a \sim R\mathrm{Pe}^{1/2}$, where R is colony radius and Pe is the Péclet number, which is the standard measure of competition between advection and diffusion (dimensionless; Guyon et al. 2001). The Péclet number can be expressed in terms of a typical flow velocity U (the velocity of the flow generated by the flagellated cells in the colony), the sphere (colony) diameter ($2R$), and a diffusion constant D for a molecule such as O_2 (2×10^{-5} cm^2/s; $\mathrm{Pe} = 2RU/D$).

R and U can be expressed as a function of cell number and proportion of somatic and germ cells. The colony radius R is proportional to the number of flagellated cells arrayed at the surface of the colony, $n_F = ns + n(1 - s)(1 - g) = B$ (the supply variable in the model); $R \propto B^{1/2}$ if cell concentration stays constant as size increases (Solari et al. 2006*b*). Since Short et al. (2006) showed that $U \propto R$, the supply of nutrients to the colonies is $I_a \sim B^{1/2}(B^{1/2}B^{1/2})^{1/2} \sim B$. On the other hand, the demand of resources depends on the number of cells in the colony and the total cells it has to produce for the next generation (the cost of reproduction $C = n^2(1 - s)$). Therefore, the constraint on the growth rate r for the Volvocales in the model becomes

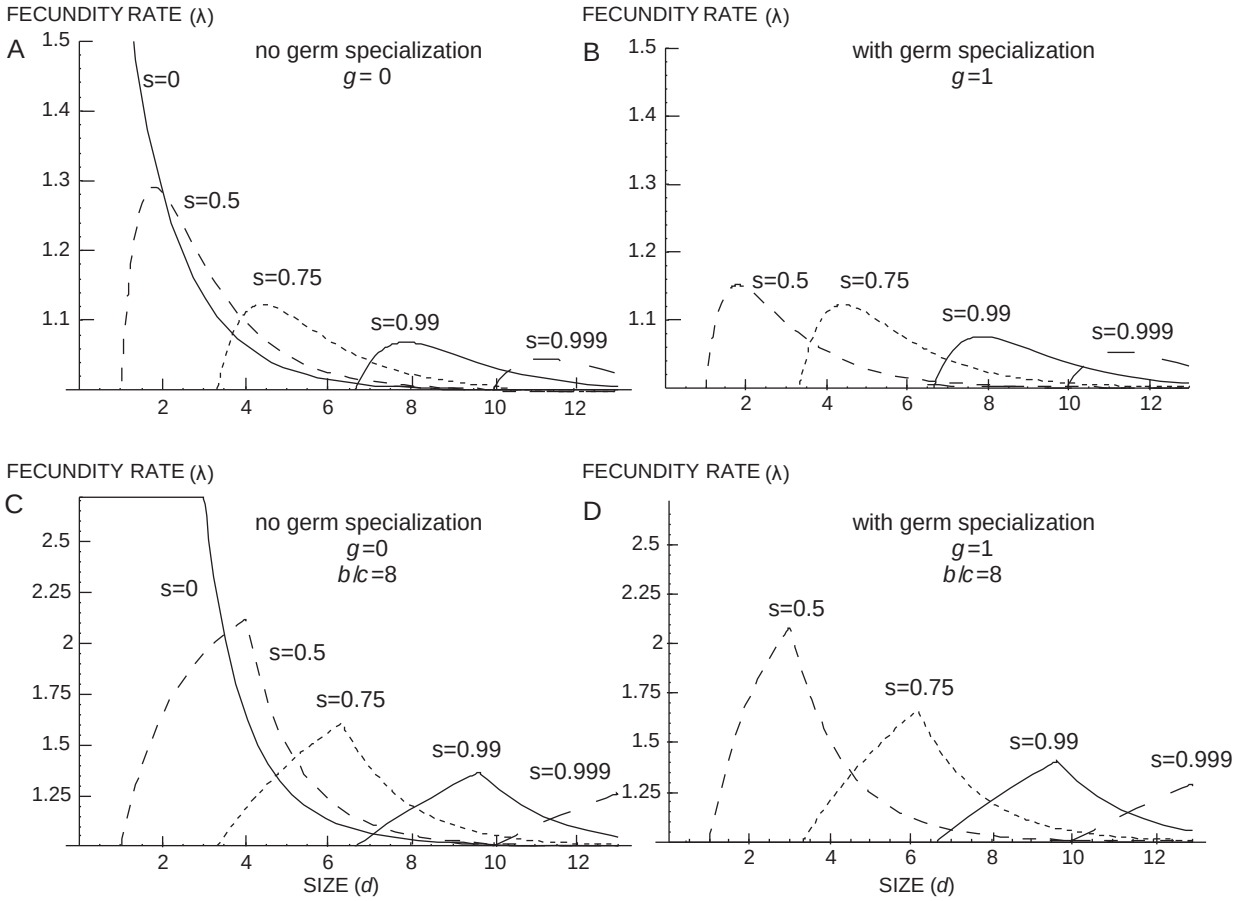

Figure 5: Fecundity rate of colonies λ as a function of size d ($\log_2 n$) for different proportions of somatic cells s (the unicellular growth rate $r_0 = 1$, scaling exponents $\alpha = \beta = 1$; eq. [6]). *A*, Plot of λ versus d without germ specialization ($g = 0$). For each proportion of somatic cells s, there is a size that optimizes the fecundity rate λ. As s increases, the fecundity rate peaks shift to larger size. *B*, Plot of λ versus d with germ specialization ($g = 1$, $u_g = 0.1$). Colonies with a low proportion of somatic cells s have lower fecundity rate peaks than colonies with no specialized reproductive cells because only a low proportion of cells contribute to the supply of resources. As s increases, the peaks of the colonies with specialized germ cells may be higher because the vegetative functions are met by the somatic cells and specialized reproductive cells have higher growth rates. *C*, *D*, Plot of λ versus d without germ specialization (*C*; $g = 0$) and with germ specialization (*D*; $g = 1$, $u_g = 0.1$), both with the ratio between the supply and demand constants $b/c = 8$. This allows the size constraint to shift to larger size and higher fecundity rate peaks.

$$BC_r \sim \frac{bB}{cC}, \tag{7}$$

Viability

where c and b are the normalization constants for the metabolic demand and the absorption rate for the unicell, respectively (e.g., *Chlamydomonas reinhardtii* in Volvocales). As size increases in the Volvocales, colonies have to invest in somatic cells to increase advection at the surface of the colony (bB) to meet the increasing metabolic demand (cC). If $cC > bB$, then the growth rate is limited by an insufficient inflow of nutrients via diffusion, thereby decreasing the fecundity rate.

Viability v gives the proportion of colonies that will survive to reproduce the next generation. Because somatic cells specialize in vegetative functions, they contribute to viability functions such as motility, while totally specialized germ cells ($g = 1$) spend all their energy in reproductive-related functions (i.e., fecundity), thereby decreasing the viability of the colony. For the sake of argument, we also model viability as the ratio between the contribution B and the cost C of the cells in the colony to survival:

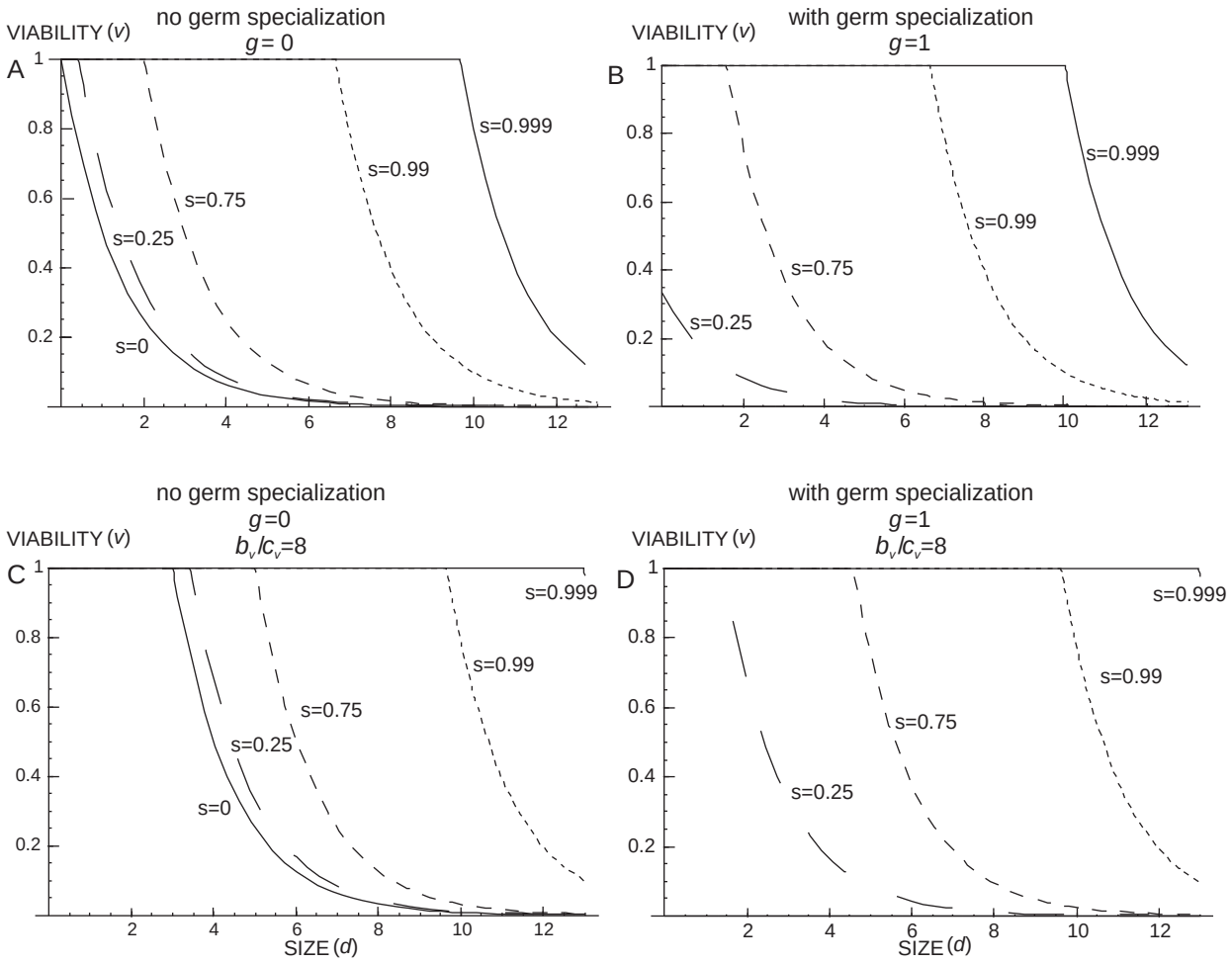

Figure 6: Viability of colonies v as a function of size d ($\log_2 n$) for different proportions of somatic cells s (all scaling exponents equal 1). *A*, Plot of v versus d without germ specialization ($g = 0$). *B*, Plot of v versus d with germ specialization ($g = 1$). *C*, Plot of v versus d without germ specialization ($g = 0$) with $b_v/c_v = 8$. *D*, Plot of v versus d with germ specialization ($g = 1$) with $b_v/c_v = 8$. In general, the increase in the cost of reproduction C decreases viability as size increases; an increase in the proportion of somatic cells shifts the constraint to larger size. Germ specialization decreases viability because specialized reproductive cells do not contribute to the viability of the colony, but this is compensated as the proportion of somatic cells s increases. Finally, an increase in b_v/c_v shifts curves to larger size.

$$\text{if } \frac{b_v B^{\gamma}}{c_v C^{\delta}} \geq 1, \; v = 1,$$

$$\text{if } \frac{b_v B^{\gamma}}{c_v C^{\delta}} < 1, \; v = \frac{b_v B^{\gamma}}{c_v C^{\delta}}, \tag{8}$$

where c_v and b_v are the normalization constants and δ and γ are the scaling exponents for the cost and the contribution to viability. Equation (8) goes from 0 (no survival) to 1 (100% survival). As in the growth rate function, the ratio between the constants (b_v/c_v) gives an indication of the size threshold at which viability may be lower than 1. Figure 6 shows the viability rates of colonies as a function of germ-soma specialization and size constraints. Colonies that invest in germ specialization decrease their viability, but that can be compensated by increasing the proportion of somatic cells.

In Volvocales, the flagellated cells are used for self-propulsion, to avoid sinking, and to reach light and nutrients (Koufopanou 1994; Hoops 1997; Kirk 1998; Solari et al. 2006*b*). Because of the importance of motility for survival in these algae, we can use the motility of colonies as a proxy for viability in the model and assume that colonies that sink strongly compromise their survival (viability v). The contribution to motility for self-propulsion and to avoid sinking depends on the flagellar force F generated by the biflagellated cells n_F arrayed at the colony surface

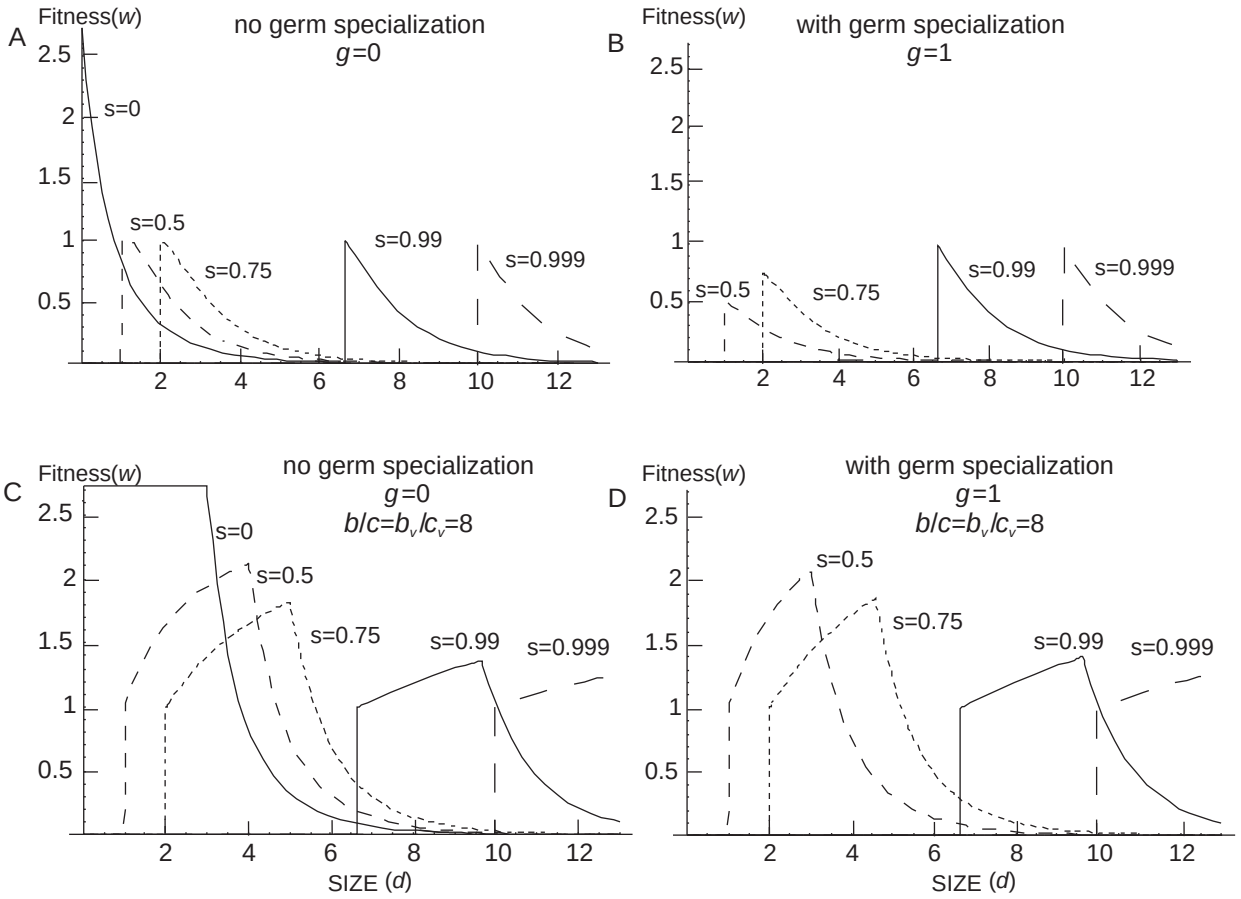

Figure 7: Fitness of colonies w as a function of size d ($\log_2 n$) for different proportions of somatic cells s (all scaling exponents equal 1, $u_g = 0.1$). *A*, Plot of w versus d without germ specialization ($g = 0$). For each proportion of somatic cells s, there is a size that optimizes fitness. As s increases, fitness peaks shift to larger size. *B*, Plot of w versus d with germ specialization ($g = 1$). Colonies with a low proportion of somatic cells s have lower fitness peaks than colonies with no specialized reproductive cells because only a low proportion of cells contribute to the supply of resources and viability. As s increases, colony peaks with specialized germ cells may be higher because the vegetative functions are met by the somatic cells and specialized reproductive cells have higher growth rates. *C*, *D*, Plot of w versus d without germ specialization (*C*; $g = 0$) and with germ specialization (*D*; $g = 1$), both with the ratio between the supply and demand constants $b/c = 8$. This allows shifting soma differentiation to larger size and higher fitness peaks.

(both somatic and undifferentiated reproductive cells). It was previously shown that in Volvocales $F \propto n_F^{3/4}$ (Solari et al 2006*b*). Because $n_F = ns + n(1 - s)(1 - g)$, then $n_F = B$. The cells in Volvocales are denser than water; therefore, the downward gravitation rate of colonies depends on the number of cells and the proportion of reproductive cells because embryos grow inside the mother colonies (the cost of reproduction $C = n^2(1 - s)$; Solari et al 2006*b*). The viability (motility) constraint in the model becomes

$$v \approx \frac{b_v B^{3/4}}{c_v C}, \tag{9}$$

where B is composed of all the cells performing motility functions, C is composed of all the cells in the colony, and b_v and c_v are the normalization constants for the flagellar force and the downward gravitation rate of the unicell, respectively (e.g., *C. reinhardtii*). If $v < 1$, the negative gravitational force of the colony ($c_v C$) is higher than the flagellar force generated by the flagellated cells for propulsion ($b_v B^{3/4}$), making colonies sink. As volvocine colonies increase in their cell number, they have to invest more in somatic cells for self propulsion and to avoid sinking.

Fitness

The overall fitness w of colonies is the product of their fecundity λ and viability v rates, $w = \lambda v$. We investigate

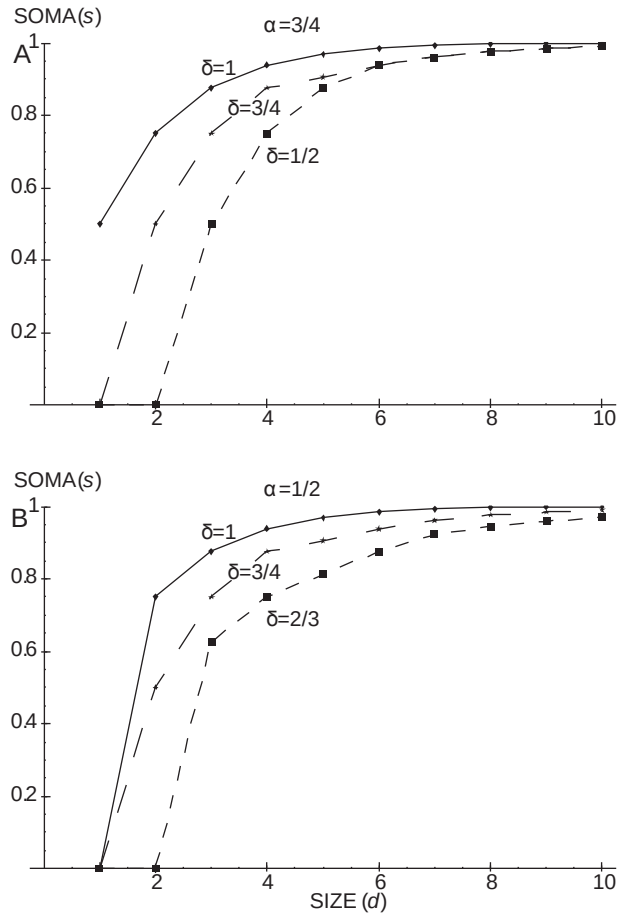

Figure 8: Proportion of somatic cells s that optimizes fitness as a function of size d for a sample of scaling exponents for the cost of reproduction for fecundity (α) and viability (δ; $b/c = 1$, $g = 0$). *A*, s versus d for $\alpha = 3/4$ for different values of δ. *B*, s versus d for $\alpha = 1/2$ for different values of δ. When the scaling exponents on the cost of reproduction decrease for fecundity and viability, the need to invest in soma decreases, lowering the proportion of somatic cells to a point where somatic cells are not needed (e.g., $\alpha = \delta = 1/2$ or lower).

the fitness landscape of the different colony types and calculate which strategy optimizes fitness as size increases. Figure 7 shows that if we compare the fitness of colonies for specific sizes, increased soma differentiation (i.e., increased ratio of somatic to reproductive cells) is favored as colony size increases. As with the fecundity rate, depending on the proportion of somatic cells s, the germ specialization benefit ($1 + u_g g$), and both threshold sizes (b/c and b_v/c_v), colonies with specialized germ cells ($g = 1$; fig. 7*B*, 7*D*) might have higher fitness than colonies with nonspecialized reproductive cells, but in general they have lower fitness when the proportion of somatic cells is low. As b/c and b_v/c_v increase, the needs of the smaller colonies are met, shifting the transition of soma differentiation to larger size (fig. 7*C*, 7*D*).

As shown in the fecundity and viability components, the Volvocales, with their spherical design and autocolony life cycle, clearly exemplify how the costs of reproduction affect fitness as size increases. But, by lowering the scaling exponents on the cost of reproduction on fecundity and viability (α and δ), we can relax the assumption of autocolony reproduction and investigate what happens when the size constraints are eased. We can simulate what might have happened in other lineages that did not have such high costs of reproduction or that had other solutions to deal with them. If the scaling exponents are equal or close to 1, soma differentiation quickly increases with size (fig. 8). Lowering the exponents decreases the cost of reproduction pressure on fitness, decreasing the pace at which the proportion of somatic cells increases with size, up to a point where soma differentiation is never needed (with scaling exponents for the cost of reproduction on fecundity and viability $\alpha = \delta = 1/2$ or lower; fig. 8).

Also, depending on the supply and demand scaling exponents, germ specialization is favored once soma evolves

Table 1: Size threshold (d) at which germ specialization ($g = 1$) is favored for colonies with proportion of somatic cells s optimizing fitness

	Viability exponents $\gamma = \delta$			
Fecundity exponents $\alpha = \beta$	.25	.50	.75	1
$u_g = .1$, $b/c = 1$:				
.25	6	7	8	8
.50	6	7	8	8
.75	7	8	8	9
1	7	8	9	9
$u_g = 1$, $b/c = 1$:				
.25	3	3	3	4
.50	3	3	4	4
.75	3	4	4	5
1	3	4	5	5
$u_g = .1$, $b/c = 8$:				
.25	6	6	7	8
.50	6	6	7	8
.75	6	6	7	8
1	6	6	7	8
$u_g = 1$, $b/c = 8$:				
.25	2	2	3	3
.50	2	2	3	3
.75	2	2	3	3
1	3	3	3	3

Note: The higher the size constraint (higher scaling exponents), the larger the size at which germ specialization evolves. An increase in the ratio between supply and demand in cells (b/c) and the germ specialization benefit (u_g) decreases the size at which germ specialization evolves.

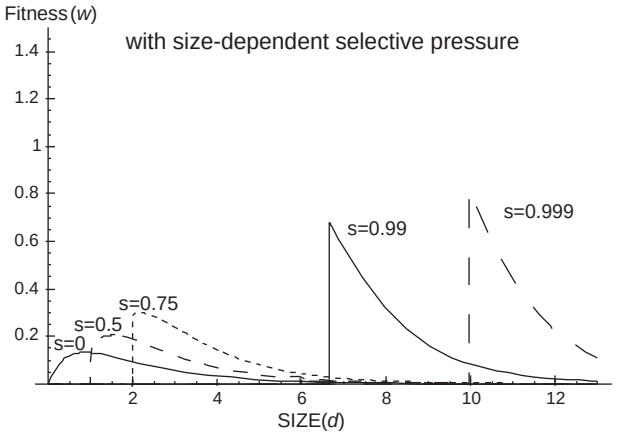

Figure 9: Fitness with size-dependent mortality added, $p = 1 - z(n)^{-\mu}$ ($z = 1$, $\mu = 0.25$). The fitness of undifferentiated colonies is depressed by the size-dependent selective pressure; thus, larger colonies with germ-soma differentiation have higher fitness than smaller undifferentiated ones.

and takes care of the vegetative functions. Table 1 shows the size threshold at which germ specialization is favored; as the size constraint decreases (i.e., lower scaling exponents), germ specialization originates at a smaller size because there is less need to invest in vegetative functions. If the germ specialization benefit (u_g) is higher, germ specialization also originates on a smaller size.

Because the model does not have an explicit advantage to size, if we compare specific colony sizes, increased germ-soma differentiation is favored as colony size increases, but if we compare fitness between sizes, the smallest unit (i.e., the unicellular organism) always has the highest fitness (fig. 7). To illustrate the size benefit, we can envision a size-dependent selective pressure $p = 1 - z(n)^{-\mu}$, where z is the mortality coefficient and μ is the size-dependent scaling exponent. For example, p could represent predation pressure (e.g., size thresholds for zooplankton filter feeders; Porter 1977; Morgan 1980) or resource availability (e.g., migration capabilities through the water column to obtain nutrients; Sommer and Gliwicz 1986), where small colonies have a higher predation rate or lower resource availability than larger ones. The fitness of colonies becomes $w = \lambda vp$. Figure 9 shows how larger colonies with germ-soma differentiation can now have a higher fitness than unicells and undifferentiated colonies that have their fitness level lowered by size-dependent selective pressure.

Discussion

Using life-history theory and allometry, we have produced a model inspired by the volvocine green algae that describes the dynamics of the emergence of germ-soma differentiation as size increases in multicellular organisms. The results show that the cost of reproducing an increasingly larger group has likely played an important role in the evolution of complexity and individuality in the transition to multicellularity. As selective pressures first pushed multicellular organisms to increase in size, the costs of reproducing an increasingly larger group also increased, having negative effects on their fitness. At some threshold size, fitness decreased dramatically, and overcoming this threshold might have required the separation of reproductive and vegetative functions between two cell types, which resulted in increased complexity.

Unlike previous models for the evolution of multicellularity, this model can be illustrated with the Volvocales, the only group of closely related organisms that retains extant species with intermediate colonial organization. The autocolony life cycle and spherical design in this group allow us to clearly observe how the cost of reproduction increases with size. Germ-soma separation was one of the solutions the Volvocales used to deal with this problem. Innovations—such as a different life cycle with continuous instead of discrete reproduction, changes in geometry to increase diffusion rates and/or to increase drag to decrease sedimentation speed, the formation of intracellular gas vacuoles to float, enhanced storage capabilities, and so on—have surely also helped emerging multicellular individuals in other lineages decrease their costs associated with size (in algae, there are many examples; Graham and Wilcox 2000). Nevertheless, we argue that to a large extent in Volvocales, and probably to some extent in other lineages, germ-soma differentiation was a solution (in parallel to other ones) to counteract these increasing costs, helping to create new levels of individuality.

The model shows first that the cost of investing in soma decreases with size, regardless of any size constraint or benefit (eq. [4b]; fig. 2). Second, for lineages such as the Volvocales, as reproduction costs increase with size in undifferentiated colonies, soma specialization can benefit the colony indirectly by decreasing such costs (fig. 3*A*) and directly by helping the reproductive cells acquire resources for their metabolic needs (fig. 3*B*). Third, germ specialization is favored once soma evolves and takes care of vegetative functions (fig. 7; table 1). As the ratio of somatic to reproductive cells increases, the benefit of having undifferentiated reproductive cells declines since the vegetative functions are taken care of by the somatic cells; a specialized germ cell can invest more resources in reproduction and benefit the colony's fecundity. In Volvocales, specialization in reproductive and vegetative functions (i.e., germ-soma separation) characterizes the large members of this lineage, and the ratio of somatic to reproductive cells increases with colony size, mimicking the results of the model presented here with scaling exponents

close to 1 (fig. 8; Koufopanou 1994; Kirk 1998; Solari et al. 2006*b*). If the autocolony assumption is relaxed and the scaling exponents on the cost of reproduction are lowered enough, soma is not favored in any proportion or size because the needs of undifferentiated colonies as size increases are always met.

The trade-offs between fecundity, viability, and size recently studied in Volvocales (Short et al. 2006; Solari et al. 2006*a*, 2006*b*) show in detail how metabolic (BC_r) and viability constraints (v) as colonies increase in size might be strong enough to push the organism design to cellular specialization, germ-soma differentiation, and higher complexity. Each degree of specialization and differentiation might counteract the higher costs associated with larger size by increasing the viability and/or the productivity (fecundity) of the larger organism, therefore allowing it to reach fitness levels impossible to attain without increased complexity. In short, we believe that the higher cost of reproducing a larger organism was an important driving force for the evolution of increased complexity (i.e., cellular differentiation) and individuality during the transition to multicellularity in Volvocales and probably to some degree in all extant multicellular lineages.

Acknowledgments

We thank two anonymous reviewers for greatly improving the manuscript, and we thank R. Michod and A. Nedelcu for important discussions over many years on the subject. This research was supported in part by Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina; the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Science and Engineering, contract DE AC02-06CH11357; the Biotechnology and Biological Sciences Research Council; and European Research Council Advanced Investigator grant 247333.

Literature Cited

Gavrilets, S. 2010. Rapid transition towards the division of labor via evolution of developmental plasticity. PLoS Computational Biology 6:e1000805.

Graham, L. E., and L. W. Wilcox. 2000. Algae. Prentice Hall, Englewood Cliffs, NJ.

Grosberg, R. K., and R. R. Strathmann. 2007. The evolution of multicellularity: a minor major transition? Annual Review of Ecology, Evolution, and Systematics 38:621–654.

Guyon, E., J. P. Hulin, L. Petit, and C. D. Mitescu. 2001. Physical hydrodynamics. Oxford University Press, New York.

Herron, M. D., and R. E. Michod. 2008. Evolution of complexity in the volvocine algae: transitions in individuality through Darwin's eye. Evolution 62:436–451.

Hoops, H. J. 1997. Motility in the colonial and multicellular Volvocales: structure, function, and evolution. Protoplasma 199:99–112.

Kirk, D. L. 1997. The genetic program for germ-soma differentiation in *Volvox*. Annual Review of Genetics 31:359–380.

———. 1998. *Volvox*: molecular-genetic origins of multicellularity and cellular differentiation. Cambridge University Press, Cambridge.

Koufopanou, V. 1994. The evolution of soma in the Volvocales. American Naturalist 143:907–931.

Larson, A., M. M. Kirk, and D. L. Kirk. 1992. Molecular phylogeny of the volvocine flagellates. Molecular Biology and Evolution 9: 85–105.

Michod, R. E., Y. Viossat, C. A. Solari, M. Hurand, and A.M. Nedelcu. 2006. Life-history evolution and the origin of multicellularity. Journal of Theoretical Biology 239:257–272.

Morgan, N. C. 1980. Secondary production. Pages 247–340 *in* E. D. Le Cren and R. H. Lowe-McConnell, eds. The functioning of freshwater ecosystems. International Biological Programme Synthesis Series 22. Cambridge University Press, Cambridge.

Niklas, K. J. 1994. Plant allometry: the scaling of form and process. University of Chicago Press, Chicago.

———. 2000. The evolution of plant body plans: a biomechanical perspective. Annals of Botany 85:411–438.

Nozaki, H., F. D. Ott, and A. W. Coleman. 2006. Morphology, molecular phylogeny and taxonomy of two new species of *Pleodorina* (Volvoceae, Chlorophyceae). Journal of Phycology 42:1072–1080.

Porter, K. G. 1977. The plant-animal interface in freshwater ecosystems. American Scientist 65:159–170.

Short, M. B., C. A. Solari, S. Ganguly, T. R. Powers, J. O. Kessler, and R. E. Goldstein. 2006. Flows driven by flagella of multicellular organisms enhance log-range molecular transport. Proceedings of the National Academy of Sciences of the USA 103:8315–8319.

Solari, C. A., S. Ganguly, J. O. Kessler, R. E. Michod, and R. E. Goldstein. 2006*a*. Multicellularity and the functional interdependence of motility and molecular transport. Proceedings of the National Academy of Sciences of the USA 103:1353–1358.

Solari, C. A., J. O. Kessler, and R. E. Michod. 2006*b*. A hydrodynamics approach to the evolution of multicellularity: flagellar motility and the evolution of germ-soma differentiation in volvocalean green algae. American Naturalist 167:537–554.

Sommer, U., and Z. M. Gliwicz. 1986. Long-range vertical migration of *Volvox* in tropical Lake Cahora Bassa (Mozambique). Limnology and Oceanography 31:650–653.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.

Willensdorfer, M. 2009. On the evolution of differentiated multicellularity. Evolution 63:306–323.

Editor: Mark A. McPeek
Editor: Ruth G. Shaw