

Fitness, Life-history, and the Evolution of Complexity in Volvoclean Green Algae

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Abstract

The fitness of any evolutionary unit can be understood in terms of its two basic components: fecundity and viability. As embodied in current theory, the trade-offs between these fitness components drive the evolution of a variety of life-history traits in extant multicellular lineages. Here, we argue that the evolution of germ-soma separation and the emergence of individuality at a higher level during the unicellular-multicellular transition are also consequences of these trade-offs. We consider the green algal lineage Volvocales to develop a model to study how these trade-offs change as colony size increases, using hypothetical colony types of increasing size with different degrees of germ-soma differentiation. The results of our model show that the evolution of soma is the first expected outcome of reducing the cost of reproduction in order to realize the benefits associated with increasing size. As size increases further, the fitness benefits of a large size can be further increased by the evolution of a specialized germ; as a result, increased levels of complexity and individuality are achieved.

Introduction

Fitness, Life-history and Complexity

The fitness of any evolutionary unit can be understood in terms of its two basic components: fecundity and viability. As embodied in current theory, the trade-offs between these fitness components drive the evolution of life-history traits. In unicellular individuals, the same cell must be involved in both fitness components, typically these components being separated in time. However, in multicellular organisms, under certain circumstances, cells may specialize in one component or the other, the result being a division of labor, leading to the differentiation of germ and soma. The evolution of a specialized and sterile soma can increase viability and indirectly benefit fecundity but, all things being equal, must directly cost fecundity by reducing the number of cells producing offspring. On the other hand, the evolution of a specialized germ will benefit fecundity (by reducing the generation time and/or increasing the quality of offspring), but must directly cost viability by reducing the number of cells participating in viability-related functions.

A variety of selective pressures put a benefit on larger size and may push unicellular organisms to form groups (colonies) and evolve into multicellular individuals. Large size can be beneficial for viability (e.g. in terms of predation avoidance, ability to catch bigger prey, a buffered environment within a group), as well as for fecundity (e.g. higher number or quality of offspring). Nevertheless, a large size can become costly, both in terms of viability (e.g. increased need for local resources) and fecundity (e.g. increased generation time). As size increases, such costs increase and reach a point at which the fitness of the emerging multicellular individual is negatively affected. Consequently, to maintain positive levels of fitness at a given size, as well as to allow for further increase in size, the benefits have to be increased and/or the costs have to be reduced.

The various trade-offs between viability and fecundity are reflected in the variety of life-history traits among extant multicellular lineages. Here, we argue that the evolution of germ-soma separation and the emergence of individuality and increased complexity at a higher level during the unicellular-multicellular transition are also consequences of these trade-offs. The results of our model show that the evolution of soma is the expected outcome of reducing the cost of reproduction in order to realize the benefits associated with increasing size. As size increases further, the viability and fecundity benefits can be better achieved via the specialization of germ and the complete germ-soma separation; as a result, increased levels of complexity are achieved. In short, we suggest that in volvocalean green algae and possibly in other groups, the emergence of higher levels of complexity during the unicellular-multicellular transition is a consequence of life history evolution. The relationship of our results to the multi-level selection approach to germ soma separation and the evolution of individuality is considered in the discussion.

Our model and argument are based on the following three premises: (i) There is a benefit of increasing size (although we do not explicitly model this benefit here), (ii) as cell-group size increases, the cost of reproducing an increasingly larger group also increases, and (iii) most variation in fitness exists at the colony level and there is little variation among cells within colonies. The latter assumption is likely to hold in volvocalean algae because of their mode of reproduction and colony formation discussed below. If significant within colony variation in cell fitness exists, a multi-level selection approach would be more appropriate (Michod 1996; Michod 1997; Michod 1999; Michod and Roze 1999; Michod et al. 2003). We consider the green algal lineage Volvocales as a case study and use their biology to instantiate the model so as to understand the principles that drive evolutionary transitions in complexity and individuality in this group.

Volvoclean Green Algae as a Model System

Volvoclean green algae are especially suited to studying the evolution of complexity because they comprise a group of closely related lineages with different degrees of complexity which seem to represent “alternative stable states” (Larson et al. 1992), yet the underlying genetics of their cellular differentiation is simple (Kirk 1998). Volvocales are flagellated photosynthetic organisms with coherent glycoprotein cell walls. They range from unicellular (i.e. *Chlamydomonas*) and multicellular forms with no cell differentiation (i.e. *Gonium*), to multicellular forms with complete germ-soma separation (i.e. *Volvox*) (Kirk 1998). It is believed that all multicellular volvoclean algae have evolved from a common ancestor similar to the extant *Chlamydomonas reinhardtii* (Coleman 1999; Larson et al. 1992). Nevertheless, phylogenetic analyses show that the transition from less complex forms such as *Gonium* to more complex forms such as *Volvox* occurred more than once in this lineage (Coleman 1999; Larson et al. 1992; Nozaki et al. 1999). In addition, the mechanism for cell differentiation in Volvocales may not involve many genetic steps (Kirk 1997), which may be the reason for the multiple and independent evolution of germ-soma separation in this group. One of our main goals is to understand the underlying fitness landscape for these stable states in complexity and individuality in this group.

Below we present two volvoclean algae features that are critical to the evolution of multicellularity in this group. First, because of a coherent glycoprotein cell wall, the position of flagella is fixed and thus, the basal bodies cannot move laterally and take the position expected for centrioles during cell division while still remaining attached to the flagella (as they do in naked green flagellates). Consequently, during cell division motility capabilities are negatively affected (Koufopanou 1994); this inability to both divide and maintain flagellar activity is referred to as the “flagellation constraint”. Second, cells do not double in size and then undergo binary fission. Rather, each cell grows about 2^d -fold in size, and then undergoes a rapid, synchronous series of d divisions

(under the mother cell wall). This type of cell division is known as palintomy with multiple fission. Palintomy occurs in *Chlamydomonas reinhardtii* (unicellular), *Gonium* and *Eudorina* (multicellular forms with no cell differentiation; 8-32 cells), *Pleodorina* (multicellular forms with incomplete germ-soma differentiation; 64-128 cells), and in all the members of the Meriliosphaera *Volvox* group (multicellular forms composed of 500-2048 cells with complete germ-soma differentiation), and is considered a primitive feature in this group (Desnitski 1995).

Palintomy with multiple fission has likely predisposed these algae to multicellularity (Kirk 1998). In *Chlamydomonas*, the cells (2^2 - 2^4 cells) separate from each other after division. However, in many species, the cluster of 2^n cells does not disintegrate, and colonial forms are produced. In this type of colony, the number of cells is determined by the number of cleavage divisions that take place during their initial formation (parameter d in our model below), and cell number is not augmented by accretionary cell divisions (Kirk 1997). In colonies without germ-soma separation (i.e., *Gonium*, *Eudorina*), each cell gives rise to a daughter colony (this has been termed autocolony; Kirk 1998).

The volvocalean way of colony formation means that the cells in the adult colony are clonally derived 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 $d = 8-12$ for *Volvox*). We have previously studied the level of within group variation created for this kind of cell group and the conditions under which within and between group variation may select for systems of conflict mediation that reduce the threat of within group selection and, by so doing, enhance the cooperativeness and individuality of the group (Michod 1996; Michod 1997; Michod and Roze 1997; Michod and Roze 1999; Michod 1999; Michod et al. 2003). We have found that the level of within group variation created by mutation depends critically on the number of cell divisions, d , as the number of mutations increases with the number of DNA replication events. Thus, within-group variation is

expected to be low for small groups like *Gonium* and *Eudorina* and to be more significant for larger groups like *Volvox*.

However, within-group variation is likely to be of less consequence in the larger *Volvox* species because of “parental control” on the cell phenotype. Our previous multi-level selection models assume “offspring control” of behavioral phenotype, that is, the genotype of the cell determines the cell’s phenotype (i.e. whether the cell is cooperative, hence, somatic, or not). In contrast, the model presented here assumes “parental control”, that is the behavioral phenotype (i.e., the cell fate) is determined during development, under the control of the “mother” cell. This is indeed the case in *Volvox 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, because the costs of cooperation are spread over the different genotypes present in the cell group (see, for example, Michod (1982)).

Volvocales are found in transient, turbid bodies of water, in which multiple species of volvocalean algae (which in this life-cycle phase are haploid and reproduce asexually) compete for essential resources such as light, carbon dioxide, nitrogen, and phosphorous (Kirk 1998). However, in eutrophic ponds, the larger and more complex Volvocales are found in a higher proportion of the total algal biomass (Koufopanou and Bell 1993). When the nutrients are depleted or the pond dries out, volvocalean algae go through the sexual phase, forming gametes that fuse and produce resistant zygospores that remain dormant until the necessary conditions for viability return again.

Our model is constructed with two specific features of the volvocalean green algae in mind: (i) the flagellation constraint (i.e. motility is negatively affected during cell division), and (ii) palintomy with multiple fission (i.e. cells do not divide by binary fission; rather, cells grow d -fold and then divide d times). The model also embodies the

following three considerations: (i) eutrophic conditions: we assume that there are no nutrient limitations that may prevent larger Volvocales from evolving (Volvocales of higher complexity and size are not found in low nutrient conditions); (ii) viability depends on motility only: we ignore predation, since predation likely depends on a threshold size (Bell 1985) and there is no evidence to support the idea that larger, more complex Volvocales suffer a decrease in predation rates compared to smaller undifferentiated colonies above that threshold size (which is approximately $d = 3$ in our model); (iii) asexual stage: we focus on the vegetative and reproductive functions during the asexual phase of the life-cycle, because the population interactions and growth occur during this phase.

The Model

Basic Approach

The coexistence of stable and diverse volvocalean green algae forms, in spite of very simple genetics and labile colony form, suggests that these alternative stable states represent peaks in a fitness landscape. Consequently, our basic approach involves understanding the fitness landscape of these different forms and the factors that may push populations to reach alternative fitness peaks.

We compare, as the size of the colony increases, the fitness of four hypothetical volvocalean colony types with different degrees of complexity, as represented by differing degrees of germ-soma differentiation. The four colony types are: (i) GS, undifferentiated colonies (comprised of cells performing both germ, G, and somatic, S, functions); (ii) GS/S, colonies with a specialized soma (composed of GS cells and specialized somatic cells S); (iii) GS/G, colonies with a specialized germ (composed of GS cells and specialized germ G cells); (iv) G/S, colonies with complete germ-soma specialization (composed of specialized G and S cell-types). The fecundity and viability

rates of these four colony types change as a function of colony size and the proportion of cells specializing in either germ or soma, or both.

Specialized somatic cells (S) always cost the fecundity of the colony since they do not reproduce. Nonetheless, S cells may increase the fecundity of the colony by helping the reproductive cells, regardless of whether the reproductive cells perform motility functions (GS or G cells). In contrast, specialized germ cells (G) increase fecundity by specializing in reproductive functions. The benefit that S cells give to the fecundity of the colony is proportional to the number of S cells in the colony (this benefit reaching its maximum with the maximum amount of S cells), but the benefit that G cells give to the colony is intrinsic to the G cell, and therefore it does not depend on the proportion of G cells in the colony. To calculate the benefits given to fecundity by S or G cells we used specific information from *Volvox carteri* wild type (with total germ-soma separation; i.e., a G/S colony), and *V. carteri* mutants (with disrupted germ-soma separation) as detailed in the Appendix. Somatic cells (S) increase the viability of the colony since they perform only motility functions. On the other hand, germ cells (G) decrease the viability of the colony since they perform only reproductive functions.

The product of the two fitness components

Table 1. Terms used in the model.

F	Fecundity
T	Generation time
λ	Fecundity rate = $F^{1/T}$
V	Viability
W	Fitness = λV
C	Cost of reproduction (to viability)
δ	Cost of reproduction threshold
r	Rate at which the cost of reproduction increases with colony size
d	Number of cell divisions to make colony
s	Proportion of somatic cells in colony
g	Proportion of germ cells in colony
B, b	Soma benefit to the generation time (realized and maximal)
x	Soma benefit to viability
k_G	Parameter describing germ benefit to the generation time
a	Exponent to make the soma benefit on motility nonlinear

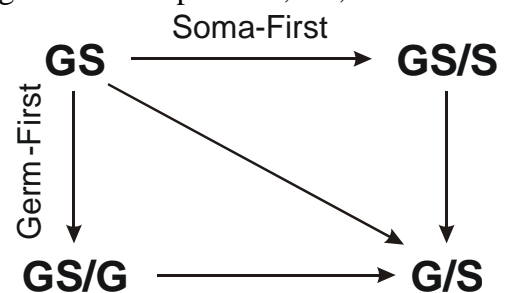


Figure 1. The possible transitions leading to germ-soma differentiation. GS: undifferentiated colonies; GS/S: soma-differentiated colonies; GS/G: germ-differentiated colonies; G/S: germ-soma differentiated colonies.

defines the fitness level that a colony can achieve. For any given colony size, we try to find out what strategy and what degree of specialization maximizes fitness in order to be able to predict how the transition from undifferentiated to germ-soma differentiated colonies in Volvocales was achieved. Figure 1 describes the possible transitions leading to germ-soma differentiation. The definitions of all variables and terms are given in Table 1.

Fecundity

In a GS colony, each cell performs d divisions to form a daughter colony with the same number of cells as the mother colony. Therefore, fecundity (F) is proportional to the number of divisions, d , and increases exponentially as a function of size as given in Equation 1.

$$F(d) = 2^d \quad \text{Equation 1}$$

In Volvocales generations are discrete since the mother colonies break down after the daughter colonies hatch. Thus, we obtain the per-time-unit fecundity rate (λ) given in Equation 2 by dividing d by generation time (T), which also increases as a function of size (d):

$$\lambda(d) = F(d)^{1/T(d)} = 2^{d/T(d)} \quad \text{Equation 2}$$

We assume that size itself does not give a direct benefit or cost to the fecundity rate of undifferentiated GS colonies, because evidence from the Volvocales indicates that generation time increases linearly with the number of cell divisions (d) (Equation 3 below). As explained in the Appendix, the smallest GS colony (i.e., 2^3 cells) has a generation time of 1 day ($d = 3$, $F = 8$ colonies, $T = 1$ day), and the largest one (i.e., 2^{12} cells) has four times the number of cell divisions and generation time ($d = 12$, $F = 4096$ colonies, $T = 4$ days). Therefore, generation time (T) increases linearly as a function of d as in Equation 3.

$$T(d) = d / 3. \quad \text{Equation 3}$$

Since T increases linearly as a function of d , in Equation 2 λ stays constant as the size, d , of GS colonies increases: $\lambda(d) = 8$ colonies/day.

We define soma-first colonies (GS/S) as colonies in which specialized somatic cells evolved first in a GS colony. GS/S colonies have a proportion of S cells (s) that are motile for the life span of the colony and do not reproduce, and $(1 - s)$ cells that undergo the ancestral GS pathway, performing both motility and reproductive functions. The fecundity of GS/S colonies ($F_{GS/S}$) decreases as s increases as given in Equation 4.

$$F_{GS/S}(d, s) = F(d) (1 - s) \quad \text{Equation 4}$$

Somatic cells can benefit the fecundity rate of GS/S colonies, $\lambda_{GS/S}$, by providing nutrients to GS cells (Bell 1985; Koufopanou and Bell 1993; Kirk 1998), thereby lowering the generation time of GS/S colonies, when compared to GS colonies of the same size. We assume that this benefit (B) is small in small colonies, and that it increases as colony size increases. We use information from the Volvocales presented in the Appendix to calculate B and scale it to size d and proportion of somatic cells s . As explained in the Appendix, if a GS/S colony with $s = .99$ and $d = 12$ decreases its generation time ($T_{GS/S}$) from 4 to 3 days compared to a GS colony of the same size (d), then by using the power function and assuming that an 8-cell colony has a generation time of 1 day, the slope of $T_{GS/S}$ is 0.8; therefore, the difference as compared to T is 0.2 ($b = 0.2$). Since b is the maximum benefit possible, the realized benefit B should be made proportional to s as in Equation 5.

$$B(s) = 1 - b s \quad \text{Equation 5}$$

Equation 5 is used to adjust the generation time as given in Equation 6.

$$T_{GS/S}(d,s) = T(d)^{B(s)} \quad \text{Equation 6}$$

Taking into account the cost of soma, fecundity becomes $F(d)(1-s)$, and using Equation 2 the fecundity rate is given in Equation 7.

$$\lambda_{GS/S}(d,s) = F_{GS/S}(d,s)^{\frac{1}{T_{GS/S}(d,s)}} \quad \text{Equation 7}$$

Figure 2A shows how the fecundity rate, $\lambda_{GS/S}$, changes as size (d) increases for different s . Due to the soma benefit (B) on generation time, $\lambda_{GS/S} > \lambda$ for some s at larger d values.

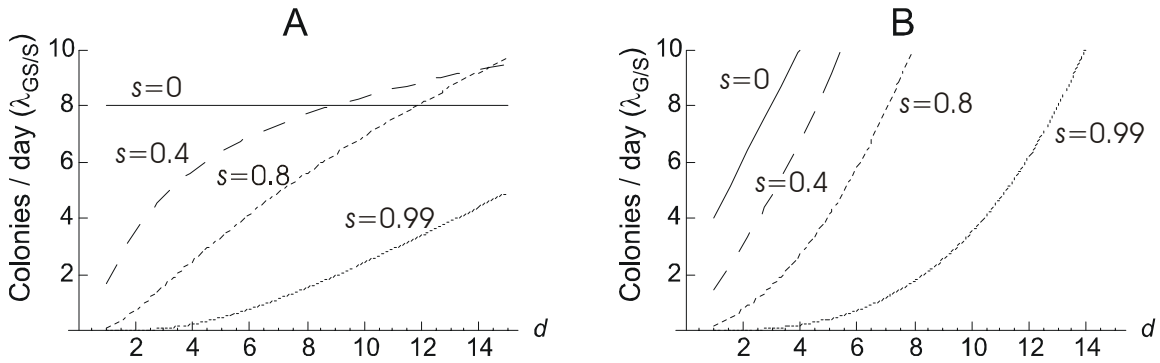


Figure 2. Fecundity rate of colonies as a function of size (d) for different values of s . A- GS/S colonies; solid line is the fecundity rate of GS colonies. B- G/S colonies.

We define germ-first colonies (GS/G) as colonies in which specialized reproductive cells evolved first in an undifferentiated GS colony. GS/G colonies have a proportion of G cells (g) which are immotile for the life span of the colony and perform reproductive functions, and $(1-g)$ cells that undergo the ancestral GS pathway, performing both motility and reproductive functions. A specialized G cell has the benefit of dedicating its total energy to reproduction, thus lowering its generation time and increasing the total fecundity rate of the colony. As the proportion of germ specialized cells, g , increases, the fecundity rate ($\lambda_{GS/G}$) of GS/G colonies increases to a certain extent, since G cells increase their fecundity rate by decreasing their generation time (T_G). For scaling T_G to colony size, we again use the *V. carteri* framework explained in the Appendix. If a G/S colony with $s = .99$ and $d = 12$ decreases its generation time from 3 days -as in a GS/S colony- to 2 days due to the specialization of G cells, then, by using the power function

and assuming that an 8-cell colony has a generation time of 1 day, the slope of T_G is $k_G = .64$. In this case the benefit does not depend on the proportion of G cells (g), since it is intrinsic to the specialized reproductive cell:

$$T_G(d) = T(d)^{k_G} \quad \text{Equation 8}$$

Given that GS/G colonies have two types of cells that reproduce at different rates, $\lambda_{GS/G}$ can be calculated using a Leslie matrix approach, as given in the Appendix.

We define G/S colonies as colonies composed strictly of specialized cells, i.e., reproductive (G) and somatic (S) cells. In G/S colonies, a proportion of G cells (g) are immotile for the life span of the colony and perform reproductive functions, and the rest of the cells (s) are motile for the life span of the colony and do not reproduce. As in the GS/S model, fecundity ($F_{GS/S}$, Equation 4) is the same for G/S colonies since $g = (1 - s)$. But in a G/S colony, G cells have both the benefit of being totally specialized as in a GS/G colony, and the help of S cells in nutrient uptake and storage as in a GS/S colony. Therefore, the generation time of G/S colonies ($T_{G/S}$) as size increases depends both on $T_{GS/S}$ and k_G :

$$T_{G/S}(d, s) = T_{GS/S}(d, s)^{k_G} \quad \text{Equation 9}$$

Thus, the fecundity rate of G/S colonies ($\lambda_{G/S}$) is:

$$\lambda_{G/S}(d, s) = F_{GS/S}(d, s)^{\frac{1}{T_{G/S}(d, s)}} \quad \text{Equation 10}$$

Figure 2B shows how $\lambda_{G/S}$ increases as d increases for different s values. Note that the equations for all fitness components for all colony types are summarized in Table 2.

Table 2. Fecundity and viability equations for the four colony types.

	Generation time	Fecundity	Viability
GS	$T(d) = \frac{d}{3}$	$F(d) = 2^d$ $\lambda(d) = F(d)^{\frac{1}{T(d)}}$	$\delta = 5$ $V(d) = \frac{1}{1 + 10^{-\delta} e^{rd}}$
GS/S	$T_{GS/S}(d,s) = T(d)^{B(s)}$	$F_{GS/S}(d,s) = F(d)(1-s)$ $\lambda_{GS/S}(d,s) = F_{GS/S}(d,s)^{\frac{1}{T_{GS/S}(d,s)}}$	$\delta(s) = 5 + xs$ $V_{GS/S}(d,s) = \frac{1}{1 + 10^{-\delta(s)} e^{rd}}$
GS/G	$T(d) = \frac{d}{3}$ $T_G(d) = T(d)^{k_G}$	$F_{GS/G}(d,g) = F(d)(1-g) + F(d)g$ $\lambda_{GS/G}(d,g)$ (from Leslie matrix)	$\delta(g) = 5(1-g)$ $V_{GS/G}(d,g) = \frac{1}{1 + 10^{-\delta(g)} e^{rd}}$
G/S	$T_{G/S}(d,s) = T_{GS/S}(d,s)^{k_G}$	$F_{G/S}(d,s) = F_{GS/S}(d,s)$ $\lambda_{G/S}(d,s) = F_{GS/S}(d,s)^{\frac{1}{T_{G/S}(d,s)}}$	$\delta(s) = (5+x)s$ $V_{G/S}(d,s) = \frac{1}{1 + 10^{-\delta(s)} e^{rd}}$

Viability and the Cost of Reproduction

The main determinant of viability is the cost of reproduction, $C(d)$, which is a function of colony size, d . We believe it is reasonable to assume that the cost of reproduction is initially small as colony size starts increasing, but increases dramatically once colonies reach a specific threshold size (given by δ below). We assume that the cost of reproduction function follows a simple logistic equation in which the ‘‘carrying capacity’’ is taken as the maximum cost possible, unity (viability can not be negative), $\frac{\partial C}{\partial d} = rC(1-C)$, with the initial cost of reproduction for single cells $C(1) = C_0$. This equation may be integrated using separation of variables to give Equation 11, the cost of reproduction at any colony size d ,

$$C(d) = \frac{1}{1 + K_0 e^{-rd}}, \quad \text{Equation 11}$$

with $K_0 = \frac{1 - C_0}{C_0}$.

The cost of reproduction in Equation 11 is subtracted from unity to give viability. As a result, the viability component of fitness (V) declines with group size, d , as given in Equation 12,

$$V(d, \delta) = \frac{1}{1 + 10^{-\delta} e^{rd}}. \quad \text{Equation 12}$$

We use K_0 in Equation 11 to tune the threshold colony size at which the cost of reproduction increases rapidly. For simplicity and without loss of generality, in Equation 12 we take $K_0^{-1} = 10^{-\delta}$, where δ is a dummy or replacement variable that represents the threshold size at which the cost of reproduction increases dramatically and viability declines rapidly. Its precise definition changes for the different colony types. In *Volvocales*, we assume that this threshold results from one of two different biological constraints on the motility of the colonies, the flagellation constraint or the enlargement constraint, as discussed further below. In Equation 12, parameter r defines the rate at which the cost of reproduction, and thus the mortality rate, increases as d increases.

Viability and the Flagellation Constraint

As GS colonies increase in size, reproduction becomes more costly due to the flagellation constraint discussed above. As size increases, the time spent in the division phase increases, and hence the motility function so basic to viability is increasingly compromised. Green algae are negatively buoyant and will sink away from the euphotic zone during development if the immotile phase is too long (Koufopanou 1994). Because the flagellum may beat for up to 5 cell divisions without the basal bodies attached, $d = 5$ is the critical threshold at which the costs of reproduction increase dramatically and viability is severely compromised (Koufopanou 1994). Thus, in Equation 12 we set $\delta = 5$ and $r = 2$, giving Equation 13 (we assume $r = 2$, because it allows viability rates to

decrease within a range of sizes that can be reached by GS colonies in laboratory cultures; i.e. *Eudorina elegans* can reach a size of 128 cells, $d = 7$, (Goldstein 1967); see Appendix for discussion on different r values).

$$V(d) = \frac{1}{1 + 10^{-5} e^{2d}} \quad \text{Equation 13}$$

Figure 3 shows how viability decreases sharply once $d > 5$ and how it nearly reaches 0 once $d = 8$.

Viability and the Enlargement Constraint

The viability of GS/S colonies always increases as s increases. However, as colonies increase in size, the cost of reproducing an increasingly larger mother cell (needed to make the increasingly larger daughter colonies) demands the help of proportionally more S cells. This is the second cost of reproduction encountered as colony size increases; we term it the “enlargement constraint”.

This constraint is consistent with the empirical observation that in modern Volvocales the somatic to reproductive cell, or S/R, ratio increases as size (d) increases (Table 3). Koufopanou (1994) showed that the investment in somatic tissue increases twice as fast with size as the investment in germ tissue. Presumably this is due to the fact that more swimming force is needed to maintain the colonies with increasingly larger germ cells in the euphotic zone. It has also been shown that in experimentally manipulated *Pleodorina californica* colonies the S/R ratio increases with the number of cells in the colony (Kikuchi 1978). Our experimental data on motility shows that there is a size threshold at which colonies start sinking (the threshold depends on the radius of the colonies and number of motile cells), and a hydrodynamics model being developed supports the enlargement constraint hypothesis (manuscript in prep.). The palintomy mode of development, as reflected in the negative correlation of germ cell size to number of germ cells (Koufopanou 1994), determines the maximum number of germ cells that can be maintained by a colony of a specific size.

To reflect the viability benefit given by somatic S cells in the context of the enlargement constraint, we return to the original viability curve representing the cost of reproduction in GS colonies (Equation 12), and assume the viability curve is shifted to a larger size as s increases. By so doing, colonies can survive to a larger size depending on s , but the reproduction costs resulting from the enlargement constraint ultimately determine a size limit for the colony. Thus, the threshold δ at which reproduction becomes costly is shifted to a larger size as a function of s and a new parameter x , which represents the motility benefit of the soma, as given in Equation 14.

Table 3. S/R ratios of a sample of extant volvocalean algae of different sizes which share the primitive developmental program assumed in the model (*Volvox* species in the table are only from the Meriliosphaera group). The species and data reported here have been taken mainly from Koufopanou (1994) to span the size distribution from *Gonium* to *Volvox*. In the table n is the number of cells in the colony ($n = 2^d$) and GS, S, and G, the different cell types. A blank in a cell type column indicates a zero value or that the cell type is not relevant. The codes in the species column are: a- Our observation; b- Identified in laboratory (Goldstein 1964; Goldstein 1967) , c- (Kikuchi 1978). d- (Starr 1969).

Species	$n = 2^d$	S/R	GS	G	S
<i>Gonium multicoccum</i>	7		1		
<i>G. Pectorale</i> (a)	8		1		
<i>Eudorina elegans</i>	24		1		
<i>E. elegans</i> (b)	32	.14	.87		.13
<i>Pleodorina californica</i>	45	.64	.61		.39
<i>P. californica</i> (c)	128	.79	.56		.44
<i>Volvox powersi</i>	388	8		.11	.89
<i>Volvox africanus</i>	534	57		.02	.98
<i>Volvox gigas</i>	554	18		.05	.95
<i>Volvox observus</i>	731	104		.01	.99
<i>Volvox tertius</i>	965	91		.01	.99
<i>Volvox carteri</i>	1328	128		.01	.99
<i>V.spermatosphaera</i>	1390	151		.01	.99
<i>Volvox carteri</i> (a,d)	2048	127		.01	.99

$$\delta = 5 + x s \quad \text{Equation 14}$$

In Figure 4 below, we compare the different cost of reproduction threshold curves for the different colony types including Equation 14.

If we let $x = 5$ and $s = 1$ in Equation 14, $\delta = 10$, indicating that the viability curve of GS/S colonies ($V_{GS/S}$) shifts to values of d similar to those reached by the larger extant Volvocales with $s = .99$ (Table 3; see Appendix for discussion on different x values).

Figure 3A shows how Equation 14 shifts the basic viability Equation 12 to larger sizes as s increases in GS/S colonies.

The viability of GS/G colonies ($V_{GS/G}$) decreases as g increases, since motility is diminished. In particular, G cells, being immotile, do not help the colony to surpass the flagellation constraint threshold (as S cells do in GS/S colonies by shifting the viability curve to higher d values). Therefore, the motility of these colonies goes from zero when $(1 - g) = 0$ (no GS cells) to the motility level of GS colonies when $(1 - g) = 1$. So δ is replaced by the cost of reproduction threshold function of GS/G colonies in Equation 12 to give Equation 15:

$$\delta = 5(1 - g) \quad \text{Equation 15}$$

The cost of reproduction threshold (δ) decreases as g increases, shifting $V_{GS/G}$ to lower values of d until it reaches zero (Figure 3B).

The viability rate of G/S colonies ($V_{G/S}$) when $s = 0$ is zero for any size (d) as in GS/G colonies ($V_{GS/G}$) when $g = 1$ (Figure 3B) because the two colony types are totally

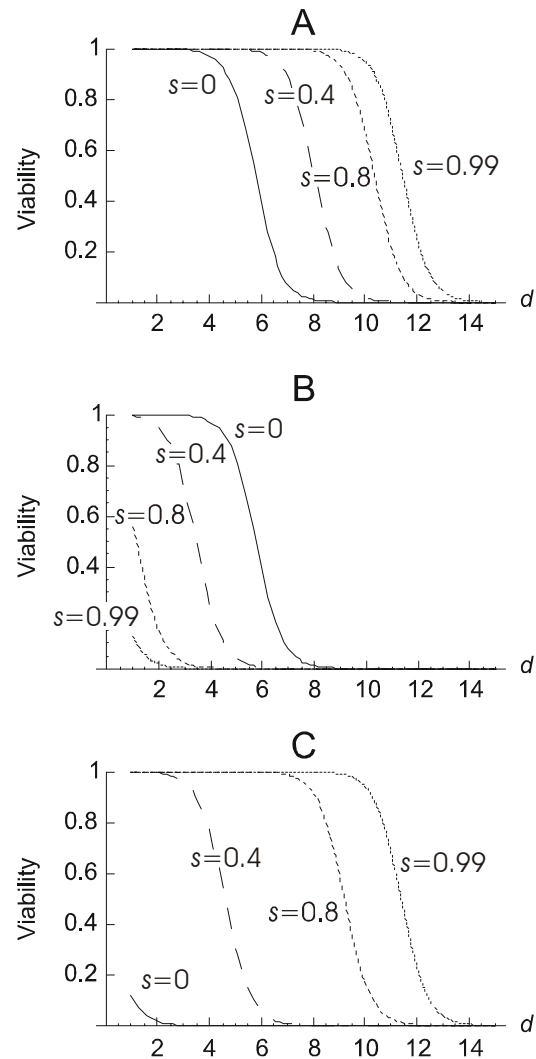


Figure 3. Viability rates of colonies as a function of size (d) for different values of s . A- GS/S colonies; solid line GS colonies. B- GS/G colonies. C- G/S colonies

composed of immotile G cells. As s increases, the cost of reproduction threshold (δ) function causes $V_{G/S}$ to approach $V_{GS/S}$, up to a point where $V_{G/S} = V_{GS/S}$ (Figure 3C) because the two colony types are totally composed of motile S cells. Thus, in Equation 12 δ is replaced by Equation 16:

$$\delta = (5 + x) s \quad \text{Equation 16}$$

Figure 4 summarizes how the threshold, δ , at which the cost of reproduction increases dramatically, is shifted to a different colony size (d) as a function of s or $(1 - g)$ for the four colony types. The GS/S colonies threshold is shifted from the GS threshold d value to the maximal possible (x) depending on s ; the GS/G colonies threshold goes from zero to the GS threshold d value depending on $(1 - g)$; and finally the G/S colonies threshold goes from zero to the maximal possible (x) depending on s .

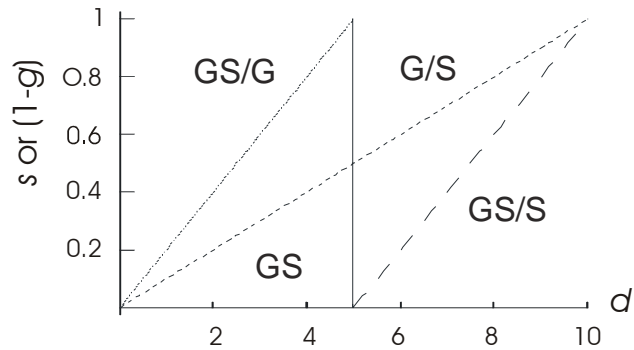


Figure 4. Cost of reproduction threshold curves defining the size of the colony at which viability is strongly affected, as a function of the proportion of cells performing motility functions (s or $(1-g)$) for the four colony types (GS, GS/S, GS/G, G/S).

Results

Fitness

The overall fitness of colonies (W) is the product of their fecundity and viability rates:

$$W(d) = \lambda(d) V(d) \quad \text{Equation 17}$$

Thus, under the assumptions of the model, smaller GS colonies will be driven to increase in size due to the selective pressures mentioned earlier, but the size that these colonies may attain is limited by the cost of reproduction, which involves the increase in time of the immotile stage (Figure 5A, solid line).

In GS/S colonies, since viability at higher d values increases, the fitness curves of GS/S colonies form adaptive peaks that shift to larger size as s increases (the $s = 0$ curve is the same as the GS colony curve). The absolute fitness of GS colonies is still higher when colonies perform 5 divisions or less (32-cell or smaller colonies), but, of course, we are not including the benefit of larger size, we are just investigating the consequences of larger size on life history evolution and complexity as represented by cell differentiation of function. Nevertheless, as size increases, GS/S colonies have a higher fitness over GS colonies of the same size (d) (Figure 5A).

In this particular model and using the Leslie matrix approach given in the Appendix for calculating $\lambda_{GS/G}$, the fitness of GS/G colonies is less than the fitness of GS colonies for all the values of g and d (see Appendix for results and discussion of different parameter values for the viability curve). GS/G colonies can never increase in size and have higher fitness than GS/S colonies since G cells do not give colonies any additional motility that will allow them to overcome the flagellation constraint. Moreover, since GS/G colonies produce two types of cells with different generation times reproducing at

different rates, these colonies would have asynchronous development and reproduction, what would probably deteriorate their viability even more (i.e. *Volvox carteri* Reg mutant (Starr 1970; Tam and Kirk 1991; Kirk 1998)).

Finally, at higher values of d and s , increased specialization allows G/S colonies to have higher fitness than GS and GS/S colonies, since the benefit that germ specialization gives to the fecundity rate outweighs the cost it gives to viability (Figure 5B; see Appendix for discussion of results with different parameter values).

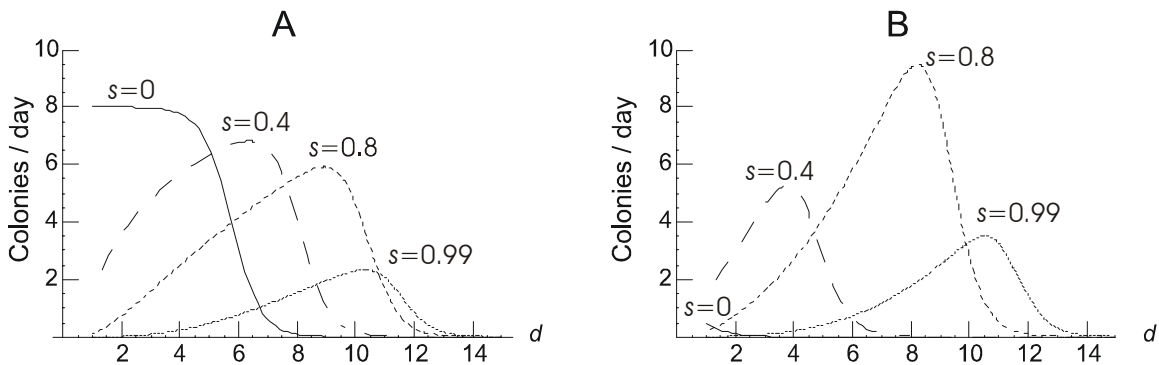


Figure 5. Fitness of colonies as a function of size (d) for different values of s . A- GS/S colonies; solid line is fitness of GS colonies. B- G/S colonies

Understanding the Volvocales

So far in our model, increasing the proportion of somatic cells, s , shifts the viability curve (by changing the cost of reproduction threshold) as a linear function of s (recall Equation 14 and Equation 16). If we assume that when s is small the benefit that S cells give to motility is less significant, but as s increases the benefit increases, then we could use an exponent a on s to express that the cost of reproduction threshold, δ , changes as a nonlinear function of s . If, for example $a = 2$, the cost of reproduction threshold equations become $\delta = 5 + x s^2$ (instead of Equation 14) and $\delta = (5 + x) s^2$ (instead of Equation 16). Table 4 shows the proportion of soma, s , that maximizes fitness in GS/S and G/S colonies and the winning strategy for each colony size d value with the linear and nonlinear form ($a = 2$) of the threshold functions. For nonlinear effects of soma on the cost of

reproduction, higher levels of fitness are attained and the transitions to higher levels of complexity are achieved at higher values of s (Table 4) which more accurately fits with the colony forms of extant Volvocales as shown in Figure 6.

Figure 6 summarizes the results concerning the transitions in complexity by showing the strategy (GS, GS/S, G/S) predicted by the model and extant Volvocales for differing values of colony size, d , and proportion of somatic cells, s . Among smaller size colonies ($d < 5$), GS colonies with general purpose

d	Linear	Nonlinear	Winning Strategy	
	s	s	Linear	Nonlinear
3	0	0	GS	GS
4	0	0	GS	GS
5	.13	0	GS/S	GS
6	.63	.52	G/S	GS/S
7	.71	.84	G/S	G/S
8	.79	.88	G/S	G/S
9	.86	.92	G/S	G/S
10	.91	.96	G/S	G/S
11	.96	.98	G/S	G/S
12	.97	.99	G/S	G/S

Table 4. Linear and non-linear benefits. The table shows how the soma-first transition in the linear approach is gradual (from a GS colony with $d = 4$ and $s = 0$ to a GS/S colony with $d = 5$ and $s = 0.13$), where the soma-first transition in the nonlinear approach is abrupt and achieved by GS/S colonies investing in a greater proportion of somatic cells (from a GS colony with $d = 5$ and $s = 0$ to a GS/S colony with $d = 6$ and $s = 0.52$). The nonlinear approach is consistent with the fact that in natural populations GS/S colonies are not found with s values smaller than 0.4 (i.e. *Pleodorina*; Table 3). The table also shows how the GS/S \rightarrow G/S transition in the nonlinear approach is only achieved by colonies with high s values (G/S colony with $d = 7$ and $s = 0.84$) as compared to the linear approach (G/S colony with $d = 6$ and $s = 0.63$). Since G/S colonies achieve higher fitness with higher s values in the nonlinear approach, this may explain why larger colonies in natural populations have so few G cells (i.e. *Volvox* species; Table 3).

cells have higher fitness, outperforming GS/S and G/S colonies of the same size with all s combinations. In contrast, among larger size colonies ($d > 5$), GS/S colonies with low or intermediate s values have higher fitness than G/S and GS colonies of the same size. G/S colonies have higher fitness compared to the others only for the highest values of s and d .

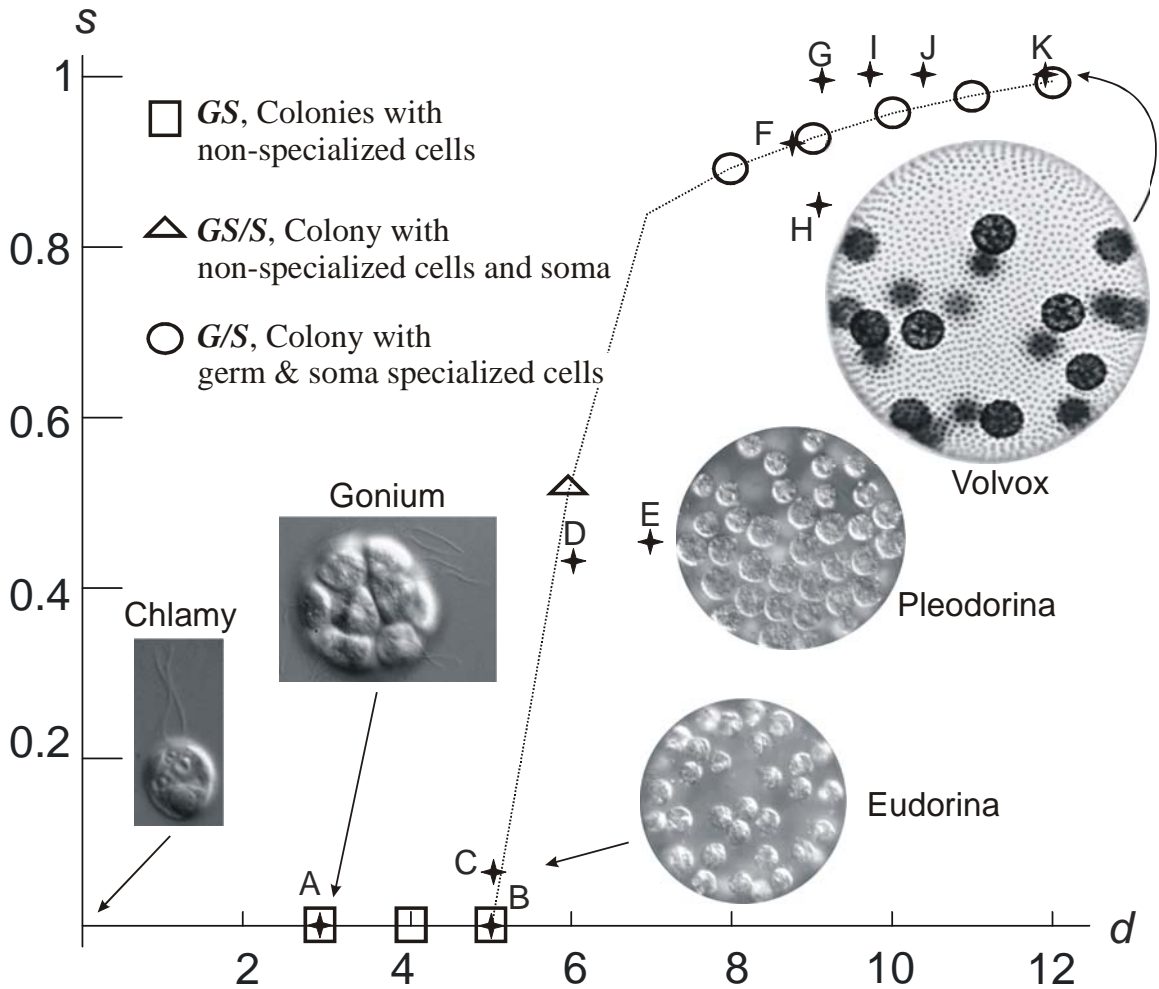


Figure 6. Fit of predictions of life history model and extant Volvocales. The proportion soma, s , plotted on the ordinate and colony size on the abscissa. Size is represented by d , the number of cell divisions required to produce a colony of N cells. Model predictions using the non-linear values in Table 4 are open square, triangle and oval shapes. Extant volvocalean species are solid star shapes (data from Table 3 which were taken mainly from Koufopanou (1994)): A- *G. multicoccum* and *G. pectorale*; B- *E. elegans*; C- *E. elegans* (a laboratory GS/S strain (Goldstein 1964)); D - *P. californica*; E - *P. californica* (Kikuchi 1978); F - *V. powersii*; G-*V. africanus* ; H - *V. gigas*; I -*V. observus*; J - *V. tertius* and *V. carteri*; and K - *V. carteri* (Starr 1969; Kirk 1998; our observations). Pictures provided care of Dr. Kirk.

In Figure 6 we have plotted the extant Volvocales of different sizes and degrees of complexity using the non-linear results of Table 4. The results for the linear model also fit the extant Volvocales, although the increase in the proportion of somatic cells, s , with colony size is not as steep as in the extant species. The species follow the critical curves of the model instead of existing in the interiors of the corresponding fitness regions

(results not shown). This suggests that the model may explain the major factors leading to transitions in complexity in this lineage. The results (Figure 6 and Table 4) agree with the data on extant Volvocales which show that as size increases, Volvocales first invest in somatic cells (S), while the undifferentiated cells remain unchanged (GS) (Table 3; i.e. transition from *Eudorina* to *Pleodorina*). Moreover, the data on extant large *Volvox* species, which have the highest d values and are totally differentiated, agree with the results that show that G/S colonies outperform the other colony types for high d values (Table 3 and Figure 6).

Discussion

Overview

A model has been developed to investigate the transition from groups of undifferentiated cells to multicellular organisms with germ-soma separation, as a means to understand the increase in complexity and the emergence of individuality during evolutionary transitions. We have shown that during the transition from unicellular to multicellular life, increased complexity and individuality can be a consequence of trade-offs between the two basic fitness components—fecundity and viability. We have studied how these trade-offs change as colony size increases, using four hypothetical colony types of increasing size and with different degrees of germ-soma differentiation. Our results indicate that (i) for colony size to increase above a threshold, a specialized and sterile soma has to evolve first, and (ii) for the size to increase further, a complete germ-soma specialization has to be achieved. We have used the volvoclean green algae to instantiate the model, so as to test in this lineage the general principles and assumptions upon which the model is based (these primarily involve trade-offs between viability and fecundity as size increases). The results argue that the cost of reproducing an increasingly large group

likely played an important role in the evolution of complexity and individuality in the Volvocales.

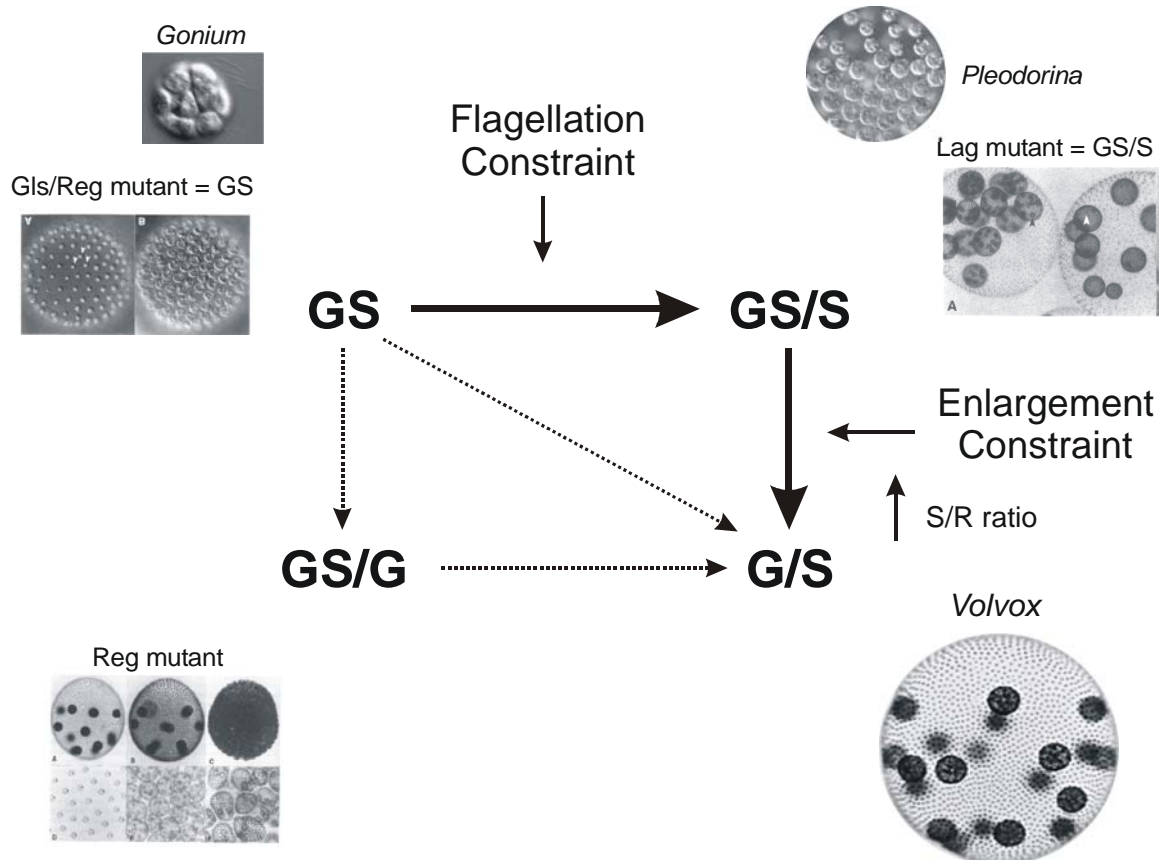


Figure 7. Summary of transitions and Volvocine forms used in the analysis. See Figure 1 and text for discussion. Heavy arrows indicate the scenario predicted by the model. Dotted arrows indicate transitions not predicted by the model. Mutants derived from *V. carteri* show alternative strategies as size increases but are not viable according to the model and not represented by extant Volvocales. The mutants are used to scale generation time as a function of size for the different strategies (see Appendix). Pictures of Volvocine species care of Dr. Kirk. Pictures of *V. carteri* mutants taken from Kirk (1998) permission pending.

In Figure 7, we summarize using the structure of Figure 1 the central results of our analysis as they bear on the volvoclean green algae. As selective pressures first pushed multicellular organisms to increase in size, the costs of reproducing an increasingly larger group also increased, having increasingly negative effects on viability. At some threshold

size (δ in our model), viability decreased dramatically and, according to our model, overcoming this threshold required the separation of reproductive and motility functions between two cell types, which resulted in increased complexity.

In undifferentiated (GS) Volvocales, as a result of the flagellation constraint, the first cost of reproduction threshold is reached rather soon, after just five cell divisions, $\delta = 5$, while the size at which the second cost of reproduction threshold is reached (the enlargement constraint) depends upon the proportion and benefit of soma, s and x , respectively, according to $\delta = 5 + x s$. By investing in somatic tissue (GS/S, and later G/S colonies), differentiated colonies are able to reach a fitness level that is impossible to attain without specialization and increased complexity. Germ first specialization (GS \rightarrow GS/G colonies; Figure 7) is not supported by the particular fitness landscapes operating in Volvocales, because initially the cost of reproduction is best alleviated by improving vegetative, not reproductive, functions, and vegetative functions may benefit both the fecundity rate and viability.

The first cost of reproduction stemmed from the flagellation constraint, and was overcome by the evolution of a specialized soma (GS \rightarrow GS/S colonies; Figure 7). The second cost of reproduction stemmed from the enlargement constraint, and was overcome by the increased somatic to reproductive cells ratio (S/R). Thus, as the S/R ratio increases, the viability benefit of having motile reproductive cells (GS) declines due to the decrease of the proportion of reproductive cells in the colony. In contrast, if reproductive cells specialize, as d increases, there is an increase of the benefit given to the fecundity rate by the decrease in the generation time resulting from germ cell specialization. Therefore, the increased division of labor (GS/S \rightarrow G/S colonies; Figure 7) allows even larger colonies to reach an even higher fitness level by enhancing the fecundity rate (through decreased generation time)—with a small cost to viability stemming from the loss of motility in the germ cells.

The first transition, $GS \rightarrow GS/S$, is achieved through lowering the cost of reproduction associated with a large size by increasing the motility of the colonies, and therefore increasing survival. In contrast, the second transition, $GS/S \rightarrow G/S$, is achieved through increasing the benefits associated with larger size by decreasing the generation time of the colony, and therefore increasing the fecundity rate. The model shows that in Volvocales, the motility capability of the colonies may have been the main driving force during the transitions to more complex forms. The flagellation constraint, the enlargement constraint, and the costs and benefits that motility provides to both components of fitness as size increases is what determines which colony type maximizes fitness (Table 4).

Increased nutrient uptake and phosphate storage in the extracellular matrix are additional benefits brought about by increased size (Bell 1985; Koufopanou and Bell 1993; Kirk 1998), but these benefits are only enjoyed if ways to maintain the motility of these large colonies (i.e., through the evolution of a sterile but permanently motile soma) have already evolved. The source-sink hypothesis developed by Bell (1985) to explain germ-soma separation in Volvocales is not intended to take into account the viability constraints imposed by the peculiar type of development in these organisms, which disrupts the motility of the colonies as size increases. In our model, if we take away the somatic benefit on the fecundity rate ($B(s)$ in Equation 5), the transitions are nonetheless achieved, but if we take away the motility benefit, or the benefit of germ specialization on the generation time, the transitions are blocked. To support our model's emphasis on motility is also the fact that *V. carteri* mutants that lose their motility during the reproductive phase are not found in nature. For instance, although *V. carteri* Reg mutants (Figure 7; see Appendix) appear spontaneously with high frequency, they do not establish populations in the wild; because these mutants enjoy the benefit of a germ-soma separation (and thus of a source-sink) for at least part of their life-cycle, it is likely that they owe their inability to succeed to the lack of motility during the reproductive phase.

Consequently, the results of our model indicate that somatic benefits on fecundity, such as those postulated by the source-sink hypothesis (Kirk 2003; Kirk 1998; Bell 1985), while they may occur, are not sufficient to explain germ-soma separation and the increase in individuality in this lineage.

The results of the model are consistent with Koufopanou's (1994) conclusions, namely that in Volvocales soma may have evolved to prevent sinking of the developing germ. Therefore, germ specialization is only possible once soma specialization has been achieved. Other evidence that shows the importance of motility for Volvocales is the dramatic change in the flagellar apparatus between *Chlamydomonas* and *Volvox* (Hoops 1997).

Complexity and Individuality

In the present work, the evolution of germ and soma is approached in the context of colony fitness maximizing strategies of functional differentiation and division of labor. The model illustrates the importance for fitness of functional differentiation among cells and so shares elements with other functional differentiation hypotheses, such as the source-sink hypothesis mentioned above. However, our model is unique in that it shows how the life history trade-off between reproduction and survival as colony size increases may lead to a functional differentiation of reproductive functions, and when this happens a new level of fitness and evolutionary transition may result.

In previous work studying transitions in individuality, we treated the evolution of an early-segregated germ in the multi-level selection context of enhancing cooperation and reducing conflict (Roze and Michod 2001; Michod 1999; Michod and Roze 1999; Michod 1997; Michod 1996). As a result of palintomy and the multiple fission type of reproduction, all cells in a volvoclean colony are clonally descended from a single cell. For smaller colonies like *Gonium*, *Eudorina* and some species of *Pleodorina*, this form of reproduction makes for high relatedness among cells and decreases the chances of within

group change allowing selection to act on the colony level as we have envisioned here. In addition, as discussed above, parental control of cell phenotype in the larger members of this lineage (such as *Volvox*) reduces the consequences of within colony variation. Still, we applied the multi-level selection approach to the evolution of an early-segregated germ-line in *V. carteri* and interpreted certain details of the timing and of sequestration of the germ line in *V. carteri* as a compromise between conflict mediation and the need to maintain a large group size and high ratio of somatic to germ cells so as to promote cooperation and optimize fitness for the group (Michod et al. 2003). All factors probably operate to some extent in most lineages. Whether for conflict mediation, division of labor, or both, germ-soma specialization enhances individuality and complexity at the new higher level during the transition from unicellular to multicellular life.

Once cell-groups start forming, to attain the many benefits of group living and larger size, certain processes are set in motion. As already mentioned, there is the problem of defection and conflict mediation (Michod 1996; Michod and Roze 1997; Michod and Roze 1999) as well as of the re-organization of fitness components at the higher level (Nedelcu and Michod 2003; Michod et al. 2003; Michod and Nedelcu 2003).

Emphasized in the present paper is the evolution of life-history traits as means of dealing with the fitness trade-offs of survival and reproduction under the selective pressure of forming increasingly larger adult groups. We have subsumed these problems under the 'cost of reproduction' and argued that in coping with them the evolution of germ and soma will also lead to enhanced complexity and individuality at the new higher level.

In short, we have argued that the higher costs of reproducing a larger organism can be an important driving force for the evolution of life history-traits and increased complexity (i.e., cell differentiation) and individuality during the transition to multicellularity. Each degree of specialization and differentiation may counteract the increase in reproduction costs associated with a larger size by increasing the viability and/or fecundity of the larger organism and therefore reaching fitness levels not possible

without increased complexity. Two general principles derived from this model may also apply to the evolution of germ-soma separation in other multicellular lineages: (i) for soma to evolve, a cell-group has to reach a specific number of cells to overcome the high cost of soma specialization on the fecundity rate, and (ii) soma, as the first specialization step, contributes to the integrity and individuality of the organism and may in certain conditions studied here increase viability, whereas germ, as the first specialization step, disrupts the integrity and individuality of the organism (by creating groups of cells that reproduce at different rates) and decreases viability.

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Appendix

Allometric scaling of generation time as a function of size using Volvox carteri wild type and mutants

To calculate generation time as a function of size for the different colony types, we use specific information from the *Volvox carteri* wild type (which is a G/S colony since it has total germ-soma separation) and *V. carteri* mutants with disrupted germ-soma separation. Under standard laboratory conditions (unlimited nutrients, and a 16/8 hours light/dark cycle), *V. carteri* germ cells perform 12 divisions to create a colony of 4096 cells with a generation time of 2 days (Starr 1969; Kirk 1998). The *V. carteri* Lag mutant varies from the wild type in two respects: (i) the germ cells perform motility functions before reproducing, and (ii) generation time is increased from 2 to 3 days as compared to the wild type (i.e., a GS/S colony; Kirk 1998). Finally, the *V. carteri* Gls/Reg gonidialess mutant (Tam and Kirk 1991) differs from the wild type in that it lacks specialized somatic or germ cells, performing 8 divisions to produce a daughter colony of 256 cells in 3 days ($F(8) = 256$ colonies; all cells perform vegetative functions first and then differentiate into reproductive cells; i.e., a GS colony). Using this information, for simplicity we assume that the generation time of a GS colony of the size of the *V. carteri* wild-type (i.e., $F(12) = 4096$ colonies) is 4 days. Table 5 summarizes the information of the *V. carteri* wild type and mutants discussed above.

Since 99% of the cells in the Lag mutant (GS/S) are S cells, we assume that the benefit that S cells give to the colony's fecundity rate by decreasing the generation time is the maximum possible. Therefore, we assume

Table 5. Summary of the information of the different *V. carteri* forms to scale generation time as a function of size for the different colony types.

<i>V. carteri</i> forms	Colony Type	d	S (%)	Generation time (days)
Wild Type	G/S	12	99	2
Lag mutant	GS/S	12	99	3
Gls/Reg mutant	GS	8	0	3
Hypothetical GS	GS	12	0	4

that the decrease in generation time from 4 days in a GS colony with 2^{12} cells to 3 days in a GS/S colony with the same number of cells but with 99% of S cells is the maximum benefit that S cells can give to the fecundity rate of a colony of that size.

On the other hand, we assume that the decrease in generation time from 3 days in a GS/S colony to 2 days in a G/S colony (*V. carteri* wild type) with the same number of cells and proportion of S cells is due to the fact that the G cells of G/S colonies do not perform motility functions as the undifferentiated cells of GS/S colonies do, thus decreasing their own generation time and consequently also the colony's generation time.

By assuming that under standard laboratory conditions also the smallest GS colony has as generation time of 1 day (e.g. *Gonium pectorale*; $d = 3$, $F(3) = 8$ colonies, $T = 1$ day; our observations), and that the two specialized cell types (S and G) would not significantly affect the generation time of the smallest colony, by using the power function $T[d] = c d^k$ and solving for k with the information presented above, we generate a generation time function as a function of size (d) for each colony type.

Leslie Matrix Approach to Determine the Benefit of Germ Specialization in GS/G Colonies

In GS/G colonies we have a population of two cell types (GS and G) reproducing at different rates. We determine the fecundity rate for these colonies by calculating the dominant eigenvalue (λ) of the Leslie matrix assuming a stable age/stage distribution. The Leslie matrix is a convenient representation for calculating the growth rates of age- and stage-structured populations (Stearns 1992). Thus, we constructed a square matrix (L) where the columns represent hours. The total number of rows and columns is defined by the generation time of GS colonies (T) times 24 (the longer generation time of the 2 cell types). The age-specific fecundities are included in the top row. Thus, the fecundity (F) of each cell type ($G \rightarrow 2^d g$ and $GS \rightarrow 2^d (1 - g)$) is included in the first row in column positions $L[1, (T_G * 24)]$ and $L[1, (T * 24)]$ respectively. Since we assume 100% viability

for each cell type, a diagonal line with probability of surviving $p = 1$ is included from $L[2,1]$ to $L[(T * 24), (T * 24) - 1]$. All the other entries in the matrix are zero. The dominant eigenvalue (λ) of L gives the GS/G fecundity rate per hour assuming a stable age distribution; λ^{24} gives the fecundity rate per day of GS/G colonies ($\lambda_{GS/G}$).

Robustness Analysis

The basic results given in Table 4 and described in Figure 6 and Figure 7 are robust to several variations in the model. First we studied the effect of the slope (determined by parameter r) of the survival function given in Equation 12 and graphed in Figure 3. We also studied the effect of the benefit of soma to size, x . Results for alternative values of r and x for the linear model are given in Table 6. The qualitative nature of the transitions remain the same, so long as the cost of reproduction is significant.

Table 6. Robustness analysis. See Appendix text for explanation.

	$r = 2.5$		$r = 1.5$		$x = 4$		$x = 6$	
d	Colony Type	s	Colony Type	s	Colony Type	s	Colony Type	s
3	GS	0	GS	0	GS	0	GS	0
4	GS/S	.13	GS/G	$g = .13$	GS	0	GS	0
5	GS/S	.31	G/S	.44	GS/S	.16	G/S	.50
6	G/S	.75	G/S	.52	G/S	.69	G/S	.58
7	G/S	.83	G/S	.58	G/S	.77	G/S	.66
8	G/S	.91	G/S	.64	G/S	.85	G/S	.73
9	G/S	.96	G/S	.70	G/S	.91	G/S	.80
10	G/S	.97	G/S	.76	G/S	.95	G/S	.86
11	G/S	.98	G/S	.81	G/S	.97	G/S	.92
12	G/S	.98	G/S	.86	G/S	.98	G/S	.96

Parameter r defines the rate at which the cost of reproduction, and thus the mortality rate, increases as d increases. Increasing r increases the rate at which the cost of reproduction increases as d increases which means that motility, and thus viability, decreases at a faster rate as size increases. Increasing r changes the transitions to lower d values, but the transition sequence envisioned in the paper remains unaltered and is still consistent with

extant *Volvocales*. On the other hand, decreasing r relaxes the cost of reproduction and thus the viability constraints as size increases, therefore changing the transition dynamics. This allows for the GS/G to be the winning strategy for $d = 4$, and larger colonies having a G/S winning strategy with a low S/R ratio. This scenario is not consistent with extant *Volvocales* and would only be possible if the costs that affect viability associated with size are low and increase gradually as size increases. Low viability cost means that the fecundity rate benefits of germ specialization dominate the transition dynamics. Low viability costs mean that there is less need to invest in soma.

Parameter x represents the motility benefit of soma, and allows the colony size where the cost of reproduction does not affect viability to increase. Lowering x to 4 increases the effect of the cost of reproduction on viability as size increases and does not change the transition dynamics significantly. Increasing x to 6 decreases the effect of the cost of reproduction on the cost of viability as size increases, and allows G/S colonies to be the winning strategy at smaller size ($d = 5$). The same results and model behavior were obtained using a piecewise linear function with different slopes (r) and soma benefit (x) as a replacement of the logistic-based sigmoidal viability function.

In conclusion, if the constraints on motility as size increases have a high effect on viability, the transitions behave in the same way as reported in the text. If the constraints on motility as size increases are relaxed, the transition dynamics change because the fecundity benefits have more effect on the transitions than the viability costs. Extant *Volvocales* species and a hydrodynamics model being developed are consistent with a high constraint of motility, and thus viability, as size increases.

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