Fitness and Complexity in Volvocalean Green Algae

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Abstract

As a means to understand the emergence of individuality at a new higher level, a model about the transition from undifferentiated cell-groups to multicellular organisms with germ-soma separation is developed. We argue that the increase in complexity is a consequence of the trade-offs between the two basic fitness components –fecundity and viability– as size increases. We use volvocalean green algae as a model system to compare the fitness, as size increases, of four hypothetical colony types with different degrees of germ-soma differentiation and show that soma evolves first, and, as size increases further, complete germ-soma specialization is achieved. Our results show that the cost of reproduction plays an important role in the evolution of multicellularity in Volvocales. Two general principles emerge from our work that may apply to other lineages: a cell group has to reach a specific number of cells to overcome the high cost of soma specialization, and soma, as the first specialization step, contributes to the integrity and individuality of the organism and increases viability, whereas germ, as the first specialization step, disrupts the integrity and individuality of the organism and decreases viability.

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. In unicellular individuals, the same cell must take care of both fitness components, typically these components being separated in time. However, in multicellular organisms 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 the emergence of individuality at a higher level is also a consequence 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.

Our model for the emergence of new levels of individuality is based on the following two premises: (i) There is a benefit of increasing size (we do not explicitly model this benefit here), and (ii) as cell-group size increases, the cost of reproducing an increasingly larger group also increases.

Volvocalean green algae as a model system

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 volvocalean 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).

Below we present two volvocalean algae features that are critical to the evolution of muticellularity in this group. First, 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 and multiple fission. Palintomy is considered a primitive feature in this group (Desnitski 1995). Multiple fission has likely predisposed these algae to multicellularity (Kirk 1998). 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).

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). Larger Volvocales with higher degree of cell differentiation are found in higher proportion in eutrophic conditions (Koufopanou and Bell 1993). Volvocalean algae go through a 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, and (ii) palintomy. The model also embodies the following three considerations: (i) eutrophic conditions; (ii) viability depends on motility only; (iii) asexual stage: we focus on the vegetative and reproductive functions during the asexual phase of the life-cycle.

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. Therefore, 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 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.

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.

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

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):

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

Viability and the Cost of Reproduction

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. As a result, the viability component of fitness (V) declines with group size, d, as given in Equation 3,

$$V(d) = \frac{1}{1 + 10^{-\delta} e^{rd}} \qquad \text{Equation 3}$$

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. 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 3, parameter r defines the rate at which the cost of reproduction, and thus the mortality rate, increases as d increases.

Results

Fitness in undifferentiated GS colonies

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 4 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).

$$T(d) = d/3 \qquad \text{Equation 4}$$

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.

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
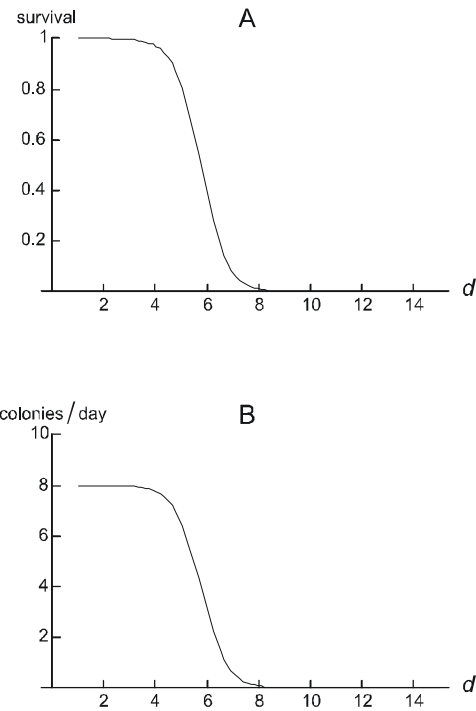

Figure 1. GS colonies viability rate and fitness curves as a function of size (d). A- Viability rate. B- Fitness.

phase increases, and hence the motility function so basic to viability is increasingly compromised. Because the flagellum may beat for up to 5 cell divisions without the basal bodies attached, $d = 5$ is the critical threshold value. Thus, in Equation 3 we set $\delta = 5$ and $r = 2$, giving Equation 5 (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)).

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

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

$$W(d) = \lambda(d) V(d) \qquad \text{Equation 6}$$

Figure 1 shows that W and V behave in the same way, since λ stays constant as size increases. 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.
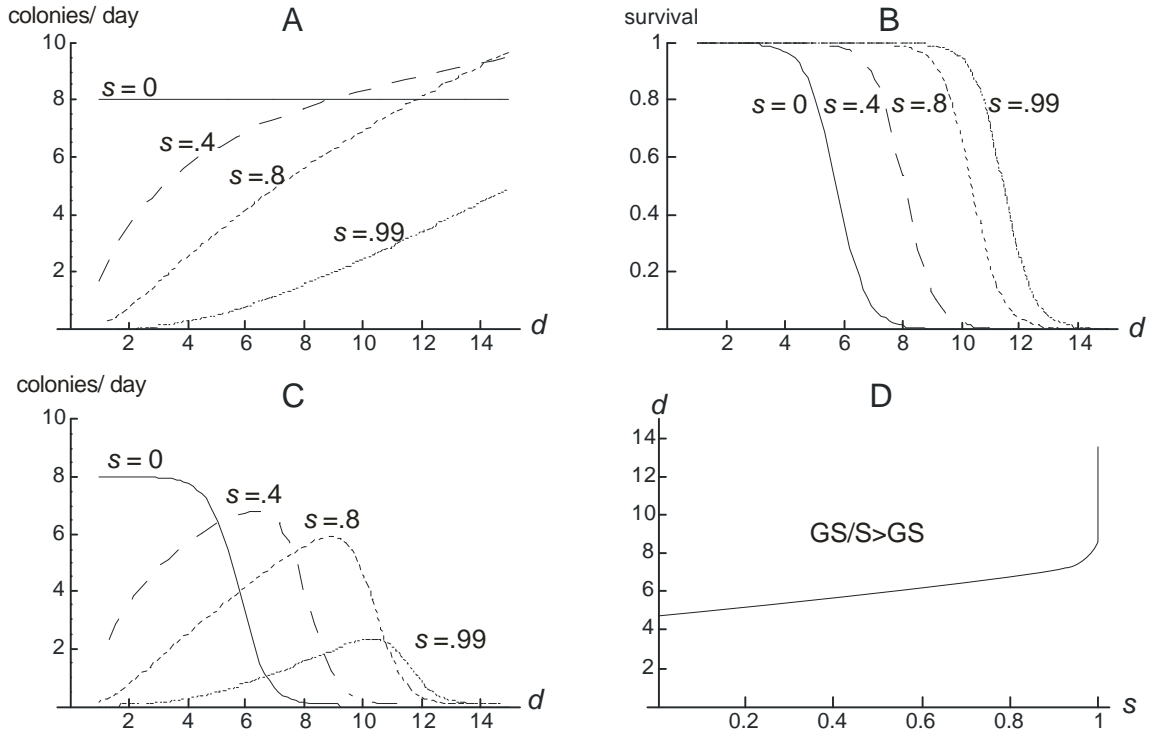
Soma-first Transition: GS→GS/S

Figure 2. GS/S colonies fecundity rate, viability rate, and fitness curves as a function of size (*d*) for different values of *s*, and the condition of *d* and *s* in which $W_S > W$. A- Fecundity rate. B- Viability rate. C- Fitness. D- $W_S > W$ threshold curve.

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_S) decreases as *s* increases as given in Equation 7.

$$F_S (d, s) = F(d) (1 - s) \qquad \text{Equation 7}$$

Somatic cells can benefit the fecundity rate of GS/S colonies, λ_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 use information from the Volvocales presented in the Appendix to calculate the benefit (*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_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_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 8.

$$B(s) = 1 - b\, s \qquad \text{Equation 8}$$

Equation 8 is used to adjust the generation time as given in Equation 9.

$$T_S(d,s) = T(d)^{B(s)} \qquad \text{Equation 9}$$

The fecundity rate is given in Equation 10.

$$\lambda_S (d,s) = F_S(d,s)^{\frac{1}{T_S(d,s)}} \qquad \text{Equation 10}$$

Figure 2A shows how the fecundity rate, λ_S, changes as size (*d*) increases for different *s*. The exponent on (1 − s) in Equation 10 eases the cost of investing in somatic cells for larger size colonies since it decreases as generation time increases. Moreover, due to the soma benefit (*B*) on generation time, $\lambda_S > \lambda$ for some *s* at larger *d* values.

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 (Koufopanou 1994). 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.

To reflect the viability benefit given by somatic S cells in the context of the enlargement constraint, we shift the viability curve to a larger size as s increases. 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 11.

$$\delta = 5 + x\,s \qquad\qquad \text{Equation 11}$$

If we let $x = 5$ and $s = 1$ in Equation 4, $\delta = 10$, indicating that the viability curve of GS/S colonies (V_S) shifts to values of d similar to those reached by the larger extant Volvocales with $s = .99$ (Koufopanou 1994).

The overall fitness of GS/S colonies (W_S) is the product of their fecundity (λ_S) and viability (V_S) rates, as is the case for GS colonies as stated in Equation 6. Figure 2B shows how Equation 11 shifts the basic viability Equation 3 to larger sizes as s increases in GS/S colonies. Figure 2C shows that 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). Nevertheless, as size increases, GS/S colonies have a higher fitness over GS colonies of the same size (d).

Figure 2D shows the condition for the transition from undifferentiated GS to soma-first GS/S colonies as a function of colony size, d, and the proportion of somatic cells, s (the transition occurs when $W_S > W$ as plotted).

Germ-first Transition: GS→GS/G

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. In contrast, the colony as a whole incurs a cost for having specialized reproductive cells, since G cells perform no motility functions.

As the proportion of germ specialized cells, g, increases, the fecundity rate (λ_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:

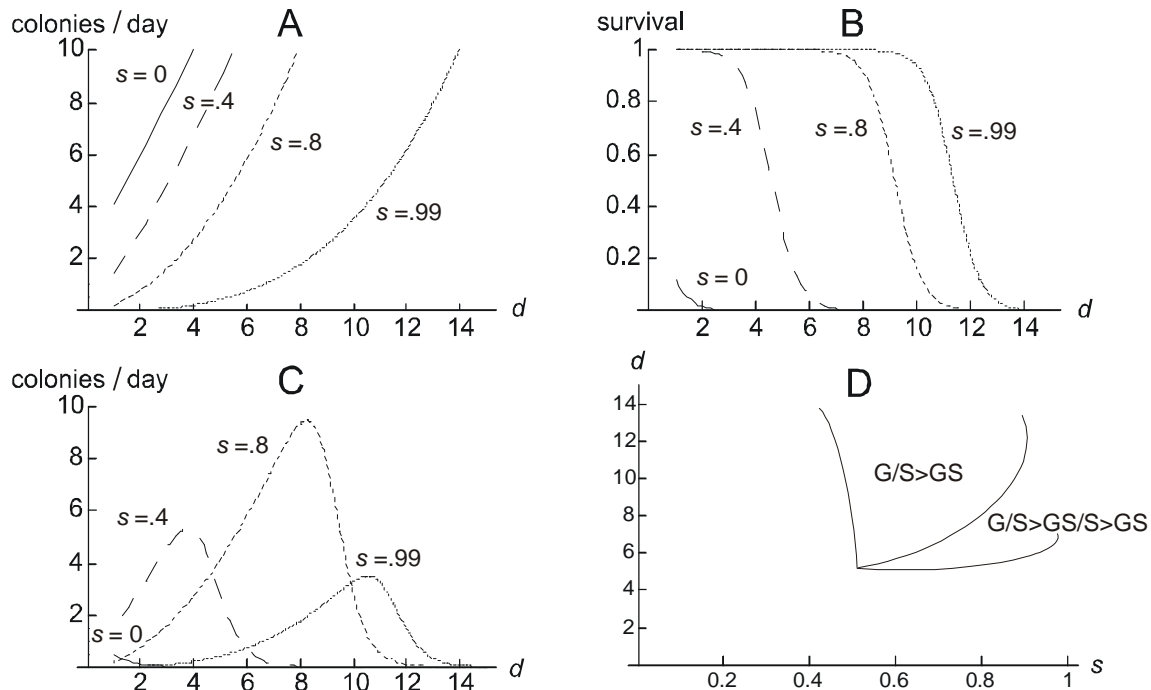$$T_G(d) = T(d)^{k_G} \qquad\qquad \text{Equation 12}$$

Figure 3. G/S colonies fecundity rate, viability rate, and fitness curves as a function of size (d) for different values of s, and the condition of d and s in which $W_{G/S} > W$ and $W_{G/S} > W_S$. A- Fecundity rate. B- Viability rate. C- Fitness. D- $W_{G/S} > W$ and $W_{G/S} > W_S$ threshold curves.

Given that GS/G colonies have two types of cells that reproduce at different rates, λ_G can be calculated using a Leslie matrix approach (Stearns 1992).

The viability of GS/G colonies (V_G) decreases as g increases, since motility is diminished. 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 3 to give Equation 13:

$$\delta = 5\,(1 - g) \qquad\qquad \text{Equation 13}$$

The cost of reproduction threshold (δ) decreases as g increases, shifting V_G to lower values of d until it reaches. Again, the fitness of GS/G colonies (W_G) is the product of their fecundity (λ_G) and viability (V_G) rates as in Equation 6 for GS colonies. In this particular model and using the Leslie matrix approach for calculating λ_G, $W_G < W$ for all the values of g and d. 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.

The Complete germ-soma Transition: GS/S → G/S

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_s, Equation 7) 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_S and k_G:

$$T_{G/S}(d, s) = T_S(d, s)^{k_G} \qquad \text{Equation 14}$$

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

$$\lambda_{G/S}(d, s) = F_S(d, s)^{\frac{1}{T_{G/S}(d,s)}} \qquad \text{Equation 15}$$

Figure 3A shows how $\lambda_{G/S}$ increases as d increases for different s values.

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_G) when $g = 1$ (Figure 3B) because the two colony types are totally composed of immotile G cells. As s increases, the cost of reproduction threshold function causes $V_{G/S}$ to approach V_S, up to a point where $V_{G/S} = V_S$ (Figure 3B) because the two colony types are totally composed of motile S cells. Thus, in Equation 3 δ is replaced by Equation 16:

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

The fitness of G/S colonies ($W_{G/S}$) is the product of their fecundity ($\lambda_{G/S}$) and viability ($V_{G/S}$) rates as before (Equation 6). Figure 3C shows the fitness curves of G/S colonies as d increases for different values of s, and Figure 3D shows the area where G/S colonies outperform GS and GS/S colonies for values of d and s. As s increases the viability of G/S colonies also increases, allowing G/S colonies to outperform GS/S colonies due to the increase in the fecundity rate. Therefore, at higher values of d and s, increased specialization allows G/S colonies to have higher fitness than GS and GS/S colonies.

Explaining Diversity in the Volvocales

Figure 4 summarizes the results concerning the transitions in complexity by showing the regions where the three different types of colonies (GS, GS/S, G/S) outperform the others for differing values of colony size, d, and proportion of somatic cells, s. Among smaller size colonies ($d < 5$), GS colonies with general purpose cells
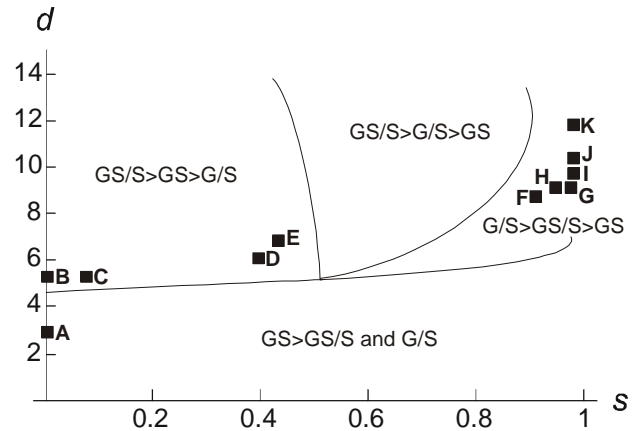

Figure 4. Areas where the different colony types outperform the others for same values of d and s plotted against the extant Volvocales data (data taken mainly from Koufopanou 1994). A- *Gonium multicoccum* and *Gonium pectorale*; B- *Eudorina elegans*; C- *Eudorina elegans* (identified in laboratory (Goldstein 1964)), wild type has no differentiation); D- *Pleodorina californica*; E- *Pleodorina californica* (Kikuchi 1978); F- *Volvox powersii*; G- *Volvox africanus*; H- *Volvox gigas*; I- *Volvox observus*; J- *Volvox tertius* and *Volvox carteri*; K- *Volvox carteri* (Starr 1969; Kirk 1998; our observations).

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.

In Figure 4 we have plotted the extant Volvocales of different sizes and degrees of complexity. The species follow the critical curves of the model instead of existing in the interiors of the corresponding fitness regions. This suggests that the model explains the major factors leading to transitions in complexity in this lineage. The results

(Figure 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 (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 (Figure 4).

Discussion

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, 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.

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) 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). 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) allows the even larger colonies to reach a fitness level not possible without increased specialization by enhancing the fecundity rate (decreased generation time)- at a decreasing cost to viability (the loss of motility by germ cells).

The first transition, GS$\rightarrow$GS/S, is achieved by lowering the cost of reproduction associated with a large size by increasing the motility of the colonies, and therefore increasing the viability rate. In contrast, the second transition, GS/S$\rightarrow$G/S, is achieved by 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 is the main driving force during the transitions to more complex forms. 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.

In short, we believe 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) 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. Two general principles derived from this model may also apply to transitions in other 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.

Appendix

Information for scaling generation time

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.

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 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.

Symbols Used in the Model

F	Fecundity
T	Generation time
l	Fecundity rate $= F^{1/T}$
V	Viability
W	Fitness $= l\, V$
C	Cost of reproduction (to viability)
d	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

References

Bell, G. 1985. The origin and early evolution of germ cells as illustrated by the Volvocales. Pages 221-256 *in* H. O. Halvorson and A. Monroy, eds. *The origin and evolution of sex*. Alan R. Liss, Inc., New York.

Coleman, A. W. 1999. Phylogenetic analysis of "volvocacae" for comparative genetic studies. *Proceeding of the National Academy of Science,* USA 96:13892-13897.

Desnitski, A. G. 1995. A review on the evolution of development in *Volvox*--morphological and physiological aspects. *European Journal of Protistology* 31:241-247.

Goldstein, M. 1964. Speciation and mating behavior in Eudorina. *Journal of Protozoology* 11:317-344.

Goldstein, M. 1967. Colony differentiation in eurodina. *Canadian Journal of Botany* 45:1591-1596.

Kikuchi, K. 1978. Cellular differentiation in *Pleodorina californica*. *Cytologia* 43:153-160.

Kirk, D. L. 1997. The genetic program for germ-soma differentiation in Volvox. *Annu.Rev.Genet.* 31:359-380.

Kirk, D. L. 1998. *Volvox: Molecular-genetic origins of multicellularity and cellular differentiation*. Cambridge University Press, Cambridge.

Kirk, D. L., M. R. Kaufman, R. M. Keeling, and K. A. Stamer. 1991. Genetic and cytological control of the asymmetric divisions that pattern the *Volvox* embryo. *Dev.Suppl* 1:67-82.

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

Koufopanou, V. and G. Bell. 1993. Soma and germ - an experimental approach using Volvox. *Proceedings of the Royal Society of London, Biological Sciences* 254:107-113.

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

Nozaki, H., N. Ohta, H. Takano, and M. M. Watanabe. 1999. Reexamination of phylogenetic relationships within the colonial volvocales (chlorophyta): an analysis of *atp*B and *rbc*L gene sequences. *Journal of Phycology* 35:104-112.

Starr, R. C. 1969. Structure, reproduction and differentiation in *Volvox carteri f. nagariensis* Iyengar, strains HK 9&10. Archiv fur Protistenkunde 111:204-211.
Starr, R. C. 1970. *Volvox Pocockiae*, a new species with dwarf males. *Journal of Phycology* 6:234-239.

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

Tam, L. W. and D. L. Kirk. 1991. The program for cellular differentiation in *Volvox carteri* as revealed by molecular analysis of development in a gonidialess/somatic regenerator mutant. *Development* 112:571-580.