

## On the transfer of fitness from the cell to the multicellular organism

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**Abstract.** The fitness of any evolutionary unit can be understood in terms of its two basic components: fecundity (reproduction) and viability (survival). Trade-offs between these fitness components drive the evolution of life-history traits in extant multicellular organisms. We argue that these trade-offs gain special significance during the transition from unicellular to multicellular life. In particular, the evolution of germ–soma specialization and the emergence of individuality at the cell group (or organism) level are also consequences of trade-offs between the two basic fitness components, or so we argue using a multilevel selection approach. During the origin of multicellularity, we study how the group trade-offs between viability and fecundity are initially determined by the cell level trade-offs, but as the transition proceeds, the fitness trade-offs at the group level depart from those at the cell level. We predict that these trade-offs begin with concave curvature in single-celled organisms but become increasingly convex as group size increases in multicellular organisms. We argue that the increasingly convex curvature of the trade-off function is driven by the cost of reproduction which increases as group size increases. We consider aspects of the biology of the volvocine green algae – which contain both unicellular and multicellular members – to illustrate the principles and conclusions discussed.

### Evolutionary transitions in individuality (ETIs)

Transforming our understanding of life is the realization that evolution occurs not only through mutational change in populations but also during *evolutionary transitions in individuality* (ETIs) – when groups of individuals become so integrated that they evolve into a new higher-level individual. Indeed, the major landmarks in the diversification of life and the hierarchical organization of the living world are consequences of a series of ETIs: from genes to gene networks to the first cell, from prokaryotic to eukaryotic cells, from cells to multicellular organisms, from asexual to sexual populations, and from solitary to social organisms. It is a major challenge to understand why (environmental selective pressures) and how (underlying genetics, population structure, physiology and development) the basic features of an evolutionary individual, such as fitness heritability, indivisibility, and evolvability shift their reference from the old to the new level.

To fully understand ETIs, a set of interrelated questions must be answered. *The Fitness Question*: Heritable variation in fitness is the basis of natural selection. How do new levels of fitness heritability arise? *The Individuality Question*: How does a group become an individual? *The Major Transitions Question*: Units capable of independent replication before the transition only replicate as part of a larger whole after the transition. How does this larger whole evolve, and how is the replication of parts regulated? *The Cooperation Question*: Cooperation exports fitness from lower to higher levels. Under what conditions will cooperation evolve and be stable? *The Complexity Question*: How do new emergent properties arise at the higher level? *The Germ–soma Question*: Under what conditions will a group of individuals specialize in reproductive and vegetative functions? *The Life History Question*: As group size increases, when is it better to gain in one fitness component at a cost to another? To answer these questions we have developed a general framework based on fitness reorganization (Table 1). Natural selection requires *heritable variation in fitness*. During ETIs, the heritability of fitness for the new higher level must increase, while, at the same time, it must decrease for the lower-level units. This requires the *reorganization of fitness* (Table 1), by which we mean the transfer of fitness from the lower-level units to the new higher-level unit and the specialization of lower-level units in the fitness components of the higher-level unit.

Several related forces drive fitness reorganization in cell groups. The most general issue is group size and its advantages and costs. Larger groups may be advantageous for a variety of reasons, e.g. a lower risk of predation or a more buffered environment within the group, as well as because of the advantages that may emerge from specialization and cooperation among the members of the group. While group living can provide substantial benefits, there remain problems that must be solved for the group to emerge into a new higher-level individual. For example, the surface to volume ratio becomes a problem as group size increases. Furthermore, the spatial organization of a locally compact group of increasing size can adversely affect the type and extent of interactions with the environment, diminishing, or perhaps exhausting, aggregate resource availability, with profound consequences for metabolism,

Table 1. Reorganization of fitness during ETIs.

|  |   |
|--|---|
| Fitness components                     | Viability (vegetative/somatic functions).<br>Fecundity (reproductive functions).  |
| Definition of fitness reorganization   | Transfer of fitness from lower to higher level.<br>Lower levels specialize in fitness components.<br>Heritability of fitness emerges at higher level. |
| Means of fitness reorganization        | Benefits and costs of increasing group size.<br>Cooperation, conflict & conflict mediation.<br>Fitness trade-offs. Germ–soma specialization.          |
| Consequences of fitness reorganization | Individuality at the new higher level.<br>Functionality. Complexity. Evolvability.  |

growth rate, viability and fecundity. In addition, there are the related costs of reproducing an increasingly large group.

Specialization among the members of the group may help to reduce these costs so as to enhance the benefits of larger group size. The most basic form of specialization involves the separation of reproductive and vegetative functions, what we refer to here as 'Germ-Soma' or 'G-S' specialization. By specializing, cells relinquish their autonomy in favor of the group; as a result, fitness and individuality are transferred from the level of the cell to the level of the group. The group, by virtue of G-S specialization of its component cells, becomes more indivisible and, hence, becomes an individual.

Throughout our discussion, we use the terms 'Germ' (G) and 'Soma' (S) to refer to cells specialized at reproduction and viability functions. We don't mean to imply anything about the timing or mode of specialization. While in some lineages G-S specialization is total – and may occur early in development – in other lineages reproductive cells differentiate late in ontogeny, from undifferentiated cells or even somatic cells. These differences in mode and timing of G-S specialization are reflected more widely in the developmental and life-history traits exhibited by the major groups (see Nedelcu and Michod 2003, Figure 3).

To become an individual, a group must settle the issue of reproductive rights among its members; otherwise, the members of the group compete with one another and disrupt the functioning of the group. This was Maynard Smith's basic insight when he first discussed evolutionary transitions (Maynard Smith 1988, 1991). In multicellular organisms, germ cells are specialized at reproduction, and somatic cells are specialized at the different vegetative functions. Similarly specialized sub-populations of individuals exist in groups of social insects.

During the multi-level selection phase in the origin of multicellularity, specialization at vegetative functions benefits the group, yet it is costly to individual cells, because these cells have less time and energy available for cell division and reproduction. Consequently, somatic functions start out as costly cooperative behaviors, so they are altruistic. The evolution of cooperation is fundamental to ETIs, because it exports fitness from the lower level (e.g., its costs to cells) to the higher level (its benefits to the group) and in this way cooperation may create new levels of fitness (Michod 1999; Lachmann et al. 2003; Michod 2003). With the evolution of cooperation comes the opportunity for competition and defection at the lower level. As with all forms of Altruism, there is a 'temptation' (read immediate selective advantage) for somatic cells to defect and cooperate less by reducing their effort at vegetative functions to the detriment of the group. Hence, with the evolution of cooperation and somatic specialization comes the need (from the group perspective) for the evolution of mechanisms that reduce conflict and competition among cells and that by so doing enhance cooperation for the benefit of the group.

Using a levels of selection approach, Maynard Smith categorized the kinds of evolving units in the living world: genes, gene networks, prokaryotic and eukaryotic cells, multicellular organisms, social groups, species, and groups

with cultural inheritance (Maynard Smith 1988, 1991). A guiding theme in his analysis was conflict mediation, or as he put it, why is it that selection at the lower level does not disrupt integration at the higher one? The need for conflict mediation drives evolutionary change during the transition between evolutionary individuals; indeed conflict mediation may be a major factor in the evolution of life's diversity. Our theoretical work proposes that the successful integration of previously independent evolutionary units into a new higher-level individual involves a cycle of cooperation–conflict–conflict mediation. The point at which the cycle is entered depends on the nature of the initial ecological interaction associated with each ETI (Michod and Nedelcu 2003). For example, the interaction may be conflictual to begin with, as with parasitic theories for the origin of the eukaryotic cell, or conflict may arise as a result of the evolution of selfish mutants, as can occur during the origin of multicellular groups. Furthermore, the nature of the subsequent interactions may differ among transitions; for example, kin selection may operate as a conflict mediator during the origin of multicellularity but not during the origin of the eukaryotic cell. In spite of these and other differences, a common framework involving cooperation, conflict and fitness reorganization can be used to understand ETIs (Table 1).

## **The evolution of multilevel selection 2**

### *Multilevel selection 1 and 2 (MLS1 and MLS2)*

During the emergence of multicellular organisms, the relationship between fitness components of single cells and the fitness components of groups changes. We assume that, as cells first began associating in groups, the fitness of the group was a simple, perhaps additive, function of the fitnesses of the component cells. In the terminology of Damuth and Heisler (1988), this corresponds to multi-level selection 1 (MLS1), in which the fitness of the group is defined as the average of the fitnesses of its members. At some point during an ETI, this definition of group fitness no longer applies and we must define fitness of the group in terms of survival and reproduction of descendent groups – what these authors term MLS2.

The distinction between MLS1 and MLS2 is profound and is another way of discussing the transfer of individuality from the cell to the cell group (Okasha 2006). Consider the case of multicellular organisms with complete G-S specialization. The germ cells specialize completely in reproductive functions and the somatic cells specialize completely in vegetative functions. The cell fitness of all cells must be zero (since fitness is the product of viability and reproduction and one of these is zero by the assumption of complete G-S specialization). Therefore, the fitness of the group is zero under MLS1, yet group fitness may be quite high under MLS2. Even something as fundamental as cell division itself, so fundamental to cell fitness and to MSL1 initially, eventually

gets co-opted to contribute no longer to MLS1 but to growth of the multicellular individual and in so doing contributes to MLS2 (Michod et al. 2003, see especially Section 5; Nedelcu and Michod 2003, see especially Figure 2).

We are concerned here with the gray area between MLS1 and MLS2 and with the role of fitness trade-offs and multi-level selection in changing MLS1 into MLS2. Recall, when Damuth and Heisler (1988) defined MLS2, they simply assumed the existence of a new function at the group level unrelated to fitness of the lower level individuals (their  $\Omega$  function). Simply put, the challenge of ETI theory is to explain how fitness at the group level in the sense of MLS2 emerges out of fitness at the group level in the sense of MLS1. We have modeled this issue in several ways using a population genetics approach based on multilevel selection theory and an optimization approach based on life history theory.

Using a multilevel selection approach we have studied the evolution of propagule size  $N$  (Michod and Roze 2000; Roze and Michod 2001). Under spore reproduction ( $N=1$ ), there are well-defined lineages traced through the spore cell. When the propagule is not a single cell, but a sample of cells in the parent group ( $N>1$ ), it is still possible to distinguish parent and descendent groups, but there is now greater opportunity for conflict and selection among cells because of the potential for reduced kinship, and consequently more opportunity for MLS1 in the presence of MLS2. Spore reproduction helps tilt the balance from MLS1 to MLS2. In our models, for all modes of propagule formation, we assume a characteristic of MLS2 which is that fitness of the group is not a simple average of the fitness of its members. In particular, we assume that group fitness is a product of two group properties: size and functionality. These group properties are functions of the properties of the cells in the group, and cell fitness may affect group fitness. For example, if cells replicate fast, the group size will be larger. However, functionality depends on the level of cooperation among cells and, as we assume a cost of cooperation on the rate of cell division, large groups tend to be less functional as they tend to be composed of less cooperative cells, thus decreasing the second aspect of group fitness. So, in these models, we are clearly in a gray area between MLS1 and MLS2.

All things being equal, the smaller the propagule, the smaller the group, so there is a direct cost of smaller propagules on the first component of group fitness. Nevertheless, single cell reproduction can evolve if the opportunity for selfish mutations is great; selfish mutations benefit the cell but harm the group. Although the evolution of single cell reproduction is clearly a step towards MLS2, we are not all the way there until group fitness is decoupled from cell fitness. This requires cell specialization at reproductive and vegetative functions, or G-S specialization.

We have studied the evolution of G-S specialization using a multi-level selection conflict mediation framework. Mathematical models (Michod 1996, 1997a, 1997b, 1999; Michod and Roze 1999, 2001; Michod et al. 2005) based on verbal arguments (Buss 1987; Maynard Smith and Szathmary 1995) predict

G-S specialization will evolve to reduce the conflict created by deleterious mutations by reducing the mutation rate and/or the opportunity for proliferation of selfish mutations. These models employ standard two-locus population genetic modifier techniques and assume several different modes of cell group formation, including aggregation, fragmentation, and groups that are clonally derived from a single cell. We have also considered sexual and asexual reproduction. Our results show that the threat of deleterious mutation (through the occurrence of MLS1) is lower with early germ sequestration and fewer divisions in the germ line. Other features of the multicellular organism may be understood as adaptations to reduce conflict among cells (Michod 2003; Hudson et al. 2002). In particular, creating the cell group by clonal cell division from a single cell (as compared to fragmentation or aggregation) increases cooperation by increasing genetic relatedness among cells (Smith and Szathmary 1995; Queller 2000; Michod and Roze 2000; Roze and Michod 2001).

In addition to these conflict mediation multi-level selection models, we have taken a life-history approach to the evolution of G-S specialization (Michod et al. 2005). In this model, the group is assumed to be the focal level of selection and we study how synergistic interactions between cells specialized at different functions benefit the group. We now discuss these life history models in more detail.

#### *Life history approach to MLS2*

In our life history model, we begin with MLS1 by assuming that the fitness components of the group are additive functions of the fitness components of the cells (Equation 1), so that the viability of the group,  $V$ , is an additive function of the effort put into viability functions by the component cells,  $v_i$  (Michod et al. 2005). Likewise, for group reproduction, or fecundity,  $B$ , we assume it to be an additive function of the reproductive efforts of single cells,  $b_i$ .

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

We assume that group fitness,  $W$ , is the product of viability and fecundity, as given in Equation 2. This is appropriate when generations are discrete, as is the case with the volvocine green algae considered below.

$$W = VB \quad (2)$$

There are interesting implications of combining the fitness components at the group level after first summing the cell contributions (as assumed in Equation 1 and Equation 2). Most importantly (and critical to the analysis of our model) is

the fact that, if one cell has a high fecundity (and hence a low viability, so that it would have a low fitness by itself), this may be compensated for if another cell has a high viability (and hence low fecundity). Consequently, even though each of these cells by itself would have a low fitness, together they can bring a high fitness to the group (especially under conditions of convexity of the trade-off). This kind of joint effect is a first step towards integration of the group, and would not be possible if we used as group fitness the average cell fitness,  $\frac{1}{N} \sum_{i=1}^N v_i b_i$ .

More formally, the normalized fitness,  $VB/N^2$ , is greater than the average cell fitness by the negative of the covariance between the two fitness components. Since in our case the covariance is itself negative, the normalized fitness associated with Equation 2  $VB/N^2$  is greater than the average cell fitness,  $\frac{1}{N} \sum_{i=1}^N v_i b_i$ , by the magnitude of the covariance between fitness components. This covariance effect at the group level appears to be quite general. Its contribution to a group property like fitness depends on the property being a multiplicative function (or some other function requiring a strong balance) of two components (e.g., viability and fecundity) which themselves covary so that higher values of one component bring about lower values of the other (trade-offs). Of course, if there is no variance in these components among the lower level units (cells), then there is no covariance and no effect at the group level. What factors might produce variance among the lower level units? We can think of two factors: noise and a convex curvature of the trade-off function. We have focused on the curvature of the trade-off function in our work (Michod et al. 2005).

Although single cells are constrained by a trade-off relation assumed to be identical for all the cells, the fitness components of the group may break through this constraint when there is an advantage to specialization. Let us consider in more detail the trade-off between reproduction,  $b$ , and viability,  $v$ , at the cell level. For single cells living alone,  $v$  and  $b$  are the fitness components of the cell. During the MLS1 phase, the properties of the group are functions of the cell properties; therefore, for cells that join groups, we assume that  $v$  and  $b$  are the contributions by the cell to the fitness components of the group. During the origin of multicellularity and the increase in size of cell groups, we expect the trade-off relationship between  $v$  and  $b$  to change with the size of the group. However, for the moment we consider the implications of a simple linear relation given in Equation 3.

$$v = v_{\max} - \alpha b \quad (3)$$

With a linear intrinsic function, the cell will invest in both reproduction and viability functions, as there is no incentive to specialize. In the case of a linear trade-off (Equation 3), the ratio of viability to fecundity at the group level is determined directly by the trade-offs at the cell level (as represented by  $\alpha$ ) and is given in Equation 4, where  $N$  is the number of cells in the group.

$$V \propto Nv_{\max} - \alpha B \quad (4)$$

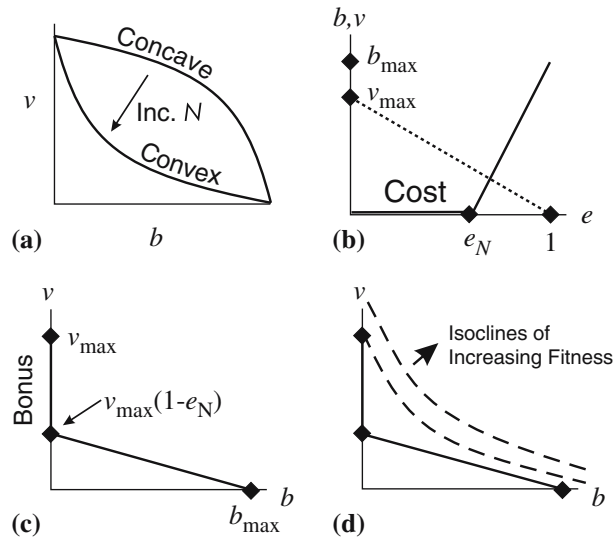
The curvature of the trade-off between fitness components is a basic issue in life history theory (Levins 1968; Schaffer 1974; Michod 1978; Reznick 1985; Stearns 1992; Benkman 1993; Carriere and Roff 1995; Benson and Stephens 1996; Takada and Nakajima 1996; Strohm and Linsenmair 2000; Kisdi 2001; Roff 2002; Sato 2002; Blows et al. 2004; Rueffler et al. 2004). For a convex (concave) function  $v(b)$ , the second derivative is positive (negative), so if we take a particular point, say  $b^*$ , and two points equidistant below and above  $b^*$ , say  $b^-$  and  $b^+$ , respectively, then  $v(b^-) + v(b^+) > (<) 2v(b^*)$ . If  $b$  is reproduction and  $v(b)$  viability, then convexity of  $v$  implies there is an advantage to specializing in the two components of fitness.

Fixed reproductive costs which must be paid to receive any reproductive success exist in many organisms (cost of a flower, a placenta, mating displays, etc.) and they tend to create a convex relationship between fecundity,  $b(e)$ , and the time, energy and resources invested in reproduction, or what has been termed reproductive effort,  $e$ . We now consider the implication of there being a fixed cost to reproduction that increases with the size of the group,  $N$ ,  $e_N$ .

A fixed cost,  $e_N$ , means no reproduction,  $b(e)=0$ , if reproductive effort is below this cost or  $e < e_N$ . As shown in Figure 1b–c this tends to make the trade-off curve convex in a piecewise linear fashion. The increasing cost of reproduction as group size increases changes the basic relationship between survival and reproduction at the group level from that given by Equation 3 into a convex function.

Somatic cells that specialize in zero reproductive effort,  $e=0$ , contribute a bonus to the group because these cells do not have to pay the fixed reproductive cost,  $e_N$ . In Figure 1c the convex trade-off curve from panel (b) is plotted with isoclines of the additional fitness to the group contributed by a newly added cell. The construction of Figure 1c illustrates a prediction of our model (Michod et al. 2005), which is that the greater the cost of reproduction ( $e_N$ ) relative to the maximum viability  $v_{\max}$ , the more likely the isocline touches the trade-off curve at  $v_{\max}$  (meaning the new cell will be soma specialized;  $b=0$ ) as opposed to touching at an intermediate value  $0 < b < b_{\max}$ .

In our model (Michod et al. 2005), we consider how cells should change their allocation to reproduction,  $b$ , and viability,  $v$ , as colony size increases so as to maximize the fitness of the group. We predict that in unicellular organisms the trade-off curve between viability and reproduction should be concave, or else the single cell state would not be stable to group formation. We argue that, as groups form and increase in size, the curve becomes increasingly convex. With increasing convexity of the curvature between fitness components, there is an increasing advantage to functional specialization as the number of cells in the



*Figure 1.* Fitness trade-offs. In (a) a concave curve changes to a convex curve as group size increases. Piece-wise linear reproduction curve (b, solid) with linear viability curve (b, dotted) approximates a convex trade-off curve (c) at cell level. In (d) isoclines of group fitness are plotted with this convex trade-off curve at the cell level. The reproductive effort  $e_N$  is the cost of reproduction increasing with group size  $N$  and  $v_{\max} - v_{\max}(1 - e_N)$  is the ‘bonus’ of soma specialization. This bonus can be obtained only by groups.

group increases (Figure 1a). With cell specialization comes increasing individuality and a shift from MLS1 to MLS2.

Note in Figure 1c the vertical portion of the intrinsic curve running along the  $v$ -axis from  $v_{\max}(1 - e_N)$  up to  $v_{\max}$ ; its length represents the benefit to viability of soma specialization stemming from not having to pay the cost of reproduction. There are four points about this benefit of soma specialization (or cost of reproduction). First, it is only obtainable through group living and is only expressed at the group level; it is not an option open to solitary cells. Second, it changes the relationship that governs the fitness components at the group level (that is, the trade-offs between fitness components change during an ETI). Third, the benefit of specialization allows the group to break through the constraints that governed the fitness of single cells living alone. Fourth, the benefit of specialization will likely change with the size and organization of the group. For example, if there are already many somatic cells in the group, the benefit of a new cell specialized at somatic functions may be small, while the benefit to the group may be larger if the cell were to specialize at reproductive functions.

This conclusion that convexity of trade-offs selects for specialization is similar to an argument in life history theory that convex fitness trade-offs select for specialization in reproductive function as organisms increase in age. That

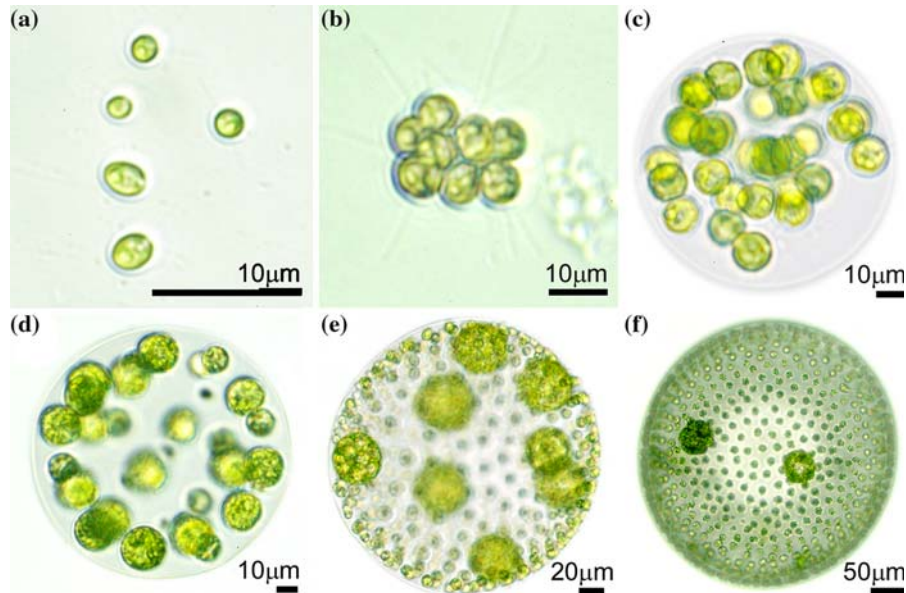


Figure 2. Subset of volvoclean species which show an increase in complexity, cell number, volume of extracellular matrix, division of labor between somatic and reproductive cells, and proportion of somatic cells. (a) *Chlamydomonas reinhardtii*; (b) *Gonium pectorale*; (c) *Eudorina elegans*; (d) *Pleodorina californica*; (e) *Volvox carteri*; (f) *Volvox aureus*. Where two cell types are present (d, e and f), the smaller cells are the vegetative somatic cells, whereas the larger cells are the reproductive cells (germ cells are called gonidia). Picture credit: C. Solari.

is, convex fitness trade-offs select for semelparity or ‘big bang’ reproduction (Schaffer 1974; Charlesworth and Leon 1976). Big bang reproduction is analogous to cell specialization in the sense that the age classes specialize in either no reproduction or complete reproduction (for the last class).

In summary, early during an ETI, while MLS1 is operative, the fitness trade-offs at the lower level directly constrain the trade-offs at the group level. However, as the ETI proceeds and cells specialize, the fitness trade-offs at the group level depart from those that once governed evolution at the cell level. The advantage of division of labor allows the group to break through the trade-offs that once constrained fitness at the lower level. Once the specialization is complete and the lower level units are specialized in one of the two major fitness components (viability or fecundity), they have no fitness by themselves and so group fitness in the sense of MLS1 is null, while group fitness in the sense of MLS2 may be quite high. In short, as a result of specialization at the lower level, the individuality of the group is enhanced while that of the lower level is reduced and there is a shift from MLS1 to MLS2.

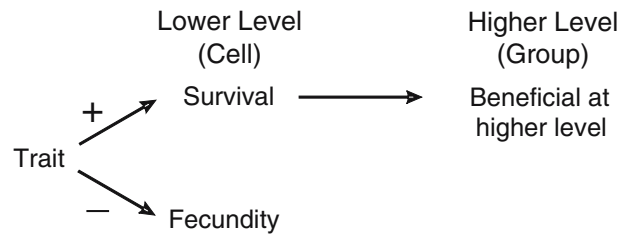


Figure 3. Trade-offs at lower level translate into altruism at the higher-level when groups are

### **Volvocine green algae as a model system**

#### *Introduction to Volvocine algae*

The premier example of the integration of lower level units into a new higher-level individual is the evolution of multicellular organisms from unicellular and colonial ancestors. Unfortunately, for the major multicellular lineages, the factors underlying their origin lie hidden deep in their evolutionary past, obscured by hundreds of millions of years of evolution. The volvocine green algae (Figure 2), according to one estimate (Rausch et al. 1989) just 35 million years old, provide a unique window into this major ETI. Within this closely related monophyletic (Larson et al. 1992; Buchheim et al. 1994; Coleman 1999; Nozaki et al. 2000, 2002, 2003; Nozaki 2003) group, significant evolutionary transitions have occurred repeatedly within a relatively short time, possibly as short as 35 million years (Rausch et al. 1989), suggesting strong selective pressures driving the transitions. The transitions which have occurred in this group are: (1) from unicellular to colonial, (2) simple clumps of cells to structured hollow spheres, (3) from undifferentiated cells to partial specialization, to (4) complete G-S specialization, (5) among four distinct developmental programs in *Volvox* (Desnitski 1995), (6) among various kinds of sexual reproduction (isogamy, anisogamy and oogamy, and homothallic or heterothallic sex) involving different forms of sexual communication (for example, with or without sexual inducer pheromones) and (7) colony sizes where cell number spans 5 orders of magnitude.

Volvocine algae consist of both uni- and multicellular flagellated photosynthetic facultatively-sexual haploid eukaryotic organisms with varying degrees of complexity stemming from differences in colony size, colony structure, and G-S specialization (Figure 2). Although several model experimental systems are being used to investigate the origins of multicellularity, including choanoflagellates (King and Carroll 2001), cellular slime molds (Strassmann et al. 2000; Foster et al. 2002; Queller et al. 2003) and myxobacteria (Shimkets 1990; Velicer et al. 2000), volvocine algae exhibit a number of features that make them especially suitable for our project on fitness deconstruction. They

can easily be obtained from nature and maintained in the lab under realistic conditions that allow for an eco-physiological framework. Uni- and multicellular forms coexist in transient, quiet bodies of water and in large, eutrophic lakes (during early summer blooms). Many aspects of their biology have been studied (Kirk 1998) (including their cytology, biochemistry, development, genetics, physiology, natural history, ecology and life-history). Unlike other model systems (cellular slime molds, myxobacteria), volvocine algae develop from a single cell, so the cells in the group are related. This aspect of the life cycle is basic to the questions we wish to investigate and is shared by the more complex multicellular forms we wish to understand. *V. carteri*'s 120MB genome is being sequenced and the genome project for *C. reinhardtii* is complete, so there is some hope of discovering the genetic basis for the variation in complexity and individuality in this group. Several 'social' genes necessary for group living and fitness reorganization have already been identified (Kirk 1998) and it is known that the mechanism for cell differentiation in Volvocales does not involve many genetic steps (Kirk 1997), which may be the reason for the multiple and independent evolution of G-S separation in this group.

The volvocine algae range from unicellular (*Chlamydomonas*) and multicellular forms with no cell differentiation (*Gonium* and *Eudorina*; 8–32 cells) or incomplete G-S differentiation (*Pleodorina*; 64–128 cells) to multicellular forms with complete G-S separation (*Volvox*; up to 50,000 cells) (Kirk 1998). In multicellular species, the number of cells is determined by the number of cleavage divisions that take place during the initial formation of the embryo, and cell number is not increased after that (Kirk 1997). In colonies without G-S separation (i.e., *Gonium*, *Eudorina*), each cell gives rise to a daughter colony. It is believed that all multicellular volvocine algae have evolved from a common ancestor similar to the extant *Chlamydomonas reinhardtii* (Larson et al. 1992). 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 (Larson et al. 1992; Nozaki et al. 1999).

In summary, volvocine green algae are especially suited to studying fitness trade-offs during the evolution of individuality 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). A general goal of our research with this group is to understand the underlying fitness landscape for these stable states in complexity and individuality.

### *Applying the theory*

We now consider the assumptions and conclusions of the theory discussed above in the volvocine algae. The important assumptions in the model are discrete generations, the operation of group selection in a multi-level selection context, the existence of cooperative and selfish behaviors, an advantage to

larger group size, costs of larger group size (especially the increasing cost of reproduction as colonies increase in size) and finally, these costs being surmountable by cell specialization. MLS1 and the additivity of fitness assumed above in the life history model would apply, for example, to the simpler forms of volvocine algae considered (Figure 2) in which cells remain together after cell division.

Flagellar motility is an example of a trait which has positive effects on viability but detracts from reproduction (Buss 1987; Kirk et al. 1997; Koufopanou 1994). For unicells, like *C. reinhardtii* (Figure 2a), we may assume that individual selection will produce a level of flagellar motility that optimally balances the conflicting needs of motility and reproduction. However, when cells join groups, like in *G. pectorale* (Figure 2b), flagellar action will enhance the motility of the group, and this group benefit may select for a level of flagellar action by the cells that is greater than would be optimal for cells living alone. Because motility detracts from cell division, enhanced cell motility becomes an altruistic behavior – a costly form of cooperation – when expressed in a group setting (since cells with lower flagellar action would divide at a higher rate). This illustrates the general point that a trait which has positive and negative effects on fitness may become cooperative and altruistic when expressed in a group setting (Michod 1999; Michod and Roze 2001).

Flagellar motility is critical for the volvocines. In lakes they perform daily vertical migrations in the water column to access resources that are heterogeneously distributed in space (surface/bottom) and time (day/night) (Sommer and Giliwicz 1986). In addition, flagellar beating likely facilitates the transport of nutrients and removal of wastes by local mixing (Niklas 1994; Niklas 2000).

Flagellar action is an example of a trait which has conflicting effects on the components of fitness at the lower level and which benefits the higher level (Figure 3). Whether such conflicted traits become altruistic in the group context depends on exactly how the costs and benefits at the cell level are translated into fitness effects at the group level (Michod 1997a). Fitness trade-offs play central roles in other evolutionary transitions; for instance, during the origin of eusociality in insects, group level traits (division of labor) emerge from the existing life-history traits (Fewell and Page 1999).

Group selection is likely to be important in these colonial algae because of their mode of reproduction and colony formation, in which all cells in the group are derived clonally from a single cell after a specific number of cell divisions,  $d$  ( $d=3$  for *Gonium*,  $d=5$  for *Eudorina*,  $d=6-7$  for *Pleodorina* and  $d=8-15$  for *Volvox*). We have previously studied the level of within group variation created for this kind of cell group, and 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 (Michod 1996, 1997a, 1999; Michod and Roze 1997, 1999; Michod et al. 2003). 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*. Most of the larger colonial forms have germ-soma

specialization which, according to the theory, helps mediate conflicts within the group in addition to benefiting the group through division of labor.

We expect within-group variation to be less in *Volvox* species than predicted by our population genetic multi-level selection models because of ‘parental control’ of the cell phenotype. Our population genetic models assumed ‘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 models we have used to study division of labor (Michod et al. 2005) assume ‘parental control’; that is, the behavioral phenotype (i.e., the cell fate) is determined during development, under the control of the group or the ‘mother’ cell. This mode of control of cell behavior is used in the more complex members of the group such as *V. carteri*, as the cell fate (somatic or germ) is established early in development through a series of asymmetric cell divisions of the anterior blastomeres (for discussion see, Michod et al. (2003)). It is well known that it is easier for cooperation to be maintained in a group under parental control than under offspring control, because the costs of cooperation are spread over the different genotypes present in the cell group when there is parental control.

Our previous theoretical work (Michod 1996, 1997a; 1999; Michod and Roze 1997, 1999; Michod et al. 2003) assumed strict genetic control of behavioral phenotypes and has been criticized on the grounds that the assumption of unconditional cooperation or selfishness (depending, as we assumed, only on cell genotype) is not general (Queller 2000). In our models, we assumed that the phenotype of the cell, whether it cooperated or not, depended only on its genotype. We did not consider conditional forms of expression of the trait as would be reasonable during development when the expression of a cell – its behavior – may depend on its particular time and place in the developing organism. For example, in *V. carteri*, whether a cell becomes somatic or germ depends on cell size, which is determined by an asymmetric division at the fifth cell division; smaller cells below a threshold size become cooperative somatic cells and larger cells become reproductive germ cells. Our assumption of unconditional expression of the altruism is clearly a simplification made for mathematical convenience.

However, the assumption of unconditional cooperation/selfishness does apply to the cooperative somatic cells in *V. carteri* in the following important sense. The cell group expressing the cooperative behavior (which is flagellar activity), the ‘action group’ in Queller’s (2000) terminology, is nearly the entire cell group of some 2000–4000 cells (with the exception of the 8–16 germ cells), rather than some smaller, more local cell group. Indeed in the selfish *regA*<sup>-</sup> mutants of *V. carteri*, all of the somatic cells redifferentiate as reproductive germ cells and do so at great cost to the motility capacity of the group.

The benefits of increased group size in volvocine algae are likely to involve reduced predation (Porter 1977; Morgan 1980; Pentecost 1983; Reynolds 1984; Shikano et al. 1990). The costs of larger size stem from five sources: (i) the ‘flagellation constraint’ (Koufopanou 1994), (ii) the ‘enlargement constraint’,

which refers to the costs of moving an increasingly large colony (Solari et al. 2006a; Solari et al. 2006b), (iii) increased resources needed to provision the growing gonidium or embryo (Bell 1985), (iv) decreased efficiency of resource uptake and waste removal, and (v) increased generation time (Bell 1985). Costs (i)–(iii) affect viability, cost (iv) could affect both fecundity and viability, and costs (iii) and (v) affect the fecundity rate.

The ‘flagellar constraint’ in this algae is a consequence of the coherent glycoprotein cell wall, which does not allow the basal bodies to 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 constraint sets an upper limit of 5 for the number of times a cell can divide while still maintaining an active flagellum and thus becomes critical at about the 32 cell stage.

The ‘enlargement constraint’ stems from the way in which volvocine algae reproduce (discussed above) (Solari 2005). Because postembryonic cell divisions are not possible (although the young colonies do increase in size after their release from the mother colony through increase in cell size and volume of extracellular matrix), the embryo must contain all the cells present in the adult. Consequently, the larger the colony, the larger the embryo that must develop and be supported by the mother colony. This cost of reproduction is especially acute in species in which cells do not double in size and then undergo binary fission, but rather each reproductive cell grows about  $N = 2^d$  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’ and occurs in many volvoclean algae, including *Chlamydomonas*, *Gonium* and *Eudorina*, *Pleodorina* and in all the members of the Meriliosphaera *Volvox* group (e.g., *V. carteri*), and is thought to have predisposed these algae to multicellularity (Kirk 1998). For these species, the cost of reproduction ( $e_N$  in Figure 1) is directly related to the group size  $N$  which the reproductive cell must produce, and thus to the cell size the reproductive cell must attain before initiating the rapid series of embryonic divisions. This investment in reproductive cell growth is an example of a cost that must be paid for a cell to produce a daughter colony. Somatic cells need not undertake this investment, and all their energy and time may be spent at flagellar activity which enhances motility and feeding (Solari et al. 2006a; Solari et al. 2006b). Besides resource issues, a larger embryo also increases the volume, mass and drag of the mother colony, which requires more swimming force per embryo; this cost underlies the increase in the somatic/reproductive (S/R) cell ratio in completely differentiated *Volvox* species as colony size increases (Solari et al. 2006a; Solari et al. 2006b).

Our work shows that the evolution of soma (and the increase in the S/R ratio just discussed) provides the benefits that compensate for the cost of reproduction in increasingly larger colonies (Solari 2005; Solari et al. 2006b). Such benefits include: (i) colony motility while reproductive (overcoming the flagellation constraint), (ii) motility while large (overcoming the enlargement

constraint), (iii) increased resource uptake due to the ‘source-sink’ effect (in which somatic cells transfer resources to germ cells which act as a sink) (Bell 1985; Koufopanou and Bell 1993; Solari et al. submitted 2006), and (iv) enhanced uptake of resources and removal of waste by flagellar beating (Niklas 1994, 2000; Solari et al. submitted 2006).

Once G-S separation is complete, germ specialization can also provide additional benefits, such as decreased generation time, increased productivity by specialization at photosynthesis, and organizational advantages stemming from the location of germ cells in the interior of the colony which lowers drag (Solari et al. 2006a). There are also direct costs of germ and soma specialization which must be overcome by the identified benefits: germ reduces the number of cells available for vegetative functions, and soma reduces the number of reproducing cells.

Single gene mutations in life history traits can be a powerful approach to understanding the cost of reproduction and trade-offs between life history traits, both long standing topics of considerable interest in evolutionary ecology (Reznick 1985; Roff 2000, 2002). We have been studying *V. carteri* mutants that differ in the basic factors hypothesized in our models (Michod 1999; Michod et al. 2003; Solari et al. 2003; Solari et al. 2006a) for the origin of multicellularity: group size, *S/R* ratio, type and timing of germ–soma specialization, motility, and development; yet they differ in just one or a few genes. These mutants include *lag*<sup>-</sup> (gonidia perform motility functions before reproducing; these mutant colonies are similar to *Volvox* species such as *V. aureus* and *V. rousselletti*), *regA*<sup>-</sup> (once-cooperative somatic cells become selfish by regenerating into reproductive cells), and *glsA*<sup>-</sup>/*regA*<sup>-</sup> (all cells perform vegetative functions first and then become reproductive; this mutant is similar to *Eudorina*) (Kirk 1998).

These mutants are especially useful for studying fitness decomposition at the cell and group levels, because a certain known number of cells have changed their reproductive effort. We can measure the consequences of this change at the colony level, and in this way estimate the contribution to the group fitness of the changed effort at the cell level as is required by our model in Figure 1. For example, in the *regA*<sup>-</sup> mutants ~235 cells have changed their phenotype from somatic (S) to unspecialized (GS); in *lag*<sup>-</sup> ~9 cells have changed their phenotype from germ (G) to GS; and in *glsA*<sup>-</sup> *regA*<sup>-</sup> there are ~561 GS cells, similar to a *Eudorina* colony but larger. As a result of these changes in reproductive effort at the cell level, the size, productivity and motility of the group have changed (Solari et al. 2006a; Solari et al. 2006b). These data show that as once-specialized S cells ( $b=0$  in Figure 1) begin exerting reproductive effort ( $b>0$ ) there is not only a large decrease in colony motility, there is also a large decrease in the motility contributed by a single changed cell. For example, the average force exerted for group motility for a single motile cell is about half in the *regA*<sup>-</sup> mutant and a quarter in the *glsA*<sup>-</sup> *regA*<sup>-</sup> mutant of what it is in wild type (Solari et al. 2006a). The cost of reproduction which underlies the convex nature of the fitness trade-offs (Figure 1) is real and directly

measurable in these organisms and attributable to a change in the effort exerted by single cells within the cell group.

In applying our theory to the development of *V. carteri*, we (Michod et al. 2003) have attempted to understand the issue of cell division (the example Okasha (2006) discusses as initially contributing to MLS1) and how it becomes decoupled from MLS1 in favor of MLS2. We take MLS2 fitness to be proportional to the product of group size and functionality (the latter depending on the frequency of cooperative cells in the group) and try to understand the evolution of G-S specialization in terms of the conflict mediation framework. To apply our theory, we must represent the cost of the germ line to the group, because when  $v$  cells are put aside to make the germ line, these cells are no longer available for somatic-like functions (Michod et al. 2003, see especially pp. 98–100). We take two approaches to this cost which predict different outcomes for the evolution of G-S specialization (outcomes given in Figures 3 and 4 of Michod et al. 2003). We think of the  $v$  germ-line sequestered cells in terms of two descendent populations of cells according to the conditions either of their new existence as differentiated germs or their prior existence as members of an undifferentiated cell group. In the case of their prior existence, the  $v$  cells would have given rise to a descendent population of size, say,  $k_v$  cells undifferentiated with respect to reproductive or vegetative functions. In the case of their new existence as differentiated germs, the germ sample (the  $v$  cells) replicates for perhaps a different period of time with a different mutation rate giving rise to a descendent population of size, say,  $K_v$  germ cells. The cost of the germ-line can be seen as stemming from either the new  $K_v$  germ cells or the missing  $k_v$  cells unavailable for vegetative (somatic) functions.

We can think of these two representations as involving MLS1 and MLS2, respectively. The missing cells approach belongs in the realm of MLS1 (since the changed size of the group is directly affecting fitness) and the new germ cells approach to the cost belongs in the realm of MLS2 (since the changed size of the group is not affecting fitness). The MLS1 approach predicts an intermediate optimum time to sequester the germ line, while the latter MLS2 predicts sequestration of the germ line as early as possible. This interpretation of our model's prediction is consistent with the view that organisms with early germ line sequestration (*Drosophila*, *C. elegans*, etc.) are fully emerged individuals. The fact that *V. carteri* behaves according to the MLS1 approach suggests the view that *V. carteri* is not yet a fully emerged multicellular individual. This same conclusion has been argued for by Niklas on the basis of the lack of intracellular bridges in the adult form of *V. carteri* (Niklas 1994, 2000).

The deconstruction and reorganization of fitness has never been accomplished in any system for any evolutionary transition, yet this is how new evolutionary units must emerge. Volvocine algae are a good model system for this work; they span significant transitions and can be approached theoretically, experimentally and in the field. The general goal of our research with this group is to understand fitness, fitness trade-offs, and fitness reorganization as colonies increase in size and complexity (Table 1). Many factors affect fitness,

however, the efficient exchange of nutrients, metabolites and waste are among the most basic. Understanding the effect of resource delivery and exchange on metabolic rate as organisms increase in size is leading to rapid advances across disparate fields of the life sciences, from comparative biology to ecosystem ecology (West et al. 1997, 1999; Enquist and Niklas 2001; Niklas and Enquist 2001). However, this work has not been addressed in the context of the ETIs which underlie the hierarchy of life. During ETIs, as lower-level units (cells) associate to form groups, the constraints of surface to volume ratio and of spatial organization adversely affect the type and extent of interactions with the environment, with profound effects on metabolism, growth rate, viability and fecundity.

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