On the Reorganization of Fitness During Evolutionary Transitions in Individuality[1]

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SYNOPSIS.   The basic problem in an evolutionary transition is to understand how a group of individuals becomes a new kind of individual, possessing the property of *heritable variation in fitness* at the new level of organization. During an evolutionary transition, for example, from single cells to multicellular organisms, the new higher-level evolutionary unit (multicellular organism) gains its emergent properties by virtue of the *interactions* among lower-level units (cells). We see the formation of cooperative interactions among lower-level units as a necessary step in evolutionary transitions; only *cooperation* transfers fitness from lower levels (costs to group members) to higher levels (benefits to the group). As cooperation creates new levels of fitness, it creates the opportunity for *conflict* between levels as deleterious mutants arise and spread within the group. Fundamental to the emergence of a new higher-level unit is the *mediation of conflict* among lower-level units in favor of the higher-level unit. The acquisition of heritable variation in fitness at the new level, via conflict mediation, requires the reorganization of the basic components of fitness (survival and reproduction) and life-properties (such as immortality and totipotency) as well as the co-option of lower-level processes for new functions at the higher level. The way in which the conflicts associated with the transition in individuality have been mediated, and fitness and general life-traits have been re-organized, can influence the potential for further evolution (*i.e.,* evolvability) of the newly emerged evolutionary individual. We use the volvocalean green algal group as a model-system to understand evolutionary transitions in individuality and to apply and test the theoretical principles presented above. Lastly, we discuss how the different notions of individuality stem from the basic properties of fitness in a multilevel selection context.

INTRODUCTION

The word "individual" comes from the Latin word *individuus* meaning "not divisible." Individuals are wholes and cannot be divided into smaller parts that maintain critical properties of the whole. In philosophy, individuals are defined as entities that are distinct in space and time. In biology, there are several different senses of individuality based on genetic homogeneity, genetic uniqueness, or physiological autonomy and unity (Santelices, 1999). There are merits and shortcomings with each of these concepts of individuality. Clonally reproducing organisms (such as bacteria, many protists and fungi, and some animals and plants), organisms with high levels of within organism change (such as long lived plants) and highly social organisms (such as wasps and insects) pose counter examples to the biological concepts of genetic uniqueness, genetic homogeneity, and physiological autonomy, respectively, as necessary defining characteristics of individuality. These organisms seem to be individuals, yet they fail to satisfy one of the criteria. An evolutionary perspective may help resolve some of these difficulties, after all, biological individuals must be units of selection and so possess the properties discovered by Darwin of heritable variation in fitness.

Here, we embrace Darwin's principles in addressing the concept of individuality from an evolutionary perspective, but, do so in a multi-level selection context. The multilevel selection approach to evolutionary transitions seeks to understand how a group of pre-existing individuals may become a new evolutionary individual, possessing heritable fitness variation at the group level. According to a theory we have developed, the transition to a new higher-level is driven by the *interactions* among lower-level units, for example, in the case of the unicellular-multicellular transition, the benefits to the group of cooperation among cells and the benefits of a larger group-size may push cells to form colonies and evolve into multicellular organisms (Michod, 1996, 1997, 1999; Michod and Roze, 1997, 1999, 2000). Cooperation is fundamental to the emergence of higher level units as discussed in a recent Dahlem Conference (Michod, 2003; Hammerstein, 2003; Lachmann *et al.*, 2003). The flip side of *cooperation* is defection and selfishness leading to *conflicts* among lower-level units; such conflicts must be mediated for the new higher-level unit (the multicellular group) to become a true individual and possess *heritable variation in fitness* at the new level (Michod, 1999). *Conflict mediation* is, thus, instrumental to the emergence and stability of individuality at the higher level. The acquisition of heritable variation in fitness at the new level through conflict mediation requires the *re-organization of fitness components* (survival and reproduction) and *general life-traits* (such as totipotency and immortality) as well as the *co-option of lower-level processes for new functions at the higher level* (Nedelcu and Michod, 2003). The way in which the conflicts associated with the transition in individuality have been mediated, and the basic life-traits have been re-organized, can influence the potential for further evolution (*i.e., evolvability*) of the newly emerged individual (Michod *et al.*, 2003; Nedelcu and Michod, 2003).

In our previous work we have focused on the origin of multicellularity, however, the principles involved are applicable to the other evolutionary transitions in individuality, for example, the transition from single replicating genes to groups of cooperating genes (Michod, 1983, 1999), the endosymbiotic origin of the eukaryotic cell (Michod and Nedelcu, 2003), and the transition from solitary organisms to social groups (Michod and Abugov, 1980; Michod, 1982, 1999; Michod and Sanderson, 1985). Here, we review these principles and present applications of these principles to the unicellular-multicellular transition in the green algal group, Volvocales, as a study-case. Lastly, we discuss how the different notions of individuality stem from the basic properties of fitness in a multilevel selection context.

MULTI-LEVEL SELECTION AND INDIVIDUALITY

The basic problem in an evolutionary transition is to understand how a group of individuals becomes a new kind of individual, possessing the properties of *heritable variation in fitness* at a new level of organization. During evolutionary transitions, preexisting individuals associate in groups, within which interactions occur that affect the fitnesses of both the individuals and the group. For example, under certain conditions bacteria associate to form a fruiting body, amoebae associate to form a slug, solitary cells form a colonial group, normally solitary wasps breed cooperatively, birds associate to form a colony, and some animals form societies. In addition, about 2 billion years ago, archaebacteria-like cells began alliances with other bacteria to form the first eukaryotic-like cell. Such associations may persist and reform with varying likelihood depending on properties of the group and component individuals.

A multi-level selection approach to evolutionary transitions begins by partitioning the total change in frequency of phenotypes of lower-level units (and their underlying genes) into within and between-group components. Groups are defined by a group property, usually the group frequency of a phenotype or genotype (or some other property reflecting group composition). Groups are quasi-independent of each other in the sense that they are "the smallest collection of individuals within a population defined such that genotypic fitness calculated within each group is not a (frequency-dependent) function of the composition of any other group" (Uyenoyama and Feldman, 1984).

During evolutionary transitions, the new higher-level evolutionary units (*e.g.,* multicellular organisms, eukaryotic cells) gain their emergent properties by virtue of the *interactions* among lower-level units (*e.g.,* cells). We see the formation of *cooperative interactions* among lower-level units as a necessary step in evolutionary transitions, even if the groups initially form via antagonistic interactions, as may have been the case during the origin of the eukaryotic cell (*e.g.,* (Maynard Smith and Szathmáry, 1995; Michod and Nedelcu, 2003).

The benefits of cooperative interactions usually depend upon the frequency with which they occur in the group or population, while the costs of performing a cooperative behavior will usually be an inherent property of the behavior itself, not depending on trait frequency. To the extent that cooperators are frequent in the population, it may pay a particular individual to forgo providing benefits, thereby reaping the benefits bestowed by others, while not paying the cost. For these reasons there is always a "temptation" to defect, that is, not help others, and so gain an advantage within the group or population relative to cooperators.

Because there are two levels of selection, the cell and the cell group, there is the opportunity for both *within* and *between-group* selection. Fitness at the cell level involves two main components, the replication and death rate of the cell, which in turn depend upon cell behavior. We consider two kinds of cell behavior, cooperation and defection. We have studied both synergistic and costly forms of cooperation; synergistic forms of cooperation benefit both the cell and the group (cheating is not possible), while costly forms of cooperation benefit the group at some cost to the replication rate and/or survival of the cell. If cooperation is costly to cell replication, the evolution of cooperation within cell groups may be seen as the evolution of soma. Deleterious mutation may occur during cell division leading to the loss of cooperative cell functions and a decrease in fitness of the adult group. Uniformly deleterious mutations are assumed to be disadvantageous at both cell and group levels, while selfish mutations are assumed to be advantageous for cells and disadvantageous for groups.

As cooperation creates new levels of fitness, it creates the opportunity for *conflict* between levels as, for example, deleterious mutants arise and spread within the group. Fundamental to the emergence of a new higher-level unit is the *mediation of conflict* among lower-level units in favor of the higher-level unit resulting in enhanced cooperation among the lower-level units, until, eventually, the group becomes a new evolutionary individual in the sense of possessing heritable variation in fitness (at the new level of organization) and being protected from the ravages of within-group change by adaptations that restrict conflict and the opportunity for defection (Michod, 1999). Of course, no evolutionary individual ever rids itself from the threat of change within, as evidenced by the numerous examples of evolutionary conflict (Hurst, 1990, 1992; Hurst *et al.*, 1996; Partridge and Hurst, 1998).

We have previously focused on the role of conflict mediation and the re-organization of fitness and basic-life traits during the unicellular-multicellular transition (Michod, 1999; Michod *et al.*, 2003; Nedelcu and Michod, 2003; Michod and Nedelcu, 2003); below, we present the general framework that we have been using in understanding this transition, and in the next section we present applications of these principles to the transition in individuality during the evolution of multi-

cellularity in the green algal group, Volvocales. We are also in the process of developing a multi-level selection framework for understanding the prokaryotic-eukaryotic transition (for preliminary applications see Michod and Nedelcu, 2003). Our framework for the prokaryotic-eukaryotic transition is based on understanding the selective and population processes acting during initiation, establishment and integration of the initial association so as to understand the emergence of a new unit of evolution with heritable variation in fitness (unpublished work). We argue that conflict and conflict mediation have also been instrumental to the emergence of individuality during the prokaryotic-eukaryotic transition (Michod and Nedelcu, 2003).

CONFLICT MEDIATION

We think that conflict and conflict mediation are fundamental to the emergence of individuality at a higher level, irrespective of the type of ecological interaction (mutualism, competition or exploitation) associated with the initial formation of the group (Michod and Nedelcu, 2003). Both antagonistic (as may have been the case during the origin of the eukaryotic cell) and mutualistic interactions involve conflict; the former, because of their very nature, and the latter because all cooperative types of interaction create the opportunity for the spread of defection. As discussed below, adaptations that restrict the opportunity for conflict between higher and lower-levels (what we term "conflict modifiers") are instrumental in the conversion of the group to a new evolutionary individual.

In the transition between interacting genes and the first cell, an example of a conflict mediator is the chromosome because it aligns the interests and evolutionary prospects of linked genes with each other. In the case of the cell associations that pre-dated the eukaryotic cell, conflict mediation may have involved the amelioration of the initially exploitative interactions such as predation or parasitism (Michod and Nedelcu, 2003). Ultimately, uniparental inheritance is thought to mediate conflict among the genetic interests of organelles coming from different cells (Hastings, 1992). In the case of multicellular groups, conflict mediation may involve the spread of conflict modifiers producing self-policing, maternal control of cell fate, decreased propagule size, determinate growth of the organism, apoptotic responses, or germ line sequestration discussed below (Michod, 1999, 2003).

To study how evolution may shape development and the opportunity for selection at the two levels we assumed a second modifier locus that affects the parameters of development and/or selection at the primary *cooperate/defect* locus. These conflict mediators are the first emergent functions that serve to turn the group into a new higher-level individual. Conflict mediators are assumed to be determined by a genetic modifier locus that may affect virtually any aspect of the model, such as propagule size (Michod and Roze, 1999, 2000; Roze and Michod, 2001), and adult size (whether it is determinate or indeterminate, Michod and Li, unpub-

lished results). To study the evolution of self policing, we assumed the modifier affects the parameters of selection at both levels, reducing the temptation to defect at some cost to the group (Michod, 1996; Michod and Roze, 1999). In the case of the evolution of programmed cell death, we assume the modifier directly decreases the replication rate of mutant cells (Michod and Nedelcu, 2003). In the case of the evolution of germ/soma differentiation, we have considered a two step process (Michod, 1996, 1997; Michod and Roze, 1999). The first step is the evolution of costly forms of cooperation, interpreted as the evolution of somatic-like functions, in which the cooperative somatic-like cells replicate more slowly (and so cost the group in terms of fecundity) but benefit the survival of the group. The evolution of the modifier allele takes these cooperative groups and converts them into groups with two cell lineages: germ cells which beget the next generation and somatic cells which benefit the group but do not contribute genes to the next generation. Initially, we assumed in our models that the germ-line developed from a single cell that was sequestered very early (after the first cell division) during the formation of the group. Using similar two-locus population genetics techniques, we have relaxed this assumption, and have studied the effect of the time of separation of the germ and soma (early *versus* late in development) and number of cells that are sequestered (Michod *et al.*, 2003).

RE-ORGANIZATION OF FITNESS AND LIFE-TRAITS

The acquisition of heritable variation in fitness at the new level, via conflict mediation, requires the reorganization of the basic components of fitness (survival and reproduction) and life-properties (such as immortality and totipotency) as well as the co-option of lower-level processes for new functions at the higher level. Furthermore, the way in which the conflicts associated with the transition in individuality have been mediated, and fitness and general life-traits have been re-organized, can influence the potential for further evolution (*i.e.,* evolvability) of the newly emerged evolutionary individual as diagrammed in Figure 1 (Michod *et al.*, 2003; Nedelcu and Michod, 2003).

Re-organizing fitness during the unicellular-multicellular transition

Group fitness is, initially, taken to be the average of the lower-level individual fitnesses; but as the evolutionary transition proceeds, group fitness becomes decoupled from the fitness of its lower-level components. Indeed, the essence of an evolutionary transition in individuality is that the lower-level individuals must "relinquish" their "claim" to individual fitness in favor of the survival and reproduction of the new higher-level unit. The lower-level units still survive and may multiply, but in so doing they contribute to the fitness of the new higher-level unit. This transfer and reorganization of fitness components from lower to higher-levels occurs through the evolution of cooperation and
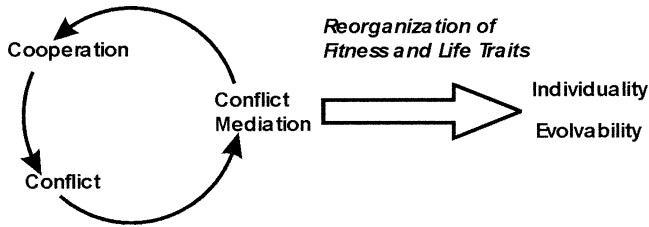
FIG. 1. Conflict and conflict mediation in evolutionary transitions. Stability of the group, and ultimately the emergence of individuality at a higher level, requires the mediation of conflict. Conflict may arise directly in response to cooperation as defection spreads within the group. Conflict mediation through the re-organization of fitness components (survival and reproduction) and general life-traits (immortality and totipotency) ads to further increases in cooperation and individuality at the group level. A successful mediation reflects in the continued evolvability of the new higher-level unit, which is fueled by new modes of cooperation and new ways to mediate conflict among component entities leading to new adaptations at the higher-level.

mediators of conflict that restrict the opportunity for within-group change and enhance the opportunity for between-group change.

It is thought that the unicellular-multicellular transition has been triggered by the survival benefits of larger size, such as escape from predators or improved homeostasis within the group (Stanley, 1973; Bell, 1985; Shikano *et al.*, 1990; Gillott *et al.*, 1993; Boraas *et al.*, 1998). In spite of these obvious benefits, a large size can be costly to fitness, in terms of reproduction, increased generation time, increased occurrence of mutations within the group, and increased need for resources. As size increases, the trade-off between the benefits and the costs of an increasingly larger size reaches a point at which overall fitness is negatively affected and size cannot increase further. In the volvocalean green algae, studied below, this point is reached rather early, by the 32-cell stage (because, as discussed below, during the reproductive phase, flagellar motility—vital to the survival of these algae, can be sustained only for the time required to form a 32-cell colony). There are two ways to overcome this impasse: by increasing the direct benefits of multicellularity and larger size (*e.g.*, via specialization and the realization of additional benefits of larger size), and/or by decreasing the costs of reproducing larger groups (so that the survival benefits have an increased relative contribution to the overall fitness).

We have modeled the evolution of germ and soma in colonial cell groups (Solari *et al.*, 2003) and think that germ/soma separation in multicellular organisms accomplishes both; germ/soma specialization (i) reduces the cost of reproduction to survival and (ii) allows for further increases in the survival component of group fitness. Here, we use the terms "germ" and "soma" to indicate specialization for reproductive and somatic functions, respectively, regardless of the time in development when the germ differentiates (for example, early—in many animals, and late—in plants). Initially, because all cells are performing both survival

and reproduction functions, the two fitness components are coupled at the cell level. However, by suppressing the reproductive component in some cells (*i.e.*, the evolution of soma), these cells perform only survival-related functions and thus may increase the survival of the higher-level unit during the reproductive phase. In addition to the benefits of decreasing the cost of reproduction to survival, the re-organization of fitness components between soma and germ allows for an improved and more independent optimization of the two components.

Such de-coupling of the two fitness components are necessary for continued evolution, because, although the evolution of soma decreases the costs of larger size to survival, the sterile soma creates a new cost in terms of the other fitness component, reproduction (due to the decrease in the number of cells that participate directly in reproduction and thus in producing offspring). In other words, the trade-off between the benefits and costs of a large size initially resolved in favor of the survival benefits created a cost in fecundity. This new fecundity cost may be ameliorated by the continued improvement of the functionality of the soma.

The release of the soma from direct participation in the reproduction of the group allowed the specialization of somatic cells for new survival-related functions (Solari *et al.*, 2003). Consequently, survival becomes not anymore a strict function of size (*i.e.*, the benefit of large size is not the only benefit associated with multicellularity) but rather of the capacity and efficiency in using (new) resources and environments (through the evolution of various cell types). Soma thus can counteract the loss in fecundity by increasing the survival component of fitness. Furthermore, soma can contribute to the reproductive component of fitness by decreasing the generation time and increasing the quality of offspring.

Because survival and reproduction-related functions can be realized simultaneously and the two fitness components are now quasi-independent, the trade-off between survival and reproduction in lineages with a germ/soma separation gained new dimensions; these are reflected in differences in life-history strategies among lineages, with respect to generation time, number and quality of offspring, the extent of care for the offspring, amount of resources put in survival *vs.* reproduction, etc. Furthermore, the different developmental pathways of germ/soma separation among lineages might reflect different strategies in overcoming specific trade-offs between the costs and the benefits of multicellularity in different lineages. For instance, a germ-line that is sequestered early in development (*e.g.,* in many animals as well as the green alga *Volvox carteri*) might reflect an increased mutation rate in these lineages and thus the need to reduce the cost of these mutations, especially when mutations are "selfish" and threaten the individuality of the higher level; in these cases, in addition to the direct survival benefits associated with the evolution of soma, the germ-line acts a "conflict mediator."
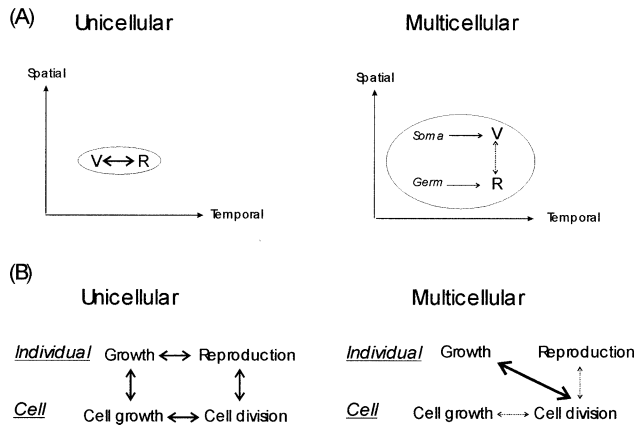
FIG. 2.   Relationships between vegetative (V) and reproductive (R) functions and spatial and temporal contexts (panel A), in unicellular *versus* multicellular individuals, and between processes and properties at the level of the cell and the individual, respectively (panel B); broken arrows denote relationships in which the two components are not necessarily dependent on one another.
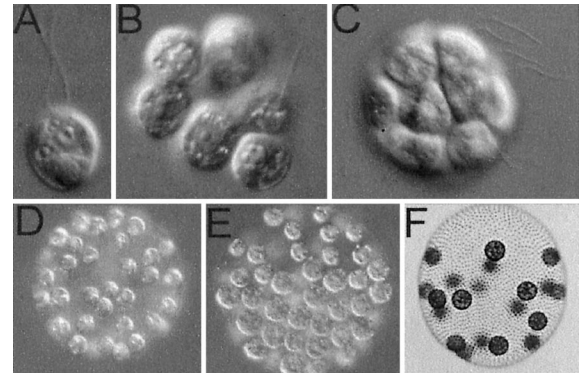

FIG. 3.   The volvocine lineage. A subset of colonial volvocalean green algae that show a progressive increase in cell number, volume of extracellular matrix per cell, division of labor between somatic and reproductive cells, and proportion of vegetative cells. A: *Chlamydomonas reinhardtii*; B: *Gonium pectorale*; C: *Pandorina morum*; D: *Eudorina elegans*; E: *Pleodorina californica*; F: *Volvox carteri*. Where two cell types are present, the smaller cells are the vegetative/somatic cells, whereas the larger cells are the reproductive cells (gonidia). Images kindly provided by David L. Kirk.

The re-organization of fitness, and thus the separation of soma and germ, requires the re-organization of some general life-functions and life-traits as well as certain cellular processes. Below we summarize the functions, traits and processes that become re-organized during the evolution of multicellularity and germ/soma separation. In the next section, we exemplify these ideas using the volvocalean green algal group as a study-case.

RE-ORGANIZING GENERAL LIFE-TRAITS

Decoupling vegetative from reproductive functions

Any biological entity features two main sets of functions, vegetative and reproductive, corresponding to the two basic components of fitness, survival and reproduction. These basic biological functions are coupled at the level of the individual, as a functional/physiological unit. However, the two sets of functions are realized differently between a unicellular and a multicellular individual (Fig. 2). In unicellular forms, the same cell is responsible for both vegetative and reproductive activities (*i.e.,* they are coupled at the cell level), but these functions do not take place simultaneously (*i.e.,* they are typically dissociated in time). In multicellular individuals with germ/soma separation, the two sets of functions are un-coupled at the cell level; some cells specialize in vegetative functions, whereas other cells are specialized for reproductive functions. Consequently, the expression of vegetative and reproductive functions changes from a temporal to a spatial context and the two sets of functions can take place simultaneously (*i.e.,* they need not to be separated in time anymore) (Fig. 2A). As discussed above, one major implication of this re-organization is that it allows for increased overall fitness at the higher level and for the two components of fitness, survival and reproduction, to be realized simultaneously and each optimized more independently.

Re-organizing immortality and totipotency

During the transition to multicellularity, and the emergence of individuality at the higher level, immortality (*i.e.,* the capacity to divide indefinitely) and totipotency (*i.e.,* the ability of a cell, such as zygote or spore, to create a new individual) become restricted to one or a few specific cell lineages, namely those involved in the reproduction of the higher level. This is rather important because it results in the separation of the immortal and totipotent germ from the mortal and differentiated soma, and thus satisfies one of the premises for the emergence of individuality at a new level, namely the indivisibility of the group.

De-coupling cell division from cell reproduction

In unicellular forms, every cell division results in the reproduction of the individual (cell division is strictly coupled with reproduction). In multicellular individuals, however, the division of most cells is uncoupled from the reproduction of the individual (*i.e.,* cell divisions do not necessarily result in the reproduction of either the higher or the lower level units); and in some cells, cell division was co-opted for the reproduction of the higher level (the group). The ability to reproduce the group can be achieved either by all or only some members of the group.

The case in which all cells can reproduce the higher-level is best exemplified by a reproductive mode called autocolony, in which when the group/colony enters the reproductive phase, each cell within the colony produces a new colony similar to the one to which it belongs; cell division no longer produces unicellular individuals but multicellular groups. This mode of reproduction characterizes some lineages in our study-case discussed later, the volvocalean green algal group, namely those without a germ/soma separation (Fig. 3B, C and D). Although the stability, heritability and

the reproduction of the higher level are ensured in this way, its individuality is not; because every member can be separated from the group, live independently and create a new group, such a group is not the smallest physiological and reproductive autonomous unit, thus it is not a true individual (in the sense used here).

The case in which only some cells have higher-level reproductive capabilities characterizes lineages with a separation between germ and soma. To achieve this, the coupling between cell division and reproduction is broken in most cells, namely the somatic cells; they reproduce neither themselves (as former free-living unicellular individuals) nor the higher-level unit; cell division is de-coupled from the reproduction of both the lower and higher levels. In this way, somatic cells loose their individuality as well as the right to participate in the next generation; but in doing so they contribute not only to the emergence of individuality at the higher level but also to the emergence of a new level of organization, the multicellular soma. Soma is thus the expected consequence of uncoupling cell division from reproduction in order to achieve individuality at the higher level.

Co-opting cell division for growth at the higher level

By de-coupling cell division from reproduction, cell division became available for new functions. We have suggested that this event was paralleled by the co-optation of cell division for a new function at the higher level, namely the growth of the multicellular individual (Nedelcu and Michod, 2003). Later, the use of cell division for more than cell multiplication, (i.e., which "gives rise to more entities of the same kind"; (Szathmáry and Maynard Smith, 1997) may have provided the multicellular lineages with an additional advantage, namely cell differentiation; indeed, in many multicellular lineages cell differentiation is associated with asymmetric cell divisions.

De-coupling cell division from cell growth and immortality

In unicellular individuals, cell division is strictly dependent on cell growth (cells do not divide unless a specific set size is achieved). In many multicellular forms, however, this is not always the case: factors other than cell size (such as intercellular or systemic signals) can trigger cell division. In addition, in unicellular forms cell division is strictly coupled with immortality, whereas in multicellular individuals, cell division has a limited and variable potential in most cell lineages (i.e., they are mortal), and is under the control of the higher-level individual.

INDIVIDUALITY IN THE VOLVOCALEAN GREEN ALGAL GROUP

Overview

We use the volvocalean green algal group as a model-system to understand evolutionary transitions in individuality and to apply and test some of the theoretical principles discussed above. The volvocalean green algae comprise both unicellular (*Chlamydomonas*-like) algae as well as colonial forms in different stages of organizational and developmental complexity (Fig. 3). All volvocalean algae share the so-called "flagellation constraint" (Koufopanou, 1994) (which has a different structural basis than the one invoked in the origin of metazoans; Margulis, 1981); as a consequence, cell division and motility can take place simultaneously only for as long as flagella can beat without having the basal bodies attached (i.e., only up to five cell divisions). Volvocalean algae also share a particular type of cell division, referred to as multiple fission and palintomy (cells do not double in size and then undergo binary fission, rather, each cell grows about 2^n-fold in volume, and then a rapid, synchronous series of n divisions under the mother cell wall). Because clusters, rather than individual cells, are produced in this way, this type of division is suggested to have been an important precondition facilitating the formation of volvocacean colonies (Kirk, 1998).

Two selective pressures are thought to have contributed to the increase in complexity in all volvocalean lineages; these are: (i) the advantages of a large size (potentially to escape predators, achieve faster motility, homeostasis, or better exploit eutrophic conditions) and (ii) the need for motility (e.g., to access to the euphotic/photosynthetic zone) (Bell, 1985). Interestingly, given the background offered by the volvocalean type of organization presented above, namely the flagellar constraint and the multiple fission type of cell division, it is difficult to achieve the two selective advantages simultaneously. As the colonies increase in size and number of cells, also does the number of cell divisions (up to 15–16 in some *Volvox* species); consequently, the motility of the colony during the reproductive phase is negatively impacted for longer periods of time than are acceptable in terms of the need to access the euphotic zone. In other words, the cost of reproduction to survival increases with increase in size, and the overall benefits of large size are counteracted by the increased cost of reproduction. This cost can be reduced by cellular specialization/division of labor: some cells specialize in survival-related functions (i.e., motility), while the rest of the cells become specialized for reproduction. In *Volvox*, the division of labor is complete: the motile (somatic) cells are sterile, terminally differentiated, and are thought to be genetically programmed to undergo cellular senescence and death once the progeny was released from the parental colony (Pommerville and Kochert, 1981); only the reproductive cells (the gonidia) undergo cleavage to form new colonies (Pommerville and Kochert, 1982). In some *Volvox* species, including *Volvox carteri*, the two types of cells are set apart by asymmetric divisions early in the embryonic development.

Conflict mediation and the evolution of an early segregated germ-line

Many multicellular organisms have a germ that is segregated early in the development (for a list see Ta-

ble I.1 in Buss, 1987). Sometimes the term ''germ'' is used only for these early segregating types. However, in the present paper we have used the term regardless of the time in development the germ cells are separated, and used ''germ line'' when the germ develops from a cell-line that is sequestered in the development and has a distinct developmental history relative to the somatic cell-lines. It has been suggested that one of the advantages of having an early segregating germ line comes from the reduction in the mutation level either by lowering the number of cell divisions (Buss, 1987; Michod, 1996) or by lowering the mutation rate per cell division (Michod, 1996, 1999; Michod and Roze, 2000). Our previous analysis of these hypotheses using a two-locus modifier model indicates that the germ-line acts as a conflict mediator. Recently, we have applied these models to the evolution of an early-segregated germ-line in the multicellular green alga *V. carteri* (Michod *et al.*, 2003).

The model predicts that the germ line evolves easier when mutation is a threat, either because mutations are selfish or frequent (because of a high mutation rate or a high number of cell divisions). When mutations are frequent, cooperation is harder to maintain, and so the within-group advantage for the mutant cells has to be lower for groups to be maintained at all. Under a lower mutation rate, a germ line can evolve only when the within-group advantage of mutant cells is rather high. These results are generally consistent with the fact that in *V. carteri* many mutations that affect the somatic cells are selfish. For instance, in the somatic regenerator mutants, or Reg mutants, the somatic cells start out as small flagellated cells (wild type-like) and then enlarge, loose flagella and re-differentiate into gonidia. In conditions where motility and access to light are strong selective pressures, the higher-level is negatively affected by the occurrence of these ''selfish'' mutant cells. To argue for the negative effects at the higher-level of these types of selfish mutations in the environments where wild-type forms of *V. carteri* are usually present is the fact that neither of these mutant forms are found as established populations in nature, although the Reg mutants occur spontaneously at a rather high rate (Kurn *et al.*, 1978). Interestingly, however, when access to light and the need for motility are not strong selective pressures (*i.e.,* in lab settings or possibly shallow waters), the fitness of these mutant forms is higher than the fitness of the wild-type (Koufopanou and Bell, 1991).

Concerning the higher mutation rate predicted by our model it is worth noting that volvocalean green algae seem to feature levels of nucleotide substitution (as suggested by the differences in branch length observed in phylogenetic trees based on nuclear rRNA sequences [*e.g.,* Friedl, 1997; Nakayama *et al.*, 1998]) that are higher than those in other green algae as well as in their close relatives, the land plants, which, incidentally, do not have an early-defined germ line.

Re-organization of fitness components

The evolution of germ/soma separation requires the re-organization of the basic components of fitness (survival and reproduction) at the two levels of selection (cell and cell group). As we mentioned above, in volvocalean green algae, motility and thus survival are negatively affected during the reproductive phase; in other words, reproduction has a direct cost on survival. Due to the flagellation constraint, the cost of reproduction (in terms of the amount of time spent in an immotile stage) increases with increasing colony size. At some point, the time spent in an immotile phase becomes too costly for survival. We suggest that germ/soma separation in this group of algae evolved as means to decrease the direct cost of reproduction to survival, under the selective pressures to both achieve a larger size but remain motile throughout the life cycle.

Interestingly, the same selective pressure (*i.e.,* large size) affects fitness via both fitness components, but in opposite ways (a large size increases survival during the vegetative phase, but it has a direct negative effect on survival during the reproductive phase); therefore, an overall increase in fitness at the higher cell-group level is difficult to achieve. The level of direct interference between the two components of fitness—survival and reproduction, can be reduced by either de-coupling fitness from flagellar motility (as may have been the case in other green algae in which flagellar motility has been lost) or by de-coupling the two components of fitness at the lower level. The latter can be accomplished by having some cells ''relinquish'' their direct contribution to reproduction of the higher level and perform survival-related functions such as motility throughout the entire life-cycle. In this way, the degree of direct interference between survival and reproduction is reduced and the two fitness components can be optimized more independently, possibly resulting in enhanced flexibility and evolvability for the new unit of organization. However, as discussed below, in *V. carteri*, the way in which the interference between survival and reproduction was reduced, and thus germ/soma separation evolved, ended up restricting the evolvability of the group by not allowing for the continued improvement of the survival component of fitness.

Although a germ/soma separation can, on the one hand, decrease the direct cost of reproduction to survival, it is, on the other hand, costly to the overall fitness of the higher level because, all other factors being equal, it must reduce fecundity. In our models, somatic function is represented as cooperation and the cost of somatic function to reproduction is represented in the cost of cooperation. The cost of soma to reproduction may be mitigated by decreasing the generation time and/or enhancing the quality of offspring. Both can be achieved through cell specialization and cooperation: somatic cells specialize in providing the reproductive cells with resources needed for the production of offspring (Bell, 1985). This additional benefit

can be very important in environments where the direct loss in fecundity associated with the evolution of a sterile soma is disadvantageous, and seems to have been achieved by *V. carteri*, in which the generation time is significantly reduced relative to that of other species of *Volvox* (from three to two days).

The paradox of the dual effect of germ/soma separation on overall fitness sets the premise for new trade-offs between various factors affecting the components of fitness (such as generation time, number and quality of offspring, care for offspring); these are reflected in the variety of life-history strategies among multicellular lineages. We have argued elsewhere that the particular way in which germ/soma separation is achieved in *V. carteri* can be explained as a consequence of the need to produce the maximum number of offspring and reach the largest number of somatic cells, under the constraint of palintomy (Michod, *et al.*, 2003).

Reorganization of general life-traits

As discussed earlier, general life-traits and cellular processes become re-organized during the evolution of multicellularity and germ/soma separation; these include: (i) the re-organization of vegetative and reproductive functions, (ii) the re-organization of basic life-traits (such as immortality and totipotency) between and within cell lineages, (iii) de-coupling of processes at the lower level (*e.g.,* cell division from cell growth), (iv) de-coupling of certain cellular processes from general functions and traits (*e.g.,* cell division from reproduction and immortality) and (v) the co-option of lower-level processes for new functions at the higher level (*e.g.,* the co-option of cell division for multicellular growth). Although the two components of fitness became re-organized at the higher level, and an early-segregated germ line and a soma have evolved in *V. carteri*, the latter was achieved in a rather peculiar way in this lineage.

Immortality and *totipotency* are two complex life-traits that become re-organized during the evolution of germ/soma separation. However, these two sets of traits are still very linked in *V. carteri*; they are either both fully expressed (in gonidia) or both completely suppressed (in somatic cells). Although immortality and totipotency have become fully restricted to the germ line (and individuality at the higher level emerged), somatic lineages have no mitotic or differentiation potential; as a consequence, somatic cells have very limited prospects for contributing to the survival of the new multicellular individual.

To ensure the emergence of individuality at the higher level and the reproduction of the new multicellular individual (*i.e.,* the heritability of the group-level traits), in some cells (*i.e.,* the soma), cell division is de-coupled from reproduction of both the lower and higher levels. As a consequence, cell division becomes available for a new function—growth of the multicellular individual. Interestingly, in *V. carteri*, although the coupling between cell division and reproduction has been broken in the somatic cells, cell division was not co-opted for the post-embryonic growth of the higher-level individual; rather, cell division was simply turned-off in somatic cells. Consequently, the survival of the lower level is limited and so is its contribution to potential increase in fitness at the higher level. Furthermore, the way in which cell division is suppressed in the somatic cells is by acting on a trait that is still very linked to it, that is cell growth (Nedelcu and Michod, 2003).

By suppressing cell growth in somatic cells, cell division is repressed and the potential for re-gaining immortality and totipotency is "under control"; in other words, potential conflicts between cell lineages, as to gaining access to the germ line, are avoided. A direct implication is that "soma" in *V. carteri* differs from the soma of other multicellular organisms. Because somatic cells do not divide, the post-embryonic growth and/or regeneration of the individual are not possible. Due to this unique type of soma, *V. carteri* is missing more than the ability to grow, regenerate, or live longer. Without a mitotically active multipotent stem cell lineage or secondary somatic differentiation there is less potential for cell differentiation and further increases in complexity.

We think that somatic growth and differentiation are important for the evolvability of a multicellular lineage (Michod *et al.*, 2003; Nedelcu and Michod, 2003). Without these features, *V. carteri* did not and will likely not attain higher-levels of complexity. We argue that, although potential conflicts are mediated through the early-sequestration of the germ-line, and individuality at the higher-level emerged, the way in which the mediation and the re-organization of fitness and basic life-traits were achieved in the lineage leading to *V. carteri*, might have interfered with the long-term evolvability of the lineage.

INDIVIDUALITY RECONSIDERED

Returning to the various notions of individuality introduced at the beginning of the paper—distinctness in time and space, indivisibility of wholes, genetic homogeneity, genetic uniqueness, and physiological autonomy and unity—we may see how they stem from the processes of multilevel selection and conflict mediation inherent in creation of a new evolutionary individual.

The multilevel selection approach to evolutionary transitions seeks to understand how a group may become an evolutionary individual, possessing heritable fitness variation at the group level. The basic evolutionary notion of a group, for example, as "the smallest collection of individuals within a population defined such that genotypic fitness calculated within each group is not a (frequency-dependent) function of the composition of any other group" (Uyenoyama and Feldman, 1984) is clearly enhanced by distinctness and cohesion in time and space. In addition, such distinctness enhances the between-group variance so essential to group selection and emergence of a higher-level unit. Conflict mediation serves to make the group in-

divisible, for example, a group with specialized germ and somatic cells is no longer divisible, since certain cells, the soma, are not totipotent, that is they can no longer reproduce the group. The restriction of reproductive authority to a specialized group of cells is essential because it removes the temptation to defect from the large somatic population of functional cells. Conflict modifiers also restrict the opportunity for within-group change (thereby making the group more genetically homogeneous within) and enhance the opportunity for between-group change (making each group more distinct and unique. By using a Price equation analysis of multilevel selection, we have shown elsewhere that the modifiers invade the population by virtue of increasing the covariance of fitness at the group level with gene frequency within the founding propagule (Michod and Roze, 1997). The modifiers serve to mediate conflict in favor of the higher-level and in so doing increase both the level of cooperation among cells (thereby making the group more of a physiological unit) and the heritability of fitness at the group level; thereby increasing the evolvability of the new unit of selection (Michod and Roze, 1997).

The physiological and organizational unity of the multicellular group stems from the reorganization of basic life functions during the transition from the single cell to multicellular state in response to the forces of multilevel selection and conflict mediation. As discussed briefly above and in more detail elsewhere (Nedelcu and Michod, 2003), vegetative and reproductive functions once performed by a single cell (these functions usually being separated in time) are distributed spatially among cells in the multicellular group. Totipotency and immortality (continued cell division) are also reorganized and redeployed during an evolutionary transition. The ways in which these basic functions and properties are reorganized in the multicellular group have profound implications for the evolvability of the new individual (Nedelcu and Michod, 2003).

Evolutionary individuals are units of selection and so must satisfy Darwin's conditions of the struggle to survive and reproduce, variation and heritability. In short, individuals must posses heritable variation in fitness. Lewontin said that "evolution by natural selection should explain 'fitness,'" but what is fitness? In a recent book one of us has adopted the following adequacy criteria for understanding fitness (Michod, 1999). To understand "fitness" we must understand its origin during the transition from the nonliving to the living realms and its creation at a new higher-level during evolutionary transitions. Fitness does not necessarily increase in evolution, yet, new levels of fitness can be created. We have tried to understand how this may occur. According to the ideas reviewed here, cooperative interactions trade fitness from lower to higher-levels. This sets the stage for conflict as defection may spread within groups. Evolvability is based on new adaptations at the higher-level and these are frustrated by conflict between fitness effects and selection at the group and the lower cell level. Through the evolution of conflict modifiers, developmental programs evolve so that heritability of fitness at the group level may increase leading ultimately to the creation of a new evolutionary individual.

REFERENCES

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

Boraas, M. E., D. B. Seale, and J. E. Boxhorn. 1998. Phagotrophy by a flagellate selects for colonial prey: A possible origin of multicellularity. Evol. Ecol. 12:153–164.

Buss, L. W. 1987. *The evolution of individuality.* Princeton University, Princeton, New Jersey.

Friedl, T. 1997. The evolution of green agae. Pl. Syst. Evol. 11:87–101.

Gillott, M., D. Holen, J. Ekman, M. Harry, and M. E. Boraas. 1993. Predation-induced *E. coli* filaments: Are they multicellular? *In* G. Baily and C. Reider (eds.), *Proceedings of the 51st Annual Meeting of the Microscopy Society of America.* San Francisco Press, San Francisco, California.

Hammerstein, P. 2003. Genetic and Cultural Evolution of Cooperation, Dahlem Workshop Report 90. MIT Press, Cambridge, Massachusetts.

Hastings, I. M. 1992. Population genetic aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction. Genet. Res. 59:215–225.

Hurst, L. D. 1990. Parasite diversity and evolution of diploidy, multicellularity and anisogamy. J. Theor. Bio. 144:429–443.

Hurst, L. D. 1992. Intra-Genomic Conflict as an Evolutionary Force. Proc. Roy. Soc. B. 248:135–140.

Hurst, L. D., A. Atlan, and B. O. Bengtsson. 1996. Genetic conflicts. Q. Rev. Biol. 71:317–364.

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

Koufopanou, V. and G. Bell. 1991. Developmental mutants of *Volvox*: Does mutation recreate the patterns of phylogenetic diversity. Evolution 45:1806–1822.

Kurn, N., M. Colb, and L. Shapiro. 1978. Spontaneous frequency of a developmental mutant in *Volvox*. Developmental Biology 66:266–269.

Lachmann, M., N. W. Blackstone, D. Haig, A. Kowald, R. E. Michod, E. Szathmáry, J. H. Werren, and L. Wolpert. 2003. Cooperation and conflict in the evolution of genomes, cells, and multicellular organisms. *In* P. Hammerstein (ed.), *Genetic and cultural evolution of cooperation*, p. xx. MIT Press, Cambridge, Massachusetts.

Margulis, L. 1981. *Symbiosis in cell evolution.* W. H. Freeman, San Francisco.

Maynard Smith, J. and E. Szathmáry. 1995. *The major transitions in evolution.* W. H. Freeman, San Francisco.

Michod, R. E. 1982. The theory of kin selection. Ann. Rev. Ecol. Syst. 13:23–55.

Michod, R. E. 1983. Population biology of the first replicators: On the origin of the genotype, phenotype and organism. Amer. Zool. 23:5–14.

Michod, R. E. 1996. Cooperation and conflict in the evolution of individuality. II. Conflict mediation. Proc. Roy. Soc. B. 263:813–822.

Michod, R. E. 1997. Cooperation and conflict in the evolution of individuality. I. Multi-level selection of the organism. Am. Nat. 149:607–645.

Michod, R. E. 1999. *Darwinian dynamics, evolutionary transitions in fitness and individuality.* Princeton University Press, Princeton, New Jersey.

Michod, R. E. 2003. Cooperation and conflict mediation in the evolution of multicellularity. *In* P. Hammerstein (ed.), *Genetic and*

cultural evolution of cooperation. MIT Press, Cambridge, Massachusetts.

Michod, R. E. and R. Abugov. 1980. Adaptive topography in family structured models of kin selection. Science 210:667–669.

Michod, R. E. and A. Nedelcu. 2003. Cooperation and conflict in the origins of multicellularity and the eukaryotic cell. *In* A. Moya and E. Font (eds.), *Evolution: From molecules to ecosystems.* Oxford University Press, Oxford.

Michod, R. E., A. M. Nedelcu, and D. Roze. 2003. Cooperation and conflict in the evolution of individuality IV. Conflict mediation and evolvability in *Volvox carteri.* BioSystems 69:95–114.

Michod, R. E. and D. Roze. 1997. Transitions in individuality. Proc. Roy. Soc. B. 264:853–857.

Michod, R. E. and D. Roze. 1999. Cooperation and conflict in the evolution of individuality. III. Transitions in the unit of fitness. *In* C. L. Nehaniv (ed.), *Mathematical and computational biology: Computational morphogenesis, hierarchical complexity, and digital evolution,* pp. 47–92. American Mathematical Society, Providence, Rhode Island.

Michod, R. E. and D. Roze. 2000. Some aspects of reproductive mode and the origin of multicellularity. Selection 1:97–109.

Michod, R. E. and M. J. Sanderson. 1985. Behavioural structure and the evolution of social behaviour. *In* J. J. Greenwood and M. Slatkin (eds.), *Evolution—essays in honour of John Maynard Smith,* pp. 95–104. Cambridge University Press, Cambridge.

Nakayama, T., B. Marin, H. D. Kranz, B. Surek, V. A. R. Huss, I. Inouye, and M. Melkonian. 1998. The basal position of scaly green flagellates among the green algae (chlorophyta) is revealed by analyses of nuclear-encoded SSU rRNA sequences. Protist 149:367–380.

Nedelcu, A. and R. E. Michod. 2003. Evolvability, modularity, and individuality during the transition to multicellularity in volvocalean green algae. *In* G. Schlosser and G. Wagner (eds.), *Modularity in development and evolution.* Univ. Chicago Press, Chicago.

Partridge, L. and L. D. Hurst. 1998. Sex and conflict. Science 281:2003–2008.

Pommerville, J. and G. Kochert. 1981. Changes in somatic cell structure during senescence of *Volvox carteri.* Eur. J. Cell Biol. 24:236–243.

Pommerville, J. and G. Kochert. 1982. Effects of senescence on somatic cell physiology in the green alga *Volvox carteri.* Exp. Cell Res. 140:39–45.

Roze, D. and R. E. Michod. 2001. Mutation load, multi-level selection and the evolution of propagule size during the origin of multicellularity. Am. Nat. 158:638–654.

Santelices, B. 1999. How many kinds of individual are there? Trends Ecol. Evol. 14:152–155.

Shikano, S., L. S. Luckinbill, and Y. Kurihara. 1990. Changes of traits in a bacterial population associated with protozoal predation. Microbial Ecol. 20:75–84.

Solari, C., A. Nedelcu, and R. E. Michod. 2003. Fitness, life-history and the evolution of complexity in Volvocalean green algae. Submitted manuscript.

Stanley, S. M. 1973. An ecological theory for the sudden origin of multicellular life in the Late Precambrian. Proc. Natl. Acad. Sci. U.S.A. 70:1486–1489.

Szathmáry, E. and J. Maynard Smith. 1997. From replicators to reproducers: The first major transitions leading to life. J. Theor. Bio. 187:555–571.

Uyenoyama, M. K. and M. W. Feldman. 1984. Theories of kin and group selection: A population genetics perspective. Theor. Popul. Biol. 38:87–102.