

# Cells, Colonies, and Clones: Individuality in the Volvocine Algae

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I saw a very many great round particles, of the bigness of a great corn of sand drive and move in the water . . . This was to me a very pleasant sight, because the said particles, as often as I did look on them, did neither lye still, and that their motion did proceed from their turning round; and that the more, because I did fancy at first that they were small animals, and the smaller these particles were, the greener was their colour.

VAN LEEUWENHOEK (1700, 511)

## Introduction

Life is organized hierarchically, with genes in genomes, organelles within eukaryotic cells, cells within multicellular organisms, and organisms within societies. At various points in evolutionary history, the higher-level units in this hierarchy (collectives) must have emerged from interactions among the lower-level units (components). These transitions, for example from prokaryote to eukaryote and from unicellular to multicellular organisms, have led to entirely new kinds of biological individuals with new, emergent properties. Collectively they are known as “major transitions” (Maynard Smith and Szathmáry 1997) or “evolutionary transitions in individuality” (hereafter = ETIs; Michod and Roze 1997). In each case, groups are formed by previously existing individuals, either by coming together or by staying together (Tarnita et al. 2013), scenarios that Queller (1997) referred to as egalitarian and fraternal transitions, respectively. Over evolutionary time, such groups begin to take on characteristics of individuals, perhaps eventually emerging as individuals in their own right. If we conceive of ETIs as a process in which groups evolve new levels, or at least increased degrees, of individuality, it is worth thinking about what we mean by individuality.

One particularly common type of ETI is the transition from single-celled organisms to multicellular organisms, which in some cases have evolved cellular differentiation (i.e., multiple functionally differentiated cell types). Cellular differentiation is a prerequisite for large, complex body plans, as scaling laws dictate that large organisms will face problems not experienced by their

smaller relatives (Bonner 2004). Large motile organisms need specialized cells to move (Kirk 1998), large sessile organisms need specialized structures to stay put (Butterfield 2000; Rozhnov 2001), and any large organisms with substantial three-dimensional structure face problems of nutrient transport due to a reduced surface area to volume ratio (Bonner 2004). In fact, the number of cell types in a multicellular organism has frequently been used as a metric of complexity (e.g., McShea 1996; Bell and Mooers 1997; Bonner 2003). Multicellularity has evolved independently in at least 25 separate lineages, including the Eubacteria, Archaea, and several lineages spanning the deepest divergences within the eukaryotes, but only a handful of these lineages have evolved cellular differentiation (Bonner 1998; Grosberg and Strathmann 1998; Herron et al. 2013).

Although the origins of differentiated multicellularity have been few in number, the consequences have been immense. Each of the major macroscopic groups had its origin in such a transition, and each has subsequently diversified into thousands to millions of species. Aside from their own diversification, the multicellular red, green, and brown algae, land plants, animals, and fungi have profoundly impacted the evolution of other lineages as well, drastically altering their environments with often dramatic effects on their evolutionary trajectories.

Here, I use the volvocine green algae as a case study to consider the emergence of a new level of individuality during the transition to multicellularity. Although a distinction is sometimes made between organisms and individuals (e.g., Queller and Strassmann 2009), I will treat these terms as synonyms in the context of individual multicellular organisms. After briefly reviewing efforts to define individuality, I introduce the volvocine algae as a model system, review the evolutionary history of developmental changes in this group, and consider how these changes relate to the emergence of a new kind of individual.

### What Is an Individual?

The deceptively simple question of what kinds of biological units should be considered individuals in fact has a long and complex history. A number of criteria have been proposed or implemented, and no universal agreement exists. Various authors have presented structural, genetic, ecological, physiological, reproductive, functional, and behavioral criteria as decisive (for a more comprehensive review, see Clarke 2010). Among the many criteria that have been proposed for individuality, most can be classified into a few major categories.

Santelices (1999) proposed a classification with three categories: genetic

uniqueness (individuals are genetically distinct from others of the same species), genetic homogeneity (all of the parts of an individual are genetically identical or nearly so), and physiological autonomy. The presence or absence of these attributes defines eight possible combinations, most of which are represented on Earth. For example, “unitary” individuals, typified by vertebrates, possess all three attributes. Clonal reproducers, such as some plants and multicellular protists, are genetically homogeneous and physiologically autonomous but lack genetic uniqueness. The genetic criteria contribute to the conception of an individual as a unit of selection, while physiological autonomy principally relates to the ecological interactions of an individual with its environment and with other individuals.

Recent efforts to understand individuality have tended to focus on evolutionary factors, treating individuals as units of selection or units of adaptation. Michod and Nedelcu (2003) treat individuals as units of selection as determined by heritable variation in fitness. A division of labor between reproductive (germ) and vegetative (somatic) functions limits within-organism genetic variation, thereby preventing within-organism conflicts that might otherwise disrupt organismal function. By exporting fitness from the components to the collective, such a germ-soma distinction maintains the unity of the individual (Michod 1997; Michod et al. 2006).

In the view of Queller and Strassmann, individuals are characterized by high levels of cooperation and low levels of conflict among their component parts (Queller and Strassmann 2009; Strassmann and Queller 2010). High cooperation and low conflict indicate that organisms are highly functionally integrated and allow them to function as “bundles of adaptation” (Strassmann and Queller 2010, 605). The two factors are treated as independent and continuous, setting up a two-dimensional space with organisms occupying one quadrant. Germ-soma specialization and single-cell bottlenecks (Hamilton 1964), which for some other authors are decisive, are for Strassmann and Queller merely mechanisms that contribute to the crucial criteria of high within-organism cooperation and low within-organism conflict.

Folse and Roughgarden (2010) argue that the crucial criteria for individuality are alignment of fitness interests of the lower-level units so that little or no within-organism conflict occurs, interdependence of the parts due to germ-soma differentiation, and functional integration as evidence of adaptation. As in the view of Queller and Strassmann (2009; Strassmann and Queller 2010), genetic homogeneity and unicellular bottlenecks are ways of preventing conflict, but not necessarily the only ways. Adaptive functional integration at the organism level indicates that the organism is a unit of fitness. Folse and Roughgarden envision these traits arising in order during

ETIs: first high genetic relatedness among components, then a germ-soma division of labor, and finally adaptation at the new, emergent level.

In the framework of Godfrey-Smith (2009) and Clarke (2012), individuals are defined by their membership in Darwinian populations, those that are capable of adaptive evolution. At a minimum, such populations must possess Lewontin's (1970) criteria for evolution by natural selection: heritable variation in phenotypes that affect fitness. Other attributes are sometimes associated with the capacity for adaptive change, but it is the capacity itself that is central. Godfrey-Smith recognizes a continuum of Darwinian and Darwinian-like processes, from marginal cases that meet only the minimal criteria to "paradigm" cases that are capable of producing complex adaptations. Populations vary more or less continuously along several axes, including the amount of phenotypic variation present, the reliability of inheritance, the strength of intra-specific ecological interactions, the extent to which fitness depends on intrinsic features, and the smoothness of the adaptive landscape (i.e., the degree to which small changes in phenotype cause small changes in fitness). Populations that possess all of these features in high degree have the potential for sustained and complex adaptive change, while those with low degrees of one or more criteria are at best capable of less interesting evolutionary outcomes. Other criteria may also be important for particular kinds of organisms. For collective reproducers, those that are composed of parts that are themselves capable of reproduction, high degrees of individuality are characterized by development that includes a bottleneck, a germ-soma division of labor, and functional integration. High degrees of these criteria indicate that Darwinian processes will be more powerful, and thus adaptations will tend to occur, at the level of the collective rather than of its components.

The criteria of these various systems are largely overlapping, as for example genetic homogeneity (Santelices 1999) aligns fitness interests (Folse and Roughgarden 2010), which is likely to reduce within-individual conflict (Queller and Strassmann 2009; Strassmann and Queller 2010). Functional integration among components plays a role in all of them, either as evidence of adaptation at the level of the collective (Queller and Strassmann 2009; Folse and Roughgarden 2010; Strassmann and Queller 2010) or by contributing to the evolvability of the collective (Michod and Nedelcu 2003; Godfrey-Smith 2009). Finally, all of these concepts share a diachronic view of individuality (Okasha 2005), viewing individuality as a derived trait (Buss 1987) in the context of major transitions (Maynard Smith and Szathmáry 1997; or ETIs, Michod and Roze 1997).

With their focus on change over time, these recent syntheses lend themselves well to the diachronic ETI tradition, treating the levels of the biologi-

cal hierarchy as outcomes rather than starting conditions of the evolutionary process (Buss 1987). In addition, the criteria they propose were derived without reference to any particular taxonomic group, a crucial advantage if we want to draw non-trivial conclusions about the evolution of individuality (Herron et al. 2013). For these reasons, the discussion that follows will privilege these recent, evolutionary views, while occasionally referencing more traditional criteria.

### The Volvocine Algae

The independent origins of differentiated multicellularity are replicate experiments with the potential to reveal general principles involved in this transition. Unfortunately, for most lineages the evidence of the intermediate stages has been obscured by extinction and by an inadequate fossil record. In the volvocine green algae (Fig. 2.1), though, abundant evidence remains

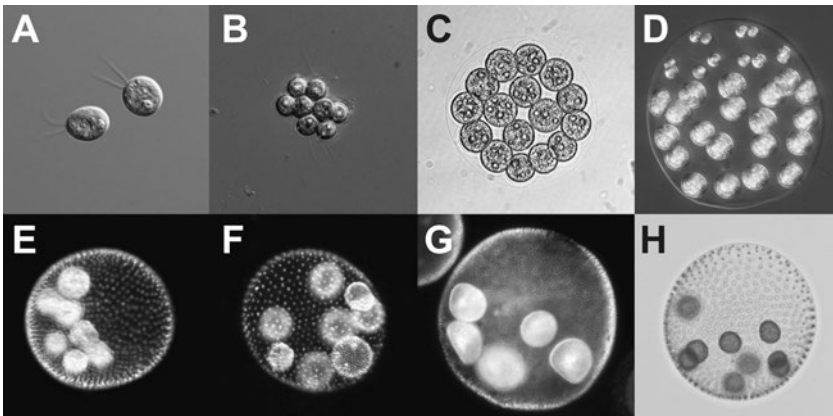


FIGURE 2.1. Representative volvocine algae. A: *Chlamydomonas reinhardtii*, a unicell with two flagella at the anterior. C. *reinhardtii* diverged from the multicellular volvocine algae around 250 million years ago. B: *Gonium pectorale*, a flat or slightly curved plate of 8 to 32 cells (8 in this example), all oriented in the same direction (photos A and B by Deborah Shelton). C: *Eudorina elegans*, a spheroid with up to 32 undifferentiated cells (16 in this example). D: *Pleodorina starrii*, a partially differentiated spheroid with up to 64 cells (32 in this example). The small cells near the anterior pole (top) are terminally differentiated somatic cells specialized for motility; the larger cells perform both reproductive and motility functions. E: *Volvox carteri*, a spheroid with ca. 2000 small somatic cells arranged at the periphery and a handful of much larger reproductive cells (gonidia). F: *Volvox tertius*, a spheroid with ~1000 small somatic cells arranged at the periphery and a handful of much larger reproductive cells. The germ cells in this colony have begun to develop into daughter colonies, and one is in the process of inversion. G: *Volvox barberi*, a spheroid with ca. 30,000 small somatic cells arranged at the periphery and a handful of much larger reproductive cells. The germ cells in this colony have begun to develop into daughter colonies, and some are in the process of inversion. H. *Volvox aureus*, a spheroid with up to ~2000 small somatic cells arranged at the periphery and a handful of much larger reproductive cells.

in the form of extant species with various mixtures of derived and ancestral traits.

The volvocine algae (Chlorophyceae: Chlamydomonadales) are an informal grouping of facultatively sexual, haploid, photosynthetic algae found mostly in freshwater. Following the taxonomy of Nozaki and Itoh (1994), I will take this grouping to include the families Volvocaceae (*Eudorina*, *Pandorina*, *Platydorina*, *Pleodorina*, *Volvox*, *Volvulina*, *Yamagishiella*), Goniaceae (*Astrephomene*, *Gonium*) and Tetrabaenaceae (*Basichlamys*, *Tetrabaena*) along with the few most closely related unicells in the genera *Chlamydomonas* and *Vitreochlamys*.

The ~50 species of volvocine algae exhibit nearly every conceivable intermediate between unicellular and fully differentiated multicellular life, including single-celled forms and multicellular forms with and without cellular differentiation. This diversity makes the volvocine algae a uniquely useful model system for understanding the evolution of multicellularity and cellular differentiation. I will refer to the members of the families Tetrabaenaceae, Goniaceae, and Volvocaceae as colonial, and to their physically discrete units as colonies; these same units are sometimes referred to as spheroids or coenobia (Cohn 1875). I intend this usage not to imply any particular degree of individuality, but only as a term of convenience.

### Partitioning Individuality

The volvocine algae illustrate two of the classic problems plaguing discussions of individuality. First, a typical volvocine life cycle involves many rounds of asexual reproduction punctuated by occasional rounds of sexual reproduction (Fig. 2.2), and so many genetically identical colonies may descend from a single zygote. This is an instance of the ramet vs. genet problem: the genetically unique and (largest) genetically homogeneous units are the descendants of a given zygote (i.e., a genet; Sarukhán and Harper 1973), which may include a large number of physiologically discrete and autonomous colonies (i.e., ramets; Stout 1929). This is a much-discussed problem in plants and colonial invertebrates (e.g., White 1979; Cook 1980; Harper 1980; Tuomi and Vuorisalo 1989; Clarke 2012; Gorelick 2012), but it is relevant for any life cycle that includes both sexual and asexual phases. If we consider genetic homogeneity to be central to individuality, we should bear in mind that the clone or genet, not the colony, is the largest genetically homogeneous unit.

The second problem is whether we should consider a colony of a given species to be a group of individuals (the cells) or an individual in its own right. This question has deep historical roots, as Ehrenberg, contrary to

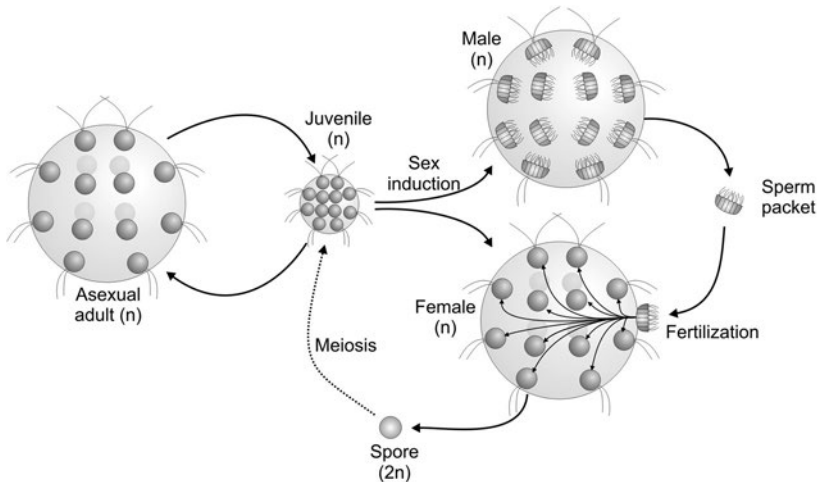


FIGURE 2.2. Example of a volvocine life cycle (based on that of *Eudorina*). Each cell in a haploid asexual spheroid undergoes a series of rapid cell divisions early in development, eventually producing a juvenile spheroid, which is eventually released from the mother spheroid. In species with cellular differentiation, only the reproductive cells divide. Juveniles escape from the parental spheroid possessing all of the cells they will have as adults; continued growth occurs by increases in cell size and in the volume of extracellular matrix rather than by cell division. The trigger to enter the sexual cycle varies among species, including low nutrient levels, heat shock, and chemical signals released by colonies. In isogamous species, cells differentiate into gametes of opposite mating types. In anisogamous species, cells differentiate into motile sperm packets or immotile eggs. In either case, fertilization results in a diploid zygote that eventually matures into a dormant, desiccation-resistant spore. Spores germinate through meiosis upon the return of optimal growth conditions. In some species, germination results in four viable offspring; in others only a single offspring and three polar bodies are produced.

van Leeuwenhoek (1700) and Linnaeus (1758), considered a *Volvox* spheroid a colony of hundreds or thousands of individuals (Ehrenberg 1832). Some form of this problem will be relevant for any ETI. For example, in eusocial insects the problem is whether to consider a colony a group of individual animals or a superorganism composed of numerous animals. Similarly, certain obligately endosymbiotic bacteria with extremely reduced genomes can be thought of as individuals in their own right or as organelles of the host cells (Andersson 2000).

Within the colonial volvocine algae, there are three kinds of biological units that can contend for at least a degree of individuality: the cells, the colonies, and the clones (or genets). Previous discussions have tended to ask (implicitly or explicitly), “Is a *Volvox* spheroid an individual?” or at least “How much of an individual is a *Volvox* spheroid?” I suggest that the relevant question is rather “How much individuality is present at each of the three (or more) levels?”

## Cells and Colonies

The transition to differentiated multicellularity in the volvocine algae has been broken down into a series of developmental changes (Kirk 2005), each plausibly adaptive, as Darwin did for the vertebrate eye (Darwin 1872; see also Nilsson and Pelger 1994; Dawkins 1997). The history of these and other developmental changes has been reconstructed in a phylogenetic framework, showing that some traits had multiple, independent origins, as well as reversions from derived back to ancestral states (Herron and Michod 2008; Herron 2009; Herron et al. 2010). Timing of developmental changes was inferred from a fossil-calibrated molecular clock analysis (Herron et al. 2009).

Here I review the evolutionary history of developmental changes in the volvocine algae and consider how these changes relate to various concepts of individuality. In this section, I propose adaptive explanations, or at least adaptive values, for several of the derived traits of volvocine algae. Bearing in mind the pitfalls of inferring adaptive origins of traits (Gould and Lewontin 1979), these speculations should be considered only as plausible adaptive hypotheses.

Volvocine life cycles include both asexual and sexual reproduction, with many rounds of asexual reproduction often preceding a single round of sexual reproduction (Fig. 2.2). In the discussion that follows, I consider the asexual developmental changes identified by Kirk (2005) in roughly chronological order (Fig. 2.3). The retention of cytoplasmic bridges in adults (Herron et al. 2010) is appended to this series as step 13.

### CONVERSION OF THE CELL WALL INTO EXTRACELLULAR MATRIX (STEP 5)

A requirement for the evolution of multicellularity in any origin of multicellularity is a mechanism for keeping cells attached to each other (Abedin and King 2010); in the volvocine algae, this is accomplished by the formation of an extracellular matrix (ECM). In unicellular volvocines, the protoplast is surrounded by a cell wall with two distinct layers. The outer cell wall has a highly organized, quasi-crystalline structure and provides structural support for the cell (Imam et al. 1985), while the inner layer has an amorphous structure. Following one or more rounds of mitotic cell division within the cell wall of the mother cell, the cell wall ruptures, releasing the daughter cells. In one of the smallest colonial volvocines, *Basichlamys*, four-celled colonies are formed when the daughter cells remain attached to the mother cell wall (Stein 1959; Iyengar and Desikachary 1981). In the other four-celled species,



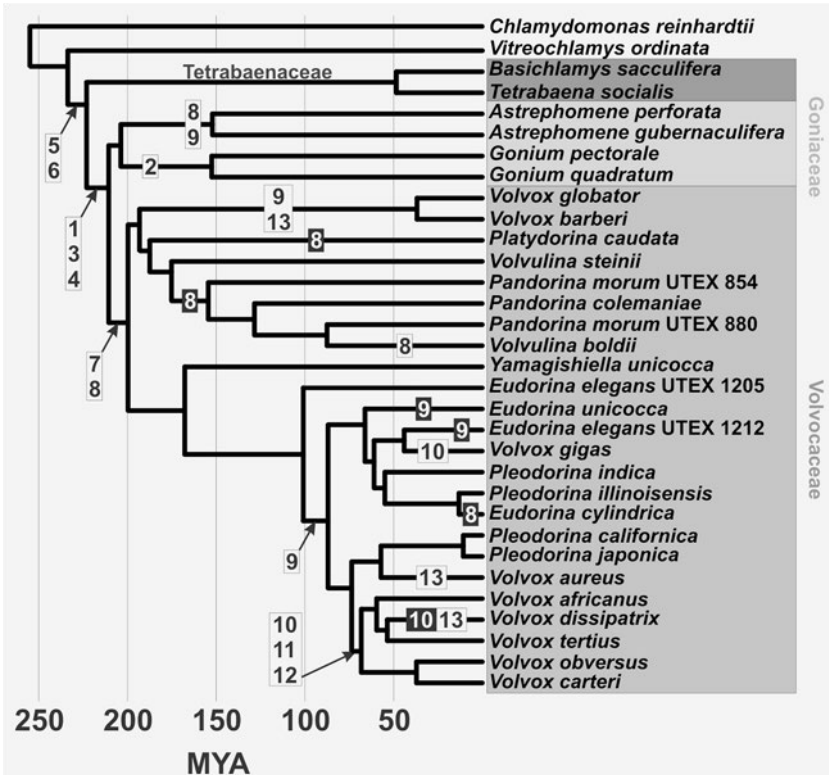


FIGURE 2.3. Evolutionary relationships and estimated divergence times among volvocine algae (adapted from Herron et al. 2009). Shaded boxes identify the 3 multicellular families; species not highlighted in this manner are unicellular. Character state changes are those supported by hypothesis tests in Herron and Michod (2008) and Herron et al. (2010). 1: incomplete cytokinesis; 2: partial inversion; 3: rotation of the basal bodies; 4: establishment of organismal polarity; 5: transformation of the cell wall into extracellular matrix; 6: genetic control of cell number; 7: complete inversion; 8: increased volume of extracellular matrix; 9: sterile somatic cells; 10: specialized germ cells; 11: asymmetric division; 12: bifurcated cell division program (steps 11 and 12 may have had 2 separate origins in the clade including *V. africanus* and *V. carteri*); 13: small gonidia, growth between divisions, and retention of cytoplasmic bridges in adult spheroids; 14: slow divisions and light-dependent divisions. Steps 1–12 are described in Kirk (2005), and I have retained Kirk's numbering for consistency. Step 13 is described in Herron et al. (2010).

*Tetrabaena*, daughter cells remain attached to the mother cell wall as in *Basichlamys*, but they are also connected to each other by connections between their own outer cell walls, as in *Gonium* (Stein 1959; Nozaki 1990). In the Volvocaceae, the outer layers of the daughter cell walls have fused to become a colonial boundary, and individual cells are surrounded only by a homolog of the inner layer, now referred to as an extracellular matrix (D. Kirk et al. 1986).

The simple step of daughter cells remaining attached to each other has important implications for their degree of individuality, and colonies formed

in this way already meet several of the traditional criteria. Structurally, such a colony is a discrete, contiguous, and spatially bounded unit (Hull 1980), which passes through a single-celled bottleneck during development (Huxley 1912, p. 45) and is genetically homogeneous (Weismann 1904, p. 347) or nearly so. In addition, this initial shift to a colonial lifestyle aligned the fitness interests of the component cells (Folse and Roughgarden 2010). For example, relative to independently living offspring, the cells in a colony are likely to realize the same viability, living or dying together as a group. Finally, the development of an ECM may have increased physiological integration: simply by keeping cells together, the ECM forces the cells in a colony to share an environment in which each is affected by the others' physiological activities. *Basichlamys*, in which the ECM is the only thing keeping the colony together, may be the least physiologically integrated volvocine colony, but it is still more integrated than the independent offspring of a mother *Chlamydomonas* or *Vitreochlamys* cell.

#### GENETIC CONTROL OF CELL NUMBER (STEP 6)

Except for a few species of *Volvox*, volvocine algae share an unusual pattern of cell division known as palintomy or multiple fission (Sleigh 1989; Desnitski 1992). Rather than repeated rounds of two-fold growth followed by a single cell division (binary fission), palintomic division consists of many-fold growth followed by multiple rounds of cell division without substantial growth between rounds of division. Thus, a cell grows  $\sim 2^N$ -fold then divides  $N$  times without intervening growth to produce  $2^N$  daughter cells. Because each colonial species appears to have a genetically fixed maximum cell number, the number of rounds of cell division has been inferred to have undergone a shift from environmental control (in unicells) to genetic control (in colonies) (Kirk 2005). However, in both unicellular and colonial species,  $N$  is controlled by both genetic and environmental factors. Just as in the colonial species, each species of *Chlamydomonas* and *Vitreochlamys* has a characteristic maximum number of rounds of cell division, suggesting a genetic influence (e.g., Lien and Knutsen 1979; Iyengar and Desikachary 1981; Ettl 1983). It is possible that the same mechanism that controls maximum offspring number in the unicellular species controls cell number in the colonial species, as suggested by Koufopanou (1994).

Genetic control of cell number could play a role in maintaining genetic homogeneity among the cells in a colony (by limiting the number of cell divisions and thus the opportunity for mutation). Should a larger colony be considered less of an individual than a small one, all else equal? Strassmann and

Queller (2010) seem to suggest that it should, since large colonies are more likely to deviate from perfect genetic homogeneity and therefore to experience conflicts among cells. Genetic homogeneity is an important criterion in many treatments of individuality (e.g., Santelices 1999; Folse and Roughgarden 2010), and factors that increase genetic homogeneity are also likely to reduce intra-organismal conflict (thereby aligning the fitness interests of the components; Folse and Roughgarden 2010).

INCOMPLETE CYTOKINESIS (STEP 1) AND RETENTION  
OF CYTOPLASMIC BRIDGES IN ADULTS (STEP 13)

In the Goniaceae and Volvocaceae, cells in the developing embryos remain connected by cytoplasmic bridges due to incomplete cytokinesis (Hoops et al. 2006). In most species, these bridges are lost later in development, and in the Tetrabaenaceae they never form (Stein 1959). Cytoplasmic bridges play an important role in the developmental process of inversion (steps 2 and 7) (Viamontes 1977; Viamontes et al. 1979; Green et al. 1981), and in three independent lineages of *Volvox* they are retained in adult spheroids (step 13) (Hoops et al. 2006; Herron et al. 2010).

The development of cytoplasmic bridges may represent a substantial increase in physiological integration, although little is known about the nature or extent of the resulting interactions. The cytoplasmic bridges in the adults of some *Volvox* species could provide a means for direct cell-cell interaction or communication, but as far as I am aware there is no evidence for or against this.

ROTATION OF THE BASAL BODIES (STEP 3) AND  
ESTABLISHMENT OF ORGANISMAL POLARITY (STEP 4)

Most colonial volvocine algae have a discernable anterior-posterior polarity. In the unicellular *Chlamydomonas* and *Vitreochlamys*, the two flagella are oriented, and beat, in opposite directions. As a result, the cell is pulled through the water in a breaststroke, and the flagella-bearing end of the cell is the anterior (as defined by direction of movement). This arrangement would be suboptimal for a colony with more than a few cells, and in the Goniaceae and Volvocaceae, the flagella are rotated to beat in the same direction (Gerisch 1959; Greuel and Floyd 1985; Hoops 1993). As a result of this rotation, *Gonium* has a center-to-edge polarity defined by cells with the two different orientations, while the spheroidal colonies have an anterior-posterior polarity. Other manifestations of organismal polarity are apparent in at least some

members of the Volvocaceae, and these have presumably evolved after the rotation of the basal bodies. In many species, cell size increases from anterior to posterior, and the eyespots are graded in both size and their relative position in the cell (Kirk 2005). In some species of *Eudorina*, the anterior-most cells facultatively differentiate as soma, depending on environmental conditions (Goldstein 1967).

Although rotation of the basal bodies is a change that occurs within the cells, the resulting center-edge or anterior-posterior polarity is an emergent trait that is defined, and makes adaptive sense, at the colony level. Without this reorganization, spheroidal colonies would be incapable of swimming. This is a particularly clear example of functional integration and thus evidence of a colony-level adaptation in the sense of Folse and Roughgarden (2010).

#### PARTIAL (STEP 2) AND COMPLETE (STEP 7) INVERSION

The embryos of *Gonium* and the Volvocaceae are all inside-out to some degree at the end of cell division, with the flagella on the concave side of a bowl shape or completely inside of a rough sphere. As with the ancestral flagellar orientation, this situation is likely suboptimal for motility, and so the developing colonies turn themselves partially (*Gonium*) or completely (Volvocaceae) inside-out, ending with the flagella on the convex or outer surface. Partial inversion in *Gonium* and complete inversion in the Volvocaceae may have evolved independently (Herron and Michod 2008), or partial inversion may have been an intermediate step preceding complete inversion (Kirk 2005).

Inversion in the Volvocaceae results from cells moving relative to the cytoplasmic bridges arising from incomplete cytokinesis (Marchant 1977; Viamontes 1977). This process requires a remarkable degree of functional integration among cells, as the cells in an inverting embryo change in shape and move relative to one another in a pattern that is both spatially and temporally coordinated (Höhn and Hallmann 2011).

#### INCREASED VOLUME OF ECM (STEP 8)

In contrast to the relatively compact colonies of *Gonium*, *Pandorina*, and *Platydorina*, in which the majority of the colony volume is made up of cells, in the remaining members of the Volvocaceae and in *Astrephomene*, the majority of the colony volume is made up of ECM. This gives the largest spheroids the appearance of a hollow ball, with the cells arranged on the periphery. In the various species of *Volvox*, ECM makes up >99% of the colony vol-

ume. The volume of ECM appears to be an evolutionarily labile trait, having undergone several expansions and contractions throughout the history of the group (Herron and Michod 2008).

The increased volume of ECM in large spheroids would be one way of increasing organismal size without the long growth periods or extremely large reproductive cells that would be required to achieve the same size by increasing cell number. ECM may serve as a storage medium for nutrients, which are shared by all the cells in a colony (Bell 1985). If either of these factors played a role, evolutionary changes in ECM volume could reasonably be considered colony-level adaptations in the sense of Folse and Roughgarden (2010).

PARTIAL (STEP 9) AND COMPLETE (STEP 10)  
CELLULAR DIFFERENTIATION

One of the most-studied aspects of volvocine biology, both from a theoretical and from a molecular-genetic perspective, is the development of sterile somatic cells. Obligate somatic cells (that is, cells that develop as soma regardless of environmental conditions) are present in *Astrephomene*, *Pleodorina*, and *Volvox* and are thought to be important for these large colonies to maintain motility throughout the life cycle (Koufopanou 1994; Solari et al. 2006). As a form of cellular differentiation, somatic cells are relevant to the evolution of complex multicellularity. As an extreme form of altruism, in that somatic cells sacrifice their reproductive capacity, their evolution is of broad interest to biologists and theoreticians interested in the evolution of cooperation. Somatic cells have arisen in at least three separate lineages within the volvocine algae, and they appear to have been lost in some lineages of *Eudorina* (Fig. 2.3).

In *V. africanus*, *V. carteri*, *V. gigas*, *V. obversus*, and *V. tertius*, the reproductive cells (gonidia) lack flagella and do not contribute to motility (Kirk 1998; Herron et al. 2010). In contrast, in *Astrephomene*, *Pleodorina*, and the remaining species of *Volvox*, the reproductive cells contribute to motility for at least part of the life cycle (Kirk 1998). In this sense, the former *Volvox* species are completely differentiated, with one set of cells (the soma) specialized for motility and not contributing to reproduction, and the other set (the gonidia) specialized for reproduction and contributing nothing to motility (Kirk 1998). This trait had two independent origins and was apparently lost in *V. dissipatrix* (Fig. 2.3).

Michod (2003) considers this type of germ-soma specialization to be crucial to individuality. In addition, the changes related to cellular differentiation

(steps 9–12) have increased interdependence, as reproductive cells became dependent on somatic cells for motility and somatic cells became dependent on gonidia for reproduction (Michod et al. 2006; Folse and Roughgarden 2010). Finally, the changes related to cellular specialization have been interpreted as colony-level adaptations to maintain or increase motility (Koufopanou 1994; Solari et al. 2006).

ASYMMETRIC DIVISION / BIFURCATED CELL  
DIVISION PROGRAM (STEPS 11 AND 12)

In *V. carteri*, *V. obversus*, and *V. africanus*, cell fate is determined by a series of asymmetric divisions, after which the smaller division products continue dividing to produce somatic cells, while the larger products stop dividing and differentiate into gonidia (Starr 1969, 1970; M. Kirk et al. 1993; Ransick 1993; Herron et al. 2010). Cell fate is strictly determined by cell size: cells larger than ~8  $\mu\text{m}$  in diameter differentiate as gonidia, smaller cells as soma (M. Kirk et al. 1993). It is unclear whether these traits arose independently in *V. africanus* or if they were lost in the ancestor of *V. tertius* and *V. dissipatrix* (Fig. 2.3).

Buss (1987) and Michod et al. (2003) have interpreted early germ-line segregation as an adaptation to maintain individuality in the face of this countervailing pressure as cell number increases. Michod has expanded on this idea and considers the bifurcated cell division program observed in *V. carteri*, *V. obversus*, and *V. africanus* as an adaptation to reduce or prevent intra-organismal conflict by reducing the number of cell divisions in the germ line and thus the genetic heterogeneity among cells (Michod and Nedelcu 2003).

### Colonies and Clones

A number of authors concerned with individuality see individuals as the units of evolution. In the views of Godfrey-Smith (2009), Clarke (2012), and Michod and Nedelcu (2003), this is reflected in heritable variation in fitness among individuals. In the colonial volvocine algae, the way in which heritable variation in fitness is partitioned within a particular population depends on the developmental program, the mutation rate, and the demography of the population.

At one extreme, we can imagine a pond in which a population is founded by a single colony, which begins reproducing asexually. This is biologically plausible, for example if a pond is colonized through long-distance dispersal. Heritable variation will arise through new mutations and will reside primarily among colonies. Any genetic variation among cells will be fleeting: a

mutation arising during development will give rise to a chimeric colony, but each of that colony's offspring will be genetically homogeneous (some with and some without the mutation), since each daughter colony derives from a single cell.

At the opposite extreme, we can imagine a pond in which the founding population is large and genetically diverse. This situation too is biologically plausible, for example when a summer bloom is initiated by descendants of sexually produced spores from the previous year's population. In this case, heritable variation in fitness will be found mainly among the clonal lineages descending from different spores. Depending on the mutation rate, additional genetic variation will eventually arise within these lineages due to new mutations.

Considering these two scenarios as extremes along a continuum, we see that the individuals-as-units-of-evolution view implies that the degree of individuality of a given unit is not entirely an inherent property of the unit itself. Rather, the degree of individuality is contingent on the particular ecological and demographic circumstances. Furthermore, as these circumstances change over ecological time, the proportion of heritable variation in fitness found at each level changes as well. This is not only an epistemological distinction. Heritability itself, and not just measurements of heritability, really does change from one generation to the next as allele frequencies change (Lynch and Walsh 1998). As it does, the partitioning of heritable variation in fitness among cells, colonies, and clones will change as well. Contrary to our intuitions, then, the units-of-evolution view suggests that the degree of individuality can change not only through long-term evolutionary change but also through short-term changes in population structure.

## Conclusions

Most recent discussions of individuality recognize that the relevant criteria are continuous rather than categorical and, as a result, that intermediate degrees of individuality are possible (Santelices 1999; Pepper and Herron 2008; Godfrey-Smith 2009; Queller and Strassmann 2009; Folse and Roughgarden 2010; Strassmann and Queller 2010; Clarke 2012). This recognition is crucial to understanding the emergence of individuality at a new, higher level. The outcome of an ETI is a new individual composed of what, before the transition, were individuals in their own right. But this dichotomous view ignores the gray areas characterized by the intermediate steps in the transition. The volvocine algae give us a unique view of these gray areas through the existence of living species with intermediate degrees of individuality. The point

of using the volvocine algae as a model system is that we can see intermediate stages of individuation (or “organismality”; Pepper and Herron 2008; Queller and Strassmann 2009).

There is no line we can draw, or at least none that everyone will agree on, “below” which colonies are groups of cells and “above” which they are individuals in their own right. However, evolutionary reconstructions show how the degree of individuality of volvocine colonies has changed over time in some lineages. By most criteria, the initial formation of simple clusters of cells was a crucial step, creating a new spatially contiguous unit with fitness interests largely aligned among its members. The extant Tetrabaenaceae have retained this condition for ca. 200 million years while their sister group, the Goniaceae + Volvocaceae, underwent further developmental changes (Herron et al. 2009). Several of these changes—especially organismal polarity, inversion, and cellular differentiation—increased the functional integration of volvocine colonies and likely represent adaptations at the colony level.

Groups that are undergoing an ETI should be expected to have intermediate levels of individuality (Pepper and Herron 2008). This may even be an indication that such a transition is in progress, although the alternative, that the group in question is evolutionarily stable at an intermediate level of individuality, should be considered as well (Herron et al. 2013). Several lineages of volvocine algae are living fossils that have undergone little change in 100 million years or more (e.g., *Astrephomene perforata*, *Gonium pectorale*, *Volvulina steinii*, *Yamagishiella unicocca*; see Fig. 2.3). Reversals from derived to ancestral states, for example losses of cellular differentiation, indicate that the path to a new level of individuality is not, or at least not always, straightforward. Along with the long-term existence of taxa with intermediate degrees of individuality (Herron et al. 2009), this suggests that transitions in individuality, once begun, do not lead inevitably to fully individuated wholes.

Individuality in the volvocine algae can, in principle, be partitioned among at least three levels of the biological hierarchy: cells, colonies, and clones. This is likely to be true for any clonally developing multicellular organisms that undergo facultative sexual reproduction. Furthermore, the ways in which heritable variation in fitness is partitioned among these three levels will vary among species and according to ecological and demographic circumstances.

The volvocine algae, of course, represent only one modestly large clade, and so we should be cautious about generalizing. However, a survey of Earth’s biodiversity shows that this group is not a pathological case. Possibly nothing matches the ideal of a unitary individual: mutation inevitably disrupts genetic homogeneity (Otto and Hastings 1998; Pineda-Krch and Lehtilä 2004), and any group that alternates sexual and asexual reproduction will have some



form of the ramet/genet problem (Santelices 1999). Finally, all extant living things are products of ETIs and include subunits that once reproduced independently. We should not be surprised when the ghosts of previous transitions lead these subunits to retain some degree of individuality.

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