Volvocine Algae: From Simple to Complex Multicellularity

Matthew D. Herron and Aurora M. Nedelcu

Abstract The evolution of multicellularity provided new ways for biological systems to increase in complexity. However, although high levels of complexity have indeed been attained in several multicellular lineages, natural selection does not necessarily favor complex biological systems. Why and how, then, has complexity increased in some lineages? We argue that the volvocine green algae (*Volvox* and its relatives) are a uniquely valuable model system for understanding the evolution of multicellular complexity. Using a general framework for the evolution of complexity, we discuss the various levels of morphological and developmental complexity achieved in this group, and consider both the why and the how underlying the changes in complexity levels that took place in this group.

Keywords Cell differentiation · *Chlamydomonas* · Chlorophyta · Complexity · Genetics · Multicellularity · Natural selection · *Volvox*

The Issue of Complexity

> It is absurd to talk of one animal being higher than another.—C. R. Darwin (1837, p. 74)

Darwin famously reminded himself against using such value-laden terms as 'higher' and 'lower', but their use has continued (including in Darwin's own writing; see Richards 1988) right up to the present. Ideas of progress have been present in the literature of evolutionary theory as long as there has been such a literature. These ideas have changed over time and differed among authors, but generally include the notion of improvement over time as either a passive or an actively (selectively) driven trend. "Improvement", of course, implies some standard against which organisms are measured, and it is in the choice of standard that subjectivity is introduced (Ayala

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1988). Large, complex, intelligent organisms are considered 'advanced' or 'higher' and therefore are thought to represent an improvement over the 'primitive' or 'lower' organisms, which lack these presumed benefits.

These ideas are the intellectual descendants of the great chain of being (Lovejoy 1936), which was in turn based on the self-evident truth that humans are the pinnacle of evolution (Hull 1988). This framework, not surprisingly, leads to the circular conclusion that humans are the most advanced species, and the criterion for judging other species as advanced or primitive effectively reduces to degree of similarity to humans.

In spite of Darwin's well-known doubts, and those of numerous subsequent authors, assumptions of progress in evolution persist. Extant organisms are referred to as more or less 'primitive' or 'advanced', or even as basal to other extant organisms (Krell and Cranston 2004). The misconceptions that lead to these statements are not always merely semantic, and they can have substantial misleading influence on the interpretation of historical evidence, typically when the ancestors of a species-rich clade are assumed to have been similar to extant members of a species-poor sister clade (for example, that early mammals must have been monotreme-like). The influence of this misconception on ideas of ancestral mammals and angiosperms was recently reviewed by Crisp and Cook (2005).

Recent years have seen efforts to recast discussions of progress in terms of explicit, objective, *a priori* criteria. An example of this trend is the shift in focus from progress, which requires value judgments (Ayala 1988), to increases in complexity, which are in some sense quantifiable. This leaves, of course, the problem of how complexity is to be defined and measured. As McShea (1996) pointed out, investigations of trends in complexity can only provide nontrivial answers when the criteria for complexity are set in advance. To get meaningful answers to questions about how and why, and even if, complexity has increased through time in a given clade, we must estimate complexity based on criteria that are defined independently of the members of the clade (McShea 1996).

In this chapter, we first discuss several aspects relevant to defining and measuring complexity in biological systems as well as to understanding how and why complexity increased in some (but not all) lineages. Then we focus on a particular type of complexity—that is, multicellular complexity; we provide an overview of multicellular systems and discuss the proximate and ultimate causes for their increase in complexity. Lastly, we focus on a specific multicellular lineage—the volvocine algae—and argue that this group is a uniquely valuable model system for understanding the evolution of multicellular complexity. Using the general framework for the evolution of complexity introduced earlier, we review the various levels of morphological and developmental complexity achieved in this group, and consider both the why and the how underlying the changes in complexity levels that took place in this group.

What is Complexity?

Complexity itself is a complex concept, which has proven rather difficult to define (see also Chap. 15). Dozens of definitions have been offered, all with specific shortcomings. Going back to the origin of the word, '*complexus*' in Latin means 'entwined' or 'twisted together.' The term implies the existence of two or more components that are difficult to separate. In other words, complexity requires two attributes: the existence of (i) distinct parts and (ii) connections. Heylighen (1999) defines 'distinction' and 'connection' as the two aspects that characterize complexity in any system. '*Distinction*' corresponds to variety, heterogeneity, and different behaviors; '*connection*' corresponds to constraint and dependency. In this framework, complexity increases when the variety (distinction) and dependency (connection) of parts increase in at least one of the following dimensions: space, time, spatial scale, or temporal scale (Heylighen 1999). The process of increase in variety corresponds to *differentiation*; the process of increase in the number or strength of connections defines *integration*. The complexity produced by differentiation and integration in the spatial dimension can be called '*structural*', in the temporal dimension '*functional*', in the spatial scale dimension '*structural hierarchical*', and in the temporal scale dimension '*functional hierarchical*'. The scale dimension reflects in the number of hierarchical levels at which structure or function can be detected. For example, a multicellular organism has one more spatial hierarchical level than a unicellular individual—that is, cells within the organism. Similarly, multicellular development adds a level of functional hierarchy not found in unicells, namely interactions among cells, such as during embryonic development.

Similarly, McShea (1996) defines four facets of complexity, which have the potential to vary at least somewhat independently. Complexity is viewed in terms of objects or processes, either of which can be hierarchical or nonhierarchical. In terms of objects, hierarchical complexity refers to the number of levels of nestedness (parts within wholes; e.g., organelles within cells, cells within tissues, tissues within organs, organs within organisms), while nonhierarchical complexity refers to the number of different part types at a given level of nestedness (e.g., the number of cell types within a multicellular organism). Process complexity refers to causal relationships, which can be hierarchical (the number of levels of a causal hierarchy) or nonhierarchical (the number of independent interactions among parts at a given level of the hierarchy). In biological terms, McShea uses object complexity to address morphological questions and process complexity to address questions related to development.

Criteria

Complexity is not only a concept that is hard to define, but also a trait that is hard to measure. Several criteria have been used to compare biological systems in terms of complexity levels (see Szathmáry et al. 2001 for discussion and references). Complexity has been evaluated in terms of the number of cell types, the amount of DNA

content, the number of genes, the number of transcription factors, and the number of transcriptome states. All of these criteria have proven to be incommensurable across the entire range of biological systems. For instance, although vertebrates have a higher number of genes than invertebrates, within invertebrates the simple worm *Caenorhabditis elegans* has more genes than the more complex (in terms of number of cell types) fly *Drosophila melanogaster* (Carroll 2001). More recently, the number of transcription factors together with the number of genes they regulate (i.e., connectivity) has been proposed as a better indicator of complexity, but such estimates are harder to achieve (Szathmáry et al. 2001).

How Biological Systems Increase in Complexity (Proximate Causes)

Several processes and mechanisms can be identified as responsible for the observed increases in complexity in some biological systems. Major increases in hierarchical complexity have been achieved during transitions in individuality—through symbiosis (e.g., during the evolution of the eukaryotic cell) and cooperation and division of labor (such as during the evolution of multicellularity and eusociality; see Chap. 9). More subtle and gradual increases in complexity levels throughout evolution involved gene duplication followed by functional diversification, domain shuffling, alternative splicing, and changes in gene regulation— in other words changes in genome complexity.

As in this chapter we are concerned with the evolution of multicellularity, here we are only focusing on some aspects relevant to the proximate causes responsible for the increases in morphological complexity that took place in multicellular lineages. As mentioned earlier, there is no perfect/universal direct correlation between the number of total genes in a genome and morphological complexity (i.e., number of cell types); thus, changes in the number of genes are not likely to be fully responsible for the observed increases in complexity. Nevertheless, it has been suggested that an increase in the number of specific genes (e.g., genes involved in particular developmental functions) might be relevant to achieving increased morphological complexity (Carroll 2001). Among these, transcription factors (TFs) are key regulators of cell differentiation (by affecting cell-specific expression of genes), and as such they are likely involved in changes in complexity levels. Yet, although an expansion in the number of TFs offers the potential for an increase in complexity, it is not necessary for the evolution of increased morphological complexity (Carroll 2001). In fact, the number of TF genes in a genome is rather small; also, a small number of TFs can be responsible for large differences in gene expression patterns among cell types (see Carroll 2001, de Mendoza et al. 2013, Chaps. 15 and 18 for further discussion and references).

Since differences in morphological and developmental complexity cannot be solely attributed to differences in gene content, increases in complexity are likely due to changes in regulatory elements that act in *cis* to control gene expression. The

expansion of regulatory elements in a genome can be interpreted as an increase in genomic complexity in all four senses described above—in the number of different parts (i.e., regulatory elements) in a regulatory system (*structural complexity*), in the number of different interactions of these parts (*functional complexity*), in the number of levels in developmental hierarchies (*functional hierarchical*), and in the number of parts and interactions at a given spatial scale (*structural hierarchical*) (Carroll 2001).

Why Biological Systems Increase in Complexity (Ultimate Causes)

There is no *a priori* reason that more complex systems would be preferred by natural selection. Evolution selects for increases in fitness, but high fitness can be achieved both by very complex (e.g., animals) and very simple systems (e.g., bacteria). So, why did complexity increase in some lineages? Below, we describe a general framework based on cybernetics principles (proposed by Heylighen 1999), which emphasizes the role of the environment in driving the evolution of biological systems.

High fitness can be achieved if a system is very stable and/or if it is likely that many copies of that system will be produced (Heylighen 1999). Thus, a system will be selected if: (1) its parts 'fit together', i.e., form an intrinsically stable whole ('intrinsic' fitness), and (2) the whole 'fits' its environment, i.e., it can resist external perturbations and profit from external resources to reproduce ('extrinsic' fitness). Variation will result in differentiation, and selection of fit relationships will simultaneously result in integration by adding or strengthening connections between parts; the end result will be an increase in *structural complexity*.

As the environment changes, the system needs to maintain an invariant configuration in spite of variable disturbances; that is, *homeostasis*. In cybernetics terms (Ashby 1956), homeostasis can be achieved by control, i.e., "the compensation of external perturbations by the appropriate counter-actions so that a desired goal is reached or maintained" (Heylighen 1999). Ashby's (1956, 1958) Law of Requisite Variety states that "in order to achieve control (and maintain homeostasis), the variety of actions a control system is able to execute must be at least as great as the variety of environmental perturbations that need to be compensated." The larger the variety of actions performed by the system, the larger the range of disturbances that can be counteracted and the set of environmental situations in which the system can survive. All other things being equal, greater control variety implies greater fitness. A larger repertoire of possible actions allows the system to survive in a larger variety of situations. As evolution through natural selection would tend to increase control, internal variety will also increase. This can be interpreted as a functional differentiation, which will result in an increase in *functional complexity* (i.e., the emergence of more diverse activities or functions).

The variety of an evolving system will slowly increase towards, but will never actually match, the limitless variety of the environment. Depending on the variety of perturbations in its environment, the evolving system will reach a trade-off level

where further increase in complexity will be costly; for instance, for viruses this trade-off level is characterized by a low functional variety (Heylighen 1999). However, as the environment of a system consists itself of evolving systems, the increase in variety in one system generates a stronger need for variety increase in the other since it will now need to control a more complex environment (cf. Waddington 1969). This self-reinforcing interaction is an illustration of the Red Queen Principle (Van Valen 1973), which states that a system must change continuously in order to merely maintain its fitness relative to the systems it co-evolves with. The end result is that many evolutionary systems that are in direct interaction with each other will tend to grow more complex as they need to control more complex environments (Heylighen 1999). Nevertheless, not all evolutionary systems will increase in complexity; those that have attained a good trade-off point and are not challenged by an environment putting more complex demands on them will maintain their current level of complexity. A shift to a less variable environment, as often accompanies a parasitic or endosymbiotic lifestyle, can even lead to a reduction in complexity.

To sum up, although fitness is relative to the environment, it has two components that can increase in an absolute sense, (i) intrinsic fitness (stability) and (ii) extrinsic fitness (control). Selection for increased stability and control, when unopposed by trade-offs, will thus tend to be accompanied by respective increases in structural complexity (number and strength of linkages between components) and functional complexity (the number of environmental perturbations that can be counteracted) (for more discussion, see Heylighen 1999).

Multicellular Complexity

Overview

Here, we define multicellularity as a category of phenotypes that are based on more than one cell. Such phenotypes can be stable and represent the longest part of a life-cycle or be transient (induced in response to external stimuli) and represent a small (or facultative) portion of a life cycle—as in, for instance, myxobacteria (Chap. 22), cellular slime molds (Chap. 21) and some choanoflagellates (e.g., Bonner 2003; Velicer and Vos 2009; Dayel et al. 2011). Multicellular phenotypes can consist of cells that are identical in terms of differentiation potential (here, referred to as simple multicellularity since the parts are identical; i.e., low structural/functional complexity) or a mixture of 2 or more cell types with distinct differentiation potentials (complex multicellularity; high structural and functional complexity); note that this distinction differs from that of Knoll (2011) (also Wolpert and Szathmáry 2002; Schaap et al. 2006; Butterfield 2009) who restricts the term "complex multicellularity" to multicellular organisms displaying intercellular communication and differentiated tissues.

In most lineages, multicellularity develops from a single cell (spore or zygote) whose mitotic products fail to separate (clonal/unitary development). However,

multicellular forms that develop via the aggregation of single-celled individuals (aggregative development) are also known (e.g., myxobacteria, Filasterea and cellular slime molds; see Chaps. 6, 21 and 22). These two developmental pathways result in multicellular phenotypes that differ with respect to the degree of relatedness among cells and the level of complexity they achieved; lower cell relatedness and lower complexity levels characterize lineages in which multicellularity involves aggregation (for a detailed treatment of the issues associated with the two types of development see Grosberg and Strathmann 2007). In this chapter, we are mainly concerned with the evolution of complex multicellularity in lineages with clonal development.

Although multicellularity has evolved independently in at least 25 separate lineages from all three domains of life—Archaea, Eubacteria and Eukaryota (see Chap. 1, King 2004, and Grosberg and Strathmann 2007 for examples and references), multicellular forms with differentiated cell types are only known in a handful of groups (e.g., cyanobacteria and myxobacteria; ciliates; cellular slime molds; red, green and brown algae; land plants; fungi; animals). How and why complex multicellularity evolved, and why some multicellular lineages increased in complexity more than others, are still challenging questions.

Proximate Causes

The transition to multicellularity requires a series of specific mechanisms to ensure (i) the physical unity/stability of the multicellular individual (though generally referred to as adhesion, such mechanisms are rather different among multicellular lineages; see Abedin and King 2010 for a discussion), (ii) communication and recognition among cells (to ensure functional unity/stability), and (iii) regulation of cell growth, proliferation and differentiation (to ensure reproductive unity/stability). Current data (see below) indicate that components of many of these mechanisms were already present in the unicellular ancestors of multicellular lineages.

Indeed, the evolution of simple multicellularity appears to mainly have involved the co-option of existing mechanisms rather than the invention (de novo) of multicellular-specific genes and pathways. For instance, genes that code for proteins associated with adhesion (e.g., integrins, cadherins), cell signaling and cell-cell communication (e.g., tyrosine kinases) predate the evolution of Metazoa (e.g., King et al. 2003, 2008; Abedin and King 2010; Sebé-pedrós et al. 2010, Suga et al. 2013; Chaps. 5, 14, 20). Similarly, in volvocine algae, genes coding for components of the extracellular matrix (which ensures the physical unity and structural stability of the group) have evolved from genes already present in their unicellular ancestors (Prochnik et al. 2010).

Multicellular development and cell differentiation pathways (ensuring functional and reproductive unity and resulting in an increase in structural and functional complexity) have also evolved from pathways present in unicellular lineages—as in, for instance, the cellular slime mold *Dictyostelium discoideum* (Schaap 2011; Chap. 21) and the green alga *Volvox carteri* (Nedelcu 2009b). Likewise, genes involved in

moss development have been found in the closest unicellular relatives of land plants (Nedelcu et al. 2006). Lastly, programmed cell death—thought to be an important developmental mechanism in multicellular lineages—is widespread in the unicellular world (e.g., Nedelcu et al. 2011); and many programmed cell death genes have been found in the genomes of single-celled species (e.g., Nedelcu 2009a). Nevertheless, some gene families coding for proteins involved in multicellular development have evolved specifically during the evolution of multicellularity (e.g., some transcription factor familes in metazoans; Degnan et al. 2009; also see Sebé-Pedrós et al. 2011, Suga et al. 2013, Chap. 18). Relative to simple multicellularity, the evolution of complex multicellularity typically entailed an increase in the number of genes involved in signal transduction and transcriptional regulation (through duplication followed by diversification), the evolution of new protein domains and/or the shuffling of pre-existing domains, and tinkering with the basic genetic toolkit via the modification of patterns of gene expression (through the evolution of new *cis*-regulatory elements) (see Rokas 2008 for further discussion and examples).

Ultimate Causes

Various benefits have been put forward to explain why complex multicellularity evolved. Discussions are mainly centered around the evolution of cell differentiation (used here to refer to spatial cell differentiation resulting in specialized cell types and an increase in complexity), especially in the context of the evolution of specialized reproductive (germ) and somatic cells. These include conflict mediation (i.e., by restricting access to the germ line), division of labor, or overcoming life history trade-offs associated with reproducing a large body (e.g., Buss 1987; Maynard Smith and Szathmáry 1997; Michod 2006; Michod et al. 2006). In addition to explanations involving selective forces, non-adaptive scenarios invoking thermodynamic laws (Otsuka 2008), genetic drift (Lynch and Conery 2003), or passive outcomes of local environmental effects (Schlichting 2003) have also been proposed to explain the increase in complexity during the evolution of multicellularity.

Volvocine Algae as a Model System

Overview

The volvocine algae are a group of green algae (in the Chlorophyceae) comprising both single-celled species, such as *Chlamydomonas*, and multicellular species with various numbers and types of cells (Table 1 and Fig. 1). Volvocine algae have fascinated biologists ever since Antonie van Leeuwenhoek first saw *Volvox* "drive and move in the water" (van Leeuwenhoek 1700, p. 511). In his *Systema Naturae*, Linnaeus, impressed with their ability to roll around without limbs ("*Volvendo*

Table 1 Taxonomy of colonial volvocine algae. Numbers of species are approximations, as the validity of many described species is questionable. Numbers of cells are restricted to powers of 2 (with the possible exception of *Volvox*), so, for example, "8–32" should be understood as 8, 16, or 32 cells

Family	Genus	# of species	# of cells	Differentiated cells	% somatic	Colony morphology
Tetrabaenaceae	*Basichlamys*	1	4	No	0	Cluster
	Tetrabaena	1	4	No	0	Cluster
Goniaceae	*Astrephomene*	2	32–128	Somatic	6–12	Spheroid
	Gonium	10	8–32	No	0	Flat/bowl
Volvocaceae	*Colemanosphaera*	2	16–32	No	0	Spheroid
	Eudorina	8	16–32	No	0	Spheroid
	Pandorina	6	16–32	No	0	Spheroid
	Platydorina	1	16–32	No	0	Flattened[a]
	Pleodorina	6	32–128	Somatic	12–50	Spheroid
	Volvox	25	500–50,000	Somatic & germ	98 +	Spheroid
	Volvulina	4	8–16	No	0	Spheroid
	Yamagishiella	1	16–32	No	0	Spheroid

[a] *Platydorina* develops as a spheroid, undergoing complete inversion, but is secondarily flattened

seque rotando celeriter movens absque artubus!"), gave van Leeuwenhoek's "great round particles" the formal name *Volvox* ("to roll") (Linnaeus 1758, p. 821). An additional 11 genera have been described since: *Gonium* (Müller 1773), *Pandorina* (Bory de Saint-Vincent 1824), *Eudorina* (Ehrenberg 1832), *Pleodorina* (Shaw 1894), *Platydorina* (Kofoid 1899), *Volvulina* (Playfair 1915), *Astrephomene* (Pocock 1954), *Yamagishiella* (Nozaki and Kuroiwa 1992), *Colemanosphaera* (Nozaki et al. 2014), *Basichlamys* and *Tetrabaena* (Nozaki and Itoh 1994; Nozaki et al. 1996). Genus-level taxonomy within the Volvocales is badly in need of revision, as most nominal genera are polyphyletic. It is hard to give an exact number of volvocine species, since many described species are almost certainly synonymous, but by the end of the twentieth century the number of valid described species was probably on the order of 50. In the twenty-first century, new volvocine species are being described at a rate approaching one per year, nearly all by Hisayoshi Nozaki and colleagues (e.g., Nozaki and Krienitz 2001; Nozaki et al. 2006, 2014; Hayama et al. 2010; Nozaki and Coleman 2011; Isaka et al. 2012).

Collectively, the volvocine algae have a cosmopolitan distribution, although the known ranges of particular species can be anywhere from a single pond to multiple continents. The most common habitat is warm, eutrophic freshwater ponds and pools (Kirk 1998), but some species are also found in oligotrophic lakes (Coleman 2001), rivers (Kofoid 1899, 1900; Znachor and Jezberová 2005), rice paddies (Nozaki 1983), damp soils (Bold 1949), snow, and ice (Nozaki and Ohtani 1992; Hoham et al. 2002). Most species are obligate photoautotrophs, but a few, notably *Astrephomene*

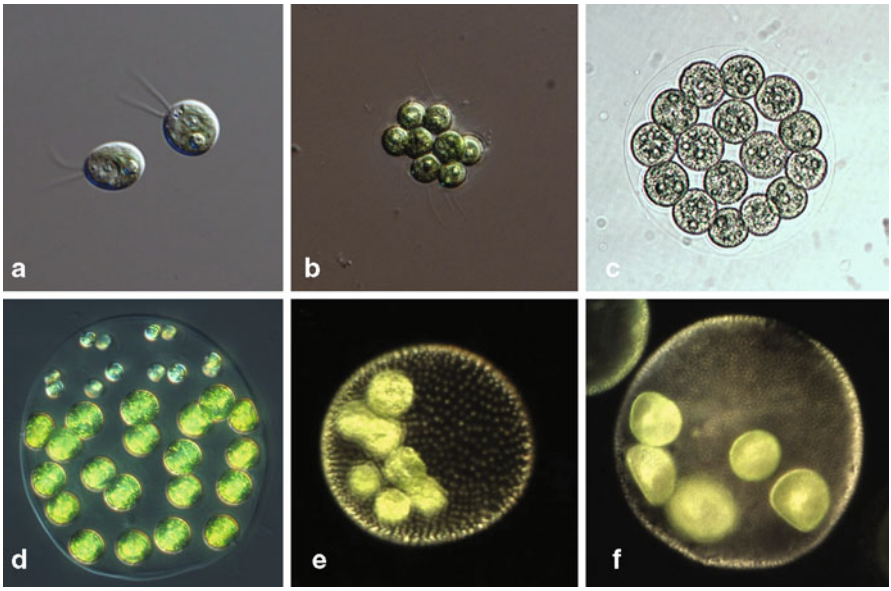

Fig. 1 Representative volvocine algae. **a** *Chlamydomonas reinhardtii*, a unicell. **b** *Gonium pectorale*, a flat plate of 8–32 cells (A and B by D. Shelton). **c** *Eudorina elegans*, a spheroid with up to 32 undifferentiated cells. **d** *Pleodorina starrii*, a partially differentiated spheroid with up to 64 cells. The small cells near the anterior (top) are somatic cells specialized for motility; the larger cells perform both reproductive and motility functions. **e** *Volvox carteri*, a spheroid with ~2000 small somatic cells and a few much larger reproductive cells. **f** *V. barberi*, a spheroid with ~30,000 somatic cells and a few reproductive cells

spp. and some species of *Chlamydomonas*, are mixotrophs that can consume acetate (Pringsheim and Wiessner 1960; Brooks 1972).

The life histories of all known volvocine algae are facultatively sexual, with asexual reproduction occurring in the haploid phase. Like most multicellular organisms, multicellular volvocine algae develop clonally through mitosis from a single cell. However, cell division in asexual development takes an unusual form, called palintomy, in which cells grow to many times their original size, and then undergo several rounds of division without intervening growth (Coleman 1979; Sleigh 1989). In single-celled species (e.g., *Chlamydomonas* spp., *Vitreochlamys* spp.), palintomy is followed by the release of up to 32 daughter cells. In multicellular species, the mitotic products of a given reproductive cell form a daughter colony, which is subsequently released; this process is known as autocolony formation.

The sexual phase of the life cycle is triggered by environmental conditions (nitrogen-deprivation in *Chlamydomonas* and some colonial volvocine algae, or heat-stress in *V. carteri*) or, in some cases, by signaling molecules released by "spontaneously" developed sexual colonies (Kirk and Kirk 1986). In anisogamous and oogamous species, asexual colonies are indistinguishable; the differences between males and females only become apparent upon entry into the sexual phase. Volvocine

sexual reproduction spans a range from isogamy (equally-sized flagellated gametes) to anisogamy (flagellated gametes of unequal size) to oogamy (one gamete is large and non-motile), with larger species tending toward more unequal gametes (Nozaki 1996). Both heterothallic (genetically determined mating types or sexes) and homothallic (both mating types or sexes within an isogenic strain) species exist, and, in homothallic species, individual colonies may produce both types of gametes (monoecy) or there may be separate male and female colonies (dioecy). Although here we are not concerned with aspects related to sexual development, this group is also an ideal system to investigate the evolution of sexes and sexual reproduction (e.g., Nozaki 1996, 2014; Hiraide et al. 2013).

The volvocine algae are an ideal model system for understanding the evolution of multicellular complexity. The origin of multicellularity in this group was probably around 200 million years ago (MYA), much more recent than those of complex multicellular taxa such as animals, land plants, fungi, and red algae (Herron et al. 2009). Furthermore, the years have been kind to this group, as many species with intermediate degrees of complexity survive to this day. For example, the basic body plans of *Astrephomene*, *Gonium*, and *Yamagishiella* appear to be unchanged from their origins ~ 150 MYA (Herron et al. 2009). The two species of volvocine algae that have been intensively studied are at the two extremes of the complexity range in this group: *Chlamydomonas reinhardtii*—a unicellular relative of the colonial volvocine algae (Harris 2001, 2009), and *Volvox carteri* forma *nagariensis*—a multicellular species with ~ 2000 cells and a complete germ-soma division of labor (Kirk 1998, 2005; Fig. 1). Both of these species have sequenced genomes (Merchant et al. 2007; Prochnik et al. 2010), and work is progressing on several other volvocine species (Umen and Olson 2012).

Here, we advocate the development of the entire volvocine clade, that is, the families Volvocaceae, Goniaceae, and Tetrabaenaceae along with closely related unicellular algae, as a model system with which to study the evolution of complexity. Doing so leverages what, in our opinion, is the most attractive feature of this group, the existence of extant species with nearly every conceivable degree of complexity from single cells to differentiated multicellular organisms. Taking this broad view encourages comparative analyses, and this approach has already been successful in addressing questions related to the evolution of cooperation, multicellularity, cellular differentiation, morphology and development, and anisogamy, as well as questions about biomechanics and hydrodynamics. Furthermore, many important traits have evolved convergently, allowing questions about how similar the genetic and developmental mechanisms underlying these traits in different lineages are. In addition, some traits vary within as well as among species, allowing studies that bridge micro- and macro-evolution. Lastly, experimental evolution studies have demonstrated that simple multicellularity can be easily evolved in the lab from various unicellular species, including *C. reinhardtii* (Boraas et al. 1998; Ratcliff et al. 2012, 2013), and volvocine algae can also be used to experimentally evolve cell differentiation. Such studies will allow for experimentally addressing a variety of issues related to major evolutionary transitions, such as biological scaling and multilevel

selection. The results of these experiments can be interpreted comparatively in the context of the extensive extant diversity of volvocine life histories.

What do the Volvocine Algae tell us About the Evolution of Complexity?

The evolution of the volvocine algae has often been viewed in the framework of the 'volvocine lineage hypothesis' (Lang 1963; Van de Berg and Starr 1971; Pickett-Heaps 1975)—the idea that the group members represent a progressive increase in size and complexity from unicellular *Chlamydomonas* to multicellular *Volvox* and that the phylogeny of the group reflects this progression. Within the colonial species, *Gonium* was considered the most 'primitive' (Pickett-Heaps 1975), and *Volvox* was a 'culminating member' of the volvocine lineage (Nozaki and Itoh 1994) and ' . . . the ultimate expression of colonial development' (Pickett-Heaps 1975). Within the genus *Volvox*, *V. powersii* and *V. gigas* were viewed as the most 'primitive', and either *V. carteri* and *V. obversus* or the members of the section *Volvox* (e.g., *V. barberi, V. globator*) were thought to be the most 'advanced' (Desnitski 1995). Nevertheless, we now know that the volvocine lineage hypothesis is an oversimplification of volvocine phylogeny; in fact, complex multicellularity evolved independently in several '*Volvox*' lineages (Herron and Michod 2008; Herron et al. 2010; Fig. 2).

Below, we discuss morphological and developmental complexity in this group, using Heylighen's and McShea's frameworks. In doing so, we address both why and how complexity increased in this group (in several lineages).

Morphological/Structural Complexity

Regardless of developmental type, the cells in a multicellular organism must have some way of adhering to each other; in Heylighen's (1999) framework, the parts/cells have to fit together to form an intrinsically stable whole. Various multicellular groups achieve this in different ways (Abedin and King 2010). In the volvocine algae, cells are held together by an extracellular matrix (ECM) that is homologous to the cell wall in unicellular relatives (Kirk et al. 1986). ECM ensures both physical unity and structural stability ('intrinsic' fitness). Compared to unicellular algae, whose daughter cells are free to realize independent fates, the fates of daughter cells in multicellular algae are inextricably bound. The daughters of a given reproductive cell share a physical location and a set of internal and external conditions. As parts of the newly formed multicellular group, the daughter cells contribute to the increase in the *structural hierarchical complexity* of the system.

Interestingly, transitions between compact colonies such as *Pandorina*, which contain very little ECM, and larger colonies such as *Eudorina* (Fig. 1), with large volumes of ECM, have occurred multiple times in both directions (Herron and Michod 2008; Fig. 2). The volume of ECM scales allometrically with colony size,

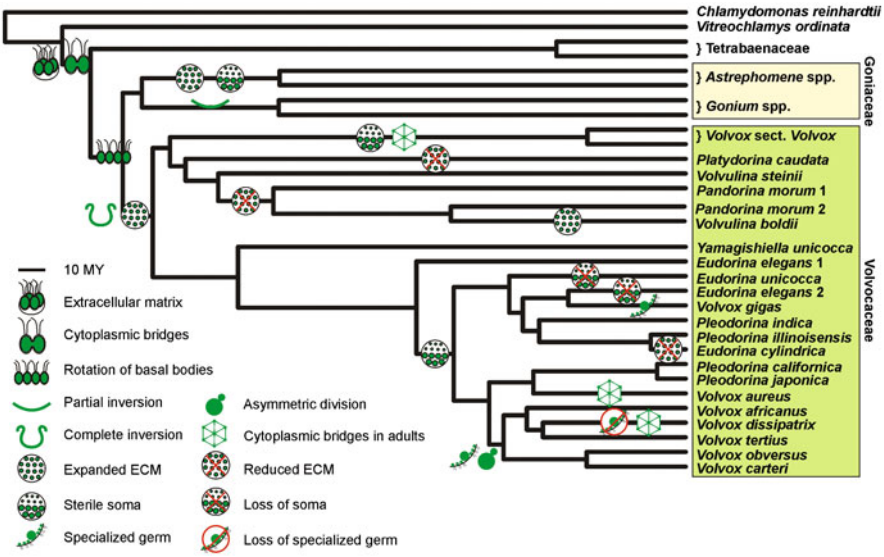

Fig. 2 Reconstructed changes in ancestral character states. Developmental changes shown are those supported by hypothesis tests in Herron and Michod 2008, Herron et al. 2009, 2010. Note that some nominal genera and species are polyphyletic and thus appear in more than one place in the tree

with the largest colonies (*Volvox* spp.) consisting of > 99 % ECM. This allometric increase suggests that the ECM might serve additional roles or have additional selective benefits. Indeed, in the larger species, the ECM is a complex organ with a great deal of internal structure that varies among species (Kirk et al. 1986; Nozaki and Kuroiwa 1992). Functionally, the ECM plays important roles in sex induction (Starr 1970; Gilles et al. 1983), inversion (discussed below; Ueki and Nishii 2009), and possibly nutrient storage (Koufopanou and Bell 1993; also see Chap. 11). In the *V. carteri* lineage, expansion of the ECM was accompanied by expansions of two of the gene families involved in its construction (Prochnik et al. 2010; Umen and Olson 2012). Thus, although the ECM evolved from a pre-existing structure (the cell wall) and a pre-existing set of genes, its expansion and differentiation have contributed to both *structural and functional complexity*.

In all members of the Goniaceae and Volvocaceae, during early embryonic development cells are connected through cytoplasmic bridges resulting from incomplete cytokinesis (Gerisch 1959; Bisalputra and Stein 1966; Gottlieb and Goldstein 1977; Marchant 1977; Fulton 1978; Green et al. 1981; Iida et al. 2013). These bridges ensure the stability of the early embryo. In most species, the bridges break down later in development, leaving cells physically unconnected; nevertheless, the stability of the system (and its 'intrinsic' fitness) is maintained by the ECM. In some species of *Volvox*, though, cytoplasmic bridges are retained in adult colonies, a paedomorphic trait (juvenile trait retained in adults) that has apparently arisen convergently

in three independent lineages (Herron et al. 2010; Fig. 2). By nearly any definition, the cytoplasmic bridges contribute importantly to the complexity of volvocine colonies. In structural terms, cytoplasmic bridges increase the connectedness of cells (and the stability of the system), at least during early development. In *Gonium* and in the Volvocaceae, the cytoplasmic bridges are essential for inversion, a process that requires a high degree of functional integration (nonhierarchical process complexity, in McShea's terminology). Whether or not the cytoplasmic bridges in any or all colonial volvocine algae play other integrative functional roles, e.g., in cell-cell communication, remains unknown. Similarly, the functional significance of the retention of cytoplasmic bridges in the adults of some *Volvox* species is unknown, though its convergent evolution in three independent lineages (Herron et al. 2010; Fig. 2) suggests that some functional role exists.

Increased complexity through cell differentiation (*structural complexity* or *object nonhierarchical complexity* in Heylighen's or McShea's fameworks, respectively) occurs only in species that have at least 32 cells; that is, in the genera *Astrephomene*, *Pleodorina*, and *Volvox* (Table 1). This trend is consistent with the general view that increase in organismal size through increase in the number of cells increases the potential for increase in diversity of cell types (and thus increase in complexity) (Carroll 2001). *Astrephomene* and *Pleodorina* species possess only one specialized cell type. In these species, initially, all cells look alike (identical parts); later, most cells lose flagella and become reproductive, while several cells (at the posterior and anterior pole in *Astrephomene* and *Pleodorina,* respectively) remain flagellated and act as terminally differentiated, non-replicative somatic cells (Table 1). Two specialized cell types—terminally differentiated somatic and germ cells (gonidia) lacking flagella—are only present in *Volvox* species, which are also the largest in terms of number of total cells (Table 1).

Developmental/Functional Complexity

In addition to the broad range of morphological complexities, the volvocine algae also exhibit varying degrees of functional complexity, which are apparent in their developmental processes, including organismal polarity, inversion, and cell differentiation.

In the Goniaceae and Volvocaceae, organismal polarity is established through rotation of the basal bodies, which are attached to the flagella. In single-celled volvocine algae such as *Chlamydomonas*, the basal bodies are arranged in such a way that the two flagella beat in opposite directions (Kirk 2005). As a result, the cell swims in a "breast stroke," and the cell has an anterior-posterior polarity defined by the direction of swimming (Kirk 2005). This arrangement is retained in the four-celled species, *Tetrabaena socialis* and *Basichlamys sacculiferum*, which are the only known members of the family Tetrabaenaceae (Stein 1959; Nozaki and Itoh 1994). In *Gonium*, however, the basal bodies of the peripheral cells are rotated such that the flagella beat in the same direction, toward the periphery of the colony (Greuel and Floyd 1985; Kirk 2005). The four central cells retain the ancestral

orientation, giving *Gonium* colonies a center-to-edge polarity at the colony level (Kirk 2005). The resulting difference between cells in the center and those on the periphery constitute an increase (relative to colonies without basal body rotation) in structural and functional differentiation. In spheroidal colonies, rotation of the basal bodies results in all flagella beating in roughly the same direction, establishing an anterior-posterior polarity, as in *Chlamydomonas*, based on the direction of swimming (Kirk 2005).

In *Gonium* and in the Volvocaceae, embryos at the end of cell division find themselves in an awkward configuration. Embryos at this stage are shaped as shallow bowls or spheres, but in either case, the flagella are on the wrong (concave or interior) surface for locomotion (Kirk 2005). Through a process of partial (*Gonium*) or complete inversion (Volvocaceae), the embryos change their topology so that the flagella end up on the convex (*Gonium*) or exterior (Volvocaceae) surface (Stein 1965; Fulton 1978; Kirk 2005). Inversion requires a high degree of functional integration among cells, as it is the movement of individual cells relative to the their cytoplasmic bridges that generates the emergent phenomenon of coordinated collective movement (*functional/process hierarchical complexity*) (Green et al. 1981).

Cellular differentiation occurs in three volvocine genera: *Astrephomene*, *Pleodorina*, and *Volvox*. In *Pleodorina* and *Astrephomene* embryos, all cells start at the same size, but a subset near the anterior (*Pleodorina*) or posterior (*Astrephomene*) either fail to grow or grow at a slower rate, resulting in adult colonies with two cell sizes. The larger cells behave as cells in undifferentiated species, initially providing flagellar motility but then eventually reproducing and losing the flagella. The smaller cells, though, perform vegetative functions only and never reproduce. The evolution of cellular specialization in these genera constitutes an increase not only in variety/differentiation (*structural complexity*), but also in functional integration (*functional/process non-hierarchical complexity)*, as cells become completely dependent on each other for the basic life-history functions of survival and reproduction.

In some *Volvox* species, as in *Pleodorina* and *Astrephomene*, at the end of embryogenesis all cells are similar in size. Some cells then grow slightly or not at all and differentiate as somatic cells, while other cells lose their flagella and grow to thousands of times their original size before producing a new generation. In a few closely related species of *Volvox*, though, during embryogenesis some cells undergo several rounds of asymmetric divisions, resulting in an embryo with two cell sizes (Fig. 2). The smaller of these cells will differentiate as somatic cells, while the larger cells become specialized reproductive cells (gonidia), with no flagella and no contribution to the motility of the individual. The evolution of asymmetric divisions (which take place only in half of the embryo, by an unknown mechanism) contributed to increased functional/process complexity in these lineages.

Both somatic cells and gonidia became specialized cells by losing functions that were present in the ancestral, generalist cells: somatic cells have lost reproductive functions, while reproductive cells have lost motility/survival functions. This is consistent with the pattern described by McShea (2002) that lower-level entities that have combined to form a higher-level entity tend to undergo a reduction in their own complexity.

Genomic Complexity vs. Morphological and Developmental Complexity

Despite the fact that *V. carteri* is morphologically and developmentally more complex than *C. reinhardtii,* a comparison between these two volvocine genomes did not reveal a significant difference in the total number of predicted genes (14,520 in *V. carteri vs.* 14,516 in *C. reinhardtii*) (Prochnik et al. 2010). Nevertheless, the number of genes involved in specific multicellular structures and developmental functions appears to be higher in *V. carteri* relative to *C. reinhardtii*. Specifically, *V. carteri* possesses a higher number of genes involved in ECM and cell-cycle regulation; furthermore, the *V. carteri* genome is enriched in volvocine-specific genes of unknown function, some of which might be involved in complexity-related traits specific to this group (Prochnik et al. 2010). Also, the TAZ family of transcription factors is more represented in *V. carteri* relative to *C. reinhardtii* (our unpublished data).

Interestingly, orthologs of *regA*—the gene responsible for the differentiation of somatic cells in *V. carteri*—have been recently found in several distantly related *Volvox* species (e.g., *V. gigas*), suggesting that this gene was already present in a volvocine ancestor without specialized somatic cells (Hanschen et al. 2014; Fig. 2). This scenario implies that the evolution of somatic cell differentiation and the increase in complexity observed in the lineage leading to *V. carteri* involved changes in the regulatory elements of *regA* (whether *regA* has a role in somatic cell differentiation in the other *Volvox* species is not yet known). Furthermore, *regA* appears to have evolved from a *regA*-like gene already present in the unicellular ancestor of volvocine algae; its co-option might have involved changing its expression pattern from a temporal context (in response to environment) into a spatial (developmental) context (Nedelcu and Michod 2006; Nedelcu 2009b).

Similarly, two other genes involved in complexity-related traits in *V. carteri* also have orthologs in the unicellular *C. reinhardtii*. These are the *glsA* gene involved in the asymmetric divisions responsible for setting aside the large cells that will develop into gonidia (Kirk et al. 1991); and the *invA* gene involved in the process of embryonic inversion (Nishii et al. 2003). In both cases, the *C. reinhardtii* ortholog can rescue a *V. carteri* mutant (Kirk et al. 1986; Kirk 2005), indicating that the difference in function between the two orthologs does not involve changes at the protein level.

Altogether, the available genomic information supports the idea that overall gene content is not a good indicator of organismal complexity, and points towards an increase in genome complexity through gene duplication and co-option via changes in regulatory elements as being mainly responsible for the observed increase in morphological and developmental complexity in this group. These findings are consistent with the general trends identified during the evolution of morphological complexity (discussed earlier).

The sequencing of additional volvocine genomes from lineages with different grades of morphological and developmental complexity (which is underway in several labs) will make it possible to further investigate the relationship between genomic complexity and organismal complexity among closely related species, thus avoiding some of the confounding factors associated with comparing species that have diverged a long time ago. The significance and relative contribution of mutations in

coding regions vs. changes in gene regulation to the genetic basis for the evolution of new morphological traits is currently an issue of heated debate (Hoekstra and Coyne 2007; Carroll 2008). Due to their relatively low but variable levels of complexity, simpler underlying genetics and recent evolutionary history, volvocine algae have the potential to provide significant insights into this debate.

Independent Increases in Complexity

Interestingly, multicellular volvocine algae are monophyletic, suggesting that simple multicellularity evolved only once in this group (Nozaki 2003). However, complex (differentiated) multicellularity appears to have evolved independently several times. For instance, sterile somatic cells, specialized germ cells, and retention of cytoplasmic bridges in adult spheroids each occurred independently, multiple times, within the volvocine algae (Herron and Michod 2008; Herron et al. 2010; Fig. 2). Nevertheless, despite the apparent ease of evolving complexity-related traits, the number of cell types remained low in all volvocine species. Furthermore, it appears that in some cases, morphologically-complex lineages evolved towards simplification. For instance, both forms of cellular differentiation have apparently been lost in some lineages within the Volvocaceae (Herron and Michod 2008; Fig. 2). These observations raise a number of questions. What were the factors that contributed to the independent increases and decreases in complexity in this group? Why did not all volvocine species reach the complexity levels attained by some *Volvox* species? And why did none of the *Volvox* lineages reach even higher complexity levels?

Following his analysis of morphological complexity, Carroll (2001) concluded that "the observed limits of form seem to be due to a combination of both chance and necessity, a product of historical contingency and imposed by external agents (for example, selection) and internal rules (for example, constraints)". He further argues that selection cannot be the whole story, and that the internally imposed constraints also shape the range of possible morphologies and can themselves evolve. Volvocine algae exemplify these statements very well; the evolution of complexity in this group is likely a reflection of (i) historical contingencies associated with the specific cellular and genetic background of the *Chlamydomonas*-like ancestor, (ii) specific developmental constraints, and (iii) diverse selective pressures.

For instance, the evolution of multicellularity in the volvocine algae is thought to have been facilitated by the specific type of cell division (palintomy) that was inherited from the *Chlamydomonas*-like ancestor (Kirk 1998). In addition, the mechanistic basis for the evolution of somatic cell differentiation (at least in *V. carteri*) can also be traced back to the ability of single-celled volvocine ancestors to temporarily repress their reproduction to increase survival, as part of their general photo-acclimation response to limiting or stressful conditions (Nedelcu and Michod 2006; Nedelcu 2009b). However, the very same factors that allowed some lineages in this group to achieve high levels of complexity may have affected how complex they could become. For instance, although palintomy has been replaced by binary fission in some *Volvox* lineages (Herron et al. 2010), other *Volvox* species have retained palintomy,

which has limited their potential to evolve increased numbers of cells (to produce *n* cells, gonidia need to grow 2^n-fold in volume). More importantly, the fact that the evolution of somatic cells involved the permanent suppression of cell division (instead of its temporal and/or spatial regulation) has likely limited its potential to evolve new cell types and thus affected the evolvability of this lineage (Nedelcu and Michod 2004).

The independent increases and decreases in size and complexity observed in this group suggest that strong selective pressures to increase or decrease complexity levels did (do) exist and that such pressures differ among the environments or the ecological niches these algae inhabit. These pressures are thought to include predator avoidance, motility, and nutrient availability (see Chap. 11), but a detailed analysis looking for a correlation between specific environmental factors, organismal size and complexity levels displayed by the volvocine algae inhabiting a specific environment/ecological niche has not been performed. Based on Ashby's Law of Requisite Variety (discussed above) we predict that in lineages that evolved large colony sizes (in response to selection for large body size) increased levels of complexity correlate to increased variability in their environment/niche. In other words, we suggest that more variable environments selected for more complex forms. This relationship between complexity increase and environmental variability might also be used to address why complex volvocine algae evolved from a *Chlamydomonas reinhardtii*-like ancestor; or why complex multicellularity (with specialized somatic and reproductive cells) did not evolve from other unicellular volvocalean ancestors. Specifically, like the extant *C. reinhardti*, it is possible that such an ancestor was adapted to variable environments and already possessed mechanistic and genetic factors that allowed control over the environment. Lineages that evolved multicellularity in response to selection for increased size have co-opted these mechanisms and use them to control variable environments in new ways. That not all lineages reached the complexity levels achieved by some *Volvox* species might reflect the fact that some species attained a good trade-off point and are not challenged anymore by their environment.

Concluding Remarks

Volvocine algae have been studied for a long time and used to address various questions from very diverse fields. Here, we argue that because complexity levels have increased or decreased independently in several lineages, this group is an ideal model-system to investigate the evolution of complexity. The independent acquisitions and losses of traits associated with complexity in this group represent an unprecedented opportunity to (i) explore the genetic basis and the selective pressures responsible for such changes in complexity levels, (ii) distinguish between the mechanisms that have been proposed to explain increases in complexity in biological systems—passive (random) vs. active/"driven" (non-random) processes, and external (affected by selection, ecology, environment) vs. internal (under genetic, developmental, biomechanical control) (McShea 1994; Carroll 2001), and (iii) identify general principles underlying the evolution of complexity.

Summary

1. Complexity is a complex concept, which has proven rather difficult to define and measure.
2. There is no a priori reason that more complicated systems would be preferred by natural selection. Evolution selects for increases in fitness, but high fitness can be achieved both by very complex and very simple systems.
3. Selection for increased stability and control over the environment will tend to be accompanied by respective increases in structural/morphological complexity and functional/developmental complexity.
4. How and why complex multicellularity evolved, and why some multicellular lineages increased in complexity more than others, are still challenging questions.
5. The volvocine algae—comprising species with nearly every conceivable degree of complexity from single cells to differentiated multicellular organisms—are an ideal model system for understanding the evolution of multicellular complexity.
6. The independent acquisitions and losses of traits associated with complexity in the volvocine group represent an unprecedented opportunity to (i) explore the genetic basis and the selective pressures responsible for such changes in complexity levels, (ii) distinguish between the mechanisms that have been proposed to explain increases in complexity in biological systems, and (iii) identify general principles underlying the evolution of complexity.

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