

The origins of multicellular organisms

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SUMMARY Multicellularity has evolved in several eukaryotic lineages leading to plants, fungi, and animals. Theoretically, in each case, this involved (1) cell-to-cell adhesion with an alignment-of-fitness among cells, (2) cell-to-cell communication, cooperation, and specialization with an export-of-fitness to a multicellular organism, and (3) in some cases, a transition from “simple” to “complex” multicellularity. When mapped onto a matrix of morphologies based on developmental and physical rules for plants, these three phases help to identify a “unicellular \Rightarrow colonial \Rightarrow filamentous (unbranched \Rightarrow branched) \Rightarrow pseudoparenchymatous \Rightarrow parenchymatous” morphological transformation series that is

consistent with trends observed within each of the three major plant clades. In contrast, a more direct “unicellular \Rightarrow colonial or siphonous \Rightarrow parenchymatous” series is observed in fungal and animal lineages. In these contexts, we discuss the roles played by the cooptation, expansion, and subsequent diversification of ancestral genomic toolkits and patterning modules during the evolution of multicellularity. We conclude that the extent to which multicellularity is achieved using the same toolkits and modules (and thus the extent to which multicellularity is homologous among different organisms) differs among clades and even among some closely related lineages.

INTRODUCTION

One of the most remarkable events in evolutionary history was the emergence and radiation of eukaryotic multicellular organisms (Valentine 1978; Bonner 1998, 2012; Maynard Smith and Szathmáry 1995; Knoll 2011). Perhaps even more remarkable is that this “event” occurred independently in different clades. Estimates of the exact number vary depending on how multicellular is defined. When defined simply as cellular aggregation, a conservative estimate is that multicellularity evolved over 25 times (Grosberg and Strathmann 2007). More stringent definitions requiring sustained cell-to-cell interconnection and communication obtain an estimate of ten eukaryotic events, that is, once in the Animalia, three in the Fungi (chytrids, ascomycetes, and basidiomycetes), and six in the three major plant clades (twice each in the rhodophytes, stramenopiles, and chlorobionta).

Regardless of the number, the multiple origins of multicellularity and their subsequent consequences evoke a number of biologically important, but largely unanswered, questions. For example, do multicellular lineages share a common morphological transformational series? What if any are the selection barriers to (and the drivers toward) multicellularity? Were the ancestors of some lineages predisposed to engender multicellular organisms, or is multicellularity the result of random events leading toward larger organisms. Put differently, are the morphological motifs that emerge in multicellular lineages the result of adaptive evolution, or the inevitable consequences of

physical laws and processes? Indeed, are the multiple origins of multicellularity truly independent given that all life ultimately shared a last common ancestor?

These and other questions about multicellularity have been addressed in different ways (e.g., Bonner 2012; Niklas 2000; Kirk 2005; Newman and Bhat 2008, 2009; Newman 2011; Knoll 2011). However, all perspectives share three features: (1) a comparative approach (because of the multiple origins of multicellularity, sometimes even within the same clade); (2) a treatment of how “information” is exchanged among cells and between cells and their external environment (because coordinated signaling among cells is one of the defining characteristics of multicellular biology; see Mian and Rose 2011); and (3) a consideration of functional morphological features (because these govern energy–mass exchange rates between an organism and its environment; see Gates 1980).

We address these features as well. However, our primary objective is to determine, as best as currently possible, whether the evolutionary trajectory toward multicellularity manifests a common trend across as well as within clades and, if so, whether this trend is the result of genomic or physical commonalities among otherwise diverse organisms. Although we discuss trends in the fungal and animal clades, our focus is primarily on plants, which we define broadly as eukaryotic photoautotrophs (Niklas 1997, 2000) to encompass the algae as well as the monophyletic land plants (embryophytes). This phyto-centrism is adopted because (1) multicellularity evolved independently at least six times in the three major plant clades, which permits extensive

interphyletic comparisons, and (2) the origins of plant multicellularity have been largely neglected in a primarily zoo-centric literature. A third reason for focusing on plants is that all plant clades evolved cell walls that, in contrast to animals, can restrict intercellular aggregation and communication (and requires somatic embryogenesis in multicellular plants).

In the following, we (1) characterize multicellular organisms in terms of intercellular adherence and cell-to-cell and cell-to-environment communication, (2) explore the requisite transition from fitness defined at the level of individual cells to fitness defined at the level of a truly multicellular entity (Wolpert and Szathmáry 2002; Michod et al. 2003; Grosberg and Strathmann 2007; Folse and Roughgarden 2012), (3) assess the transition from simple to complex multicellularity (*sensu* Knoll 2011), (4) compare character polarities among the different plant, fungal, and animal clades, and (5) discuss whether the evolution of multicellular organisms was instigated by physically based patterning modules mobilized by shared or unique molecular toolkits (Newman and Bhat 2009; Hernández-Hernández et al. 2012).

We will affirm that the evolution of multicellular organisms typically involved intermediate body plans that were achieved by similar developmental mechanisms in different lineages, but not necessarily by mechanisms sharing the same physical or biochemical components. Much like the plant organs collectively called “leaves,” which evolved independently in different lineages, multicellularity is a recurrent feature of morphological evolution that was reached in many different ways. Consequently, the extent to which multicellular organisms are developmentally homologous at the most basic levels requires careful analyses, particularly since selection acts on functional traits and not on their underlying generative mechanisms, enabling different mechanisms to achieve the same functional traits (Marks and Lechowicz 2006).

EVOLUTIONARY PHASES

Multicellularity has been defined in different ways because of different taxonomic, developmental, morphological, physiological, or genomic frames of reference. Here, instead of attempting to define this condition, we focus on two features that enter into virtually all discussions of multicellular organisms—cell-to-cell adherence, the *condicio sine qua non* of multicellularity across all clades, and cell-to-cell communication, the basis of multicellular development. However, despite these commonalities, the cellular and molecular bases of the modes of adherence and communication differ markedly among clades. Thus, the Ca²⁺-rhamnogalacturonic-dominated composition of the middle lamella that binds embryophyte cell walls together differs chemically from the Type-1 transmembrane cadherin proteins responsible for animal cell adhesion (Hulpiau and van Roy 2009), or the glycoprotein-based glues produced by many fungi

(Epstein and Nicholson 2006). Likewise, the intercellular interconnections in the green alga *Volvox* differ significantly from embryophyte plasmodesmata, mammalian gap-junctions, or fungal intercellular septal pores that all nevertheless provide avenues for cell-to-cell communication. Just as in cladistics, “cell-to-cell adhesion” and “intercellular communication” can be thought of as “characters” that assume different “character states” depending on a lineage’s phyletic legacy.

If cell-to-cell adhesion and communication are requisite for the evolution of multicellular organisms, their basic elements must have ancestral unicellular character states but not necessarily manifesting the same functionalities. For example, molecular analyses identify a diversity of cadherins in the unicellular choanoflagellate *Monosiga brevicollis* (a descendent of the unicellular metazoan progenitor) that likely function in environment-responsive intracellular signal transduction, for example, tyrosine kinase and hedgehog signaling (Abedin and King 2008). Likewise, adherens junctions tethering metazoan cells occur in sponge epithelia, albeit in a rudimentary form (Abedin and King 2010; Suga et al. 2012). One pathway to fungal multicellularity illustrated by dictyostelid slime molds (Bonner 2012) shares elements with the evolution of animal multicellularity. Upon starvation, a developmental cascade is instigated involving diverse cell-to-cell-to-substrate adhesion, for example, membrane bound DdCAD-proteins facilitating cell contact play a role similar to that of animal cadherins (Nelson 2008; Bonner 2012). Indeed, the evolution of multicellularity in the fungi may have been rapid. Using differences in settling velocities to separate unicellular and clustered *Saccharomyces cerevisiae* cells, Ratcliff et al. (2012) isolated isogenic “snowflake” genotypes with a simple cellular division of labor and multicellular propagules.

Multicellular organisms can evolve along different pathways even within the same clade. Consider the chlorobionta. In the volvocine algae, multicellularity likely evolved by differential modifications of cell wall layers in a *Chlamydomonas*-like progenitor (Kirk 2005). Specifically, the walls of unicellular volvocines (e.g., *Chlamydomonas*) are composed primarily of hydroxyproline-rich glycoproteins and are separated into a structured outer layer and a more amorphous inner layer (Fig. 1). Among colonial volvocines (e.g., *Gonium*), adhering cells are interconnected by struts that preserve the features of the outer cell wall layer (Fig. 1). However, among multicellular volvocine algae (e.g., *Pandorina* and *Volvox*), the entire plant is surrounded by a layer that preserves features of the ancestral outer wall layer by virtue of an unusual pattern of cell division (palintomy) in which cells undergo multiple divisions without increasing in total cytoplasmic volume (Herron et al. 2010). High protein sequence similarities among unicellular and multicellular volvocines support this scenario; for example, homologs of the *Chlamydomonas* outer cell wall protein GP2 occur in *Pandorina* and *Volvox* (Adair and Appel 1989). In contrast, cell adhesion in the evolutionarily related embryophytes involves a

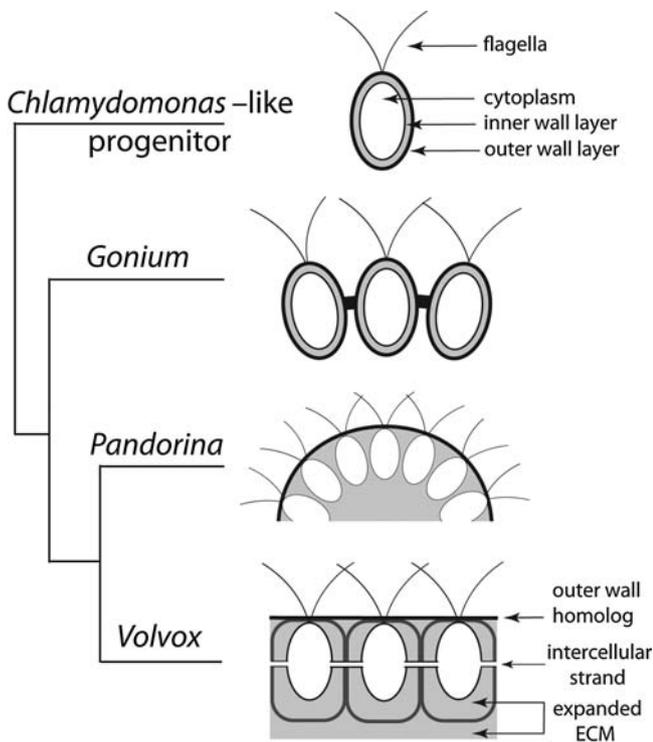


Fig. 1. Putative evolution of multicellularity in the volvocine algae (adapted from Kirk 2005). The inner cell wall layer of a unicellular *Chlamydomonas*-like progenitor is modified into an expanded extracellular matrix (ECM) wherein multicellularity is achieved by intercellular cytoplasmic strands (e.g., *Volvox*). The outer cell wall layer adheres cells in colonial organisms (e.g., *Gonium*).

middle lamella enriched with pectins, a functionally and structurally diverse class of galacturonic acid-rich polysaccharides (Caffall and Mohnen 2009). Chemical modification of pectins contributes to changing the mechanical properties of cell walls (Peaucelle et al. 2011; Wolf and Greiner 2012), which includes cell wall relaxation in concert with indole-3-acetic-acid (Niklas and Kutschera 2012). Because similar systems are reported for unicellular green algae (Musatenko 2005), it is possible that embryophyte cell adhesion evolved by modifications of the fundamental mechanism of cell wall expansion operating in unicellular progenitors.

Under any circumstances, cell-to-cell adhesion in one form or another participates in the life cycles of nearly every unicellular organism (e.g., *Chlamydomonas* gamete-aggregation) and there is ample evidence that cell adhesion (and even limited cellular cooperation) is ancient; for example, the expression of the flocculation *FLO1* gene resulting in *S. cerevisiae* cell clustering (Smukalla et al. 2008) and *Chlorella* (a unicellular green alga) genome homologues of *Arabidopsis* auxin receptors (Blanc et al. 2010). However, although it is a necessary condition for a multicellular body plan, cell adhesion is insufficient. What

prevents palmelloid *Chlamydomonas*, colonial choanoflagellates, and other similar “cellular aggregate” life-forms from being multicellular organisms is the failure to constitute biological entities possessing sustained cell-to-cell communication and cooperative interactions among formerly individualized cells (Buss 1987; Bonner 1998), a feature that permits opportunities for the evolution of cellular specialization (Wolpert and Szathmáry 2002; Michod et al. 2003). In multicellular plants and fungi, this capacity may have evolved by the co-option of prokaryotic two-component signaling pathways involving histidine kinases, response regulators, and in some cases histidine-containing phosphotransfer proteins (see Schaller et al. 2011), which have been identified in a broad spectrum of eukaryotes including the centric diatom *Thalassiosira*, *Chlamydomonas*, *Dictyostelium*, a variety of fungi, and *Arabidopsis* (Anatharaman et al. 2007). Indeed, the molecular basis for cell-to-cell adhesion and communication may have evolved simultaneously in some cases; for example, metazoan tetraspanin-enriched protein/membrane microdomains participate in cell–cell adhesion and communication as well as membrane fusion and cell migration (Bailey et al. 2011; Wang et al. 2012).

But how does a cellular aggregate achieve individuality? One suggestion is that two evolutionary stages are required—an *alignment-of-fitness* phase in which genetic similarity among cells prevents cell–cell conflict and an *export-of-fitness* phase in which cells become interdependent and collaborate in a sustained effort (reviewed by Folse and Roughgarden 2012). The first phase can be achieved by any “unicellular bottleneck” in an organism’s life cycle, for example, spores or zygotes. The second phase requires intercellular cooperation sufficient to shift selection from the level of individual cells to the level of a multicellular entity that reproduces similar entities with a heritable fitness, typically followed by some degree of cellular specialization. Although the separation of soma and a germ-line sometimes occurs, obligate sexual reproduction is not required to override the conflict between the individual and its constituent cells (Buss 1987; Michod 1997; Nanjundiah and Sathe 2011). Certainly, in the absence of somatic mutations, the presence of a zygote in any life cycle assures genetic homogeneity by providing a unicellular bottleneck regardless of the type of life cycle (Fig. 2). Likewise, it can be difficult for asexual organisms to escape the consequences of Muller’s ratchet (the inevitable accumulation of deleterious mutations). However, asexual multicellular organisms also experience an alignment-of-fitness by means of unicellular or cloned propagules (Fig. 2).

Likewise, multicellularity is not required for the evolution of cellular specialization. Unicellular bacteria, algae, yeast, and amoeba exhibit alternative stable states of gene activity and even morphology during their life cycles, often as a result of competing processes, for example, motility versus mitosis. The origin of cellular differentiation therefore may reside in the inherent multistability of complex gene regulatory networks

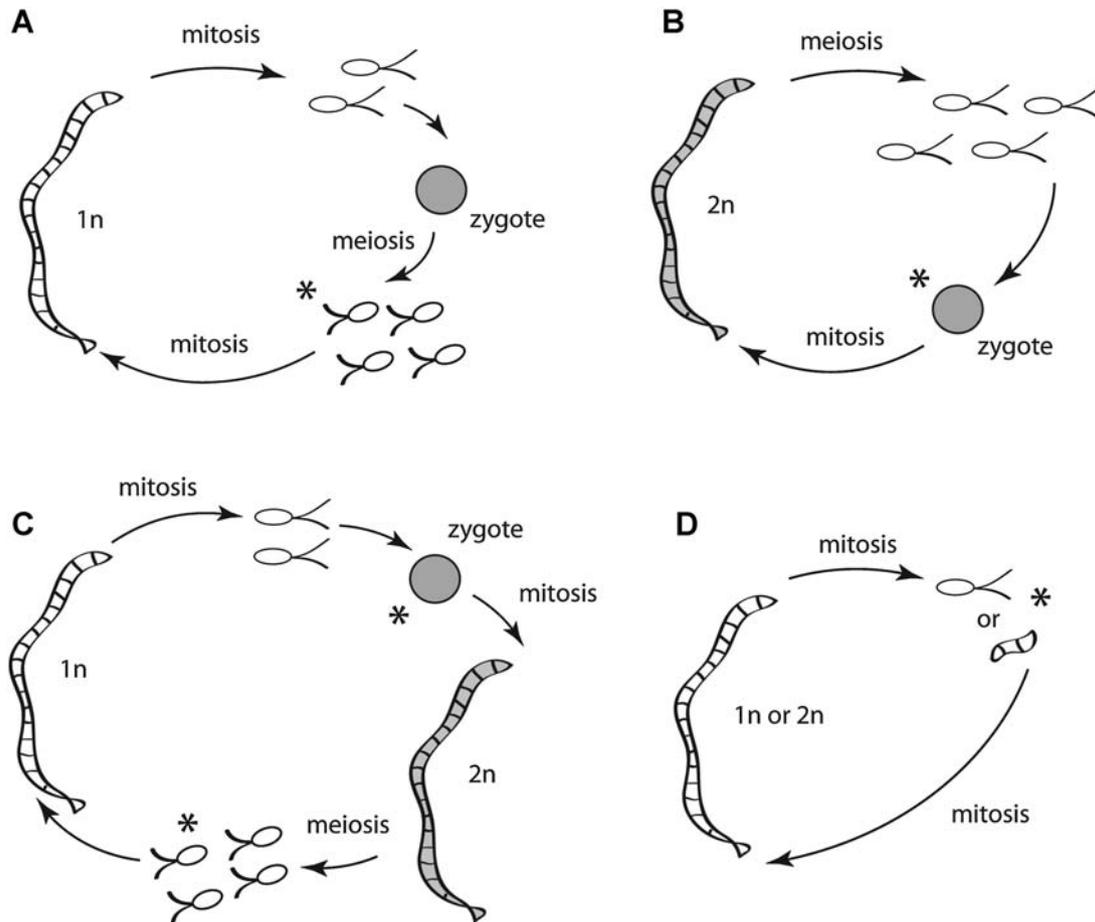


Fig. 2. Schematics of four contrasting life cycles each with one or more “unicellular bottlenecks” (indicated by an asterisk). Diploid cells and organisms are shaded; haploid cells and organisms are unshaded. For simplicity, each organism is depicted as a hermaphrodite. (A) The haplobiontic-haploid life cycle in which the multicellular phase develops after zygotic meiosis. (B) The haplobiontic-diploid life cycle in which the multicellular phase develops from zygotic mitosis. (C) The diplobiontic life cycle containing two multicellular individuals, one developing after zygotic mitosis and another developing from meiosis. (D) An asexual life cycle in which the multicellular organism and its unicellular (or cloned) propagules resulting from mitotic cellular division (or fragmentation).

(Laurent and Kellershohn 1999) with somatic or reproductive functional roles for different cell-types possibly established ad hoc by natural selection. Thus, mathematical models indicate that cellular differentiation can emerge among genetically identical cells as a response to poor compatibility among competing physiological processes (Ispolatov et al. 2012). In more derived lineages, an alignment of fitness can compensate for conflicts of interest among cellular components such that a division of cellular labor becomes possible and even necessary.

SIMPLE VERSUS COMPLEX MULTICELLULARITY

Some authors distinguish between “simple” and “complex” multicellularity (Butterfield 2000; Schlichting 2003). This distinction has focused recently on whether all cells make

contact with their external environment, or whether some are internalized (Knoll 2011), for example, unbranched filamentous morphologies and organisms with tissue systems, respectively. This difference is important because (1) it is correlated with differences in cell specialization, energy consumption per gene expressed, and increases in non-protein-coding DNA (Bonner 2004; Lane and Martin 2010; see, also, Lozada-Chavez et al. 2011), (2) it helps to assess the likelihood that a multicellular organism can evolutionarily revert to a unicellular state (e.g., a complex multicellular to unicellular transition is far less likely than a colonial to unicellular transition), and (3) it helps to identify when a simple multicellular organism evolutionarily reaches a size or morphology that necessitates tissue specialization for the bulk transport of nutrients.

Consider the consequence of size on passive diffusion as shown by a variant of Fick’s law of diffusivity, which estimates the time required for the concentration of a neutral molecule i

initially absent within a cell to reach 50% of the external concentration, that is, $t_{0.5} = [V/(AP_i)] \ln(c_{t=0}/c_{t=0.5})$, where V is cell volume, A is cell surface area, P_i is the permeability coefficient of i , and $c_{t=0}$ and $c_{t=0.5}$ are the concentrations of i at time zero and at time 50% concentration (Niklas and Spatz 2012). This formula shows that the time it takes i to diffuse into a spherical cell from all directions and reach $c_{t=0.5}$ is linearly proportional to cell radius, increases with increasing volume, and decreases with increasing surface area. Consequently, passive diffusion can be sufficient for the metabolic demands of small or attenuated cells, filaments, or thin sheets of cells, but it becomes increasingly insufficient as cells or cell aggregates increase in size.

Indeed, diffusion is a mode of communication that can drive cell specialization because increasingly steep gradients in nutrient-availability resulting from increasing size or distance can give rise to a reaction–diffusion (R–D) morphogenetic system. For example, an *Anabena* or *Nostoc* filament is a one-dimensional R–D system. Heterocysts manufacture a diffusible inhibitor that prevents heterocyst formation unless its concentration falls below a specific threshold (Wilcox et al. 1973; Risser et al. 2012). As the distance between two heterocysts increases (due to intervening vegetative cellular divisions), the first undifferentiated cell to be triggered midway develops into a heterocyst, releases the inhibitor, and reiterates the process. Analogous R–D systems operating in two- or three-dimensions are posited for the development of stomata and root hairs (Torii 2012). Even more complex examples can be drawn from the evolution of land plants or extant macroscopic algae. Thus, as early embryophytes evolved greater height, passive diffusion eventually failed to provide water to aerial tissues at sufficient rates, thereby requiring bulk water flow via xylem (Niklas 1997). Likewise, the inner cortex of the stipes of the great kelp *Macrocystis* contain specialized “trumpet” cells that transport photosynthates much like the phloem of vascular plants (Bruggeln et al. 1985).

Nevertheless, the organization of differentiated cells into two- and three-dimensional patterns, shapes, and forms need not reflect the immediate consequences of selection (Gilbert 2010). Although the adaptive advantage of a morphological novelty is clear (such as in the development of plant and animal vascular systems), morphological or behavioral novelties can also arise via phenotypic plasticity (West-Eberhard 2003; Niklas 2009), or as a consequence of the inherent physical properties of embryonic tissues and condition-dependent developmental systems (Newman 1994; Newman and Müller 2000; Manning et al. 2010), only to become assimilated subsequently into the organism’s developmental program (see, e.g., Palmer 2004). The former may help to explain why colonial life-forms often presage the appearance of multicellularity in some lineages since phenotypic plasticity among genetically identical cells contributes to fitness (Yokota and Sterner 2010). Likewise, the colonial body plan has distinct advantages over the unicellular life form; for example, non-defective cells can compensate for the defects

of neighboring cells, or provide resources to other cells that are then free to specialize.

CHARACTER POLARITIES WITHIN MULTICELLULAR CLADES

The preceding provides some insight into how unicellular organisms evolved into simple (and later complex) multicellular forms because it establishes a hypothetical morphological transformation series, viz., unicellular \Rightarrow colonial \Rightarrow simple multicellular life forms. For plants, this series can be refined further based on simple developmental and physical principles that identify four major body plans, that is, unicellular, siphonous/coenocytic, colonial, and multicellular (Fig. 3). Among variants of the plant multicellular body plan, an unbranched filament of cells is the simplest biophysically because it requires a single plane of cell division while reducing drag forces, maximizing light interception, and maintaining a constant surface area to volume relationship (Niklas 2000). Further elaboration requires cell division in two and then three planes of reference, which respectively gives rise to branched filaments (and the potential for a pseudoparenchymatous tissue construction) and a parenchymatous tissue construction (Fig. 3). (We define parenchymatous tissues as those in which cells can divide in all three planes of reference.) Accordingly, for plants, the evolution of multicellularity is predicted to conform to a unicellular \Rightarrow colonial \Rightarrow filamentous (unbranched \Rightarrow branched) \Rightarrow pseudoparenchymatous \Rightarrow parenchymatous transformation series. This hypothesis can be evaluated empirically by mapping the different body plans and tissue constructions onto current phylogenies for plants, fungi, and animals. In theory, unicellular, colonial, and simple multicellular morphologies should map onto ancestral (basal) branches within each clade, whereas more complex tissue constructions should map onto more derived branches.

Although recent plant phylogenies inform this hypothesis, the cladistic resolution within some clades currently lacks sufficient precision to identify the ancestral body plans even among some closely related lineages. Perhaps the most difficult clade to resolve is the red algae (rhodophytes). Molecular data identify two divergent lineages (one extending from the Porphyridiophyceae and another from the Bangiophyceae) that share the unicellular Cyanidiophyceae as their sister taxon (Saunders and Hommersand 2004; Yoon et al. 2009). The porphyridian lineage contains unicellular, colonial, and what are generally believed to be more derived multicellular algae, whereas the Compsopogonophyceae contains filamentous algae (Fig. 4). The taxonomic relationships of the Rhodochaetales and Erythropeltiales with the Compsopogonophyceae and other related rhodophytes remain unresolved (Yoon et al. 2009). However, in the filamentous Bangiophyceae, the immediate ancestral condition is unicellular and the derived condition in the “floridean” red

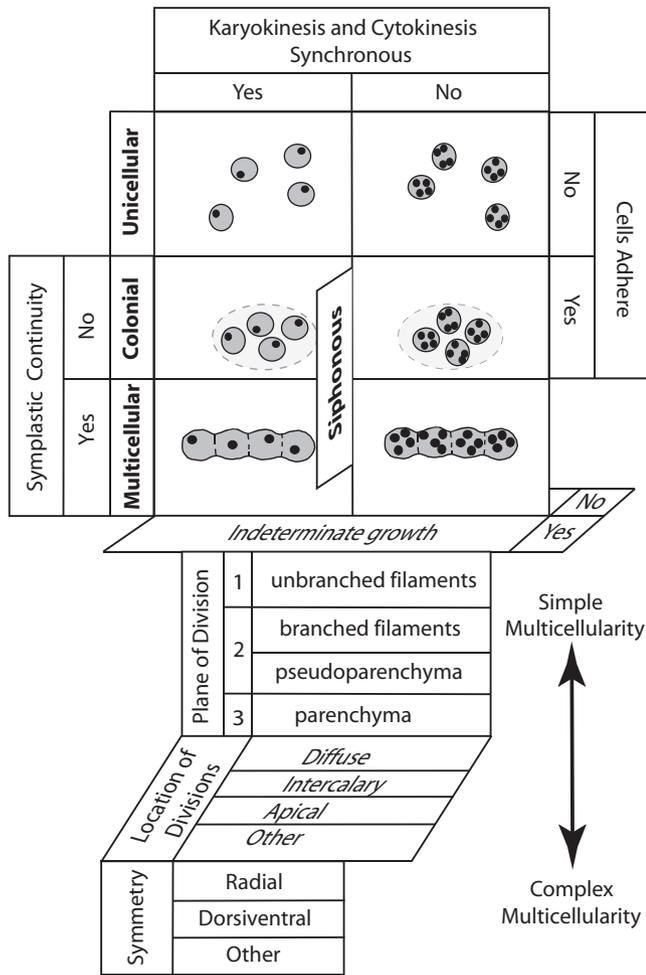


Fig. 3. The four major plant body plans shown in bold (unicellular, siphonous/coenocytic, colonial, and multicellular) configured in a matrix of the developmental phenomena that give rise to them, two of which are critical for the evolution of multicellularity: cell-to-cell adherence and cytoplasmic (symplastic) continuity among adjoining cells. Note that the siphonous/coenocytic body plan may evolve from a unicellular or a multicellular progenitor. This body plan can become multicellular directly. The lower panels dealing with the plane of cell division, localization of cellular division, and symmetry pertain to the evolution of complex multicellular organisms. Adapted from Niklas (2000).

algae is pseudoparenchymatous. Molecular analyses of the monophyletic stramenopiles (Anderson 2004; Maistro et al. 2009) identify three major lineages—the eustigmatophytes, dictyochophytes, and the pelagophytes (Fig. 5). Within the eustigmatophyte and the dictyochophyte lineages, the colonial body plan is derived and the filamentous body plan is observed only among the most derived genera. The various branches emerging from the pelagophyte lineage have a complex pattern. Nonetheless, the unicellular ⇒ colonial ⇒ filamentous (unbranched ⇒ branched) ⇒ pseudoparenchymatous ⇒ parenchymatous transformation series is consistent with current views regarding the phylogenetic relationships within the brown

algae (phaeophytes) and especially within the yellow-green algae (xanthophytes; see Cocquyt et al. 2010) (Fig. 5). Perhaps the strongest evidence among the three major plant clades for the unicellular ⇒ colonial ⇒ filamentous (unbranched ⇒ branched) ⇒ pseudoparenchymatous ⇒ parenchymatous transformational series comes from the molecular phylogenetics of the monophyletic green plants (chlorobionta) (Lewis and McCourt 2004; Leliaert et al. 2012). This series occurs within the “green algae” and within the streptophytes (the charophycean algae and the embryophytes) (Fig. 6).

In contrast to the plants, the transformational series observed for the fungi and animals are more truncated. Molecular analyses indicate that unicellular or siphonaceous (coenocytic) fungi gave rise to the multicellular condition in the asco- and basidiomycetes. Although the monophyletic status of the chytrids and the lack of resolution at the base of fungal phylogeny make it difficult to resolve the details of the last common ancestor (Lutzoni et al. 2004; Schoch et al. 2009) (Fig. 7), the colonial body plan is absent, whereas multicellular fungi consist of unbranched filaments that achieve a pseudoparenchymatous tissue construction in asco- and basidiomycete crown-taxa, that is, fungal evolution conforms to a unicellular or siphonous ⇒ unbranched filament ⇒ pseudoparenchymatous transformation series. Among animals, analyses identify the choanoflagellates as basal (Fig. 7). The phyletic relationships among the Placozoa, Ctenophora, and Cnidaria remain uncertain (e.g., Halanych 2004; Dunn et al. 2008). Within the monophyletic choanoflagellates, the unicellular body plan is traditionally interpreted as ancestral and the colonial body plan as more derived. Although the phylogenetic relationship between the choanoflagellates and the Holozoa requires extensive revision, analyses indicate that the choanoflagellates and Metazoa shared a last common unicellular ancestor (Carr et al. 2008). Within the Metazoa, the colonial body plan evolved independently (corals and bryozoans), although it may be a derived condition. The phyletic relationships among the Placozoa, Ctenophora, and Cnidaria remain uncertain (e.g., Halanych 2004; Dunn et al. 2008). Thus, within the Holozoa, we see a comparatively simple and direct unicellular ⇒ colonial ⇒ parenchymatous transformational series (Fig. 7). Subsequent, evolution gave rise to organisms with successively inclusive morphological motifs, that is, functionally differentiated cell types (sponges), multilayering (placozoans), interior cavities (ctenophorans, cnidarians), and eventually Bilateria with additional layers and body cavities (ectoderm, mesoderm, etc.) and segments (annelids, etc.) (Newman 2012) (Fig. 8).

The foregoing polarities clearly need to be examined using finer levels of taxonomic resolution (e.g., Cocquyt et al. 2010) at the ordinal and family levels. However, they indicate that the colonial body plan is a consequence of cell adherence mediated by typically lineage specific molecules with functionalities that predate the multicellular condition. In the following section, we argue that other motifs are similarly the result of the automatic mobilization of physical processes and effects by appropriate

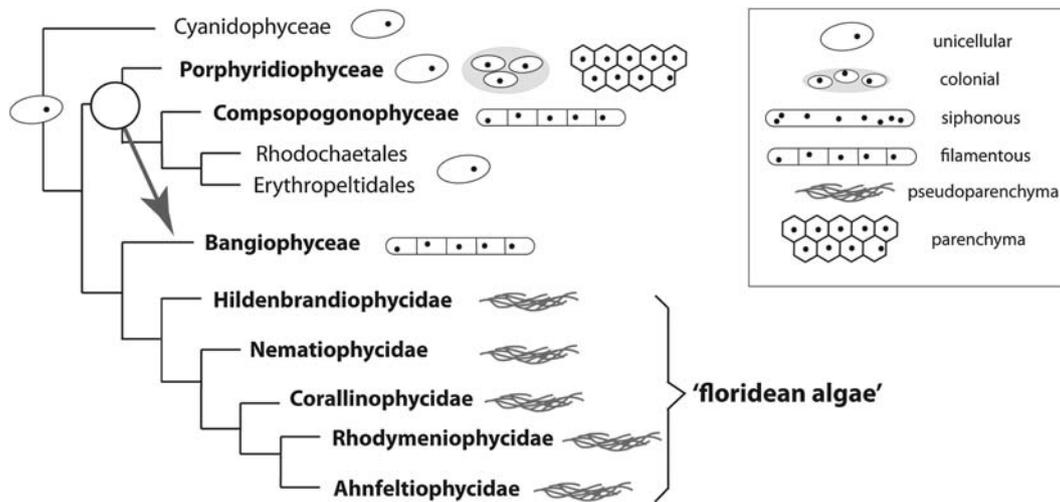


Fig. 4. Tentative phylogeny of the monophyletic red algae (rhodophytes) based on the phylogenetic analyses of Saunders and Hommersand (2004; see also Graham et al. 2009, pp. 310–311). The body plans characteristic of terminal branches in the phylogeny are indicated by schematics (see insert); all groups with multicellular body plans are indicated in bold. The grey arrow indicates that the uppermost branch in the red algal clade is traditionally placed in the Bangiophyceae.

cell-interaction-mediating molecules within the multicellular state. The self-organizational capabilities of these “dynamical patterning modules” (Newman and Bhat 2008, 2009) are realized in cell clusters arising by aggregation, by failure to separate after division, or by subdivision of an enlarged cell (palintomy in plants, cleavage in animals). In evolutionary terms, genetic uniformity of entities that exhibit developmental

activity can arise therefore before or after the appearance of the relevant morphogenetic mechanisms (Newman et al. 2006). Since adaptive selection has little to do with such physically based morphogenetic effects, framing the origin of multicellular body plans exclusively in terms of an alignment-of-fitness phase and an export-of-fitness phase is perhaps unduly restrictive, an issue that we return to in our concluding remarks.

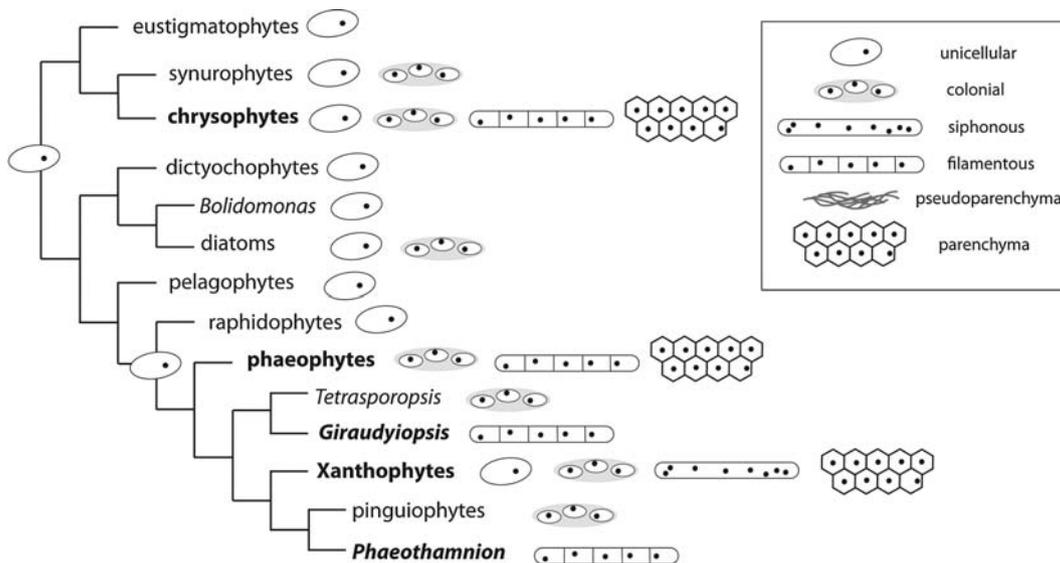


Fig. 5. A phylogeny of the monophyletic stramenopiles (brown and golden-brown algae) based on the analyses of Anderson (2004). The body plans characteristic of terminal branches in the cladogram are indicated by schematics (see insert); all groups with multicellular body plans are indicated in bold.

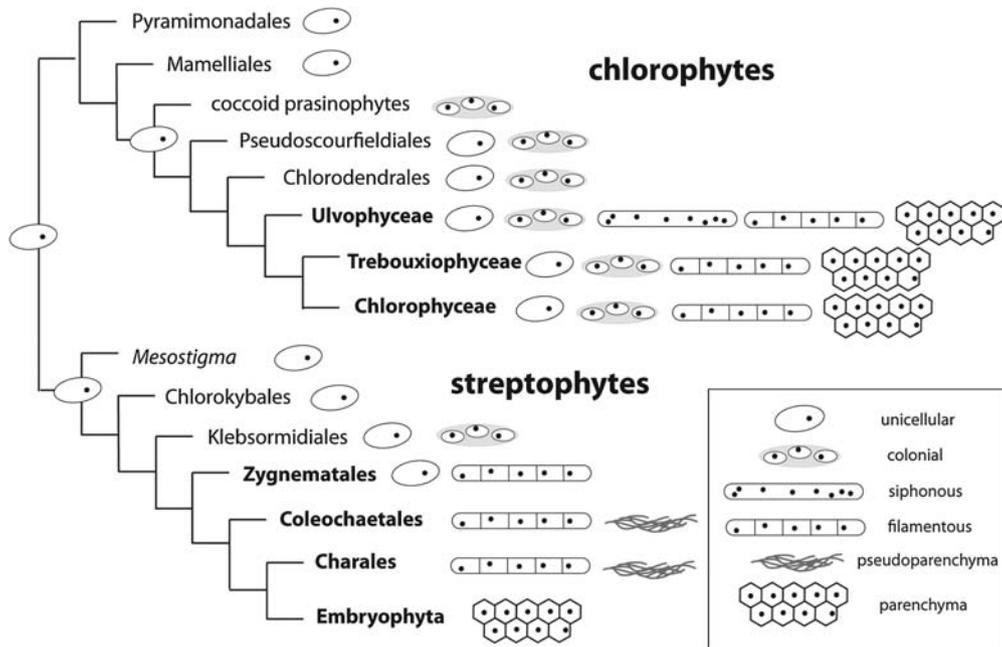


Fig. 6. A phylogeny of the monophyletic chlorobionta based on the analyses of Lewis and McCourt (2004) and Leliaert et al. (2012). Two major lineages are recognized: the green algae (chlorophytes) and a lineage containing the charophycean algae and the land plants (streptophytes). The body plans characteristic of terminal branches in the cladogram are indicated by schematics (see insert); all groups with multicellular body plans are indicated in bold.

SHARED AND UNIQUE TOOLKITS AND PATTERNING MODULES

We have emphasized five evolutionary features of multicellular organisms: cell adhesion, cellular communication, cellular

specialization, tissue pattern formation and morphogenesis, and “individuality” at the level of a multicellular entity. Molecular analyses indicate that each of the major multicellular clades contains a characteristic set of developmental “toolkit” genes, some of which are shared among disparate lineages (e.g.,

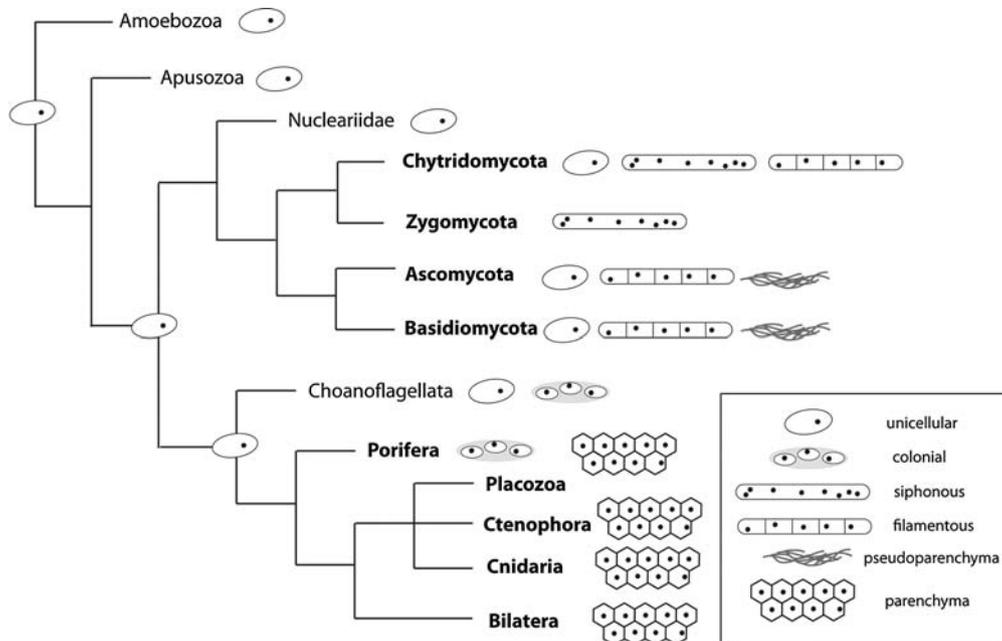


Fig. 7. Redacted phylogeny for the Fungi and Animalia and their respective putative ancestors. The body plans characteristic of terminal branches in this phylogeny are indicated by schematics (see insert); lineages with multicellular body plans are indicated in bold.

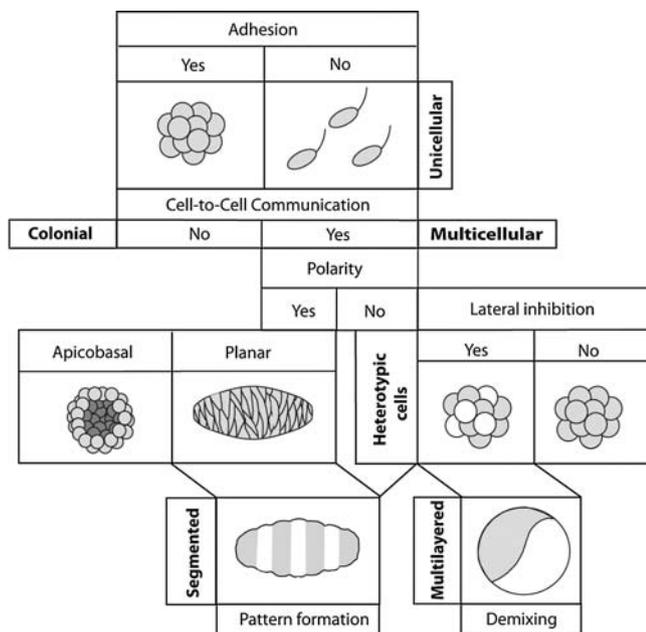


Fig. 8. The elaboration of the animal body plans (shown in bold) involved additions of successive morphological motifs based on the mobilization of newly relevant physical effects once the multicellular state evolved. These effects were harnessed by molecules that had first evolved to serve unicellular functions but that later became developmental “toolkit” molecules. While aggregates of cells with the relevant genes would have been capable of generating these motifs in a heritable fashion, blastulae resulting from cleavage of an enlarged cell (a “proto-egg”) would have been genetically uniform thus exhibiting a greater alignment-of-fitness (Newman 2011). Adapted from Newman and Bhat (2009).

tetraspanin genes in plants, protozoa, insects, fungi, and mammals; Wang et al. 2012), either by virtue of sharing a last common ancestor, or as a result of lateral gene flow (e.g., Sun et al. 2010). However, many are unique to their respective clades (e.g., cell polarity in plants and animals involves PIN- and PAR-polarity modules, respectively; Gelder 2009) and some even differ among closely related lineages (e.g., cell division mechanisms in unicellular and filamentous ascomycetes; Seiler and Justa-Schuch 2010).

A substantial number of toolkit genes in each clade are developmental transcription factors (DTFs), such as MADS box gene products in plants and Hox gene products in animals, which, along with their *cis*-acting target sequences on other such genes, form multistable regulatory networks whose alternative stationary states determine cell type identity (Furusawa and Kaneko 2002; Kaneko 2011). Others mediate cell–cell interaction and communication and constitute an “interaction toolkit” (Newman et al. 2009). Although DTFs cross cell borders only rarely during animal embryogenesis (Prochiantz and Joliot 2003), they do so more typically in plants, making them part of the interaction toolkit in that clade. Other interaction plant toolkit molecules include the adhesive components described previously and phytohormones

such as auxin (Niklas and Kutschera 2009; Boot et al. 2012). In animals, these include cadherins, Notch and Wnt and their respective ligands, BMP, Hedgehog, and extracellular matrices. Regardless of the roles they may have played in unicellular ancestors, these molecules assume entirely new roles in pattern formation and morphogenesis in the multicellular state, typically by virtue of the newly relevant physical processes they mobilize at the mesoscale (Forgacs and Newman 2005; Hamant et al. 2008; Newman and Bhat 2008, 2009; Manning et al. 2010; Müller 2012). This has led to animal body plans that are (as noted) variously, multilayered, hollow or with nested cavities, elongated, segmented and appendage-bearing, and to organs with similar morphological motifs (Fig. 8). Among embryophytes, interaction toolkits are implicated in branching, phyllotaxy, apical dominance, and the development of vascular tissues.

Considering the shared and specific interaction toolkits of the various clades in relation to the physical forces and effects they mobilize helps explain how phylogenetically different organisms use genetically homologous components to construct phenotypically dissimilar but functionally similar (analogous) structures often without common ancestors exhibiting the character (Newman 2006). Subtle variations in the physical environment under which cell aggregation occurs, or minor genetic changes in adhesion molecules, can result in alternative forms such as solid or hollow spheroids, filaments, or multilayered tissues. With the advent of complex multicellularity, the physical and physiological context and microenvironments of distinct subpopulations of cells (e.g., interior vs. exterior) can yield different developmental fates. Eventually the conditional effects that contribute to such morphological variability may become developmentally canalized under selection when their host organisms find suitable ecological niches. Random gene-based modifications in body mass or dimension likewise would alter a priori the rates or magnitudes of size-dependent physical phenomena, such as passive diffusion and mechanical stresses, in ways that would allow natural selection to filter from a variety of possible forms those better adapted to available or novel ecological settings.

FUTURE DIRECTIONS

The origination of multicellular organisms required the evolution of mechanisms capable of achieving and maintaining cellular differentiation temporally as well as spatially. The unicellular progenitor of each multicellular lineage therefore must have had the capacity to express alternative states of gene expression temporally in response to changing environmental conditions and the pre-existing regulatory mechanisms responsible for this developmental variability were co-opted to provide the capacity to differentiate spatially in the multicellular context. This new phenotypic context, mediated by preexisting attachment and matrix molecules, led to the mobilization of newly relevant physical effects and self-organizing dynamics that

provided the early basis for spatiotemporal regulation in multicellular organisms (Newman and Bhat 2008, 2009; Hernández-Hernández et al. 2012).

The evolutionary expansion of pre-existing gene families encoding regulatory proteins in combination with novel physical and regulatory interactions resulting from such expansions also played critical roles and may even have driven the evolution of multicellular complexity (e.g., Feller et al. 2011; Pires and Dolan 2012), as illustrated by the basic helix-loop-helix (bHLH) protein family involved in diverse cellular developmental processes in both plants and animals (reviewed by Feller et al. 2011). Indeed, many intracellular functions involve bHLH–bHLH interactions as well as synergistic interactions with other regulatory proteins, such as MYB, to form complexes that either repress or activate the expression of sets of target genes (Ramsay and Glover 2005; Feller et al. 2011). Future research is required to identify these targets and to determine their participation in developmental processes. However, as noted, it is critical to determine the extent to which the details of transcription factor regulation and gene network architecture carry over from one organism to another, since sequence homologies do not necessarily imply the conservation of function. Likewise, functional homologies are not invariably the result of genomic or developmental homology, as is evident from the broad spectrum of molecules providing cell adhesion and intercellular communication. Nevertheless, we believe that future research will show that that three vastly different plant clades achieved multicellularity along a similar morphological transformation series, e.g., unicellular \Rightarrow colonial or siphonous \Rightarrow filamentous (unbranched \Rightarrow branched) \Rightarrow pseudoparenchymatous \Rightarrow parenchymatous.

Finally, we have side stepped the question of whether multicellularity confers any selective advantage. Certainly, the current abundance of multicellular organisms gives the impression that it does. Likewise, it allows an organism to exceed the size limits imposed by passive diffusion. Although we are sympathetic to the notion that major evolutionary innovations will not be retained within a lineage if they are incompatible with survival, it is not always the case that every transition requires a large or even measurable advantage (Grosberg and Strathmann 2007), nor is it the case that phenotypic responses to selection invariably are in the direction of an adaptive advantage (Bonduriansky and Day 2009). Thus, a recent theoretical model for filamentous bacteria shows that strains with the same fitness can produce genotypes differing in cell-number as a result of differences in cell division and death rates, or as a result of changes in the environmental carrying capacity (Rossetti et al. 2011). This model, which has empirical support, also shows that differences in fitness attributable to morphology are not required a priori for the evolution of life cycles with multicellular entities (Rossetti et al. 2011), although advantages may arise subsequently (Koschwanez et al. 2011). The retention of multicellularity in some lineages therefore may reflect

“diffusion from the left (unicellular) wall of life” (*sensu* Gould 1989; Marcot and McShea 2007). Indeed, although examples of a unicellular organism descending from a multicellular organism are known (Velicer et al. 1998; Schirmermeister et al. 2011), once an organism passes through the export-of-fitness phase and achieves complex multicellularity, its capacity for contingent evolutionary reversion to a simpler state is reduced for reasons that have little or nothing to do with selection on fitness.

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