

Life cycles as a central organizing theme for studying multicellularity

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Abstract

Multicellular organisms exhibit a wide diversity in multicellular life cycles that differ in how development and reproduction are achieved. Every multicellular life cycle comprises group formation (via staying together or coming together, i.e. clonal or aggregative multicellularity) and group propagation. Many of these life cycles contain facultative life stages that are only expressed under specific environmental conditions. While a life cycle-centered approach is crucial to understanding the evolution of multicellularity, many open questions remain. In order to answer these questions, a bottom-up approach to the evolution of multicellularity—which aims to characterize evolutionary trajectories towards multicellularity as they unfold from a specific unicellular starting point—can be a useful complement to the traditional top-down approach. As an explicit example, we discuss a recent theoretical model that shows that diverse multicellular life cycles can readily emerge from a unicellular ancestor, shaped by ancestral constraints and environmental conditions. We envision that future progress will depend on sustained dialog between the bottom-up and top-down perspectives.

4.1 Introduction

Repeated evolutionary transitions from unicellular to multicellular life gave rise to an extraordinary diversity of multicellular life forms (Bonner 1998; Grosberg and Strathmann 2007; Herron et al. 2013; Ratcliff et al. 2017; Claessen et al. 2014; Lyons and Kolter 2015). Yet, our thinking of multicellularity is mostly shaped by a few paradigmatic, macroscopic examples. We begin this chapter by discussing several examples that showcase the broader diversity. Subsequently, we use the concept of the life cycle as a tool to systematically categorize multicellular diversity and study its evolutionary origin. We then review how recent advances in both empirical and theoretical research have improved our understanding of the evolution of multicellular life cycles, discuss the types of questions that still remain unanswered, and distinguish the conceptual approaches—bottom-up versus top-down—that can be used to

investigate those questions. Finally, we show how a bottom-up approach can be employed theoretically to explore the origin of multicellular life.

When thinking about multicellularity, animals almost immediately jump to mind. Unsurprisingly, then, much of our intuition regarding multicellularity is derived from the animals. For example, among the textbook examples of multicellular development are the mammals (Fig. 4.1). Mammals develop from a single cell (zygote) that repeatedly divides and ultimately gives rise to a mature multicellular individual. Reproduction takes place sexually: each individual produces gametes, which, after fusion, form a zygote capable of recapitulating the same developmental process. The mammalian form of multicellularity can be well understood as repeated cycles of development and reproduction: a zygote gives rise to a multicellular individual through repeated cell divisions (development), and this multicellular individual then generates single-celled gametes that again form a zygote (reproduction), thereby closing the cycle. There are, however, also animals for which multicellularity takes strikingly different forms than for the mammalian textbook example, such as the almost undifferentiated placozoa, the “moss animals” (Bryozoa), and the coral-forming “flower animals” (Anthozoa) (Buss 1983; 1987). Unlike mammals, these animals may reproduce asexually without a single-celled intermediate, form sessile colonies that can merge together, or exhibit diverse morphological forms depending on environmental conditions (Harvell 1991; Todd 2008; Hughes 1989; Simpson et al. 2020). Thus, even though animals represent a single evolutionary transition to multicellularity (meaning that they share a common multicellular ancestor), they are multicellular in diverse ways (Minelli and Fusco 2010; Moran 1994; Cavalier-Smith 2017).

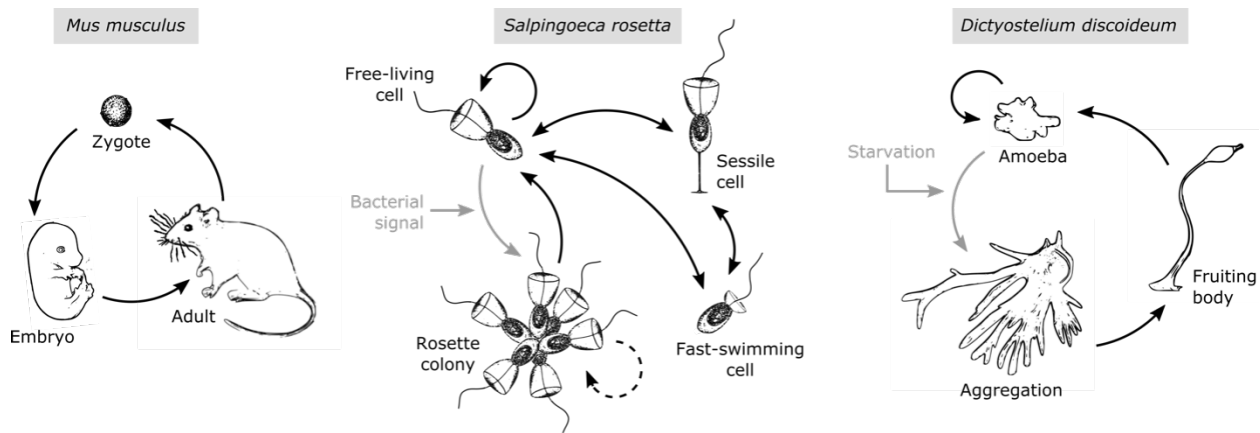


Figure 1. Extant multicellular organisms have diverse life cycles. Simplified depictions of the life cycles of the mouse *Mus musculus*, the choanoflagellate *Salpingoeca rosetta* (Dayel et al. 2011) and the cellular slime mold *D. discoideum*. Arrows indicate life stage transitions. Environmentally induced group formation is indicated with a gray arrow for *S. rosetta* and *D. discoideum*; the dashed arrow indicates colony fission for *S. rosetta*. For simplicity, not all life stages are shown; for example, the *S. rosetta* life cycle does not show chain colonies or the sexual cycle.

Right next door from the animal kingdom we find the choanoflagellate *Salpingoeca rosetta*, a marine eukaryote and one of the closest animal relatives (Fig. 4.1) (Fairclough et al. 2010;

Brunet and King 2017). *S. rosetta* exhibits a simple¹ form of multicellularity, consisting of small colonies of cells. Like animals, *S. rosetta* becomes multicellular via repeated cell divisions. Unlike animals, however, *S. rosetta* is facultatively multicellular, which means that it can also exist and reproduce as a unicellular organism. What's more, it can adopt three different unicellular life forms that are so different from one another that they were believed to belong to different genera until it was discovered that they were, in fact, different life stages of a single organism (Dayel et al. 2011). The different life stages are adapted to different conditions: one allows surface-attachment whereas the other two are free-living. Of the two free-living forms, one is a fast swimmer specialized for dispersal and quick swimming towards patches of prey bacteria and the other is a slow swimmer that can become multicellular (Dayel et al. 2011; Koehl 2020; Miño et al. 2017). Two different types of multicellular structures can be formed: linear “chain colonies” and spherical “rosette colonies”. Although much remains uncertain about the regulatory mechanisms through which *S. rosetta* switches between its different possible unicellular and multicellular forms, it has become clear that the environment plays an important role. For example, formation of rosette colonies from single cells can be caused by a specific environmental trigger: the detection of lipids secreted by bacteria in the environment (Alegado et al. 2012; Woznica et al. 2016). The reverse transition from colonies to single cells is less well understood, although it has been observed that colonies can split in two through fission (Dayel et al. 2011). This would suggest that while *S. rosetta* is strictly unicellular in the absence of the environmental trigger that induces colony formation, it may be strictly multicellular in its presence—with colonies giving rise to new colonies without the need for a single-celled intermediate. Thus, *S. rosetta* can express a range of different forms, both unicellular and multicellular, and some of these forms are induced by the environment.

Yet different types of multicellularity can be found in more distant branches of the tree of life, in organisms that evolved multicellularity independently from animals and choanoflagellates. The cellular slime molds provide one example (Fig. 4.1). Cellular slime molds are soil-dwelling microbes that, like *S. rosetta*, are facultatively multicellular: they can live and reproduce as unicellular amoebae and only become multicellular in response to specific environmental conditions—in their case, the trigger for multicellularity is starvation (Bonner 2009; Schaap 2011). Whereas animals and choanoflagellates become multicellular by repeated cell divisions, cellular slime molds exhibit aggregative multicellularity, meaning that individual amoebae aggregate to form a multicellular organism. The multicellular stage is transient and short-lived, culminating in the formation of a fruiting body consisting of a stalk and a head filled with spores that is raised in the air (Schaap 2011; Bonner 1957). The fruiting body facilitates survival and dispersal. The starvation-resistant spores will germinate upon encountering favorable conditions and continue their life as unicellular amoebae. This type of multicellularity can be understood as an “emergency response” to harsh environmental conditions, allowing cells to quickly join forces to achieve efficient dispersal (Brunet and King 2017). Thus, the cellular

¹ Multicellular complexity is difficult to define. Accordingly, there is no commonly accepted definition for what constitutes “simple” or “complex” multicellularity. Here, we use these terms to capture the difference between microscopic multicellular organisms with few differentiated cell types (“simple”), such as cyanobacteria, choanoflagellates or volvocine algae and macroscopic multicellular organisms with many differentiated cell types (“complex”), such as most plants and animals. Many multicellular organisms do not fit neatly in either of these categories.

slime molds are facultatively multicellular, with a short-lived aggregative multicellular stage that is induced by the environment.

Animals, choanoflagellates, and cellular slime molds are but a few examples of the immensely diverse ways to be multicellular. Here, we employ the concept of the life cycle to organize this diversity. Broadly speaking, an organism's life cycle describes the life stages between which an organism transitions in time. One of the prominent advocates for taking a life cycle-centered approach to the study of multicellularity was John Bonner, who argued that a dynamic view of all the life stages of an organism is more meaningful evolutionarily than a static view that emphasizes a specific stage of the life cycle (e.g., the adult at maturity) (Bonner 1965; 1993; van Gestel and Tarnita 2017). As the above examples highlight, these different life stages need not occur in a predictable succession and some life stages may only be expressed sporadically, under specific conditions. What all multicellular life cycles have in common, however, is that they involve *group formation* (the construction of multicellular groups, often starting from single cells) and *group propagation* (the process by which multicellular groups beget new multicellular groups). Therefore, these two processes—together with the intrinsic mechanisms (i.e., development) and extrinsic factors (e.g., environmental cues) that regulate them—offer a meaningful starting point for cataloging multicellular life cycles.

Group formation occurs in one of two principal ways (but hybrids of these are also possible, see (van Gestel and Tarnita 2017): either groups are formed by cells staying together after division (also known as clonal multicellularity), as in animals or choanoflagellates, or groups are formed by cells coming together via aggregation (also known as aggregative multicellularity), as in the cellular slime molds (Bonner 1998; Grosberg and Strathmann 2007; Bonner 1965; Tarnita et al. 2013). The staying together–coming together dichotomy encapsulates a wide range of possible grouping mechanisms. For example, staying-together multicellularity can be achieved by means of a shared cell wall, an extracellular matrix, adhesive molecules on the cell surface, or coenocytic growth (Suga and Ruiz-Trillo 2013; Abedin and King 2010), while coming together can be achieved through aggregation via chemotaxis or binding to a common surface (van Gestel and Tarnita 2017). Both staying together and coming together have evolved multiple times and in evolutionarily distant clades (Bonner 1998; Grosberg and Strathmann 2007; Fisher et al. 2013). While staying-together multicellularity includes both facultative and obligate forms of multicellularity, coming together is always associated with facultative multicellularity, with the multicellular stage being transient and induced by specific (typically adverse) environmental conditions (Brunet and King 2017; Brown et al. 2012; Sebé-Pedrós et al. 2013; Du et al. 2015; Sebé-Pedrós et al. 2017; Fisher et al. 2013).

Much like there are multiple mechanisms by which group formation can be achieved, there are also multiple ways for groups to propagate (Pichugin et al. 2017; Angert 2005). In many cases, group propagation requires reversal from the multicellular to the unicellular stage. For example, mammals have a very brief unicellular stage that comprises just the haploid gametes (sperm and egg) and the fertilized egg cell (zygote). Group propagation—the production and release of gametes—can occur repeatedly throughout the mature organism's reproductive lifespan. Cellular slime molds, in contrast, have a life cycle where the multicellular stage ends with a single group propagation event: the release of spores from the mature fruiting body. Finally, multicellular groups of *S. rosetta* propagate through fission, whereby the group splits in two (Dayel et al. 2011). Group propagation modes in which a group splits into two groups allow a life cycle to proceed without a single-cell intermediate.

Not only do life cycles differ in the mechanisms used to achieve group formation and group propagation, they also differ in the *regulation* of these processes. For example, while mammals are obligately multicellular organisms with a life cycle that is under tight developmental control, the facultative multicellularity of choanoflagellates and cellular slime molds is more flexible. In these organisms, the multicellular stage is expressed conditionally on the presence of a specific environmental trigger, highlighting an important role for the environment in the regulation of the life cycle. Environmental triggers involved in life cycle regulation can be both abiotic (e.g., nutrient or drought stress (King 2004; Ritchie et al. 2008)), as in the case of cellular slime molds, or biotic (e.g., quorum-sensing, predator-prey interactions (Alegado and King 2014; Waters and Bassler 2005; Woznica et al. 2017)), as in the case of choanoflagellates, and they ensure that life stages (in particular, the multicellular stage) are only expressed under the appropriate environmental conditions (Woznica and King 2018). Sensitivity to environmental conditions is not unique to facultatively multicellular organisms; the life cycles of many obligate multicellular organisms also depend strongly on environmental conditions (Nagy et al. 2018; Schlichting 1986; Walbot 1996).

Two general points emerge from a broad outlook on multicellular life cycles (Fig. 4.1). First, evolution has been extraordinarily creative in inventing different ways for multicellular organisms to develop and reproduce, leading to a plethora of multicellular life cycles. Second, a life cycle can only be properly understood in its ecological context, as the environment may provide crucial signals that govern life stage transitions. The extent to which the life cycle depends on the environment varies widely across life cycles: at one extreme are facultatively multicellular organisms, for which specific environmental conditions are required to trigger the multicellular stage; at the other extreme are life cycles that require limited environmental input, in which different life stages occur in a predictable succession (e.g., zygote → embryo → adult, for mammals).

4.2 Life cycle evolution

A life cycle-centered approach can also shed light on the evolutionary origins of multicellularity. A transition from unicellular to multicellular life requires the evolution of mechanisms by which cells can attach to each other (group formation) as well as the evolution of mechanisms by which multicellular groups can propagate themselves (group propagation) (Libby and Rainey 2013). Group formation alone is not sufficient for the evolution of multicellularity. For example, a mutation that blocks cell separation after division may quickly lead to the formation of multicellular groups, but will not lead to multicellular life if cells stay attached indefinitely—in this case, the multicellular group is an evolutionary dead end because it lacks the ability to propagate. Thus, while unicellular organisms may frequently undertake opportunistic experiments with multicellularity in which multicellular groups are formed, these experiments can only lead to the evolution of a multicellular lineage when a primitive multicellular life cycle is established. This renders the question of how multicellularity originated *identical* to the question of how a multicellular life cycle originated (Bonner 1993; van Gestel and Tarnita 2017; De Monte and Rainey 2014; Black et al. 2020).

The empirical understanding of how multicellular life cycles evolve has greatly expanded in the past two decades. First, work in comparative genomics has reshaped our thinking about the genomic underpinnings of transitions to multicellularity. Most strikingly, this research has revealed that the unicellular ancestors at the stems of multicellular lineages are, from a genomic perspective, more complex than previously appreciated, and that much of the genetic toolkit for

multicellularity predates the evolution of multicellularity (Sebé-Pedrós et al. 2017; Prochnik et al. 2010; Glöckner et al. 2016; Rokas 2008; Brooke and Holland 2003; Hanschen et al. 2016; Suga et al. 2013). The last unicellular ancestor of animals, for instance, was already equipped with genes employed in animal cell adhesion, with transcription factors used in animal development, and with some components of developmental signaling pathways—although the cell signaling repertoire also greatly expanded with the advent of multicellularity (Sebé-Pedrós et al. 2017; King et al. 2008; Srivastava et al. 2010; Sebé-Pedrós et al. 2018). The fact that many multicellularity genes are of ancient, unicellular origin implies that understanding the unicellular ancestor is key to understanding the first emergence of a multicellular life cycle: multicellularity may more readily evolve from preadapted unicellular organisms equipped with genes or behaviors that can be co-opted for multicellular organization (King 2004). The evolution of facultative multicellularity, for example, is often contingent upon ancestral mechanisms that allow the ancestor to sense its external environment and to express different cellular behaviors in response (Ritchie et al. 2008; Kawabe et al. 2015).

Second, recent progress in experimental evolution has made it possible to study the emergence of multicellular life cycles in the laboratory (Boraas et al. 1998; Ratcliff et al. 2012; 2013; Herron et al. 2019; Hammerschmidt et al. 2014). This approach makes use of experiments in which a unicellular organism is subjected to an artificial selective pressure that favors multicellularity, such as a selective pressure for increased size. For example, in an evolution experiment in which the unicellular green alga *Chlamydomonas reinhardtii*² was subjected to selection for rapid settling in liquid medium (which favors larger clusters of cells), a life cycle evolved that had alternating unicellular and multicellular life stages (Ratcliff et al. 2013). Experimental evolution studies reinforce the idea that transitions to multicellularity can readily be made, provided that a unicellular organism is equipped with the right preadaptations and that a selective pressure exists that favors multicellularity.

Despite such major empirical advances, there are also aspects of the evolution of multicellular life cycles that remain largely inaccessible empirically, at least for now. While we can use comparative genomics to make inferences about the genome of unicellular organisms that underwent transitions to multicellularity, characterizing the ancestral functions of these genes in the life cycle of the unicellular ancestor is challenging. And while the emergence of a multicellular life cycle can be studied experimentally under an artificial selective pressure, we have little idea of what selective environments drove the evolution of multicellularity in nature. These gaps in our empirical knowledge currently leave unanswered many questions about the evolution of multicellular life cycles: Why did multicellular life cycles emerge in some lineages, but not in others? Why did so many different types of life cycles evolve and how much of that diversity was present early on? And how does the life cycle facilitate (or impede) the emergence of multicellular innovations and the evolution of multicellular complexity?

Different conceptual approaches have been used to make progress on these open questions. For instance, a “top-down” approach starts by identifying the common features of many different multicellular life forms—such as high levels of cooperation and coordination between cells in

² *C. reinhardtii* is a close relative of the multicellular genus *Volvox*, a model organism for germ-soma differentiation.

multicellular groups, the individuality³ of the multicellular organism, and the prevalence of a single-cell bottleneck in the life cycle (Ratcliff et al. 2017; De Monte and Rainey 2014; Godfrey-Smith 2009; Queller and Strassmann 2009; West et al. 2015). Next, the top-down approach aims to explain how multicellular groups could have evolved these key features. For example, a clear pattern among extant multicellular organisms is that all examples of complex multicellularity, such as animals and plants, are associated with a staying-together life cycle (Fisher et al. 2020). In comparison, the multicellular complexity of cellular slime molds and other organisms with coming-together life cycles has remained limited; these organisms invariably have a transient and short-lived multicellular stage (Brunet and King 2017). A potential explanation for this pattern is that coming together can lead to genetically heterogeneous groups and hence evolutionary conflict (where the evolutionary interests of cells within a multicellular group are misaligned). In the absence of mechanisms to prevent genetically heterogeneous groups, such conflict could impede the evolution of multicellular complexity beyond a transient multicellular life stage (Rainey and De Monte 2014; Michod and Herron 2006; Michod and Roze 2001; Queller 2000). Conversely, in the absence of conflicts, staying together would allow for longer-lived groups and facilitate the evolution of multicellular innovations (e.g., the evolution of cell specialization and complex morphologies), which could lead to complex forms of multicellularity.

The top-down framework has been successful in providing potential evolutionary explanations that do not hinge on the particularities of any individual transition to multicellularity. However, there are also limitations to the top-down perspective. First, when identifying the features that multicellular organisms have in common (and could be of importance for their evolutionary origin), the top-down approach runs the risk of introducing biases towards the paradigmatic examples of complex multicellularity that we are most familiar with, even if the resulting collection of organisms is not representative of the actual diversity of multicellular life (van Gestel and Tarnita 2017). Second, within the top-down framework it can be challenging to select among multiple competing explanations. For example, proposed top-down explanations for the prevalence of a single-cell bottleneck range from its ability to reduce conflicts of interests between cells (Ratcliff et al. 2015; Grosberg and Strathmann 1998; Roze and Michod 2001) to its ability to purge deleterious mutations (Grosberg and Strathmann 1998) or to ensure coherent development (Wolpert and Szathmary 2002), and it is not immediately clear which of these explanations should carry the most weight. In fact, none of these explanations may be required at all, as empirical work shows that life cycles with a single-cell bottleneck can arise rapidly, without requiring selection, through co-option of the ancestral unicellular form (Ratcliff et al. 2013), and mathematical modeling suggests that a single-cell bottleneck may simply be favored because it maximizes growth under a wide range of conditions (Pichugin et al. 2017). Such studies highlight the fact that the explanations provided by the top-down framework are best seen as working hypotheses that require independent testing, both empirically and theoretically.

³ What constitutes a biological individual is a challenging conceptual question. Various definitions have been proposed, which may, for example, require indivisibility, genetic homogeneity, physiological autonomy, and/or satisfying the Lewontin conditions for evolution by natural selection (i.e. variation, reproduction and heritability) (Buss 1987; van Gestel and Tarnita 2017; De Monte and Rainey 2014; Santelices 1999; Michod 2007; Godfrey-Smith 2009).

The second conceptual framework is the bottom-up approach. In contrast to the top-down approach, which starts by considering extant multicellular organisms, the bottom-up approach asks what features of multicellularity might evolve given a certain unicellular ancestor and its environmental setting (van Gestel and Tarnita 2017). In doing so, it aims to obtain a mechanistic understanding of the way in which the unicellular ancestor and its ecology shape nascent multicellular life. The bottom-up approach can distinguish between features of multicellularity that emerge spontaneously, as a direct consequence of the way in which multicellularity arises from the unicellular ancestor, and features of multicellularity that require subsequent selection, beyond the origins of multicellularity, in favor of a particular multicellular organization. Moreover, by considering explicit evolutionary trajectories from a unicellular ancestor to a multicellular organism, the bottom-up approach is ideally suited to test specific hypotheses—such as the hypotheses that the top-down framework extracts. Relative to the top-down approach, a bottom-up perspective has the advantage that it explores the full potential of transitions to multicellularity and minimizes bias towards the actually realized or most paradigmatic multicellular lineages (van Gestel and Tarnita 2017). Just like the top-down approach, however, the bottom-up approach has its limitations. By explicitly considering the ancestral starting point and the mechanistic underpinnings of an evolutionary trajectory towards multicellularity, the scope of the bottom-up is more limited than that of the top-down approach, and the explanations it offers may generalize less broadly.

While the theoretical literature on the evolution of multicellularity has historically largely employed the top-down framework, these top-down insights have recently been complemented by bottom-up models that explore specific scenarios for the origin of multicellularity (Solé and Valverde 2013; Solé and Duran-Nebreda 2015). These scenarios range from public goods sharing (Biernaskie and West 2015) or collective motion (Garcia et al. 2014; 2015) in microbial populations to filament formation (Rossetti et al. 2011) or surface colonization (van Gestel and Nowak 2016) in bacteria. What makes studying the evolution of multicellularity with bottom-up models exciting is that these models enable us to do something that is very difficult to do empirically: directly study how the unicellular ancestor and the selective pressures it faces shape the evolution of multicellularity. And, by explicitly accounting for the cell-cell and cell-environment interactions that shape multicellularity, bottom-up models shed mechanistic light on the transition to multicellularity in the process.

4.3 A bottom-up approach to life cycle emergence

To give a concrete example, we recently constructed a mechanistic model to study how a multicellular life cycle first emerges from a unicellular ancestor during a transition to multicellularity (Staps et al. 2019). Our goal was to explore (1) what kinds of multicellular life cycles could emerge and (2) what features of the ancestor and its environment would shape these life cycles at the origin of multicellularity. The evolutionary starting point is a unicellular organism that is able to sense its environment and express different genes in response. We assumed a simple (but non-constant) environment that fluctuates back and forth between two different states, mimicking, for example, the diurnal cycle faced by photosynthetic algae or the feast-and-famine cycles faced by soil-dwelling amoebae. Motivated by the empirical observation that co-option of ancestral functions underlies multiple transitions to multicellularity (Hanschen et al. 2016; Kawabe et al. 2015; Olson and Nedelcu 2016), we introduced a potential for multicellularity by allowing an ancestral gene to start causing some degree of cell adhesion, leading to daughter cells staying attached after division. We then investigated whether and what

multicellular life cycles could arise through evolutionary changes in gene regulation. Thus, the bottom-up model makes the ancestral ecology and ancestral regulatory mechanisms explicit, and studies what life cycles may emerge from this starting point.

This bottom-up approach provided two important insights. First, despite the simple setup, this model produced a surprising diversity of life cycles. What life cycle evolved depended on two factors: the biophysical constraints on group formation (i.e., the strength of the attachments between cells, which determines the maximum group size that could theoretically be reached by cells that express the cell adhesion gene), and the benefits of multicellularity (specifically, the minimum size that groups need to reach for multicellularity to provide a benefit) (Fig. 4.2a,b). Among the several evolved life cycles, we recovered simple analogs of the mammalian one—an obligately multicellular life cycle in which groups are dependent for their propagation on the occasional release of cells from the group (Fig. 4.2b, life cycle III)—and the cellular slime molds one—an environment-dependent life cycle in which groups regularly propagate by dissolving completely into solitary cells (Fig. 4.2b, life cycle IV). The obligately multicellular life cycle arises when cell stickiness is sufficiently high for group formation to reap the benefits of multicellularity, but low enough to have the occasional release of single-cell propagules that ensure group propagation. In contrast, the environment-dependent life cycle arises when high cell stickiness renders accidental detachment of cells unlikely, and instead relies on environmentally induced active down-regulation of adhesion for the group to dissolve and ensure propagation. Thus, our simple bottom-up model reveals that diverse multicellular life cycles can evolve already at the origin of multicellularity through co-option of ancestral regulatory mechanisms.

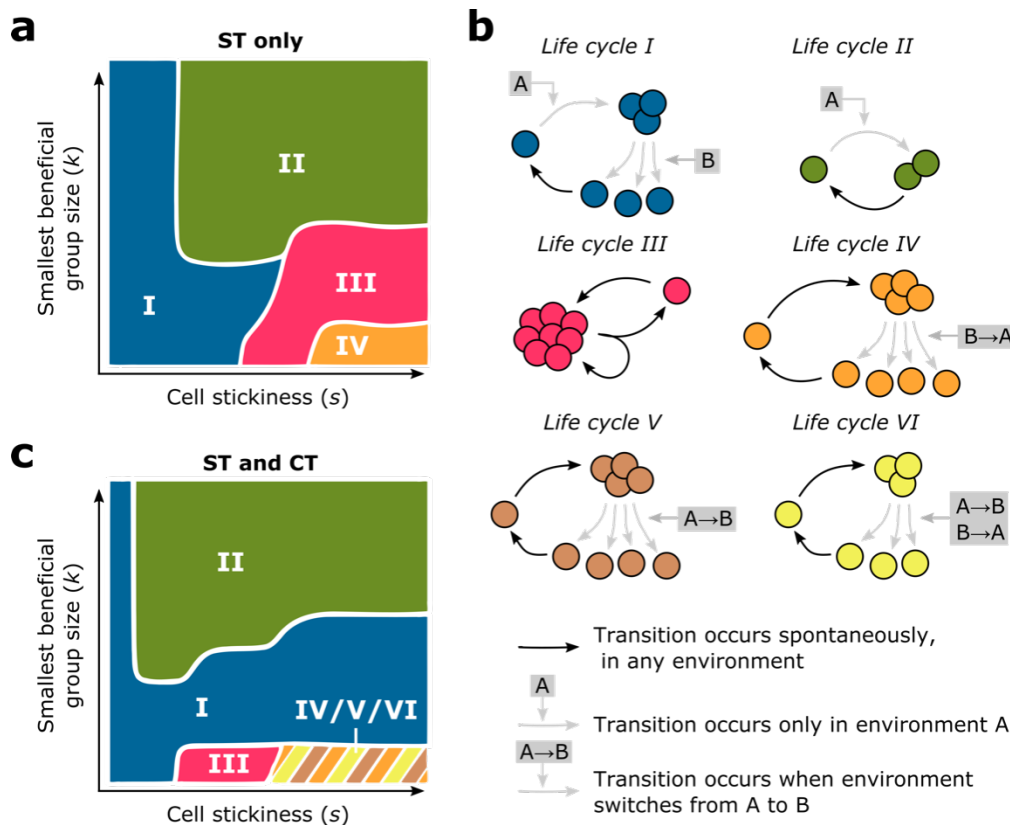


Figure 2. Modeling the emergence of multicellular life cycles. a, Evolved multicellular life cycles in simulations of the evolution of multicellularity. What life cycle evolves depends on the ability of cells to stay attached to each other (cell stickiness, s , ranging from virtually instantaneous detachment on the left to indefinite attachment on the right) and the minimum group size at which being in a group provides a benefit to constituent cells (smallest beneficial group size, k , range 2–10). Different colors indicate different life cycles. b, Cartoons depicting the evolved life cycles. Arrows indicate life stage transitions; gray arrows indicate life stage transitions that are under environmental control. The environment switches back and forth between two states A and B. In life cycle I, groups are formed in environment A, and groups fall apart in environment B. In life cycle II, groups may occasionally form in environment A but groups fall apart immediately. In life cycle III, group formation is obligate, but occasionally cells detach from their group. In life cycles IV—VI group formation is also obligate, but groups propagate by dissolving into solitary cells in response to specific environmental triggers. c, The evolved multicellular life cycle in simulations in which coming together (CT) is possible in addition to staying together (ST). See (Staps et al. 2019) for a full description of the model and the original data figures.

Second, in an extended version of the model, we allowed cells to join groups via coming together, in addition to their capacity to form groups via staying together. Allowing for group formation via coming together favored the evolution of multicellular life cycles with a short-lived multicellular stage and frequent dissolution into solitary cells (Fig. 4.2b, life cycles I, IV, V, VI). How can this be explained? Since coming together is not limited by the rate of cell division, it allows groups to assemble faster from single cells than staying together alone (Pentz et al. 2020). Faster group formation also enables groups to propagate more frequently, and thus results in a shorter life cycle. Thus, our simple bottom-up model provides an alternative explanation for the short-lived multicellular life stages observed in many organisms with aggregative multicellularity: it might not necessarily be that the potential for within-group conflict *prevents* longer-lived multicellularity, but rather that the potential for fast group formation and propagation *allows* for short-lived multicellular life stages.

Our life cycle model showcases the potential of the bottom-up approach. Explicitly accounting for the life cycle and ecological context of the unicellular ancestor can readily generate new insights: a surprising diversity of life cycles could have emerged at the origin of multicellularity and the transience of coming-together life cycles might be explained without needing to invoke conflicts among constituent cells. Interestingly, these insights emerge even though the model makes several simplifying assumptions about the ecology (the fluctuating environment) and development (the regulatory architecture underlying cell adhesion). This simplicity reflects purposeful abstraction as well as a (partially inevitable) ignorance about the life history and ecology of organisms that underwent transitions to multicellularity. Current empirical developments pave the way for a next generation of models that can more realistically account for the biology of early multicellular organisms and their unicellular predecessors. Indeed, studies of unicellular relatives of multicellular lineages enhance our understanding of the relevant regulatory mechanisms at the origin of multicellularity (Sebé-Pedrós et al. 2018; 2016; Arenas-Mena 2017; Arenas-Mena and Coffman 2015; Levin et al. 2014). At the same time, progress in biophysics and single-cell biology helps reveal the physical, metabolic and behavioral constraints on early multicellular organisms (Dexter et al. 2019; Goodwin 1989;

Kempes et al. 2012; Larson et al. 2020). And finally, largely overlooked knowledge from paleobiology can inform the environmental conditions that selected for transitions to multicellularity (Erwin 1993; Valentine and Marshall 2015; Butterfield 2000; 2009).

4.4 Conclusion

Life cycles are central to studying the evolution of multicellularity. They are a useful tool to catalog the variety of ways different branches of the tree of life have found to achieve multicellularity, and, because they encapsulate development and reproduction, they determine how evolution can act on nascent multicellular life. Understanding the evolution of multicellularity, and of multicellular organizing principles such as cell differentiation or morphogenesis, therefore requires life-cycle-centered approaches that explicitly consider the life cycle and its evolutionary origin. Future progress depends on the sustained dialog between two complementary approaches: a historically dominant top-down approach that aims to provide general evolutionary explanations that transcend the particularities of individual transitions to multicellularity, and a complementary bottom-up perspective that emphasizes specific evolutionary trajectories. The top-down approach can inform the bottom-up approach by identifying general organizing principles of multicellularity and by suggesting underlying evolutionary explanations. In turn, the bottom-up approach is able to inform the top-down approach by critically evaluating these explanations, adding mechanistic insight, and elucidating specific evolutionary trajectories as they unfold starting from a unicellular ancestor. It is by integrating the top-down and bottom-up perspectives that we can paint a fuller picture of the evolutionary transition to multicellularity: starting with how multicellular life arose when the first multicellular life cycle emerged from a unicellular ancestor and building towards an understanding of why multicellular life as we see it around us today looks the way it does.

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