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Nascent life cycles and the emergence of higher-level individuality

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Evolutionary transitions in individuality (ETIs) occur when formerly autonomous organisms evolve to become parts of a new, 'higher-level' organism. One of the first major hurdles that must be overcome during an ETI is the emergence of Darwinian evolvability in the higher-level entity (e.g. a multicellular group), and the loss of Darwinian autonomy in the lower-level units (e.g. individual cells). Here, we examine how simple higher-level life cycles are a key innovation during an ETI, allowing this transfer of fitness to occur 'for free'. Specifically, we show how novel life cycles can arise and lead to the origin of higher-level individuals by (i) mitigating conflicts between levels of selection, (ii) engendering the expression of heritable higher-level traits and (iii) allowing selection to efficiently act on these emergent higher-level traits. Further, we compute how canonical early life cycles vary in their ability to fix beneficial mutations via mathematical modelling. Life cycles that lack a persistent lower-level stage and develop clonally are far more likely to fix 'ratcheting' mutations that limit evolutionary reversion to the pre-ETI state. By stabilizing the fragile first steps of an evolutionary transition in individuality, nascent higher-level life cycles may play a crucial role in the origin of complex life.

This article is part of the themed issue 'Process and pattern in innovations from cells to societies'.

1. Introduction

Few biological phenomena have created more scope for evolutionary innovation than the creation of new 'levels of selection', and the resulting rise of new types of biological individuals. All known organisms that populate Earth today are the result of at least one such evolutionary transition in individuality (ETI [1,2]). Notable ETIs include the origin of membrane-bounded protocells encapsulating chemical replicators, the aggregation of genetic replicators into chromosomes, the domain-spanning symbiotic origins of eukaryotic cells, the origin of multicellular organisms from unicellular ancestors, and the evolution of colonial 'super-organisms' from solitary multicellular organisms [2]. Like layers to an onion, Earth's organisms maintain the signature of their multilevel evolutionary history.

Despite the profound differences in these evolutionary transitions, they appear to proceed in an analogous manner. Extant individuals (e.g. single-celled organisms) first form a new unit of selection—this typically occurs through tight spatial coupling between cooperating individuals in a collective (e.g. a cluster of cells). Increased complexity subsequently arises as the result of adaptation taking place in collective-level traits, not in the traits of the lower-level individuals [2]. Such a shift in evolutionary process would appear to be susceptible to evolutionary conflict, with contrasting Darwinian dynamics playing out at the lower- and higher levels. Indeed, lower-level units would appear to have numerous advantages, including a shorter generation time, a larger population size and greater trait heritability. This rationale will sound

familiar to many evolutionary biologists, as it forms the core of the argument made against group selection since the 1960s [4,5].

Unfortunately for ETIs, it gets worse. Perhaps the largest obstacle they must overcome is an organizational asymmetry. Lower-level units tend to be fully fledged organisms that have long been evolving as the primary unit of selection, gaining adaptations that enhance fitness at their organismal level. In fact, some philosophers consider this to be a defining feature of biological individuality [6,7], though it is important to remember that not all traits that are beneficial at level X are the result of selection acting at level X [8]—they may have arisen through non-adaptive means [9]. In the terminology of Godfrey-Smith, 'Darwinian individuals' are the members of populations that are capable of adaptive evolution, i.e. those that possess heritable variation in traits that affect fitness [6,10]. Their long history as Darwinian individuals gives lower-level units ample opportunity to evolve traits that make them more effective Darwinian individuals (e.g. by increasing robustness [11,12] and evolvability [13], or by mitigating conflicts between levels of selection [2,14,15]), while novel collectives have no such advantage. Thus, during an ETI, novel collectives face a daunting challenge: they must overcome these systemic biases in favour of lower-level adaptation in order for the higher-level unit to be the 'dominant' Darwinian individual. Interestingly, it appears very difficult to fully remove the potential for Darwinian individuality from an entity that once had it: cells in multicellular organisms readily mutate and grow in an unchecked manner, causing cancer [16,17]; non-functional mitochondria take over yeast cells when given the opportunity [18]; and 'selfish' genetic elements reproduce at the rest of the genome's expense [19,20]. Still, in each case, the balance of selection, and corresponding adaptation, is clearly on the higher-level individual.

In this paper, we examine how nascent life cycles arise and drive the origin of new biological individuals. We examine how critical elements of the life cycle necessary to satisfy the Darwinian algorithm arise 'for free' as a side effect of physical interactions among particles within the collective. Specifically, we focus on how collectives gain the capacity to act as Darwinian individuals: that is, how heritable collective-level traits emerge from particle-level traits, and how key elements of the life cycle potentiate collective-level evolvability. We examine the role of life cycles in collective-level adaptation by modelling the spread of beneficial mutations across various life cycles. Finally, we examine how mutations that epistatically increase collective-level fitness while reducing the fitness of particles can de-Darwinize lower-level units, reinforcing the ETI. Taken together, our results show that biological consortia readily form, grow and reproduce in a manner that catalyses the emergence of higher-level individuals, facilitate selection for beneficial mutations at this new biological level and can fix mutations that stabilize the ETI by stripping lower-level units of their evolutionary autonomy.

2. Life cycles

For conceptual and empirical simplicity, we will focus on the transition from uni- to multicellularity, but our arguments should apply to other ETIs that occur through an analogous process of multilevel selection (e.g. symbiosis or the evolution of super-organisms). Life cycles in well-established multicellular organisms (e.g. plants and animals) describe the process through which individuals grow and reproduce. Similarly, we may describe the process through which any multicellular collective forms, grows and reproduces as its 'life cycle', even if the collective is not organismal (e.g. a bacterial biofilm).

One of the most important consequences of nascent life cycles is the extent to which they partition cellular variation among groups [21]. Life cycles that reduce within-group genetic diversity and increase between-group diversity help establish the collective as a Darwinian individual in a number of key ways (box 1). While there are many routes through which microbial collectives form and reproduce, there are two key elements that affect within-group genetic diversity: (i) Is growth clonal, or do growing collectives merge or incorporate cells from other lineages? (ii) How genetically diverse are propagules? The latter depends both on propagule size (smaller propagules are less diverse) [24] and on the physical structure of cells within collectives. Multicellular clusters that develop clonally via branching (such as filamentous bacteria or snowflake yeast) spatially partition genetic variation, and hence even multicellular propagules generated by fragmentation tend to have low genetic diversity [25].

Extant microbes display an extensive variety of nascent multicellular life cycles. While a comprehensive review is beyond the scope of this paper, we will examine several representative examples (figure 1). Perhaps the most ubiquitous multicellular collectives formed by microbes are biofilms. There are many ways to form a biofilm [26,27], but in general, they require the production of adhesive polymers. When biofilms grow by aggregation and reproduce via multicellular propagules (figure 1), it is difficult for selection to act on biofilm-level traits, as this growth form leaves them susceptible to within-group genetic conflict and reduces the heritability of collective-level traits [25,28]. One notable exception is that of *Pseudomonas fluorescens* 'wrinkly spreaders'. In free-swimming *Pseudomonas*, mutations cause the bacteria to begin producing a cell–cell adhesive [29]. This wrinkly spreader mutant then forms a multicellular mat at the air–water interface through clonal division, and produces unicellular propagules when mutations cease production of the cellular adhesive. In principle, this life cycle includes single-cell bottlenecks at each life stage transition (dictated by the mutational steps that alternate between unicellular and multicellular growth), and experimental work shows that it is capable of multicellular adaptation [30]. Although initially unstable, due to a reliance on *de novo* mutations to complete the multicellular life cycle, the formation of such a 'proto-life cycle' may set the stage for developmental control which could arise via an epigenetic mechanism that enables switching between multicellular and unicellular states [30–35].

Experimentally evolved 'snowflake' yeast have an obligately multicellular life cycle, caused by a loss-of-function mutation at the gene *ACE2* [25]. As a result, daughter cells remain attached to mother cells after mitosis, forming a fractal-like branched growth form. Propagules are produced whenever a cell–cell connection is severed. Despite the rarity of unicellular propagules [36], the physical structure of snowflake yeast introduces regular genetic bottlenecks, as every cell in a propagule is clonally derived from the cell at its base

> **Box 1.** The importance of limiting within-collective variation.
>
> 'We designate something as an organism, not because it is n steps up on the ladder of life, but because it is a consolidated unit of design, the focal point where lines of adaptation converge. It is where history has conspired to make between-unit selection efficacious and within-unit selection impotent.'—David Queller [22, p. 187].
>
> Life cycles that strongly partition genetic variation (e.g. through clonal development and a unicellular bottleneck in ontogeny) help make among-collective selection efficacious through three key steps: (i) *Limiting the potential for evolutionary conflict between levels of selection.* Within-collective cellular evolution cannot occur if there are no heritable differences among those cells for selection to act on. (ii) *Facilitating the emergence of heritable multicellular traits.* When the cells in a collective are genetically identical, selection on multicellular traits may correspond directly with genes affecting those multicellular traits. Within-collective genetic diversity should lower the heritability of multicellular traits if the genetic composition of collectives changes across generations (the logic here is identical to why epistatic variation does not contribute to standard measures of narrow sense heritability). (iii) *Increasing among-collective variation, accelerating collective-level adaptation.* As long as cellular genotypes produce heritable multicellular phenotypes, the variance of collective-level traits in the population will be maximized when each group is formed by a single genotype. Applying Fisher's fundamental theorem [23], this accelerates collective-level adaptation. Taken together, life cycles that limit within-group genetic diversity should produce more effective multicellular Darwinian individuals.

(figure 1) [25]. Simple multicellular traits, such as cluster size, are highly heritable ($H^2 = 0.84$) [25], and snowflake yeast readily respond to selection on multicellular traits [36,37].

The volvocine green algae and their unicellular relatives possess a cell cycle that has decoupled growth and reproduction. Individual cells grow, sometimes many times larger than their starting size, then rapidly divide to produce 2, 4 or 8 daughter cells [38]. In unicellular *Chlamydomonas*, daughter cells can remain attached after division, forming multicellular palmelloids [39]. Regardless of whether these collectives disperse via unicells or small clusters of cells, each dispersing unit experiences a unicellular genetic bottleneck (figure 1).

The transition to a multicellular life cycle in the volvocine algae appears to have occurred primarily through the co-option of existing genes rather than through the origin of *de novo* genes [40,41]. Genomic comparisons among unicellular *Chlamydomonas reinhardtii*, undifferentiated *Gonium pectorale* and germ/soma differentiated *Volvox carteri* show that few genes are uniquely shared between *G. pectorale* and *V. carteri*, i.e. that few genes are specific to the multicellular members of the clade [40]. Direct experimental evidence of the importance of co-option comes from a complementation experiment: replacement of the cell cycle regulator *mat3*, a retinoblastoma homolog, with the *G. pectorale* version of the gene causes *C. reinhardtii* to form colonies of 2–16 cells [40]. Thus, a change to the coding sequence of a cell cycle regulator is sufficient to cause a shift to a multicellular life cycle.

Choanoflagellates are a group of unicellular and colony-forming aquatic eukaryotes. They have generated intense interest among evolutionary biologists because they are the closest known living unicellular relatives of animals [42]. Some species possess extensive developmental plasticity, switching between unicellular and multicellular growth ([43]; figure 1). Multicellular rosettes typically develop from unicells via clonal reproduction [44], but these bottlenecks are not strict, as rosettes can generate additional rosettes via multicellular propagules [43].

While genetic conflict is rightfully seen as a major impediment to ETIs, the above examples demonstrate that diverse microbes readily form collectives with little within-group genetic diversity. In the case of small, relatively short-lived collectives such as these, clonal development and regular genetic bottlenecks should be sufficient to maintain this low diversity state, largely immunizing them from within-collective genetic conflict. Conflict, of course, is not the only issue ETIs face: in the next section, we examine how heritable multicellular traits emerge from the properties of cells.

3. Origin of higher-level traits: volvocine algae as a case study

Individuals have traits, and adaptive phenotypic change results from selection on those traits. The outcome of an ETI is a new kind of individual, which has traits that did not exist before the transition. Selection on these novel traits results in adaptations at the new, higher level, but where do the new traits come from?

A *Volvox* colony (or spheroid), for example, has a diameter, a behavioural response to light, and an anterior–posterior polarity. A *Volvox* cell, and for that matter a *Chlamydomonas* cell, also has these traits, but in each case the colony-level trait is not the cell-level trait. In the most recent unicellular ancestor of *Volvox*, these traits were defined at the cell level, but in *Volvox* we can define them at both the cell level and the colony level. Somehow, during the transition from a unicellular to a multicellular life cycle, the colony-level traits came into existence. How did these new traits arise, and how are their values determined?

The initial transition to a multicellular life cycle necessarily begins with some mechanism of keeping (or bringing) cells together [45,46]. In the volvocine algae, this was accomplished through modifications to the cell wall that resulted in the formation of an extracellular matrix [47,48]. The resulting colonies may have been similar to those of the modern *Basichlamys* [49], in which four *Chlamydomonas*-like cells are held together by a common extracellular matrix.

By forming simple multicellular structures, the ancestors of *Basichlamys* acquired traits that are defined at the colony level, such as colony diameter and number of cells. In McShea's [50] terminology, they underwent an increase in hierarchical object complexity, adding an additional hierarchical level (the colony) while retaining all those nested within it (the cell and lower levels). The new, colony-level traits could conceivably affect fitness and vary in heritable ways, thus meeting Lewontin's criteria for adaptive evolution [10].
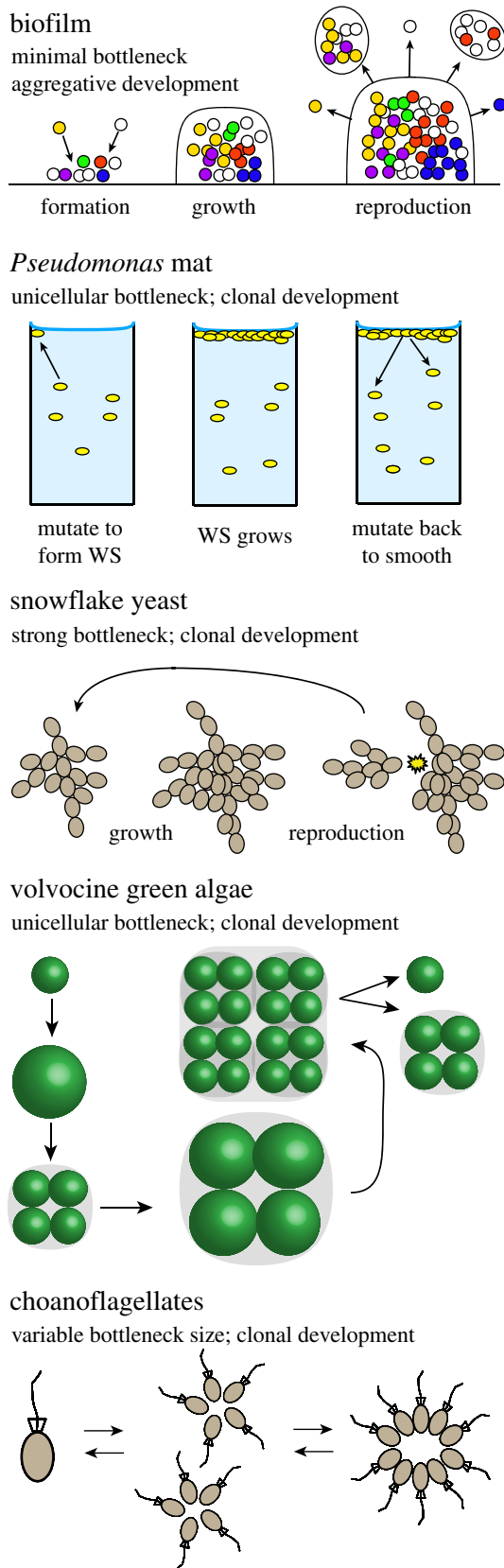
4

biofilm
minimal bottleneck
aggregative development

formation   growth   reproduction

Pseudomonas mat
unicellular bottleneck; clonal development

mutate to   WS grows   mutate back
form WS   to smooth

snowflake yeast
strong bottleneck; clonal development

growth   reproduction

volvocine green algae
unicellular bottleneck; clonal development

choanoflagellates
variable bottleneck size; clonal development

Figure 1. Nascent microbial multicellular life cycles in extant microorganisms.

Colony diameter is meaningless in the context of unicells. Although unicells have a cell number, they have no heritable variation in cell number. The formation of multicellular structures automatically generates new traits that are potentially capable of adaptive evolution.

In both cases, these traits are simple functions of cell-level traits. Colony cell number (N) is determined by the number of rounds of cell division (n) each cell undergoes to form a

daughter colony: $N = 2^n$. The colony-level trait N potentially meets Lewontin's criteria for adaptive evolution, but it is completely and uniquely determined by the cell-level trait n. Genetic variation in n generates genetic variation in N, which is potentially subject to selection, for example, if small N colonies reproduce more quickly than large N colonies.

Colony diameter (D) is also potentially subject to selection, for example, if a gape-limited predator preferentially consumes colonies smaller than a threshold diameter. For a spheroidal colony such as *Eudorina*, D is a function of n, cell volume (v) and the volume of extracellular matrix produced by each cell (e): $D = 2\sqrt[3]{\frac{n(v+e)}{4\pi}}$. Genetic variation in n, v and/or e generates genetic variation in D. The colony-level trait D is completely determined by the cell-level traits, but different combinations of n, v and e values can generate the same value of D.

Colony diameter and cell number are colony-level traits that come into existence as a necessary consequence of the transition to a multicellular life cycle. Although they are simple functions of cell-level traits, neither is defined at the cell level. Rather, they emerge from the properties of the cells. These colony-level traits have the potential to meet Lewontin's criteria for evolution by natural selection at the colony level, and we can expect that selection *on* the colony-level traits will drive adaptive change *in* the colony-level traits (provided there is genetic variation).

The functions relating colony diameter and cell number to cell-level traits are among the simplest such functions possible. We now consider a colony-level trait whose relationship to cell-level traits is more complicated and more difficult to define. In the volvocine family Volvocaceae, which includes *Volvox* and a number of smaller spheroidal genera, the process of embryogenesis includes a complete inversion of the developing daughter colony. After cell division, the flagella of the cells are oriented towards the inside of the colony, a situation not conducive to efficient motility. Over the course of an hour or so, the embryos turn themselves inside out, moving the flagella to the outside surface of the colony.

Although the details of the inversion process vary among Volvocaceaean species, the fundamentals are similar. Inversion involves a combination of changes in cell shape and movements of the cytoplasmic bridges that connect cells during embryogenesis [51]. Cells elongate to become spindle-shaped, and the cytoplasmic bridges migrate to the narrow ends of the cells, causing local changes in the curvature of the cell sheet. These changes propagate through the embryo in a spatially and temporally coordinated wave, eventually reversing the curvature of the entire cell sheet and inverting the embryo.

How this process is coordinated is not known; cells could be responding to mechanical signals (e.g. stresses from local curvature) [52] or to chemical signals transmitted through the cytoplasmic bridges. Regardless, inversion is driven by cell-level developmental processes, possibly influenced by plastic responses to local environmental cues. In principle, the colony-level process of inversion could be described as a function of cell-level traits, with arguments possibly including the degree of cell elongation, the number of cytoplasmic connections formed by each cell, and the shapes of reaction norms describing cellular responses to mechanical or chemical signals. The likely complexity of such a function does not

change the fact that the colony-level process of inversion is entirely controlled by cell-level traits.

The analogous functions underlying many colony-level traits will be even more complex, perhaps even inscrutable. They may include signalling, positional information, feedbacks and more complicated cell–cell interactions. However, their obscurity and complexity do not imply their non-existence. Traits of multicellular organisms must emerge from the traits of their cells; there is no other source.

4. Heritability of higher-level traits

Predicting the magnitude of a response to selection requires estimates of both the strength of selection and the heritability of the trait under selection. This relationship is expressed in the breeder's equation of quantitative genetics: $R = h^2 S$, where R is the response to selection (the difference between mean trait value before and after selection), h^2 is the narrow-sense heritability and S is the selection differential. Narrow-sense heritability is the ratio of additive genetic variance to total phenotypic variance [53], i.e. $\mathrm{Var}(A)/\mathrm{Var}(P)$. In addition to additive genetic variance, the denominator may include environmental effects and the effects of dominance, epistasis (interactions among genes), genotype by environment interactions, maternal effects, etc.

For asexual reproduction, the appropriate expression uses broad-sense heritability H^2 [53]: $R = H^2 S$. Broad-sense heritability is the ratio of total genetic variance to total phenotypic variance: $\mathrm{Var}(G)/\mathrm{Var}(P)$. In this case, genetic effects that are not additive (dominance, epistasis, etc.) are included in the numerator. Because these effects persist in subsequent generations in asexual reproduction, broad-sense heritability, rather than narrow-sense heritability, correctly predicts the response to selection in this case.

Both forms of the breeder's equation succinctly capture the basic insight that heritability is just as important as the strength of selection in predicting the magnitude of a response to selection. This is important for any process that involves multilevel selection. Regardless of the strength of selection on a collective-level trait, no adaptive response is possible unless there is heritable variation in the collective-level trait.

Since colony-level traits are functions of cell-level traits, the heritability of colony-level traits can, in principle, be related to that of cell-level traits. For complex functions, estimating this relationship may be intractable, but for simple functions it can be calculated. Herron & Ratcliff [54] derived an analytical solution for the relationship between cell-level and collective-level heritability for traits for which the colony-level trait is a linear function of the cell-level traits. Under reasonable assumptions, the heritability of a collective-level trait is never less than that of the cell-level trait to which it is linearly related. This asymmetry is driven by an advantage groups have over cells: emergent group-level traits depend on the sum of constituent cell phenotypes, which cancels out (by averaging) much of the heritability-lowering effects of cellular phenotypic noise. For more complicated functions relating cell-level to colony-level traits, collective-level heritability is higher under most (but not all) conditions [54].

A crucial assumption underlying these models is that the development of collectives is clonal, i.e. that particles reproduce asexually within a collective. This roughly corresponds to Queller's 'fraternal' major transitions (Tarnita's 'staying together'), in which collectives consist of genetically

similar (or identical) particles [22,46,55], and it characterizes most multicellular organisms. Land plants, animals, multicellular fungi, red algae, ulvophyte and chlorophyte green algae, and brown algae all develop clonally.

Clonal development ensures that within-collective genetic variability is low; the only source of such variability is *de novo* mutations during development. For a particular trait, especially for small collectives (as are probable early in a transition), it will usually be zero. Nevertheless, phenotypic variability among particles within a collective is inevitable, as stochastic and micro-environmental effects will influence particle phenotypes (both sources of non-genetic variation are treated as 'environmental' components in quantitative genetics models). As long as phenotypic variability is randomly distributed around the genetic mean, though, collectives benefit from an averaging effect, which reduces their non-heritable phenotypic variation relative to the particles that comprise them [54].

Although collective-level heritability has sometimes been considered a hurdle that must be overcome during an ETI [1,56], these results show that it comes 'for free' when development is clonal [54]. Heritability of collective-level traits does not have to 'arise' during the transition to a multicellular life cycle (given clonal development)—it must necessarily exist if the underlying cell-level traits are heritable. This is probably true also for other 'fraternal' transitions.

Next, we quantitatively examine how nascent multicellular life cycles affect the ability for evolutionary innovation. Specifically, we examine the spread of beneficial mutations across three canonical simple multicellular life cycles and consider the implications of key differences.

5. The spread of a beneficial mutation across different life cycles

The structure of a life cycle may affect its capacity to harness beneficial mutations. To explore this idea, we introduce a modelling framework that enables direct comparison of the fixation dynamics of beneficial mutations within different nascent multicellular life cycles (figure 2). In each life cycle, we assume that a mutation arises in a single group during the group stage of a multicellular life cycle. For life cycles that alternate between group and single cell stages, we assume that the mutation occurs right at the end of the group stage so that it begins at some low frequency x_0 within the single cell population. For the life cycle that forgoes a unicellular stage, we assume that, for comparison, the mutation occurs in a group of size N at relative frequency x_0. In each case, we compute the relative frequency of the mutation in the group's lineage over the course of many life cycles.

The beneficial aspect of a mutation can potentially occur at two levels: cell and group. At the cell level, a beneficial mutation may increase the frequency of the mutant in a population of single cells or within the group depending on the structure of the life cycle. At the group level, the mutation may improve the ability for the group to leave offspring. To explore these different aspects and potential interactions between them, we use two parameters, s_c and s_g, that correspond to the fitness benefit conferred to cells and groups, respectively. In the following sections, we determine how a beneficial mutation spreads in three canonical multicellular life cycles.
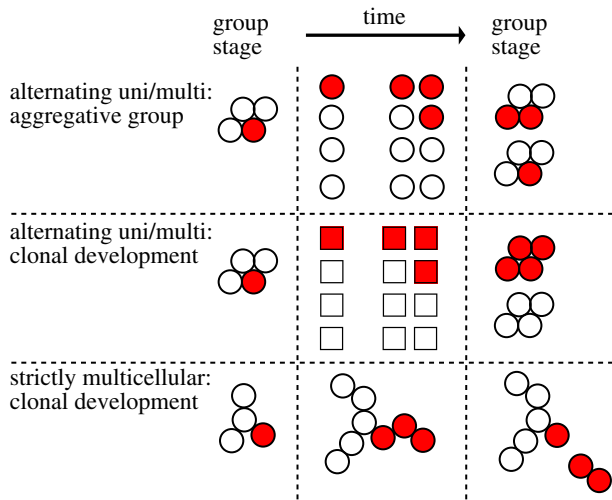
Figure 2. Schematics of canonical early microbial multicellular life cycles. We depict three multicellular life cycles in which groups of cells replicate. The top two life cycles alternate between unicellular and multicellular stages. The primary difference between them is how they form groups. In the aggregative group life cycle, cells form groups through random binding similar to flocculating yeast. The groups eventually dissociate, releasing cells so as to return to the unicellular phase. In the clonal development alternating life cycle, groups are formed from single cells, similar to the formation of wrinkly mats by smooth cells in the *Pseudomonas fluorescens* experimental system [29]. Groups release single cells, usually through a phenotypic switch, indicated by the box- and circle-shaped cells. Finally, there is the strictly multicellular life cycle in which there is no unicellular phase. Cells reproduce within groups and groups eventually split into smaller groups, similar to snowflake yeast [36]. (Online version in colour.)

(a) Model: aggregative life cycle

To compute the spreading dynamics of a beneficial mutation in the aggregative life cycle, we split the life cycle into three phases: (1) growth as single cells, (2) formation of aggregates and (3) survival of aggregates followed by the release of single cells.

During the unicellular phase, cells reproduce, causing the population to expand. We assume that if there is a benefit during this phase, i.e. $s_c > 0$, then the relative frequency of the mutants should increase in the population. Hence if the mutants start at a certain proportion, x_0, in the population, then they will increase to x_1 by the end of this first phase where $x_1 > x_0$. The new proportion will depend on many factors including x_0, s_c and the population growth structure. For simplicity, we assume that the new proportion x_1 is a simple function of x_0 and s_c, called $f_c(x_0, s_c)$, where $f_c(x_0, s_c) = (1 + s_c)x_0/(1 + s_c x_0)$. This form of $f_c(x_0, s_c)$ follows from a simple model of an exponentially growing population; equation (5.1) shows the derivation of $f_c(x, s_c)$, where λ is the growth rate of non-mutant single cells and we assume that $e^{s_c}t = (1 + s_c)$. We use the assumption that $e^{s_c}t = (1 + s_c)$ so that the relative frequency of the mutant compared to the non-mutants increases by $1 + s_c$. Choosing this time enables us to more easily compare between s_c and s_g. We could choose a different time but would then need to rescale s_g so that their effects would be comparable.

$$\frac{x_0\,e^{(\lambda+s_c)t}}{x_0\,e^{(\lambda+s_c)t} + (1 - x_0)\,e^{\lambda t}} = \frac{x_0\,e^{s_c t}}{x_0\,e^{s_c t} + (1 - x_0)}$$
$$= \frac{x_0(1 + s_c)}{x_0(1 + s_c) + (1 - x_0)}$$
$$= \frac{(1 + s_c)x_0}{(1 + s_c x_0)}. \tag{5.1}$$

After the single cell growth phase, there is an aggregation phase. We assume that cells randomly aggregate to form groups of size N. If we assume that the populations of mutants and non-mutants are very large, then the binomial distribution approximates the distribution of aggregates with different proportions of mutants. Thus, a group with proportion $x = i/N$ of mutants has probability $\binom{N}{i} x_1^i(1 - x_1)^{N-i}$ of forming, which we denote as $p(x; N, x_1)$ for $x \in [0/N, 1/N, \ldots, N/N]$, and 0 otherwise.

In the last phase, aggregates compete for survival so as to release single cells and complete the life cycle. For simplicity, we assume that cells do not reproduce while in the aggregate phase. If the mutation confers a fitness benefit to the group, i.e. $s_g > 0$, then this benefit increases the ability of the group to release single cells, either via increased fecundity or increased survival. We do not need to specify the precise mechanism by which the mutation confers a benefit. Instead, we only need a measure of fitness that can be used to translate the distribution of groups with different proportions of mutants $p(x; N, x_1)$ into a scalar corresponding to the population proportion of single-celled mutants, x_0. To this end, we define a group fitness function $f_g(x)$ that assumes that the fitness of groups only depends on the frequency of the mutant within the group and groups with higher proportions of mutants are fitter. We assign a group that only contains mutants, $x = 1$, with fitness $f_g(1) = 1 + s_g$ and a group that has no mutants, $x = 0$, with fitness $f_g(0) = 1$. For intermediate proportions, we consider a simple linear fitness function: $f_g(x) = 1 + s_g x$. The new population proportion of the mutant following this final phase is simply:

$$\frac{\int_0^1 x f_g(x) p(x; N, x_1)\partial x}{\int_0^1 f_g(x) p(x; N, x_1)\partial x}$$

where the denominator is a normalization term.

Equation (5.2) shows the combined effect on the population proportion of the mutant ($x_0 \to x_0'$) after the three phases of the life cycle:

$$x_0' = \frac{\int_0^1 x f_g(x) p(x; N, f_c(x_0, s_c))\partial x}{\int_0^1 f_g(x) p(x; N, f_c(x_0, s_c))\partial x}. \tag{5.2}$$

(b) Model: alternating life cycle (clonal development)

We can determine the spreading dynamics of a beneficial mutation in the alternating life cycle with clonal development by using a similar approach as before with the aggregative life cycle. Again, we split the life cycle into three phases: (1) growth as single cells, (2) formation of groups and (3) survival of groups so as to release single cells. The approaches for phases 1 and 3 are the same as with the aggregative life style. The main difference is in the second phase where groups are formed.

In the aggregative life cycle, groups form randomly such that different types of chimeras are possible. In the case with clonal development, all groups grow from a single cell. This means that there are no chimeric groups and there are only two possibilities: groups with $x = 0$ and groups with $x = 1$. The proportion of groups with $x = 1$ and $x = 0$ is the same as the proportion of mutant and non-mutant cells in the population, respectively. As before, we use the function p to characterize the distribution of groups. We omit the parameter N for group size because it has no effect in the context of this life cycle. The result is $p(x; x_1)$ where $p(1; x_1) = x_1$,

$p(0; x_1) = 1 - x_1$ and $p(x; x_1) = 0$ for $0 < x < 1$. We note that although there is growth during the group stage, we assume that the function f_g, as described in the aggregative life cycle, adequately encapsulates the combined process of growth in the group stage and selection on groups in the alternating life cycle with clonal development.

(c) Model: strictly multicellular life cycle

In the strictly multicellular life cycle, there is no unicellular phase. Instead, groups of cells grow and reproduce via fission. Nonetheless, we can adopt a similar approach to that used to model the two alternating life cycles. Again, we break the life cycle into three phases analogous to the other life cycles: (1) growth within the group, (2) group fission and (3) group survival.

In the previous life cycles, we were able to model the spreading dynamics of a beneficial mutation via x_0, the proportion of mutants in the general population. However, in the strictly multicellular life cycle cells are always members of groups and their distribution across groups may be important to the spreading dynamics. Thus, we use $P(x)$ to track the relative frequency of groups with different proportions of mutants, e.g. $P(0)$ is the proportion of groups with no mutants. If the groups are the same size, then we can relate the proportion of mutants across all cells to the distribution across groups through $x_0 = \int_0^1 xP(x)\partial x$.

The actual structure of the group plays a key role in determining the spread of a beneficial mutation in the same way that population structure does in the other models. It is outside the scope of this paper, however, to consider the gamut of group morphologies. Hence, for simplicity, we will only consider the simplest (and one of the earliest evolving, within the cyanobacteria) life cycles: a linear cellular filament. Cells are each connected in linear chains and all cells can reproduce. Eventually, filaments fragment into smaller filaments and thereby complete the life cycle (figure 3). For simplicity, we assume that a beneficial mutation occurs at a terminal cell in a group of size N. As a consequence, all new mutant cells will be connected to each other and only the original mutant will be connected to a wild-type cell.

The manner in which cells grow within the filament makes it difficult to apply both the same form of $f_c(x, s_c)$ from equation (5.1) and its underlying theoretical framework. As mutant and non-mutant cells reproduce at different rates, if all groups reproduce via fission after some fixed time then the filaments will be of different lengths. Moreover, depending on the choices for parameters, the length of one type of filament (either mutant or non-mutant) would perpetually increase or decrease. To circumvent this issue, we consider two cases: one that uses the same form as $f_c(x, s_c)$ as in the other models and one that uses the same underlying theoretical model. For the first case, we assume that the fragments all grow to reach the same size prior to fragmentation, at which point they all reproduce simultaneously. During the growth phase of the filaments, the proportion of mutants in a group increases according to $f_c(x, s_c)$ from equation (5.1). While this model is directly comparable to the other life cycles, it invokes a mechanism other than simple exponential growth. For the second case, we assume that the cells are all growing exponentially and filaments reproduce whenever they reach a size N—this will occur at different times for
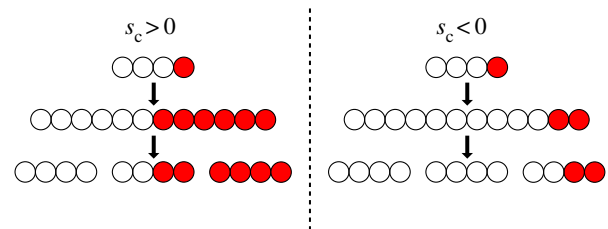

Figure 3. Filament reproduction. Filaments reproduce through binary fission. The mutant (shaded red) increases in relative frequency within the filament when $s_c > 0$ and decreases when $s_c < 0$. In either case, because the mutant increases in absolute numbers, this can lead to offspring filaments with high proportions of mutants. (Online version in colour.)

mutant and non-mutant filaments. The different timescales for the life cycles of non-mutants and mutants means that group reproduction will not be synchronous and so the methodology must be modified. As a result, the spreading dynamics are not directly comparable to the two alternating life cycles. The mutation can still fix in the population even when $s_c < 0$ but the analysis is more involved and thus considered in the electronic supplementary material.

Following growth within filaments, there is a second phase of the life cycle in which groups reproduce through fission. We assume that the filament breaks evenly such that all new filaments are the same size. Hence, if the filament splits into k smaller filaments, then every $1/k$th segment of the large filament is a group offspring. This process results in three possible types of offspring depending on the proportions and the number of offspring: homogeneous with all non-mutant cells, homogeneous with all mutant cells, and one possible heterogeneous filament. If the mutant makes up proportion x_1 of a large filament, then the number of homogeneous mutant offspring filaments are $\lfloor kx_1 \rfloor$ (or floor(kx_1), which returns the largest preceding integer to kx_1). Similarly, the number of homogeneous non-mutant filaments is $\lfloor k(1 - x_1) \rfloor$. If x_1 cannot be divided evenly by $1/k$, then there is a heterogeneous filament that contains proportion $(kx_1 - \lfloor kx_1 \rfloor)/(kx_1 - \lfloor kx_1 \rfloor + k(1 - x_1) - \lfloor k(1 - x_1) \rfloor)$, which we label $\hat{x}_{x_1}$. We define a distribution function $p_G(x; x_1, k)$ that describes the fraction of group offspring with mutant proportion x produced by a group with mutant proportion x_1. Equation (5.3) shows the possible values of $p_G(x; x_1, k)$. We use a subscript G to denote that this p function is different in character from the previous ones. Here, p_G describes the distribution of types of groups following fission from a single type of group, while the previous p functions described the distribution of types of groups in the population.

$$p_G(x; x_1, k) \begin{cases} \dfrac{\lfloor kx_1 \rfloor}{k}, & \text{for } x = 1, \\ \dfrac{\lfloor k(1 - x_1) \rfloor}{k}, & \text{for } x = 0, \\ \dfrac{1}{k}, & \text{for } x = \widehat{x_{x_1}}, \\ 0, & \text{otherwise.} \end{cases} \tag{5.3}$$

The third and last phase of the life cycle has groups with different distributions of mutants competing for survival and reproduction. We can apply the same functional form, $f_g(x)$, as used earlier in the other life cycles. The effect of the life cycle on the distribution of groups is shown in equation (5.4). The primary difference in form from equation (5.2) is
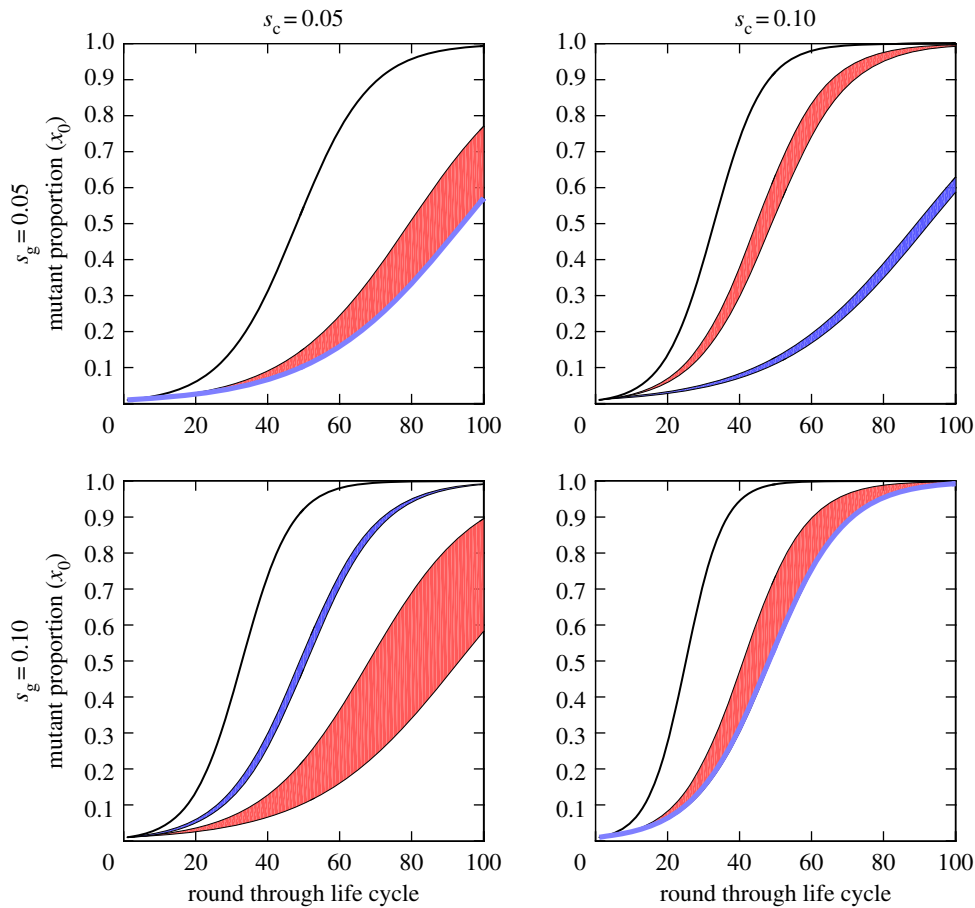
Figure 4. Spreading dynamics of mutations beneficial to both cells and groups in different life cycles. The plots show the proportion of the mutation in a population as a function of the number of rounds through different life cycles for different values of $s_c > 0$ and $s_g > 0$. The aggregative life cycles are shown in the red area (spanning $N = 5$ to $N = 100$), the alternating clonal life cycle is in black and the strictly multicellular life cycles are in the blue area (spanning $k = 2$ to $k = 50$). In all cases, the mutation spreads fastest in the alternating clonal life cycle. When $s_g \leq s_c$, the mutation spreads faster in the aggregative life cycle than the strictly multicellular life cycle.

a consequence of the shift in focus from x_0 to $P(x)$.

$$x_0' = \frac{\int_0^1 x \int_0^1 f_g(x) p_G(x; f_c(\tilde{x}, s_c), k)) P(\tilde{x}) \partial \tilde{x} \partial x}{\int_0^1 \int_0^1 f_g(x) p_G(x; f_c(\tilde{x}, s_c), k)) P(\tilde{x}) \partial \tilde{x} \partial x} \quad (5.4)$$

(d) Comparison of spreading dynamics

With our modelling framework, we can now directly compare the spread of mutations in different life cycles. Figure 4 shows the spreading dynamics for mutations with different values of $s_c, s_g > 0$ (see electronic supplementary material, figure S3 for a broader set of parameter sweeps). In all cases, the mutation spreads the fastest in the alternating life cycle with clonal development. Between the other two life cycles, the mutation spreads faster in the aggregative life cycle in 3 of the 4 cases corresponding to $s_c \geq s_g$. One reason the mutation spreads slowest in the strictly multicellular life cycle is the manner of the s_c fitness benefit. The s_c benefit manifests such that the mutant has a competitive advantage to the wild type. This is important in life cycles with a unicellular phase because the different cell types are in direct competition as single cells. In the strictly multicellular life cycle, the cell types are only in direct competition within heterogeneous groups. As heterogeneous groups (filaments) make up a small proportion of the population, the s_c advantage is effectively masked. Interestingly, the heterogeneity of groups explains why the mutation spreads slower in the aggregative life cycle than in the alternating

clonal life cycle. The heterogeneity of aggregative groups dilutes the s_g benefit of the mutation and inhibits its spread.

If we compare the spread of a mutation that has opposite group-level and cell-level effects, i.e. $s_g > 0, s_c < 0$, then we find different spreading dynamics. These mutations spread fastest in the strictly multicellular life cycle (figure 5; see electronic supplementary material, figure S4, for a broader set of parameter sweeps). This is a result of the same phenomenon that made $s_c > 0$ mutations spread more slowly: this life cycle is shielded from the effects of cell-level fitness, which in this case is negative. As a result, mutations that improve group-level fitness can spread even when they are costly to the fitness of individual cells. This mutation is generally prevented from spreading when the life cycle includes a unicellular stage: it never spreads in the aggregative life cycle and does so only in the clonal life cycle when $s_g > -s_c$, $s_g > 0$. While the $s_g > -s_c$ mutation should confer a net benefit, selection could only act on it in the clonal life cycle where group-level fitness benefits were not shared with non-mutant competitor cells.

6. The evolutionary stability of multicellularity

Mutations where $s_c < 0$ and $s_g > 0$ are of particular interest because they may act to increase the stability of the multicellular collective and facilitate the evolution of increased multicellular complexity [57,58]. The reason for this can be seen by imagining the fitness effect of such a mutation if that
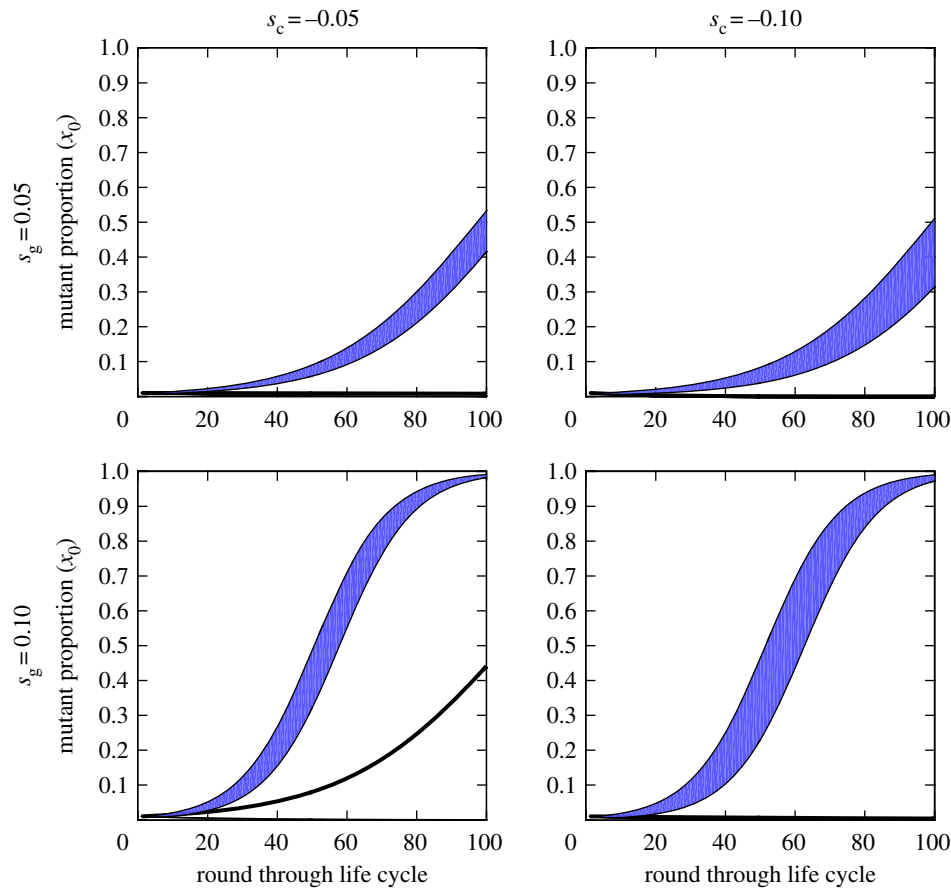
Figure 5. Spreading dynamics of mutations beneficial for groups but deleterious for cells in different life cycles. The plots show the proportion of the mutation in a population as a function of the number of rounds through different life cycles for different values of $s_c < 0$ and $s_g > 0$. The colouring is the same as in figure 4. In all cases, the mutation spreads fastest in the strictly multicellular life cycle. It does not spread in the aggregative life cycle and only spreads in the alternating clonal life cycle when $s_g > -s_c$. (Online version in colour.)

genotype were to revert to a purely unicellular lifestyle (this is similar to the 'counterfactual fitness' approach developed by Shelton & Michod [57]). With the group context eliminated, competition occurs in a way analogous to phase 1 of the aggregative life cycle with a global population of cells multiplying according to equation (5.1). In such a scenario, the beneficial effects of s_g never manifest and mutant cells with $s_c < 0$ would be expected to be driven extinct. This differs from the case of uniformly beneficial mutations (where $s_c, s_g > 0$), because even if a genotype were to revert back to unicellularity, it would have fitness higher than its ancestor.

Libby *et al.* [59] previously studied the effect of mutations that are beneficial in the multicellular context but deleterious in the unicellular context, which they referred to as ratcheting mutations, in populations of genotypes that could switch between unicellular and multicellular states [59]. They found that longer periods of time spent in an environment favouring multicellularity led to the fixation of more ratcheting mutations; this made it more difficult for groups to revert to unicellularity even when environmental conditions favoured single cells. Furthermore, the fixation of ratcheting mutations was shown to favour lower rates of switching between multicellular and unicellular states. This suggests that ratcheting mutations can promote further commitment to the multicellular lifestyle. However, this study did not consider alternating multicellular life cycles, and the deleterious consequences of the ratcheting mutations did not manifest unless a mutation caused reversion back to unicellularity.

Here, we find that the spreading dynamics of ratcheting mutations ($s_c < 0$ and $s_g > 0$) vary dramatically depending

on the details of the multicellular life cycle. Strictly multicellular life cycles are able to fix ratcheting mutations for some value of k under all conditions tested in which $s_g > 0$ (electronic supplementary material, figures S2 and S4). Alternating clonal life cycles can also fix ratcheting mutations, but only under restrictive conditions (where $s_g > -s_c$ and $s_g > 0$). Clonality appears to be essential for the spread of ratcheting mutations, as we did not observe their spread in the aggregative life cycle under any of the conditions tested. However, we note the possibility that mutations exhibiting magnitude epistasis (where $s_c, s_g \geq 0$ and $s_g \gg s_c$) could also behave in a ratchet-like manner, although this would not result in cells that are maladapted in the unicellular phase. Collectively, our modelling suggests that ratcheting mutations fix most easily in clonally developing life cycles that do not exhibit a persistent unicellular phase, which is consistent with the observation that all lineages that have evolved complex multicellularity (e.g. metazoans, plants, brown algae and large multicellular fungi) possess this life cycle [60].

7. Summary/concluding remarks

One of the most astonishing facts about life on Earth is the remarkable fluidity of biological individuality: life, since its inception more than 3.5 Gyr ago, has experimented endlessly with novel collaborations, some of which have resulted in new kinds of organisms and paved the way for transformative adaptive radiations. These ETIs have been surprisingly common, occurring repeatedly in diverse lineages [2]. In this

10

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paper, we examine how simple, emergent life cycles can provide a critical scaffold supporting an ETI during its fragile beginning.

At least in principle, ETIs would appear to be exceptionally restrictive. During an ETI, novel collectives must form and become the focal point of adaptation while not being undone by adaptations occurring among lower-level units. This is challenging, because lower-level units should possess numerous evolutionary advantages (e.g. larger population size, shorter generation time, direct expression of traits that are heritable and prior adaptations that enhance evolvability). Using the transition to multicellularity as a model to explore ETIs in general, we find that the structure of nascent multicellular life cycles can mitigate these factors.

Life cycles that restrict within-group genetic variation through frequent cellular bottlenecks and clonal development evolve readily in diverse taxa (e.g. figure 1), in some cases (e.g. *Pseudomonas* [29], snowflake yeast [25] and unicellular relatives of volvocine algae [40]) through a single mutation. These life cycles limit the potential for within-group evolution and facilitate the emergence of heritable multicellular traits (box 1). As a result, selection shifts to the higher level, efficiently acting on mutations that increase multicellular fitness, even if these mutations reduce single-cell fitness (figure 5) and can restrict the lineage's ability to revert back to strict unicellularity. Given sufficient time, the accumulation of 'ratcheting' mutations can erode cellular autonomy and transform cells into mere parts of the multicellular individual. Taken together, it appears trivially easy for unicellular organisms to form multicellular collectives that grow and reproduce in a manner that is ideal for spurring an ETI.

We are not the first to note that multicelluarity appears to evolve readily—Grosberg & Strathmann [61] labelled it a 'minor major transition', but our life cycle-focused results provide additional insight into how and why multicellularity has evolved so many times. Our argument also extends beyond multicellularity, applying to any ETI that evolves through the creation of a new level of selection. The same features that make a multicellular life cycle efficacious at spurring an ETI (box 1) apply to the origins of cells, super-organisms and novel organisms emerging from symbiosis. For example, monogamy is ancestral to eusocial hymenopterans [62], super-organismal siphonophores are composed of clonal individual animals [63] and the symbiotic origins of cellular plastids occur readily when symbionts are vertically transmitted [64] (a process facilitated by a uniparental bottleneck at fertilization [65]). While much less is known about the origin of cells, when particle movement between cells is limited and subcellular replicators reproduce mainly through protocellular fission, this simple life cycle efficiently allows for selection to act on cell-level fitness [66], minimizing within-cell conflict, improving cell-level heritability and promoting cell-level adaptation. In each case, the life cycle involves a strong ontogenetic bottleneck (or, in the case of symbiosis and protocells, a mechanism that ensures partner fidelity across multiple generations) that limits the potential for within-collective conflict and increases the heritability of collective-level traits.

Observations of extant multicellular organisms are consistent with the idea that clonal development and unicellular bottlenecks facilitate the evolution of complex multicellularity. All extant clades that have evolved complex multicellularity (in the sense of Knoll [60]) develop clonally and have strong genetic bottlenecks, though not necessarily every generation. Unfortunately, this hypothesis is difficult to test. Modern life cycles cannot be assumed to represent ancestral life cycles, and most origins of multicellular life are ancient, with little or no fossil evidence that illuminates the first steps in the transition. However, an increased focus on small, soft-bodied, ancient fossils provides reason for optimism that this situation will improve. Some such fossils are sufficiently abundant that they can be arranged into a developmental series. For example, the large number of fossils of the red alga *Bangiomorpha* preserved at different developmental stages allows a nearly complete reconstruction of their ontogeny [67]. Our results suggest a prediction: if clonal development and single-celled bottlenecks are prerequisites for complex multicellularity, we should expect that future fossil discoveries will show that the ancestors of complex multicellular groups had these traits.

The evolution of complex life on Earth provides us with a model for how complexity might evolve elsewhere in the Universe. Taking Darwinian evolution as a necessary step for the origin of life [68], we see no reason that independently derived replicators would be prevented from forming collectives characterized by life cycles that potentiate higher-level adaptation, especially over planetary scales of size and time. While other factors may limit the origin of complex life [69], the potential for evolutionary innovation is probably not a major constraint.

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