Hypotheses

Cheats as first propagules: A new hypothesis for the evolution of individuality during the transition from single cells to multicellularity

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The emergence of individuality during the evolutionary transition from single cells to multicellularity poses a range of problems. A key issue is how variation in lower-level individuals generates a corporate (collective) entity with Darwinian characteristics. Of central importance to this process is the evolution of a means of collective reproduction, however, the evolution of a means of collective reproduction is not a trivial issue, requiring careful consideration of mechanistic details. Calling upon observations from experiments, we draw attention to proto-life cycles that emerge *via* unconventional routes and that transition, in single steps, individuality to higher levels. One such life cycle arises from conflicts among levels of selection and invokes cheats as a primitive germ line: it lays the foundation for collective reproduction, the basis of a self-policing system, the selective environment for the emergence of development, and hints at a plausible origin for a soma/germ line distinction.

Keywords:
biological complexity; conflict; cooperation; experimental evolution; multi-level selection

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Abbreviations:
MLS, multi-level selection; **WS**, wrinkly spreader.

Introduction

The panoply of plant and animal form that defines life owes much to the rise of multicellularity [1]. From a genetically diverse range of starting positions, independent unicellular lineages have made the transition to multicellularity [2]. The most ancient transitions occurred in the major lineages of large multicellular eukaryotes approximately 1,000 million years ago [3]. Multicellularity has also arisen in the ciliates, slime molds, diatoms, and certain groups of prokaryotes [2, 4–7]; most recently it has occurred in the volvocine algae [8–11].

The evolution of multicellularity involved a hierarchical shift in Darwinian individuality during which individual cells relinquished their capacity to reproduce as independent units and came to reproduce as part of a larger whole [12, 13]. Explaining this shift in selection – from individual cells to groups of cells – poses a range of significant problems. Okasha [13] summarizes: "*The challenge is to understand [. . .] transitions in Darwinian terms. Why was it advantageous for the lower-level units to sacrifice their individuality and form themselves into a corporate body? And how could such an arrangement, once first evolved, be evolutionarily stable?*" Equally, one might focus on the higher level and ask how individuality emerges at the level of the corporate body. In placing the emphasis on individuality at the higher level [14]

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there is recognition that individuality is a derived character and one that requires an evolutionary explanation [15]. The key issue is to explain how variation in lower-level individuals generates a corporate entity with Darwinian characteristics [16]. In this context we argue that the critical problem is the evolution of a means of collective reproduction.

The obvious solution is a life cycle: life cycles involving single-cell bottlenecks are a ubiquitous feature of multicellular life [15, 17, 18]: life cycles allow collectives to produce offspring. Despite their biological significance, the evolutionary origins of life cycles are unclear [15, 19]. Here, informed by experimental studies, we draw attention to critical issues and mechanistic problems that lie at the heart of life cycle evolution. We suggest solutions – albeit of an unconventional sort – and even go so far as to suggest that one route to a proto-life cycle may have been fueled by the tension inherent in levels of selection and may have involved cheating genotypes as propagules.

The multi-level selection framework

Multi-level selection (MLS) theory [13, 20–22] provides a powerful theoretical framework within which to consider major evolutionary transitions. During initial stages of the transition from single cells to multicellularity, the focus is individual cells. Given appropriate ecological conditions [23–25], selection favors the evolution of simple undifferentiated groups – arising, for example, from the production of adhesive glues [25–28]. The cause of cooperation (production of adhesive glues) is the property of the individual cells. Selection at the higher (group) level affects the spread of the trait, but group fitness is nothing more than the average (or sum) of the fitness of the individual cells that comprise the group. From a formal perspective the spread of cooperation is readily explained by kin selection and traditional group selection theory and is encompassed by MLS-1 theory. Within this MLS-1 framework, the fittest groups are those that contribute the greatest number of *individual cells* to the next generation [13, 20].

However, the transition to multicellularity is far more than the evolution of cooperation. Critical for the evolution of multicellular organisms is the evolution of group level adaptations including group reproduction, mechanisms to suppress cheating, and the emergence of development and differentiation. The focus of attention thus shifts from traits that are defined by the properties of individual entities to traits that are the properties of *groups* of cells. This shift marks a significant alteration in perspective and a move to the MLS-2 framework [20]. However, in MLS-2, group fitness is defined independently of particle fitness. The most successful groups are those that contribute the greatest number of group offspring to the next generation irrespective of the number of cells those groups contain. Thus, fitness in MLS-1 and MLS-2 contexts is different: in the MLS-1 context, fitness is the number of offspring particles, whereas, in MLS-2, the number of offspring collectives defines fitness. While this makes intuitive – and theoretical – sense [13], it does not amount to an explanation: just how individuality transfers from particles to collectives is a profound problem.

Theoretical studies of Michod and Nedelcu have made important contributions, particularly the concept of fitness decoupling: the need – during an evolutionary transition – for fitness at the higher level to become decoupled from the fitness of lower level [29]. While being a seminal insight, the mechanism by which it comes about is unclear. For example, Michod [14] uses a simple model for the evolution of multicellularity that begins with ''adult'' organisms comprised of two cell types (cooperate and defect). Although the adult organisms are capable of producing offspring propagules, the production of propagules is not a consequence of adult functionality, but rather is dependent on the average fitness of the individual entities of which each adult is comprised. As Okasha [13] remarks, this is ''*a sort of gray area between MLS-1 and MLS-2*''. Gradually, as the transition proceeds, fitness becomes ''decoupled'' from the lower level and with this, individuality emerges at the level of the adult, to the point where the capacity to leave offspring is a product of adult functionality and independent of the reproductive properties of the individual cells. While such a scenario describes plausible changes, the model assumes that the capacity to leave group offspring is already in place. But how such a new level of reproduction emerges requires explanation.

The evolutionary emergence of group reproduction

From a theoretical perspective the shift from MLS-1 to MLS-2 encapsulates an evolutionary transition in individuality. The transition completes when the higher-level entities become Darwinian individuals, that is, when populations of these organisms display variation, heritability, and reproduction. Thus, one critical trait that marks individuality at the higher level is the capacity for groups to leave offspring groups.

Reproduction of collectives requires development and a life cycle, which is not something that newly formed groups are necessarily born with [15, 19, 30]. When considering the evolutionary origins of such a capability – particularly *via* natural selection – problems arise. The evolution of traits adaptive at a given level of biological organization requires the existence – at that level – of the necessary prerequisites for Darwinian individuality [16, 31–34]. When the trait whose origin we wish to explain is reproduction we face a dilemma: appeals to natural selection would seem to presuppose the existence of collective reproduction – the very trait whose evolution requires explanation. Griesemer foresaw precisely this problem when he argued that explaining the emergence of a new level of organization is necessary before invoking the evolution of adaptations specific to that new level [30].

Below we outline two adaptive solutions in which individuality emerges at the very same moment that the capacity for groups to leave collective offspring evolves. However, we also recognize the potential for non-adaptive solutions. A third possibility is that evolution of a means of collective reproduction is not necessary and that selection on group viability alone is sufficient.

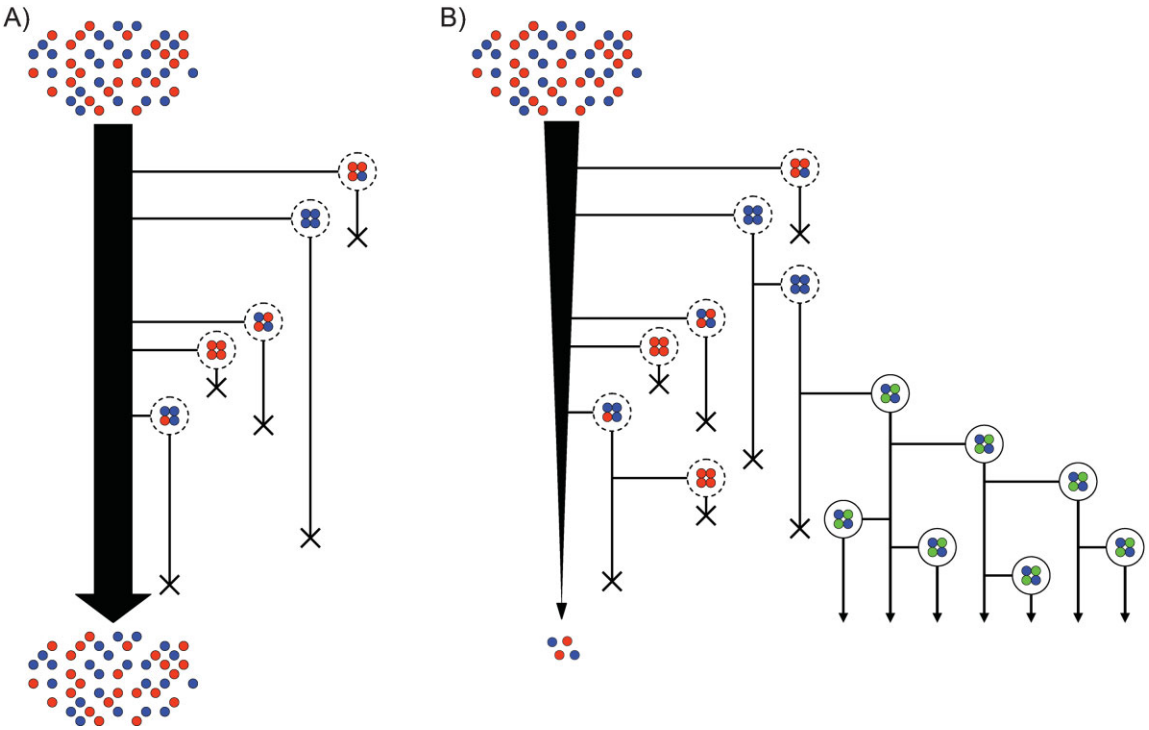

Figure 1. The role of group reproduction in group adaptation. **A**: A scenario is shown in which loose groups form from individual cells (given as red and blue circles). These groups do not beget new groups, nor do they contribute individual cells back to the cell population. Natural selection can certainly act on these groups. For example, in the picture, groups with more blue cells live longer and therefore the frequency of blue cells within groups remains high (this occurs even though the blue cells are at a frequency equal to the red cells within the ''free cell'' population). However, there is no way for evolutionary innovations at the group level to propagate through this form of group viability selection (given finite group lifetimes). For example, it is not the case that groups with blue cells are more likely to form in future generations because they have a viability advantage at the group level. **B**: A scenario is shown where group reproduction occurs. This opens the door for fecundity selection at the level of groups. In this picture, if a group possesses an innovation improving its survival or reproduction, then the innovation can be passed on to daughter groups. For example, the production of specialized cell types (shown in green) leads to a proliferation of groups with these specialized cells. Such a scheme requires both group reproduction and heredity of the developmental program. In this figure we surround the constituent cells with a solid outer circle as they now have some of the properties associated with a higher-level individual (*i.e.* differentiation of parts and capacity to reproduce). If these groups compete with their free cell cousins and group formation confers advantages, then this population could shift from lower-level individuals to higher-level individuals, thereby accomplishing a major transition.

This final option we consider unlikely and explain why in the next section.

The inadequacy of viability selection

The absence of a means of collective reproduction does not mean that selection cannot act on collectives, but its capacity to do so is limited to selection at the level of collective *viability*. Provided that simple undifferentiated groups can evolve repeatedly from the ancestral state (which is readily envisaged), then selection will favor the most viable groups (Fig. 1A). Although such groups are seen by selection, the connection between the consequences of selection at the level of groups at one point in time and the properties of groups at a latter point in time is lacking. The only connection is *via* the lower-level entities. It is difficult to see how viability selection alone could result in the evolution of true group-level traits such as the capacity for group reproduction, let alone, self-policing, development, and differentiation.

Imagine, however, that the viability process operates in tandem with a process by which groups are created from the lower-level parts of pre-existing groups (Fig. 1B). For selection to work creatively – and potently – on the higher level it is crucial for groups to beget groups. But this returns us to the paradoxical situation described above: namely, that the capacity of groups to beget groups requires groups to have evolved this capacity.

Insights from experiments

Our experimental work uses populations of the bacterium *Pseudomonas fluorescens*. When propagated in a spatially structured environment, the ancestral bacterium diversifies producing a range of niche specialist genotypes [35]. Among the numerous emergent forms is a class of genotypes collectively known as wrinkly spreader (WS), which form a self-supporting mat at the air-liquid interface (Fig. 2).

WS genotypes arise from a wide range of simple mutations that result in over-activation of adhesive factors (a cellulosic polymer and a proteinaceous factor) [36–38]. The overproduction of ''glues'' causes cells to remain

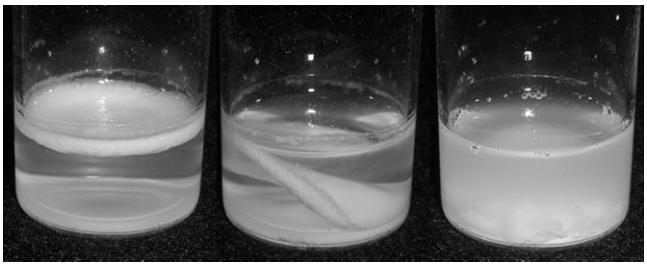

Figure 2. The rise, fall, and destruction of a simple undifferentiated group. Left: The wrinkly spreader mat is the cumulative product of the cooperative interactions of millions of cells. By working together the cells in the mat colonize the air-liquid interface – a niche that is unavailable for the ancestral (broth-colonizing) type. In colonizing this new niche the cells of the mat are rewarded with an abundance of oxygen. Middle: When the mat becomes too heavy, it collapses into the broth (it is not buoyant). The collapse is hastened by the presence of cheating genotypes that grow like a cancer within the mat adding no structural strength, but reaping the benefits (access to oxygen). Right: A mat is far more than the sum of the individual parts. This photo was taken immediately after disturbing (with a brief shake) a microcosm with an intact mat. The mat breaks into many pieces (just visible on the bottom) and does not spontaneously reform. While a mat will eventually re-emerge, it will do so by a process of growth and development from a limiting inoculum.

attached after cell division. While there is a significant fitness cost to each individual WS mutant [25, 39, 40], WS cells nonetheless increase in frequency ultimately out-competing the ancestral genotype. They achieve this because the cost to individual cells is traded against a benefit that accrues to the group of WS cells. It works as follows: the production of adhesive glues means that upon binary fission, daughter cells remain linked. Continuing cell division causes the population of cells to expand in a single-cell layer across the air-liquid interface ultimately joining and becoming attached to the edge of the glass vial. Once the surface is colonized, the mat grows in thickness, becoming a robust structure that is the cumulative product of the cooperative interactions of many millions of cells. By working together, the cells in the mat colonize a niche unavailable to the ancestral type. In colonizing this new niche the cells of the mat are rewarded with an abundance of oxygen [25].

The evolution of a WS mat involves the evolution of cooperation – *de novo* and in real time – from an ancestral state that is asocial and unicellular. The spread of polymer production is readily explained by kin selection [41, 42]. Baring mutation, clonal reproduction means that WS mats are comprised of individuals whose relatedness is complete, the mat being a clone of genetically identical cells. Given mutation, the evolution of cheating (selfish) types is to be expected. Such types evolve and grow as a cancer within the mat. Cheats do not produce adhesive polymers and therefore grow rapidly – they are also highly motile. Provided they arise within the fabric of the mat then they reap the benefits of group membership (access to oxygen) while forgoing the cost of polymer production: in doing so they make no contribution to the network of polymeric strands required for maintenance of mat integrity. As might be anticipated, the cancerous growths compromise the WS mat, and it ultimately collapses [25] (Fig. 2): a classic tragedy of the commons [43].

The emergence of groups leads to questions as to their further evolution. At this point, standard (MLS-1) group selection models are invoked, but it becomes apparent that such models fail to fit with the biological reality of newly formed WS groups. Standard group selection models effectively explain the maintenance of cooperation in the face of selfish types that emerge as a consequence of selection at the lower level. In the absence of population structure, selfish types ultimately outcompete cooperating types causing their extinction. If population structure exists, then cooperating types can be maintained provided there is periodic dispersal of cells into a global population, reassortment, followed by the formation of new groups [44, 45]. This requires that the cells within each group periodically switch off traits that determine social behavior and then reactivate their expression to form new groups. This requires the existence of developmental control – a group-level trait – the evolution of which raises the problems discussed above. In the absence of a means of regulating social behavior, newly formed groups are driven extinct by selfish types.

One way forward would be for group reproduction to be effected by an external factor, for example, stochastic disturbance of microcosms. Individuality of a kind would therefore be endowed to the groups, but it is difficult to see how this haphazard means of reproduction would be effective. Dawkins [34] comes to a similar conclusion regarding the difficulty of organismal adaptation given reproduction through a type of slapdash fissioning.

Life cycles: Solutions and transitions

For Dawkins, adaptive evolution at the level of the multicellular organism requires a developmental cycle (*e.g.* multicellular differentiation from a single-cell origin each generation). However, to avoid the pitfall of invoking group reproduction as a precondition for its own evolution any adaptive solution to the evolution of a life cycle would appear to require the emergence of a life cycle concomitant with the transition in individuality. While seemingly improbable, we outline two scenarios, the first arising directly from experimental studies.

Consider the model *Pseudomonas* populations: the moment the number of WS cells become sufficient to form a mat the stage is set for the evolution of cheating types. Cheats, while being the nemesis of the mat, are also its potential savior. Cheats have characteristics of propagules: they can disperse from the mat – like a germ line they can regenerate WS, albeit upon further mutation (Fig. 3). Indeed, in the case of *Pseudomonas*, the modular nature of the genetic architecture underlying the evolution of WS genotypes provides considerable evolutionarily flexibility [46, 47]. Ancestral genotypes readily give rise to WS genotypes, which in turn

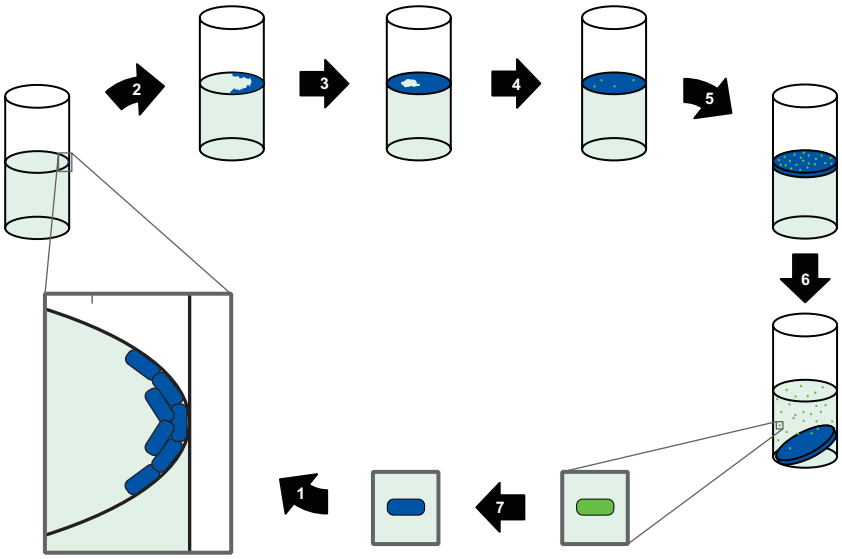

Figure 3. A putative life cycle for mat-forming bacteria. We start with a single bacterium (given in blue) capable of producing an extracellular adhesive. (1) It reproduces at the interface between liquid and air (in the case shown, starting at the inner surface of a glass tube). Daughter cells stick together because of the adhesive they produce. (2, 3) The resulting mat spreads over the liquid's surface as a single-cell layer. (4) Due to prime access to oxygen, a robust mat forms. Mutation generates ''cheats'' (green cells that do not produce any adhesive polymer and grow faster as a consequence). (5) These cheats spread like a cancer within the mat and contribute to (6) the collapse of the mat. Because the cheats do not produce the adhesive, they are liberated from the mat upon collapse. (7) Back mutation from one of these cheats to a mat-producing cell completes the life cycle. Of course, we do not imagine such a life cycle playing out in an environment where only a single mat can form (like a single tube). Rather, the back mutants from the liberated cheats could establish mats in different locations from their parent mat. Here the cell type leading to the death of the group also leads to its rebirth. The cheats amount to propagules (''germ line''), arising *de novo* from the mat-forming ''soma'' of an incipient multicellular individual.

lose the mat-forming phenotype by simple mutations that suppress production of the adhesive glues. The effects of these suppressor mutations can be readily reversed by mutations at additional loci [48]. Thus, from the tension among levels of selection, a proto-life cycle emerges spontaneously (given appropriate ecological conditions) and with no requirement to invoke group-level reproduction as a precondition.

A life cycle that requires mutation to transition the emerging ''organism'' between phenotypic states is a far cry from a developmentally regulated life cycle; however, its existence is sufficient to allow selection to operate at the level of the collective. Indeed, we suggest that the proto-life cycle might provide the basis for the evolutionary emergence of development – a ''kick-start'' – that establishes the ecological conditions necessary for the eventual integration of ''life cycle'' phases within a single cohesive organism (Boxes 1 and 2). Indeed, a recent experiment in which *P. fluorescens* cells were ''forced'' to transition between groups gives reason for optimism. After just four cycles, in two (of twelve) replicate lines, genotypes arose that evolved the capacity to switch stochastically between states by an epigenetic mechanism [48].

The emergence of such phenotype switching is a critical event in the evolution of developmental control [49, 50]. While the end product remains to be experimentally realized, we envisage developmental control emerging as a multi-step process, the first stage being the realization of a novel phenotypic state (the mat-forming phenotype) – the result of selection in an ''extraordinary environment'' [51]. Mutation brings the existing pathway to an expression (and phenotypic) state inaccessible to the ancestral genotype [52]. While adaptive in the new environment, the trait is not environmentally responsive. Critical evolutionary events are thus required to ''rewire'' the organism, such that mat formation comes under developmental control. We suggest that continued selection in an environment that favors alternate phenotypic states (mat-forming and cheating) provides such an opportunity. In outlining this scenario we recognize both parallels and differences with traditional and emerging ideas surrounding the evolution of developmental control [19, 49, 51, 53–56].

Additional scenarios for the evolution of life cycles that might effect the transition from MLS-1 to MLS-2 can be envisaged. Before considering non-adaptive models for life cycle evolution, we describe an alternative hypothesis in which, unlike the model above where the ''germ line'' is *interrupted* by mutation, here the germ line is *uninterrupted* by mutation. From the outset such a model is appealing because it removes the potentially restrictive requirement of mutation for the transition between stages of the life cycle.

Once again we make use of the model *Pseudomonas* populations as a vehicle for our ideas, but this time we take as the focus of interest the lower-level (cheating) entities. Consider the cheating type as a totipotent germ line. Imagine that during the course of its growth it produces, by chance mutation, a cell type with which it interacts, either directly, or indirectly, and which, *via* that interaction, aids its own reproductive output. We might consider this a ''helper'' type; indeed, we might consider the WS genotype an exemplar of such a helper, although in so doing we add a level of complexity (and selection) that is not necessary: the helper may be any kind of reproductive altruist. An interesting example is provided by the suicidal altruists of *Salmonella typhimurium* that die while preparing the ground for infection [57].

Nonetheless, returning to the familiar WS: as the mat forms it becomes infiltrated by cells of the germ line which reap the advantage that accrues from growth at the air-liquid interface. Eventually the mat collapses and the WS

Box 1

Model for the development of a single mat

Here we develop a simple discrete-time model to track differentiation within a mat and its eventual collapse. The model follows two cell types: mat formers and cheats. We begin by describing the population dynamics within a single mat. Assuming that every mat is initialized by a single mat former cell, then over time, mutation generates cheats. Let $m(t)$ and $c(t)$ be the sizes of the mat former and cheat populations, respectively, in a single mat at time t. Populations within a mat grow according to the following branching process [70]:

$$m(t+1) = \sum_{i=1}^{m(t)} [X_i - F_i(X_i)] + \sum_{j=1}^{c(t)} G_j(Y_j), \quad (1)$$

$$c(t+1) = \sum_{j=1}^{c(t)} [Y_j - G_j(Y_j)] + \sum_{i=1}^{m(t)} F_i(X_i). \quad (2)$$

The sets $\{X_1, X_2, X_3, \ldots\}$ and $\{Y_1, Y_2, Y_3, \ldots\}$ contain independent and identically distributed (i.i.d.) Poisson-distributed random variables ($X \sim \text{Poisson}(\beta_m)$ and $Y \sim \text{Poisson}(\beta_c)$). The ith mat former has X_i offspring cells, whereas the jth cheat has Y_j offspring cells. In this model, β_m and β_c are the average number of offspring cells per mat-forming cell and cheat, respectively, per unit of time (β's are birth factors). Because cheats reproduce without contributing to the integrity of the mat, we assume that these cells have a birth rate advantage, *i.e.* $\beta_c > \beta_m$.

The sets $\{F_1, F_2, F_3, \ldots\}$ and $\{G_1, G_2, G_3, \ldots\}$ contain i.i.d. binomially-distributed random variables ($F(n) \sim \text{Binomial}(n, \mu_{m,c})$ and $G(n) \sim \text{Binomial}(n, \mu_{c,m})$). Of its X_i offspring, the ith mat former has F_i cheating mutants, and of its Y_j offspring, the jth cheat has G_j mat former mutants. For simplicity, we let the probability of mutation from mat former to cheat ($\mu_{m,c}$) and from cheat to mat former ($\mu_{c,m}$) be equal: $\mu_{m,c} = \mu_{c,m} = \mu$ (Box 2).

The cell dynamics within a microbial mat are given by equations (1) and (2). In addition, we assume that any mat has a finite lifetime (t^*). The probability that a mat collapses at time $t^* = T$ is given by:

$$\Pr(t^* = T) = 1 - \exp\{-(\alpha_m m(T) + \alpha_c c(T))\} \quad (3)$$

Thus, as the number of cells in a mat increase, the mat is more likely to collapse. Again, because cheats do not contribute to mat integrity, they have a disproportionate negative effect on the lifetime of the mat, *i.e.* $\alpha_c > \alpha_m$.

lineage goes extinct; nonetheless, the germ line remains and in time gives rise to further WS types which it again exploits for its own advantage. Such a scenario captures aspects of an earlier hypothesis in which the germ line originates as a consequence of "*other cell lineages altruistically removing themselves from the reproductive line to perform some somatic benefit to the organism*" [58]. From one perspective, the WS is an extreme altruist, sacrificing its life for the germ line (altruism being an indirect consequence of the short-term advantage gained from colonization of the oxygen-replete air-liquid interface). From another perspective, the WS is an unfortunate pawn, sacrificed by the germ line.

Thus from different starting positions we arrive at essentially the same end point: in both, interrupted and uninterrupted models, there exists potential for the evolution of a life cycle and with that exists potential to arrest in the germ line stage: individuality in an MLS-2 sense is apparent. There are, however, some differences. For example, the interrupted model carries with it the initially burdensome requirement for mutation to mediate the transition between different stages of the life cycle, whereas the uninterrupted model requires only one-way mutation (to dead-end helper cells). The uninterrupted model thus seems to offer a lower hurdle for an evolutionary transition. However, things get more complex when one considers a second distinguishing feature, namely the origin of multicellular differentiation. The uninterrupted model requires the emergence of extreme altruism *via* mutation in the presence of would-be cheats. On the other hand, the interrupted model involves nothing more than the advent of cheats in the face of cooperation. We do, however, note that for both models, the reliance on mutation sets a lower limit to the number of cells that comprise each collective.

In outlining these two models our intention has been to portray possible scenarios for the evolution of life cycles, particularly the selective conditions favoring ecologically distinct phenotypes, that might eventually evolve to come under regulatory (developmental) control. The molecular details by which such control could emerge are unknown but are likely to depend on non-adaptive processes such as mutation and genetic drift [59], opportunities for co-option [60, 61] (facilitated by mutation and drift) and the existence of plasticity [49, 62]. Under some circumstances it is even possible that the plasticity inherent in the genomic and regulatory organization of certain unicellular entities might be sufficient to produce a simple life cycle with minimal involvement from selection. For example, single cells driven to group formation as a mechanism of predation-avoidance might – given an appropriately organized and pre-prepared regulatory system – be capable of utilizing gradients generated across the colony as a means of, for example, regulating the transition between clumping and dispersing behaviors [63].

An idea like this involving co-option of a life history gene has been suggested to explain the evolution of reproductive altruism in the higher volvocine algae [64]. The central idea is that in the ancestral (unicellular) state expression of the life history gene is

Hypotheses

Box 2

Adaptive developmental programs in mat populations

A life cycle initially dependent upon mutation and fueled by conflicts among levels of selection appears understandably restrictive. In particular, there is the thorny issue of heritability, which arises from the mutational lottery that determines the fate of cells. However, this problem is not as great as it may first seem: the critical issue is the rate of transition between states and this rate is heritable. Indeed, the way a mat consigns cells to different categories *via* mutation defines its *developmental program*. In turn, this yields the life history of the mat. Thus, we focus on how changes in mutation rate (μ) affect mat fitness. Here we show how the developmental program can be adaptively tuned to specific ecological conditions.

Mat-level fitness is the ability of the mat to generate offspring mats and is proportional to the number of cheats contained in the mat upon its collapse. This fitness metric is fully adequate if mats always have the same generation time. However, the generation time of a mat is specified (at least probabilistically) by its developmental program [Eq. (3) in Box 1].

All else being equal, a shorter generation time is beneficial within a growing population of mats. However, because cheats simultaneously contribute to mat reproduction and expiration, all else is not equal. For instance, if a slightly longer-lived mat can have many more cheats upon collapse, then it may be advantageous to live longer.

Different ecological circumstances will favor different developmental programs. Here, we consider two ecological conditions. In the first (*r*-selection), sites for mat formation are always available, so there is a premium on a short mat generation time. Production of cheats should be adjusted as to maximize growth rate within an expanding *population* of mats. In the second condition (*K*-selection), sites for mat formation are rarely encountered and there is pressure to lengthen mat generation time to maximize the *absolute number* of cheat cells produced by a mat. We use the model from Box 1 to identify the optimal mutation rate under *r*- and *K*-selection.

As in Box 1, assume a given mat collapses at t^*. There are $c(t^*)$ cheats at this time, which we label c^*. Under *r*-selection, we maximize growth rate of mats within a mat population. To do this, we consider the joint distribution of t^* and c^*. Specifically, for any mat, we have:

$$\Pr(t^* = T \text{ and } c^* = C) = \pi(T, C)$$

Armed with this distribution, the long-term growth rate (r) of a mat population with a specified developmental program is given by the solution to the Euler-Lotka equation [71–73]:

$$\sum_{T=0}^{\infty}\sum_{C=0}^{\infty} \pi(T, C)\mu C e^{-rT} = 1$$

For simplicity, we assume that a fraction μ of the cheats mutate back to mat formers directly after the mat collapses.

We use a Monte Carlo simulation approach to generate the joint distribution π. Specifically, we generate 50,000 points $(t^*, c^*)_i$ using equations (1)–(3). An example of this joint distribution is shown in Fig. 4. In the figure we see the life history tradeoff faced by the mat: higher fecundity requires a longer generation time. With this joint distribution, we solve the following equation for r:

$$\sum_{i=1}^{50,000} \frac{\mu c_i^* e^{-rt_i^*}}{50,000} = 1$$

We then look for the mutation rate (μ) that maximizes r.

For *K*-selection, we search for the mutation rate that maximizes c^*. We employ the same Monte Carlo approach to generate 50,000 c^* values, then we look for the mutation rate that maximizes the average c^* value.

Figure 5 shows the results of this analysis: under *r*-selection, high mutation rates are favored; under *K*-selection, lower mutation rates are favored. Under *r*-selection, longevity is sacrificed for a quick investment in cheats allowing a rapid explosion of mats. Under *K*-selection, longer-lived mats are selectively favored to maximize cheat output.

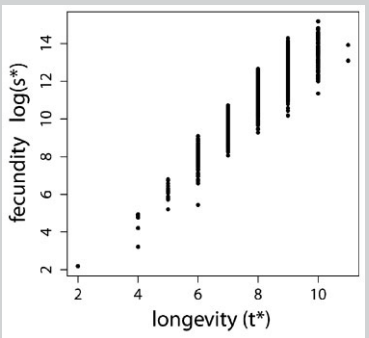

Figure 4. The joint distribution of mat longevity and mat fecundity. These points were generated from simulations of mat development given by Eqs. (1), (2), and (3) (β_m = 4.0, β_c = 6.0, $\alpha_m = 10^{-6}$, $\alpha_c = 10^{-5}$).

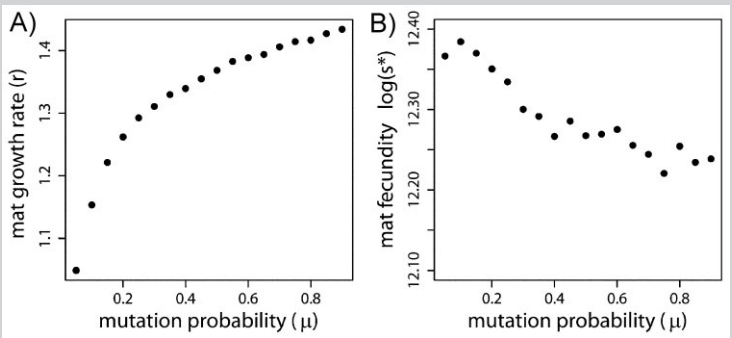

Figure 5. Optimal mutation rates in mat development. **A**: Long-term growth (in an *r*-selected environment) is shown as a function of the mutation probability. Here we see higher mutation rates yielding faster growth of a lineage of mats. **B**: Mat fecundity (favored in a *K*-selected environment) is maximized at lower rates of mutation. In parts A and B, the parameters of the model are the same as those in Fig. 4.

conditioned on an environmental cue, but during the transition to multicellularity it evolves to come under the control of spatial (developmental) signals. Such a scenario makes a good deal of sense and is even supported by studies of *regA* expression (a regulator of chloroplast expression) in unicellular *versus* multicellular volvocine algae [64]. However, just how such a change comes about – particularly the change necessary to bring differentiation under the control of endogenous signals – still requires an evolutionary explanation (see Ref. [65, 66] for a possible mechanism based on a viability-fecundity tradeoff).

Conclusion

Darwinian transitions in individuality, particularly those originating from a fraternal alliance among lower-level entities [58], pose some of the most tantalizing problems in biology. Here we have drawn attention to the need to explain, in mechanistic terms, how variation in lower-level individuals generates a corporate entity with Darwinian characteristics [16]. Our emphasis on this issue stems from the recognition that any explanation for the evolution of multicellularity from unicells – for the transition between MLS-1 and MLS-2 – is dependent upon explaining how collectives evolve the capacity to leave collective offspring. The life cycle, we argue, is the critical innovation: life cycles decouple fitness – they transition individuality.

The unconventional life cycles that span the MLS-1 to MLS-2 juncture are founded in experimental reality. The interrupted life cycle model can operate in experimental *Pseudomonas* populations and, *via* its operation, WS mats can assume the role of ''organisms'' – organisms whose fitness is measured, not by the number of bacterial cells within each mat, but by the number of mat offspring left by parents. In advocating this model as one route to a proto-life cycle we recognize the irony. Tensions between levels of selection are typically viewed as significant impediments to evolutionary transitions [12, 67, 68], but our altered perspective reveals a creative role for conflict. This conflict generates in a single step a means of collective reproduction, a life cycle, the basis of a self-policing system (Boxes 1 and 2), and ecological circumstances possibly conducive to the eventual emergence of development. In addition, the hypothesis provides a plausible scenario for the origin of a soma/germ line distinction, and for sequestration of the germ line by soma – the latter arising from the fact that WS ''soma'' is under strong selection to check increased replication of cheating germ line types. In this context it is interesting to note recent ideas on the evolution of ageing as a deprivation syndrome driven by the tension between soma and germ line [69] – a tension that perhaps, at least for some evolutionary transitions, may have an ancient past.

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