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A conceptual framework for the evolutionary origins of multicellularity

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Abstract

The evolution of multicellular organisms from unicellular counterparts involved a transition in Darwinian individuality from single cells to groups. A particular challenge is to understand the nature of the earliest groups, the causes of their evolution, and the opportunities for emergence of Darwinian properties. Here we outline a conceptual framework based on a logical set of possible pathways for evolution of the simplest self-replicating groups. Central to these pathways is the recognition of a finite number of routes by which genetic information can be transmitted between individual cells and groups. We describe the form and organization of each primordial group state and consider factors affecting persistence and evolution of the nascent multicellular forms. Implications arising from our conceptual framework become apparent when attempting to partition fitness effects at individual and group levels. These are discussed with reference to the evolutionary emergence of individuality and its manifestation in extant multicellular life—including those of marginal Darwinian status.

 Online supplementary data available from stacks.iop.org/PhysBio/10/035001/mmedia


1. Introduction

The evolution of multicellular organisms occurred on multiple occasions, millions of years ago, and in a past that afforded essentially unknowable ecological conditions [1–3]. A standard and general account takes the following form: collections of single cells, by virtue of heritable differences in reproductive output, exist as members of Darwinian populations [4–6]. As members of such populations, cells participate in the process of evolution by natural selection, that is, they are units of selection [4]. During the transition to multicellularity, individual cells became components of groups that eventually evolved the capacity for autonomous reproduction [6]. The point at which this occurred—even to the most marginal extent—marked the moment at which selection was afforded opportunity to operate at the higher (group) level. In some instances this led to the emergence of groups as

Darwinian individuals—as units of selection—in their own right.

Thinking about multicellular organisms in terms of units and levels of selection has become increasingly important. In the 1980s ‘multilevel selection’ (MLS) theory formalized a way to partition fitness effects among individuals and groups [6–10]. The idea is akin to a set of matryoshka dolls with lower levels nested within higher levels, each level replete with Darwinian properties. Recognition of this organizational structure has implications for understanding its consequences, in particular, consequences that arise from the contrasting effects of selection operating simultaneously at higher and lower levels [11].

While primarily a theory of organizational hierarchies, the MLS framework is often extended to questions pertaining to origins [6, 12–14]. In terms of the origins of multicellular organisms, it is usual to make a distinction between MLS1 and MLS2 theory [6, 8, 9]. The former encompasses early stages in the formation of groups, where groups are formed from single cells, by, for example, production of adhesive glues. Kin selection theory and trait group models fall within

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this MLS1 context and describe conditions for the origin and maintenance of cooperation [6]. In each case the focus of selection is on individual entities that happen to evolve within a group context. The fitness of these groups is the average (or sum) fitness of the component cells.

MLS2 theory applies to groups that have made the transition to multicellularity and exist as Darwinian individuals. From an MLS2 perspective, group fitness is defined as the number of collective offspring each group produces. For the most part this is likely to be independent of (decoupled from) the fitness of the individual cells that comprise the group [6, 14].

With a focus on the evolutionary origins of multicellular life—and drawing upon the MLS framework—it makes sense to consider the transition from cells to collectives as the transition from MLS1 to MLS2; that Darwinian individuality transitions from cells to groups along with a concomitant shift in the level at which selection acts [2, 3, 5, 6]. Theoretically this makes sense. The difficulty is that MLS theory fails to explain *how* the transition from MLS1 to MLS2 comes about. It is not sufficient to assume that Darwinian properties inherent in the lower level units are simply ‘moved up’ to the higher level. Variation, heritability and reproduction are derived properties and their emergence at the group level requires an evolutionary explanation [5, 15, 16]. Somehow, during the transition to multicellularity, groups evolved Darwinian characteristics. Just how these emerged is a problem of seminal significance.

Here we consider the earliest stages in the evolutionary emergence of multicellularity, that is, those stages during which unicellular forms gave rise to the simplest groups. In so doing we sidestep the MLS framework and strict notions of Darwinian individuality in order to operate free of assumptions that might limit concepts surrounding the form and evolutionary properties of the earliest groups. There is no doubt that transitions in Darwinian individuality are central to the emergence of multicellular life; however, explaining the emergence of Darwinian properties—particularly the evolution of group level reproduction—requires consideration of a set of starting states that may have been devoid of such properties, but nonetheless, from which Darwinian characteristics might plausibly have emerged.

From this position we outline a logical framework that identifies a set of routes by which groups and individual cells might interact to bring forth group level reproduction. Central to this framework is recognition of a limited set of pathways by which genetic information can be transmitted between individual and group states. Each pathway defines the form and organization of a primitive multicellular organism. This in turn defines its evolutionary properties and, ultimately, opportunities for selection to transition individuality. Finally, we consider each pathway from the MLS perspective and draw attention to issues in the partitioning of fitness effects at individual and group levels: issues that have consequences for the emergence of individuality, and for explaining the diverse array of extant multicellular life—including those of marginal Darwinian status.

2. Sufficient conditions for the evolutionary emergence of multicellularity

Before considering the evolution of multicellularity we establish a set of criteria that define the earliest templates. Extant multicellular organisms are more than a diffuse collection of cells. Indeed, even the simplest exhibit division of labor, developmental programs, morphogenic life cycles, and spatial patterning. While such complexity is characteristic of current multicellular life, it need be neither defining nor essential for all—including the earliest templates. In order to encompass the broadest swathe of plausible founding states (and extant types) we adopt a permissive definition that requires the earliest cellular collectives (groups) to be units of evolution in only a loose sense.

Defining collectives of cells that possess the minimal set of criteria to function as units of evolution implies two sufficient conditions.

- (1) *Existence*. There must be a stage during the life cycle of the organism where a group state is clearly recognizable.
- (2) *Evolution*. Groups must be able to multiply and share heritable information with newly created groups.

The first condition establishes the need for an identifiable group state. This could be a collection of cells that exists in close proximity as a consequence of a defined boundary (which may be exogenously or endogenously defined), or due to, for example, adhesive polymers, incomplete cell division, flocculation, or taxis. It is not necessary that the collective exist solely in a group state. Organisms such as the slime mold *Dictyostelium discoideum* that express a life cycle alternating between unicellular and multicellular states readily satisfy the first condition [17].

While the first sufficient condition establishes the existence of a collective phase, the second condition establishes minimal requirements for the evolution of groups. According to this condition a group of cells existing within a population of groups can be considered a higher level unit of evolution if groups have some capacity to multiply, if offspring groups resemble their parents, and if there is variation among groups. This condition is intentionally permissive and includes the possibility for non-Darwinian groups. By not making explicit a requirement for collective-level reproduction (where groups directly beget groups) and avoiding the need for variation to be linked to reproductive output, we permit the possibility that even improbable, non-Darwinian groups might constitute—if only temporarily—primordial units of evolution (proto multicellular organisms). This does not preclude the possibility that variation among groups might be directly linked to reproductive output.

3. A framework for the evolution of multicellularity

The two sufficient conditions for evolutionary emergence of multicellularity identify a primordial multicellular organism when it arises in a population, but these conditions say nothing about its organization or evolutionary fate. To this end, we model the first appearance of cells that form groups

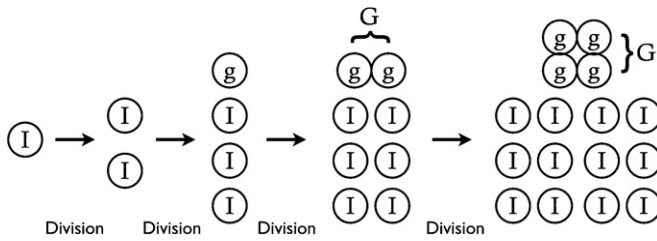


Figure 1. Schematic for the origin of group G from unicellular organism I . In an unstructured environment, independent unicellular organisms (I) divide and occasionally produce a mutant g cell. The g cells form a group G such that when a g cell divides the offspring remain a part of the same group G . This conceptual model provides a template for the evolution of multicellular organisms.

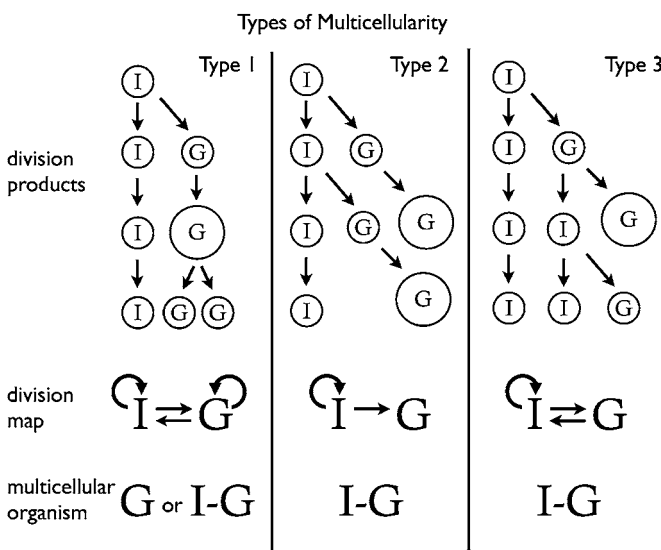


Figure 2. Three types of primordial groups. This framework categorizes the earliest self-replicating groups into three types that satisfy the two sufficient conditions. The first type (type 1) is the origination of a group G (comprised of g cells) capable of multiplication without need of the unicellular I state. The organism in this case is simply the G state though there is the possibility of cycling between individual I and group G states. In type 2 the group is perpetually produced by the I state. While the groups can increase in size through the growth of g cells, G groups cannot reproduce. The only way for a new G group to arise is via the I state. Finally, in type 3, groups multiply via an I state, but unlike type 2 the groups can produce I cells themselves. Here, the g cells in a group occasionally produce an I . Because the genetic distance is small, the I cells produced by g still have the genetic material capable of forming groups G . In types 2 and 3 the multicellular organism is a combination of I and g cells.

and consider the conditions that permit groups to persist and evolve. We assume a spatially unstructured world of unicellular organisms (I for individuals) free to move about and capable of reproduction. With each division there is a probability that a mutation will produce a new type of cell (g). The g cells form a cohesive group G and thereby satisfy the first sufficient condition (figure 1). This group state may be due to any number of different mutations such as those that lead to production of glue on a cell surface or cause failure of cells to separate post division. As individual g cells reproduce, the offspring initially remain part of the same

group so that groups grow in size. Within this framework there are three possible routes for groups to multiply and satisfy the second sufficient condition (figure 2, also supplementary material for an explanatory model (available from stacks.iop.org/PB/10/035001/mmedia)). The difference between these routes depends on whether groups have an innate capacity to produce new groups (type 1), or rely solely on unicellular states to reproduce (type 2), or rely on both group and individual states for reproduction (type 3). As a consequence of these differences, each route presents unique opportunities and challenges to the success of the nascent multicellular organism.

3.1. Type 1

The first route to multicellularity (type 1) stems from groups G that emerge with a capacity to multiply that is independent of the unicellular ancestral state. This category encompasses groups that multiply as a result of fragmentation as wrought by some external environmental factor, as well as groups that have an intrinsic capacity for division. While an intrinsic capacity for multiplication (concomitant with group formation) might seem improbable, such possibilities can be envisaged. Indeed, we can conceive the emergence of groups G that at the moment of origin have the capacity to cycle between collective and dispersing states.

Consider the possibility a g cell, arising by mutation from I , expresses a gene that encodes an adhesive polymer and thereby forms a group. Furthermore, by chance, the gene is under the control of an environmental factor that accumulates as the number of g cells grows. Once a threshold level of factor is exceeded, the gene expressing the polymer is repressed which leads to dispersal of the g cells.

A further example of *de novo* multiplication, although seemingly less probable, was recently observed in experiments with yeast populations subject to selection for sedimentation [18]. Here, the most successful types formed snowflake-like groups that reproduce via a form of fission. Initially groups reproduce via fragmentation and later as a result of increased rates of localized cell death. As cells die within the group, connections between cells are severed, giving rise to new snowflake groups. Given that cell death is a natural feature of yeast it seems that expression of this trait in newly formed groups coincidentally (and fortuitously) allowed group reproduction.

Irrespective of the mechanism by which G groups multiply, such groups will be maintained within a population provided the expected number of offspring per organism over its life time (W_G) is at least one ($W_G \geq 1$).

While it is straightforward to think of the success of the type 1 organism in terms of expected offspring per life time, there is an implicit notion that the group has a finite ‘life time’ ending with death of the collective. This has significant implications for the evolutionary success of nascent multicellular organisms. Manifestation of death at the group level sets the time frame within which the group must reproduce. Consider the snowflake phenotype in which death of individual cells facilitates group division and cell division

increases group size. The only way such a G organism can die is if all individual cells die before any one cell divides. This significantly increases the life span of the group and makes it more likely to satisfy the condition $W_G \geq 1$. If instead the organism died upon death of just a small subset of cells (as is the norm for many extant multicellular organisms), then it would be prohibitively less likely to survive. Thus, as a new type I organism emerges, so too do the conditions that determine its death.

For completeness it is necessary to consider the possibility that a complex life cycle could emerge under type 1 in which group and individual states cycle with each state capable of reproduction. Any time a group G arises from an individual I cell, it faces the same pressures to survive as though there were no route back to I . Since G states can divide on their own, there exists the evolutionary opportunity to forego production of the I state and end the cycling between types.

3.2. Type 2

In the second (type 2) route to multicellularity groups cannot reproduce independently of I cells. The only way that groups propagate is for I cells to continually generate g cells and thereby G groups. This dependence means that unlike type 1 organisms, the organism in type 2 is a combination of G and I states. Without the I state the G state cannot multiply and without the G state the I state is not multicellular.

Considering the breadth of possible configurations within this category there exist two extremes, both of which constitute multicellular organisms, but only one of which offers the possibility for Darwinian evolution of groups. Both extremes involve a lineage of I cells that produce g cells analogous to a perpetual 'germ line'. For one extreme, in which Darwinian evolution of groups is not possible, the I cells give rise stochastically to g cells that detach and drift away, conferring no adaptive benefit to the I cells. These g cells form G groups with finite life spans but exert no influence on the fitness of the unicellular state, I . Any evolution at the group level would be solely a byproduct of selection on I cells combined with differences in survival rates of groups. Nonetheless, an outside observer would notice the presence of groups and even witness changes in the frequency of groups. Of course it is not beyond possibility that future evolution of the g cells might result in groups with some capacity for autonomous multiplication thus transitioning to a type 1 state.

At the opposite extreme, and readily envisioned, is the normal state for metazoan reproduction, in which the dependence between I and G states represents the basic germ/soma distinction [19]. Soma is an evolutionary dead end since it cannot reproduce itself directly and must rely on the germ line. In return, soma offers some benefit, such as increased likelihood of survival, to the germ line [13, 14].

The difference between these two extremes—both of which share the reliance on the germ line for the production of groups—highlights an important feature, namely, the degree of feedback between the cell I and group G states. The moment there is some dependence of I states on G states, the G state is likely to acquire the capacity for differential reproduction thus becoming a unit of selection.

Even though the production of soma G from germ I is usually fixed in the genome via developmental programs, at its origination, the initial production of g cells from I cells is likely to have been caused by a random mutation. Before this mutation, I cells give rise solely to other I cells, but afterwards, the mutation might create a line of I cells that has some tendency (stochastic or deterministic) to produce g cells. Furthermore, I cells that produce groups (call them I_G) are likely to compete with other I cells not producing groups (I_I). As only a small genetic distance separates I_G from I_I , there exists the possibility for I_G to revert to I_I , leading to disappearance of the G state and by extension the proto multicellular organism. The success of such an organism therefore depends on conditions that favor I_G over I_I .

In the extreme case where there is no feedback between groups G and the I_G cells that give rise to them, it is unlikely that conditions will favor I_G over I_I . When an I_G cell divides it either produces a g cell and thereby a group G , or another I_G cell. The consequence of this decision depends on the fitnesses of the two cell types (I_G and g). If g cells have a selective advantage compared to I cells then they will drive the I cells extinct. This will leave a population of g cells that form one G organism with no way for the group G to reproduce. Since the groups cannot multiply, this organism does not constitute a unit of evolution because it violates the second sufficient condition. If, on the other hand, g cells are less fit than I_G (and I_I) cells, then I_I cells will be favored over I_G because they avoid the cost of producing maladapted g types. Ultimately there will be no emergence of G and no multicellular organism.

For I_G cells to have a selective advantage over I_I , the production of groups G must be beneficial. Groups might, for example, offer protection against predation, or allow occupancy of a new niche, or increase the fecundity of I cells by some means. If the advantage of the group is not preferentially conferred on the I_G types, but rather to I cells in general, then the advantage would be a public good and therefore subject to exploitation. Under such conditions, I_I cells are likely to outcompete I_G cells because they do not suffer the costs associated with producing groups. Thus, to stabilize the production of groups, the benefit groups afford must preferentially accrue to I_G types.

Multicellular organisms with germ/soma distinctions often have a soma that encompasses the germ line thereby ensuring that those I_G that give rise to a group G benefit directly from its production. As long as the expected offspring per I_G is not less than I_I , $W_G \geq W_{I_I}$, then the production of G is expected to be maintained.

3.3. Type 3

Lastly, we consider the type 3 route to multicellularity, which combines elements of the other two types. Like type 2, G groups reproduce via I cells. Unlike type 2, g cells within the group G can produce I cells. By enabling g cells to produce I cells, groups are no longer evolutionary dead ends but rather can divide independently of their I progenitors, similar to type 1. Here, the multicellular organism is a combination of g and I cells in which genetic information is transmitted between

both types. This is different from type 2 in which only I cells pass the genetic information for creating groups. Thus, for a group G to survive it must reliably produce I cells that in turn reliably produce g cells. A biological example of an organism that alternates between individual and group states is the ciliate *Sorogena stoianovitchae* whose I cells can divide on their own but go through a group phase (aggregation and fruiting body) to colonize new environments [20].

It has been suggested that the mutual dependence between individual I and group G states may be important for the evolution of cooperating groups in the face of destructive cheating types [21]. The I cells can be viewed as cheats, because they do not act as members of group G . Although cooperation is usually undone by cheats, the cheating types (I) carry the genetic information necessary to generate groups. From the perspective of the g cells, the I cells are its nemesis, and yet from the perspective of the G groups, I cells are its savior [15].

On first emergence, switching between states is likely to rely on mutation, which means that g cells and the I cells they produce (and vice versa) will have subtly different genetic constitutions. Heritability is thus marginal, but nonetheless, the rate of switching is likely to be heritable. Significantly though, switching via mutation may be sufficient to provide the opportunity for a subsequent mutation to bring the capacity to transition between states under epigenetic and ultimately developmental control [22], thus solving the problem of heritability. Such an outcome has been observed in experimental bacterial populations that have experienced continual selection for the ability to transition between two states [23].

Although heritability constitutes a difficulty for the nascent type 3 organism, the fact that G groups can arise from single I cells means a clear divide—in the form of a bottleneck—between states. Such a bottleneck ensures that each new G group is founded by a single g cell which reduces within-group variation and thus minimizes conflicts within nascent groups.

The success of a type 3 organism depends on the selection for both g and I cells. This distinguishes it from the other two forms of multicellularity that require the selection of only one type of cell: g in type 1 and I_G in type 2. To maintain type 3 multicellularity, selection must maintain two phenotypes. The problem is analogous to the maintenance of bet hedging, stochastic switching, and bacterial persistence [24–27]. In all of these circumstances, organisms generate multiple responses/phenotypes in the face of unpredictable environmental fluctuations. In the case of type 3 multicellularity, if newly emergent organisms inhabit a single environment in which g and I cells compete, then either g or I will win, but not both. If, on the other hand, selective conditions fluctuate between favoring groups G and individuals I , then organisms that tune the rates at which they transition between types in order to maximize geometric mean fitness will dominate. The optimal rates of transition between types depends on the characteristics of the environments and their frequency of fluctuations [24–27].

If we consider the example of *S. stoianovitchae*, then those organisms that fail to generate the fruiting body

(group) will be compromised in their capacity to disperse to new environments. The first environment (E_I) constitutes a phase during which unicellular organisms compete for limited resources. Once resources are depleted, there is selective pressure to form a fruiting body and with this, the opportunity to disperse to new environments. Since groups are favored (because of the benefits associated with dispersal), the environment shifts from E_I to E_G . Those cells that do not form a group have limited opportunity to access new resources and likely face extinction. Under this scenario the probability of producing a group must be greater than 0 in order for an organism to compete in combinations of E_I and E_G . These conditions select for stable production of both I and G and thus ensure maintenance of a type 3 organism.

3.4. The MLS1-to-MLS2 divide and fitness decoupling

The framework outlined above defines three distinct pathways by which multicellularity might evolve from unicellular precursors and does so without explicit reference to levels of selection or the focus of selection. As we have argued, this enables consideration of plausible primordial groups that may have marked the earliest stages in the evolution of groups and from which groups with Darwinian properties may subsequently evolve. With such groups in mind it is interesting to consider their place within the MLS framework, where the transition to multicellularity is represented as the shift from MLS1 to MLS2 [6]. Central to this shift is the notion of fitness decoupling, that is, a recognition that as the higher (group) level entity emerges as a unit of selection, fitness at the higher level becomes decoupled from the fitness of the cellular entities that comprise the ‘lower level’ [13, 14].

The question of fitness decoupling in type 1 organisms depends on g cells since they determine both individual and group fitnesses. In the example of the snowflake phenotype of yeast, faster growing g cells produce faster dividing G groups (assuming a fixed probability of cell death). Such a correlation between growth of individual cells and rate of group multiplication implies an MLS1 framework because group fitness is determined by cell fitness. Consider, however, the possibility that the rate of cell death increases. This would lead to fitness decoupling because group fitness would oppose cell fitness. Thus multicellularity evolving by the type 1 pathway might fall within either MLS1 or MLS2 frameworks.

Interestingly, the appropriate MLS framework can be relative (figure 3). Consider a snowflake phenotype composed of g cells defined by only two parameters: a growth rate and a death rate. Imagine that over time growth rate increases, followed by an increase in death rate. Ultimately this results in a genotype that can generate more g cells and more G groups in a fixed amount of time as compared to the original ancestor. An outside observer witnessing the beginning time points (T0–T2 in figure 3) might claim that the MLS1 framework applies, because the growth rate is higher for individual g cells (cell fitness is higher). Another observer who witnessed only the ending time points (T2–T4 in figure 3), in which death rate increased, would argue that the MLS2 framework

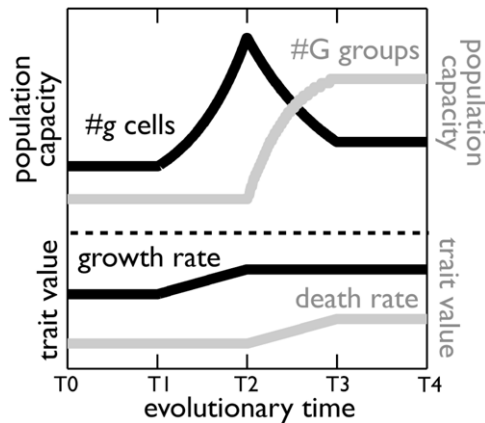


Figure 3. Fitness decoupling in types 1 multicellularity. An example type 1 multicellular organism (a simplified model inspired by the yeast snowflake phenotype, details in supplementary material (available from stacks.iop.org/PB/10/035001/mmedia)) produces new groups when g cells die. The bottom partition of the graph shows the change of growth and death rates of g cells over time. The top partition shows the corresponding abundance for g cells (black) and G groups (gray). Comparing T2 to T0, the number of g cells and groups has increased and selection thus seems to focus on the individual level (MLS1). However, when comparing T3 to T2 it is apparent that fitness decoupling has occurred (MLS2). Following the trajectory from T0 to T4, one would conclude that an MLS1 to MLS2 transition has occurred. If, instead the sections T1–T2 and T2–T3 were reversed, one would conclude that an MLS2 to MLS1 transition occurred. Thus, the exact trajectory needs to be known in order to determine the type of MLS multicellularity even though the starting and ending points (T0 and T4) are identical.

applies, because increased death rate sacrifices individual cell fitness for group fitness. A third outside observer witnessing the entire course of evolution (T0–T4 in figure 5) would see firstly an increase in growth rate, followed by an increase in death rate. Such an observer would argue evolution involved a shift from MLS1 to MLS2. If, however, evolution resulted in firstly an increase in death rate, followed subsequently by an increase in growth rate, then an observer would conclude evolution involved a shift from MLS2 to MLS1. Thus, although the beginning and ending states of the system are identical, transitions would have occurred in opposing directions. The classifications therefore depend on previous known historical states in order to determine the directionality of the evolutionary transition, and consequently the appropriate MLS framework.

The application of MLS1 and MLS2 frameworks to type 2 multicellularity depends on how the lower (individual) level is defined. While type 2 multicellularity embodies both g and I_G cells, success of the nascent organism hinges on the success of I_G cells. In its simplest form, the fitter the I_G cells, the greater the number of both groups and individuals (both I_G and g cells) produced (figure 4). Not only does this result in an alignment of group and individual fitnesses, but the group is only created so long as it enhances the individual I_G . Yet, if the relationship between group fitness and fitness of the g cells that comprise the G group is considered, then there is likely to be decoupling. Here there is no direct effect of g cell growth (or number) on G group fitness, but there are indirect effects. Earlier, we reasoned that type 2 evolves only if groups

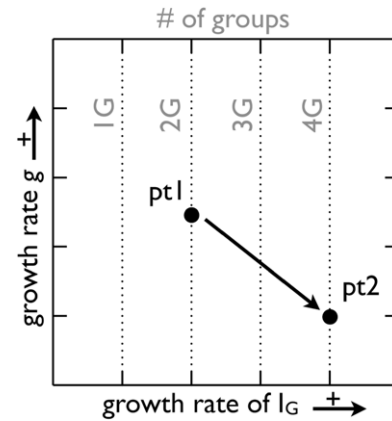


Figure 4. Fitness decoupling in type 2 multicellularity. Contour plot for the number of G groups of a type 2 organism versus growth rates of I_G and g cells (details in supplementary material (available from stacks.iop.org/PB/10/035001/mmedia)). Since groups are produced via I_G cells, the number of groups is only affected by the growth rate of I_G . In contrast, the growth rate of g cells has no effect on the number of groups. If an organism with growth rates defined by pt1 evolves to pt2, then the fitness of groups increases while the fitness of g cells decreases and the fitness of I_G cells increases. The occurrence of fitness decoupling between group and individual levels depends on which cell type (g or I_G) represents the individual, 'lower', level.

offer a benefit to I_G cells. In the specific circumstance that the benefit is directly proportional to the number of g cells, or their growth rate, then fitness decoupling would not occur. In all other cases, fitness of the higher level likely decouples from the lower. So for type 2 organisms, if I_G cells are the individual entities then the system is MLS1, but if g cells are considered the individual entities then the organism resides in MLS2 territory. By extension, if both I_G and g cells are classified as the individual entities, then the system is either MLS1 or MLS2 depending on the proportions of each type of cell.

At first glance, type 3 organisms appear to reside within MLS1 territory. Groups reproduce via I cells, so for a fixed rate of transition between I and g cells, groups that produce more I will yield more offspring. Since the conditions that maintain type 3 multicellularity select for maximum geometric mean fitness via transitions between I and g cells, it is possible to conceive of G groups or g cells as merely a way to increase the propagation of I cells in the population and advantages common to g and I cells would rapidly fix. In a simple model in which g and I cells switch randomly with some probability, then the groups with the largest number of g cells are likely to produce the largest number of I cells, which are themselves group offspring. Thus, type 3 organisms are seemingly MLS1 in nature.

Although type 3 multicellularity appears to be MLS1, it may evolve according to an MLS2 framework. The key to seeing the MLS2 context involves recognition of the fact that group offspring are valued more than individual cells. I cells that increase the probability of producing a g cell exchange I offspring for group offspring. If this increased productivity of g cells occurs in an environment that favors I cells (E_I) then the total cell number decreases but group number increases.

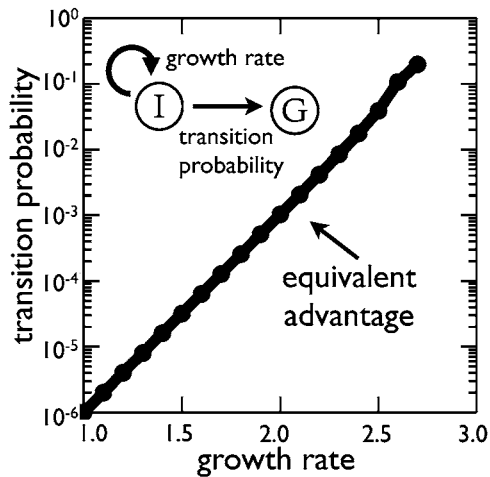


Figure 5. Fitness decoupling in type 3 multicellularity. The advantage in group offspring a type 3 organism gains from increasing transition probability, or cell growth rate, in comparison to a reference organism that has divided ten times and produces g cells with probability 10^{-6} (details in supplementary material (available from stacks.iop.org/PB/10/035001/mmedia)). Increasing transition probability to $\approx 10^{-3}$ exceeds the advantage of doubling the growth rate. Since increasing transition probability is potentially easier than growing twice as fast, the organism is likely to evolve to tune its transition probability. Increasing the transition probability decouples fitness (MLS2) while changes wrought via alterations in growth rate retain the organisms within the MLS1 state.

In this case, type 3 multicellular organisms operate within the MLS2 framework.

To determine whether MLS1 or MLS2 is more likely, we model the population growth of type 3 organisms that begin in the I state and grow until the environment switches to favor the G state—recall that for type 3 multicellularity to evolve the environment must fluctuate between favoring G groups (environment E_G) and I cells (environment E_I). If we assume there is an optimal number of groups G that must be produced by the time the environment switches to favor the G state (E_G), then this can be achieved by either increasing the growth rate of I , or by increasing the probability of producing a g cell from I (the transition probability). From this, we create an equivalence using the common currency of the number of g cells produced (figure 5). Whether the growth rate or transition probability evolves to achieve a certain number of groups depends on the likelihood of the two events. Experimental observations show that after 20 000 generations *E. coli* cells propagated in the same environment, while increasing in fitness, have not doubled their growth rate [28]. In contrast, organisms can increase the transition probability between states by several orders of magnitudes in just 100 s of generations [23]. Thus, the optimal number of group offspring is more likely to be obtained by manipulating transition probability, as opposed to increasing growth rate. So, while type 3 organisms may appear to exist within an MLS1 framework, selection is more likely to operate on the rate of transition between cell types, thus moving the organism into the MLS2 arena. Furthermore, this selection on transition probability is likely to lead to fitness decoupling.

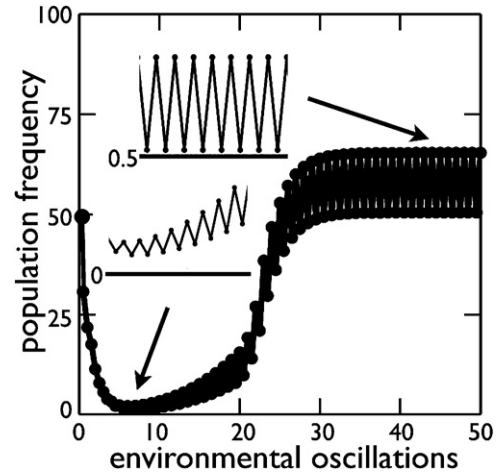


Figure 6. Emergent fitness in type 3 multicellularity. The frequency of a type 3 organism in competition with one that grows 5% faster (both I and g cells) but has different transition probabilities between cells. The frequency of the slow growing type drops close to 0 before the tenth environmental cycle (where $E_G \leftrightarrow E_I$ is one cycle) and yet subsequently enters into a stable oscillation in which it becomes the dominant organism by the 50th environmental cycle. The fitness of the slower growing organism is not dependent on the numbers or fitness of either I or g cells, but rather their interplay.

In type 3 organisms, fitness can be an emergent property, not residing at either group or individual level. To illustrate this point, we compete two type 3 multicellular organisms, one of which grows 5% faster in both the I and g states compared to the other (figure 5). The organisms have different probabilities/rates of transition between the two states. We propagate the two organisms in regularly oscillating environments (environments switch when the population of cells reaches a carrying capacity of 10^9) in which a subpopulation (2×10^3) passages through the fluctuations (details in supplementary material (available from stacks.iop.org/PB/10/035001/mmedia)). For the first five environmental oscillations the slower growing types decrease in frequency in both states until they are almost extinct. They then increase in frequency until they reach a stable oscillation in which both slower and faster growing organisms co-exist. Such behavior shows that once there is an advantage to produce opposing types, population dynamics may diverge from monotonic extinction or dominance and take more complex trajectories. The fitness of the collective type 3 organism does not rest in either group or individual states, nor in their average, but rather in the interplay between them.

4. Conclusions

Multicellularity has evolved on multiple independent occasions, but the starting states and conditions are unknown [1–3]. Each transition began with unicellular organisms that gave rise to groups that subsequently evolved Darwinian properties. Of these Darwinian properties, group level reproduction is arguably seminal. Once groups evolved the capacity to transmit heritable variation to group offspring—no matter how marginally—there was opportunity for selection to act upon nascent groups. Indeed, the particular mode of

reproduction has implications for the partitioning of variation (heritability) among offspring. Here, with interest in these earliest stages, we have outlined a logical framework for understanding the set of possible routes from unicellular organisms to primordial, self-reproducing groups. Among these routes are some that deliver groups that lack rudimentary Darwinian properties, but which may nonetheless evolve these properties by non-Darwinian means. Other routes, by virtue of fortuitous events of chance and history, may, even at the moment of origin, deliver groups capable of participating directly in the process of evolution by natural selection.

Central to our framework is recognition of the need for fluidity between individual (unicellular) and group states. This is not only a prevalent feature of many extant multicellular types, but was likely relevant for the initial (primordial) stages in the evolutionary emergence of multicellularity. It is tempting to think of the transition to multicellularity as cataclysmic: a moment of abrupt change that delivered self-replicating groups ready formed and replete with Darwinian properties at the very moment at which they diverged from their unicellular ancestors. Although we consider this possible, for example, via the type 1 route, it is more probable that the first groups lacked any capacity for autonomous replication beyond that inherent in the unicellular state from which they emerged. In this context, type 2 and 3 multicellularity have special significance as these routes permit fluidity between individual and group states. This fluidity, while permitting a return to the unicellular state, allows for repeated opportunities to transition Darwinian individuality. However, any such opportunity is likely to be dependent on specific ecological conditions: the longer each nascent group is maintained, the more opportunity selection has to shape the emergence of Darwinian characters.

In considering the earliest stages in the formation of self-replicating groups, constraints associated with the MLS framework—particularly the notion that evolutionary transitions begin within an MLS1 framework and then transition to MLS2—become apparent. MLS theory was inspired by the hierarchical organization of extant multicellular forms, characteristic of organisms with both a high level of cellular integration and clearly marked divides between generations (typically a consequence of a dedicated germ line involving a bottleneck phase) [6–10]. In these organisms the nesting of levels (cells within organisms) is a defining feature. Consequently it makes sense to think of one level of organization where groups beget groups and another (lower level) where cells beget cells. The difficulty is that the earliest multicellular forms—and many extant forms—do not comfortably fit this framework. For example, among the earliest groups it is conceivable that some did not always beget groups (but beget unicellular organisms, which then beget groups). Initially, such reproduction would have likely been driven by mutation or some other stochastic mechanism, resulting in unreliable life cycles. The MLS framework does not readily accommodate these situations where groups beget individual cells or individual cells beget groups—particularly when they do so in a noisy fashion. Indeed, even among extant multicellular organisms, such as slime molds (where cells beget groups and groups beget cells) the MLS framework sits with some difficulty.

While establishing plausible initial stages for the emergence of multicellularity, our framework makes no predictions as to the likely course of evolution for each form. It could be that some starting conditions and states are better suited for the evolution of complex traits such as developmental programs, division of labor, differentiation, and life cycles, than others. Indeed, types 2 and 3 encompass forms that arguably show primitive division of labor, a nascent soma / germ line distinction, and developmental programs. Yet the selective conditions required for the maintenance of these two types are likely to be stringent in comparison with groups that emerge via the type 1 route. Further, the fluidity permitted by our framework means that while an organism may begin as type 1, it may shift to a type 2 group by virtue of a *g* cell that differentiates into a dedicated germ line. Additionally a type 2 group may evolve into type 3 should a *g* cell randomly give rise to an *I* cell. In addition, it is conceivable that a type 3 group *G* might evolve a means of multiplication thereby foregoing the *I* state and shifting to a type 1 organism. Thus, primitive multicellularity could transition between forms and states numerous times before settling into a stable configuration.

Just as our framework offers no long-term predictions for the final form of multicellularity, it makes no specific predictions concerning the most probable starting state. Ecological circumstances (along with chance and history) are likely to be critical in determining whether groups emerge via type 1, 2, or 3 routes. However, it is possible that certain configurations are more likely than others. For example—given appropriate ecological conditions—type 1 organisms can emerge, in principle, via just a single event (production of *g* cells with specific properties). Similarly type 2 maintains *I* cells but simply requires that at some point they produce *g* cells with appropriate characteristics. Type 3, on the other hand, requires that *I* cells not only produce special *g* cells but that these *g* cells in turn produce *I* cells. There is experimental evidence that this is possible [23] but it is likely that the type 3 route is more demanding, both in terms of genetic architecture and ecological conditions, than types 1 and 2.

Although we argue that type 1, 2 and 3 pathways logically encompass the full swathe of early multicellular forms, there are important underlying assumptions. First of all, we assume that autonomy of individual cells is maintained. Cells remain independent entities and cannot merge to form new individuals. If a multicellular organism were to form as a consequence of cell fusion, such that the group was not composed of distinct individual cells, then the framework outlined here would not be applicable. One reason for requiring autonomy is that the specific way cells fuse or lose identity is likely to have a significant impact on evolution of the collective. This makes it difficult to logically construct a distinct, small number of types. Additionally, cell fusion is likely to create chimeric organisms thus impacting negatively on the emergence of group heritability.

There are some interesting connections between the evolution of multicellularity as outlined here and other areas of evolutionary research. The selective conditions necessary to maintain type 1 multicellularity are analogous to those required for any newly evolved species [29]. In type 2 organisms, I_G

cells engage in a costly behavior by producing g cells. Thus in order to be favored over I_I cells, I_G cells need to reap some selective advantage. This same challenge is present in explanations for the evolution of public goods, cooperation, altruism, and germ line sequestration [19, 30]. Type 3 multicellularity has similarities with bet-hedging strategies like bacterial persistence [24–27] since both require fluctuating selection. Thus, these well-recognized problems in evolution are united in our framework as describing the challenge of maintaining groups in different types of multicellularity.

Finally, our framework connects to recent thinking on the emergence of biological individuality, and particularly issues surrounding the assignment of individuality to non-paradigm status multicellular organisms [5, 31]. Many extant multicellular organisms, including slime molds and algae, exist in multiple states, i.e., a combination of I and G . Take for example the brown alga *Ectocarpus siliculosus*, which exists as a sporophyte, gametophyte, and parthenophyte [32]. Our framework shows that for organisms that exist in multiple states at different stages of the life cycle, the fitness or success of the organism does not rest in any one state nor a sum of states. Consider two genotypes of the brown alga, one of which produces more sporophytes, but fewer parthenophytes, than the other. It is not clear which of the two is the more fit. Indeed, our framework makes this clear. In this example (and also the example shown in figure 5), individuality does not rest in one state or in a sum of states, but rather is an emergent property. As an emergent property, it exists in the complex interactions between states and is not reducible to a single state or level.

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