Trade-offs Drive the Evolution of Increased Complexity in Nascent Multicellular Digital Organisms

Peter L. Conlin¹ and William C. Ratcliff²

The transition to multicellularity was a major step in the evolution of large, complex life on Earth (Maynard Smith and Szathmáry 1995). Unlike other major evolutionary transitions, which have occurred only once (e.g., prokaryotes to eukaryotes), multicellularity has evolved multiple times in diverse lineages including archaea (Jahn et al. 2008), bacteria (Velicer and Vos 2009; Overmann 2010; Schirrmeister et al. 2011), and eukaryotes (Bonner 1998; King 2004; Grosberg and Strathmann 2007; Herron et al. 2013). Prior work suggests that the formation of simple clusters of cells, the first step in the transition to multicellularity, may be adaptive under a number of distinct ecological scenarios. For example, clusters may provide protection from predation (Kessin et al. 1996; Boraas et al. 1998), protection from environmental stress (Smukalla et al. 2008), or improved utilization of diffusible nutrients (Pfeiffer and Bonhoeffer 2003, Koschwanez et al. 2011; Koschwanez et al. 2013). Nevertheless, how and why nascent multicellular lineages evolve increased complexity remains a fundamental question in evolutionary biology. Progress has been impeded by a lack of experimental systems due to the fact that most nascent multicellular lineages have been lost to extinction.

To sidestep this historical limitation, we (and colleagues) have been using experimental evolution to re-create this major transition under controlled laboratory conditions (reviewed in Ratcliff and Travisano, 2014). Starting with outbred diploid unicellular yeast, we selected for cluster formation by favoring yeast that settle rapidly through liquid medium. In all ten replicate populations, cluster-forming ‘snowflake’ yeast readily evolved and displaced their unicellular ancestors. Snowflake yeast consist of daughter cells that remain attached after mitotic division, forming spherical branched structures of genetically-identical cells. Over the next several hundred generations, several traits of interest evolved as snowflake yeast further adapted to this selection regime.

¹Department of Biology and BEACON Center for the Study of Evolution in Action, University of Washington
²Department of Biology, Georgia Institute of Technology
In response to selection for rapid settling, snowflake yeast first evolved to form clusters that contain more cells. Later, snowflake yeast evolved a 2.1-fold increase in the volume of individual cells, further increasing cluster biomass and thus settling speeds (Ratcliff et al. 2013). Large-bodied yeast also evolved higher rates of programmed cell death, hereafter referred to as apoptosis. Prior experiments suggest that these dead cells act as ‘weak links’ in the chains of cells that make up the cluster, resulting in greater reproductive asymmetry (i.e., smaller propagules relative to cluster size). This conclusion is based on comparisons between high and low-apoptosis strains, direct experiments modifying the frequency of apoptosis chemically, and the observation that dead cells are found at the site of propagule scission ~12 times more frequently than is expected by chance (Ratcliff et al. 2012).

Fitness trade-offs, while central to all of life history theory (Roff 2001), are thought to take on a particularly important role during major evolutionary transitions such as the evolution of multicellularity (Michod et al. 2006). Specifically, trade-offs between survival and reproduction may drive increases in complexity and cellular differentiation (Michod et al. 2006). Perhaps the best-known example comes from the evolution of multicellularity in the volvocine algae: individual cells cannot reproduce and phototax simultaneously (Koufopanou 1994), favoring the evolution of divided labor through germ-soma differentiation (Koufopanou 1994; Solari et al. 2006). More generally, simple clusters of cells may benefit from increased size (e.g., reduced consumption by predators [Boraas et al. 1998; Becks et al. 2012]), but cellular clusters face greater diffusional limitation than single-cells, impeding resource uptake from their environment (Lavrentovich et al. 2013). Our experimental results suggest that trade-offs also play a role in the evolution of multicellularity in snowflake yeast. Evolving larger clusters increases settling speed, but decreases growth rates, likely because cells in the interior of large clusters become resource limited as a result of greater diffusional impedance (Ratcliff et al. 2012; Lavrentovich et al. 2013). Similarly, increasing cell size may decrease the rate at which individual cells are produced, again because larger cells have a proportionally greater surface area to volume ratio (but see Jorgensen et al. 2002). The effects of apoptosis are a bit more complicated. A small fraction of the cells in the cluster (~1.5-2.5%) die, a direct viability cost. However, by producing proportionally smaller propagules, large clusters produce offspring that are less diffusional-limited. Thus, apoptosis increases growth rates but decreases survival during settling selection, and will only be adaptive when the sum of these effects is positive.

Here we investigate the role of simple trade-offs during the evolution of increased multicellularity in snowflake yeast by modeling the evolution of simple multicellular digital organisms. We find that apoptosis, which results in the production of smaller propagules at the expense of the acting cell’s life, is adaptive under a broad suite of conditions. This is because it can increase growth rates enough to compensate for the loss of apoptotic cells and reduced survival during settling selection. In our models, competition for faster settling results in an evolutionary arms race that drives a modest (maximum of 150 cells) increase in cluster size and apoptosis. Much larger clusters only evolved if the size required for surviving settling selection was increased through time. Using a two-player tournament-style evolutionary algorithm, we find that snowflake yeast that are initially mismatched in size will niche partition, with the smaller strain evolving into a growth specialist and the
larger strain a settling specialist. Finally, we find that increasing the dimensionality of the multicellular trait space from two (cells per cluster and apoptosis) to three (adding cell size) increases the degree to which competing strains in a single population will diverge. This work demonstrates that multicellular complexity readily arises when trade-offs between group size and growth rates are ameliorated by the evolution of novel multicellular traits.

Model Description

Cluster growth and reproduction

The model we develop here considers competition occurring between two genetically distinct snowflake yeast strains within a single population. As in our laboratory experiments, the transfer cycle involves two discrete phases: growth and settling selection (summarized in Figure 1). For each time step, clusters grow by adding cells in proportion to their initial number following the equation:

\[ n' = n(2 - nd) - na, \]  
\[ (1) \]

where \( n \) is cell number per cluster, \( d \) is the diffusional limitation cost (ranging from 0.001 to 0.002) and \( a \) is the rate of apoptosis (see Table 1 for a summary of model parameter). In comparison to single-cells, clusters are diffusionally-limited, and thus grow less rapidly (Ratcliff et al. 2012). For simplicity, we model the cost of diffusional limitation as a linear trade-off between cluster size (# of cells) and growth rate, such that a single cell doubles during each time step, and larger clusters grow to size \( n(2 - nd) \) cells. Cluster growth is offset by apoptosis where the number of cells that undergo cell death at each time step is calculated as:

\[ n(a) = \frac{n(\alpha - 0.5)}{50}, \]  
\[ (2) \]

Table 1: Summary of model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Base value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a )</td>
<td>Rate of apoptosis</td>
<td>0.001</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>Reproductive asymmetry</td>
<td>0.55</td>
</tr>
<tr>
<td>( d )</td>
<td>Cost of diffusional limitation</td>
<td>0.001</td>
</tr>
<tr>
<td>( n_{min} )</td>
<td>Minimum number of cells within a cluster</td>
<td>1</td>
</tr>
<tr>
<td>( n_{max} )</td>
<td>Maximum number of cells within a cluster</td>
<td>2000</td>
</tr>
<tr>
<td>( N )</td>
<td>Population size</td>
<td>( 8 \times 10^6 )</td>
</tr>
<tr>
<td>( r )</td>
<td>Size at reproduction</td>
<td>150</td>
</tr>
<tr>
<td>( s )</td>
<td>Size threshold for settling selection</td>
<td>140</td>
</tr>
</tbody>
</table>
Figure 1: **Model schematic.** The model is separated into two distinct phases. First, clusters grow, competing for finite resources. Larger clusters face greater diffusional limitation and thus gain proportionally fewer cells during each time step. If a cluster’s growth causes it to exceed its reproductive size $r$, then propagules are produced sequentially until cell number $n < r$. Settling selection is applied once resources are exhausted. All clusters above size threshold (thresh.) $s$ settle to the bottom of the tube. Not all cells at the bottom are large, however: 6.6% of the clusters in the population simply start out there by chance. Finally, clusters are transferred to fresh medium. To allow for sufficient growth between rounds of settling selection, we transfer 1/20 of the stationary phase biomass to fresh medium. Clusters are transferred in proportion to the biomass of each strain in the pellet.

where $\alpha$ is reproductive asymmetry, a parameter that specifies the propagule size when a cluster undergoes reproduction (discussed below). For most of our simulations, the rate of apoptosis is equal to $-0.001$ unless otherwise noted. For a 148-cell cluster growing up from a 10-cell propagule during a single culture cycle, this corresponds to a cumulative death rate of 1.93%, which is similar to what we observe in our experiments (Ratcliff et al. 2012). Importantly, this step occurs before new cells are added so only cells $\geq$ one generation old are capable of dying from apoptosis.

When a cluster grows larger than $r$ cells, they split, producing daughter propagules sequentially until they are smaller than the reproductive threshold. Offspring size depends on the cluster’s reproductive asymmetry, $\alpha$. Specifically, mean propagule size is $n(1 - \alpha)$. Because snowflake yeast produce offspring that vary in size, we implemented a stochastic smoothing function into our model, such that asymmetry at each reproductive event is drawn from a uniform probability distribution bounded by $(\alpha - 0.05, \alpha + 0.05)$. Similarly, a cluster’s size at reproduction is drawn from a uniform probability distribution bounded by $(r - 5, r + 5)$. This has the effect of preventing the accumulation of many clusters of exactly the same size, which can result in simulation artifacts (e.g., abrupt changes in fitness with small changes in traits). For all simulations, asymmetry was bounded between 0.5 and 0.9.

**Population growth and settling selection**
We model our experimental regime as two distinct phases, a growth phase and a settling phase. During the growth phase, resources are consumed by both strains of yeast until they are exhausted (in most simulation runs, we allow for $8 \times 10^6$ cellular reproductions) with each yeast strain growing and producing propagules according to the equations given above. After resource exhaustion, settling selection is applied. In our laboratory experiments, we transfer the cells found in the lower 100 µl of a microcentrifuge tube after settling selection to 10 ml of fresh medium. There are two ways that clusters can get to the bottom of the test tube: first, a small fraction of clusters (~6.6%) simply start out there after the tube is mixed. These clusters need not be large – they are simply lucky. Second, clusters can settle rapidly enough to sink to the bottom of the tube. Here, we impose a simple threshold, such that clusters containing more than $s$ cells make it to the bottom of the test tube, and those smaller do not. In our laboratory experiments, settling selection results in a 20 to 25-fold dilution per day for relatively fast-settling snowflake yeast. We model this by selecting clusters from each strain (in proportion to their biomass in the pellet) until 1/20 of the carrying capacity of the population is met. These clusters are then transferred to fresh medium and the cycle repeated.

In reality, settling selection is less precise: small clusters starting out just above the lower 100 µl may still join the pellet, while larger cluster starting at the very top of the tube may fail to make it to the bottom. Still, this simplifying assumption does not change the basic dynamics of size selection favoring larger sized clusters. Selection can be made more or less stringent by increasing or decreasing the threshold cluster size, $s$.

**Results**

**Local fitness landscapes reveal the conditions favoring elevated apoptosis**

We first examined the snowflake yeast fitness landscape under different nutrient diffusion regimes, $d$, as a function of both the rate of apoptosis and cluster size at reproduction. In each case, we competed a single strain (demarcated by the black circle in Figure 2a-d) against 1554 different competitor strains that varied in these traits. We measure relative fitness as the change in frequency of strain 1 cells relative to strain 2 cells between stationary phase in transfer two and stationary phase in transfer three. The threshold for surviving settling selection $s$ was 140 cells, which is similar to what we have observed in early snowflake yeast (1-3 weeks of evolution). Competition between smaller clusters with little diffusional limitation ($d=0.001$) favors larger clusters with negligible apoptosis (Figure 2a). Increasing the severity of diffusional limitation ($d = 0.002$) favors elevated apoptosis (Figure 2c). We note, however, that the genotype with the highest fitness under these conditions still has the lowest rate of apoptosis. Among clusters that are much larger than the size necessary to survive settling selection (Figure 2b and 2d; which start out at size 225, but need only be 140 cells in size to survive selection), smaller size is beneficial. Importantly, higher
rates of apoptosis can be selectively advantageous among these larger clusters, even when diffusional limitation is mild \((d = 0.001)\).

**Figure 2:** Fitness landscapes vary depending on the extent of resource diffusion and on cluster size. In each fitness landscape plotted above, a single strain (filled circle) competes against 1,554 competitor strains varying in cluster size at reproduction and apoptosis. Large size and low apoptosis are favored in small clusters with little diffusion limitation (A), while increasing the growth cost of diffusion favors smaller clusters with higher rates of apoptosis (C). Increasing cluster size at reproduction by 100 favors smaller cluster size (B, D). Apoptosis provides more of a benefit to larger clusters (lower region of B, D). Here \(s = 140, d = 0.001\), and the growth phase contains sufficient resources for the production of \(8 \times 10^6\) cells. The dashed line demarcates a relative fitness of 0.

Snowflake yeast compete in two key arenas: for resources during the 24 h of batch culture, and for a spot at the bottom of the tube during settling selection. It is in these two arenas where fitness trade-offs are realized. For example, large clusters settle quickly but grow slowly. By increasing reproductive asymmetry (reducing propagule size), increased rates of apoptosis should increase growth rates at the expense of survival during settling selection. Whether or not these traits are adaptive for a given environmental context depends on the benefit of faster growth relative to the cost of reduced survival during settling.
Figure 3: Disentangling fitness contributions from growth (a) and settling (b). We calculated the relative fitness consequences (as selection rate constants) during growth and settling for the landscape shown in figure 2c. Smaller cluster size at maturity and apoptosis increases fitness during growth at a cost to settling. Here $s = 140$, $d = 0.002$, and the growth phase contains sufficient resources for the production of $8 \times 10^6$ cells. The dashed line demarcates a selection rate constant of 0.

directly compare the magnitude of this fitness trade-off, we calculated the selection rate constants (following Travisano and Lenski 1996) for growth and settling for the landscape in Figure 2c. This approach allows us to compare each phase of competition using fitness as a common currency. As expected, elevated apoptosis increased growth rates, but reduced survival for settling selection, and larger cluster size was uniformly favored during settling (Figure 3). The benefits of faster growth out-weighed the cost of slower settling for part of the trait space (asymmetry between 0.62 and 0.75 and cluster size between 200-220 cells; Figure 2c). This result also highlights the fine-line being walked during the evolution of elevated apoptosis: mutations of large effect may produce strains with too much apoptosis, such that the costs of slower settling exceed the costs of faster growth.

**Competition Drives an Evolutionary Arms Race for Increased Cluster Size**

Static fitness landscapes (e.g., Figures 2 and 3) are useful for examining the interaction between traits and fitness over only a limited range of conditions, because relative fitness is contextual and changes over evolutionary time along with the traits of the two competitors. To examine how cluster size and apoptosis rates might coevolve as the competitor also changes, we implemented a two-player evolutionary algorithm. For each time step, 10 derivatives of each snowflake yeast genotype were generated, each with a 90% chance of mutation in cluster size at maturity or reproductive asymmetry. Each mutation was drawn from a normal distribution, with the mean being the former trait value with standard deviation of 1 (for cluster size at reproduction) or 0.003 (for reproductive asymmetry).
**Figure 4: Arms races and niche partitioning.** Larger clusters with higher rates of apoptosis evolve when both starting strains are similarly sized (a, b). If the initial size difference is substantial, arms-race dynamics are prevented, and instead the smaller strain evolves smaller size, becoming a growth specialist (c). The frequency of niche partitioning declines linearly as strain (Str.) 1’s starting size increases from 150 to 160 (c, insert). Plotted are 100 simulations for each strain (strains 1 and 2 are demarcated by dark X’s and light circles, respectively) over 150 transfers. Here $s = 140, d = 0.0015$, and the growth phase contains sufficient resources for the production of $2 \times 10^6$ cells.

All 10 variants of strain 1 were competed against last-round’s strain 2 winner, then all 10 variants of strain 2 are competed against the best strain 1 variant. The strain with the highest relative fitness after three transfers was selected as the parent strain for the next round. For all simulations, the size threshold for surviving settling selection ($s$) was 140.

When competition occurs between two similarly-sized strains, both readily evolve larger cluster size and higher rates of apoptosis (Figure 4a and b). In contrast, when we compete two strains that vary substantially in size (150 vs. 200 cells at reproduction), snowflake yeast rapidly partition their niches (Figure 4c). Specifically, the 150-celled strain evolved to form clusters that were ~15 cells smaller than its ancestor over 150 rounds of competition against a 200-celled strain, while the same starting genotype evolved to form clusters that were an average of 67 cells larger when competed against another 150-celled strain (Figure 4a vs. 4c; $t_{157} = 114, p < 0.0001$, Bonferroni-corrected two-way t-test). This effect appears to be due to competitive exclusion during settling selection, driving the smaller strain to evolve smaller size and increased competitiveness during the growth phase of competition. We examined the size difference required for niche partitioning to occur, varying strain 1’s starting size from 150 to 160 cells while leaving strain 2’s starting size at 200 cells. For each competition, we ran 100 simulations for 150 transfers. The percent of runs in which strain 1 evolved to be a growth specialist declined linearly as their size increased ($y = 1503.9 - 9.3x, r^2 = 0.97, F_{1,10} = 254.9, p < 0.001$; Figure 4c, insert). Interestingly,
we also found that the 200-celled strain evolved to form clusters that were ~30 cells larger when competing against another 200-celled strain, but not against a 150-celled strain (Figure 4b vs. 4c; $t_{196} = 30$, $p < 0.0001$, Bonferroni-corrected two-way t-test), further illustrating the importance of coevolution in our model. One caveat of this simulation is that it ensures coexistence of the two competing strains. It is possible that extinction in real populations would limit the ability for the evolution of niche partitioning.

The results of the two-player games (Figure 4) illustrate the importance of arms-race dynamics among similarly-sized competitors in the evolution of increased cluster size. The extent of directional change is limited, however, as the trade-off between settling and growth components of fitness result in stable coexistence at modest (250-340 cell) cluster size and low apoptosis (reproductive asymmetry $\approx 0.57$, Figure 5a). To examine how size and apoptosis coevolve in response to different size-selection thresholds, we simulated competition in environments where the size threshold for surviving settling selection, $s$, varied from 50 to 750. We initialized each competition with two identical strains whose maximum size was just 10 cells larger than that required for settling, and then allowed them to come to equilibrium (1000 transfers per competition). While larger clusters readily evolved, they rarely got more than 150 cells larger than the threshold for surviving settling selection (Figure 5a, insert). Larger size clearly evolves in response to selection for faster settling, but imposing severe selection for rapid settling on small clusters can be counterproductive: selection cannot favor faster settling clusters if none survive. How then do large clusters evolve? In our experiments, we periodically increased the strength of settling selection (Ratcliff et al. 2013), favoring the progressive evolution of faster settling. We thus reran the above simulation, starting with a small snowflake yeast ($r = 140$), and increased the size of settling selection by 1 cell every 10 time steps. Here, much larger (>900 celled) clusters evolved, along with maximal rates of apoptosis (reproductive asymmetry $\approx 0.9$ in strain 1, Figure 5B).

In our experiments, in addition to evolving an increased number of cells per cluster, snowflake yeast also evolved a 2.1-fold increase in cell size within two months (~400 generations, Ratcliff et al. 2013), increasing the settling speed of clusters. We thus modified the model to allow for the evolution of increased cell size, changing the settling selection step to count cluster biomass equivalents in addition to cell number. Further, we imposed a linear growth penalty for larger cells, assuming that cells with twice the volume grow at 98% the speed of a wild type cell. We repeated the two-player tournament simulations, allowing for mutations that increase cell size to occur with 90% probability (the distribution of mutational effect sizes was identical to that of reproductive asymmetry). Increasing the strength of settling selection was again essential for the evolution of both large and many-celled snowflake yeast (Figure 6).

Increasing the dimensionality of the multicellular trait space makes it possible for genetically and phenotypically distinct strains to arrive at the same multicellular solution (i.e., strains differing in cell size and cell number can nonetheless evolve the same overall cluster biomass). To examine how allowing a third multicellular trait to evolve affected within-population divergence, we calculated the Euclidian distance of normalized trait values between competitors in each microcosm, either with or without cell size mutations, after equilibrium was reached (1000 time steps, $s = 140$). Increasing the dimensionality of
Figure 5: **Pushing the envelope—the evolution of large clusters.** (a) When the environment is constant ($s = 140$ for all 7,600 transfers), equilibrium dynamics rapidly establish themselves with the evolution of modest cluster size and low apoptosis. For a range of $s$ from 50–750, cluster size at equilibrium (1,000 transfers, purple circles in 5a inset) is only modestly larger than required for surviving settling selection (black line, inset). (b) Slowly ratcheting up the threshold for settling to the bottom of the tube ($s$ starts at 140 and increases by 1 every 10 transfers) results in the evolution of very large clusters with high rates of apoptosis. Filled circles and triangles refer to the two strains in competition. In these simulations the growth phase contains sufficient resources for the production of $2 \times 10^6$ cells and $d = 0.001$.

The multicellular trait-space increased the opportunity for within-population diversification: competitors were an average of 32% more phenotypically divergent ($t_{196.7} = 3.07$, $p = 0.0024$, two-sided t-test) when cell size was allowed to evolve (Figure 7). Because there was only a 1% difference in cluster biomass (mean of 272 and 279 wild type cell equivalents for 2D and 3D simulations, respectively) and no difference in apoptosis (mean of 55.9% for both 2D and 3D simulations), it appears that yeast capable of evolving both greater cell number per cluster and larger cell size took different, though ecologically equivalent, paths to increased cluster size. This demonstrates that the evolution of additional multicellular traits may, as a side effect, increase the population’s capacity to support the coexistence of ecologically equivalent (though genetically and phenotypically distinct) isolates.

**Discussion**

One of the most surprising results from our yeast experiments is the rapidity of adaptation after simple multicellularity evolves. After just ~400 generations, snowflake yeast evolve to form clusters containing twice as many cells, higher rates of apoptosis, and cells that are more than twice as large as the ancestor. These clusters settled 28% faster, on average
Figure 6: **Evolution of larger cell size.** Coevolution in a static ($s = 140$; a) or gradually more stringent size-selective environment ($s$ starts at 140 and increases by 1 every 10 transfers; b). Increasing the strength of settling selection favors the evolution of all three key multicellular traits: large cluster size at reproductive maturity, high rates of apoptosis, and large individual cells. Here the growth phase contains sufficient resources for the production of $2 \times 10^6$ cells, $d = 0.001$, and marker shade reflects the yeast strain.
Figure 7: **Divergence in 2-D versus 3-D games.** Competing pairs of snowflake yeast evolved more divergent multicellular traits, measured as the Euclidian distance of each competitor after 1,000 generations. Plotted are the results of 2-D simulations where cell size was held constant (A) and 3-D simulations where cell size was allowed to evolve (B).

(Ratcliff et al. 2013). Further, we have found that after a similar length of time (but in a different experiment), 9/10 replicate populations contained at least two strains that varied in size (Rebolleda-Gomez et al. 2012). The modeling results presented above help to explain both of these observations.

In both this model and in evolving populations of yeast (Ratcliff et al. 2012; Ratcliff et al. 2013), initially-small clusters of cells are under strong selection for increased size. The model described here shows why: even small increases in settling speed dramatically improve relative fitness, and at this small size, diffusional limitation is not yet very restrictive. As a result, competition among co-evolving yeast results in a short-term arms race for increased size. As larger cluster size evolves, apoptosis becomes increasingly beneficial, allowing large-bodied yeast to produce proportionally smaller offspring that are temporarily freed from strong diffusional limitation. Competition in a static environment (no change in $s$) thus results in a modest increase in both size and apoptosis. However, we find that for very large, high apoptosis clusters to evolve, the strength of settling selection must increase through time. Long-term experiments running in our lab suggest that this increase in the strength of settling selection is also required for the in vitro evolution of large snowflake yeast clusters (unpublished).

Apoptosis readily evolves in this simple simulation model, ameliorating the growth rate cost incurred by large body size. This effect appears to be due to the fact that apoptosis produces proportionally smaller offspring that have a growth rate advantage when resource diffusion is more limiting. Specifically, increased rates of apoptosis are favored under conditions of low resource penetration into the cluster (high $d$), or for low values of $d$, as a consequence of selection favoring the evolution of progressively larger slower growing clusters (but see Duran-Nebreda and Solé 2015 for a non-adaptive explanation for elevated rates of apoptosis). Apoptosis may therefore be a general solution to the biophysical constraint of diffusionally-limited growth so long as: i) selection favors larger clusters of
cells, ii) larger clusters grow less rapidly than smaller clusters, iii) cell death results in propagule production, and iv) propagules that are produced by apoptosis are related (high Hamilton’s r) (Hamilton 1964) to apoptotic cells. Both iii and iv likely require that clusters are formed through incomplete mother-daughter cell separation. Finally, it is also important that propagules have sufficient time to grow large enough to survive the next round of size-based selection.

Programmed cell death (PCD, a category of cell death mechanisms that includes apoptosis) plays a critical role in the evolution of multicellular complexity. In independently-evolved multicellular lineages, PCD is used to modify multicellular form during development (Jacobson et al. 1997; Pennell and Lamb 1997; Umar and Van Grienven 1997), plays a central role maintaining the multicellular body (e.g., removal of damaged [Jacobson et al. 1997], infected [Lam 2004] or cancerous cells [Lee and Bernstein 1995]), and can be useful in coordinated multicellular behaviors (e.g., leaf abscission [Bleecker and Patterson 1997]). Indeed, PCD plays such an important role in multicellular organization that it is difficult to imagine complex multicellular life in its absence. PCD, however, is not a multicellular invention: many diverse unicellular organisms possess active, genetically regulated cell death mechanisms (Nedelcu et al. 2011). Comparative work demonstrates that some PCD pathways in multicellular organisms arose in their unicellular ancestors (Nedelcu 2009), suggesting they were co-opted for novel use after the transition to multicellularity. The work presented here provides a simple, general explanation for how unicellular PCD can be co-opted for a novel multicellular purpose. See Duran-Nebreda et al. (this volume) for a discussion of how multicellular complexity can emerge as a consequence of interactions among the component cells.

Within-population diversity is present in both our lab experiments (Rebolleda-Gomez et al. 2012), and this simulation model. The model predicts that some of this diversity may simply be the result of different lineages taking divergent trajectories during adaptation (Figures 5 and 6). This may be especially important when the multicellular trait-space is high dimensional, because, all else equal, these contain a greater number of ecologically equivalent trajectories. There are a number of potential multicellular traits that we did not include in the model, but which may be relevant and would further increase the dimensionality of the multicellular trait-space. For example, the architecture of snowflake yeast clusters (Libby et al. 2014) might change, affecting both d and how size relates to surviving during settling selection. This can be due to simple alterations, like the shape of individual cells, or more complex modifications. Indeed, after 227 days of selection, we see the evolution of more spherical, hydrodynamic clusters that settle 35% faster per increase in unit mass (Ratcliff et al. 2013). We do not yet know the mechanistic basis of this trait, but it is potentially due to a modified branching pattern, or changes in the location and timing of cellular apoptosis.

How multicellular complexity arises in evolution is a fundamental question in biology. In combination with our experimental work (Ratcliff et al. 2012; Rebolleda-Gomez et al. 2012; Ratcliff et al. 2013; Ratcliff et al. 2015), the results described here demonstrate that multicellular complexity readily arises as a solution to a trade-off between selection for fast growth and large size. While selection for rapid sedimentation is likely not a good proxy for natural systems, selection for large size is certainly common in microbial populations (e.g.,
cluster formation increases survival in the face of predators [Kessin et al. 1996; Boraas et al. 1998; Becks et al. 2012], but cluster formation slows growth [Yokota and Sterner 2011; Becks et al. 2012]). This work, along with other pioneering work experimentally evolving novel multicellularity (Boraas et al. 1998; Rainey and Rainey, 2003; Koschwanez et al. 2011; Koschwanez et al. 2013; Ratcliff et al. 2013), has shown that the first steps in this transition readily occur. The challenge before us is to determine how more complex, functionally integrated multicellular individuals (e.g., an organism that consists of multiple cell types whose multicellular life cycle is developmentally regulated) evolve from simple multicellular ancestors.

Acknowledgements

We would like to thank Ben Kerr, Vidyamand Nanjundiah, Kayla Peck, and Jennifer Pentz for thoughtful comments on this manuscript and the Konrad Lorenz Institute for funding/hosting the meeting where this work was first presented. This work was supported by NASA Exobiology grant #NNX15AR33G.

References


