Foundations of Complex Systems and Biological Complexity
DOI: http://dx.doi.org/10.7551/978-0-262-31709-2-ch164

Using Reproductive Altruism to Evolve Multicellularity in Digital Organisms

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Abstract

The processes by which multicellular organisms first emerged from their unicellular ancestors are fundamental to the biology of complex, differentiated life forms. Previous work suggests that reproductive division of labor between specialized germ and soma cells was central to this evolution in some cases. Here, we assess the potential of the digital life platform Avida to examine the trade-off between survival and replication in multicellular organisms. Avida uses a grid of self-replicating computer programs capable of mutation and evolution to address biological questions computationally. We model our digital organisms after the Volvocales, a flagellated order of photosynthetic green algae that includes both unicellular and multicellular species. We show that, given selective pressures similar to those experienced by the Volvocales in nature, digital organisms are capable of evolving multicellularity within the Avida platform. The strategies we observed that best handled the trade-off between survival and replication involved germ cells producing sterile, somatic offspring. These strategies are similar to those observed in volvocine algae, which suggests that digital platforms, such as Avida, are appropriate to use in the study of reproductive altruism.

Introduction

How and why multicellular organisms developed are central questions in developmental biology. In life's history, multicellularity has emerged from unicellularity on at least 25 separate occasions (Grosberg and Strathmann, 2007). The fact that this type of specialization and cooperation between cells has emerged independently and repeatedly in organisms ranging from algae to fungi suggests that this phenomenon is not a statistically unlikely event, but is the result of selective pressures experienced by various types of life. Previous theoretical and experimental work has shown multicellularity to be selectively advantageous in several circumstances (Rokas, 2008). In *Chlorella vulgaris*, for example, multicellular forms have evolved from their unicellular counterparts in the presence of a predator within 100 generations, suggesting that a multicellular existence might be advantageous to combat predation (Boraas et al., 1998). Here, we focus on the potential benefits of reproductive division of labor in multicellular forms.

Both reproduction and survival are vital for life to propagate. Differentiation between reproductive germ cells and purely functional soma cells is observed in the Volvocales, a flagellated order of photosynthetic green algae (Kirk, 2001). We chose to model our experimental parameters after the Volvocales specifically because they include multicellular organisms of varying colony size, each of which displays a different degree of complexity and specialization (Koufopanou, 1994).

The primary trade-off Volvocales address is between mobility and reproduction. An algae colony's ability to photosynthesize effectively is dependent upon its depth within the water column, and vertical traversals of entire colonies are common (Sommer and Gliwicz, 1986). A colony's capacity for mobility is primarily determined by the total functionality of its members' flagella.

In the Volvocales, however, cell division damages flagella. When a cell replicates, its flagella continue to function, but "not as strongly or as well coordinated as when [a cell is] not dividing" (Marchant, 1977). After a cell replicates several times its flagella become completely nonfunctional. The number of divisions until a cell loses all flagellar function is generally assumed to be about five (Koufopanou, 1994; Michod et al., 2006). Previous literature suggests that differentiation between germ and soma "may have evolved as a solution to this problem: by denying reproduction to some cells, a parental colony can maintain functional flagella on these cells, which will enable it to maintain its position in the water column while the rest of its cells are dividing" (Koufopanou, 1994). We refer to this constraint as the flagellation constraint.

Another consideration regards the physical volume of germ cells. Reproductive cells in differentiated volvocine colonies tend to have much greater volume than their sterile counterparts (Figure 1). This size differential results from the fact that post-embryonic cell division is not possible (Michod et al., 2006). For the purposes of designing our Volvocale-inspired digital organisms, however, establishing an association between physical volume and replication is adequate. When a colony's total volume increases, its mobil-

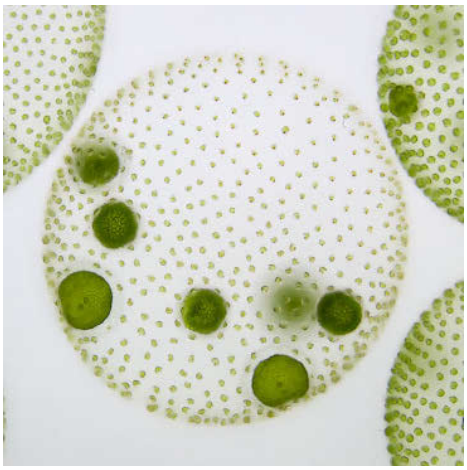

Figure 1: *Volvox*, a species of the Volvocales, exhibiting full germ-soma differentiation. The larger cells within the colony are germ cells, while the smaller cells are soma. Image courtesy Frank Fox of www.mikro-foto.de.

ity decreases because of increased mass and drag, and more total flagellation is required for colonial motion. Previous work suggests that overcoming this enlargement constraint is yet another benefit to germ-soma specialization (Michod et al., 2006).

It has been shown that reproductive altruism, cells voluntarily producing sterile, somatic offspring for the benefit of their kin, emerges in volvocine green algae. This process involves the expression of an altruistic gene within the parental cell in response to environmental cues (Nedelcu and Michod, 2006; Michod et al., 2006). We aim to implement the enlargement and flagellation constraints in a digital life platform and compare the strategies evolved by digital organisms to those evolved by the Volvocales in nature. While our model could be improved upon, our primary goal is to assess the potential of a digital life platform to address questions of reproductive altruism.

The Avida Platform

Avida is a software platform that maintains a grid of self-replicating and mutating computer programs, and can be used to address biological questions computationally. Unlike the slow progress of organic evolution, digital evolution allows researchers to conduct experiments relatively quickly; tens of thousands of generations can be executed in a single day. Additional benefits of using a digital platform like Avida include the ability to repeat experiments exactly, specify all environmental parameters, and measure population statistics with precision. Avida is not meant to perfectly model any specific biological system, but supports the basic evolutionary processes of "replication, variation, and differential fitness." These three conditions create an environment in which evolution will occur (Dennett, 2002). It's also worth noting that experimentation within Avida is "not a simulation of a particular evolutionary theory but ... an experimental study in its own right" (Ofria and Wilke, 2004).

The function of a digital organism in Avida is specified by a sequence of low-level computer instructions, in the same way that a genome composed of DNA encodes the form and behavior of a biological organism. The default Avida genomic language contains instructions that perform logical and mathematical operations, functions specific to replication such as allocating and copying memory, and flow control commands that modify the execution order of an individual's genome. The control commands include instructions that cause small modifications to execution order such as `IF-LESS`, which may skip the immediately following instruction based on a numerical comparison between stored values, and instructions that allow for larger changes such as `MOV-HEAD`, which could cause execution to jump to an instruction anywhere in the genome.

Each Avida organism runs on its own unique set of simulated hardware, including a virtual CPU, three registers, two stacks, input/output functionality, and memory. An organism is allocated memory to hold its own genome of instructions plus extra initially "blank" memory it can use to store the genome of any child it produces. The virtual CPU executes the genome as a continuous sequence of instructions, simply starting again with the first instruction after executing the last. This hardware combined with the instructions in the default Avida language is Turing complete.

In the past, Avida has been used to examine fundamental evolutionary principles in great detail. Notable examples include an examination of the origin of complex features in biological organisms (Lenski et al., 2003) and a study of the relationship between genomic complexity and robustness (Lenski et al., 1999). Because of Avida's demonstrated capacity to improve our understanding of evolution in general, we believe it is a worthwhile endeavor to assess its potential to address questions of reproductive altruism.

Avida in particular is well suited for our experiments because we are interested in the specific strategies digital organisms might evolve to address the flagellation and enlargement constraints. While a mathematical model or a less complex evolutionary algorithm could offer some insight and might be easier to analyze, it likely would not yield the same depth of information. Some of the more interesting strategies we observed, for instance, would not have appeared in a simpler system.

Avidian Life-cycle

The Avida life cycle is defined as follows. First, an organism generally executes a sequence of instructions that allocates memory for its child within its own memory space, and copies each of its instructions into this newly allocated memory. Replication is asexual, and occurs when an individual executes an `H-DIVIDE` command, creating a new organism

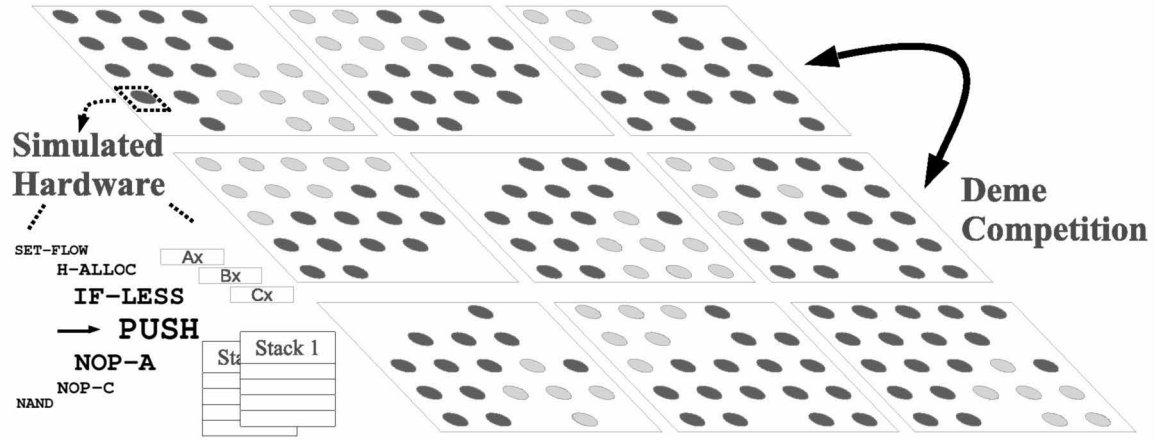

Figure 2: Avida population partitioned into demes of size 25 and an illustration of a subset of the simulated hardware

from the genome in the child memory space. The parent is reverted to its initial state with blank child memory, and begins execution again with its first instruction. In Avida several instructions in a relatively precise arrangement are required for an organism to replicate, and it is standard to seed starting populations with organisms that already have a hand-coded replication loop in their genomes. However, these instructions are subject to the same mutations and copy errors as the rest of the genome, and organisms frequently evolve modified methods of replication.

Mutation is accomplished through probabilistic instruction addition, deletion and modification within a child's genome prior to its insertion into the greater population. When an organism is copying its instructions into the memory it has allocated for its child, for instance, there is a user-defined probability that the instruction it is attempting to copy will be replaced with a random instruction from the valid set. Avida mutation rates are generally much higher than those found in the natural world; around one mutation on average per child genome is standard.

Organism death occurs either through old-age, or when an organism is overwritten by another's child. All organisms are allowed to execute the same number of instructions on average per update, though the allocation of cpu cycles is stochastic. Therefore organisms that successfully copy themselves using fewer total instructions than average generally have a higher relative fitness. However just as in the nature, many other factors affect a genome's long-term success, such as its robustness to the relatively high mutation rate.

Multicellularity and Digital Platforms

The origins of multicellularity have previously been investigated in several contexts using artificial life models. Furusawa and Kaneko (1998) used an artificial chemistry model to examine multicellular emergence on a simulated two-dimensional grid. Their focus was largely on exploring the mechanisms that cause cell differentiation during development. Other researchers have studied task-related division of labor, wherein individuals cells cooperate to perform specialized tasks efficiently, as a mechanism by which multicellularity can arise (Michod, 2007). Goldsby et al. (2010) investigated task-related division of labor within the Avida platform and found that digital organisms are capable of selecting specialized roles in groups using both spatial information and inter-organism communication.

In this paper we focus solely on the potential benefits of *reproductive*, as oppose to task-related, division of labor.

Finally, Schlessinger et al. (2006) provided an excellent investigation into the emergence of multicellularity in which they extended the Mosaic World software system to allow for optional organism aggregation into multicellular units. While the authors do include the ability for organisms to forfeit their reproductive capacity, individuals that join a multicellular unit lose their autonomy entirely, as tasks carried out by an aggregation are decided "democratically" through a poll of all constituents. Our investigation, in this context, is orthogonal, utilizing forced aggregation and optional autonomy.

Extensions to the Avida Platform

We extend the Avida platform to incorporate flagellation and enlargement constraints similar to those exhibited by the Volvocales by adding two variables to each digital organism, the *flagella* value and the *physicalSize* value.

flagella is a numerical variable that takes on values between $[0, 1]$. This variable represents the functionality of a cell's flagella. When a new cell is created, we assume that its flagella are fully functional, and assign it a *flagella* of 1. If and when a cell executes a divide command, we model the flagellation constraint by decreasing this value. We decrease a cell's *flagella* value either linearly, by subtracting .25 upon replication, or exponentially, by dividing by 2 upon replication, depending on the specific experiment.

To model the enlargement constraint, we use the *physicalSize* variable, which represents the physical volume of a cell. While many species of volvocine algae replicate through a more complex process known as palintomy (Michod et al., 2006), we found a simpler binary fission model to be acceptable for our organisms.

In accordance with our binary fission model, we increment a cell's *physicalSize* value each time it executes a copy command such that *physicalSize* increases linearly from 1 to 2, until the parent has copied its entire genome to the child. Upon division, this value is reset to 1.

Because our experiments dealt with cooperation, we were particularly interested in the fitness and replication of groups of cells. The Volvocales ultimately specialize and become multicellular forms, so it was necessary to implement multilevel selection and evaluate groups of organisms in addition to individual organisms. We utilized Avida's population partitioning functionality to group organisms into distinct and separate subpopulations, called demes. In our experiments, each deme represented a potential multicellular organism. To evaluate and replicate demes, we modified Avida's CompeteDemes framework to accommodate for user-defined deme-level fitness functions and Volvocale-inspired deme replication.

Our CompeteDemes implementation computes the fitness of each deme semi-periodically and executes a fitness proportional tournament selection with five demes per tournament to determine the next generation of colonies. The timing of each CompeteDemes execution is offset by a random value from a uniform distribution. We implemented this deviation because, in early testing, organisms evolved an understanding of perfectly periodic timing and commonly developed undesirable behaviors.

While colonial algal reproduction is considerably more complex, we base our replication implementation on the concept that, in germ-soma differentiated species, all cells in a colony are closely related, and usually derived from the same original germ cell (Michod et al., 2006; Kochert, 1975). To replicate a colony, we select the organisms with the smallest and largest *physicalSize* values, and designate them as the founders of the appropriate demes in the next colonial generation. The cell with largest *physicalSize* is likely in mid-replication during selection, while the cell with the smallest *physicalSize* is likely not replicating during selection. By selecting the physically largest and smallest organisms, we aim to allow a single germ and a single soma cell to found colonies in the next generation, if their parental colony were germ-soma differentiated. While this process differs slightly from naturally observed reproductive mechanisms, our goal was to mimic the selective pressures experienced by Volvocine algae, not create a perfect model of their biology.

Experimental Design

For our experiments, we used a population of 400 demes, each of which contained a maximum of 25 organisms. Real CPU cycles were assigned to each deme according to their living population size, and then randomly to members of that deme.

The unit of time in Avida is the "update," in which an average organism executes 30 instructions. Our experiments were run for 10^5 "updates," and terminated after about 10 hours. The initial seed organism executed 390 instructions before reproducing, which translates to an average of 13 updates per generation. The number of instructions executed before reproduction varied considerably between populations, as well as over time and between organisms in a single population, however all values fell within the range [200, 2000].

We designed our fitness function to reward greater total deme flagellar function, and penalize greater deme physical size. With this objective in mind, we decided to limit the space of potential fitness functions to linear combinations of these two deme-level statistics. While this limitation is somewhat arbitrary, our goal was not to perfectly model Volvocale colonial fitness, and the following simple, linear trade-off function worked as well as, or better than, any other in evolving populations with interesting, diverse germ-soma differentiated strategies.

$$F = max(0, \sum flagella - \frac{\sum physicalSize}{2})$$

We also implemented a floor on the minimum number of living cells a deme could contain and still be considered viable. We assign a fitness of 0 to demes with less than 15 cells.

Mutation rates were kept at the default Avida settings; for a given command being copied, there was a .75% chance of random instruction replacement. The expected number of mutations depends upon the genomic length of an individual. For a default organism with length 100, for instance, we would expect .75 swap mutations. Additionally, for a single replication event, there was 5% chance that an instruction would be added or deleted from the child's genome upon cell division.

Results

Before discussing the specific mechanisms digital organisms developed to accomplish germ-soma differentiation, we aim to establish that producing sterile organisms is, in general, a solution to the flagellation and enlargement constraints. We compare the final dominant deme fitnesses from our 46 exponential flagella decay trials to the proportion of somatic cells in the final population for each trial. We find a positive correlation between these variables (Figure 3). This positive correlation indicates that, in general, populations that display higher degrees of germ-soma differentiation produce more fit dominant demes.

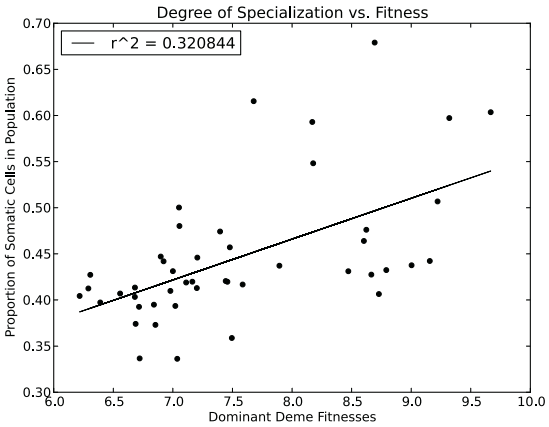

Figure 3: Least squares regression of deme fitnesses versus somatic proportion in exponential trials

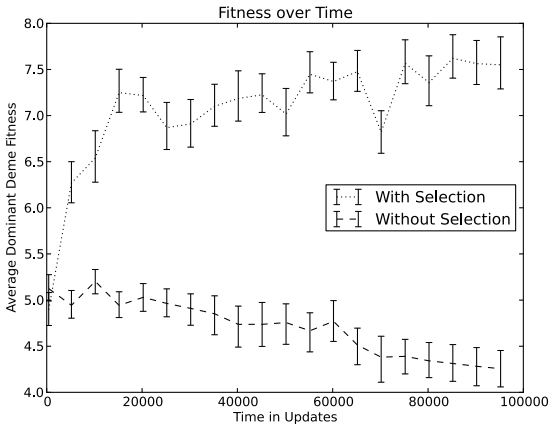

Figure 4: Average dominant deme fitness over time with bootstrapped 95% confidence intervals using 10^5 re-samples

Using an order statistic such as dominant deme fitness in this analysis is appropriate because our fitness function is truncated at 0; the distributions of deme fitnesses are non-normal because of this truncation. A mean, in this case, would not be an accurate reflection of overall population performance.

Strategic Analysis

Within the exponential flagellar decay experiments, organisms evolved several different strategies, some of which resulted in germ-soma differentiation. Here, we will present these strategies in decreasing order of observed frequency. First, however, it's worth noting that within a single Avida trial, we observed some cases where multiple strategies coexisted. In these trials, it was usually the case that the deme with the highest fitness utilized one of the more complex and interesting strategies we present here, while the most common replicator in the entire population did not.

Most commonly, no complex, germ-soma differentiated strategy emerged at all. In about 70% of our trials neither the most common nor the dominant replicator utilized a strategy other than adjusting its gestation time and genomic sensitivity to mutation, or "brittleness." The concept and prevalence of brittleness will be addressed in a later section.

The most common germ-soma differentiated strategy, which produced the dominant deme in about 13% of our trials was probabilistic replication. Organisms using this strategy produced children that were accurate copies of themselves a fixed proportion of the time. Probabilistic replicators exhibit phenotypic plasticity, which is the ability to change one's phenotype in response to environmental cues. The Avida platform inputs random numbers to cells to simulate changing environmental conditions. These inputs can be stored and operated on by organisms, if they evolve such behavior. In this case, organisms used these random numbers to determine whether or not to replicate. Those that did not replicate were basically somatic cells in the colony, with the maximum flagella value and minimum physical size. Thus the colony as a whole gained the benefits of germ-soma cell specialization though all constituent cells had identical genomes.

We also observed more complex manifestations of probabilistic replication. In one trial, for instance, a cell replicated with a probability of about 64% but did not always attempt to make an exact copy. Very rarely, this organism produced a purely somatic cell with a separate genotype. The probability of this occurrence was too low to estimate accurately, as a population of several thousand parental cells was required to observe tens of these somatic cells.

The other most common germ-soma differentiated strategy we observed involved parental organisms deterministically producing a tiered set of offspring. For example, in one of our trials, a replicative organism deterministically produced a second, different organism, and this new organism produced sterile offspring. The observed frequency of this behavior in our sample of trials was exactly the same as the probabilistic replicator.

Tiered sets of size three and four were observed, each of which contained exactly one somatic genotype and two or three germatic genotypes, all with similar gestation times. Inherently, these tiered replicative structures encode an expected proportion of sterile cells at the time of deme replication. While the best deme produced by either of these tiered strategies was produced by a three-tiered cell, the proportion of nonviable demes within the three-tiered trial was greater than the proportion of nonviable demes within the four-tiered trial ($p < .05$, Student's t-test) indicating that the four-tiered strategy might be more stable.

Of note was one of our trials that could be considered both a tiered and a probabilistic replicator. This particular indi-

vidual produced a tiered set of offspring, where each tier had a statistically unique, associated replicative probability that ranged from about 15% to about 25%.

The least common multicellular strategy that emerged involved cells producing both exact, germatic copies and sterile, somatic cells in deterministic sequences. This behavior was only observed in about 4% of trials. An example of an observed sequence-replicator was a cell that always produced exactly one soma before attempting to replicate itself indefinitely. We also observed genomes that encoded more complex sequences; another cell first produced a single soma, followed by a repeating sequence of one copy of itself followed by two somatic cells.

Because each of these strategies could manifest in an infinite number of ways, however, constructing a definitive ranking of strategic superiority from our data is impossible. We argue that it is only appropriate to compare the ultimate effectiveness of specific strategic manifestations, rather than the strategies themselves.

It is clear, however, that strategies which emerged in trials where selection was applied perform better than no strategy at all. When selection is not applied, mean dominant deme fitnesses are consistently lower, according to data collected from 46 trials with selection and 50 trials without selection (Figure 4).

Trials with "no selection" were still subject to some experimental constraints which selected for certain behaviors. In no selection trials, all demes were assigned identical fitnesses, making deme-level tournament selection random. From demes that were randomly selected for replication, two random organisms were selected as founders. The most common behavior that emerged within these trials was decreased gestation time. Organisms replicating increasingly quickly with no respect to the flagellation and enlargement constraints decreased average deme fitness over time.

Brittleness

Replicative cells within trials where selection was applied also tended to evolve heightened sensitivity to lethal mutations, increasing their genotypic brittleness; germ cells that developed this behavior ultimately increased the proportion of their offspring that were sterile. This behavior was common in successful replicative organisms that did not exhibit any of the germ-soma differentiated strategies.

The Avida platform supports several types of mutation, and it's likely that cells evolved increased brittleness with respect to each. However, measurement of total brittleness is a computationally expensive process; accounting for every possible combination of legal mutations is impossible. Here, for the sake of computational complexity, we restrict our consideration to the most common mutation type: single-swap.

For each locus in a given organism's genome, each instruction within the legal set was swapped in, holding the rest of the genome constant. The resulting genotype was then analyzed for viability, and the fraction of single-swap mutations that rendered an organism sterile was recorded.

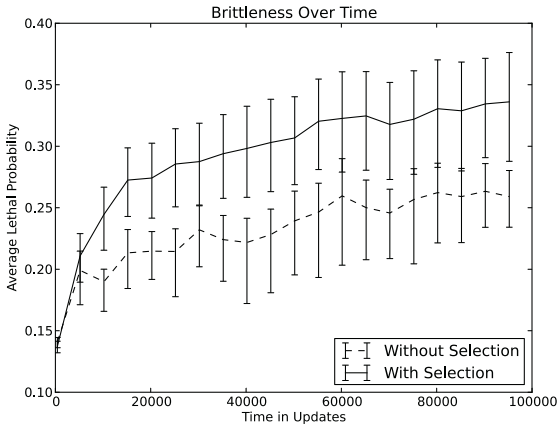

Figure 5: Average single-swap mutation brittleness over time in selection and no selection trials with bootstrapped 95% confidence intervals using 10^5 re-samples

Here, we examine the most common replicative genotype in each of our trials and compare the effect of applying Volvocale-inspired selection on average brittleness. Even in trials where multicellular strategies emerged, however, it was usually the case that the most common replicator did not exhibit a germ-soma differentiated strategy. By examining these typically undifferentiated cells, it was our goal to establish that increased brittleness alone commonly emerges as strategy for addressing the flagellation and physical size constraints.

We find that the average brittleness of the most common replicator in trials where we applied selection was always greater than in trials where we did not (Figure 5). The difference is more consistent early in trials, indicating that increased brittleness likely emerges quickly as an "easy" solution to the flagellation and enlargement constraints prior to the emergence of more complex, differentiated strategies.

In general, the brittleness of organisms in Avida tends to increase naturally over time as genome length and organism complexity increase, which accounts for the increased brittleness we observe in the trials with no applied selection.

Linear versus Exponential Decay

Up to this point in our analysis, we have not addressed our linear decay trials. We observe that implementing linear decay results in less germ-soma differentiation than exponential decay ($p < .05$, Student's t-test). Michod et al. (2006) note that a higher initial cost of reproduction yields a larger benefit from soma specialization to population viability. The Volvocales fitness can be represented in general as a multi-objective problem with an indirect relationship between via-

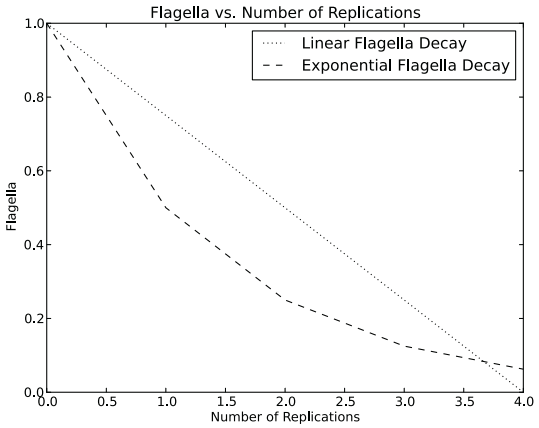

Figure 6: Flagellar function versus number of replications for exponential and linear flagella decay trials.

bility and fecundity, as a cell has limited resources to invest in each. Multi-objective problem theory predicts that if the trade-off between viability and fecundity forms a concave curve, generalists will evolve, while a convex curve will lead to specialists (Deb, 2005). Increasing the initial cost of reproduction pushes that curve to be more convex. In our experiments, an exponential decay of flagella yielded a much higher initial cost of reproduction than a linear decay, and thus more often led to the evolution of specialists (Figure 6).

Conclusions and Future Work

We have shown that digital organisms are capable of evolving multicellularity as a solution to the flagellation and enlargement constraints within the Avida platform. The wide range of effective strategies involving germ-soma specialization we observed indicates that digital platforms can be appropriate for studying reproductive altruism. Avida, specifically, was well suited for our experiments because it offered detailed insight into novel strategies digital organisms might use to accomplish germ-soma differentiation.

In the future, we aim to improve upon our model in several ways. For example, in the Volvocales, inter-deme competition is negligible because all organisms result from the same germ cell. In our experiments, however, this type of competition exists, particularly prior to differentiation. We believe that inter-deme competition in Avida could be eliminated by implementing a more dynamic, less periodic deme replication. For instance, a deme could replicate when it contains a given number of cells, rather than at a specific time.

Another potential experimental extension would be to incorporate more physically-inspired parameters into our models. Our deme-level fitness function, for instance, might be better informed by a more careful consideration of the interplay between cell radius, volume and drag. Such considerations would likely not alter our core findings, but might produce new types of organism behavior.

Finally, previous work suggests that, as colonial size increases, multicellularity becomes more advantageous (Michod et al., 2006). This theoretical result is supported by biological observation. In *Volvox*, a multicellular species of the Volovocales, colonies can contain thousands of cells (Kirk, 1998). Allowing for greater colonial size would increase the computation time required for our experiments, but would likely result in a higher proportion of trials evolving multicellularity.

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