Evolving Multicellularity in Digital Organisms through Reproductive Altruism

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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 use 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 are appropriate to use in the study of reproductive altruism.

Categories and Subject Descriptors

J.3 [**Life and Medical Sciences**]: Biology and Genetics; I.2.11 [**Distributed Artificial Intelligence**]: Coherence and Coordination

General Terms

Experimentation

Keywords

Artificial Life, Digital Evolution, Avida, Multicellularity, Volvox

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1. 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 [4]. The fact that 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 [13]. 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 [1]. 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 [6]. 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 [8].

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 [14]. Flagella are whip-like organelles responsible for algal motion, and a colony's capacity for mobility is determined by how many functional flagella it has.

In the Volvocales, however, cell division damages flagella. When a cell replicates, it's flagella continue to function, but "not as strongly or as well coordinated as when [a cell is] not dividing" [9]. After a cell replicates several times (the upper limit is generally assumed to be five divisions) its flagella become completely nonfunctional [8, 10]. 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" [8]. 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. This size differential results from the fact that post-embryonic cell division is not possible [10]. 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 mobility decreases because of increased mass and drag; more flagellation is required for colonial motion. Previous work suggests that overcoming this enlargement constraint is yet another benefit to germ-soma specialization [10].

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 [11, 10]. 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 is somewhat limited and could be improved upon, our primary goal is to assess the potential of a digital life platform to address questions of reproductive altruism.

2. THE AVIDA PLATFORM

Avida is a software platform that maintains a grid of self-replicating and mutating computer programs, and is used to address biological questions computationally. Because organic evolution tends to occur extremely slowly, considering digital evolution allows researchers to conduct experiments relatively quickly. Digitizing the study of evolution carries with it additional benefits, too; with digital platforms, it is easy to repeat experiments, specify environmental parameters exactly, and measure population statistics with precision. While no biological system can be perfectly modeled within the platform, Avida supports "replication, variation, and differential fitness," three conditions that, when met, create an environment in which evolution will occur [3]. 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" [12].

Just as biological organisms use DNA to encode individuality and functionality, the genotype of an Avidian digital organism consists of a sequence of instructions. Each of these assembly-like commands operates on an organism's unique set of simulated hardware. Every organism is allocated a virtual CPU, memory to hold instructions, three general purpose registers, two general purpose stacks, and input/output functionality.

The Avida life cycle is defined as follows. Replication occurs when an organism executes a divide command, and mutation is accomplished through probabilistic instruction addition, deletion and modification within a child's genome prior to its insertion into the greater population. Organism death occurs either through old-age, or when an organism is overwritten by another's child. Differential fitness is achieved on the organism level by allocating more real CPU cycles to more fit organisms. This allows more fit organisms to execute a greater number of instructions and ultimately function and reproduce more successfully.

We believe that Avida is well suited for our experiments because we are interested in determining if digital platforms are appropriate for studying reproductive altruism. While a simpler mathematical model could offer some insight and would be easier to analyze, we concern ourselves with the specific strategies digital organisms evolve to solve the flagella and enlargement constraints.

Extensions to the Avida Platform

We extend the Avida platform to accommodate for the flagellation and enlargement constraints by adding two variables to each digital organism. In our experiments, each cell is assigned a *flagella* value and a *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 [10], 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 multi-level 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. In order 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 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 fact that, in germ-soma differentiated species, all cells in a colony are derived from the same original germ cell [10, 7]. 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

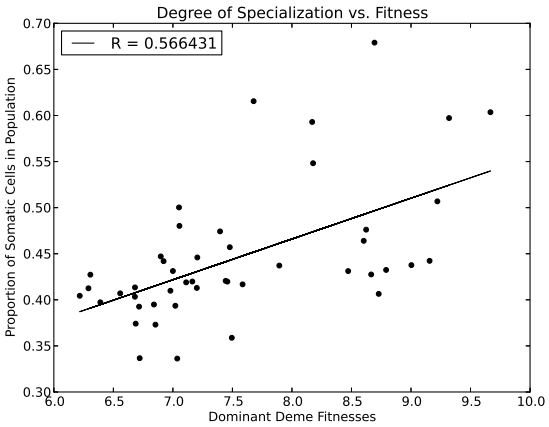

Figure 1: Least squares regression of deme fitnesses versus somatic proportion in exponential trials

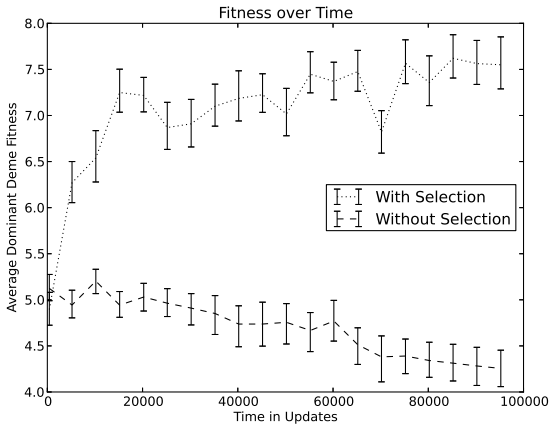

Figure 2: Average dominant deme fitness over time with bootstrapped 95% CI (10^5 re-samples)

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. The somatic cell is included for increased colonial stability.

3. 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. Experiments were run for 10^5 "updates," which act as a unit of time within Avida. In a single Avida update, an average organism executes 30 assembly-like instructions. Individual trials terminated after roughly 10 hours, on average.

Our fitness function was designed to reward greater total deme flagellar function, and penalize greater deme physical size. After experimenting with more complex, nonlinear functions, we decided to use the following function to assess deme fitness.

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

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.

Inter-deme cell replication was accomplished by preferentially inserting new cells into empty locations. If a deme was full and replacement was inevitable, a new cell's parent was not a candidate for replacement.

4. RESULTS

Before discussing the specific mechanisms digital organisms developed to accomplish germ-soma differentiation, we aim to establish that designating organisms as sterile is, in general, a solution to the flagellation and enlargement constraints. We compare the final dominant deme fitnesses from our 46 exponential decay trials to the proportion of somatic cells in the final population for each trial. We find a moderate positive correlation between these variables (Figure 1). This positive correlation indicates that, in general, populations that display higher degrees of germ-soma differentiation produce more fit demes.

Using an order statistic 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, for example, 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 present these strategies in decreasing order of observed frequency.

The strategy we observed with the greatest frequency did not involve germ-soma specialization. Individual organisms commonly looped through several junk instructions many times before replicating. This behavior made organisms appear sterile during loop execution, but ultimately did not represent differentiation.

The 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. Tiered sets of size three and four were observed, each of which contained exactly one somatic genotype and two or three germatic genotypes 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 emerged within the three-tiered trial, the proportion of nonviable demes within the three-tiered trial was greater than the proportion of nonviable demes within the four-tiered trial, indicating that the four-tiered strategy might be more stable (95% CI for $p_3 - p_4$ [.0198, .1442]).

Another multicellular solution that emerged involved cells producing both exact, germatic copies and sterile, somatic cells in deterministic sequences. For example, we observed one cell that always produced exactly one soma before attempting to replicate itself indefinitely. We also observed 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.

The final strategy, which we observed in only one of our trials, was probabilistic replication. We observed a cell that attempted to replicate itself only 58% of the time ($p = .5793$, $n = 10^5$, 95% CI $[0.570, 0.589]$). This organism exhibits phenotypic plasticity, which is the ability to change one's phenotype in response to environmental cues. In this case, the environmental cues are random numbers input to the organism by the Avida platform.

Notably, replicative cells within all of these strategies also tended to evolve heightened sensitivity to lethal mutations, increasing their genotypic brittleness. Germ cells that developed this behavior effectively increased the proportion of their offspring that were sterile.

Comparing the best trials exhibiting each of the three germ-soma differentiated strategies, we see that the probabilistic replicator likely emerged early and immediately improved dominant deme fitness, while the deterministic and tiered strategies displayed slower, incremental improvement.

Because each strategy can 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 any of these germ-soma differentiation strategies performs better than no strategy. When deme-level 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 2).

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. A 95% CI for the difference in average proportion of sterile cells between exponential and linear trials is given by $[.0887, .0213]$ ($n_{lin} = 18$, $n_{exp} = 46$). Michod et al [10] 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 viability 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 [2]. 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.

5. 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 are appropriate for studying reproductive altruism.

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 prior to differentiation. We believe that inter-deme competition in Avida could be eliminated by implementing a more dynamic, less periodic deme replication. For example, a deme could replicate when it contains a given number of cells, rather than at a specific time.

Finally, previous work suggests that, as colonial size increases, multicellularity becomes more advantageous [10]. For example, in *Volvox*, a multicellular species of the Volovocales, colonies can contain thousands of cells [5]. 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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