Evolution of Group Reproduction in the Transition to Multicellularity: A Bottom-Up Modeling Approach

Using ABMs to Advance ETI Theory in the Evolution of Multicellularity, Inspired by Volvocine Algae

Complex Adaptive Systems MSc

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Abstract

Evolutionary transitions in individuality (ETIs)—in which new biological units emerge from groups of simpler entities—pose deep theoretical and empirical challenges. While mathematical models have explored the selective conditions and traits that might allow groups to evolve reproductive division of labor, few, if any, simulation-based approaches have attempted to model this transition from the bottom up, allowing group-level fitness to emerge explicitly from individual-level interactions.

In this thesis, I develop an agent-based model in PhysiCell to explore early stages of multicellularity, focusing on the evolution of group-level reproduction inspired by transitions observed in photosynthetic volvocine algae. The model simulates simple single-celled agents with evolvable gene regulatory networks controlling reproduction, motility, and adhesion in response to environmental inputs and internal energy states. These behaviors unfold within ecological contexts involving light competition, shading, periodic death cycles, and dispersal events—allowing group reproduction to emerge from modifications of single-cell life cycles. The aim was to test whether this process could precipitate a ratcheting effect, as described by Maliet et al., ultimately leading to reproductive division of labor and a complete ETI.

Although reproductive division of labor did not evolve, multicellular clusters capable of coordinated group reproduction did. I conclude that the ratcheting effect was inhibited not by ecological constraints, but by the absence of accessible evolutionary pathways to restructure energy relationships within the group. Specifically, cells remained energetically autonomous and followed fixed energy allocation strategies, both as individuals and within clusters. This suggests that both energy sharing and flexible control over energy allocation are necessary conditions for ratcheting toward full ETIs. Energy sharing enables functional interdependence among cells, establishing the metabolic basis for collective organization. Equally critical, however, is internal regulatory control over how energy is distributed between costly cellular functions—such as growth, division, and motility. This control introduces the degrees of freedom required for evolution to explore divergent allocation strategies, which are a prerequisite for specialization. Without both metabolic interdependence and allocation flexibility, the evolutionary pathway toward reproductive division of labor—and thus full individuality—remains inaccessible.

Keywords: Evolutionary transitions in individuality (ETIs), Multicellularity, Agent-based modeling, Energy allocation, Metabolic interdependence, Group selection, Group reproduction, PhysiCell, Gene regulatory networks (GRNs), Evolutionary simulation, Volvocine algae, Computational biology

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In a field where rapid publication often takes precedence over long-term, foundational exploration, he gave me the space to pursue a conceptually ambitious and computationally demanding model of evolutionary transitions in individuality. Where others might have insisted (and to be fair, he did gently suggest once or twice) that I consider something more conventional, simple, or guaranteed, he instead encouraged my curiosity, respected my process, and stood by me through both theoretical and practical hurdles. This work would not have been possible without his critical insight, steady encouragement, and his remarkable patience with a project that wandered well beyond typical timelines and expectations.

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Cyrena Howland, Gothenburg, July 2025

List of Acronyms

Below is the list of acronyms that have been used throughout this thesis listed in alphabetical order:

ABM	Agent Based Model
ECM	Extracellular Matrix
ETI	Evolutionary Transition in Individuality
GRN	Genetic Regulatory Network
NN	Neural Network
ODD	Overview, Design concepts and Details
ODE	Ordinary Differential Equation
PDD	Probability Density Distribution

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1

Introduction

For my master's thesis project, I set out to simulate an evolutionary transition in individuality (ETI) from the bottom up using an agent-based model (ABM). Agent-based modeling is uniquely suited for exploring emergent phenomena—complex, system-level behaviors that arise from simple, localized interactions. Evolutionary transitions in individuality (ETIs) are among the most compelling examples of such emergence in evolutionary biology: they involve the transformation of formerly independent units into integrated wholes, where the fitness of the collective takes precedence over that of its individual components.

Yet, despite the theoretical significance of ETIs and their central place in major transitions theory, no prior work—at least to the best of my knowledge or that of the researchers I consulted—had used an ABM to simulate such a transition in detail. Why not? My advisor suspected computational limitations were to blame. But with the recent availability of high-performance simulation environments, I was less convinced. I began to wonder: was it finally possible to simulate an ETI from the ground up?

I turned to the evolution of multicellularity, one of the best-studied ETIs in both empirical and theoretical contexts. Unlike some other transitions, multicellularity has evolved independently at least 25 times across the tree of life, and has been experimentally observed in laboratory settings with organisms like yeast and volvocine algae. The theoretical work of Richard Michod and colleagues—especially their mathematical models of the transition to multicellularity in systems like Chlamydomonas and Volvox—offered a foundational conceptual framework. Their studies of fitness decomposition, germ-soma differentiation, and the reproduction-survival trade-off gave me a rich theoretical basis to adapt for a bottom-up, simulation-based approach.

The availability of robust biological simulation platforms such as PhysiCell also proved crucial to surmounting computational challenges. This framework provided the computational tools and biological primitives—such as cell division, metabolism, and environmental sensing—necessary to model individual cells and their interactions over evolutionary time. By leveraging these tools, I could focus on the emergent properties of multicellular organization, rather than building every component from scratch.

Because a key stage in any evolutionary transition in individuality (ETI) is the decoupling of fitness from the individual level to the group level, this project focused on the critical evolutionary point at which a unicellular life cycle transitions into a coordinated group-level life cycle. This transformation—where reproduction becomes a property of the collective rather than the individual—is widely regarded as the pivotal innovation that enables group-beneficial traits to be inherited, even if they reduce individual fitness. It is at this stage that a proposed "ratcheting effect" (Maliet et al., 2015) can emerge, wherein once group-level reproduction evolves, it becomes increasingly difficult for evolution to revert to individual-level selection, due to the structural and functional interdependence

that develops within the collective.

In the mathematical model developed by Maliet et al. (2015), the evolution of a multicellular life cycle is conceptualized as a reorganization of the phases of a unicellular life cycle. Framed within life-history theory, the model employs pre-defined fitness equations to represent a key trade-off believed to underlie the evolution of multicellularity: the balance between survival and reproduction. Rather than simulating individual organisms, the model explores trait dynamics across a fitness landscape, using analytical tools to infer the evolutionary conditions under which group-level reproduction becomes advantageous.

By explicitly modeling how fitness varies across the survival-reproduction axis, the framework identifies which traits must co-evolve to facilitate a shift from individual-level to group-level selection. Crucially, it suggests that once group cohesion and coordinated reproduction arise, a ratcheting effect can occur—making the transition effectively irreversible as the collective begins to function as a new, higher-level unit of selection.

Inspired by these inferences I tried to build my ABM to reflect the insights of the mathematical model proposed, while incorporating explicitly energy and resources, and other elements necessary for my agents to be complete Darwinian individuals.

I did successfully simulate the evolution of group reproduction; however, the shift in fitness from the individual level to the group level proved more nuanced and difficult to detect, and the expected ratcheting effect wasn't expressed as predicted. I believe the beginnings of a shift in individuality did emerge, but not one that would ultimately lead to a full evolutionary transition in individuality (ETI) or the complete evolution of multicellularity.

I concluded that in energy-explicit systems—where fitness emerges from lineage persistence rather than predefined fitness equations, evolutionary transitions in individuality may not manifest through life-history trait shifts, as predicted by earlier mathematical models such as Maliet et al. (2015). Instead, fitness and individuality may arise as properties of energetic organization, not as scalar traits like growth duration that are typically measurable in laboratory systems.

The key limitation, I believe, was that the evolvable trait space in my model did not include the axis necessary for a full transition—specifically, strategies for energy allocation between cellular functions, and intercellular sharing among attached clonal cells. In this way, the model's inability to produce a full ETI was itself revealing: it suggested that for such transitions to occur, the system must allow evolution to act on how energy flows and is managed within collectives.

From this perspective, the earliest signals of individuality may not arise from changes in reproductive timing and the evolution of life-history traits, but from emerging patterns in how energy is acquired, shared, and constrained within a group. Individuality, in this framework, is no longer defined by shifts in scalar value traits assumed to be crucial to individual fitness, but by the emergence of energetic interdependence—cells coordinating and constraining their energy use as a unified metabolic entity.

This insight underscores the value of agent-based models in this field. ABMs offer a uniquely powerful tool to investigate *how* these transitions occur by capturing emergent processes and energetic dynamics that are not easily observable in empirical or experimental systems. They enable us to ask questions and discover possible underlying mechanisms that drive these transitions that would be inaccessible through traditional experimental or trait-based mathematical models.

Ultimately, this project demonstrates that with modern computational abilities and ac-

cessible high performance ABM platforms, simulating evolutionary transitions in individuality from the bottom up is now feasible. The real barrier is no longer computational, but conceptual: we must evolve our models, frameworks, and metrics to better capture the energetic, relational, and organizational dimensions that underpin the emergence of new evolutionary individuals, with the help of agent based modeling.

2

Theory: What is an ETI?

2.1 Evolutionary Transitions in Individuality: the Emergence of Life's Hierarchy

The evolution of life on Earth can be understood through the lens of a limited set of evolutionary transitions in individuality (ETIs). These transitions mark pivotal moments in life's history when individual units of selection, the entities on which natural selection operates—such as genes, cells, or organisms— cooperated and became integrated into higher-order individuals. The result is the hierarchical structure of life, where biological organization builds upon itself through evolutionary processes.[3]

This perspective, first formalized by Maynard Smith and Szathmáry in their landmark book *The Major Transitions in Evolution* (1995), transformed how biologists conceptualize the emergence of biological complexity and served as the genesis of an entire field of study. [3] This field focuses on understanding the processes and principles underlying the emergence of new levels of biological organization, from molecular cooperation to complex societies [3],[4]]. Life's nested organization, from molecules to multicellular organisms to eusocial societies, is a direct outcome of repeated ETIs, which reorganized evolutionary individuals into increasingly cooperative and interdependent systems.

2.1.1 Defining ETIs and the Role of the Darwinian Individual

At the core of ETIs is the concept of the Darwinian individual, an entity that exhibits heritable variation in fitness—the fundamental requirement for natural selection to act [[5]]. Traditionally, selection has been framed in terms of individual organisms, yet evolution does not privilege any particular level of biological organization [[3],[6]]. Instead, selection acts wherever variation, differential success, and inheritance occur [[7]].

A **Darwinian individual** is an entity that meets the necessary conditions to be subject to natural selection. In other words, it is an agent that can evolve through differential reproduction over generations. The key traits of such an entity are:

1. **Variation:** The population must have heritable differences among individuals. These differences may arise from genetic mutations, recombination, or other mechanisms that introduce diversity.
2. **Heritability:** Traits must be passed from one generation to the next. This ensures that successful variations persist over time rather than being lost after a single generation.

3. **Reproduction:** The entity must be able to make copies of itself (either clonally or through sexual reproduction), allowing the transmission of traits to offspring.
4. **Struggle for Existence (Competition):** In order for there to be differential survival, there must be some mechanism that determines which agents persist and which do not. Without some form of selective pressure, advantageous traits would not necessarily be favored.
5. **Individuality:** The entity must be identifiable as a unit of selection, meaning it has a coherent boundary and functions as a unit in evolutionary processes.

In the context of multicellularity and evolutionary transitions in individuality (ETIs)—which my project focuses on—there is an additional question of what counts as an "individual." A group of cells might transition from being a collection of Darwinian individuals (each cell being an evolutionary unit) to forming a new higher-level individual (a multicellular organism) subject to selection.

In early life, selection primarily operated at the level of molecular replicators, with self-replicating RNA molecules serving as the earliest units of evolution [[7]]. Over time, cooperative interactions between these molecules led to the first genomes, housed within primitive cells [[3],[6]]. Further ETIs saw the transition from single-celled to multicellular life[[3],[4]], and in some cases, to eusocial societies, where colonies function as integrated superorganisms [[8],[8]]. Each of these transitions reflects a fundamental shift: the prior evolutionary individuals cease to act as independent units and instead become components of a larger, more complex entity [[3],[6]].

The importance of defining an evolutionary individual lies in its role as the unit upon which selection operates [[3],[6]]. Without a clear delineation of individuality, understanding how new levels of biological organization evolve becomes challenging. Thus, ETIs provide a framework for studying the emergence of complexity in evolution, highlighting how life has repeatedly reorganized itself into new levels of selection [[3],[6]].

2.1.2 Selection Dynamics in the Evolution of Multicellularity

A recurring pattern in the evolution of multicellularity is the initial dominance of rapidly reproducing cells, followed by the evolution of mechanisms that regulate excessive cell proliferation [[9]]. Laboratory experiments have directly observed these dynamics in real time.

For example, in the evolution of multicellularity in **Saccharomyces cerevisiae** (yeast), selection initially favored fast-dividing cells that could proliferate within multicellular clusters [[9]]. However, over generations, some clusters evolved programmed cell death (apoptosis), which improved group-level fitness by preventing uncontrolled growth and promoting cooperative behaviors [[9]].

Similar patterns have been observed in bacteria. In an experiment with Pseudomonas fluorescens, early multicellular groups were dominated by hyper-proliferative mutants, disrupting cooperative integrity. Over time, new mutants evolved that stabilized group cohesion and suppressed excessive replication [[3],[7]].

Comparative studies of natural multicellular lineages, such as volvocine green algae, reinforce this trend. In Volvox, the emergence of germ-soma differentiation—where some cells specialize in reproduction while others perform structural functions, or processes necessary for survival —was crucial for maintaining group stability [[10]]. This differentiation is thought to be a key innovation that prevents evolutionary conflicts between individual and group-level selection[[10]].

2.1.3 Multi-Selection Theory: Evolution across Biological Scales

In the context of ETIs, selection occurs at multiple levels of organization simultaneously, a principle formalized in multi-selection theory [[3],[7],[6]]. This framework extends natural selection beyond just individual organisms to include genes, cells, and social groups as units of selection. This perspective is crucial in understanding why cooperation—often viewed as paradoxical under traditional Darwinian models—persists and flourishes in many evolutionary systems [[11],[8]].

For example, in the evolution of multicellularity, selection initially favors individual cell survival and rapid replication, but over evolutionary time, selection at the group level begins to favor cooperation, division of labor, and reproductive specialization [[9],[10]]. This pattern is evident in both experimental evolution studies and natural multicellular organisms. The same principles apply at higher levels, such as in eusocial insect colonies, where individual ants sacrifice their reproductive potential for the success of the colony [[8]].

Multi-selection theory clarifies that natural selection does not always act solely on individual survival and reproduction; instead, it can favor groups when cooperation enhances collective fitness [[11],[8]]. Understanding these principles is essential for explaining how complex, hierarchical life forms emerge and persist despite the inherent conflicts of interest at lower levels [[3],[6]].

2.1.4 Major ETIs

The major evolutionary transitions identified by Maynard Smith and Szathmáry [[3] which resulted in the emergence of a new and more integrated level of individuality are:

- **Replicating molecules → Populations of molecules in compartments:** The compartmentalization and cooperation among molecules which led to the emergence of primitive "cells."[[3]]
- **Independent replicators (e.g., RNA) → Chromosomes :** The organization of genetic material into chromosomes reduced competition between genetic material and enabled coordinated replication. [[3],[7]]
- **Genes cooperating → Genomes:** Genes began to function as parts of integrated systems, enabling more efficient expression and regulation. [[3]]
- **RNA as gene and enzyme → DNA and protein:** Early life utilized RNA for both genetic storage and catalysis. The division of labor between DNA (information storage) and proteins (enzymatic functions) improved efficiency and stability. [[3],[7]]

- **Prokaryotes (archaea + eubacteria) → Eukaryotes:** The transition to eukaryotic cells involved the integration of organelles like mitochondria through endosymbiosis.[[3],[6]]
- **Single-cells → Multicellular organisms:** Individual cells began to cooperate, specialize, and form complex organisms like plants, animals, and fungi.[[3],[6]]
- **Individuals → Social groups:** The evolution of eusociality in insects like ants and bees introduced specialization into castes, with some individuals foregoing reproduction entirely. [[4],[6]]

These transitions are united by two key features. First, individual units of selection cooperate to create a more complex entity, shifting the focus of natural selection to the new higher-level "individual." [[3],[4],[6]] Second, the once-independent parts become dependent on the whole, losing the ability to replicate or survive independently. [[6]]

2.1.5 Cooperation and Fitness in ETIs

A hallmark of these transitions is that the components of the new individual evolve away from maximizing their own fitness in favor of maximizing the fitness of the collective. For example:

- **In multicellular organisms**, cells may undergo programmed cell death (apoptosis) or specialize into non-reproductive roles to benefit the whole organism. [[6]]
- **In eusocial colonies,** worker castes forego reproduction to support the reproductive success of the queen. [[4],[6]]

This sacrifice raises a fundamental question: why would individuals cooperate when it seemingly contradicts their self-interest? The resolution lies in reconciling cooperation with natural selection.

2.1.6 Reconciling Natural Selection and Cooperation: a Historical Challenge

Darwin himself acknowledged that the evolution of cooperation presented a profound challenge to his theory of natural selection, which is primarily framed around competition and survival of the fittest. Cooperation, particularly in forms that appear altruistic—where an individual incurs a cost to benefit others—seemed to contradict the central tenet of natural selection: that individuals should maximize their own reproductive success.[[5],[8]]

2.1.6.1 Darwin's Concern with Social Insects:

One of Darwin's most pressing concerns, as expressed in *On the Origin of Species* (1859), was explaining the evolution of sterile worker castes in eusocial insects, such as ants and bees. [[5]] These individuals sacrifice their own reproductive potential entirely, dedicating their lives to supporting the colony, an arrangement that appears to defy natural selection.

Darwin famously called the existence of sterile castes "one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory." [[5]]

2.1.6.2 The Conundrum of Cooperation:

Beyond insects, Darwin recognized cooperation in many forms, including human morality and mutualism in other species. These behaviors often appeared at odds with the principle of "every individual for itself." [[8]] For decades, researchers following Darwin struggled to reconcile cooperative and altruistic behaviors with the fundamentally competitive framework of natural selection. [[11],[8]]

2.1.6.3 Resolution through Theory Expansion:

The apparent contradiction between cooperation and natural selection was gradually resolved by broadening our understanding of how selection operates, not only at the level of individual organisms but across multiple levels of biological organization. Several key theoretical frameworks have been developed to explain the evolution of cooperation and altruism: [[11], [8], [8]]

1. **Kin Selection (Hamilton, 1964):**

   Kin selection proposes that individuals can increase their genetic fitness not only by reproducing themselves but also by helping close relatives who share many of their genes.[[8]] A key concept in this theory is that the measure of genetic success is inclusive—a fitness that includes both direct fitness (offspring an individual produces) and indirect fitness (offspring produced by relatives as a result of an individual's help). [[8]]

   An example is a hive of worker bees that do not reproduce themselves, but instead assist the queen, who shares much of their genetic material, in producing offspring. By helping their "mother," the worker bees indirectly propagate their shared genes. [[8]]

   Kin selection explains how altruistic behaviors that seem self-sacrificial at the individual level can persist because they ensure the genetic success of the family or lineage.[[8]]

2. **Group Selection**

   Group selection focuses on the idea that selection can act at the level of groups, favoring traits that benefit the group as a whole, even if they are costly to individual members. [[5],[8]] While group selection was controversial for decades, it is now recognized as a valid mechanism when conditions favor competition between groups rather than within them. [[**?**],[8]]

   An example is a population of early Homo sapiens. A tribe in which individuals cooperated to share resources and defend against predators would be more likely to survive and pass on their genes than a tribe of purely selfish individuals, even if cooperation incurred costs or death for individual members. [[11]]

   A key difference between kin selection and group selection is that it applies even when

individuals are not closely related, provided the benefits to the group outweigh the individual costs. [[11]]

2.1.7 Theoretical Significance in ETIs

The evolution of cooperation is not merely a phenomenon isolated at the level of the animal kingdom; it is a central force driving every evolutionary transition in individuality (ETI). Each ETI represents a fundamental shift in biological organization. In each transition, from molecules to multicellular organisms to eusocial societies, cooperation among lower-level units is an essential step in forming a cohesive, higher-level "individual." And thus, in every ETI, at every level of selection, are fundamental questions that echo the challenges Darwin faced when reconciling the theory of natural selection with the pervasive cooperation evident in the natural world.

At the heart of ETIs are two intertwined questions, which underpin all studies in this field:

1. How do the fitness interests of the lower-level units become aligned or even replaced by the fitness interests of the higher-level individual?
2. How do once-independent lower-level units become interdependent components of a new, indivisible whole?

These questions are addressed through a conceptual framework that includes both the formation of cooperative groups and their integration into cohesive wholes. They also emphasize the twin themes of interdependence and reorganization of fitness.

2.1.8 A Two-Stage Process

Evolutionary transitions in individuality are broadly described as a two-stage process [[3],[4]]:

1. **Formation of cooperative groups:** Initially, individual units come together to form a loosely cooperative group. Cooperation is often favored because it provides immediate fitness benefits, such as protection from environmental pressures or improved resource acquisition.

   At this stage the “individuality” and fitness interests of the lower-level unit are still intact, and the fitness of the collective is a sum of the fitness of its constituent lower level units. Cooperation often incurs a cost to the individual units, such as resource sharing or reduced autonomy and motility. However, cooperation evolves because the collective benefits outweigh these costs.

   An example is the transient aggregation of amoebae into a slug during the life cycle of Dictyostelium discoideum, which demonstrates how cooperation can provide survival benefits under harsh conditions [12] [13] Aggregation allows some cells to form spores while others become stalk cells, which altruistically sacrifice their survival to enable spore dispersal [12] [13] [14]. This process provides a clear model of the

interplay between cooperation and fitness reorganization in ETIs [6] [12].

2. **Integration into a cohesive whole:** Once these individuals are attached as a part of a group, selection can begin to work on the group as a whole, driving evolution at the individual level to become more and more integrated at the level of the collective since a well coordinated group would do much better and outcompete a poorly coordinated one.

   It is thought that over time these groups would evolve into highly integrated units and eventually the fitness of the whole would takes precedence over that of the parts.

   This is marked by:

   (a) Interdependence: The parts become reliant on the collective for survival and reproduction.

   (b) Specialization: Different components take on distinct roles to support the higher-level individual. [12], [14]

   (c) Loss of Autonomy: The lower-level units lose the ability to function, survive, and reproduce independently.

Transition of the Unit of Selection:

In this stage, the collective transitions from being a mere aggregation to a true individual, and the unit of selection is now in the higher level individual. [3],[6]

The evolution of multicellularity is a prime example of this transition, when single-cell life cycles evolve into multicellular organisms with a life-cycle as an organism. At this point, the organism becomes more than just a collection of cells—it is a new individual with a new reproductive program. [12], [14]

Illustration of Specialization:

In multicellular organisms, cells differentiate into somatic and germ lines. Somatic cells support the organism motility, energy acquisition and general survival while germ cells ensure reproduction. Without each other germ cells would never survive, and somatic cells would never reproduce. [6]

At a larger scale, in eusocial colonies, worker and reproductive castes integrate into a single functional unit, with workers sacrificing reproduction to support the colony. [3], [4], [8]

2.1.9 Overcoming Self-Interest and Competition in ETIs

Cooperation is central and the first step to every ETI, but implicit in Darwin's original conundrum is the idea that stable cooperation requires something counteracting competition among lower-level units. Put another way: What prevents a component member that is receiving group benefits—such as shared resources—from exploiting those resources and prioritizing its own survival at the cost of the group (also referred to as *defecting*)? Competition between members of a group must be resolved, and mechanisms must develop to suppress selfish behavior or the group could not survive.

3

Theory II: The Evolution to Multicellularity

The transition from unicellular to multicellular life represents a Major Evolutionary Transition in which formerly independent unicellular entities relinquish their individual autonomy to function as a collective, coordinated unit [3]. This transition is considered one of the most consequential in evolutionary history, as it underlies the complexity and diversity of multicellular life forms [[15],[16]]. Unlike the origin of life—thought to have occurred only once on Earth—multicellularity has evolved independently at least 25 times across all three domains of life: Bacteria, Archaea, and Eukarya [[17], [18]]. This remarkable evolutionary convergence suggests that multicellularity is a highly advantageous and adaptable strategy, emerging in response to diverse selective pressures.

Empirical evidence further supports the notion that unicellular organisms can evolve multicellular lifestyles relatively quickly under appropriate selection pressures [15]. Experimental evolution studies have demonstrated that unicellular lineages, such as yeast [19] and green algae [20], [21], [22], [23], can transition to multicellularity in a relatively short time frame. This suggests that the genetic and physiological barriers to multicellular organization may not be as prohibitive as once thought [[16], [1]]. However, despite the apparent ease with which multicellularity can evolve, understanding the precise mechanisms driving this transition remains a significant challenge. The long evolutionary divergence times between extant multicellular organisms and their unicellular ancestors, along with limited experimental tools for many multicellular models, pose technical obstacles to studying this transition in detail [24].

A particularly compelling system for investigating the evolution of multicellularity is the volvocine algae, a group of chlorophyte green algae that exhibit a spectrum of organizational complexity—from unicellular species to fully differentiated multicellular forms. These algae provide a tractable model for comparative and experimental studies aimed at identifying the genetic and molecular underpinnings of multicellular evolution [24].

3.1 Defining Multicellularity

Determining the number of times multicellularity has evolved depends on how it is defined. Broad definitions encompass various cooperative and aggregative behaviors, such as those observed in social amoebae like Dictyostelium, whereas more stringent definitions require features such as division of labor, coordinated function, or irreversible cell differentiation

[18]. Multicellularity, therefore, exists along a spectrum, ranging from transient cellular collectives to highly integrated organisms with specialized cell types and developmental programs.

As Grosberg & Strathmann note, the evolutionary plasticity of the transition to multicellularity enables researchers to analyze both the selective pressures favoring this shift and the adaptive mechanisms that regulate cooperation and prevent defection within multicellular groups.[[15]] The challenge remains to decipher the precise evolutionary, genetic, and ecological factors that facilitate this transition.

To provide clarity, multicellularity is often described as evolving in three major stages, each representing distinct evolutionary processes and increasing levels of integration and complexity [18].

3.2 The Three Stages of Multicellularity

3.2.1 1. Evolution of Multicellular Groups

This first stage, often referred to as the evolution of cooperation, involves the formation of groups of cells that act collectively, gaining an advantage over single cells. At this stage:

- **Natural selection** still operates at the level of individual cells, meaning that cells join groups because it benefits their individual fitness. Cooperation arises when individuals work together for mutual benefit. This often inherently incurs a cost for the cooperating individuals. This cost is offset by the evolutionary advantages gained at the group level, such as improved resource acquisition or defense against predators [18].

  - A classic example is the formation of biofilms, where bacterial cells secrete extracellular matrices that protect the group but require individual cells to expend energy in the process.
- **Adhesion** is the key cell attribute that underlies this stage. When cells are stuck together, it's in their best interest to cooperate and are more likely to develop adaptations that facilitate this cooperation. There are two types of adhesion that occur in the diversity of life. Clonal adhesion and aggregate adhesion:
  - Clonality (failure of cell separation post-division). In clonal aggregation, cells are genetically identical. For example, volvocine algae show clonal multicellularity.
  - Aggregation (adherence of unrelated cells) slime molds exhibit aggregative multicellularity [15].

  Clonal aggregation is thought to be critical to the evolution of multicellularity because lack of genetic diversity among attached cells reduces competition among cell lineages. Grosberg and Strathmann (2007) [15] suggest that clonal multicellularity—where cells remain connected post-division—facilitates a bottleneck that ensures genetic uniformity and supports the evolution of altruistic traits like spe-

cialization, as cells are less likely to act selfishly when they share identical genetic interests.
There is good reason why almost all multicellular life forms retain unicellar bottlenecks in their life cycle. The unicellular stage ensures that mutations arising in the parent organism are replicated and distributed in later generations, diluting the effect of genetic variation and curbing selection and competition among cells of the multicellular organism. [25] [15].

3.2.2 2. Evolution of Multicellular Individuals

In this transitional stage of an ETI, the group of cells evolves into a single "Darwinian individual," meaning the group itself becomes the unit of selection. This stage involves:

- The evolution of a multicellular cell cycle. Initially, cells within a group divide independently, but for a multicellular individual to emerge, the division of constituent cells must become coordinated, giving rise to a collective cell cycle.
- Group-level reproduction and selection. At this stage, the group reproduces as a whole, rather than simply being a collection of independently reproducing cells. Mechanisms such as germ-soma differentiation often evolve to support group-level reproduction [18].
- Conflict resolution mechanisms. To achieve cohesion, selection pressures drive the evolution of mechanisms that suppress selfish behavior (e.g., defector cells) and enhance cooperation and interdependence. Mechanisms like policing or programmed cell death stabilize cooperation [18].

This stage represents a fundamental shift in individuality, as the group begins to function as a higher-level organism rather than merely a collection of cooperating cells.

3.2.3 3. Evolution of Multicellular Organisms

The final stage involves the emergence of division of labor and functional specialization among cells, cementing the multicellular organism as a new evolutionary individual. Key characteristics of this stage include:

- Specialization and differentiation. Cells take on distinct roles, such as somatic cells (non-reproductive cells that provide structural support and basic functions such as motility for the organism) and germ cells (exclusively reproductive), often at the expense of individual cellular fitness [18].
- Functional integration and interdependence. The multicellular organism becomes so highly organized that its constituent cells can no longer survive independently . Instead they can only replicate as part of the larger group [18].
- Shift in selection. Natural selection now acts at the level of the multicellular organism rather than the individual cells. As a result, adaptations that benefit the group accumulate, enhancing the fitness of the whole at the expense of the parts [26].
- Optimization of group-level traits. As group-level adaptations accumulate, such as epigenetic regulation and developmental pathways, the multicellular organism reaches higher levels of complexity.

3.2.4 Conclusion of the Stages

These three stages—cooperation, individuality, and organismality — are the accepted paradigm of multicellular evolution. While specific mechanisms and pathways vary across lineages, the underlying principles of increasing interdependence and integration remain consistent across the tree of life.

4

Theory III: Volvocine Algae Lineage

4.1 Introduction to the Volvocine Algae: A Case Study in Multicellularity

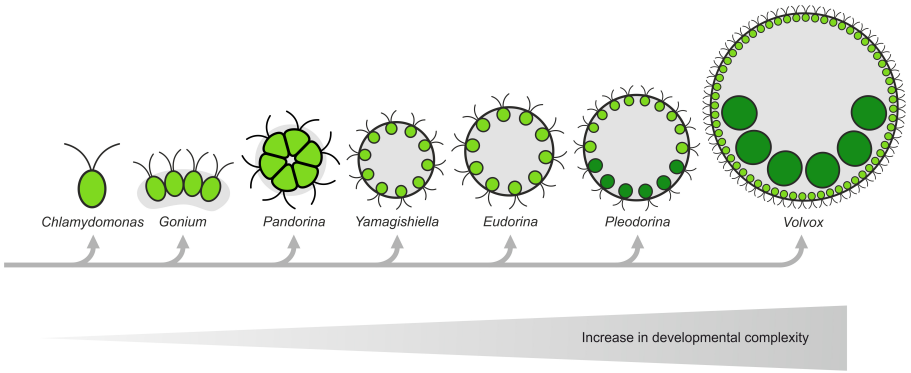


Figure 4.1: Illustration of the The volvocine algae's gradual and modular progression through the major stages of multicellularity

Volvocine algae, a family of green algae ranging from unicellular to multicellular forms, represent one of the most compelling natural examples of the evolution from unicellularity to multicellularity, and have served as a model for that transition. Their lineage provides a stepwise progression of increasing cellular organization and complexity from single cells (Chlamydomonas reinhardtii) to a fully integrated multicellular organism (Volvox), offering us "snapshots" at each stage of this evolutionary transition in individuality (ETI). By examining its genetic, ecological, and developmental changes, researchers have gained unique insights into one of life's most profound evolutionary shifts—the emergence of multicellular individuality.

Some of the reasons this lineage is so attractive to experimental biologists and genomic researchers include its remarkable phenotypic diversity, its genetic tractability as a laboratory model, and its relatively recent evolution to multicellularity, thought to have occurred in the Triassic period. [24]. Volvocine algae offers a natural laboratory for exploring one of life's major innovations—the evolutionary transition from discrete individual cells to cooperative multicellular organisms.

4.2 Clonal Adhesion and Genetic Bottlenecks: the Foundation of Multicellularity

A defining feature of multicellularity in volvocine algae is the preservation of genetic relatedness among cells within a group. This is primarily achieved through clonal adhesion—where daughter cells remain attached after division—and genetic bottlenecks, which ensure that each multicellular colony originates from a single progenitor cell. Together these mechanisms minimize internal conflict and reinforce cooperation, making them essential to the evolutionary transition from unicellular to multicellular life.

4.2.1 Clonal Adhesion: Staying Together for Collective Success

In the simplest colonial volvocine forms, such as Tetrabaena socialis and Gonium pectorale, clonal adhesion arises when daughter cells fail to completely separate after division. This results in stable, genetically identical groups, where each cell is a clone of the original. By staying together, cells maintain high genetic relatedness, which reduces competition between individual cells and increases the likelihood that cooperative traits—such as shared extracellular matrix (ECM) production or synchronized movement—are selected for.

As multicellularity evolves, adhesion becomes increasingly reinforced through ECM expansion and structural modifications. The extracellular matrix (ECM) is a complex network of proteins, polysaccharides, and other molecules that surrounds and supports cells in tissues and organs. It plays crucial roles in maintaining tissue structure, regulating cell behavior, and facilitating communication between cells. Larger colonies (Pandorina, Eudorina) invest more in ECM, strengthening the physical integrity of the group. By the time Volvox emerges, cell-cell adhesion is no longer simply an incidental byproduct of division but a genetically regulated feature that locks the group into an obligate multicellular state.

However while clonal adhesion and an ECM physically holds cells together, it does not necessarily prevent internal competition or the spread of selfish mutations within a colony over generations. This is where genetic bottlenecks become critical.

4.2.2 Genetic Bottlenecks: Resetting Genetic Identity across Generations

A genetic bottleneck occurs when a multicellular group originates from a single cell, ensuring that all cells in the next generation are genetically identical. This mechanism plays a key role in maintaining cooperation because it eliminates genetic diversity within the group at the start of each new cycle. When all cells share the same genetic material, there is little opportunity for selection to favor selfish behaviors that might undermine the stability of the group.

For example, in Volvox, each new colony arises from a single reproductive cell (gonidium), which then divides to form the next generation. This ensures that every new colony starts with a single genetic identity, reinforcing long-term cooperation. In earlier-stage colonies like Tetrabaena and Gonium, all cells retain reproductive ability, meaning that selection still acts at both the individual and group levels. However, in more complex forms (Pleodorina, Volvox), only a subset of cells retain reproductive ability (germ cells), while others become permanently specialized for motility (somatic cells). The genetic bottleneck at the start of each generation ensures that even these differentiated somatic cells—despite their reproductive sacrifice—are still genetically aligned with the success of the group as a whole.

4.2.3 Clonal Adhesion and Genetic Bottlenecks as Evolutionary Stabilizers

Although clonal adhesion and genetic bottlenecks both maintain genetic relatedness, they function at different levels:

- **Clonal adhesion** operates within a single generation, keeping genetically identical cells together and fostering cooperation.
- **Genetic bottlenecks** operate between generations, ensuring that each new colony starts from a single ancestor, eliminating genetic conflict over time.

Together, clonal adhesion and genetic bottlenecks resolve one of the fundamental challenges of multicellularity: the potential for internal competition among cells. Clonal adhesion physically holds the group together, while the genetic bottleneck ensures that cooperation remains evolutionarily stable across generations.

These mechanisms are not unique to volvocine algae. Many independently evolved multicellular lineages, from animals to fungi, exhibit similar strategies to prevent selfish mutations from undermining the integrity of the collective. In volvocine algae, they set the stage for further multicellular innovations, such as synchronized cell cycles, larger colony sizes, and division of labor. Without these foundational processes, the transition from unicellular life to true multicellularity would likely collapse under the pressures of internal conflict and selection at the individual cell level.

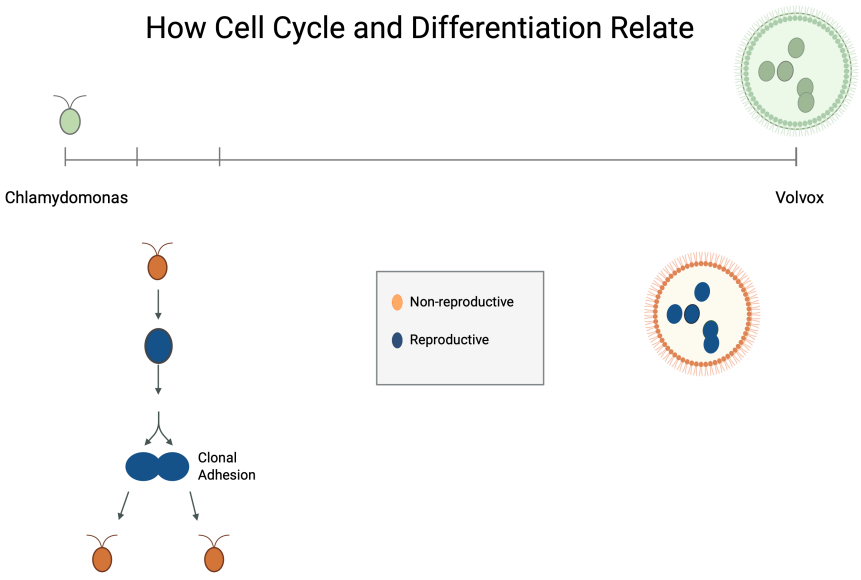


Figure 4.2: On the left, there is a representation of the unicellular Chladomynomous's cell cycle from the perspective of whether or not it is in its 'reproductive' state (in purple) vs 'non-reproductive' or survival process focused state (in orange). On the right is a visualization of the roles of the cell types in the multicellular Volvox, colored the same, as 'reproductive' also known as Germ cells and 'non-reproductive', also known as Soma cells.

4.3 Reproduction-Survival Trade-Offs: A Universal Constraint and a Driver of Multicellularity

All living organisms face a fundamental trade-off between reproduction and survival—resources allocated to one function often come at the expense of the other. This trade-off is a universal constraint that shapes biological strategies across all scales of life, from single cells to complex multicellular organisms. It plays a central role in the evolution of multicellularity by creating selective pressures that favor cooperative behaviors and the division of labor among cells.

In unicellular organisms, this trade-off is especially stark. A single-celled organism must balance its need to grow and divide with its ability to survive environmental challenges. For example, in Chlamydomonas reinhardtii, reproduction requires resorption of the flagella, temporarily sacrificing motility. This creates a biphasic life cycle, where a cell cannot both move (to access nutrients or escape predators) and divide at the same time. Similar trade-offs exist throughout nature: bacteria face a balance between rapid proliferation and stress resistance, while multicellular organisms weigh investment in offspring against self-maintenance.

The evolution of multicellularity represents a key innovation in resolving this trade-off. If cells remain attached post-division and form cooperative groups, they can specialize in different functions—some maintaining motility while others focus on reproduction. This shift allows organisms to circumvent the constraints that individual cells face, leading to functional differentiation, which is an essential step in the emergence of complex life.

In volvocine algae, this process unfolds as a progressive increase in cellular interdependence and division of labor. Early colonial forms like Tetrabaena retain full reproductive capacity in all cells, but as colony size increases (Gonium, Eudorina), group-level coordination becomes essential. In a more complex type, Pleodorina californica, a fraction of cells are dedicated entirely to motility, while others handle reproduction, demonstrating an early form of germ-soma separation. In Volvox carteri, this separation is complete—somatic cells permanently forgo reproduction in favor of collective survival, ensuring that the organism functions as an integrated whole.

This trade-off is not unique to volvocine algae; it is a fundamental principle in the evolution of complex life. From the earliest protocells to the rise of multicellular plants and animals, life has repeatedly evolved strategies to balance reproduction and survival, often leading to higher levels of organization and cooperation. In this way, the same pressures that shaped the transition to multicellularity continue to shape biological complexity across all levels of life, from microbes to metazoans.

4.4 Evolutionary Steps to Multicellularity in Volvocine Algae

The volvocine algae lineage represents a well-documented model for the evolution of multicellularity, providing a stepwise progression from unicellular ancestors to fully differentiated multicellular organisms. Each intermediate form in the lineage introduces a new level of group organization, illustrating key transitions in the evolutionary process. Below, I outline these major stages, emphasizing the emergence of multicellular traits such as clonal adhesion, division of labor, and obligate multicellularity.

Starting from a single-celled ancestor, each intermediate genus in the lineage adds a new

level of group organization:

4.4.1 Step 1: Unicellular Ancestor – Chlamydomonas reinhardtii

Key Trait: Biphasic life cycle with a trade-off between reproduction and survival

The unicellular ancestor of the volvocine algae,Chlamydomonas reinhardtii, provides a starting point for the evolutionary transition toward multicellularity. As a biflagellate unicell, Chlamydomonas exhibits a biphasic life cycle, alternating between a motile phase and a reproductive phase.

- **Biphasic Life Cycle and Reproduction-Survival Trade-Off** During reproduction, Chlamydomonas undergoes multiple fission, producing 2, 4, or 8 daughter cells at a time. However, cell division requires the resorption of flagella, rendering the cell temporarily immotile. This trade-off between motility and reproduction represents a key constraint that is hypothesized to have driven the evolution of multicellularity [10].
- **Implications for Multicellularity** The need to alternate between movement and division may have favored cooperative solutions, wherein some cells could remain motile while others specialized in reproduction, an early precursor to germ-soma differentiation.

4.4.2 Step 2: Early Colonial Forms – Tetrabaena socialis

The transition from unicellular life to simple multicellular groups is first observed in Tetrabaena socialis, a four-cell colonial species.

- **Emergence of Clonal Adhesion** In Tetrabaena, daughter cells remain physically attached following division due to incomplete cytokinesis, forming a clonal group life cycle in which the colony, rather than individual cells, reproduces as a unit.
- **Group-Level Reproduction** Unlike Chlamydomonas, where daughter cells separate and become independent, Tetrabaena exhibits a coordinated group reproductive cycle: all four cells divide synchronously to produce a new four-cell colony.
- **Incipient Multicellular Traits: Coordination and Fixed Colony Size** The orientation of flagella in Tetrabaena differs from that of unicells, suggesting the emergence of group-level motility rather than independent swimming [27]. The fixed colony size (always four cells) is genetically controlled, indicating the emergence of a developmental program at the group level (the group's cell number is regulated, rather than open-ended division) [28]

4.4.3 Step 3: Expansion of Colony Size – Gonium, Pandorina, and Eudorina

Larger colonies appear in the next stage of volvocine evolution, with species such as Gonium pectorale, Pandorina, and Eudorina forming groups of 8–64 cells. In these colonies all cells are still morphologically similar and capable of reproduction – there is no dedicated germ-soma separation yet [29].

- **Increase in Extracellular Matrix (ECM) Production** A major structural innovation in these species is the increasing role of extracellular matrix (ECM), which provides mechanical support and strengthens cell adhesion.

- Synchronized Division and Coordinated Motility Cells divide synchronously, and colonies exhibit anterior-posterior polarity, allowing coordinated flagellar beating that enhances directional swimming.
- The life cycle still proceeds through a unicellular bottleneck (a single cell or all cells of the colony divide to produce daughters), which ensures the colony's cells are clonal and cooperate with minimal internal conflict [28]. The group's cell cycle is now clearly distinct from a solitary cell's: rather than each cell perpetually growing and dividing independently, divisions are synchronized and limited in number so that the entire colony develops as a unit and then propagates as a whole. In effect, reproduction is becoming a property of the group (the colony produces new colonies) rather than just of individual cells [1].
- **Genetic Bottleneck Reinforces Cooperation** Each new colony originates from a single progenitor cell, ensuring genetic uniformity and reinforcing selection for cooperative traits [23].

4.4.4 Step 4: Partial Division of Labor – Pleodorina californica

At this stage, we observe the first evidence of incipient germ-soma differentiation, a hallmark of obligate multicellularity.

- **Specialization of Somatic and Reproductive Cells:** In Pleodorina californica, cells begin to exhibit functional specialization:
  - A fraction of cells specialize in motility (somatic cells), losing the ability to reproduce.
  - Others retain reproductive capability (germ cells), ensuring colony propagation.
- **Flexible Germ-Soma Separation** This differentiation is not yet absolute—under some conditions, somatic cells may regain reproductive potential, unlike in Volvox where the division of labor is irreversible [30].

Most cells in Pleodorina remain capable of both reproduction and motility, but a fraction of the cells (often toward the posterior of the spherical colony) become specialized somatic cells that do not divide [29]. These somatic cells are smaller and focused on flagellar beating, while the larger cells in the colony can reproduce. This represents a partial evolutionary transition in individuality: some cells have relinquished their reproductive role entirely, increasing the colony's survival (continuous motility) at the cost of their own reproduction. However, the separation is not absolute – if reproductive cells are removed or under certain conditions, somatic cells in Pleodorina may still have the potential (albeit limited) to produce offspring colonies. The colony's life cycle includes a stage in which only certain cells reproduce, foreshadowing the complete germ-soma split seen in Volvox. Notably, the proportion of somatic (sterile) cells tends to increase with colony size among volvocines suggesting that larger group size amplifies the benefits of having non-reproductive, specialized helper cells (and also the need to mitigate internal competition for resources or motility) [1].

4.4.5 Step 5: Fully Integrated Multicellularity – Volvox carteri

The final stage in the transition to multicellularity is seen in Volvox carteri, which exhibits a complete and obligate germ-soma division of labor. Unlike earlier volvocine forms, in which selection acted at both the colony level and individual cell level, Volvox represents a

stage where selection overwhelmingly favors the multicellular group rather than individual cells.

- **Permanent Specialization of Somatic and Germ Cells**
  - **Somatic cells** (2,000 per colony) are terminally differentiated, meaning they will never regain reproductive function. Their sole role is to provide motility.
  - **Germ cells** (12–16 per colony) are exclusively responsible for reproduction, forming daughter colonies.
- **Obligate Multicellularity**
  - Unlike earlier volvocine forms, individual cells of Volvox cannot survive independently, making it a true multicellular organism.
  - In species such as Pleodorina, somatic cells still retain some reproductive potential under certain conditions, but in Volvox, the germ-soma split is irreversible.
- **Dominance of Group-Level Selection**
  - Selection at the group level begins as soon as colonies reproduce as a unit (in Tetrabaena and Gonium), but in Volvox, selection at the multicellular level completely supersedes selection at the individual cell level [31].
  - This represents the completion of the evolutionary transition in individuality [32].

From an evolutionary perspective, the Volvox colony has achieved an indivisible unity: its cells are so specialized and interdependent that they cannot revert to a free-living state without fatal consequences. The somatic cells cannot leave the group or reproduce on their own, and the germ cells, while capable of dividing, rely on the somatic cells to survive and disperse. This makes the Volvox colony an integrated individual in its own right. All cells are descended from a single zygote (ensuring genetic uniformity) and remain stuck together, sharing a common fate. If you were to isolate a somatic cell, it would be maladapted outside the colony as it lacks the ability to grow or reproduce, and even its structural polarity is suited for the spherical colony context [28]. Likewise, an isolated germ cell would not be effective without the somatic support. Thus, the indivisibility criterion for a new individual is met – the group cannot simply fall apart into viable subunits [28]. The evolution of Volvox essentially locked in the multicellular organization as irreversible and obligate.

At each stage, clonal adhesion and genetic bottlenecks serve as key stabilizing forces, ensuring that multicellular cooperation is preserved despite potential selection pressures acting at the individual cell level. These stepped transitions make volvocine algae a particularly valuable system for understanding how multicellularity evolves and becomes entrenched

4.5 Step 3 and 2: Genetic Bottlenecks Become Evolutionarily Significant

Genetic bottlenecks ensuring that each new generation originates from a single progenitor cell are present in all volvocine algae, including their unicellular ancestors. However the key evolutionary transition is not the presence of the bottleneck itself, but rather how it interacts with multicellularity to reinforce cooperation.

- In unicellular species like Chlamydomonas reinhardtii, the genetic bottleneck ensures that all daughter cells produced in a single reproductive event are genetically identical. However because these daughter cells immediately separate and live independently, the bottleneck has no direct impact on group-level cooperation.
- In early colonial forms (Tetrabaena, Gonium), clonal adhesion begins to extend the benefits of the genetic bottleneck to a group setting. Cells that would have lived independently now remain attached, meaning that selection can begin to act on cooperative traits at the colony level.
- As colonies grow larger (Pandorina, Eudorina), the genetic bottleneck continues to play a crucial role in maintaining group-level cooperation. Even as selection pressures start to act at the colony level, the genetic bottleneck ensures that all members remain genetically aligned, reducing internal competition.
- By Volvox carteri, the genetic bottleneck has become essential for maintaining division of labor. Since somatic cells permanently lose the ability to reproduce, their survival depends entirely on cooperation with reproductive germ cells. The genetic bottleneck prevents evolutionary conflicts by ensuring that all cells in the colony share a common genetic interest.

Thus, while genetic bottlenecks have always been present in volvocine algae, their evolutionary significance increases as multicellularity evolves. In unicellular species, the bottleneck maintains genetic identity only at the individual level, while in colonial and multicellular species, it stabilizes group-level cooperation and ultimately allows for the emergence of specialized cell types.

4.6 Key Biological Insights from Genomics: Repurposing Outweighs Novelty

One of the most intriguing discoveries made from comparative genomics of volvocine algae is that multicellularity arose with surprisingly few novel genes. The genome of Volvox carteri (a complex multicellular organism) is similar to that of Chlamydomonas reinhardtii (its unicellular relative); the two share the vast majority of their 14,000 genes [10], as cited in [24]). The evolution of multicellularity in this lineage did not require a wholesale invention of new genetic material, but rather a new utilization of existing genes.

Researchers found an “apparent lack of master multicellularity genes" governing the transition. Instead, changes in gene regulation utilized co-option of old genes for new roles, changes in expression patterns, and protein domain tweaks for the emergence of multicellular traits. ([10], as cited in [24]) This means that the single-celled ancestor already had much of the toolkit needed for multicellular life; evolution repurposed those tools in novel ways to build a multicellular organism.

A prime example of co-option is that changes in gene regulation and cell cycle control underpin the transition, rather than brand new multicell-specific genes. For instance, all volvocine algae possess the cell-cycle regulatory gene Retinoblastoma (RB) (encoded by MAT3), which in unicellular Chlamydomonas helps control cell division. In multicellular species like Gonium or Volvox, the RB gene has undergone small modifications in its domains that alter its activity. ([33], as cited in [24]). Remarkably, experiments showed that taking the RB protein from a multicellular species and expressing it in Chlamydomonas can induce the unicells to form multicellular clumps ([28], as cited in [24]). This suggests

that evolved changes in RB helped ancestral cells stick together as a cluster of an appropriate size by adjusting the number of divisions. Indeed, multicellular volvocines tend to have RB proteins with different phosphorylation sites and linker regions, making them more permissive of additional rounds of cell division before cells separate ([28], as cited in [24]). This kind of tweak allowed one mother cell to produce multiple daughter cells that remain attached, effectively creating a multicellular group in one round of reproduction. It underscores that regulatory changes – in this case, altering the cell cycle timing and the adhesion between daughter cells – were pivotal in establishing multicellularity.

Beyond RB, researchers have noted only a few gene innovations or expansions linked to multicellularity. Some expansion of ECM protein families correlates with larger colony size ([10], as cited in [24]), but this expansion does not appear to be a major driver of the fundamental transitions towards multicellularity, such as cell adhesion or differentiation [[33], [28]]. Instead, ECM modifications primarily play a role in maintaining colony integrity and size regulation rather than initiating multicellular organization [[33], [19]] There is no one "multicellularity gene" that suddenly appeared; rather, Volvox and Chlamydomonas differ in how and when genes are expressed. Many multicellular traits seem to result from novel gene regulation circuits and interactions (e.g. new patterns of gene activation during colony development) built on a conserved genomic repertoire [[24], [10], [**?**]].

In summary, the volvocine algae demonstrate that the evolution of multicellularity was driven by the co-option and reorganization of pre-existing genes. The cells didn't require new components so much as they required new activation instructions for some of those parts. This finding aligns with the idea that multicellular life may often evolve through regulatory innovation – changes in gene expression timing, spatial patterns, and cell-to-cell signaling – rather than through the appearance of completely novel genes. It also means the single-celled ancestor of Volvox already had latent capabilities for cell adhesion, communication, and differentiation that evolution progressively activated or enhanced to achieve a multicellular organism.

5

Theory IV: ABMs in Evolutionary Biology

5.1 Introduction: Agent Based Models: Description and Function

At a fundamental level, agent-based models (ABMs) seek an answer to a profound question: *How is it possible that individual units interact in such a way as to create patterns on a larger scale?* This question is crucial in studying complex adaptive systems, where collective behaviors cannot be simply inferred from individual components.

For centuries, scientific inquiry has operated at two separate scales:

- The **granular level**, where disciplines like physics and cellular biology study individual behaviors (e.g., molecular collisions, cellular growth, or Newtonian mechanics).
- The **global scale**, where statistical physics and thermodynamics describe collective behaviors in systems with homogenous components with weak interactions in equilibrium (e.g., gases).

However, understanding the global emergent patterns of complex adaptive systems such as ecosystems, economies, and multicellular organisms requires more than simple aggregation. They exhibit:

- **Heterogeneity:** Individual components (agents) have varied traits, goals, or behaviors.
- **Dynamic, local interactions:** Components influence each other through spatial or network connections.
- **Feedback loops:** Global patterns emerge from and, in turn, influence the behaviors of individual components.
- **Emergent phenomena:** Global system-wide patterns arise that are qualitatively new and cannot be reduced to the statistical averages of individual behaviors. This occurs because it is not just the static properties of individual components, but their interactions, that generate higher-level behavior.
  - For example, rolling electrical blackouts and traffic jams are emergent phenomena. They do not result from a single, central cause—such as a downed power line or a car accident—but rather from the dynamic interactions between many individual components. You cannot fully understand a traffic jam by examining individual cars, nor can you explain a blackout by analyzing a single power line. Instead, these large-scale patterns arise from the cascading and interde-

pendent interactions of many agents, producing system-wide behaviors that are not evident at the granular level.

Traditional global-scale tools like statistical physics and equilibrium-based models, while powerful, are not designed to handle these dynamics. Traditional mathematical models often assume states of homogeneity and equilibrium that fail to capture complex system behaviors. They describe systems where aggregation suffices; where collective behavior can be represented as the sum or average of its parts. For example, in a gas, macroscopic properties like pressure or temperature are straightforward statistical results of particle motion.

ABMs provide an alternative by explicitly modeling interactions, heterogeneity, and feedback mechanisms, making them invaluable for studying emergent complexity.

5.1.1 ABMs: a Tool for Complexity Science

Throughout history, the tools at our disposal have shaped scientific discovery. The microscope allowed us to see cells and atoms; Newtonian mechanics enabled us to calculate the trajectory of a ball tossed into the air; and thermodynamics revealed the behavior of gases. These tools excelled at describing linear, predictable systems, but struggled when faced with complex interactions.

The advent of computational power allowed us to simulate systems wherein individual components follow their own rules and interact dynamically. ABMs have since emerged as a new scientific lens for studying complex systems by:

- **Simulating evolutionary transitions step-by-step**, rather than assuming outcomes.
- **Allowing fitness and selection to emerge**, rather than imposing it.
- **Testing multiple evolutionary trajectories**, something experiments and genomics cannot do.
- **Capturing the stochastic, contingent nature of evolution**, revealing whether multicellularity is inevitable or context-dependent.

5.1.2 Bridging the Gap Between Scales

While ABMs and complex adaptive systems may seem to conflict with reductionism, they are firmly grounded in its rigor. Reductionism has provided us with the tools to understand individual components: Newtonian physics for particle motion, molecular biology for cellular mechanisms; and differential equations for predator-prey dynamics. ABMs build on these foundations, combining granular-level insights with global-scale analysis to bridge the gap between scales.

However, ABMs go beyond reductionism in critical ways:

1. **Emergence, not aggregation:** While statistical physics describes how gases behave as an aggregate of particle motion, ABMs simulate systems where emergent phenomena arise from the interactions between diverse, adaptive agents.
2. **Feedback loops:** Traditional tools often treat individual components as independent of global dynamics. ABMs allow global-scale behaviors to influence individual decisions, capturing the recursive dynamics central to complex systems.
3. **Synthesis of scales:** ABMs integrate granular and global perspectives, providing

a holistic view of how local behaviors drive large-scale patterns—and vice versa.

ABMs extend reductionist rigor to new scales:

1. **Granular insights:** ABMs use reductionist principles to model individual agents with detailed, empirically grounded behaviors.
2. **Global patterns:** ABMs use computational power to simulate emergent phenomena and analyze system-wide dynamics.
3. **Bridging the gap:** By explicitly modeling interactions and feedback, ABMs reveal how individual behaviors give rise to—and are shaped by—global patterns.

This makes them uniquely powerful for understanding phenomena that reductionism alone cannot address, such as the transition to multicellularity, economic collapse, or coordinated bird flocks.

5.2 ABMs: a Tool to Study ETIs and the Evolution of Multicellularity

5.2.1 The Challenge of Evolutionary Transitions in Individuality (ETIs)

The evolution of multicellularity represents one of the most significant evolutionary transitions in individuality (ETIs), wherein selection shifts from impacting single cells to a collective as a new evolutionary unit. However, understanding how this shift occurs remains a major challenge due to the limitations of existing research approaches:

- **Mathematical models** provide insights into cooperation, group selection, and fitness trade-offs, but rely on predefined fitness equations, rather than allowing selection to emerge dynamically. They also fail to capture individual-level interactions and spatial structure, which are fundamental to early multicellular evolution.
- **Experimental evolution** demonstrates that multicellularity can evolve, but it does not reveal how it evolved step by step, nor whether an ETI is inevitable or contingent on specific conditions. Additionally generations take too long, and researchers cannot rewind evolution to test alternative paths.
- **Comparative genomics** identifies genes associated with multicellularity but does not reveal the processes by which those genes adapted—it provides a snapshot rather than a dynamic view of evolution.

Agent-based models (ABMs) offer a powerful alternative, a new approach that captures local interactions, allows selection to emerge, and simulates evolution dynamically.

5.2.2 ABMs: Uniquely Suited for Studying Early Multicellularity

ABMs explicitly simulate individual cells as autonomous "agents" that interact with each other and their environment. Unlike traditional mathematical models, which assume predefined fitness landscapes and homogeneous populations, ABMs allow fitness, cooperation, and group selection to emerge from local interactions over generations.

Key advantages include:

Individual-Level Interactions: Each cell is simulated as a separate agent with its own behaviors, such as adhesion, division, and cooperation. Instead of assuming how groups form, ABMs let cell clusters emerge dynamically from individual interactions.

Spatial Structure & Local Competition: Unlike traditional models that assume well-mixed populations, ABMs explicitly model cells in space, allowing for realistic clustering, adhesion, and resource competition. This is crucial because spatial proximity is a key driver of multicellularity, influencing cooperative and competitive dynamics.

Emergent Selection & Fitness Landscapes: In ABMs, fitness is not predefined—instead, selection acts on the evolving population, allowing cooperation and group selection to emerge naturally. This is essential to test whether multicellularity is an inevitable outcome of evolution or contingent on rare events.

Stochasticity & Evolutionary Contingency: Evolution is not deterministic; it is shaped by random mutations, genetic drift, and fluctuating environmental conditions. ABMs capture stochastic processes in a way that traditional models cannot, making simulations more biologically realistic.

Environmental Feedback & Developmental Complexity: Evolution happens in response to environmental pressures. ABMs allow cells and environments to co-evolve, capturing dynamic selection pressures like predation, oxygen levels, and resource limitations.

ABMs can also incorporate cell differentiation, signaling, and division of labor, making them well-suited for studying the transition from simple cell clusters to fully integrated multicellular organisms.

As a result, ABMs provide a unique and powerful approach for testing hypotheses about how multicellularity evolved—not just whether it could.

5.3 Conclusion: ABMs and ETIs are a Functional Fit. but Remain an Unfulfilled Promise

ETIs, and the evolution of multicellularity represent precisely the kind of emergent phenomenon that ABMs are designed to explore. Individual units of selection—each acting toward its own fitness benefit—interact and evolve in such a way that they become a part of a higher unit of selection. As this higher unit, the collective ceases to act toward the fitness goals of its individual components, and becomes a new individual in its own right. Reductionist principles help us understand how individual cells adhere, signal, and divide. But these principles alone cannot explain how cells evolve into multicellular organisms with coordinated reproduction and specialization. ABMs integrate these granular insights into a dynamic framework, allowing us to simulate how local interactions drive emergent multicellular behaviors. Through this lens, ABMs offer unparalleled insights into the origins of biological complexity.

Despite their promise, agent-based models have yet to successfully simulate a full evolutionary transition in individuality. While they have been used to explore certain aspects of these transitions—such as the evolution of cooperation, group adhesion, or collective behaviors—no model has captured the entire shift from individual-level optimization to group-level fitness domination. Why not?

ABMs or complex adaptive systems are not new fields. Agent-based modeling has been available for over 50 years, and researchers have successfully applied it to a wide range of questions in ecology, economics, and evolutionary biology. The limitations have largely

been technological. As we discussed earlier, the tools we have—whether microscopes or computational methods—shape the kinds of questions we ask. Just as magnifying lenses determine how finely we can resolve biological structures, in agent-based modeling, it's the power of our computers that determines what phenomena we can realistically simulate.

Evolutionary transitions in individuality operate on evolutionary timescales, requiring the simulation of tens of thousands of interacting agents over thousands of generations. Historically, our computational power and techniques have struggled to handle that level of complexity and provide the speeds required to allow meaningful experimentation—much less to allow graduate students to complete their research in a reasonable timeframe.

We are finally reaching a tipping point. Advances in computational efficiency, algorithms, and hardware are pushing the boundaries of what ABMs can achieve. And so, perhaps bolstered by naïve hubris, I challenged the limits of our technology to discover if even part of an evolutionary transition in individuality can be simulated.

My work builds on decades of research. Cellular-level studies convincingly identified genes and attributes critical for driving the evolution to multicellularity (e.g., the long-studied volvocine algae family), while mathematical models described how single-cell life cycles might evolve into multicellular life cycles at the population level. My ABM leverages these insights and puts them to the test in a dynamic framework, attempting to explicitly simulate the emergence of group-level fitness dominance.

To the best of my knowledge and that of my advisor's (bolstered by years in this field), no other work has attempted this. Therefore my model represents a critical step toward understanding the origins of multicellular complexity and the emergence of higher units of selection, signaling an exciting new era for the scale and types of phenomena we can study and simulate from the bottom up, in-silico.

Although I failed to simulate from the bottom up a complete evolutionary transition in individuality, I drew closer than anyone else before me. I achieved something novel: the evolution of group reproduction and explicitly simulated evolution of a multicellular cell cycle from a single cell cycle, where natural selection can begin acting on the group-level, and group-level fitness can theoretically begin to take precedence over individual-level optimization. This shift is a critical precursor to full ETIs and an essential step toward understanding multicellularity.

Moreover, the absence of the ratcheting mechanism proposed by Maliet et al. (2015) — and my hypothesis as to why it failed to emerge — offers new insight into the mechanisms required for higher-level individuality to evolve. In that sense, the missing ratchet is itself an informative result.

Importantly, I encountered no terminal impediments suggesting that a full ETI simulation is beyond reach. With continued refinement and even conservative estimates of computational advancement, it is not unrealistic to expect such a model to be realized. I believe this project lays a strong foundation — and with further development, it could take us the rest of the way. Going forward, theoretical progress will depend on models that can explicitly represent the mechanisms driving these transitions. Agent-based modeling offers a uniquely powerful framework for this task.

6

Model Inspiration

6.1 Chlamydomonas reinhardtii and the Evolutionary Transition to Multicellularity

Chlamydomonas *reinhardtii* serves as a model unicellular ancestor for the volvocrine algae lineage, exemplifying key trade-offs between survival and reproduction that set the stage for the evolutionary transition to multicellularity. As an independent, free-living organism, Chlamydomonas must balance motility, photosynthesis, and reproduction within a single cell. Understanding its cell cycle and life-history traits provides insight into how early multicellular colonies evolved from single-celled ancestors.([24], [10], [33]).

Since Chlamydomonas is a unicellular species within a lineage that evolved multicellularity, it provides a realistic starting point for modeling the transition. Its evolutionary trajectory has been extensively studied, and many genetic and ecological investigations have explored how it might have evolved multicellular traits, providing empirical data to inform agent behaviors.

Therefore this unicellular species provided a key inspiration for the design of my model's Darwinian individual agents. Many of my model design decisions and assumptions mirrored traits of Chlamydomonas reinhardtii that were crucial to its survival, its embodiment of reproductive-survival trade-offs in its biphasic cell cycle, and its reproductive paradigm of increasing size leading to more offspring and clonal adhesion through a single-cell bottleneck. ([28], [19]).

6.1.1 Cellular Traits of Chlamydomonas reinhardtii

- **Photosynthesis:** Chlamydomonas reinhardtii is a photo-autotrophic organism, meaning it primarily derives its energy from photosynthesis. It uses light energy to generate ATP and NADPH, which fuel biosynthetic pathways essential for growth and reproduction ([21]).
- **Multiple Fission:** Unlike organisms that divide at a fixed rate through binary fission, Chlamydomonas undergoes multiple fission. The number of daughter cells follows an exponential pattern, where the number of divisions (n) determines the total offspring (N):

$$N = 2^n$$

  - More growth → more divisions → more offspring.
  - Cells accumulate biomass before division rather than dividing immediately upon reaching a certain size ([22], [23]).

- **Clonal Adhesion:** After multiple fission, daughter cells remain temporarily connected by cytoplasmic bridges, remnants of the mother's cell wall or incomplete cleavage during cytokinesis. This temporary attachment, known as clonal adhesion, represents an early step toward group cohesion seen in multicellular relatives ([34]).
- **Cooperation**: Cells lacks any obligate cell-to-cell cooperation.

  The life cycle, genetic architecture, and cellular behavior of Chlamydomonas reinhardtii provide a baseline for understanding how a unicellular ancestor could evolve into a multicellular form.

6.1.2 Cell Cycle Phases and Reproductive-Survival Trade-Offs

Chlamydomonas exhibits two distinct phases in its cell cycle that highlight a fundamental reproductive-survival trade-off:

- **G1 (Non-reproductive Phase):**
  - Cells are motile, swimming with flagella.
  - Cells grow by increasing biomass and cell volume.
  - Cells photosynthesize and store energy for future division.
  - Cells do not reproduce during this phase. ([6], [35]).
- **S/M (Reproductive Phase):**
  - DNA replication and multiple fission occur, producing 2, 4, 8, or 16 daughter cells.
  - Cells become immotile, resorption of their flagella.
  - Cells stop growing and cannot photosynthesize.
  - Cells rely entirely on stored energy and biomass from the G1 phase to complete division. ([36]).

This division of labor within the cell cycle creates a reproductive-survival trade-off: when Chlamydomonas enters the reproductive phase, it forfeits motility and photosynthetic ability, making it vulnerable to environmental stressors. [37]. This trade-off likely played a pivotal role in the evolution of multicellularity, as a group of specialized cells with different functions (motility vs. reproduction) could outperform single-celled individuals. [38].

6.1.3 Implications for Multicellularity

- **Energy and Biomass Accumulation in G1:**
  - Growth in G1 is essential; a cell must photosynthesize and accumulate sufficient resources to undergo multiple rounds of division.
  - A well-fed cell in G1 can produce up to 8 to 16 daughter cells, whereas an energy-starved cell may only divide into 2 or 4 offspring or delay division ([22]).
- **Lack of Photosynthesis During S/M:**
  - Before entering the S/M phase, cells lose their flagella and become immotile.
  - While the chloroplast remains present, photosynthetic function is greatly reduced, and the cell must rely on stored resources to complete division. ([6],

[35]).

- **Key Evolutionary Step:**
  - In early multicellular groups, some cells may have remained in G1 to maintain motility and photosynthesis, while others transitioned into S/M to divide.
  - This functional differentiation would have given an advantage to groups over solitary cells, laying the foundation for a division of labor seen in multicellular Volvox colonies [38].

Chlamydomonas reinhardtii embodies the core trade-offs that drive the transition to multicellularity. Its reproductive-survival conflict provides a natural selection pressure favoring cell cooperation, eventually leading to specialized cell roles in later volvocrine species. By modeling the ancestral state of Chlamydomonas and mapping the evolutionary modifications across the volvocrine lineage from a lower to a higher level of complexity, we can gain key insights into the fundamental principles that shape evolutionary transitions in individuality. ([24], [10]).

6.1.4 Predation as a Driver of Multicellularity in Chlamydomonas reinhardtii

One of the most compelling selective pressures favoring the evolution of multicellularity in Chlamydomonas reinhardtii and its relatives was predation. Experiments have demonstrated that unicellular Chlamydomonas populations rapidly evolved multicellular forms when exposed to filter-feeding predators, such as Ochromonas and Paramecium (Boraas et al., 1998; Becks & Agrawal, 2010).

Predation Selects for Multicellularity in the following ways:

- **Size-Based Predation Avoidance**: Filter feeders are constrained by the size of prey they can ingest.. Chlamydomonas individuals are small enough to be efficiently ingested, but when cells adhere in clusters, they become too large to engulf (Boraas et al., 1998).
- **Rapid Evolution of Clustering**: Experimental evolution studies have shown that Chlamydomonas can evolve stable, heritable multicellular groups within hundreds of generations under predation pressure. (Ratcliff et al., 2012; Herron & Ratcliff, 2019)
- **Parallel Evolution across Multiple Lineages**: Similar predation-driven multicellularity has been observed in other unicellular lineages, such as yeasts, bacteria, and ciliates, reinforcing evidence that predation is a general evolutionary force favoring collective organization (Ratcliff et al., 2012).

This predator-driven selection for multicellular grouping supports the idea that cooperation first evolved as a survival strategy, rather than for cellular specialization. In Chlamydomonas, early cell groups formed as a passive defense mechanism, but over evolutionary time, these groups evolved stable adhesion, division of labor, and specialized cell types, as seen in more derived multicellular volvocine species like Volvox (Goldschmidt & Dolinšek, 2016; Baumgartner, 2016).

6.1.5 Self-Shading and Competition in Chlamydomonas Colonies: a Constraint on Multicellularity

While multicellular grouping offers protection from predation, it also introduces new evolutionary challenges, one of which is self-shading competition. As Chlamydomonas reinhardtii colonies increase in size, individual cells within these clusters experience a reduction in light availability, which is essential for photosynthesis and energy acquisition(Chua et al., 2024).

6.1.6 How Self-Shading Reduces Photosynthesis

- **Light Attenuation in Colonies:** In dense algal groups, outer cells receive the most light, while inner cells experience significant light deprivation, reducing their ability to photosynthesize efficiently (Lukeš, 2020; Sand-Jensen & Raun, 2009).
- **Decreased Energy Acquisition:** Studies have shown that colony-forming Chlamydomonas strains exhibit lower net photosynthetic output per cell than their unicellular counterparts due to internal shading (Chua et al., 2024).
- **Reduced Growth and Competition:** Colonies experiencing self-shading grow more slowly and may face competition from smaller, more light-efficient unicellular individuals (Chua et al., 2024; Abu-Ghosh et al., 2021).

6.1.7 Potential Costs of Adhesion in Multicellularity

While clonal adhesion offers protection from predation, it also traps some cells in low-light environments where they cannot efficiently generate energy. This introduces an inherent fitness trade-off:

- **Outer cells** in a colony may have an advantage in light absorption but are more exposed to predation and environmental stressors.
- **Inner cells** may be protected from predation but suffer from light limitation, which could force them into an energy-dependent state, relying on stored resources rather than active photosynthesis (Hemschemeier et al., 2009).

In some cases, selection may favor colonies with loose adhesion that allow cell rearrangement or movement, mitigating self-shading effects (Negi et al., 2020). Alternatively, the evolution of differentiated cell types—in which some cells specialize in photosynthesis while others focus on reproduction—could be an adaptive response to this problem, as seen in more complex volvocine species like Volvox (Chua et al., 2024).

6.1.8 Implications for Agent-Based Modeling

In my model, self-shading competition was explicitly incorporated as a potential cost of multicellular organization:

- Agents that formed large clusters experienced diminished energy acquisition, simulating reduced photosynthesis due to shading.
- This led to trade-offs between the benefits of adhesion (predator resistance) and the costs (reduced energy intake and competition with unicellular individuals).

By including these constraints my model not only captures the advantages of multicellularity, but also the realistic evolutionary trade offs that organisms must overcome in

order to maintain a cooperative, multicellular lifestyle during the evolutionary transition in individuality.

6.2 Cell Cycle Evolution

6.2.1 Choosing the Maliet, Shelton & Michod (2015) [1] Model as a Foundation

Once I had settled on the atrributes of Chlamydomonas and its simplest multicellular relatives as the model for my Darwinian individuals, I still needed a theoretical framework to program evolvable traits at the single-cell level capable of evolving into multicellular cell cycles. The complexity of biological and genomic literature surrounding volvocine algae's transition to multicellularity was extensive and intricate, necessitating a simpler yet robust theoretical framework that provided the following:

- defined cell-cycle traits as evolvable parameters
- provided a mathematical abstraction of life cycle evolution
- captured key biological principles of unicellular and multicellular algae

The Maliet, Shelton & Michod (2015) [1] model provided exactly this. It sought to solve a fundamental mystery in the evolutionary transition to multicellularity:

How does a group of initially independent cells, each with its own reproductive mode, evolve into a single Darwinian individual in which the group itself becomes the unit of selection?

This model, inspired by volvocine algae—the same lineage that motivated my work—compared the cell cycle of unicellular Chlamydomonas to simple multicellular relatives like Tetrabaena socialis and Gonium. It proposed a mathematical framework showing how group reproduction could evolve through the reordering of unicellular cell-cycle stages.

What struck me most was how directly this model spoke to my own core design challenge. It wasn't just offering a framework for cell-cycle evolution; it also temptingly pointed toward an answer to my deeper theoretical question:

> *How could the evolution of the cell cycle itself drive a shift in individuality—reorganizing fitness from the level of individual cells to the level of the group?*

That link between evolving life-history traits and transitions in individuality resonated strongly with the goals of my project.

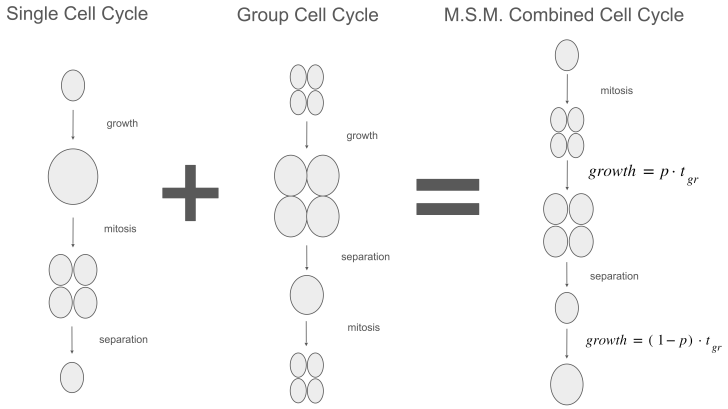


Figure 6.1

6.2.2 Evolution of the Cell Cycle in the Maliet, Shelton & Michod (2015) Model

6.2.2.1 Key Insight: a Multicellular Life Cycle as a Reordered Unicellular Cycle

The life cycle of unicellular algae follows a simple pattern:

1. A cell grows as an individual until it reaches its target size.
2. The cell undergoes mitosis and immediately separates from its offspring.
3. The new daughter cells repeat the cycle as independent individuals.

In multicellular volvocine algae, by contrast:

1. Cells remain attached to their parent group for most of their lives.
2. They detach only after reaching their target size and dividing.
3. The cycle repeats, with most of the life cycle spent in an attached state.

6.2.2.2 Two Evolvable Parameters: Growth Time (t_{gr}) & Grouping Propensity (p)

Maliet et al. formalized this transformation by defining two key parameters that determine whether cells evolve a unicellular or multicellular life cycle:

- $t_{gr} \rightarrow$ The time a cell spends growing before mitosis.
- $p \rightarrow$ The proportion of the cell cycle spent attached to offspring.

Using these parameters, it described the life cycle as follows:

- Time spent as an individual $\rightarrow (1p) \cdot t_{gr}$
- Time spent in a group $\rightarrow p \cdot t_{gr}$

This formulation allows a mathematical description of the evolution of cell cycles and incorporates it into equations that define fitness. It optimizes the fitness function and uses the results to make predictions about which conditions trigger unicellular cycles to evolve into multicellular ones.

6.2.3 Evolutionary Dynamics & Predictions from Maliet et al. Model

6.2.3.1 Selective Pressures Favor Either $p = 0$ (Unicellularity) or $p = 1$ (Fully Grouped)

Maliet et al. modeled how p and t_{gr} evolve over time by optimizing their fitness equation. They found that:

- If both p and t_{gr} were allowed to change over-time (mathematical evolution), the system has only two equilibrium states:
  - $p = 0 \rightarrow$ The unicellular cell cycle.
  - $p = 1 \rightarrow$ A fully grouped cell cycle.
- They found the equilibrium state for $p = 0$ was unstable, whereas the equilibrium state for $p = 1$ was stable. No intermediate values of p were found to be equilibrium states. Even weak selection favoring group formation is enough to drive the population to fully grouped cell cycle.

6.2.3.2 Growth Follows a Power-Law Model

Maliet et al. also assumed a power-law growth model, where:

$$\frac{\partial V}{\partial t} = kV^b$$

where:

- V is the cell's volume.
- b and k are constants.

They chose this power-law growth model because it captured slowed growth as Chlamydomonas cells get larger.

During reproduction, a series of n rapid divisions occur, producing 2^n daughter cells. The larger the parental cell , the more offspring it produces, meaning fecundity (how many offspring a cell has) is directly tied to time spent growing (t_{gr}).

6.2.4 Inspiration for my Model

Maliet et al.'s fitness equations, assumptions, and results will be explored further in the Literature Review section of this report.

Many of Maliet et al.'s assumptions about fitness maximization and reproduction-survival trade-offs were not incorporated into my model due to significant conceptual differences, particularly regarding how fitness is conceptualized and calculated. A key part of this project involves unpacking these differences, which I will explore in detail in the Literature Review and return to in my Discussion when comparing my results to their predictions.

While I do not adopt the Maliet et al. model's fitness framework, its core mathematical abstraction of cell cycle evolution provides a valuable foundation for encoding evolvable cell-cycle traits in my model.

This framework allowed me to design agents with evolvable traits capable of driving the

emergence of multicellular cell cycles, while integrating biological realism and emergent fitness — something Maliet et al.'s model did not explicitly consider.

Although the Maliet et al. model provides a foundation, this distinction is critical because my approach relies on emergent fitness arising from agent interactions within a dynamic environment, rather than mathematical fitness maximization. As I will discuss, this divergence has important implications for interpreting my results, especially regarding the reasons certain predicted shifts in individuality may not occur in my model despite theoretical expectations.

6.2.4.1 Framing the Expectation for a t_{gr} Shift and its Link to Individuality

With this foundation in place, I now turn to one of the key theoretical predictions from Maliet et al.'s model that shaped my initial expectations: the link between evolving cell cycle traits — specifically t_{gr} — and a potential shift in individuality due to the transition to group reproduction.

In the Maliet et al. model, the co-evolution of two traits—p, the proportion of the life cycle offspring spend growing together in a group, and t_{gr}, the growth time—lead to two stable evolutionary strategies: a unicellular life cycle ($p = 0$) or a fully grouped multicellular life cycle ($p = 1$). The model demonstrates that if the benefits of multicellularity (increased survival) outweigh the costs (reduced growth), natural selection drives p toward 1, evolving a group-based reproductive cycle.

A central result of the model is that if t_{gr} is a key factor influencing cell fitness, it cannot remain fixed at its unicellular optimum once cells begin reproducing as a group. This is because group living changes the balance between growth and survival, altering the fitness landscape. As p increases, fecundity and survival are affected differently due to group-specific costs and benefits. Optimizing fitness in the new context requires a corresponding shift in t_{gr}.

Maliet et al. explicitly frame this required shift in t_{gr} as a hallmark of an evolutionary transition in individuality (ETI). Their argument follows Maynard Smith and Szathmáry's (1995) [3] definition of ETIs, in which entities capable of independent reproduction before the transition can no longer replicate effectively outside the new collective:

> *Changes in a life-history trait, t_{gr}, can be detrimental to the functionality that cells would have on their own, were they to leave the group. Groups of cells that evolve away from the unicellular optimal value of t_{gr} are no longer merely spatio-temporal collections of entities with the full capacity to function on their own. These cells now require the group context, the larger whole, to reproduce most effectively" [1]*

6.2.4.2 Reflecting on the Maliet, et al Model:Interpreting the Link to Individuality

My interpretation of their results is that if t_{gr} is crucial to cell fitness because it balances growth (fecundity) and survival in Chladomynomous cells:

The model predicts that if both t_{gr} and p are free to co-evolve, and if the benefits of multicellularity outweigh the costs, then cells will evolve fully grouped life cycles (p shifting to 1) and t_{gr} will shift away from the unicellular optimum.

Once this happens, it marks a shift in individuality: cells are no longer evolving solely to maximize their own fitness, but the fitness of the group. Because t_{gr} is crucial to fitness, evolving away from the unicellular optimum would leave a cell worse off if it were to leave the group. This dynamic incentivizes continued investment in the integrity and cohesion of the group, reinforcing a new group-level identity.

6.3 Conclusion

The major elements of Maliet et al. that inspired my model design are:

1. The essential components of a Darwinian individual capable of evolution as defined by what is known as essential to Chladomynomonous.
2. The evolution of the cell cycle mathematical model.
3. The idea of genetic co-option of traits being integral to multicellular evolution as described in the background section on volvocine Algae

7

Model Design: Justification, Approach, and Core Components

7.1 Advantages of an Agent-Based Model

Modeling the evolutionary transition from unicellularity to multicellularity requires tracking individual-level behaviors to their emergent group-level consequences. An agent-based model (ABM) was chosen because:

- It allows selection to act on individual variation, enabling natural evolutionary processes to shape behavior.
- It captures emergent group dynamics, such as shading competition and spatial constraints, that are difficult to model at the individual level.
- It enables flexible simulation of genetic inheritance represented by a static neural network (NN) proxy for a genetic regulatory network (GRN).

7.2 Balancing Complexity and Simplicity in Evolutionary Models

A well-designed model includes only the elements necessary to capture the fundamental dynamics of the subject system without introducing unnecessary complexity. This requires making deliberate choices about what to include and what to simplify, ensuring that the model remains both computationally feasible and conceptually meaningful.

Although I drew inspiration from volvocine algae and modeled my agents after Chlamydomonas, the goal of this model is not to precisely replicate the biology of any one species. I strove to simulate an evolutionary transition in individuality (ETI) in a generalized way, specifically focusing on the evolution of multicellularity.

The criterion for including biological details of Chlamydomonas in my model was based on their relevance to ETI theory and the broader principles of the evolution of multicellularity. However, Chlamydomonas and its relatives elegantly embody many aspects of this theory—far more than I could have designed from scratch. Their evolutionary trajectory provides a natural framework for testing ideas about how multicellular life evolves.

7.2.1 Agent-Based Modeling Is Unique

ABM is uniquely suited to this kind of research. Biological systems are highly complex, making it difficult to isolate the essential elements that drive an evolutionary process. The ABM allows us to systematically separate necessary dynamics from incidental biological details, making it one of the few approaches that can directly test evolutionary theories in a controlled, mechanistic way.

7.3 Core Model Components

7.3.1 Darwinian Individual Capable of Evolution

To qualify as Darwinian individuals capable of evolution these agents must satisfy the following criteria:

1. **Identifiable as individuals:** Each cell is distinct with defined boundaries and interactions.
2. **Differential fitness and competition:** Cells exhibit variation in survival and reproductive success, leading to differential persistence and spread of genetic traits in the population. Selection pressures act on this variation, favoring genetic codes that enhance fitness while eliminating less advantageous versions.
3. **Reproduction, inheritance, and variation:** Cells inherit traits, reproduce, and exhibit variation in fitness that selection can act upon.
4. **Genetic encoding with controlled mutability:** The inheritance system must permit gradual adaptation without excessive randomness.

7.3.2 Translating the Cell Cycle into an Evolvable Trait

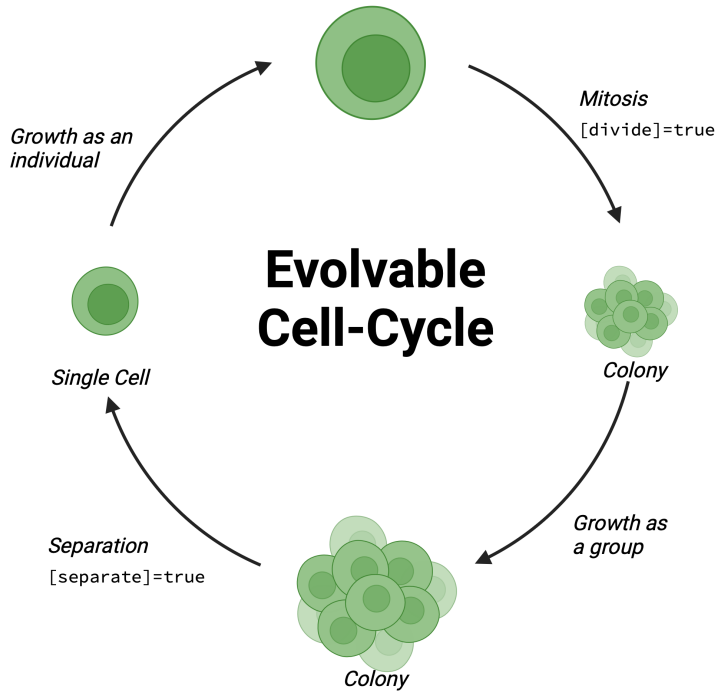


Figure 7.1

The Maliet et al. mathematical model that inspired this work describes the evolutionary transition from a unicellular to a group-cell cycle in terms of two key population-level parameters:

- t_{gr}: The length of the growth phase (how long a cell grows before division).
- p: The proportion of the growth phase a cell spends attached.

These values are defined on the population level.

These parameters are not explicitly pre-programmed at the population level in my model. They emerge as a *consequence* of individual-level behavioral decisions:

Cells dynamically decide:

- when to stop growing and start division? → `[divide] = true`
- when to detach from siblings? → `[separate] = true`

This approach allows the cell cycle to evolve dynamically under selection pressures, rather than being fixed in advance. The parameters t_{gr} and p are then measured post-hoc to assess how selection shapes division and attachment behaviors over evolutionary time.

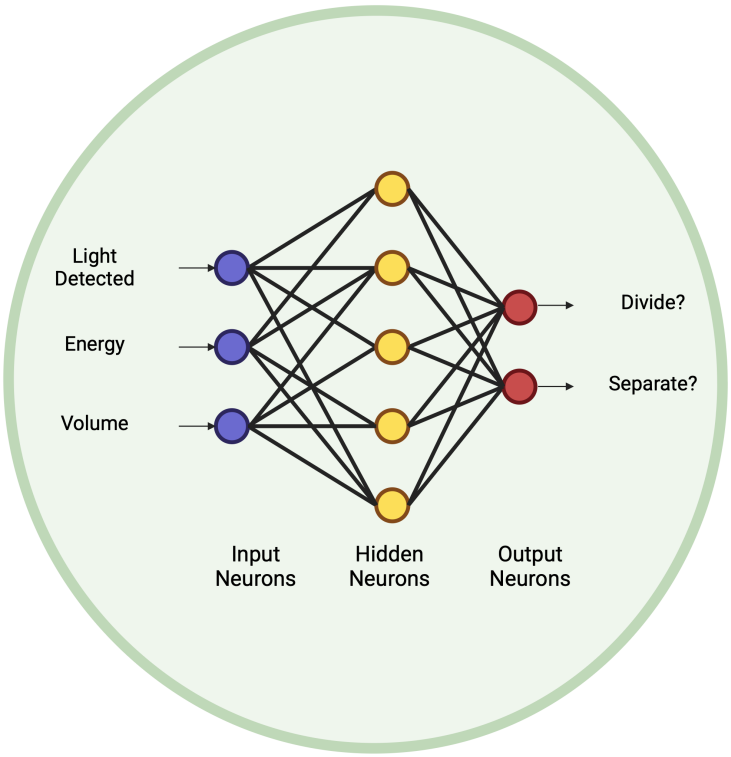


Figure 7.2

7.3.3 Genetic Regulatory Network (GRN) Proxy

Rather than pre-programming behaviors like "*If a cell has Gene X, it does Y under Condition Z,*" this model uses a static neural network (NN) as a GRN proxy to regulate cellular decision-making.

- Maps environmental inputs to behavioral outputs
- NN weights are inherited with mutations at division

Primary outputs:

- `divide`: Triggers transition to reproduction mode from growth mode
- `separate`: Determines post-division detachment

This setup enables genetic co-option, similar to how gene networks are thought to have played a crucial role in the evolution of multicellularity.

7.3.4 Bimodal Cell Cycle: Growth and Division Phases

Inspired by the bimodal phase observed in Chladomynomous, agent cells transition between two primary states, regulated by their genetic network proxy (GRN):

- **Growth Phase** (`divide = false`): Motile, absorbs light, stores energy, grows in volume.
- **Reproductive Phase** (`divide = true`): Cells become immotile, stop absorbing light energy or growing, and prepare for division following a multiple fission strategy similar to Chlamydomonas.

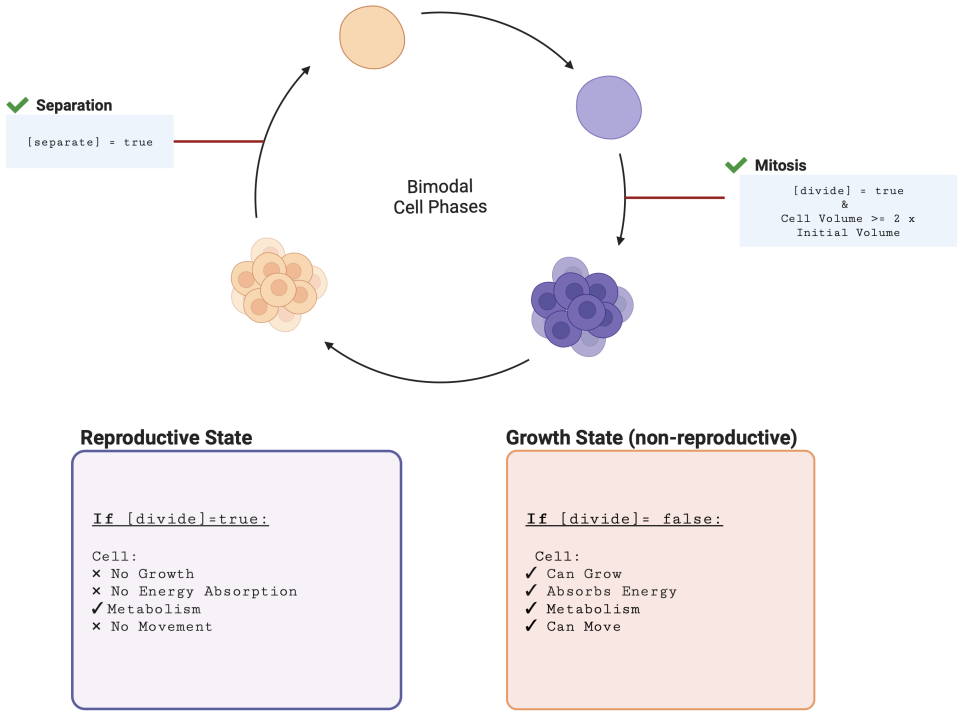


Figure 7.3: Schematic of the bimodal phase cycle of agent cells

7.3.5 Selection Pressures

7.3.5.1 Competition for Resources

Cells compete for light and space, shaping selection pressures on their positioning, attachment behavior, and division timing.

- Competition for light introduces shading effects, so that cells that are attached and tightly packed enough to pull sunlight from the same location compete for light.
- Spatial constraints limit the number of cells that can access peak light intensity.

7.3.5.2 Periodic Selection Pressures

In real photosynthetic organisms, fluctuations in light availability—such as day/night cycles—create selection pressures on energy acquisition strategies and division timing.

- The model captures this effect by implementing a periodic light cycle, where the light gradient is present for 600 minutes ("day") and absent for 600 minutes ("night").
- This places a strong selective pressure on cells to evolve energy acquisition strategies that ensure survival through light-limited periods and to coordinate division timing accordingly.

7.3.5.3 Predator Selection Pressures

To drive the evolutionary transition from a unicellular to a group-cell cycle, the model introduces time-dependent selection pressures in the form of increased death probabilities for either attached or unattached cells at different simulation phases:

- **Early Phase (0–180,000 min):** Selection Against Attached Cells
  - An extra death probability is applied to attached cells, favoring single-cell survival and reproduction.
  - This selects for a unicellular reproductive cycle.
- **Later Phase (180,000–360,000 min):** Selection Against Unattached Cells
  - The selective pressure is reversed, increasing the probability of unattached cell death.
  - This favors the persistence of cell attachments, favoring the evolution of a group-cell cycle.

This staged selection process allows the cell cycle to evolve from unicellular to multicellular, demonstrating how environmental pressures shape the emergence of group-level traits over evolutionary time.

7.4 Measuring Evolutionary Outcomes

A core challenge of any evolutionary simulation is determining how to measure evolutionary change and evaluate fitness at the population level. The challenge is multiplied in simulations involving thousands of autonomous, evolving agents. Agent-based models like mine offer a powerful advantage over mathematical models: they allow for biologically realistic simulations in which individual cells mutate, grow, reproduce, and compete over many generations. However, this complexity requires the analysis of vast amounts of data to extract meaningful evolutionary outcomes.

Unlike mathematical models which often define fitness explicitly with elegant equations and solvable functions, agent-based models allow fitness to emerge through interactions among individual cells, their environment, and the consequences of survival and reproduction over time. This generates large, messy datasets. I can track individual cell values for various parameters (like p, t_{gr}) and other traits. But simply knowing these values at every time step is not enough to understand evolution.

A strength of agent based modeling is the ability to capture a recursive, emergent, lineage-based view of fitness, but by doing so presents a challenging amount of data to evaluate for evolutionary success. There is no single function I can optimize, only patterns I can observe.

Therefore measuring evolution in my system requires identifying traits that persist, spread, or become dominant over generations, indicating that selection is acting upon them. Merely observing variation is not enough because random mutations alone generate variation. The goal is detection of non-random patterns that link environmental conditions to consistent evolutionary strategies.

Convergence as a Signal of Adaptation .The clearest signal of adaptation is convergence, when populations consistently evolve toward particular trait values under specific environmental pressures. If similar conditions lead to widely divergent strategies it can be attributed to genetic drift. But consistent evolution of similar strategies under the same

conditions indicates selection is shaping evolutionary outcomes.

To detect selective evolutionary transitions, I analyze the population-level distributions of key evolvable traits—specifically t_{gr} and p—using probability density distributions (PDDs) to determine how this distribution evolves over time. PDDs allow me to visualize not only average trait values, but also the extent and shape of variation within the population. By determining if trait distributions narrow (indicating convergence) or remain broad (indicating weak selection), I can detect meaningful adaptation.

In this way, my measurement method aligns with the recursive nature of fitness. Rather than looking at survival or reproduction in isolation, I evaluate whether certain traits reliably promote long-term success—the ability to survive, reproduce, and dominate across generations—under the selection pressures imposed in the model.

8

Model Description

8.1 Overview

This agent-based model simulates the evolutionary transition from a unicellular to a multicellular life cycle, inspired by the volvocine algae lineage. The model represents individual cells competing for light resources, reproducing through asexual division, and evolving under mutation-driven selection pressures. The primary goal in simulating the evolution of a multicellular cycle is to explore how shifts in individuality emerge in a biologically realistic system.

Cells in the model follow a bimodal life cycle, alternating between growth and division states based on environmental cues and internal energy levels. Evolution occurs as natural selection acts on variation in division timing, attachment behavior, and energy acquisition strategies. Over time, selection pressures shape the transition from a unicellular to a group-based reproductive cycle, mirroring early steps in the evolution of multicellularity that occurred in the volvocine algae lineage.

For full model parameters and implementation details, refer to the ODD document.

8.2 Model Structure

8.2.1 Agents as Individual Cells

Cells are represented as individual agents in a continuous 2D space with a small z-component, allowing for limited overlap. Each cell has:

- defined physical boundaries and volume, influencing movement and interaction
- motility enabling navigation of light gradients to optimize energy intake
- an internal genetic regulatory network (GRN) proxy, determining behavioral outputs based on environmental inputs

8.2.2 Cell Metabolism, Growth, and Energy Constraints

Cells in the model have the following attributes::

- Cells are photosynthetic, relying on light exposure to obtain the energy required for survival and growth.
- Each cell absorbs light from its local environment.
- Sunlight absorbed also translates to volume growth according to a power-law.

- Energy depletion occurs due to baseline metabolic costs, regardless of cell movement or state. Energy is continuously depleted through metabolism at a fixed rate.
- Cells grow in volume according to a power-law relationship with absorbed sunlight.
- If a cell's stored energy drops below a threshold, it dies.
- Cells have a maximum growth limit—if a cell exceeds 30 times its initial volume, it dies.

8.2.2.1 Energy Update Equation

The energy update follows these steps:

1. Absorb sunlight (if above the absorption threshold).
2. Store sunlight absorbed as energy.
3. Apply metabolic cost to simulate energy consumption.

8.2.2.1.1 Equation Formulation:

Let

- E_t = Energy at time step t
- S_t = Sunlight available at time t
- S_{min} = Absorbable sunlight threshold
- S_{abs} = Sunlight absorbed
- T_{abs} = Cell's absorption threshold
- R_{met} = Metabolic rate
- Δt = Time step

8.2.2.1.2 Energy Absorption:

$$S_{abs} = \begin{cases} min(S_t - S_{min},\ T_{abs}), & \text{if } S_t > S_{min} \\ 0, & \text{otherwise} \end{cases}$$

8.2.2.1.3 Energy Update:

$$E_{t+1} = \frac{E_t + S_{abs}}{1 + R_{met} \cdot \Delta t}$$

8.2.2.2 Growth

Similar to *Chlamydomonas reinhardtii*, the number of offspring produced during mitosis is a function of how much larger the organism becomes compared to its initial size during the growth phase. Growth depends on photosynthesis during this phase.

8.2.2.3 Volume Update Equation

Volume growth follows a power-law model:

- Growth rate scales with absorbed sunlight.
- General form:

$$\frac{dV}{dt} = k \cdot V^b$$

8.2.2.3.1 Equation Formulation: Let:

- V_t = Cell volume at time t
- k is the growth rate
- $b = \frac{3}{4}$, the growth exponent
- $k_0 = 10$ Base growth rate constant
- S_{abs} = Sunlight absorbed

8.2.2.3.2 Discrete Volume Update:

$$k = k_0 S_{abs}$$

$$V_{t+1} = V_t + k \cdot V_t^b \cdot \Delta t$$

8.2.3 Genetic Encoding, Evolution, and Inheritance

Each cell contains a static neural network (NN) that serves as a genetic regulatory network (GRN) proxy, capturing the evolutionary dynamics of gene co-option during the transition to multicellularity.

Rather than pre-programmed behavior rules, the NN maps environmental and internal state inputs (light level, energy, volume, etc.) to behavioral outputs (division timing, attachment behavior, motility speed, etc.), allowing for an abstract, evolvable genetic system.

8.2.3.1 Inheritance, Mutation, and Selection

- **Inheritance**: A cell's neural network weights encode its genetic information and are inherited by daughter cells at division.
- **Mutation**: Before each mitosis event begins, the NN weight of parent cells mutate, allowing natural selection to shape cell behavior over generations and introducing heritable variation into the lineage. All daughter cells produced in a reproductive phase share the same mutated genotype, preserving clonal identity within the group. (See ODD for mutation rates and implementation details.)
- **Selection**: Natural selection acts on this variation because differences in neural network outputs influence key fitness-related behaviors: division timing, attachment, motility, and energy acquisition. This mechanism enables gradual evolutionary change in behavioral strategies and allows for the genetic co-option of new traits, driving the emergence of multicellular life cycles.

8.2.3.2 Reproductive Investment and Energy Partitioning

When cells divide, daughter cells inherit half the parent's stored energy and volume. While cells can only initiate division when they reach at least twice their original volume, there is no energy threshold per offspring. Energy is conserved across divisions and evenly partitioned, meaning that more rounds of division reduce the initial energy available to each daughter cell.

This trade-off embeds reproductive cost directly into the life cycle and allows natural selection to shape strategies for growth, reproduction, and survival over evolutionary time. Ultimately, this framework enables the genetic co-option of new traits and supports the evolution of multicellular life cycles.

During division:

- Daughter cells inherit half of the parent's stored energy and volume.
- Division is possible only after the cell doubles its initial volume, but energy has no per-offspring threshold.
- Energy is conserved and partitioned evenly, embedding reproductive costs into the life cycle.

8.2.3.3 Neural Network Inputs and Outputs

Inputs:

- Sunlight level (energy availability)
- Cell volume (relative growth state)
- Stored energy (internal reserves)
- Time since last division (cell age)
- Attachment state (attached/detached binary)

Outputs:

- **Divide** (Boolean): When to stop growing and enter mitosis
- **Separate** (Boolean): When to detach from siblings post-division
- **Speed**: Motility speed adjustment
- **Absorb threshold**: Regulates maximum sunlight absorption, affecting shading dynamics

8.3 Reproduction & Development of Multicellularity

8.3.1 Bimodal Cell Cycle: Growth & Division Phases

In this model, each cell follows a bimodal cell cycle, alternating between two distinct states, as controlled by their GRN proxy (NN) outputs.

8.3.1.1 Growth Phase (divide = false)

- Cells absorb light, store energy, grow in volume, and are motile.
- Volume growth follows a power-law relationship with light absorption.
- Cells' motile ability translates to ability to move towards optimal light conditions via phototaxis.

8.3.1.2 Reproductive Phase (divide = true)

- Cells become immotile and stop growing or absorbing energy from light, but maintain metabolic activity to prepare for division.

- If a cell has reached a critical volume, it enters the division process, which mirrors the multiple fission strategy observed in *Chlamydomonas*:
- A cell does not divide once per cycle but instead divides sequentially, based on how many times its volume exceeds its initial size. This follows the rule:

$$N = 2^n$$

N is the total number of daughter cells produced, and **n** is the number of division rounds.

Division process:

1. All previous attachments are removed, detaching the parent from any clonal clusters.
2. Genetic material mutates once before division begins (see Section 2.3).
3. The cell divides into two daughters, each inheriting half of the parent's energy, volume, and mutated genetic code, including the value for the original *initial_volume*.
4. If the volume of a daughter cell still exceeds twice its *initial_volume*, it immediately undergoes another division, repeating until each daughter is less than 2 times its *initial_volume*.
5. After division, daughter cells remain attached, unless the GRN triggers separation via the `separate = true` output.

Key Features of the Reproductive Phase

- **Clonal Clustering**: All daughter cells are genetically identical clones (post-mutation) and remain attached unless separation is triggered.
- **Energy Partitioning**: Although cells need at least 2 times `initial_volume` in order to actually divide, energy has no such threshold. Cells can divide with any amount of energy. However, energy is evenly divided at each split, meaning additional rounds of division reduce the initial energy available to each daughter cell, embedding energetic trade-offs into the reproductive strategy.

8.4 Environment

8.4.1 Light as a Resource

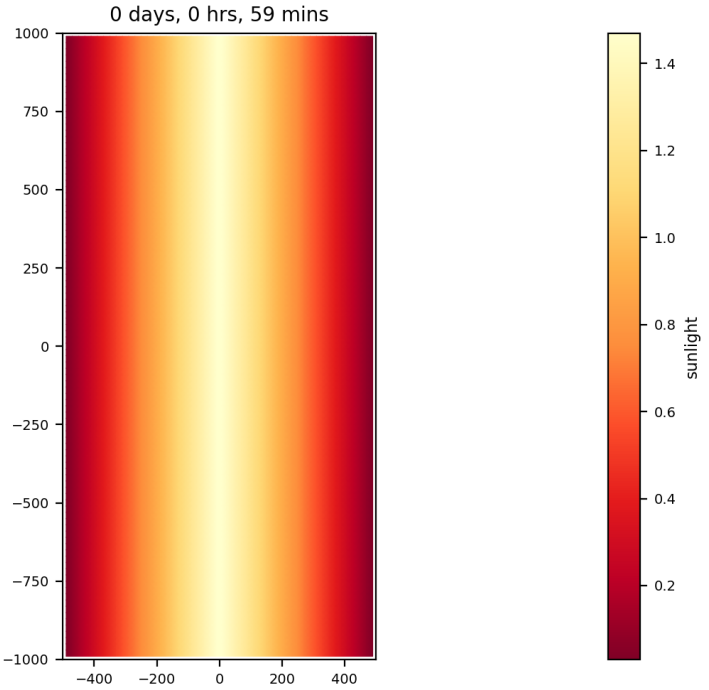


Figure 8.1: The linear light gradient experienced by a cell when its "day" for 600 mins, visualization in PhysiCell Studio. The linear gradient in the environment peaks at $x = 0$, which is visualized as white/yellow in the center (highest) to red on the vertical edges (lowest). The key for the colors to light value is the vertical legend that is titled 'sunlight' and shows values from 0.0 to 1.5.

Light is modeled as a spatial gradient in the cell microenvironment, represented as a 2D grid where each voxel contains a fixed amount of light. Unlike traditional nutrient-based resources, the amount of light available on voxels is not permanently depleted when absorbed. Instead it is momentarily depleted before being restored at the next diffusion update step, due to Dirichlet boundary conditions (see ODD for time step and Dirichlet details).

Perceived Light Availability Depends on Population Density:

- The light gradient is linear, peaking at the center of the environment.
- Because cells absorb light during phenotypes [which are less frequent than diffusion time steps], local depletion occurs, but is only registered by cells if multiple cells try to absorb light from the same voxel at the same phenotype update step. This introduces short-term, local, density-dependent competition between cells, where cells will register a reduction in light on the voxel they are located.

- Attachment intensifies shading: attached cells pack more tightly than free cells, amplifying shading effects.

This mechanism creates a direct link between cell positioning, density, attachment, and access to energy, generating biologically realistic trade-offs that shape evolutionary strategies.

8.4.2 Day/Night Cycles

A major environmental factor regulating *Chlamydomonas* cell cycles is the day/night cycle, which determines when photosynthetic growth is possible. To capture this periodic selection pressure, the model implements a periodic light cycle:

- **Day Phase (600 min ON)**: The linear light gradient is present, allowing cells to absorb light and store energy for growth.
- **Night Phase (600 min OFF)**: The gradient disappears, preventing light absorption. Cells must rely on stored energy for survival.

This cycle repeats continuously throughout the simulation, introducing a strong selection pressure on cells to evolve strategies for energy acquisition, division timing, and clustering behaviors that maximize survival across light-limited periods.

8.5 Selection Pressures and Competition

8.5.1 Predator-Driven Selection Pressures

To simulate an evolutionary transition from a unicellular to a multicellular cycle, the model applies time-dependent selection pressures that favor different cell cycle strategies at different stages of the simulation.

Early Phase (0 – 180,000 min): Selection Against Attached Cells

During this phase, cells that remain attached after division experience an increased probability of death. This simulates predator-driven selection favoring single cells, forcing the population to evolve a unicellular reproductive cycle.

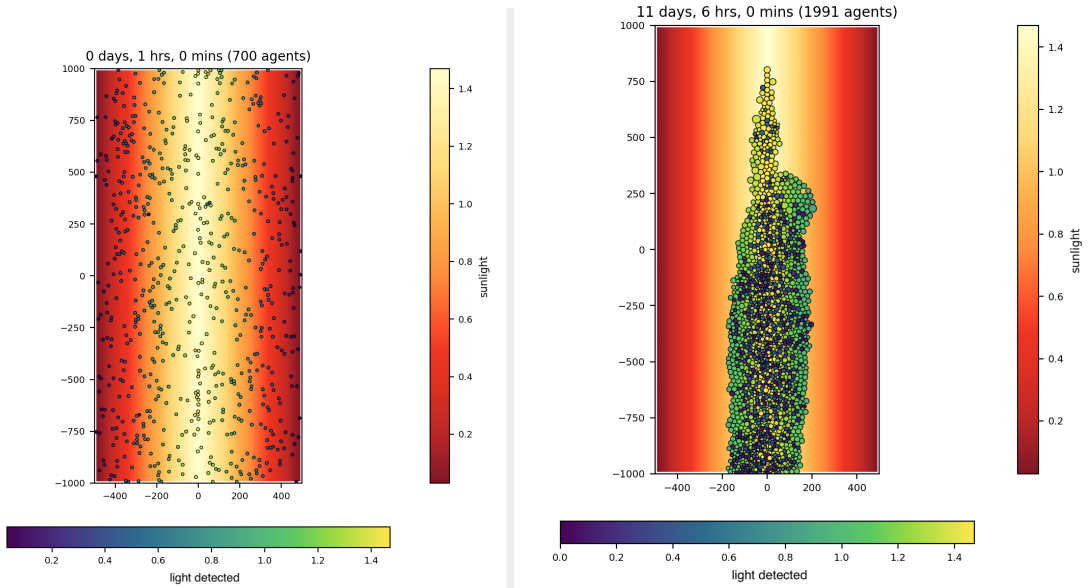
Later Phase (180,000 – 360,000 min): Selection Against Unattached Cells

After the initial selection pressure is lifted, a new pressure is applied, wherein unattached cells have a higher probability of death. This mimics a shift in selection pressure favoring multicellular group formation, allowing the evolution of a group-based cell cycle. This two-phase structure ensures that the model captures an evolutionary trajectory, transitioning from unicellular reproduction to a group-cell cycle, where multicellular organization becomes selectively advantageous.

8.5.2 Competition for Resources

8.5.2.1 Shading as a Competitive Constraint

Light availability is the primary limiting resource. Because light is momentarily depleted, shading effects introduce competition and evolutionary trade-offs:



(a) When there is low density, cells experience the light amount that corresponds to the value of the gradient.

(b) Shading effect: when there is high density, cells often experience less than the amount of light dictated by the gradient.

Figure 8.2: Shows cells in simulation and the shading effect experienced by cells. The gradient in the environment, which is white/yellow in the center to red on the vertical edges. The key for the colors to light value is the vertical legend that is titled 'sunlight' and shows values from 0.0 to 1.5. The cells are colored according to the horizontal key titled 'light detected', which ranges in values from 0.0 to 1.5 and are the colors purple to yellow. (a) A snapshot of when cells are spread apart, showing that when and where cells have low density, they experience the light amount that corresponds to the value of the gradient. (b) Shows a snapshot of when cells are in single cell cycles, in the first 180k minutes of the cell cycle, when cell density is high. You can see that where cell density is increased, individual cells experience less light than the value of the gradient would dictate according to their position.

- Cells absorb light at discrete time steps (each phenotype update), meaning momentary reductions in light availability create local competition.
- Shading is stronger when cells are attached. Because cells closely packed together share the same light-depleting region, attached cells experience a trade-off:
  - Attachment may provide survival benefits (e.g., avoiding extra death probability).
  - Attachment reduces individual access to light by increasing competition, reducing energy stored and rates of growth, which influences reproduction and survival.
- Shading effects scale with population density. As attachment behaviors evolve and the population grows, shading effects become more pronounced, affecting both growth rates and the evolution of division strategies.
- Attachment intensifies shading: attached cells pack more tightly than unattached cells, amplifying shading effects.

8.5.2.2 Space as a Competitive Constraint

Beyond shading, spatial constraints further shape competition:

- Cells navigate toward a fixed light peak, where light intensity is highest.
- Only a limited number of cells can physically fit at the peak, due to their physical volume and mechanical constraints.
- Survival depends on light acquisition, and because the night cycle depletes energy reserves, cells must reach sufficiently high light levels before nightfall to survive.

This creates a selection pressure on motility and division timing, favoring strategies that maximize energy acquisition while avoiding predator selections.

8.6 Summary

This model was designed to capture the evolutionary transition from a unicellular to a multicellular cell cycle, simulating how individual-level behaviors, such as growth, division timing, and attachment might evolve under selection pressures arising from resource competition, spatial constraints, and predator selection.

The model allows group-cell cycles to emerge through heritable changes in a genetic regulatory network (GRN proxy), driven by mutation, inheritance, and selection. It incorporates shading dynamics, periodic day/night cycles, and time-dependent selection pressures that create trade-offs between survival, energy acquisition, and group formation.

While the model successfully simulates the evolution of a multicellular reproductive cycle, it does not capture a corresponding shift in individuality within groups. As such, it demonstrates one possible pathway for the evolution of multicellular life cycles but highlights that changes in group-level individuality may require additional mechanisms or selective pressures beyond those included here.

(For technical implementation, parameter values, and full model structure, see the supplementary ODD document.)

9

Methods

9.1 Simulation and Modeling Software Platform

Computational limits were overcome by using a high performance, tissue modeling, agent-based modeling platform called PhysiCell.

9.1.1 PhysiCell Framework

The model was implemented using the PhysiCell agent-based simulation framework [39], version 1.14.2, with interactive development, exploration, and visualization performed in PhysiCell Studio [40]. PhysiCell is an open-source framework optimized for large-scale simulations capable of modeling up to up to 10^5 or more cells in 2D or 3D, on quad-core desktop workstations using OpenMP parallelization, its performance scaling linearly with the number of cells.

As summarized by [41],

> "PhysiCell is an open-source framework that allows the development of multicellular models at various spatial/temporal scales. In this tool, the cells are represented by off-lattice agents with independent behaviors, including cell cycle progression, death processes, volume changes, and motility. Some of these dynamics may be defined by substrate availability in the environment, which is represented in PhysiCell using an open-source biological diffusion solver, BioFVM [42]. PhysiCell has been used in a wide variety of multicellular problems, such as virus therapy, cancer immunology, tissue mechanics, and drug screening, among others [43, 44, 45, 46, 47]."

PhysiCell represents cells as center-based, off-lattice agents with defined interaction rules. The microenvironment, in contrast, is discretized on a grid and simulated using the open-source biological diffusion solver, BioFVM [42]. PhysiCell has been widely applied in studies of virus therapy, cancer immunology, tissue mechanics, and drug screening. [43] [44] [45] [41] [47] [46]

Additional details on PhysiCell can be found through PhysiCell documentation such as [39].

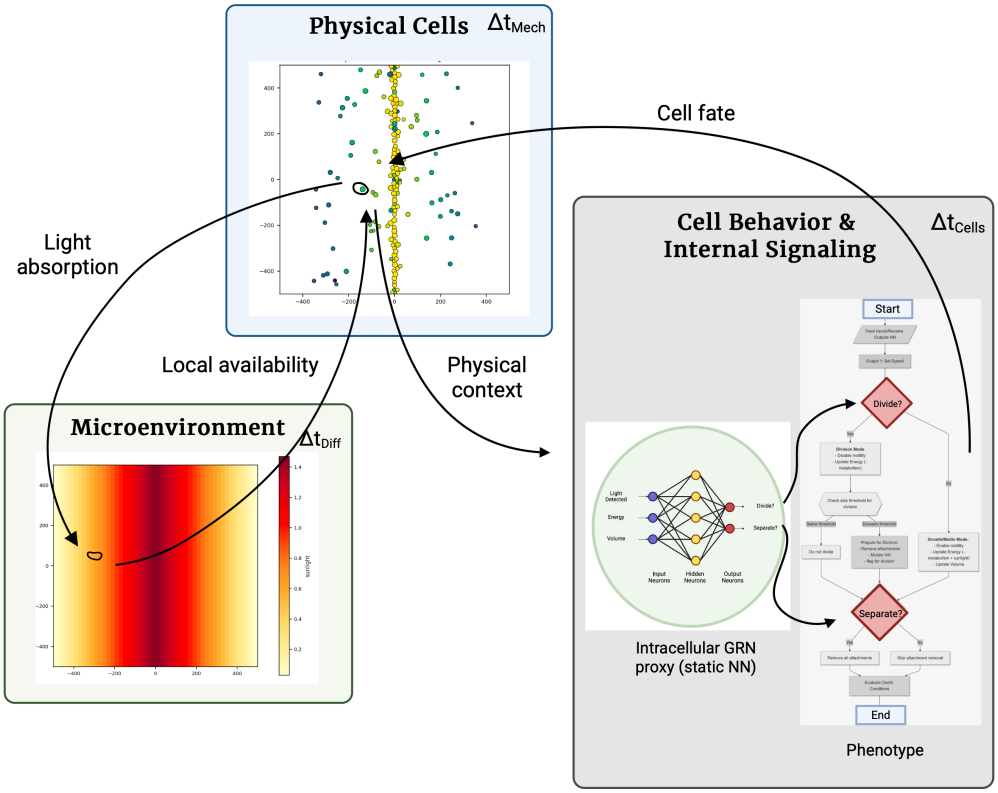


Figure 9.1: Schematic representation of PhysiCell's three main interconnected parts in my model. The three parts are: the microenvironment representation in BioFVM (green, bottom left), which models the sunlight gradient and enforces Dirichlet boundary conditions; the physical representation of cells as dynamic spheres in PhysiCell (blue, top); and the internal signaling and behavior module (grey, bottom right), which includes a GRN proxy modeled as a static neural network. This proxy governs only a subset of cellular decisions (e.g., division, separation, motility), while other processes (e.g., growth, attachment dynamics, and death) follow predefined code-level protocols. This figure is conceptually inspired by the schematic of PhysiBoSS published in [2].

Full implementation details, including model architecture, simulation parameters, and the ODD protocol, are provided in the Supplementary Materials.

10

Results

10.1 Prolog

In this simulation selection pressure shifted from favoring unattached single cells to favoring attached groups at 180,000 time steps (minutes).

The model's primary goal is to observe the evolutionary transition from a single-cell to a multicellar cycle and to determine whether this transition corresponds to a shift in t_{gr} (length of growth phase) away from the unicellular optimum, signifying a shift in individuality.

Based on the conclusions of Maliet et al. (2015), I expected the following patterns in the probability distributions of p (the proportion of time a cell spends attached during its growth phase):

1. Before the shift (first half of the simulation, when selection favors single cells): a strong peak at $p \approx 0$, indicating convergence toward a unicellular life cycle.
2. After the shift (post-180,000 time steps, when selection favors attached cells): a new peak at $p \approx 1$, signaling evolution toward a fully grouped, multicellular life cycle.

For t_{gr} (growth phase duration), I expected:

1. Before the shift: the population would converge on a clear unicellular optimum value of t_{gr}.
2. After the shift: if multicellularity evolved, we might observe a corresponding shift away from this unicellular optimum.

Before running the simulation, I had no clear prediction about the direction of any potential shift in t_{gr}—whether it would increase, decrease, or change at all.

10.2 t_{gr} and p Probability Densities

Figure **??** presents the probability density distributions of p and t_{gr} over time.

As expected, during the first phase of the simulation, the distribution of p peaks sharply at $p \approx 0$, consistent with a unicellular cycle. Following the selection shift at $t = 180{,}000$, the peak shifts to $p \approx 1$, signaling the evolution of a fully grouped, multicellular cycle.

These results align with Maliet et al., who found that when t_{gr} and p are allowed to co-evolve, the system did not stabilize at intermediate values of p. Instead, evolutionary dynamics favored either fully unicellular ($p \approx 0$) or fully multicellular ($p \approx 1$) cell cycles once multicellularity provided a net benefit.

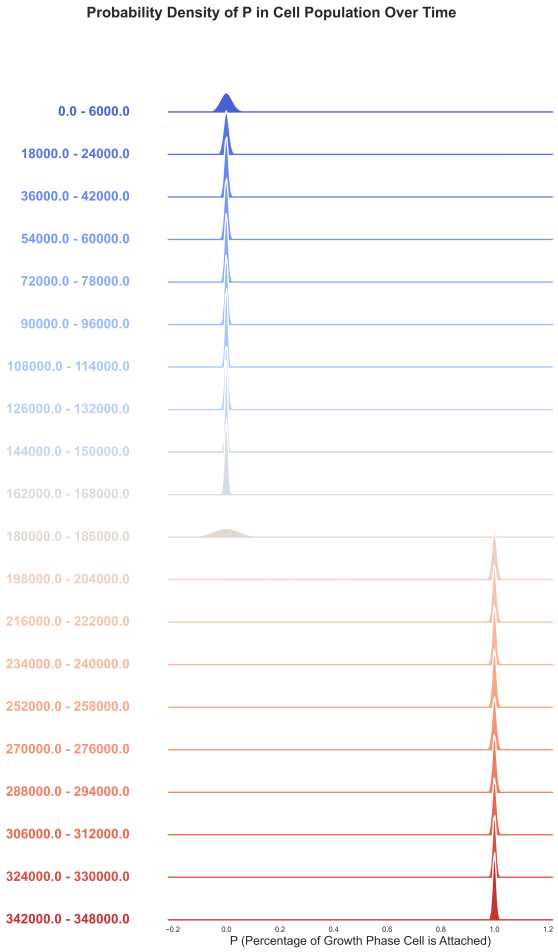


Figure 10.1: Probability density distribution of p (the percentage of a cell's cycle spent attached) over time. At $t = 180{,}000$, selection pressure switches from favoring unattached cells to favoring attached cells, leading to the evolution from a single-cell cycle ($p \to 0$) to a multicellular cycle ($p \to 1$).

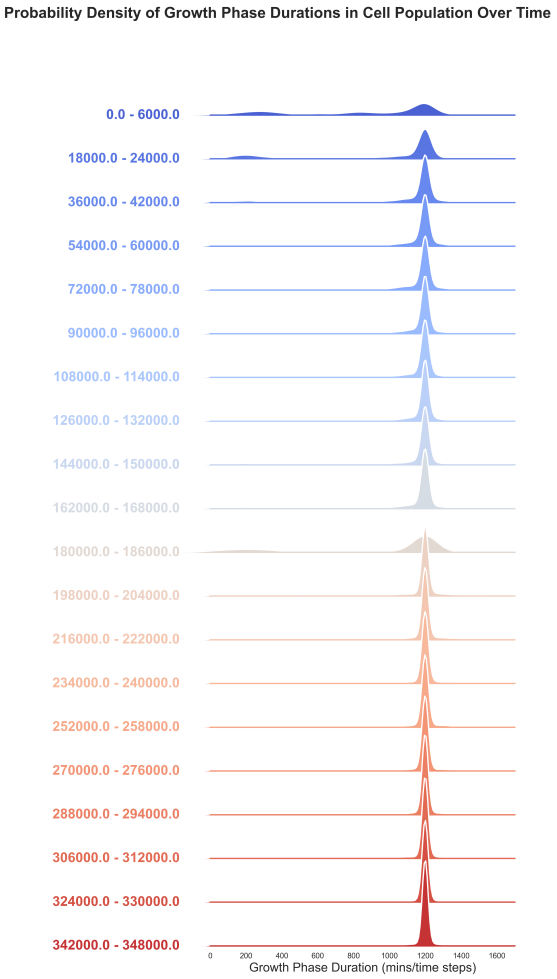


Figure 10.2: Probability density distribution of t_{gr} (growth phase duration) over time. A clear convergence occurs around $t_{gr} \to 1200$ time steps, continuing regardless of selection pressures or multicellular evolution.

Figure 10.1 presents the probability density distribution of p over time. As expected, during the first phase of the simulation, the distribution peaks sharply at $p = 0$, consistent with a unicellular cycle. Following the selection shift at $t = 180,000$, the peak shifts to $p = 1$, signaling the evolution of a fully grouped, multicellular cycle. These results align with Maliet et al., who found that when t_{gr} and p are allowed to co-evolve, the system did not go to intermediate values of p, and that evolutionary dynamics favored either fully unicellular ($p = 0$) or fully multicellular ($p = 1$) cell cycles (once multicellularity provided a net benefit).

Figure 10.2 shows the probability density of t_{gr} of the cell population throughout the simulation. There is a clear convergence to the optimum cell cycle length (growth phase length) of $t_{gr} \approx 1200$ time steps, and then a cessation of convergence as the simulation progresses despite the selection pressures or evolution of the multicellular cell cycle.

This optimal growth phase length is likely attributable to the fact that cells cannot photosynthesize when light is not present, nor when they are in their reproductive phase. Like real Chladomynomous, my agents divide at night, and prioritize their growth state when they detect light. My cells' metabolic needs are constant, and when they divide, they divide both their volume and stored energy equally into each daughter cell. Therefore the cell maximizes its offspring's viability by dividing immediately prior to (or perhaps even exactly when) the next "day" period begins. This reduces the chances that their offspring will "starve" during the night.

10.3 Population Over Time

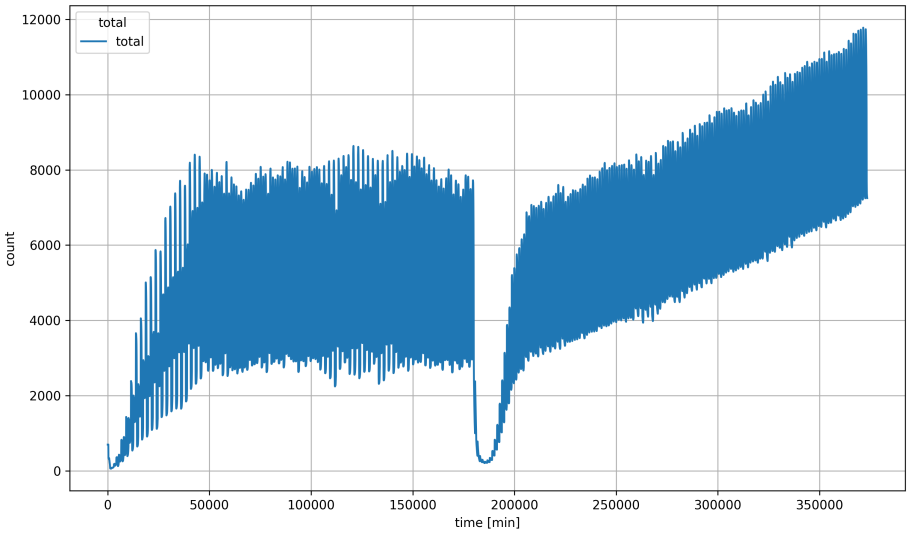


Figure 10.3: Cell count over time

Figure 10.3 shows the number of cells present in the simulation at each data save (60 min intervals), throughout the simulation.

10.3.1 Micro-trends: Day/Night Dynamics

The population exhibits boom-bust cycles every 600 minutes, corresponding to the day/night light cycle. Since the selection pressures based on energy acquisition were strict in order to motivate cells' evolution towards an optimal growth phase length, many died throughout the "night" if they did not acquire enough energy from sunlight during the "day". As discussed above, in both the real Chladomynomous cells and my agent cells, division timing becomes synchronized as surviving cells divide at roughly the same time, leading to population surges.

10.3.2 Macro-trends: Population Dynamics across the Simulation

For the first 180,000 time steps, the population steadily increases as cells evolve better energy acquisition strategies and division timing. At about 50,000 time steps, the popu-

lation appears to reach a carrying capacity: the bust cycle minimum stabilizes at around 3,000 cells, and the boom peaks at about 8,000 cells, with some fluctuation between cycles.

After the selection shift at 180,000 time steps, the population initially crashes. However, by about 200,000 time steps, it begins to recover. Boom-bust cycles become less pronounced, and by 250,000 time steps, the population exceeds the previously established unicellular carrying capacity. Growth continues beyond this point without an apparent new carrying capacity before the simulation ends.

10.3.3 Evaluating the Amplitude of Growth Rate ($\frac{dN}{dt}$)

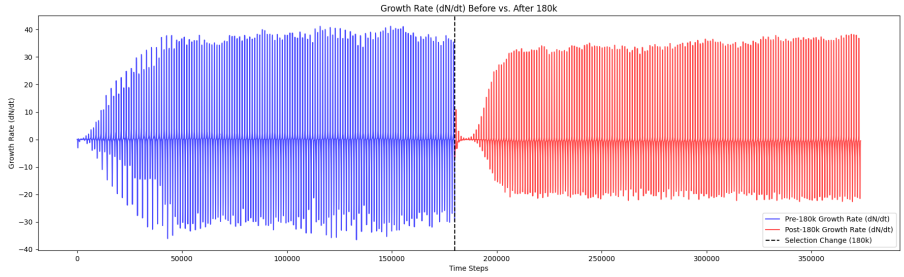


Figure 10.4: Growth Rate ($\frac{dN}{dt}$) Before vs. After 180k This figure visualizes the cell population's growth rate over time. The blue region (pre-180k) shows more restrained fluctuations, whereas the red region (post-180k) exhibits increased amplitude in $\frac{dN}{dt}$, indicating stronger growth bursts and reductions per cycle. The shift at 180k reflects a major change in growth dynamics, aligning with the observed increase in population carrying capacity.

Figure 10.4 shows the growth rate ($\frac{dN}{dt}$) before vs. after 180k.

Here, N represents the number of cells in the population at a given time step. Examining the oscillations in $\frac{dN}{dt}$ before and after the selection shift at 180,000 time steps reveals important insights into the population's growth dynamics:

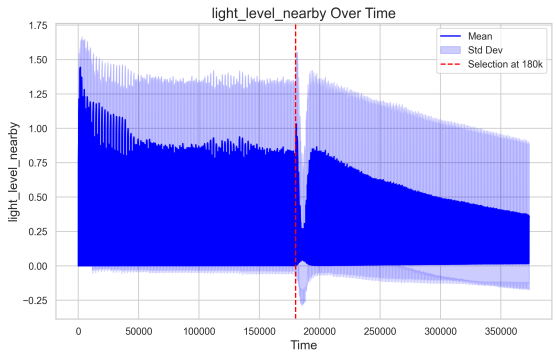
- **Pre-180k (Blue):**
  - The oscillations appear larger initially, but they are actually quite irregular.
  - The peak positive and negative growth rates vary over time and seem equally positive and negative, i.e. the death rate and the birth rate canceling each other out.
- **Post-180k (Red):**
  - The growth rate fluctuations, birthrates, seem comparable if not slightly less than pre-180-k, but it is important to note that the death rate is significantly reduced post-180k.
  - The peaks and troughs of $\frac{dN}{dt}$ appear more consistent over time.

10.4 Cell Variable Statistics

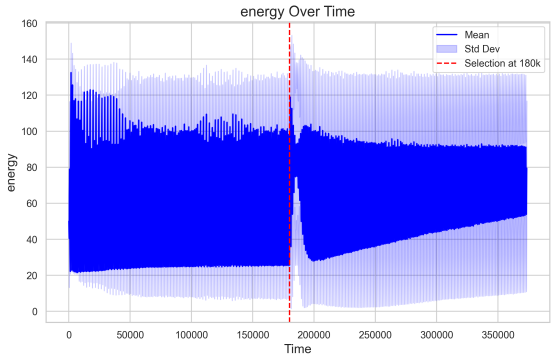
To better understand how individual cell behavior influences population trends, I analyzed the averages and variances of key cell-level variables. Additional variables are presented

in the supplementary materials.

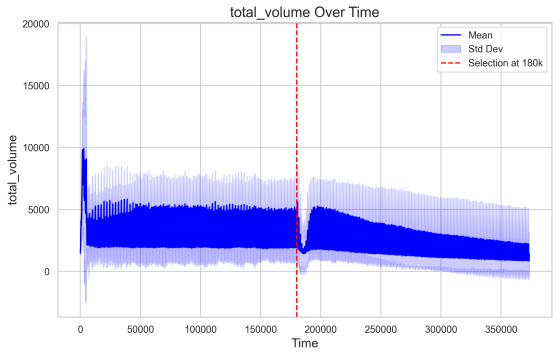
All metrics in this section were calculated at each data-save interval (every 60 minutes), averaged over the population. A red, dotted, vertical line in each plot marks the selection pressure shift from favoring single cells (unicellular life cycle) to favoring attached cells (multicellular life cycle).



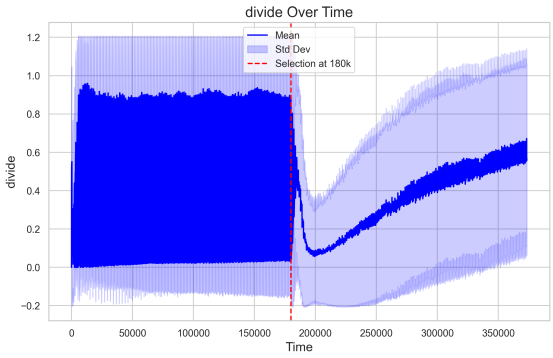
(a) Light experienced by cells



(b) Stored energy in cells



(c) Total cell volume



(d) Proportion of cells in the reproductive phase (indicated by the boolean variable divide)

Figure 10.5: Population averages and variances of key cell variables over time: (a) Light experienced (b) Stored energy (c) Total volume (d) Reproductive phase proportion

10.4.1 Key Observations

- **Figure 10.5 (a)** shows that after selection pressures switch, the average amount of light experienced by cells steadily decreases, as does the variance.
- **Figure 10.5 (b)** shows that after selection pressures switch, the average upper bound on the cells' stored energy remains relatively the same with perhaps less variation each day/night cycle compared to before selection pressures switch. However, once the group cell cycle evolves, the lower bound steadily increases.
- **Figure 10.5(c)** shows that after selection pressures switch, the average upper bound for total cell volume decreases significantly.
- **Figure 10.5(d)** shows that before the selection pressures switched at 180k mins, cells had synchronized divisions (or at least their reproductive states/ non reproductive states happened simultaneously) corresponding to the day/night light cycle (600 mins with light on/ 600 mins with light off). After the selection pressure changed at

180k mins, divisions seem to be less synchronized.

10.4.2 Interpretation:

The observed increase in carrying capacity following the evolution of multicellular life-cycles appears driven by **shading effects**:

- Cells remaining attached to one another post-division receive less light on average due to shading, which reduces individual growth rates.
- However, because attached cells can be closer to one another than two unattached cells, when grouped cell cycles evolve, they are able to pack more densely into well-lit regions, increasing the overall population capacity within the same environment.
- Importantly, the population growth post-180k is not driven by increased birth rates but by a reduction in death rates. In contrast, during the unicellular phase, balanced birth and death rates maintained a relatively stable carrying capacity.

This suggests a fundamental shift in survival dynamics after the evolution of the grouped cell cycle. While individual cell growth slows due to light competition within the colony, group structure improves collective survival on the population level and lowers death rates by allowing more cells to effectively pack into higher light regions.

Supporting this interpretation, Figure 10.5 (c) shows that individual cell volume growth is reduced after the shift. Yet the population as a whole achieves higher growth rates relative to death, leading to an overall population increase.

Figure 10.5(d): The figure show that cells' reproductive/non-reproductive states become less synchronized after the 180k mins. switch. This can likely be attributed to selection pressures favoring unattached cells, as they likely learned to time their reproductive phase to coordinate with the light conditions they experienced. Shading effects increased after group cell-cycles evolved, causing more decoupling between experiential light levels and actual day/night periods. This in turn could have caused reproductive/non-reproductive desynchronization. This also likely accounts for boom/bust cycle oscillations having smaller amplitudes and more consistent amplitude between cycles.

11

Prolog to Discussion

11.1 Classical Life History Theory and Gene-Centric Fitness

Life history theory originated in evolutionary biology, particularly as a response to the need for formal models that could explain observable variation in reproductive timing, growth, and survival across species. It posits that these differences reflect fitness trade-offs — constraints that force organisms to balance investment in survival, reproduction, and growth under finite resource conditions. [48].

The theory emerged under the broader framework of gene-centric evolutionary thinking. Formalized by Stearns, Roff, and others in the mid-to-late 20th century, it was built on the idea that natural selection ultimately favors genes that persist through generations. Because long-term evolutionary success is difficult to quantify, especially in natural populations, life history theory focused on observable, single-generation outcomes—modeling fitness as the product of survival and reproduction within an individual's lifetime. Traits like age at maturity, fecundity, and survival probability served as proxies for this lifetime reproductive output, under the assumption that selection optimizes these traits over evolutionary time [49], [50] ,[48].

In this framework, traits such as age at first reproduction, fecundity, and survival probability were treated not just as outcomes of selection, but as approximations of Darwinian fitness. The underlying assumption was that these traits mediate gene transmission, and thus reflect evolutionary success. Life history models operationalized this by formalizing fitness as a function of two main components

$$Fitness = v \cdot b$$

where v is viability (the probability of surviving to reproductive age) and b is fecundity (the number of offspring produced). These components were typically treated as separable and scalar. This mathematical formulation allowed for clear analytical predictions, cross-species comparisons, and mathematical tractability. [48] [50]

This modeling logic became foundational not only in classical evolutionary ecology but also in later models of major evolutionary transitions, such as those by Michod and Maliet et al. [51, 1]. In these models, scalar traits like t_{gr} (growth duration) and p (group retention time) are treated as the levers of evolutionary change, with fitness still analytically decomposed into viability and fecundity—even as the unit of selection is theoretically shifted from individual cells to collectives.

11.2 Trait-Based Models in the Context of ETIs

As researchers turned their attention to major evolutionary transitions—such as the shift from unicellular to multicellular life—many extended the logic of classical life history theory to these more complex systems. Among the most influential are the models developed by Michod and colleagues, including the work of Maliet, Shelton, and Michod (2015), who apply trait-based reasoning to the emergence of multicellular individuality.

These models retain the foundational structure of classical life history theory: fitness is modeled as the product of two scalar traits—**viability** (the probability of survival) and **fecundity** (the potential for reproduction). The key innovation is that these fitness components are now modeled at the group level, even as they remain functions of lower-level traits (e.g., cellular behaviors). In this way, classical life history logic is *lifted* into a multilevel framework, where the evolving unit of selection is the group, but the traits under selection are often cellular.

These models assume that traits like t_{gr} and p encode a trade-off between survival and reproduction—a trade-off presumed to stem from internal energy allocation, and treated as equivalent to a trade-off in fitness. While the models do not represent energy explicitly, they abstract this dynamic by associating certain trait values with increased viability (e.g., more time in a group reduces mortality) and others with increased fecundity (e.g., longer growth phase yields more offspring).

11.2.1 Viability × Fecundity in Multicellular Transitions

In the framework developed by Michod ([30], [31]) and expanded by Maliet et al. (2015) [1], fitness during an evolutionary transition in individuality is still defined as:

$$W = v \cdot b$$

where:

- v: viability — the probability of surviving to reproduction,
- b: fecundity — the number of offspring produced.

This decomposition assumes that survival and reproduction are *trait governed and separable*, even in emerging collectives. The traits themselves may be group-level properties, such as how long cells remain attached post-division, but their contributions to fitness are still modeled through a viability × fecundity lens.

11.2.2 Formalization in Maliet et al. (2015)

Maliet et al.[1] formalize this framework by introducing two key traits:

- t_{gr}: growth phase duration before reproduction,
- p: the proportion of the growth phase spent in a multicellular group.

They then define fecundity and survival as explicit functions of these traits. For example:

$$F = (1 + K(1 - p(1 - a))t_{gr})^{1/(1-b)}$$

$$S = \exp\left(-m(1 - p(1 - b))t_{gr}\right)$$

where:

- K: growth rate constant,
- a: cost of group living on growth,
- b: benefit of group living on survival,
- m: baseline mortality rate.

Fitness is then expressed as the per-generation growth rate:

$$r(p, t_{gr}) = \frac{\ln(F \cdot S)}{t_{gr}}$$

This formalism allows them to study how increasing group-living (via p) and extending growth duration (t_{gr}) can co-evolve to promote multicellularity. Crucially, their model assumes that the trade-off between survival and reproduction—*a trade-off in how energy is allocated between these two biological functions*—can be captured by scalar traits and directly mapped to fitness components.

11.2.3 Trait-Based Levers of Evolution

In this view, scalar traits like t_{gr} and p become *evolutionary levers*—they mediate the redistribution of fitness components from individuals to collectives. For example, increasing p shifts viability benefits to the group level, while tuning t_{gr} adjusts the fecundity payoff derived from group living.

Although this abstraction is powerful and analytically tractable, it also inherits limitations from classical trait-based modeling. It assumes that:

- fitness can be decomposed into separable components,
- traits are reliable proxies for energy allocation strategies,
- and evolutionary transitions can be captured through shifts in scalar trait values.

As we will see in later sections, these assumptions may obscure emergent processes—especially when fitness is better understood as arising from *dynamic energy flows, coordination among units, and recursive group reproduction*, rather than from optimized trait values alone.

11.3 Limitations of Trait-Based Fitness Models

Trait-based models, like those in classical life history theory offer analytical clarity and empirical accessibility. By defining fitness in terms of scalar traits—typically fecundity and viability—they allow researchers to formalize evolutionary trade-offs, estimate outcomes from measurable data, and generate general predictions across biological systems.

However, this formalism comes with conceptual and mechanistic limitations, particularly in the context of evolutionary transitions in individuality (ETIs), where fitness emerges from dynamic coordination across levels of biological organization.

11.3.1 Traits Mediate Selection, but Do Not Inherit

One central limitation is that traits do not themselves replicate; they mediate selection but are not passed on directly. Genes—or other heritable regulatory mechanisms—are

what persist across generations. As Lewontin noted, while selection acts on phenotypic variation (traits), evolutionary change is ultimately the differential persistence of replicating units [52]. Trait-based models risk conflating short-term trait optimization with long-term evolutionary success.

11.3.2 Trade-Offs Are Entangled in Material Flows

Trait models often assume that trade-offs are separable and scalar—for example, that fecundity and viability are independent and can be adjusted through discrete trait values. But in biological systems, these trade-offs are rarely so clean. Survival and reproduction often compete for the same limited resources, such as energy or biomass, and are dynamically interdependent.

In this sense, trade-offs are not simply abstract relationships between traits, but outcomes of competing demands on internal resource allocation. A trait like t_{gr} (growth duration) may appear to balance survival and fecundity, but in an energy-explicit system, its effect depends on the entire metabolic context: how much energy is acquired, how it is stored, and how it is used. Trait-based models typically abstract away these dynamics, limiting their realism in such systems [53].

11.3.3 Fitness Is Emergent, Not Assigned

Classical and ETI trait models assume fitness can be decomposed into pre-defined components (e.g., $v \cdot b$), often computed within a single lifetime or generation. This overlooks the fact that fitness is often an emergent property of long-term lineage dynamics. Particularly in agent-based models and eco-evolutionary simulations, fitness may not be measurable within a single life cycle, but only through recursive persistence over time.

In such models, fitness emerges from patterns of group coordination, environmental feedback, and regulatory inheritance. This contrasts with classical approaches where fitness is assigned analytically from predefined trait values, not emergent from system dynamics.

11.3.4 Implications for ETI Modeling

In the context of evolutionary transitions in individuality, these limitations are especially salient. During ETIs, selection is not just determining trait values—it is reconfiguring the architecture of inheritance, the structure of cooperation, and the boundaries of the individual. Trait-based proxies may still offer useful approximations, but they risk masking the deeper processes that drive individuality shifts: emergent coordination, recursive reproduction, and multilevel integration.

Recognizing these limitations motivates alternative approaches—such as mechanistic, energy-explicit models—where fitness is not assumed but emerges from how systems solve coordination and resource allocation problems over evolutionary time.

11.3.5 Ratcheting Mechanisms in the Evolution of Group Reproduction

(Adapted from Maliet et. al. [1])

One of the central theoretical challenges in explaining evolutionary transitions in individuality (ETIs) is the so-called "chicken and egg" problem: traits required for group-level reproduction often appear to both result from, and be prerequisites for, group-level selection. Maliet, Shelton, and Michod (2015) [1] offer a compelling resolution to this dilemma

by introducing a coevolutionary ratcheting mechanism, where a life cycle trait (e.g., the duration of time offspring remain grouped) co-evolves with a life history trait (e.g., growth time before reproduction). This mechanism enables a gradual, yet directional, shift toward group-level reproduction, even in the absence of fully developed group-level adaptations at the outset.

This model, inspired by volvocine algae, demonstrates how a new life cycle can emerge from incremental changes to existing unicellular cycles. The authors show that, under the right conditions, even a small increase in group living (captured by the trait p, the proportion of the growth phase spent in a group) can favor changes in traits like t_{gr}(growth duration). These changes reinforce one another: more time spent growing in a group can shift the selective optimum of growth-related traits, which, in turn, further stabilizes group living. This self-reinforcing loop constitutes a "ratcheting" dynamic: once a certain threshold is crossed, reversion to purely unicellular cycles becomes maladaptive.

As Maliet et al state it, “group reproduction does not have to be fully formed before group-level adaptations can evolve." Instead, the life cycle and the life history traits coevolve in a self-reinforcing way.

This idea is important to the argument developed in my thesis for two reasons:

1. It contextualizes the emergence of multicellular life cycles observed in the simulation: the shift from unicellular to group-based reproduction may represent the beginning of such a ratcheting process.
2. It highlights a key interpretive distinction: whereas Maliet et al. treat traits like t_{gr} as drivers of the transition (and as proxies for fitness components like viability and fecundity), my model suggests that in energy-explicit systems, such traits may function differently, not as levers for optimizing fitness per se, but as manifestations or symptoms of deeper energetic strategies.

Nevertheless, the coevolutionary logic outlined by Maliet et al. remains relevant: even in mechanistic models, a kind of ratcheting may occur, but through structural and energetic entanglements rather than through scalar trait shifts alone.

Thus, the ratcheting mechanism described by Maliet et al. serves as a conceptual bridge between classical trait-based models and emergent, energy-explicit models like the one developed in this thesis. It underscores how feedback between structure and selection—whether trait-based or energetically embodied—can generate irreversible progression toward collective individuality.

11.4 Dynamic Energy Budget (DEB) theory

Frustrations encountered during my own project led me to search out alternative theoretical frameworks. Dynamic Energy Budget (DEB) theory offers a powerful framework for modeling organismal energy acquisition and allocation across survival, growth, and reproduction, and has been successfully implemented in individual-based models (e.g., Martin et al., 2012 and 2013 [54] [55]). These approaches generate emergent life history patterns through constrained energy flows, challenging the assumptions of classical trait-based life history theory. Unlike classical life history theory (which assumes fitness is optimized via discrete trait values such as reproductive timing or growth rate) DEB frameworks model physiological constraints directly, allowing trade-offs to emerge from energy-flow dynamics ([56]). This reframing has produced predictions that diverge from classical theory,

particularly under fluctuating environmental conditions or when reproductive costs are dynamically coupled to ecological inputs [57](Kearney, 2012).

11.5 Summary

11.5.1 Fitness Modeling Assumption by Maliet et. al. and Michod

Fitness is treated as analytically separable contributions of survival probability and reproduction potential, and traits' contribution to fitness is treated as analytically separable into survival probability and reproduction potential. In reality, survival, reproduction, and traits may be entangled with resources.

11.5.2 Distinction Between Trait Outcomes and Resource Dynamics

Life history traits are **observable outcomes** of hidden resource allocation strategies. In the effort to make elegant models, Classical and ETI frameworks often **oversimplify, disregarding the crucial resource level**.

11.5.3 Summary Table: Life History Theory vs. My Mechanistic Model

Table 11.1: Trait-Based Modeling vs. Mechanistic Model

Concept	**Trait-Based Modeling (Michod)**	**Mechanistic Model (Mine)**
Resource Flow	Implied, not modeled	Explicitly modeled
Fitness Definition	Viability × Fecundity	Emergent from lineage persistence
Viability and Fecundity	Treated as separable	Materially entangled
Specialization Driver	Trait trade-offs	Energetic constraints
Modeling Goal	Analytical tractability	Mechanistic realism

12

Discussion

12.1 1. Summary of Key Results

This project explored the evolution of multicellularity by simulating an agent-based population of energy-limited, photosynthetic cells. The central research question was: *Can I simulate the evolution from a unicellular to a multicellular life-cycle under shifting selection pressures? And, will the successful evolution of group reproduction initiate a 'ratcheting' towards a full shift in individuality and group fitness prioritization similar to that described in Maliet et al. (2015) [1]?*

The simulation confirmed that a grouped multicellular life cycle *did* evolve. This transition was triggered by a change in selection pressure: at timestep 180,000, unattached cells were penalized with a higher death probability. In response, cells retained clonal attachments for the duration of their cell cycles, transitioning from single-cell to group-level reproductive cycles.

Despite the evolution of group reproduction and group-based survival strategies, this shift was **not accompanied** by a change in the trait t_{gr}— ,which was the indicator for a shift in individuality in the Maliet et al. (2015) [1] model.

12.1.1 Dynamics of the Evolutionary Shift

Simulation Progression

At timestep 180,000, the model introduced an increased death probability for unattached cells. In response, cells evolved a strategy of clonal attachment post-division, forming multicellular clusters.

This adaptation carried trade-offs. While attachment conferred survival advantages by avoiding the elevated death risk, it also increased shading, reducing access to light for individual cells. This constrained their ability to grow, store energy, and reproduce. However, clustering also brought a key spatial benefit: more cells could access high-light zones collectively, improving population-level survival and increasing the system's overall carrying capacity.

12.1.1.0.1 Phase I: $t = 0$ to 180,000: Unicellular Cell-cycle Prior to the shift in selection pressure, unattached cells competed as individuals. They evolved single cell cell cycles and prioritized maximizing energy intake and growth. Despite the low probability of achieving sufficient growth for two full divisions, cells grew as large as possible not only for fecundity but likely also to monopolize light by dominating their voxel and reduce shading effects by competitors. During this phase, the population quickly stabilized as birth and death rates equilibrated.

12.1.1.0.2 Phase II: t = 180,000 to 360,000: Multicellular Cell-cycle Following the shift in selection pressure, grouped reproduction emerged. Cells began remaining attached to their clonal offspring, forming structured colonies and shifting toward multicellular cycles. While attachment reduced individual light access, the overall spatial efficiency improved—more cells fit into illuminated regions, lowering mortality and increasing population density.

Interestingly, cells appeared to deprioritize individual energy maximization. They tolerated lower personal growth and storage in favor of strategies that increased collective viability. Rather than optimizing for individual reproductive output, the system began to optimize for population-level persistence. The population density steadily increased, and no clear carrying capacity was reached by the end of the simulation.

These dynamics suggest that cells were no longer competing for dominance within the energy landscape but were instead saturating it—surrendering high-value light access to collectively support progeny survival. The system evolved toward maximizing group persistence, even at the expense of individual performance.

12.2 2. Interpreting the Evolutionary Shift

12.2.1 The Shift: Structural Coupling and the Emergence of Shared Fate

At each time step, the code checked whether cells had attachments. During the second half of the simulation, cells without attachments faced a higher probability of death and experienced a shared evolutionary fate with those they were connected to. If one cell in a clump died, its partner lost the group's protection and became vulnerable to the elevated death risk faced by unattached cells.

This coupling suppressed exploitative behavior: a cell that monopolized light at the expense of its partner ultimately jeopardized its own survival. In this way, coupling destabilized competitive asymmetries, and a form of proto-cooperation emerged—not from altruism or signaling, but from the structural entanglement of risk.

Cooperative behavior, therefore, arose not through evolved traits or communication, but as a consequence of the coupling structure itself and its implications for energy acquisition and survival.

This dynamic satisfies several key conditions associated with the early stages of an evolutionary transition in individuality:

- **Suppression of within-group conflict**
- **Alignment of survival incentives**
- **Emergent group-level benefits**
- **Shift in selective pressure away from individual optimization**
- **Group reproduction/ reproduction as a unit**

Where cells had previously saturated the energy landscape through individual growth and survival, shared risk now incentivized saturation via population-level expansion.

> This marks not only a behavioral shift, but a reconfiguration of the system's **energetic topology**: from competitive exclusion to cooperative saturation.

In this view, the emergence of cooperation and increased population viability are not defined by trait specialization, but by how the structure of the collective reshapes the flow of energy and risk. This offers a new lens for evaluating early individuality: not by measuring trait decomposition, but by tracking changes in system utilization and energy redistribution under evolutionary constraint.

Population growth, in this context, is neither the explicit target of selection nor a passive byproduct—it is a signal of structural adaptation, and may mark one of the earliest steps in a deeper evolutionary transition.

12.2.2 Why t_{gr} Didn't Evolve: Rethinking the Trait-Based Lens

In classical life-history theory and models like Maliet et al. (2015), traits such as t_{gr}—the duration of the growth phase—are treated as proxies for the trade-off between survival and reproduction. A longer t_{gr} implies higher fecundity but greater risk of death before division. Accordingly, shifts in t_{gr} are often used as indicators of evolving life-history strategies or transitions in individuality.

In my model, t_{gr} remained static even as multicellular reproduction and structural cooperation emerged. This was not due to an absence of evolutionary pressure, but because t_{gr} was not the axis along which the relevant trade-offs were expressed. The model explicitly simulated energy acquisition, storage, and use—yet no trait governed how energy was allocated between competing demands like growth, motility, and maintenance. As such, trade-offs unfolded behaviorally and structurally, not through changes in scalar traits.

This reveals a deeper insight: in energy-explicit systems, trade-offs are not always encoded in traits—they are embedded in flows. Trait shifts only occur when those traits mediate how energy is prioritized across survival and reproduction. In the absence of evolvable energy allocation strategies, my model resolved selective pressures through emergent patterns—like structural attachment and spatial coordination—rather than through trait evolution.

This suggests that models seeking to capture evolutionary transitions in individuality (ETIs) must move beyond trait-based proxies and toward frameworks that account for how energy is managed. Where fitness emerges from dynamic resource use, individuality may first arise through energetic interdependence and shared constraints—long before trait specialization evolves.

I propose a conceptual reorientation: in energy-explicit systems, fitness trade-offs are not carried by scalar traits but emerge from the dynamics of energy acquisition and allocation.

12.3 Volvocine Algae & Energy Management Strategies

Like the agents in this model, empirical observations of unicellular *Chlamydomonas* indicate that cells time their life cycles according to diurnal light patterns. Notably, the lack of evolutionary change in t_{gr} observed in my simulation is consistent with what is seen in *Chlamydomonas* under laboratory conditions. For example, under predation pressure, *Chlamydomonas reinhardtii* can evolve multicellular life cycles ([9], [**?**]) while maintaining cell-cycle durations comparable to both its ancestral unicellular form and its multicellular relatives (such as *Gonium pectorale*) in similar settings ([**?**], [58]).

While my system allowed traits like t_{gr}, attachment propensity, motility, and light absorption thresholds to evolve, it lacked a key evolutionary axis: cells could not evolve

energy allocation strategies when balancing competing energy-intensive functions such as motility (critical for survival) and growth (linked to reproductive potential). Yet this balance is precisely what embodies the core survival–reproduction trade-off faced by all living systems.

A closer comparison of energy management across the volvocine algae lineage further illuminates this point. In *Chlamydomonas*, cellular functions are temporally partitioned across cell-cycle phases:

- G1 Phase:
  - Growing
  - Motile
  - Non-reproductive
- S/M Phases:
  - Non-growing
  - Immotile
  - Reproductive

By contrast, in multicellular *Volvox*, these same functions are partitioned spatially across cell types:

- Germ cells:
  - Reproductive
  - Immotile
  - Growing
- Soma cells:
  - Non-reproductive
  - Motile
  - Non-growing

Importantly, *Volvox* does not simply "freeze" the cell-cycle phases of its ancestor into fixed cell types. Rather, it fundamentally reorganizes how energy is allocated across cellular roles. In *Chlamydomonas*, both growth and motility occur during the G1 phase, while reproduction is restricted to S/M phases. In *Volvox*, by contrast, energy allocation is decoupled: biomass accumulation and reproduction are confined to germ cells, while motility and survival are delegated to specialized somatic cells.

This comparison suggests that *Volvox* not only co-opted inherited temporal coordination of phases from its unicellular ancestor but control over energy investment strategies across mutually exclusive costly functions.

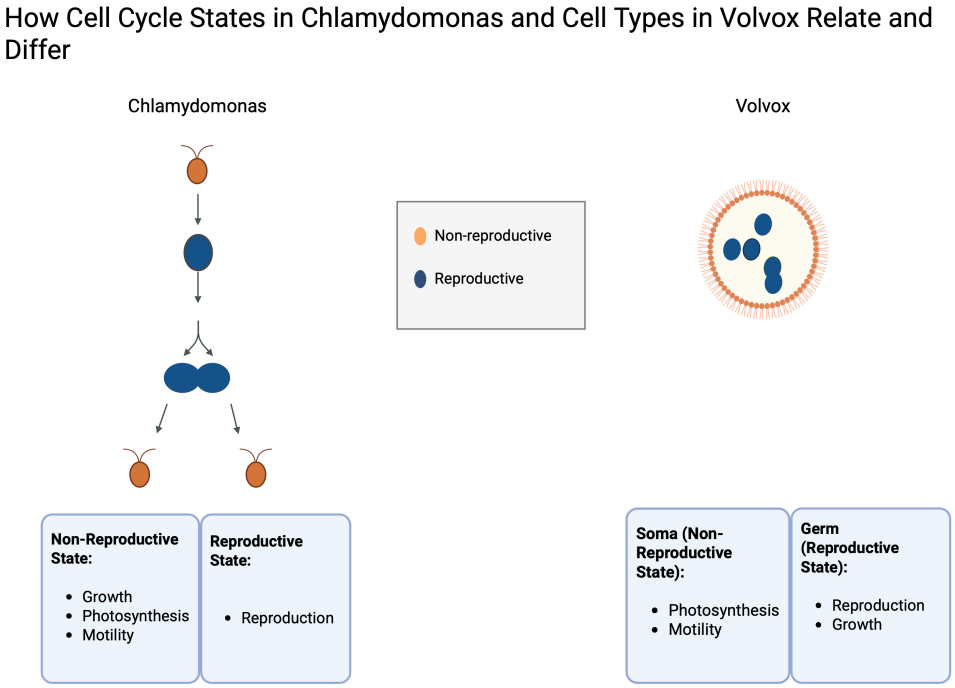


Figure 12.1: Similar to figure 4.2, where we compared the cell cycle states of the unicellular Chlamydomonas and the cell types of the multicellular Volvox, we make that comparison here again. In this figure there is also a comparison of key differences between them, namely whether growth is delegated to the reproductive or non reproductive state/cell-type.

This comparison suggests that Volvox not only co-opted control over reproductive/non-reproductive phase transitions from its unicellular ancestor in order to evolve distinct cell types, but also reorganized energy use towards different costly functions, enabling division of labor through differential energy allocation. This represents a fundamental shift in energy strategy—one that is likely crucial for a full evolutionary transition in individuality. **Without the ability to regulate internal energy allocation across functions and non-reproductive/reproductive modes, a true fitness transfer may be biologically and evolutionarily blocked.**

In my model, although energy was modeled explicitly, cells could not control energy use. There was no output to the GRN proxy for allocating energy between functions. As a result, while group-cell cycles evolved structurally, no internal division of labor or differentiated investment strategies could emerge. Future model extensions could explore whether enabling the evolution of energy allocation strategies drives shifts in life-history traits, potentially shedding light on a critical step in the evolution of multicellularity.

One of the key contributions of using agent-based modeling in this study is the ability to observe evolutionary transitions from the level of the individual—each agent making local decisions with limited information. Based on the findings of this project, I propose that successful transitions in individuality likely require individual-level regulation over three core decisions:

- When do I divide?
- When do I detach (or remain attached)?

- How do I allocate energy between competing functions like motility and growth?

12.3.1 Energy Sharing

Cells form cytoplasmic bridges during mitosis in order to distribute embryonic resources. Therefore it is plausible that these connections could be retained and co-opted as pathways for resource sharing. In other words, energy sharing potential could be inherent in a colony. In such a case, the group would no longer function as a mere spatial aggregation of cells, but as a metabolically interdependent system.

A spatial cluster is simply a group of cells that remain physically attached. In this arrangement, each cell still operates mostly autonomously. There is no coordination of internal state, no shared energetic fate, and no active redistribution of resources. Cooperation, when it emerges, does so passively—arising from structural attachment or indirect environmental effects such as shading. The group is a structure, not a system. This characterizes the progress of my simulation.

A metabolic network, by contrast, is an emergent system in which energy acquisition and use are distributed across individuals. If one cell photosynthesizes and shares energy with another—especially a neighbor in a shaded interior—cells become metabolically interdependent for survival and reproduction, creating a web of energetic dependencies. Functional roles may emerge: some cells prioritize light absorption, while others become growth or reproduction-focused. Importantly, this suggests this division of labor may not be preprogrammed—but arises as an energy resource management strategy under environmental constraints. The conditions may change, but the goal is the same: to maximize resource acquisition and optimize resource allocation to increase lineage persistence.

In this scenario, the group begins to function as a higher-level unit—a collective with internal energy regulation. **This is a hallmark of individuality**: the group exhibits metabolic cohesion, not just spatial cohesion.

Moreover, once energy can flow into and through the colony, novel life-history dynamics become possible. For instance, outer cells that are exposed to light could focus on energy harvesting and resource provision, while interior cells—shielded from light and freed from the need for motility—could specialize in growth and division. This would enable the colony to optimize total energy acquisition collectively, rather than compete for energy resources. Such specialization may even drive a decoupling of colony behavior from the diurnal light cycle: rather than all cells synchronizing reproduction to nighttime, the colony could maintain reproductive and photosynthetic activity throughout the full cycle, buffered by internal energy sharing and differentiation.

> In short, energy sharing may transform colonies from passive structural collectives into active metabolic systems—capable of role specialization, decoupled dynamics, and internal coordination. It is not merely a cooperation mechanism—it is the functional infrastructure that enables the emergence of higher-level individuality.

Thus, I suspect that incorporating intercellular energy transfer, along with energy allocation control, into the model design would be a critical next step toward simulating a full evolutionary transition in individuality (ETI). It would allow selection to act not only on energy acquisition and structural attachment, but on the allocation and flow of energy within a group—revealing whether functional integration and differentiation can evolve as emergent strategies for maximizing lineage persistence under constraint.

12.3.1.1 Biological Hypothesis

In the evolution of multicellularity in volvocine algae, the co-option of genes regulating cell-cycle timing and adhesion likely enabled the emergence of clonal group reproduction. But it may have been the inherent capacity for cytoplasmic resource sharing, together with the genetic regulation of energy allocation between motility and growth, that constituted the critical innovation driving the transition in individuality itself.

12.4 Revisiting Life-History Theory, Maliet et al., and Dynamic Energy Budget (DEB) Theory

12.4.1 An Updated ETI Framework for Energy Explicit Systems

Building on the earlier observation that the trait t_{gr} did not evolve in my model, this section reframes that result through the lens of classical life-history theory, the model of Maliet et al. (2015), and insights from dynamic energy budget (DEB) theory. The aim is not to repeat the findings, but to clarify why they matter — and to propose an updated framework for interpreting trait evolution when energy is modeled explicitly.

A common question that arises is whether a capacity like "control over energy allocation"—for example, how much energy is directed toward motility versus growth—should be considered a trait at all. If implemented in my model as evolvable, it would be genetically encoded (via the GRN proxy), heritable, and variable across lineages—meeting the evolutionary definition of a trait. But unlike classical life-history traits, it would not function as a proxy for survival or reproduction. Rather than reflecting fitness, it would shape it.

This distinction is central. In energy-explicit systems, such traits function as metabolic control parameters: they determine energetic behavior, and fitness consequences emerge from their downstream effects. Trade-offs arise not from fixed trait values, but from the strategies cells employ under energetic and environmental constraints

In Maliet et al. (2015), the evolution of life cycles in unicellular algae is modeled using t_{gr}, and the duration of the growth phase is modeled as the focal life-history trait. Within their framework, t_{gr} encodes a classical trade-off: longer growth phases increase fecundity, but also increase the risk of death before reproduction. Although their discussion acknowledges that other traits—particularly those involving energy allocation—may be relevant to life cycle evolution, these dynamics are not explicitly modeled. Even if energy allocation had been included, it would likely have been treated as another scalar parameter—an input to a fitness function—rather than a process unfolding in time.

This scalar abstraction is common across classical life-history theory. Traits like growth rate or age at first reproduction are treated as statistical proxies for unobservable strategic variations in resource allocation. Fitness is inferred from patterns across populations, rather than arising from the internal logic of how organisms behave energetically within their environments.

In contrast, my model explicitly simulates energy flow as a dynamic constraint on behavior, growth, survival, and reproduction. In its current form, cells absorb energy through photosynthesis, which simultaneously contributes to both growth and energy storage. Growth and survival draw from the same resource pool, but do so in parallel, without explicit internal competition or prioritization between them.

In hindsight I believe that a more realistic and evolutionarily meaningful design would have involved an additional step: energy gained from light would first be stored, and then allocated across competing demands:motility, growth, division, and maintenance. This allocation could have been regulated as an evolvable output of the GRN proxy, allowing cells to evolve different strategies for optimizing energy use.

In such a system, energy allocation becomes an evolvable strategy rather than a background assumption. The processes of growth, survival, and movement would compete for finite internal resources, and trade-offs would emerge directly from these behavioral dynamics, rather than being embedded in predefined trait values.

Trade-offs emerge from within the energetic system, shaped by environmental inputs and lineage survival — not inserted as fixed-cost structures or trait trade-offs. The shift is subtle, but significant: energy is not just an input; it is a dynamic driver. And modeling energy as behaviorally allocated—rather than statistically abstracted—re-frames how we detect and interpret shifts in individuality.

Traits as Proxies for Fitness

In classical life-history theory, traits like growth rate, age at first reproduction, or number of offspring are often used as proxies for the underlying resource allocation strategies that shape an organism's survival and reproductive success. These allocation strategies — rationing energy to growth, maintenance, or reproduction — are typically not observable, so traits serve as indirect signals of fitness-affecting strategies.
This modeling shortcut is summarized in works by Stearns (1992), Roff (1992), and Michod (2006), where fitness is treated as a function of life-history traits, and those traits are assumed to represent resource allocation outcomes.
But in energy-explicit agent-based systems, this shortcut breaks down. Resource allocation is not inferred; it s modeled directly. Traits in these systems no longer stand in for fitness components — they become modulators of energy flow, and fitness emerges from how well these energy strategies perform over evolutionary time. In short:

- In classical models:
  - Trait → Fitness component → Fitness (per generation, trait decomposition)
- In energy-explicit models:
  - Trait → Energy behavior → Survival & Reproduction → Fitness (lineage persistence, emergent)

This shift requires rethinking the definition and tracking of life-history traits in models that explicitly and dynamically simulate the metabolic underpinnings of fitness.

Table 12.1: Comparison of Energy Allocation in Classical Life-History Models vs. Energy-Explicit Agent-Based Models

Concept	**Classical Models (e.g., Michod, Maliet)**	**Energy-Explicit Agent-Based Model (This Work)**
Energy Allocation	Abstracted as a scalar trait (e.g., t_{gr}) influencing survival and reproduction	Emerges from real-time behavior in an energy-limited environment; not pre-defined
Life-History Trait	Stand-in for resource allocation outcomes (e.g., growth rate, division time)	Behavioral control parameters (e.g., GRN-controlled motility, division, absorption threshold)
Fitness	Explicitly calculated per generation as viability × fecundity	Emergent from lineage persistence over time; no explicit fitness function
Individuality Shift	Inferred via trait redistribution (e.g., changes in t_{gr} and p)	Reflected in energetic interdependence, shared survival outcomes, and spatial coupling

12.4.2 DEB

I am not the first to recognize the importance of modeling an organism's energy acquisition and allocation across survival, growth, and reproduction. Nor am I the first to connect this approach to life-history theory or implement it in individual-based models.

Although unaware of DEB theory when first developing my model, my conclusions extend its insights into a new domain: the evolution of individuality. While DEB focuses on physiological regulation within individuals, my model embeds energy-explicit behavior into an evolutionary, agent-based framework in which selection acts on emergent regulatory strategies.

In this sense, my work complements DEB theory but also reframes its potential: not just as a tool for simulating organismal physiology, but as a generative framework for understanding the emergence of evolutionary units from energy flow.

The goal is not simply to simulate physiological realism. It is to explore how control over energy allocation—a trait absent from most classical ETI models—may shape evolutionary outcomes. By allowing groups, not just individuals, to persist through cooperative, energy-mediated strategies, this framework offers a new perspective on the conditions fostering the evolution of individuality in energy-limited environments.

While recent studies have begun to integrate DEB theory into individual-based models with evolutionary components ([55] [59] [60]), few explore how energy-use regulation evolves—and none, to my knowledge, examine how such regulation contributes to the emergence of group-level traits or individuality transitions.

12.4.3 Conclusion: Catalyst of Individuality

Despite the constraints of my model, this exploration reveals a more profound limitation of classical life-history theory—not in its internal logic, but in what levers it allows to evolve. For true evolutionary individuality to emerge, **selection must act on internal prioritization strategies**.

In this light, energy-explicit, agent-based models offer more than just structural real-

ism—they offer a new lens for investigating how fitness emerges from dynamic, embodied systems. If scalar trait shifts no longer track transitions in individuality, the question becomes: what does?

12.5 3. Rethinking Individuality: a Theoretical Perspective on Energy-Explicit Transitions

While the results of my model suggest a clear behavioral and structural transition, the challenge lies in how to interpret that transition within existing theoretical frameworks for ETIs. In particular: what signals a shift in individuality when trait-based metrics no longer apply?"

So, How do we detect a shift in individuality in systems where fitness is not a function of traits, but an emergent property of energy flow, structural interaction, and lineage persistence?

In the theoretical frameworks developed by Michod and colleagues, evolutionary transitions in individuality (ETIs) are marked by a **reallocation of fitness components**—specifically, the redistribution of viability and fecundity from individual cells to the collective. This approach assumes that survival and reproduction can be cleanly decomposed into measurable life-history traits, and fitness can be decomposed into separable components fecundity and viability. Where fitness is defined within one generation.

$$Fitness = v \cdot b$$

But in my model, this decomposition breaks down.

12.5.1 Fitness in Energy-Explicit Systems

As discussed earlier, survival and reproduction in my model are not governed by distinct traits but are entangled outcomes of a shared energetic strategy. Cells must continually acquire, store, and allocate energy to all functions—survival, metabolism, growth, and division. These processes draw from the same energy source. Trade-offs emerge not between isolated traits, but within this dynamically contested energy pool.

In my model fitness is also not calculated per generation. Instead, it emerges from lineage persistence over time—from a cell's ability to survive environmental constraints, reproduce, and pass on its genetic architecture across many generations. In this context, fitness is not a static function of life-history traits. It is a consequence of embodied, adaptive strategies unfolding in an evolving, resource-limited environment.

To make this more concrete: in my model, a cell grows in volume based on light absorption while simultaneously storing energy. At first glance, one might assume that volume corresponds to investment in reproduction, and stored energy to investment in survival. But this distinction quickly breaks down. When a cell divides, its stored energy is distributed evenly among its offspring. Because cells incur constant metabolic costs, the survival and success of each offspring depend on how much energy the parent accumulated prior to division—and how many offspring were produced in that mitotic event. If a cell divides too many times, or without first building sufficient reserves, it spreads its energy too thinly, reducing the viability of its descendants.

These offspring not only inherit energy, they inherit the parent's strategy for acquiring

it, as well as division timing strategy. Additionally environmental context, spatial position, and light availability all interact to shape the outcome. In this system, fitness cannot be partitioned cleanly into contributions from survival and reproduction. Instead, it emerges from the energetic consequences of interaction, competition, inheritance, and environmental constraint over time.

Here, the classical viability–fecundity trade-off does not cleanly apply. The underlying trade-off remains—but it is resource-embedded, not aligned with orthogonal fitness axes. Traits do not independently modulate viability or fecundity. Instead, they contribute to a unified energy-use strategy, balancing competing demands under constraint.

In this kind of system, the limiting variable is not fitness—it is energy. And survival and reproduction are not managed by separate levers; they are deeply commingled by shared energy use. Fitness, in turn, is not decomposable into component functions—it emerges dynamically from the way lineages manage energy through time.

12.5.2 Evolutionary Shifts in Energy Use: Case Study from my Model

Thus in detecting a shift in individuality, or a fitness transfer from the individual to the group level in energy explicit systems may mean detecting symptoms of a deeper reorganization of energy flow and a shift in the axis along which evolution maximizes lineage persistence under constraint.

In my model, this shift becomes visible when comparing how the population saturated the available energy landscape before and after the selective regime changed. In both phases of the simulation, cells adapted to fill the environment as fully as possible. But the mode of saturation changed qualitatively, revealing a deeper transition in how energy was used and shared across the population.

12.5.2.1 Before 180k: Individual Optimization

When unattached cells had a survival advantage, the system quickly evolved toward **single-cell life cycles**. Cells competed to maximize their own growth and energy storage, even if this seldom translated to increased offspring. Regardless it was likely advantageous to adopt a competitive strategy, **monopolize light** at their voxel by maximizing growth and pushing others out of high-light zones.

The energy landscape was saturated through individual growth maximization, with population size limited by the exclusionary dynamics of light competition—essentially, a carrying capacity imposed by competitive exclusion.

12.5.2.2 After 180k: Collective Optimization

When unattached cells faced increased mortality, multicellular group cycles evolved. Clonal attachments enabled daughter cells to remain together, forming persistent groups that sacrificed some individual light access in exchange for structural survival benefits. These collectives packed more efficiently into high-light zones, reducing mortality and increasing the system's overall carrying capacity.

This shift was not driven by new trait values per se, but by a change in the system's strategy for energy saturation. Where cells once optimized for personal growth and persistence, they now evolved toward population-level survival—a shift from maximizing individual success to maximizing collective viability.

> **This shift reflects not just a behavioral adaptation, but a reorganization of the system's energetic topology—an emergent form of individuality embedded in patterns of energy use and survival.**

From this perspective, what trait-based models might interpret as a transfer of fitness from individuals to groups may instead reflect a deeper transition in energetic interdependence. That is, a point at which collective energy dynamics, rather than individual optimization, begin to shape evolutionary outcomes. The level at which energy is controlled, shared, and prioritized becomes the level at which persistence is maximized.

Had my model allowed cells to evolve traits—via the GRN proxy—that regulated internal energy allocation (e.g., balancing motility and growth) or intercellular energy sharing, the system might have crossed another threshold. In such a case, energy could have begun to flow within each group rather than just structurally between them. This would have allowed the colony cluster—not just the individual cell—to emerge as the true unit of selection. Such a shift could have enabled competition between collectives and driven the evolution of functional differentiation and metabolic interdependence, akin to the soma–germ division seen in Volvox.

> **This shift reflects not just a behavioral change, but a reorganization of the system's energetic topology—an emergent form of individuality embedded in the energy dynamics of the group.**

Rather than rejecting Michod's framework, I propose an extension:

> **In systems where fitness is lineage-based and energy flow is explicit, detecting shifts in individuality—particularly in the intermediate stages of ETIs—requires more than observing trait changes. It requires attention to how control and cooperation over energy use shifts across levels of organization, and how that control determines which level's persistence is prioritized. In turn, this shapes the evolutionary axis through which the system saturates the energy landscape.**

12.5.3 Coevolution and the Ratchet Toward Individuality

One of the major points of the Maliet et al. (2015) paper proposed a resolution to the classic "chicken-and-egg" problem in evolutionary transitions in individuality: that group-level reproduction requires group-level adaptations, but group-level adaptations only evolve under group-level selection. Their solution was to show that a **coevolutionary ratchet** can emerge between two traits:

- p: the probability that daughter cells remain attached
- tgr: the time cells spend growing before division

Although my model did not allow cells to evolve the key modulating traits that embody the reproduction–survival trade-off—traits considered crucial for a full evolutionary transition to multicellularity—I observed a shift in dynamics that I take to be evidence of the validity of this theory. For my system specifically, the **axis along which evolution maximized lineage persistence** changed from maximizing individual growth (fecundity) and persistence (viability) to maximizing population growth (increasing carrying capacity, perhaps a form of fecundity at the population level) and collective viability (death rate reduction)
.

I interpret this as evidence that the **'ratcheting' effect** described by Maliet et al. would have occurred given the reigh evolutionary levers had been present. In my model, the emergence of group-level reproduction in response to selection pressure marked a shift in the way persistence was maximized—from individual competitive cells to collective population increase. This shift occurred through structural adaptations that allowed groups to saturate the energy landscape more efficiently.

12.6 5. Locating my System on the Continuum of Individuality

Although my model did not produce a full evolutionary transition in individuality (ETI), but several hallmarks of early individuality shifts do emerge:

12.6.1 1. Structural Interdependence

Cells form clonal groups that are mutually entangled in survival. The risk of death is no longer carried individually, but collectively—especially when the loss of one member increases the vulnerability of the others. This **shared fate** is a fundamental prerequisite for cooperative stability and evolutionary alignment.

12.6.2 2. Suppression of Intra-Group Conflict

Exploitative behavior within attachments is disincentivized because **cheating increases self-risk**. This structural constraint acts as a **conflict mediator**, even in the absence of evolved rules or enforcement mechanisms. Evolution favors strategies that preserve attachments over strategies that maximize individual energy gain.

12.6.3 3. Evolution of a Clonal Multicellular Life Cycle

Perhaps most significantly, the system evolved a **multicellular group cell cycle**, in which clonal attachments persist post-division, and the cycle itself is subject to evolutionary change via mutations to the GRN. This qualifies as a form of **early group-level replication**: the basic reproductive unit has shifted from the individual cell to the clonal group governed by shared regulatory logic. While the model lacks explicit group-level control over morphological traits such as shape or differentiated function, the coordinated group behaviors—specifically, the duration of attachment and timing of division, and size of the cluster—are inherited through clonal propagation of GRN weights and evolve over time. In this way, the system implements an implicit form of group-level heredity, consistent with early multicellular life cycles such as those described in *Gonium*.

12.6.4 What's Missing?

In order for a full transition to occur, natural selection must shift from the individual to the group level. In order for natural selection to be fully acting on the group level, there must be heritable variation in group fitness present, and group competition. In my simulation, no such variation was detected. While group-level reproduction and clonal identity emerged, all groups functionally shared the same energy management logic. As a result, no meaningful differences arose between groups in their capacity to survive or reproduce. Without variation in group-level strategies, selection could not act to shape

group-level traits, and the transition toward full group-level individuality stalled. Key missing components include:

- Heritable variation in group fitness.
- Division of labor or trait differentiation.
- Group-level identities beyond clonality.
- Competition between groups, and thus, explicit group-level selection.

These missing components suggest clear directions for future work.

Category	Indicator / Sign	Rationale	What my model shows
Fitness Decoupling	Group fitness increases independent of the fitness of individual components.	Indicates selection is shifting toward the group level.	Partial: population-level survival increases via spatial packing, even as individual growth potential decreases. Suggests early stages of decoupling.
High Relatedness	High genetic similarity among group members (e.g., clonality, vertical transmission).	Reduces conflict and enables group-level selection.	Yes: groups are clonal; attachments form among daughter cells from a single division event.
Reproductive Division of Labor	Some cells specialize in reproduction, others in survival or support.	A hallmark of functional integration and evolved cooperation.	Not yet: no differentiation of cell roles; all cells remain generalist.
Group-Level Reproduction	The group undergoes a life cycle: growth, division, and regeneration.	Required for selection to act on group-level traits and dynamics.	Yes, partially. Clonally related cells remain attached after division, forming groups that go through coordinated life cycles involving growth, energy accumulation, and synchronized reproduction. Although group-level reproduction is not explicitly enforced as a distinct higher-level process, it emerges implicitly through the cell cycle and attachment behaviors. The group reproduces when the attached colony members separate and immediately divide, resulting in another clonal cluster colony.
Bottleneck Events	Reproduction includes a single-cell or minimal propagule stage.	Enhances heritability, reduces internal conflict, aligns fitness.	Yes. Groups form clonally from a single parent cell that undergoes multiple fission. This constitutes a single-cell bottleneck, ensuring that all group members are genetically identical. This bottleneck increases heritability and reduces within-group genetic conflict—two key ingredients for group-level evolution.
Conflict Suppression	Exploitation within groups becomes costly or suppressed.	Enables stabilization of cooperation.	Yes: exploitative shading risks breaking attachment and triggering higher mortality, disincentivizing selfish behavior.
Functional Integration	Components perform different functions and rely on one another.	Indicates the group is more than a collection of individuals.	Not yet: no functional differentiation or intercellular dependence beyond physical proximity.
Heritable Variation at Group Level	Groups vary in traits and pass these on during group reproduction (e.g., via a bottleneck).	Needed for evolution by natural selection at the higher level. Heritable variation ensures that groups—not just individuals—can evolve distinct strategies.	Yes, partially. Each group of clonally produced cells shares a common genotype inherited from a single parent via multiple fission. Mutation occurs prior to division, so all offspring in a group are genetically identical at birth. This creates group-level inheritance through a single-cell bottleneck and enables selection to act on emergent group traits. However, since the traits affecting within-group energy allocation (e.g., division of labor) are not yet evolvable, group-level heritable variation is present but limited in scope.
Autonomy & Boundary Formation	The group behaves as a coherent unit with distinct identity.	Reflects emergent individuality.	Emerging: group cycles evolve, and clonal groups persist, but group identity is not yet distinct or protected.
Environmental Scaffolding	Spatial or ecological context promotes cooperation or cohesion.	Helps stabilize proto-groups during early transitions.	Yes: the light gradient and death penalty for unattached cells create conditions that favor attachment and spatial coordination.

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Conclusion

This thesis demonstrated the bottom-up emergence of clonal group reproduction and multicellular life cycles under shifting selection pressures. With mechanisms for mutation, inheritance, and bottlenecks in place, the simulation reproduced several hallmarks of early evolutionary transitions in individuality (ETIs): clonal attachments, shared fate, suppression of intra-group conflict, and an emergent form of group-level replication.

Even though group-level reproduction evolved, life-history traits such as t_{gr} remained static. This revealed a deeper insight: in energy-explicit systems, evolutionary dynamics may not manifest as changes in trait values, but as emergent strategies embedded in energy flow and spatial structure. Trade-offs were not directly encoded in discrete traits, but negotiated through the collective reorganization of energy acquisition, use, and survival.

From this perspective, individuality is reframed—not as the redistribution of functional traits, but as a reconfiguration of energetic interdependence. In systems where energy acquisition and use are explicitly modeled, the earliest signals of individuality may appear not in traits, but in topology: a shift from autonomous optimization to interdependent metabolic constraint.

> **Individuality, in this framework, is a shift in energetic topology—from autonomous optimization to interdependent constraint.**

Although group-level reproduction evolved, the transition to full individuality—marked by reproductive division of labor—did not. The model revealed that for division of labor to emerge, cells must move from individual energy autonomy to interdependent energy topology within the group. As long as cells remained energetically self-sufficient, selection continued to act primarily at the individual level—even within cooperative structures.

This suggests that the emergence of higher-level individuality requires more than spatial cohesion or behavioral coordination. It requires a shift in the underlying metabolic interdependence—specifically, in how energy flows are structured and shared. Two key capacities must evolve to enable the ratcheting toward a full ETI, as described by Maliet et al. (2015) [1]:

- **Energy sharing**, which creates metabolic interdependence and ties individual survival to the success of the group.
- **Intracellular control over energy allocation**, which enables cells to regulate how their energy is invested—opening the door to functional differentiation, such as soma–germ specialization.

It is this second capacity—regulatory control over energy use—that determines what

roles cells can take on within a group. Without it, even shared energy cannot support division of labor. Together, these two features allow selection to act on group-level organization, function, and reproduction.

Without both, group-level selection remains weak, and the ratchet toward full ETI stalls. This offers a theoretical refinement to existing ETI frameworks, which often emphasize cooperation, communication, or differentiation, but less often focus explicitly on energy topology and governance. ETIs, in this light, depend not just on trait divergence or cohesion, but on the evolution of an internal economic system.

In any system of Darwinian individuals, evolution favors strategies that maximize lineage persistence. When fitness depends on the ability to acquire and manage limited resources, evolution explores the degrees of freedom by which resource flows can be optimized to maximize persistence.

In my simulation, when unattached cells had a survival advantage, evolution favored lineages that maximized individual resource acquisition through growth and competitive exclusion. But once selection shifted to penalize unattached cells, clonal attachments emerged, and solitary strategies were no longer viable. Lineages began maximizing persistence in a new way: not through individual success, but through collective survival and spatial coordination.

Although cells could not share energy within clumps, they could benefit from group structure by reducing collective mortality—packing more efficiently into high-light zones and expanding population size. This was not metabolic cooperation in the traditional sense, but a structural workaround: evolution exploited the one path still available to increase lineage persistence under constraint. Shared fate became the scaffolding on which selection built a new solution.

Had cells been capable of sharing energy within their groups, evolution might have taken a further step—optimizing energy flows among group members, enabling collective strategies, and introducing heritable variation between groups. This would have opened the door to true group-level selection and possibly a full evolutionary transition in individuality.

> **The emergence of individuality is not only a matter of traits or cooperation—it is a matter of energy: how it is acquired, how it is managed, and the direction it flows.**

Moreover, this work shows that computational barriers are no longer the primary constraint to simulating a full ETI. With modern agent-based platforms and appropriate design, we are now capable of simulating full evolutionary transitions in individuality from the bottom up. The challenge is now conceptual: to develop theoretical frameworks that account for the metabolic and structural dynamics through which new evolutionary units emerge.

> **We are now capable of simulating full evolutionary transitions in individuality, from the ground up. The conceptual tools—now updated—must follow suit.**

Beyond the technical and theoretical findings, one idea stayed with me throughout this project—something both biologically plausible and poetically resonant. The evolution of complex multicellularity, particularly in lineages like the volvocine algae, is thought to

begin with clonal adhesion: cells remaining physically connected after mitosis. In such contexts, energy sharing becomes not just possible, but necessary—especially during reproduction. This simple act of retaining physical connection after division may create the preconditions for metabolic interdependence and higher-level individuality.

If I am right in my hypothesis that this is a key precondition for the emergence of complex multicellularity, then one finds something quietly profound. The answer to the question, "How do many become one?"—how individual cells come to feel and function like a single organism—may lie in the shared origin of all members of the group. Because we all originate from a single replicating cell, the cells in our body share a structural basis for energy sharing. That shared origin may be what enables cells to unify—and life to form higher levels of individuality.

Many can become one—precisely because they once were.

This is not merely a matter of genetic relatedness, as emphasized in kin selection theory, but of physical continuity—an infrastructure that enables energy interdependence to emerge and evolve.

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Appendix 1

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