Contents

1 Microbial Design

> In the past, changes in gene expression and metabolic strategies across growth conditions have often been attributed to the optimization of … growth rates. However, mounting evidence suggests that cells are capable of significantly faster growth rates in many conditions. … Based on these observations, it is clear that [design] objectives other than optimization of … growth rates must be considered to explain these phenotypes.
>
> —Markus Basan[27]

Why don't microbial cells grow as fast as possible? Perhaps cells trade growth rate for other attributes of success.

One widely discussed tradeoff concerns rate versus yield. Growing faster uses resources inefficiently. Resources wasted to increase metabolic rate lower the resources available to build new biomass. Fast growth rate reduces the reproductive yield.[317,444]

Suppose we observe microbes that grow more slowly than the maximum rate that they could achieve. We see mutations that enhance growth. How can we know if the tradeoff between growth rate and yield dominates in metabolic design?

Typically, we cannot know. An observed rise in rate and decline in yield supports the tradeoff. But rejecting the rate-yield tradeoff hypothesis is difficult. For example, the microbes may produce toxins to kill competitors. If competitors are absent in our study, we may see increases in both rate and yield as the unobserved toxin production declines.

Other tradeoffs may be hidden. Perhaps growth trades off with dispersal. Maybe the microbes typically grow under iron-limited conditions and must trade growth rate for scavenging iron.

We could measure more tradeoffs. Although helpful, that approach ultimately fails. We can never estimate the many tradeoffs across the full range of natural conditions that shaped design.

Given those difficulties, how can we understand why growth rate is sometimes maximized and other times not? In general, how can we understand the forces that shape the design of microbial traits, such as dispersal, resource acquisition, defense, and survival?

I advocate comparative hypotheses. As a focal parameter changes, we predict the direction of change in a trait. For example, as the genetic heterogeneity among competitors rises, we predict an increase in growth rate.[130,317] If the predicted direction of change tends to occur, then the focal parameter associates with a causal force that shapes the trait, revealing the fundamental forces of biological design.

This book divides into two parts. The first part presents the conceptual tools for making comparative predictions. The second part develops comparative predictions for metabolic traits.

We can use this approach to make comparative predictions for the full range of microbial traits, providing a general method for the study of biological design.

1.1 How to Read

Part 1 sets the theoretical background. How does one form and test predictions about the forces that shape biological design?

Part 2 turns to unsolved puzzles in microbial metabolism. How can we use Part 1's principles for the study of design to advance the understanding of microbial evolution?

Readers primarily interested in microbes may wish to start with the second part. As particular concepts arise in that second part, one may follow the pointers to the first part to fill in the background.

Readers primarily interested in evolutionary concepts may wish to start with the first part. The second part illustrates how to turn those concepts into a fully realized program of empirical study.

Although each part stands alone, the real value comes from the synergy between parts. Full progress demands combining Part 1's evolutionary concepts and general principles for studying causality with Part 2's application to metabolism, the engine of life.

That pairing between theory and application provides the best way to study the forces that have shaped biological design.

To help readers find their preferred starting point and path through the book, the following sections briefly summarize each chapter.

1.2 Theoretical Background

Organismal traits often seem designed to solve environmental challenges. Presumably, natural processes have shaped design. However, the underlying processes can be difficult to observe.

How can we study those causal forces of design? Somehow, we must link the hidden forces to the observed traits. Part 1 develops the theoretical background to meet that challenge.

Chapter 2 defines design in relation to biological fitness, the ultimate measure of success. Three fundamental forces of design often dominate. Marginal values measure trading one design for another. Reproductive values weight different components of fitness, such as reproduction, survival, and dispersal. Generalized kin selection links the similarity of interacting individuals with the transmission of traits through time.

Chapter 3 turns to the causal analysis of design. We can rarely match organismal traits to the forces of design that shaped those traits. Many particular forces played a role. We cannot measure or infer all of them.

Instead, we must focus on change. Can we predict how change in a specific factor alters a particular trait? For example, how does increasing genetic variability between competitors alter reproductive rate?

Comparing states of a particular factor isolates partial causality, the change caused holding all else constant. Comparative prediction becomes the building block of causal understanding. How does a changed factor alter a trait, mediated by a fundamental force of design?

Chapter 4 illustrates comparative predictions. The examples link changes in environmental factors to predicted changes in the metabolic traits of microbes. Each hypothesis associates the predicted change in a metabolic trait to a causal force of biological design.

The following chapters of Part 1 fill in the theoretical background needed to develop comparative predictions. Part 2 uses that theory to make comparative predictions about organismal design, with emphasis on microbial metabolism.

Chapter 5 reviews various forces that shape biological design. Marginal values, reproductive values, and generalized kin selection play key roles, as noted above. Natural history modulates forces of design. Examples include demography and complex life cycles, the scaling of spatial and temporal environmental variability, and the different timescales over which competing design forces act.

Chapter 6 notes that biological design concerns organismal traits. However, the nature of traits often remains vague. Different problems arise when studying the evolutionary origin of traits versus the modification of traits. Some traits change within an organism in response to the environment. Other traits may be genetically fixed, varying only between individuals rather than within them.

Chapter 7 extends discussion of traits that vary within an individual. Much of evolutionary design concerns the control of such traits in response to environmental signals. This chapter reviews principles of engineering control theory as they may be applied to biological design. Error-correcting feedback is perhaps the single greatest principle of design in both human-engineered and biological systems.

Chapter 8 contrasts this book's comparative predictions with historical antecedents. Darwin developed comparison in the study of adaptation. Classic phylogenetic comparative methods extended Darwin's vision.

This book differs primarily in the scale of change. Prior analyses typically studied change between species or higher taxa. By contrast, design forces often act at smaller scales of change. Those smaller scales set the focal point for this part's theory and the following part's application to microbial metabolism.

1.3 The Design of Metabolism

In microbes, large populations and short generation times provide opportunity to observe small-scale changes in action. Progress in technology and measurement opens new windows onto those small-scale changes. Part 2 takes advantage of this new era in the study of biological design to advance the testing of comparative hypotheses.

Chapter 9 explains the focus on metabolism. Extracting and using the free energy driving force from food is a universal challenge of life. Microbial metabolism provides a good starting problem to sharpen our tools in the study of biological design.

Chapter 10 illustrates comparative hypotheses and tests by analyzing microbial growth rate, typically measured as the increase in biomass. Growth rate seemingly provides the simplest trait by which to measure fitness, the long-term contribution to the future population.

However, tradeoffs arise. Faster short-term growth may use resources inefficiently. Lower efficiency reduces reproductive yield per unit food

uptake, slowing long-term growth as food gets used up. Comparatively, decreasing the available food raises the marginal gains for yield efficiency. Enhanced gains for yield predict lower short-term growth rate, driven by the fundamental force of marginal valuations between alternatives.

This chapter lists many comparative hypotheses. Those hypotheses link changes in natural history to predicted changes in growth rate. The analysis then turns to testing comparative hypotheses. Examples illustrate the kinds of data that have recently been collected in natural and laboratory populations.

Chapter 11 develops the universal challenge of extracting free energy from food to drive the processes of life. The thermodynamic driving force of free energy comes from moving low entropy electrons in food to high entropy electrons in final electron acceptors, such as oxygen.

Metabolic design exploits the increasing entropy between food and final electron acceptors to drive coupled reactions that decrease entropy. The decreased entropy of the driven reactions creates the ordered molecules of life or the entropy disequilibria, such as ATP versus ADP, that act as storage batteries to drive subsequent order-creating processes.

Textbook descriptions of biochemical thermodynamics often fail to emphasize how the entropy disequilibria in food drive the entropy disequilibria of life.[18,47,294] Studying metabolic design requires focus on the flux of those coupled disequilibria through metabolic cascades.

Metabolic flux also depends on the resistance to reactions from chemical activation barriers. Cells modulate resistance by using enzyme catalysts or by changing the biochemical conditions. Net flux depends on the thermodynamic driving force divided by the resistance to reaction, an analogy with Ohm's law of electric current flow.

Chapter 12 describes how cells modulate flux by altering the thermodynamic driving force. The greater the displacement of a reaction from equilibrium, the greater the driving force and the rate of reaction. High driving force also causes the loss of potentially usable entropy change, typically dissipated as heat.

This chapter analyzes the design of glycolysis in terms of the thermodynamic tradeoff between reaction rate and usable entropy yield. Recent technical advances allow direct in vivo measurement of the driving force for individual reactions within the glycolytic cascade.

Those direct measurements open up new possibilities to study comparative hypotheses. For example, environmental changes in cellular

competition and genetic variability may alter the fine-scale design of metabolic flux control. Large-scale biochemical changes between alternative glycolytic pathways also pose interesting puzzles of design.

Overflow metabolism presents a key challenge. Many microbes excrete post-glycolytic products that contain most of the usable entropy in the original food source. Why overflow usable food? Disequilibria, thermodynamic driving force, and the tradeoff between rate and yield play important roles. Changed conditions alter overflow, providing a model to test comparative hypotheses about metabolic design.

Chapter 13 discusses the modulation of flux by altering the resistance of reactions. Mechanisms include varying enzyme concentration, modifying enzyme structure, and spatially separating reactants.

Changes in metabolic design may alter thermodynamic driving force or the resistance to reactions. Small changes typically occur by modulating current biochemical pathways. Larger changes may lead to different biochemical pathways. Other design goals shape pathways, such as the need for precursors to build particular molecules.

Constraining forces interact with design forces. For example, cell size constrains space for protein catalysts. Limited proteins impose tradeoffs between the potential to modulate different reactions.

Flux control has been widely discussed. However, clearly specified comparative hypotheses remain scarce with regard to the forces of design and constraint that have shaped metabolic diversity. This book sets the foundation on which to build comparative hypotheses and provides many examples of such hypotheses.

Chapter 14 turns to the observed diversity in metabolic pathways. The biochemical detail in this chapter raises many puzzles, setting a challenge for comparative predictions and tests of metabolic design.

In one example, different glycolytic pathways have different yields of ATP, NADH, and NADPH, each of which create distinct disequilibria that drive different cellular processes. In another example, the diverse final electron acceptors of catabolism create different entropy gradients, which greatly influence metabolic design. Weak gradients pose special design challenges.

Metabolic electron flow sometimes happens between cells of the same or different species. Distributed electron gradients raise novel puzzles in metabolic design. Those puzzles often depend on how particular biochemical disequilibria enhance or limit electron flow.

This chapter also analyzes the regulation of alternative sugar catabolism within cells and cellular shifts between different complex carbohydrate food sources. The chapter's conclusions synthesize puzzles of design for variant pathways.

Chapter 15 emphasizes tradeoffs, which set the basis for design. For example, faster growth reduces food use efficiency. Less permeable membranes protect against attack but slow resource uptake.

However, particular tradeoffs often fail to reveal design. Suppose growth rate, yield efficiency, and defense trade off. Less attack reduces investment in defense, potentially increasing both growth rate and yield. Without measurement of defense, one might see only the simultaneous rise in rate and yield, apparently contradicting the rate-yield tradeoff.

Comparative hypotheses about the tradeoffs themselves may help. For example, more abundant food weakens the tradeoff between growth rate and yield efficiency.

The more completely one understands the range of potential tradeoffs, the more effectively one can make comparative predictions. This chapter provides a preliminary catalog of the tradeoffs that shape the metabolic design of microbes.

Chapter 16 highlights the forces that shape overflow metabolism, the cellular excretion of usable food. Several challenges for inferring design emerge. Forces act over different timescales. Each empirical method reveals particular forces and timescales while hiding others.

Progress requires explicit consideration of the challenges and limitations in the study of biological design. The importance of clear comparative predictions and partial causation rises once again.

Chapter 17 continues the analysis of model problems in metabolic design. Part 1's forces of design play an important role as we broaden the range of metabolic traits and natural history.

When exposed to multiple foods, how do cells express alternative catabolic pathways? Sometimes, preferred foods repress pathways for other foods. Other times, cells simultaneously express different pathways. In some clonal populations, cells differ in expression patterns. Various design forces shape expression. Testable comparative predictions follow.

How do cells overcome limited access to final catabolic electron acceptors such as oxygen? Cable bacteria form filaments with electric wires. The wires pass electrons from anoxic zones to oxic zones, creating strong catabolic flux. Linked cells form various multicellular lengths, altering

life cycles, spatial competition, and the forces of design.

Other species use extracellular shuttle molecules to move electrons from cell surfaces to distant electron sinks. Shuttles, once released from producing cells, can be used by any neighboring cells. Such publicly shareable resources create special challenges. Demography and genetic mixing alter design forces in predictable ways.

When life cycles pass through habitats that prevent catabolism, how do cells store and use resources? Microbial wastewater treatment provides an interesting model system. The treatment passes bacteria through alternate anaerobic and aerobic habitats. Food is available only during the anaerobic phase. However, lack of oxygen prevents catabolism.

In that anaerobic habitat, cells transform food into internal storage. During the aerobic phase, cells catabolize the internal stores. Varying the alternative habitats changes the demographic forces of design.

Wastewater treatment and other industrial applications provide excellent model systems to test comparative predictions about the forces that shape metabolic design.

Chapter 18 revisits problems in the study of biological design.

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