On the dynamics of prebiotic evolution

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1 Introduction

The question of how life arose from non-living matter is the focal mystery where chemistry and molecular biology converge with evolutionary biology. But although it exemplifies the scientific challenge of explaining how and why complex phenomena appear in nature, it defies standard methods of scientific inquiry. The first life to assemble abiotically left no fossils, and scientists are unlikely to prove anything definite about its identity. Instead, origins of life researchers look for conditions under which non-living material assumes certain properties of living material, trying to bridge the chasmal gap that separates living and nonliving chemistry today.

In 1828, Friedrich Wohler's urea synthesis proved that organic molecules can be obtained from inorganic ones via ordinary chemical reactions [36], debunking the 17th century belief that organic materials owe their heat-sensitivity to a "vital energy" that is absent from inorganic material [37]. Wohler's work ignited a science of organic chemistry that has used simple physics to explain most unique properties of biomolecules; in particular, chemists have found many chemical conditions that promote predictable interconversions between organic and inorganic matter. In 1959, Stanley Miller proved that such conditions need not be highly artificial, synthesizing DNA and protein building blocks within an apparatus that simulated a lightening storm over a pool of common sludge [27]. James Ferris later proved that charged clay surfaces and drying conditions are enough to promote the polymerization of complex RNA chains [13]. Such work has effectively demystified the chemical split between life and non-life; although we will never know with certainty how the chemical components of life first appeared on Earth, their presence can be explained in many plausible ways. However, there are no equally satisfying explanations for the advent of self-replication and metabolism, behaviors that biochemicals only exhibit when organized into complex systems. By creating a scientific landscape where the difference between synthetic organic material and natural organic material is organizational complexity, 19th and 20th century biochemists have turned the origins of life question from a chemical synthesis problem into an information-theoretic problem.

The supposed manifestation of 17th century "vital force" in heat sensitivity was spot on in that a protein fails to reconstitute after heating because the information content of its three-dimensional fold has been destroyed. When a protein cannot assume its bio-active, water-soluble native configuration without help from the cellular machinery that its own catalytic activity helps maintain, we are faced with the sort of chicken-or-egg dilemma that creates so much of the mystique surrounding the origins of biological systems. But just as paleontologists know that eggs preceded chickens by millions of years, modern biochemists know that there exist certain proteins that are capable of reconstituting after being heated because they assume their native folds spontaneously. Furthermore, it is well understood via the theory of energy landscapes why certain proteins fold spontaneously and others do not [20].

The information contained in a spontaneously folding protein sequence can propagate itself if it encodes a structure that catalyzes its own replication, as was demonstrated by the

synthesis of a self-replicating peptide in 1996 [26], and the popular RNA World hypothesis postulates that such self-replicating catalysts were the first carriers of genetic information. This hypothesis favors self-replicating RNA molecules over self-replicating proteins because RNA catalysts tend to be simpler than protein catalysts and replicate in a more uniform and straightforward way. However, no protein or nucleic acid polymer has ever been replicated in the laboratory without the help of enzymes derived from cells. Chemists have spent decades trying to synthesize a ribozyme that catalyzes RNA-dependent RNA polymerization faithfully enough to replicate itself, and such an RNA replicase has proved elusive. In 2001, Johnston, et al. reported the synthesis of a ribozyme that is capable of RNA-dependent RNA polymerization, but found that it disintegrated after less than 20 nucleotide additions under the harsh chemical conditions that were required to maintain catalytic activity [23].

Although it is not known how many potential replicases are crippled by catalytic dependence on degradative chemicals, it is possible to mathematically derive some universal constraints on successful molecular replicators. The earliest of these constraints emerge from the *quasispecies model* that was developed in the 1970s by theoretical chemists Manfred Eigen and Peter Schuster [5, 6, 7, 8, 9, 10]. In addition to revealing constraints on the viability of an early molecular replicator, quasispecies theory describes viral population dynamics with remarkable accuracy [29]. Eigen and Schuster also discuss the origin of translation, arguing that an RNA world must have given way to an RNA-and-protein world via a chemical reaction they call a *hypercycle* [7, 8, 9]. However, they say little about chemical systems that predated the advent of virus-like replicators, and it was not until 2008 that Nowak and Ohtsuki modeled the emergence of virus-like replicators from polymers that did not replicate, assembling without templates by a process like clay surface catalysis[1] [28]. When viewed end-to-end, these three bodies of theory constitute a scenario for the transformation of disorganized biomolecules into dynamic populations of self-replicating polymers that eventually become capable of protein synthesis.

My review article will present the theory of this remarkable transformation in the order that it was published, beginning with the quasispecies in Sections 2 and 3, moving to the hypercycle in Section 4, and ending with Nowak and Ohtsuki's "prevolutionary dynamics" in Section 5. Such mathematical models form a cornerstone of origins of life research because even experimental results about the origins of life depend strongly on models of never-observed phenomena. Although a successful experiment can never prove that a certain event actually took place in the prebiotic world, a mathematical result is better suited to proving that a certain chemical process can *never* have helped bring about the origin of life. In addition to providing truly absolute knowledge about the history of life on earth, mathematical results can guide experimentalists toward types of chemical reactions that are likely to display life-like behavior and steer them away from reactions that will never display such behavior. Although it is far from a historical account of early evolution, the theory discussed here is central to our current understanding of the ontogeny of biochemistry.

2 A dynamical model of self-replication

This paper will model the emergence of life-like properties in systems of large molecules whose behavior is best described by the theory of chemical kinetics. Specifically, we consider a solution populated by n chemical species $x_1, \ldots, x_n$ whose players interconvert via chemical reactions of the form

$$a_1x_1 + \cdots + a_nx_n \rightleftharpoons b_1x_1 + \cdots + b_nx_n$$

[1] In [28], Nowak and Ohtsuki treat polymer extension and replication as abstract processes, preferring not to situate their evolutionary dynamics within a particular chemical framework. They would not identify their 'prelife' landscape with anything as concrete as the clay surface polymerization experiments reported in [13], and the viability of their models is not bound up with the viability of clay surface polymerization as a precursor to replication. However, my goal is to piece together an origins of life scenario that is as concrete as possible, and so I will take the liberty of identifying Nowak and Ohtsuki's evolutionary processes with illustrative chemical processes.

for some integers $\{a_i\}, \{b_i\}$ [5]. We will let $[A]$ denote the concentration of species A in solution, and let $\vec{k}$ denote the rate constant of the forward reaction, such that the reactants turn into products at the rate of

$$\vec{k}[x_1]^{a_1} \cdots [x_n]^{a_n}$$

moles per liter per second. Similarly, we will let $\overleftarrow{k}$ denote the rate constant of the reverse reaction, meaning that products turn into reactants at the rate of

$$\overleftarrow{k}\ [x_1]^{b_1} \cdots [x_n]^{b_n}$$

moles per liter per second. We will say that a system is *at equilibrium* whenever the set of concentrations $[x_1], \ldots, [x_n]$ is a solution to the equation

$$\vec{k}[x_1]^{a_1} \cdots [x_n]^{a_n} = \overleftarrow{k}\ [x_1]^{b_1} \cdots [x_n]^{b_n},$$

such that the forward and reverse reactions happen at equal rates and the concentrations $[x_1], \ldots, [x_n]$ remain constant over time. A system is *far to the left of equilibrium* when the chemical concentrations render the reverse reaction much slower than the forward reaction, such that the overall rates of change $d[x_i]/dt$ coincide with the forward reaction rate. Similarly, a system is *far to the right of equilibrium* when the forward reaction is much slower than the reverse reaction.

A reaction is *autocatalytic* whenever there exists x_i for which $b_i > a_i > 0$, in which case x_i is called a *self-replicating species*. When a system subject to this autocatalytic reaction is far to the left of equilibrium, the concentration of the self-replicating species x_i will increase at a rate of

$$(b_i - a_i)\vec{k}[x_1]^{a_1} \cdots [x_n]^{a_n}$$

moles per liter per second. But as a system approaches equilibrium, the rate of change

$$(b_i - a_i)(\vec{k}[x_1]^{a_1} \cdots [x_n]^{a_n} - \overleftarrow{k}\ [x_1]^{b_1} \cdots [x_n]^{b_n})$$

of $[x_i]$ approaches zero. We conclude that autocatalysis can lead to the self-replicating behavior we associate with life, but only in systems that lie far from chemical equilibrium [5].

All isolated chemical reactions fall toward equilibrium with the passage of time;[2] a truly living entity must be capable of metabolism, which pumps energy into the system in a way that keeps autocatalytic processes from nearing equilibrium. Many biologists believe that replication preceded metabolism in that the earliest self-replicating entities did nothing to stave off chemical equilibrium and were mining an existing chemical gradient that they could not maintain on their own. Although a few scientists believe that metabolism actually preceded replication [30], this paper will focus on replication-first scenarios in which the first precursors to life were passively autocatalytic.[3] The next section will construct a

[2] A few chemical reactions never stabilize near equilibrium, but instead oscillate along a limit cycle of nonequilibrium states. The first chemical oscillator reported in the literature is a mixture of potassium bromate, cerium (IV) sulfate, propanedioic acid, and sulfuric acid; as the cerium ions oscillate between an oxidized state and a reduced state, a yellow color repeatedly appears and disappears [1]. Some chemists consider this *Belousov-Zhabotinsky reaction* to be a good theoretical model for nonequilibrium biological dynamics, but it is hard to imagine how a chemical oscillator could beget the stable molecular replicators that focus discussions about the origins of life. By definition, living things must propagate themselves, and it is impossible for an oscillator trapped within a limit cycle to beget copies of itself without disassembling these copies to begin the cycle again.

[3] In a seminal paper on the dynamics of early replicators [7], Eigen lists metabolism, self-reproduction, and mutation as the three chemical prerequisites for evolution by natural selection. However, he defines metabolism to be the continuous formation and degradation of chemical structures that occurs in all non-equilibrated systems. It is more standard to define metabolism to be a mechanism for maintaining chemical non-equilibrium; we classify Eigen's model system as pre-metabolic because it does not include a mechanism for maintaining chemical gradients and will only be subject to natural selection as long as external forces keep it far from equilibrium.

model population of such passive autocatalysts, and will prove several results about the evolution of this population. This model includes no provisions for population structure or other interactions among the replicators, and thus represents the simplest possible model of replicator dynamics.

3 Primitive replicators and quasispecies theory

The work that follows will present Eigen's basic assumptions about a model population of autocatalytic entities and prove several important results that he introduced in [7]. To begin, we will let $x_1, \ldots, x_n$ denote self-replicating polymers and write down n differential equations describing the rates of change of their concentrations as they react with activated monomers to make copies of themselves. When we write out these differential equations explicitly, we will omit concentration brackets and let each variable x_i denote the concentration of a molecular species. We will assume that the reaction conditions remain constant by virtue of a constant influx of activated monomers balanced by an outflux of polymers and their degradation products. In addition, we will assume that the rates at which x_i forms and degrades depend linearly on $[x_i]$, as is the case when one polynucleotide catalyzes the synthesis of one complementary strand by acting as a template.

In a system that lies close to equilibrium, chemical reactions tend to be reversible, meaning that the conversion $A + B \rightharpoonup C$ is a microscopic reversal of the conversion $C \rightharpoonup A + B$. In contrast, systems far from equilibrium are usually dominated by irreversible reactions where the conversions $A+B \rightharpoonup C$ and $C \rightharpoonup A+B$ proceed by completely different mechanisms. Since self-replicating molecules must inhabit chemical systems that lie far from equilibrium, it follows that their formation and degradation will proceed by distinct pathways with different rate-dependencies. For example, the rate $A_i x_i$ at which x_i templates the production of new polymers generally depends much more strongly on catalytic activity than does the rate $D_i x_i$ at which x_i is degraded. Another source of the polymer x_i is the imperfect replication of related polymers x_j; when the sequences x_i and x_j differ by only a few monomer insertions, deletions, and substitutions, x_j will catalyze the production of x_i at the rate $w_{ij} x_j$. Conversely, there is a nonzero probability $1 - Q_i$ that x_i will replicate itself imperfectly and produce some mutant polymer. Finally, a solution outflux will carry away x_i at the rate $\Phi_i(\mathbf{x})$. If we neglect all other factors that affect the concentration of x_i, we conclude that a set of differential equations of the form

$$\dot{x_i} = (A_i Q_i - D_i) x_i + \sum_{k \neq i} w_{ik} x_k - \Phi_i(\mathbf{x}) \tag{1}$$

describes the evolution of the system at hand.

In addition to catalyzing its own replication at a rate of $A_i Q_i x_i$, x_i catalyzes the production of mutant polymers at a rate of $A_i(1 - Q_i)x_i$. When $\{x_1, \ldots, x_n\}$ is the complete set of polymers that inter-mutate within the chemical system at hand, all of the mutants produced by the imperfect replication of x_i are members of this set. In this case, the individual mutant production rates w_{ji} must sum to the net production of mutants $A_i(1 - Q_i)$ that occurs during the replication of x_i:

$$\sum_{i=1}^{n} A_i(1 - Q_i) = \sum_{j \neq i} w_{ji}$$

In test tube evolution experiments, it is common to regulate the flow rates Φ_i so that reaction conditions remain constant and reproducible. Specifically, it is convenient to ensure that the total replicator concentration remains constant:

$$\sum_{i=1}^{n} \Phi_i = \sum_{i=1}^{n} A_i x_i - \sum_{i=1}^{n} D_i x_i \tag{2}$$

We will assume that the Φ_i's have this property so that our system of differential equations will model a snapshot of evolution where conditions are approximately constant and selective effects can be observed most clearly. We constrain the individual Φ_i's so that polymers x_i leave the system in proportion to their representation in the pool:

$$\Phi_i = \left(\sum_{k=1}^{n} \Phi_k \right) \frac{x_i}{\sum_{k=1}^{n} x_k} \tag{3}$$

In order for the n equations of the form (1) to furnish a physically meaningful model of evolution, they must ensure that positive concentration variables never become negative as the system evolves. We will always consider initial conditions for which every x_i is nonnegative, and if we suppose for the sake of contradiction that some x_i is negative at time t, then there exists a time $t_0 < t$ at which $x_i = 0$ and $\dot{x_i} < 0$. Since Φ_i is regulated according to (3), it vanishes at time t_0, meaning that

$$\dot{x_i}(t_0) = \sum_{k \neq i} w_{ik} x_k(t_0).$$

Without loss of generality, we can assume that no x_k becomes negative before x_i does. Therefore, since each w_{ik} is nonnegative, we have a contradiction, and we can be assured that the x_i population variables remain nonnegative indefinitely.

If we assume that outflow is regulated according to (2) and (3) and let $E_i = A_i - D_i$ denote the "excess productivity" of the replicator x_i, then we can let

$$E(t) = \frac{\sum_{i=1}^{n} E_i x_i}{\sum_{i=1}^{n} x_i}$$

denote the average excess productivity as a function of time. This lets us write the replicator equations in a homogeneous form:

$$\dot{x_i} = (A_i Q_i - D_i - E(t)) x_i + \sum_{k \neq i} w_{ik} x_k$$

When the fitness values $W_i = A_i Q_i - D_i$ and the mutation probabilities w_{ik} are constant with respect to time, this system of differential equations is solvable. An exact solution is reported in [33], and good approximate solutions can be obtained using perturbation theory.

The replicator equations highlight the fact that no primitive autocatalyst could realistically drive all other autocatalysts to extinction. Even a "master sequence" x_i whose fitness value W_i is much higher than all competing W_j's continuously populates a range of nearby mutants, except in the chemically unrealistic case $Q_i = 1$. This process produces a distribution of replicators centered at x_i, and Eigen presents a mathematical argument that the replicator mixture will stabilize over time, converging to an eigenvector of the *mutation matrix* $\mathbf{W}$ whose diagonal entries are the W_i's and whose off-diagonal entries are the w_{ij}'s. If we let $\mathbf{x}_i$ denote the n-entry vector whose jth entry is $x_i \delta_{ij}$, then by construction, the replicator population will converge to the stationary state $\lim_{k \to \infty} \mathbf{W}^k \mathbf{x}_i$, which is an eigenvector of $\mathbf{W}$ by the theory of finite-state Markov chains.[4] It is thus more appropriate to say that natural selection acts on eigenvectors than on individual replicator species; for this reason, Eigen refers to the eigenvectors of $\mathbf{W}$ as *quasispecies*. His argument implicitly assumes that the spectral radius of $\mathbf{W}$ is an eigenvalue of $\mathbf{W}$ and that the corresponding eigenvector $(c_1 \cdots c_n)^T$ has nonnegative entries, such that the linear combination $c_1 x_1 + \cdots c_n x_n$ is a physically meaningful sequence distribution to which the chemical solution makeup can

[4]The convergence of a replicator population to a steady state concentration vector looks uncomfortably similar to the onset of chemical equilibrium, and we will even refer to such a steady state as an equilibrium point. However, a steady-state population lies far from chemical equilibrium as long as it depends upon a constant influx of energy-rich molecules such as activated nucleotides, which is the case for every replicator population at equilibrium that we will encounter in this paper.

converge. We can justify both of these assumptions using the following theorem, which is a standard linear algebra result proved in references like [19]:

Theorem 1 (Perron-Frobenius Theorem, [19])**.** *If $A = (a_{ij})$ is a real $n \times n$ matrix with positive entries $a_{ij} > 0$ and eigenvalues $\lambda_1, \ldots, \lambda_n$, then the following statements hold:*

1. *There is a unique positive real eigenvalue λ_i for which*

$$\max\{|\lambda_1|, \ldots, |\lambda_n|\} = \lambda_i$$

2. *λ_i is a simple root of the characteristic polynomial of A.*

3. *λ_i is associated with an eigenvector that has strictly positive entries. Conversely, there is a unique nonnegative eigenvector $(v_1, \ldots, v_n)$ of A for which $v_1 + \cdots + v_n = 1$.*

4. *The matrix entries obey the inequality*

$$\min_j \sum_k a_{jk} \leq \lambda_i \leq \max_j \sum_k a_{jk}.$$

Assuming that the x_i's are all potentially viable replicators for which $A_i Q_i - D_i \geq 0$, such that each would prosper in the absence of competitors, $\mathbf{W}$ has nonnegative entries. In addition, the mechanics of nucleic acid replication generally ensure that if there is a nonzero probability that x_i will give rise to x_j after n generations, then there is a nonzero probability that x_i will give rise to x_j after one generation. With this justification, it will be convenient to assume that $\mathbf{W}$ is a block diagonal matrix for which each block $\mathbf{W}_i$ has strictly positive entries. When W_i and W_j are entries in separate blocks, the corresponding replicators x_i and x_j do not catalyze one another's replication, but only interact by competing for resources.

If we let $\lambda_{i_1}, \ldots, \lambda_{i_m}$ be the m eigenvalues of the block $\mathbf{W}_i$, then the Perron-Frobenius theorem guarantees that there exists a real λ_{i_k} for which $|\lambda_{i_j}| < \lambda_{i_k}$ for all $j \neq k$ and that the coefficients of the eigenvector $\mathbf{y}_{i_k} = c_{i_k 1}\mathbf{x}_1 + \cdots + c_{i_k n}\mathbf{x}_n$ are nonnegative. We also define scalar concentration variables $y_1, \ldots, y_n$ such that when $\mathbf{y}_i = c_{i1}\mathbf{x}_1 + \cdots + c_{in}\mathbf{x}_n$, we have $y_i = c_{i1}x_1 + \cdots + c_{in}x_n$. When the c_{ij}'s are nonnegative, the quasispecies has physical meaning as a pool of related polymers that coexist in specific ratios. In addition, when a physically meaningful quasispecies $\mathbf{y}_k$ is associated with a positive real eigenvalue λ_k, we can see that $\mathbf{y}_k$ produces offspring at a rate of $\lambda_k y_k$, such that the size of λ_k dictates the fitness of $\mathbf{y}_k$. Thus, although it will be mathematically expedient to model the system's evolution as a competition among quasispecies that do not all have physical meaning, the fittest quasispecies will always be physically meaningful entities whose success can be experimentally measured. These quasispecies will behave as if they are competing against less fit quasispecies that do not have the same physical meaning, but these imaginary quasispecies will disappear with time.

Since the set of real $n \times n$ matrices that are not diagonalizable over $\mathbb{C}$ is the complement of the measure-zero hypersurface in $\mathbb{R}^{n \times n}$ of matrices with the property that the discriminant of the characteristic polynomial vanishes, we can perturb $\mathbf{W}$ infinitesimally if necessary and assume that it is diagonalizable over $\mathbb{C}$, such that the eigenvectors $\mathbf{y}_1, \ldots, \mathbf{y}_n$ are linearly independent. Therefore, there exist coefficients $c_1, \ldots, c_n$ for which $\sum_{i=1}^n \mathbf{x}_i = \sum_{i=1}^n c_i \mathbf{y}_i$, and if necessary, we can infinitesimally perturb $\mathbf{W}$ to ensure that the c_i's are all strictly positive. After doing so, we can choose $c_1\mathbf{y}_1, \ldots, c_n\mathbf{y}_n$ as our representative quasispecies eigenvectors and assume that $\sum_{i=1}^n \mathbf{x}_i = \sum_{i=1}^n \mathbf{y}_i$.

Proposition 2. *The average excess productivity $E(t)$ is invariant under a change to quasispecies coordinates, with*

$$E(t) = \frac{E_1 x_1 + \cdots + E_n x_n}{x_1 + \cdots + x_n} = \frac{\lambda_1 y_1 + \cdots + \lambda_n y_n}{y_1 + \cdots + y_n}.$$

Proof. Let $\sum_{j=1}^{n} (\mathbf{W}\mathbf{x}_i)_j$ be the sum of the entries of the vector $\mathbf{W}\mathbf{x}_i$, with respect to the basis where $(\mathbf{x}_i)_j = x_i\delta_{ij}$. By construction, we can see that

$$\sum_{j=1}^{n} (\mathbf{W}\mathbf{x}_i)_j = (A_iQ_i - D_i + \sum_{k\neq i} w_{ik})x_i = E_ix_i.$$

We now have

$$E(t) = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} (\mathbf{W}\mathbf{x}_i)_j}{\sum_{i=1}^{n} x_i}.$$

Since the vectors $\mathbf{y}_1, \ldots, \mathbf{y}_n$ span replicator concentration space, there exist coefficients $k_{i1}, \ldots, k_{in}$ for which $\mathbf{x}_i = k_{i1}\mathbf{y}_1 + \cdots + k_{in}\mathbf{y}_n$. Furthermore, since $\sum_{i=1}^{n} \mathbf{x}_i = \sum_{i=1}^{n} \mathbf{y}_i$, it must be true that $\sum_{i=1}^{n} k_{ij} = 1$. Therefore,

$$E(t) = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} (\mathbf{W}\mathbf{x}_i)_j}{x_1 + \cdots + x_n} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} (\mathbf{W}(k_{i1}\mathbf{y}_1 + \cdots + k_{in}\mathbf{y}_n))_j}{y_1 + \cdots + y_n}$$

$$= \frac{\sum_{j=1}^{n}\sum_{\ell=1}^{n}(k_{i1} + \cdots + k_{in}) (\mathbf{W}\mathbf{y}_\ell)_j}{y_1 + \cdots + y_n} = \frac{\sum_{j=1}^{n}\sum_{\ell=1}^{n} \lambda_\ell (\mathbf{y}_\ell)_j}{y_1 + \cdots + y_n}.$$

Letting $\mathbf{y}_i = c_{i1}\mathbf{x}_1 + \cdots + c_{in}\mathbf{x}_n$ as before, we now have

$$\frac{\sum_{j=1}^{n}\sum_{\ell=1}^{n} \lambda_\ell (\mathbf{y}_\ell)_j}{y_1 + \cdots + y_n} = \frac{\sum_{j=1}^{n}\sum_{\ell=1}^{n} \lambda_\ell (c_{\ell 1}\mathbf{x}_1 + \cdots + c_{\ell n}\mathbf{x}_n)_j}{y_1 + \cdots + y_n}$$

$$= \frac{\sum_{\ell=1}^{n} \lambda_\ell (c_{\ell 1}x_1 + \cdots + c_{\ell n}x_n)}{y_1 + \cdots + y_n} = \frac{\lambda_1 y_1 + \cdots + \lambda_n y_n}{y_1 + \cdots + y_n}.$$

□

In terms of this coordinate-invariant quantity, the equations describing the evolution of the system take the form

$$\dot{y_i} = (\lambda_i - E(t))y_i.$$

We will now state and prove a proposition implying that our system of replicators evolves toward a state populated entirely by the fittest quasispecies:

Proposition 3. *Let $\boldsymbol{W}$ be a mutation matrix with eigenvalues $\lambda_1, \ldots, \lambda_n$ and quasispecies eigenvectors $\boldsymbol{y}_1, \ldots, \boldsymbol{y}_n$. As the replicator system described by $\boldsymbol{W}$ evolves with time, we can show that*

$$\lim_{t\to\infty} y_i = 0$$

whenever

$$|\lambda_i| < \max_{k\leq n} |\lambda_k|.$$

Proof. We can describe the evolution of this system over a large time interval T up to an arbitrary level of precision by dividing T into m small time intervals $[T_0, T_1], \ldots, [T_{m-1}, T_m]$ such that

$$E(T_i + t)|_{t<T_{i+1}-T_i} \approx E(T_i)$$

can be regarded as a constant and $y_i|_{t<T_{i+1}-T_i}$ can be regarded as a simple exponential function:

$$y_i|_{t<T_{i+1}-T_i} \approx C\exp\left((\lambda_i - E(T_i))t\right)$$

If $|\lambda_1| = \cdots = |\lambda_n|$, then there is nothing to prove. Otherwise, there exists i for which $|\lambda_i| < |E(T_1)|$. When $|\lambda_i| < |E(T_j)|$, $|y_i|$ will decline exponentially over the interval $[T_{j-1}, T_j]$; similarly, $|y_i|$ will increase exponentially over T_j whenever $|\lambda_i| > |E(T_j)|$. Therefore,

$$|E(T_{i+1})| = \left| \frac{\sum_k \lambda_k y_k(T_{i+1})}{\sum_k y_k(T_{i+1})} \right| > \left| \frac{\sum_k \lambda_k y_k(T_i)}{\sum_k y_k(T_i)} \right| = |E(T_i)|.$$

If we let S_i denote the set of $i \in [n] = \{1, \ldots, n\}$ for which $|\lambda_i| > |E(T_i)|$, then we can see that

$$\lim_{t \to \infty} y_i = 0$$

for all $i \in [n] \setminus S_i$. Therefore, we need only suppose that $|\lambda_l| < \max_{k \leq n} |\lambda_k|$ and show that there exists T_j for which $|\lambda_l| < |E(T_j)|$. We have already shown that the set S_i has the property that

$$\lim_{t \to \infty} E(t) = \frac{\sum_{k \in S_i} \lambda_k y_k}{\sum_{k \in S_i} y_k}.$$

Therefore, either $\lambda_j = \lambda_k$ for all $j, k \in S_i$, or there exist $\lambda_j \in S_i$ and $k > i$ for which $|E(T_k)| > |\lambda_j|$. We deduce that $S_k \subsetneq S_i$, such that

$$\lim_{t \to \infty} E(t) = \frac{\sum_{j \in S_k} \lambda_j y_j}{\sum_{j \in S_k} y_j}.$$

Therefore, the set $\bigcap_{i=1}^m S_i$ contains exactly those λ_i that equal $\max_{j \leq n} |\lambda_j|$, and all other λ_k have the property that

$$\lim_{t \to \infty} y_k = 0.$$

□

We can think of a dominant quasispecies as a collection of "mutant sequences" that cluster around a "master sequence" whose fitness is maximal. Theory and experiment show that the "master sequence" rarely makes up more than a few percent of the replicator population [7, 29]; although each individual mutant is much less abundant than the master sequence, the vast number of possible mutants lets them dominate the population. But despite the relatively low abundance of master copies, the fitness of a quasispecies depends much more upon the fitness of the master than upon the fitness of the mutants. Using second-order perturbation theory, Eigen and Schuster compute that the largest eigenvalue λ_m of a mutation matrix without fitness level degeneracies is approximately

$$W_m + \sum_{k \neq m} \frac{w_{km} w_{mk}}{W_m - W_k},$$

where W_m is the fitness of the master sequence [7]. For polymers that replicate with low error rates, the w_{ij} terms will be much smaller than W_m, and so the fitness of the master quasispecies will be very close to the fitness of its master sequence. However, high replication error rates can lead to the success of quasispecies that are much fitter than their master sequences. We have seen that a quasispecies centered at x_m will proliferate if and only if x_m produces more "offspring" than its competitors produce on average, such that

$$A_m - D_m > \frac{\sum_{k \neq m} (A_k - D_k) x_k}{\sum_{k \neq m} x_k}. \tag{4}$$

However, it is possible for the concentration of x_m to decrease at the same time that a quasispecies centered at x_m proliferates. As mentioned earlier, the number of different mutants that can be produced by imperfect replication of x_k is so great that the abundance of any particular mutant in the resulting pool is very small. Accordingly, the amount of x_m that is produced by "back-mutation" is negligible, and so the frequency of perfect copies of

x_m will only increase with time if x_m makes perfect copies of itself at a rate that exceeds the average productivity of competing sequences, such that

$$A_m Q_m - D_m > \frac{\sum_{k \neq m} (A_k - D_k) x_k}{\sum_{k \neq m} x_k}. \tag{5}$$

In a system where the fittest quasispecies is centered around a sequence satisfying (4) but not (5), we observe a phenomenon known as the *error catastrophe*. When each individual sequence frequency x_i approaches zero as time approaches infinity, the polymers in solution distribute themselves uniformly across sequence space. In reality, a finite sequence population will sample sequence space stochastically, evolving by random drift as every sequence that appears inevitably dies out [10].

Inequality (5) dictates a sharp *error threshold* that is usually represented as a minimum replication accuracy that is required to propagate a master sequence of a given length and fitness. It is standard to assume that the replicators are assembled by adding monomers one by one to a growing chain, and that the probability of incorporating the wrong monomer during any given assembly step is q.[5] If the replicators $x_1, \ldots, x_n$ are polymer chains of length ν, then $Q_1 = \cdots = Q_n = q^\nu$. Therefore, we can let

$$\sigma_m = \frac{A_m}{D_m + \frac{\sum_{k \neq m} (A_k - D_k) x_k}{\sum_{k \neq m} x_k}}$$

denote a superiority parameter for the dominant species x_m and observe that an error catastrophe will happen if and only if

$$\nu > \frac{\log \sigma_m}{1 - q}.$$

Unlike many results in the theory of prebiotic evolution, the existence of an error threshold implies many testable claims about modern organisms. Experimentalists have created error catastrophes *in vitro* by sabotaging error-correcting mechanisms that maintain viral mutation rates below the error threshold [7]. In addition, they have measured a general inverse correlation between a genome's size and the effectiveness of its error-correcting machinery (see Figure 1). An RNA-dependent RNA polymerase like viral Qβ replicase is accurate enough to preserve the information contained in a genome about 10^4 nucleotides long, which is the maximum length of a single-stranded RNA virus genome. Double-stranded genomes allow for error checks that reduce the mutation rate by as much as three orders of magnitude; it has been observed that double-stranded virus genomes can be 10^5 nucleotides long, while bacterial genomes can be 10^7 nucleotides long [7]. Eukaryotic organisms use still more complex error-correction mechanisms, some made possible by diploidy, to achieve even smaller error rates that make larger genomes possible.

Although the error threshold furnishes a beautifully nonobvious explanation for the inverse correlation between genome size and mutation rate, it must be pointed out a genome encoding complex error-correcting enzymes must be large enough to encode such enzymes. Biologists estimate that no genome with fewer than 10^4 nucleotides could encode a system capable of faithfully translating DNA sequences into protein sequences [7]; however, we noted earlier that 10^4 nucleotides is the maximum length that single-stranded RNA genomes can achieve. It is reasonable to assume that modern RNA viruses have evolved polymerases

[5] Although both of these assumptions are standard, they represent oversimplifications. In modern DNA and RNA synthesis, purine $\rightarrow$ purine and pyrimidine $\rightarrow$ pyrimidine substitutions are much more common than purine $\rightleftharpoons$ pyrimidine substitutions [7]. It is also possible that early template-directed polymerization was less organized than modern nucleic acid synthesis and did not proceed by the sequential addition of monomers. Unpublished computer simulation data suggest that more chaotic systems also exhibit error thresholds; however, it is easiest to put a numerical value on the error threshold when we make the stated assumptions about replicator assembly mechanics.

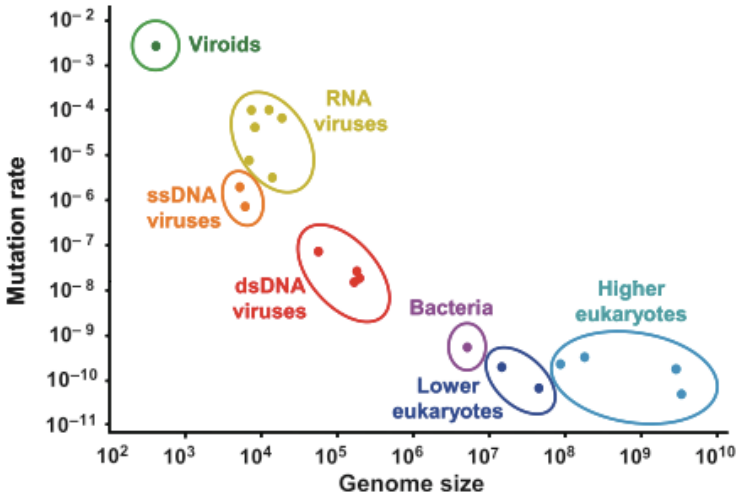

Figure 1: This graph, taken from a recent paper reporting that 399-nucleotide viroid genome has the exceptionally high error rate of 1/400 [16], illustrates the fact that the error threshold pressures large genomes to evolve high-fidelity replication mechanisms.

that are as accurate as is chemically possible, and such polymerases could not evolve without the help of a translation mechanism that intertwines the fitness of a protein enzyme with the fitness of the polynucleotide genome it replicates. We conclude that independent polynucleotide replicators that have not yet evolved to encode translation machinery can never grow long enough to encode such machinery. This hypothetical barrier for molecular evolution is known as *Eigen's paradox*.

Scientists have proposed several solutions to Eigen's paradox, the most popular being the hypothesis that RNA can catalyze high-fidelity RNA replication. An *RNA replicase* capable of reproducing sequences of 10^4 nucleotides could have maintained pools of replicators that eventually gave rise to protein translation machinery, resolving the chicken-or-egg dilemma that we described earlier. But although molecular biologists have spent decades searching for such a molecule and have created ribozymes that catalyze template-directed RNA polymerization [23], none of these ribozymes are accurate enough to replicate their own sequences. Eigen himself proposed a different solution to his paradox, and the next section will furnish a description of his solution.

4 The hypercycle

Eigen's paradox presents a serious obstacle to the acquisition of translational function by a lone self-replicating molecule. However, we have already shown that no self-replicating molecule can completely outcompete other molecules that belong to the same quasispecies, such that it is unreasonable to picture a replicator evolving in isolation. We assumed earlier that a quasispecies derives its fitness from the autocatalytic abilities of its isolated constituents, but it is natural to suppose that the related sequences making up a quasispecies could evolve cooperative behaviors in response to kin selection. Experiments where "digital organisms" compete for computer processor space have shown that the fitness of a quasispecies can depend strongly on the fitness of the mutants it harbors; a 2001 study conducted at the Caltech Digital Life Laboratory reported that quasispecies with flat fitness peaks can outcompete other quasispecies centered around higher but narrower fitness peaks [35], where a few sequences are extremely fit but their close neighbors are considerably less fit.

Cooperation among members of a quasispecies could raise the average fitness of that quasispecies even more effectively than can fitness peak flatness, partly because a cooperative

of free-living molecules can survive replication at higher error rates than can a single molecule containing the same amount of information. We can convince ourselves of this by a thought experiment, in which sequences A, B, and C form a molecular cooperative and replicate at an error rate that is below the minimum threshold at which each sequence can replicate individually, but is above the threshold at which the concatenation ABC could replicate. In a pool where A, B, and C are joined to form one molecule, the abundance of perfect ABC master sequences will decline after each replication cycle, even as the abundance of sequences containing a perfect copy of A increases. We can explain this by noting that the master sequence never makes up more than a few percent of a population replicating near the error threshold; even if a replication cycle increases the abundance of A from 5% to 6% and increases the abundance of B from 5% to 6%, it is unlikely that the perfect copies of A will be joined to the perfect copies of B, meaning that the perfect copies of ABC will decline and disappear. In contrast, a population where A, B, and C are free-living and perfect copies of these sequences can find one another in solution will see the abundance of ABC molecular cooperatives increase from 5% to 6%, and the sequence information will survive. Manfred Eigen and Peter Schuster explored different molecular cooperative structures using dynamical systems theory, varying the ways that different molecular species catalyze one another's replication. They found that some catalytic networks dissolved as key molecular species went extinct, while others stabilized and prospered [7, 8, 9]. In [8], Eigen and Schuster argue that the only stable autocatalytic network is an unbranched circular network that they call a *hypercycle*, and in [9], they describe a specific hypercycle that could account for the origin of translation in an RNA quasispecies.

The simplest natural hypercyles occur because of the fact that real nucleic acids strands are *not* autocatalytic. The only truly autocatalytic single-stranded RNA molecules are palindromes; all other single-stranded RNA molecules template the replication of complementary sequences, and these complementary sequences return the favor. Most modern catalytic RNA molecules are single strands that fold up into clover-leaf shapes with diverse tertiary structures, and such molecules must propagate themselves within catalytic cycles where complementary polymers catalyze one another's replication. Eigen envisioned the hypercycle as a generalization of this process, with n molecular species $E_1, \ldots, E_n$ interacting such that E_k catalyzes the formation of E_{k+1} for $k < n$ and E_n catalyzes the formation of E_1. A collection of ten RNA 100-mers organized into a hypercycle could theoretically replicate without the help of an optimally adapted protein polymerase, yet contains enough information to support a translation system.

We saw in the previous section that a dominant quasispecies outcompetes all other related and unrelated replicators in its environment. This selective pressure makes it unlikely for a hypercycle to evolve from out of catalytic interactions among members of different quasispecies. One might object that the information contained in ten RNA 100-mers belonging to the same quasispecies is less than the information content of a typical RNA 1000-mer, since molecules belonging to the same quasispecies have many residues in common, such that the Shannon information content of their concatenation is not maximal [32]. However, the RNA sequences of modern translation apparatuses do not have maximal Shannon information content either. Besides the ribosome that attaches a new amino acid to the end of a growing polypeptide, our cells express twenty transfer RNAs (tRNAs) that link free amino acids to the RNA codons that call for them, plus twenty aminoacyl-tRNA synthetases that attach amino acids to tRNAs according to the genetic code. Two modern tRNAs are similar enough to be well-represented in the same quasispecies, as are two modern synthetases. Eigen and Schuster postulate that the earliest translation apparatus could do without ribosomes and synthetases by substituting non-specific inorganic catalysts and modifying the structure of a tRNA [9], leaving an army of tRNAs that differ only in a few amino acid-specific residues and could easily inhabit one quasispecies. Phylogenetic analysis of modern tRNA sequences suggest that they could have diverged from a common ancestor within a quasispecies framework [11, 12]. In addition, a computer simulation of RNA evolution that assigns a selective advantage to rows of stable internal base pairs has a very high probability

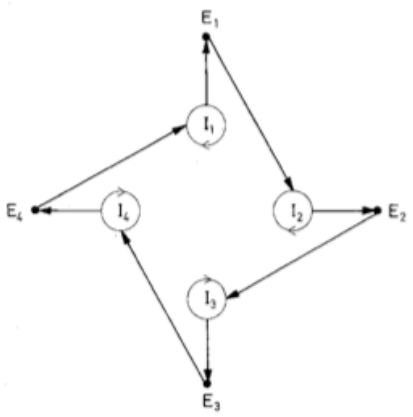

Figure 2: In this simple hypercyclic translation apparatus ($n = 4$) [9], $I_1, \ldots, I_4$ are tRNA-like molecules that double as templates for the peptide catalysts $E_1, \ldots, E_4$. When E_i is most efficient at catalyzing the replication of I_{i+1} and E_4 is most efficient at catalyzing the replication of I_1, all molecular species survive indefinitely, stabilizing at finite equilibrium concentrations. If we complicate this hypercycle by introducing additional catalytic dependencies such as $E_4 \rightarrow I_2$, then as long as these dependencies facilitate reactions with weaker rate constants than the reactions depicted above, they will alter the position of equilibrium slightly without jeopardizing the stable coexistence of all replicators [9].

of producing a clover leaf structure that resembles tRNA [5]. These pieces of evidence add support to the idea that tRNA was the first part of the translation apparatus to evolve.

Eigen and Schuster assume that the first translation systems evolved when a set of replicators $I_1, \ldots, I_n$, which belong to a single successful quasispecies, began to catalyze peptide synthesis by acting as tRNAs. With these tRNAs present in solution, the polynucleotides I_k could template the formation of polypeptides E_k, and these polypeptides would amplify the production of the I_ks. When the system can do without synthetases and E_i is a replicase specialized for the polymer $x_{i+1-\delta_{ni}n}$, we get a hypercyclic structure (see figure 2).

In theory, it is as plausible that the E_k's will act as synthetases as it is plausible that they will act as replicases. Eigen and Schuster propose two alternate causal networks where the products of translation function in this way (see figure 3).

The arrows in these schematics represent causal links that show up in the set of differential equations that govern replicator proliferation as in Section 2. The diagrams only lack causal arrows between the I_k replicators and the $I_k \rightarrow E_k$ translation process because of an implicit assumption that templates, and not tRNAs, are the limiting reagents in the translation process. This assumption will be valid at the beginning of the translation process because all I_ks are abundantly produced in the quasispecies distribution, but it will stop being valid if any concentration ratio I_k/I_j increases unboundedly as the dynamical system evolves. For this reason, a viable translation system is one where the participant concentrations $I_1, \ldots, I_n$ approach a stable equilibrium where all species coexist and the assumption of abundant tRNAs remains valid as the original quasispecies succumbs to competition and disappears. The central project of [8] is to show that a simple, unbranched hypercycle is the only catalytic network that achieves such a stable equilibrium over time, and we now proceed to discuss the mathematics of this argument.

As before, we will start with polynucleotide replicators $x_1, \ldots, x_n$ and assume that the total concentration of polynucleotides $c = \sum_{i=1}^{n} x_n$ remains constant. New polynucleotides enter the system via fitness-dependent replication as old ones leave by an outflow ϕ. The rate ϕ_i at which x_i flows out of the system is dependent only on its abundance:

$$\phi_i = \frac{x_i}{c}\phi$$

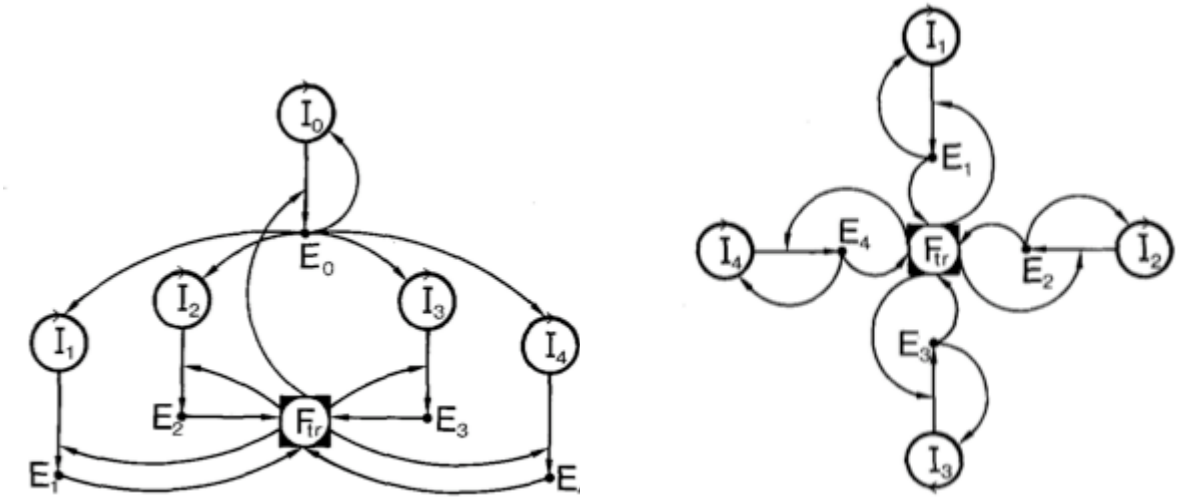

Figure 3: These hypothetical primitive translation networks are heavily branched, meaning that they are not stable hypercycles [9]. In the network on the left, peptide E_0 acts as an RNA polymerase, while all of the other catalytic peptides act as tRNA synthetases. The collection of all four synthetases is required for translation to proceed, and this collection is depicted as a separate species F_{tr}. In the network on the right, all four peptides double as polymerases and synthetases. However, the system is doomed to instability by the fact that E_i replicates its parent polynucleotide I_i, leading to excessive competition among the individual replicators.

When the x_i are evolving as part of a simple quasispecies, we assume that that they obey linear growth equations

$$\dot{x_i} = W_i x_i + \sum_{k \neq i} w_{ik} x_k - \phi_i.$$

In contrast, the advent of translation provides new ways for the x_i's to catalyze one another's production, thereby introducing non-linearities into the equations governing system growth. To reflect this fact, we will begin writing replicator functions in the general form

$$\dot{x_i} = \Gamma_i(\vec{x}) - \phi_i.$$

The condition

$$\dot{c} = \frac{d}{dt}\left(\sum_{i=1}^{n} x_i\right) = 0,$$

known as *constant organization*, means that outflow exactly balances replicator productivity, such that

$$\phi = \sum_{i=1}^{n} \Gamma_i(\vec{x})$$

and

$$\dot{x_i} = \Gamma_i(\vec{x}) - \frac{x_i}{c} \sum_{j=1}^{n} \Gamma_j(\vec{x}).$$

We have already seen that $\Gamma_i(\vec{x}) = W_i x_i$ when dealing with simple quasispecies. In the simple hypercycle pictured above, the rate equations will include more nonlinearities, with

$$\begin{aligned} \Gamma_1(\vec{x}) &= k_1 x_1 + k_1' x_n x_1 \\ \Gamma_i(\vec{x}) &= k_i x_i + k_i' x_{i-1} x_i \ \forall\ 2 \leq i \leq n \end{aligned}$$

for some rate constants $k_1, \ldots, k_n, k'_1, \ldots, k'_n$.

In general, it is difficult to solve nonlinear differential equations exactly. However, we are less interested in describing the trajectory that a primitive translation system follows toward equilibrium than in deducing whether it ends up at an equilibrium where all catalytic species coexist. Therefore, we will analyze each chemical system by fixing the total concentration of replicators and finding the critical points of the growth equations over the n-simplex whose points span all possible concentration ratios of $x_1, \ldots, x_n$, then determining which of these critical points represent stable equilibria. A critical point will represent a stable equilibrium if it is an attracting point, such that nearby states converge to it with time, and if it is in the interior of the concentration simplex, where all replicator concentrations are positive. When the growth equations are homogeneous in the x_i variables, the identities of their critical points do not depend upon the total concentration c [8]. However, this independence does not hold in general, and so there may be a restricted range of concentrations at which the interior of the simplex contains one or more fixed points.

After locating the critical points at which $\dot{\vec{x}} = 0$, we can verify that some of these critical points represent equilibria using Lyapunov's second theorem on stability:

Lemma 4 (Lyapunov's Second Theorem on Stability, [25])**.** *Suppose that $\dot{\vec{x}} = f(\vec{x})$ is an n-dimensional dynamical system and that $\vec{x_0}$ is a critical point, such that $\dot{\vec{x_0}} = 0$. If there exist a function $V(\vec{x})$ and a neighborhood $U \ni \vec{x}$ in $\mathbb{R}^n$ for which $V(\vec{x_0}) = 0$, $V(\vec{x}) > 0$ for all $\vec{x} \in U$, and*

$$\dot{V}(\vec{x}) = \sum_{j=1}^{n} \left(\frac{\partial V}{\partial x_j} \right) \frac{dx_j}{dt} < 0 \ \forall \ \vec{x} \in U,$$

then $\vec{x_0}$ is a stable equilibrium point, such that every dynamical trajectory passing sufficiently close to $\vec{x_0}$ will move toward $\vec{x_0}$ as t approaches infinity.

When $n \leq 4$, Lyapunov's Second Theorem can be used to show that the simple, symmetric hypercycle with growth equations

$$\begin{aligned}
\dot{x_1} &= k_1 x_1 + k'_1 x_n x_1 - \frac{x_1}{c} \left(k_1 x_1 + k'_1 x_n x_1 + \sum_{i=2}^{n} \left(k_i x_i + k'_i x_{i-1} x_i \right) \right) \\
\dot{x_i} &= k_i x_i + k'_i x_{i-1} x_i - \frac{x_i}{c} \left(k_1 x_1 + k'_1 x_n x_1 + \sum_{j=2}^{n} \left(k_j x_j + k'_j x_{j-1} x_j \right) \right) \ \forall \ 2 \leq i \leq n
\end{aligned}$$

has a stable equilibrium point at which all species coexist [8]. Computer approximations suggest that the same is true for larger values of n and for non-symmetric hypercycles, which do not obey the convenient constraints $k'_i = c = 1$ and $k_i = k_j \in \{0, 1\}$ for all $i, j \in \{1, \ldots, n\}$ [8].

We can see that $\vec{x_0} = (1/n, \ldots, 1/n)$ is a critical point of the dynamical system that describes a symmetric hypercycle, verifying easily that all relevant partial derivatives vanish. In addition, the function

$$V(\vec{x}) = \frac{1}{n^n} - x_1 \cdots x_n$$

vanishes at $\vec{x_0}$. We compute that

$$\begin{aligned}
\frac{dV}{dt} &= -\sum_{i=1}^{n} \frac{x_1 \cdots x_n}{x_i} \cdot \dot{x_i} = -x_2 \cdots x_n \left(k_1 x_1 + k_1' x_n x_1 - \frac{x_1}{c} \left(k_1 x_1 + k_1' x_n x_1 + \sum_{i=2}^{n} \left(k_i x_i + k_i' x_{i-1} x_i \right) \right) \right) \\
&\quad -\sum_{i=2}^{n} \frac{x_1 \cdots x_n}{x_i} \left(k_i x_i + k_i' x_{i-1} x_i - \frac{x_i}{c} \left(k_1 x_1 + k_1' x_n x_1 + \sum_{j=2}^{n} \left(k_j x_j + k_j' x_{j-1} x_j \right) \right) \right) \\
&= -x_1 \cdots x_n \left(\sum_{i=1}^{n} k_i + k_i' x_{i-1+n\delta_{i1}} - \frac{n}{c} \sum_{j=1}^{n} k_j x_j + k_j' x_j x_{j-1+n\delta_{1j}} \right).
\end{aligned}$$

When we make the substitutions $k_i' = c = 1$ and $k_i = k_j \in \{0, 1\}$ and use the fact that $\sum_{i=1}^{n} x_i = c = 1$, we find that

$$\begin{aligned}
\frac{dV}{dt} &= -x_1 \cdots x_n \left(\sum_{i=1}^{n} k_i - \sum_{i=1}^{n} k_i x_i + \sum_{i=1}^{n} x_i - n \sum_{i=1}^{n} k_i x_i x_{i-1+n\delta_{i1}} \right) \\
&= -x_1 \cdots x_n \left(1 - n \sum_{i=1}^{n} x_i x_{i-1+n\delta_{i1}} \right).
\end{aligned}$$

When $n \leq 4$, we can verify that $dV/dt < 0$ at all interior points of the concentration simplex except for the central fixed point, proving that this point is stable. For $n \geq 5$, it is not possible to prove this stability by Lyapunov's method, but numerical integration suggests that stability still holds [8].

Looking back at Figure 3, we see two hypothetical translation mechanisms that are not simple hypercycles, but branched networks. A computation very similar to the previous one locates no internal attracting points within the representation of a branched network on a replicator concentration simplex. Therefore, branched networks are inherently unstable, as are hypercycles with parasitic couplings [8]. This suggests that the earliest translation system can be represented by a cyclic set of catalytic linkages, which motivates Eigen and Schuster to favor the synthetase-free model featured in Figure 2. This model has its problems, considering that there is no biochemical example of a tRNA that can link itself to a specific amino acid without enzymatic help. Any biochemical manifestation of this process would have a nonzero error rate, which would compound with ordinary transcription and translation errors. One of the most important consequences of the mathematical analysis in [8] is the knowledge that these obstacles affect the general viability of catalytic cooperation among replicators; we have proof that we should not waste our time studying branched networks that eliminate the problems of a synthetase-free scenario, seeing that network stability constraints make these scenarios unlikely to account for the origin of translation. Instead, we must focus on the more obviously problematic cyclic networks. Serious problems with the biochemistry of cyclic translation reactions also magnify the importance of completely different solutions to Eigen's paradox, which would allow for protein translation to emerge later in the history of life among chemical species that are subject to less constrained dynamic behavior. One alternative to hypercyclic organization is a relaxation of the error threshold that is supported by experimental evidence that replication slows during the production of mutant sequences [21, 22]. If this stalling dampens the production of mutants effectively enough, then hypercyclic organization may be less crucial for the advent of long genomes than Eigen and Schuster originally supposed.

One salient feature of protein translation is its reliance on a near-universal genetic code. Scientists looking for patterns in the genetic code have noted that the simplest amino acids are specified by the codons that would have replicated most easily without complex enzymes [9], and others have noted that the genetic code maximizes the likelihood that a point mutation will induce the substitution of an amino acid that is chemically similar to the wild-type residue [15]. At the same time, critics of such pattern-finding efforts wonder how much

of the code is a 'frozen accident' that persisted because of evolutionary inertia [4]. There is mathematical evidence that the selection of a hypercycle is a 'once-forever' decision, partly because hypercycles resist branching and parasitism [8]. A hypercycle's nonlinear growth rate will help it easily outcompete any independently replicating quasispecies, eliminating any precursors to competing hypercycles from its environment. Despite our lack of definitive knowledge about the earliest translation system, it was almost certainly complex enough to grow at a nonlinear rate, making it subject to once-forever selection dynamics.

The systems that were analyzed in Sections 3 and 4 revolve around a scenario for the emergence of translation and high-fidelity transcription in a pool of polymers that are already capable of crude self-replication. Much less theory has focused upon the *de novo* emergence of self-replication, perhaps because there is no modern biological process that seems to recapitulate this event in the way that translation ribozymes hearken back to an RNA world. A 2008 paper by Martin Nowak and Hisashi Ohtsuki tackles this problem via a model where natural selection precedes replication [28]. In the next section, we present this work as a possible foundation for Eigen and Schuster's older origins of life scenarios.

5 Prevolutionary dynamics and the origin of replication

Like classical quasispecies theory, the process that Nowak and Ohtsuki have dubbed 'prevolution' is described by a set of ordinary differential equations whose variables x_i represent polynucleotide concentrations [28]. As before, we assume that the solution contains a ready supply of activated nucleotides, and for simplicity we consider only two bases denoted 0 and 1. In Eigen's theory, the activated nucleotides 0* and 1* react with polynucleotides via a black-box transcription mechanism such that the sequences x_i beget copies of themselves. In contrast, prevolutionary dynamics allows for activated nucleotides to tack themselves onto any polynucleotide strings they encounter, transforming the strand x_i into the strands x_{i0} and x_{i1}. Activated nucleotides can also become deactivated hydrolytically, begetting the monomeric strands x_0 and x_1. To compensate for excess production, the polymer x_i decays at the rate dx_i. Letting $x_{i'}$ denote the species obtained by removing the terminal nucleotide from x_i and a_i denote the rate at which $x_{i'}$ is transformed into x_i, we obtain the growth equations

$$\dot{x_i} = a_i x_{i'} - (d + a_{i0} + a_{i1})x_i.$$

Since activated nucleotides become deactivated by reacting with superabundant water molecules, we can assume that $x_{0'} = x_{1'} = 1$.

Given that prevolutionary dynamics obey a set of linear differential equations with constant coefficients, they are much simpler to analyze mathematically than either primitive replicator quasispecies or catalytic networks. Letting $\mathbf{x} = (x_{0'} = x_{1'}, x_0, x_1, x_{00}, x_{01}, x_{10}, x_{11}, \ldots)$ be the infinite vector whose entries are the finite binary strings, we can write $\dot{\mathbf{x}} = \mathbf{A}\mathbf{x}$ for the constant matrix

$$\mathbf{A} = \begin{pmatrix} 1 & & & & \cdots \\ a_0 & -d - a_{00} - a_{01} & & & \cdots \\ a_1 & & -d - a_{10} - a_{11} & & \cdots \\ & a_{00} & & -d - a_{000} - a_{001} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix}.$$

This implies that the solutions of the prevolutionary dynamics equations have the form $\mathbf{x} = \exp(\mathbf{A}t)\mathbf{x}_0$, where $\mathbf{x}_0$ is a constant vector of initial conditions. $\mathbf{A}$ is clearly diagonalizable with real eigenvalues, the largest eigenvalue corresponding to an eigenvector that is a stationary, limiting distribution of polynucleotides. The easiest way to calculate this distribution is to solve the equation system $\dot{\mathbf{x}} = 0$, which holds only at equilibrium [28]. Doing so, we obtain the equations

$$\frac{x_i}{x_{i'}} = \frac{a_i}{d + a_{i0} + a_{i1}} := b_i.$$

Given the convention $x_{0'} = x_{1'} = 1$, we can let $\sigma \in \{0, 1\}$ denote the initial nucleotide of x_i and conclude that

$$x_i = b_i b_{i'} \cdots b_\sigma. \tag{6}$$

In the super-symmetric situation where both nucleotides hydrolyze at the rate $a_0 = a_1 = \alpha/2$ and all sequence extensions proceed at the rate $a_i = a$, we compute using (6) that the sequence abundance x_i depends only on the sequence length n:

$$x_i = \frac{\alpha}{2a} \left(\frac{a}{2a + d} \right)^n.$$

We can see that super-symmetric prevolutionary dynamics, in which sequence abundance declines uniformly and exponentially with sequence length, lacks behaviors resembling natural selection. However, selective pressure begins to appear when we introduce reaction rate asymmetries, and it is enough to require that a subset of the sequence extension reactions happen faster than the others. If we keep the symmetric hydrolysis requirement of $a_0 = a_1 = \alpha/2$ but introduce numbers $s > 0$ and $0 < p < 1$ such that $a_i = a + s$ with probability p and $a_i = a$ with probability $1 - p$, then we create a system where a small fraction of sequences of length n outcompete all other sequences of length n. To prove this, we will let x_i be a sequence of length n and use (6) to calculate the probability that its abundance goes to zero as the selective pressure parameter s goes to infinity. Letting $\sigma, \rho \in \{0, 1\}$ denote the first two elements of the string x_i, we can rewrite (6) as

$$x_i = \frac{1}{d + a_{i0} + a_{i1}} \cdot \frac{a_i}{d + a_{i'0} + a_{i'1}} \cdot \frac{a'_i}{d + a_{i''0} + a_{i''1}} \cdots \frac{a_{\sigma\rho}}{d + a_{\sigma 0} + a_{\sigma 1}} \cdot \frac{\alpha}{2}.$$

When we let the ordered pair (a_{i0}, a_{i1}) range over its four possible values, we can see that

$$\lim_{s \to \infty} b_i = \lim_{s \to \infty} \frac{1}{d + (a + s) + a} = \lim_{s \to \infty} \frac{1}{d + (a + s) + (a + s)} = 0$$

with probability $p^2 + 2p(1 - p)$, while

$$\lim_{s \to \infty} b_i = \lim_{s \to \infty} \frac{1}{d + a + a} = \frac{1}{d + a + a}$$

with probability $(1 - p)^2$. Therefore, the probability that $\lim_{s \to \infty} b_i > 0$ is the probability that $a_{i0} = a_{i1} = a$, which is $(1 - p)^2$.

For a precursor x_j of x_i that has at least two elements, the four possible values of (a_{j0}, a_{j1}, a_j) lead to four possible values of $\lim_{s \to \infty} b_j$. We can see that

$$\lim_{s \to \infty} b_j = \lim_{s \to \infty} \frac{a + s}{d + (a + s) + (a + s)} = \frac{1}{2}$$

with probability p^2,

$$\lim_{s \to \infty} b_j = \lim_{s \to \infty} \frac{a + s}{d + (a + s) + a} = 1$$

with probability $p(1 - p)$,

$$\lim_{s \to \infty} b_j = \lim_{s \to \infty} \frac{a}{d + (a + s) + a} = 0$$

with probability $p(1 - p)$, and

$$\lim_{s \to \infty} b_j = \lim_{s \to \infty} \frac{a}{d + a + a} = \frac{a}{d + a + a}$$

with probability $(1 - p)^2$. Therefore, the probability that $\lim_{s \to \infty} b_j > 0$ is $1 - p(1 - p)$. Combining these results, we find that the probability that $\lim_{s \to \infty} x_i = \lim_{s \to \infty} b_i b_{i'} \cdots b_\sigma >$

0 is $(1-p)^2(1-p(1-p))^{n-2}$. Accordingly, the expected number of sequences of length n that persist in solution as s approaches infinity is $2^n(1-p)^2(1-p(1-p))^{n-2}$ [28].

These results show that a simple asymmetry in polymer extension speed can produce a distribution of sequences that looks very much like the work of natural selection. However, a successful form of prelife must eventually become subject to natural selection for traits other than polymerization speed. We already know that self-replicating molecules are subject to a full range of selective forces, and so our main task is to show that the rate-dependent selection that shapes prevolutionary dynamics is capable of selecting for replicative capacity. We can think about template-dependent polymerization in terms of our rate-dependent selection model by assuming that a certain short 'primer strand' has the tendency to base-pair with the end of a complementary sequence, and that the nonenzymatic extension of this primer proceeds faster than the free polynucleotide additions that generate other sequences. This scenario represents a new way of thinking about Nowak and Ohtsuki's master sequence model, a special case of asymmetric reproduction in which all reactions leading to the formation of some fittest sequence x_m happen faster than reactions that are not prerequisite to the formation of x_m [28].

Working with the master sequence 0^n, we can use use (6) to calculate the abundance of the sequence $x_\ell = 0^\ell \cdot 1 \cdot \sigma_{n-\ell-1}$, where $\sigma_{n-\ell-1}$ is an arbitrary string of length $n-\ell-1 \geq 0$, and compare it to the abundance of the master sequence $x_n = 0^n$. We find that

$$x_0 = \frac{\alpha}{2a}\left(\frac{a}{2a+d}\right)^n,$$

$$x_\ell = \frac{\alpha}{2b}\left(\frac{b}{a+b+d}\right)^\ell \left(\frac{a}{2a+d}\right)^m \quad \forall\ 1 \leq \ell \leq n-1,$$

and

$$x_n = \frac{\alpha}{2a}\left(\frac{b}{a+b+d}\right)^{n-1}\left(\frac{a}{2a+d}\right).$$

Given that $b > a$, we find that

$$x_0 > x_1 < \cdots < x_{n-1} < x_n,$$

and that $x_0 < x_n$. Therefore, the master sequence x_n outcompetes all other n-member sequences as expected [28]. If we fix a and let b approach infinity, we find that the abundance of the master sequence approaches a limiting value:

$$\lim_{b\to\infty} \frac{\alpha}{2(2a+d)}.$$

If we use the master sequence model to describe primitive template-directed polymerization, then it is natural to consider the effects of mutation and look for an error threshold phenomenon. We will suppose that the rate of nucleotide misincorporation during master sequence replication is u, as replication proceeds at the rate b and ordinary polymer extension proceeds at the rate a. We will assume that a single misincorporation breaks the bond between a mutant and its template, such that further extension of the mutant will proceed at the rate a. Given these conditions, reactions that happen within master sequence lineages will proceed at the rate $b(1-u)$. Reactions that produce sequences of the form $0^\ell \cdot 1$ with $1 \leq \ell \leq n-1$ will proceed at the rate $a+bu$, and all other extensions proceed at the rate a. As before, we can use (6) to calculate the abundance of the n-nucleotide polymers $x_\ell = 0^\ell \cdot 1 \cdot \sigma_{n-\ell-1}$:

$$\begin{aligned}
x_0 &= \frac{\alpha}{2a}\left(\frac{a}{2a+d}\right)^n, \\
x_\ell &= \frac{\alpha}{2b(1-u)} \cdot \frac{a+bu}{a}\left(\frac{b(1-u)}{a+b+d}\right)^\ell \left(\frac{a}{2a+d}\right)^{n-\ell} \quad \forall\ 1 \leq m \leq n-1 \\
x_n &= \frac{\alpha}{2a}\left(\frac{b(1-u)}{a+b+d}\right)^{n-1}\left(\frac{a}{2a+d}\right)
\end{aligned}$$

Our introduction of mutation does not change the limiting abundance x_n:

$$\lim_{b\to\infty} \frac{\alpha}{2a}\left(\frac{b(1-u)}{a+b+d}\right)^{n-1}\left(\frac{a}{2a+d}\right) = \frac{\alpha}{2(2a+d)}$$

To derive an error threshold relation, we first fix a and look for constraints on b and u that will guarantee that the master sequence x_n has an abundance of $1/k$ times its maximum possible abundance [28]. We rearrange the inequality

$$\frac{\alpha}{2(2a+d)}\left(\frac{b(1-u)}{a+b+d}\right)^{n-1} > \frac{1}{k}\cdot\frac{\alpha}{2(2a+d)} \tag{7}$$

to obtain

$$\left(\frac{a+b+d}{b(1-u)}\right)^{n-1} < k.$$

When $n \gg 0$ and $b \gg a+d$, $u \ll 1$ such that $u^2 \approx u\cdot\frac{a+d}{b} \approx 0$, we can simplify the left hand side as follows:

$$\left(\frac{a+b+d}{b(1-u)}\right)^{n-1} = \left(\frac{1}{1-u}+\frac{a+d}{b(1-u)}\right)^{n-1} = \left(\left(1+\frac{a+d}{b}\right)\left(\frac{1+u}{1-u^2}\right)\right)^{n-1} \approx \left(\left(1+\frac{a+d}{b}\right)(1+u)\right)^{n}$$

$$\approx \left(1+\frac{a+d}{b}+u\right)^{n} \approx 1+n\left(\frac{a+d}{b}+u\right) \approx \exp\left(n(1+\frac{a+d}{b}+u)\right).$$

Using this approximation, we can rewrite (7) as

$$\frac{a+d}{b}+u < \frac{\log k}{n},$$

and as b approaches infinity, we get the error threshold relation

$$u < \frac{\log k}{n}.$$

Assuming that the error rate u is small enough for self-replicating sequences to form in large numbers, we will start to see the kind of replicator dynamics that were introduced in Section 3. At first, replicating sequences will coexist with non-replicating sequences, and the system will be subject to a mixture of chemical kinetics and evolutionary dynamics. When the concentration of a sequence x_i can grow either by template-dependent replication or by extension of the free sequence $x_{i'}$, it obeys a differential equation of the form

$$\dot{x_i} = a_i x_{i'} - (d + a_{i0} + a_{i1})x_i + r x_i(f_i - \phi).$$

In this equation, r scales the relative speeds of template-directed replication and free sequence extension, f_i denotes the fitness of x_i, and ϕ is the outflow that balances excess replicative sequence production.

Computer simulations show that the abundance of replicators shoots up immediately above the critical value of r at which there are more copies of x_i being created by replication than being consumed by random nucleotide addition [28]. Prevolutionary dynamics become evolutionary dynamics when

$$-(d + a_{i0} + a_{i1}) + r(f_i - \phi) > 0,$$

such that

$$r > r_c := \frac{d + a_{i0} + a_{i1}}{f_i - \phi}.$$

Just as high error rates hinder a replicator's ability to outcompete other replicators, they also hinder its ability to outcompete products of random polymer extension. When

template-directed nucleotide addition proceeds with an error rate of u, we can reintroduce the parameter $q = (1-u)^n$, which is the probability that a given issue of x_i will not be a mutant. When $q < 1$, the sequence x_i will replicate faster than it is consumed if and only if

$$-(d + a_{i0} + a_{i1}) + r(f_i q - \phi) > 0,$$

which requires that

$$q > \frac{d + a_{i0} + a_{i1}}{r f_i}.$$

Nowak and Ohtsuki's model is too abstract for us to meaningfully ask whether sequences that are subject to selection before replication could spontaneously achieve a replication fidelity that is high enough to beget a landscape dominated by replication. Nowak and Ohtsuki do not address the question of how replicators might emerge in a polynucleotide population where natural selection screens only for polymerization speed [28], but experience from the laboratory tells us that template-directed polymerization happens faster than free polymerization under most reaction conditions.

Unlike the quasispecies and the hypercycle, which were described mathematically to shed light on the concrete chemical processes of transcription and translation, prelife is a theory awaiting a physical counterpart. But just as the theory of the hypercycle shows that certain models of primitive translation have inviable network structures, this theory of prelife promises results to biochemists who seek out chemicals with a well-defined set of properties.

6 Conclusion

We have now reviewed three dynamical systems that describe hypothetical stages in the early evolution of life, piecing them together into a narrative where the spontaneous polymerization of activated nucleotides gives rise to self-replicating macromolecules and eventually to a primitive translation system. All of these models use differential equations to summarize interactions among prebiotic chemicals, and exact or approximate solutions to these differential equations encode the capabilities that these interactions confer. "Prevolutionary dynamics" describes the advent of natural selection in collections of polymers that form more-or-less randomly and cannot replicate themselves, a state that experimentalists have already created in the laboratory [13]. Nowak and Ohtsuki also include equations describing a transition from prevolutionary dynamics to quasispecies, and Eigen and Schuster's earlier theory applies following the advent of polynucleotides that predictably catalyze their own replication [5, 7, 8]. Later work by Eigen and Schuster focuses on the transition from populations of autonomous molecular replicators to primitive protein translation systems [7, 8, 9]. All three models are unsustainable in requiring a continuous influx of high-energy metabolites, as it is assumed that life must have gained a certain level of organizational complexity before it became capable of regenerating these metabolites. None of these models considers the possibility that the ligation of polynucleotides together was as important as extension by mononucleotide addition, although both mechanisms have been shown to lengthen polynucleotides during nonenzymatic polymerization experiments [18]. However, experiments and intuition suggest that consideration of ligation only complicates the details of the reactions being considered, and does not qualitatively change their outcome.

It is unlikely that we will ever know whether evolution historically proceeded via the trajectory outlined here, but these mathematical models serve to combat the idea that the origin of life will never reduce to chemical kinetics. Skeptics have long doubted that the incremental modification of useful structures can account for innovations like self-replication, translation, and the vertebrate eye, but mathematics can prove that the acquisition of specific lifelike properties reduces to chemical kinetics quite concretely. Zoologists explain the evolution of the eye by citing examples of primitive photoreceptors that likely provided our ancestors with key selective advantages as they gained complexity over long periods

of time [2], but the mathematics described here presents a different kind of answer to skepticism about the spontaneous origin of replication and translation. When information-carrying molecules achieve critical levels of synthesis speed, replication fidelity, and catalytic activity, dynamical models predict sharp, dramatic changes in their behavior and explain how lifelike properties can appear very quickly. Every transition from a less lifelike state to a more lifelike state depends on a strict error threshold to proceed, and once this error threshold is met, a discrete change in molecular dynamics results from a Hopf bifurcation, which is a well-understood mathematical phenomenon. Theoretical transitions predicted by the error threshold correspond to real discrete changes in the structure and evolution of viral populations [29], and provide a new way of understanding both hypothetical and realizable biological systems.

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