HOW MUTATIONAL NETWORKS SHAPE EVOLUTION:
Lessons from RNA Models

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Abstract Recent advances in molecular biology and computation have enabled evolutionary biologists
to develop models that explicitly capture molecular structure. By including complex and realistic maps
from genotypes to phenotypes, such models are yielding important new insights into evolutionary
processes. In particular, computer simulations of evolving RNA structure have inspired a new conceptual
framework for thinking about patterns of mutational connectivity and general theories about the nature of
evolutionary transitions, the evolutionary ascent of nonoptimal phenotypes, and the origins of mutational
robustness and modular structures. Here, we describe this class of RNA models and review the major
conceptual contributions they have made to evolutionary biology.

Key Words RNA, Robustness, Evolutionary Dynamics, Fitness Landscape, Genotype-Phenotype Map

1. INTRODUCTION

1.1. Overview

Evolutionary biologists have long sought to understand the evolutionary processes that transcend any particular biological system. Models are indispensable tools for gaining such insights. During the twentieth century, evolutionary theoreticians built a powerful conceptual framework on simple mathematical models. Recently, however, thanks to startling advances in molecular biology and computational power, a new generation of higher resolution quantitative models is changing our perspectives on the origins and processes that have led to the current diversity of life on Earth.

1.2. Motivation

Detailed models of RNA structural evolution have enabled advances in evolutionary biology. The success of this model system stems partly from the biological importance of RNA and partly from our ability to rapidly and reliably predict the structures of these molecules.

1.2.1. RNA IS CENTRAL TO BIOLOGY. DNA, RNA, and proteins are the three essential biological macromolecules. Although RNA mediates information transfer from DNA genes to functional proteins and thus lies at the heart of the "central dogma of molecular biology," it has historically been overshadowed by DNA and proteins. Several recent discoveries, however, have brought RNA to center stage. RNA plays a vital regulatory role (for recent reviews see Mattick & Makunin 2006, Niwa & Slack 2007, Winkler & Breaker 2005) in many cellular processes and is the primary genetic material for a large number of viruses, including influenza and HIV. Molecular biologists are thus working hard to characterize the molecular structure of RNA and the relationship between RNA structure and biological function.

1.2.2. RNA MAKES A GREAT EVOLUTIONARY MODEL. Evolutionary biologists have harnessed the efforts of RNA molecular biologists. They have built evolutionary models that explicitly consider the relationship between RNA sequence (genotype) and RNA structure (phenotype) which are vastly more biologically realistic than traditional

mathematical models. Through computational simulations of evolutionary dynamics, these models yield rapid results, yet incorporate significantly greater biological detail than traditional mathematical models. Simulations have been used to study a wide range of evolutionary patterns and processes, such as the evolution of robustness (Ancel & Fontana 2000), the distribution of fitness effects of beneficial mutations (Cowperthwaite et al. 2005, 2006), the causes and implications of neutral evolution (van Nimwegen et al. 1999), evolutionary transitions (Fontana & Schushter 1998a, Huynen et al. 1996), and the structures of fitness landscapes (Schushter et al. 1994).

2. THE MODELS

2.1. History

This modeling framework originates in the work of Manfred Eigen and, later, Peter Schuster (Eigen 1971, Eigen & Schushter 1979). They sought to address origin-of-life questions, and, in particular, develop a general theory for the emergence of biological information and self-replicating life from "molecular chaos." Based on the assumption that early life must have undergone highly error-prone replication, Eigen sought to understand the evolutionary consequences of high mutation rates (Eigen 1971).

Two influential concepts emerged from this work. Eigen & Schuster used mathematical models to demonstrate that the balance between mutation and selection could result in a quasi-species---a population that stably includes not only the wild type (best type) but also suboptimal mutants of that wild type (Eigen & Schushter 1979). At very high mutation rates, a population may, in fact, include only very few wild-type genotypes and many less-fit variants. The quasi-species concept has often been thought to describe an entirely novel set of evolutionary principles. Recently, however, this concept has been shown to be an extension of classic mutation-selection balance theory (Bull et al. 2005, Wilke 2005). The concept has been embraced by virologists who regularly observe that rapidly mutating viral strains may achieve high levels of diversity, yet there is debate as to whether these

viruses truly evolve as quasi-species (Domingo 2002, Eigen 1996, Holmes & Moya 2002).

Eigen's second influential concept is the error catastrophe, the genetic meltdown of a population experiencing excessively high mutation rates. He showed mathematically that, under fairly reasonable assumptions, there would be a critical mutation rate below which populations would stably persist as a quasi-species and above which the wild-type and its close mutants would disappear entirely. Based on these ideas, virologists have sought to cure viral infections by using chemical mutagens to induce error catastrophes.

To test these ideas, Eigen encouraged the development of mathematical and computer models of evolving molecular structures (Eigen 1971). He recognized that such biologically grounded and highly detailed models would elucidate evolutionary dynamics at a higher level of resolution than previously possible. Many researchers have taken his charge and developed models of evolving RNA and protein molecules (see Chan & Bornberg-Bauer 2002 and references therein). Here, we focus on RNA-based evolutionary models. We describe the structures of these models, diverse methods for analyzing them, and the resulting insights into evolutionary processes.

2.2. RNA Folding

RNA molecules are composed of four nucleotides---adenine (A), guanine (G), cytosine (C), and uracil (U). Pairs of nucleotides in an RNA molecule can form stable electrostatic interactions, thus holding two parts of a molecule close together. The strength of an interaction varies with the specific combination of nucleotides, and more stable interactions tend to form at the expense of less stable interactions. Through such pairing, RNA molecules fold into secondary structures (hereafter shapes). The shape of an RNA molecule is composed of combinations of familiar motifs, such as stems (helical base-paired regions) and loops/bulges (unpaired regions) (**Figure 1**).

Parenthetical notation

((.((((.....))))..))((..((((((.......))))..(((...((..))...)))..)))...)))

Figure 1. Diagram of genotype-to-phenotype map in the RNA model system. The genotype is the primary nucleotide sequence and the phenotype is the most probable secondary structure (shape). Shape is predicted from sequence using thermodynamic folding algorithms. We label the primary RNA shape motifs. Stems are contiguous stretches of base pairs that include at least two base pairs, loops are unpaired bases that connect the two halves of a stem, and a bulge is an unpaired region in the middle of a stem. Parenthetical notation represents paired bases as matching parentheses and unpaired bases as dots. This notation contains all of the structural information given in the graphical representation.

The shape of an RNA molecule may be vital to its function, particularly for functional RNA molecules (as opposed to protein-coding RNA molecules) like ribosomal RNA, micro-RNA molecules, and ribozymes. In fact, the function of a molecule depends not only on its (two-dimensional) shape, but on its (three-dimensional) tertiary configuration, which includes additional far-reaching pairings between nucleotides already participating in secondary-structure motifs. The formation of tertiary interactions, however, is not particularly well understood. Fortunately, RNA shapes can be predicted with reasonable accuracy, and constitute most of the full structure of a typical molecule (Hofacker et al. 1994; Mathews et al. 2004, 1999; Zuker & Stiegler 1981).

Theoreticians originally developed a set of rules for predicting RNA shape, based on thermodynamic considerations (Waterman 1978). These rules assume that a

molecule will fold into the shape that releases the most energy upon formation, and thus is the most stable configuration. This is called the minimum-free-energy (mfe) shape of a molecule. The RNA folding rules are much simpler than the analogous set for proteins, largely because RNA has a smaller set of building blocks (four nucleotides versus twenty amino acids), and generally forms simpler secondary structural motifs. Michael Zuker and colleagues developed the first efficient computer algorithms to predict RNA shape using this approach (Zuker 1989, Zuker & Stiegler 1981). Their software, called mFold, is still actively developed and freely available at http://www.bioinfo.rpi.edu/applications/mfold/. More recently, Ivo Hofacker and colleagues have been developing and maintaining the ViennaRNA package, which includes many computational tools for folding and analyzing RNA structures and is freely available from http://www.tbi.univie.ac.at/~ivo/RNA/ (Hofacker et al. 1994). Researchers are continually improving the accuracy and scope of these folding algorithms. For example, new versions can predict the shapes of RNA molecules during interactions with other molecules (Bernhart et al. 2006, Mathews 2006, Mathews & Turner 2006).

These thermodynamic folding algorithms make several simplifying assumptions. Notably, they cannot predict pseudoknots (a common tertiary motif) or noncanonical base interactions (Hofacker et al. 1994). Researchers have developed comparative-genomics-based approaches that generally yield more accurate predictions of RNA shape, particularly for large RNA molecules (Gutell et al. 2002). The comparative approach, however, is much slower than the thermodynamic approach and requires large sets of homologous sequences to predict the shape of any given sequence. Thus it is not computationally tractable for evolutionary simulations.

2.3. Model Overview

RNA models typically simulate a large population of RNA molecules evolving via mutation and natural selection. The fitness of any given molecule is determined by first predicting its shape(s) and then applying a prespecified fitness function to these predictions (described in detail below). Molecules replicate in proportion to their fitnesses and, upon replication, bases mutate randomly at a prespecified rate. Some

versions assume discrete populations (Cowperthwaite et al. 2006) whereas others assume a continuous individual-based birth-death process (Ancel & Fontana 2000, Fontana & Schushter 1998a, Huynen et al. 1996, van Nimwegen et al. 1999).

Analogies can be drawn between the particulars of this model and any other evolutionary system. Each nucleotide is a genetic locus with four possible alleles (A, C, G, or U); interactions among these loci determine the phenotype; and mutations can cause a locus to switch from one allele to another, which, depending on the rest of the molecule, may alter the phenotype. These models do not make many of the assumptions often found in evolutionary models. For instance, fitness stems from a biologically grounded model of molecular folding. Thus the fitness of a given mutant does not come from an assumed probability distribution, but rather is determined organically. The likelihood that a mutation is beneficial or deleterious, and the nature of epistatic (nonadditive) interactions among loci are similarly unconstrained.

2.4. The Genotype-to-Phenotype Map

Phenotypes are produced by manifold interactions between genetic, cellular, organismal, and environmental factors. The term genotype-to-phenotype map refers to this complicated route from genotype to phenotype. The phenotypes of an organism (physiological and behavioral) collectively interact with the environment (including other organisms) to determine fitness. Ultimately, evolutionary biologists aspire to characterize these complex processes and their evolutionary consequences, but these studies have just begun.

The main advantage of RNA folding models is their realistic genotype-to-phenotype map. Unlike many traditional population genetic models, which completely ignore phenotype and assume simple one-to-one maps from genotype to fitness, the phenotypes in the RNA models result from detailed interactions among genes and their microenvironment (Eigen 1971, Fontana & Schushter 1987). In particular, the genotypes are primary nucleotide sequences and the phenotypes are the shapes predicted from these sequences via thermodynamic folding algorithms. Thus the algorithms serve as biologically motivated genotype-to-phenotype maps (Schushter et al. 1994).

The original RNA models consider only the single most-stable (mfe) shape of each molecule (**Figure 2a**). We refer to these as simple models. In reality, however, an RNA molecule may not necessarily fold into its mfe shape, and may even spontaneously switch among several thermodynamically probable shapes. Thus researchers introduced a more complex, but perhaps more biologically realistic, model in which sequences are mapped to the set lowest free energy shapes (**Figure 2b**) (Ancel & Fontana 2000). We refer to these as plastic models because they capture structural plasticity produced by Brownian motion.
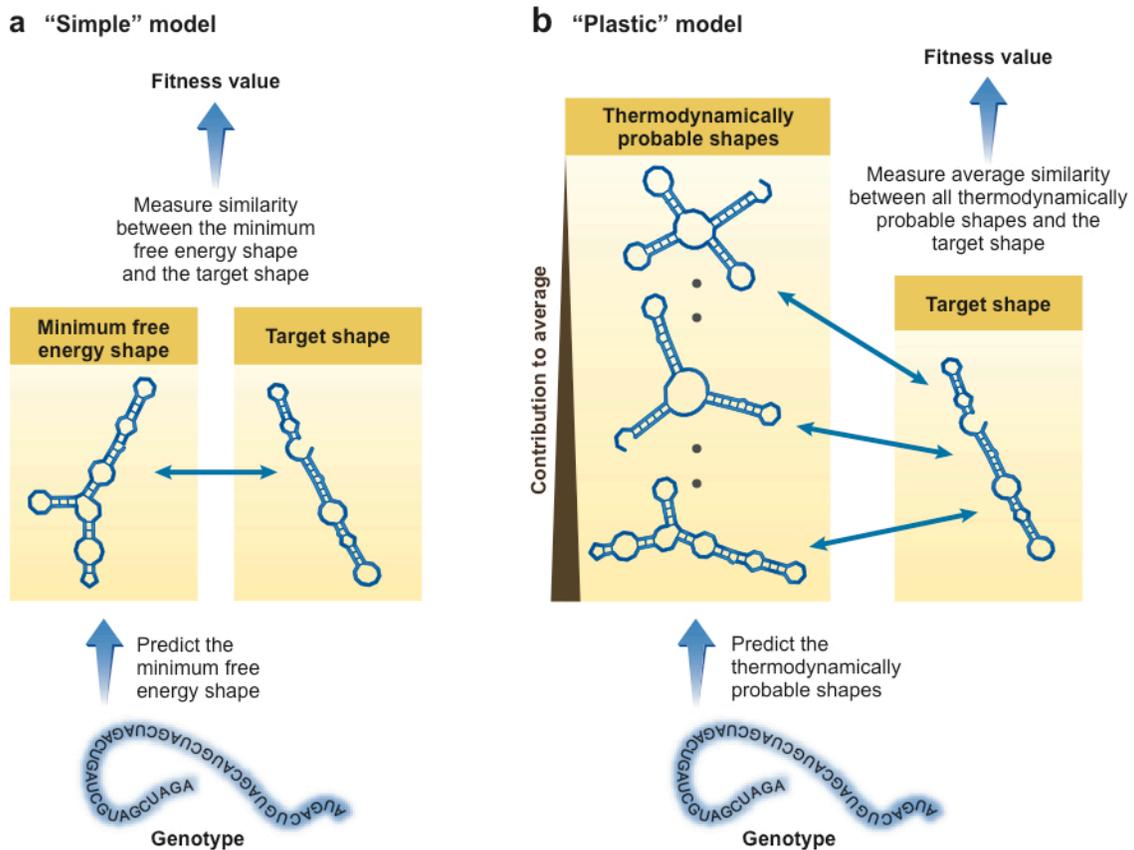
Figure 2. The two fitness models for RNA. (*a*) Under the simple model, the fitness of an RNA genotype depends only on the similarity of its mfe shape to the target shape. (*b*) Under the plastic model, the fitness of a molecule is determined by the entire ensemble of probable (lowest free energy) shapes. The similarity of any given shape to the target contributes to the final fitness in proportion to its Boltzmann factor, which is an estimate of the thermodynamic stability of a shape.

2.5. Fitness Functions

As discussed above, fitness is determined in two steps. First the shape(s) of a molecule are predicted using thermodynamic algorithms, and then a fitness value is attained via a function from shapes to real numbers. Here, we use the term fitness function to refer just to this second function from phenotype to fitness and the term fitness landscape to describe the projection of a large set of genotypes (a so-called sequence space) to their ultimate fitness values.

The fitness functions used in RNA models are often based on the similarity of a molecule's shape(s) to a predetermined ideal target shape. Fitness typically decreases monotonically as a function of the distance to the target shape. These models thereby use shape as a proxy for function and do not model function explicitly. This is justified (at least somewhat) by the dominant role typically played by shape in functional tertiary structure and the extreme conservation of shape throughout the evolutionary history of most functional RNA molecules (Doudna 2000).

In simple models that consider only the mfe shape, fitness is determined by the distance between those structures and the target shape. In the plastic models that consider the ensemble of favorable shapes, fitness is determined by the distances between all shapes in the ensemble and the target. In particular, each shape contributes to the overall fitness of the molecule in proportion to its thermodynamic likelihood, which is typically estimated by the Boltzmann coefficient (Ancel & Fontana 2000). Assuming thermodynamic equilibration, the Boltzmann coefficient of a shape estimates the fraction of time an RNA molecule spends in that shape and is calculated using an algorithm developed by McCaskill and coworkers (McCaskill 1990).

There are several methods for quantifying the structural distance between two shapes (Ancel & Fontana 2000, Stadler et al. 2001). For example, one can represent each shape in parenthetical notation, where dots stand for unpaired bases and matching parenthesis stand for paired bases (as in **Figure 2**), and then compute a Hamming distance between two such representations of shapes. Alternatively, the tree-edit distance measures the differences between the binary-tree representations

of two shapes. Although researchers have used a variety of shapes distance metrics, several studies suggest that most observations in RNA models are relatively robust to the specific choice of distance metric (Ancel & Fontana 2000, Fontana & Schushter 1998b).

The form of the fitness function, that is, how exactly fitness declines as distance to the target grows, can profoundly influence the outcome of evolution. One might naively assume that this is linear, such that any unit decrease in similarity to the target shape results in the same loss of fitness. Given that RNA structures are highly evolutionarily conserved, however, it is more likely that fitness declines faster than similarity. That is, even slight deviations from the ideal shape result in substantial loss of function. Many studies have therefore assumed hyperbolic fitness functions (Ancel & Fontana 2000; Cowperthwaite et al. 2005, 2006; Fontana & Schushter 1998a).

3. EVOLUTIONARY INSIGHTS INTO FITNESS LANDSCAPES

Since Sewall Wright introduced fitness landscapes in 1932, the concept has profoundly influenced evolutionary thinking (Wright 1932). Fitness landscapes are maps from large sets of genotypes to their fitnesses. Metaphorically, as populations evolve, they traverse the surfaces of fitness landscapes with mutation and recombination sampling new regions and natural selection pushing uphill. Though fitness landscapes are extremely high dimensional for most real biological systems, they are often illustrated as two-dimensional surfaces in three-dimensional Euclidean space. The structure of a fitness landscape is thought to constrain many micro- and macroevolutionary processes, including the rates of adaptation and speciation (Gavrilets 2004).

With the advent of high-throughput laboratory methodologies and modern computation, researchers are starting to undertake large-scale characterizations of fitness landscapes (Cowperthwaite et al. 2005 Fontana & Schushter 1998b; Gruner et al. 1996a,b; Li et al. 1996; Lunzer et al. 2005; Weinreich et al. 2006). The RNA model system offers the ideal balance of biological complexity and computational tractability for such studies. Some of the earliest and most exciting ideas about

fitness landscapes have come out of this body of work (Cowperthwaite et al. 2005; Fontana & Schushter 1998a,b; Gruner et al. 1996a,b; Schushter et al. 1994).

Technically, an RNA fitness landscape is a projection from genotype space---the set of all possible sequences of a given length---to fitness space (often the real numbers). Recall, however, that these models use shape as a proxy for fitness. Consequently, the landscapes that have been characterized are actually maps from sequence space to shape space, where the mapping functions are thermodynamic folding algorithms. All of the RNA landscape studies so far are based on the simple map from sequence to mfe shape (which ignores alternative low free energy structures).

The total number of sequences of a specific length n is 4^n. There is extensive degeneracy in the map from sequences to shapes, with many sequences folding into the same mfe shape, which means the size of the shape space will always be less than the size of the sequence space (Schushter et al. 1994). Waterman first proposed an upper bound for the number of shapes of length n--- $S_n = 1.4848 \times n^{-\frac{3}{2}}(1.8488)^n$ based on several assumptions about the nature of the shapes, such as stem length and loop size (Waterman 1978). In the first large-scale computational surveys to estimate the extent of redundancy, Gruner and colleagues folded all 30-nucleotide binary RNA molecules (composed of only A/C or G/U). Approximately one billion unique sequences folded into approximately 220,000 and 1,000 unique shapes in the G/C and A/U landscapes, respectively (Gruner et al. 1996a,b). Evidence for similar degeneracy was found in partial surveys of four-nucleotide RNA landscapes (Fontana & Schushter 1998b, Schushter et al. 1994). Recently, we characterized several complete landscapes for short RNA molecules and found that the number of unique shapes exceeds Waterman's theoretical upper bound, but we did not completely meet the assumptions of Waterman's theory (Cowperthwaite et al., submitted).

A many-to-one relationship between genotypes and phenotypes is not unique to RNA. For instance, there is considerable sequence divergence in 16S rDNA sequences, yet there is extensive functional conservation. As a result, these are key

molecules for phylogenetic analysis (Delsuc et al. 2005). Degeneracy has been observed in proteins based on lattice models of protein structure (Chan & Bornberg-Bauer 2002) and is at the heart of the neutral theory of molecular evolution---which asserts that most mutations have negligible phenotypic consequences (Kimura 1968)---and the molecular clock hypothesis (Zuckerkandl & Pauling 1962). As we discuss below, this redundancy profoundly affects the evolutionary dynamics of RNA.

3.1. Mutational Networks

Evolutionary transitions from one phenotype to another are mediated by mutations to their underlying genotypes. Historically, evolutionary biologists have thought of mutations in terms of distributions of fitness effects and have sought to measure the fractions of mutations that are typically beneficial, neutral, and deleterious. Although these distributions are critical determinants of local evolutionary dynamics, they provide little information about larger-scale processes. To this end, it is useful to think in terms of mutational paths connecting distant genotypes and, more generally, in terms of the large-scale patterns of mutational connectivity within genotype spaces.

Specifically, the space of all genotypes can be construed as a mutational network in which each genotype is a node and mutations between genotypes are edges. In other words, any two genotypes that differ by exactly a single point mutation are connected by an edge (**Figure 3**, *bottom*). One can then represent phenotypes (or fitness values) as colors. The coloration in **Figure 3** illustrates the degeneracy in the sequence-shape relationship discussed above. The colored edges represent neutral mutations that preserve the phenotype, and black edges represent non-neutral mutations that may be beneficial or deleterious. RNA mutational networks are regular graphs, that is, each genotype is mutationally connected to exactly $3L$ other genotypes, where L is the sequence length.
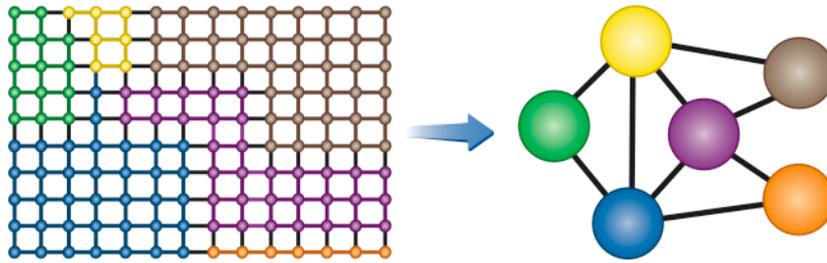
Figure 3. Mutational networks capture patterns of mutational connectivity among genotypes and phenotypes. In the bottom network, each node is a genotype and each edge is a point mutation. Colors represent phenotypes, and each group of genotypes that share the same color forms a neutral network. The top half shows a phenotype network in which each phenotype is condensed into a single node and two phenotypes are connected by an edge if there is at least one point mutation that converts one phenotype to the other.

3.2. Neutral Networks

Each colored patch in **Figure 3** is a neutral network---a mutationally connected set of genotypes that produces the same phenotype (or fitness value). This concept originated and has been studied extensively in the RNA model system (Fontana et al. 1993b; Gruner et al. 1996a,b; Huynen et al. 1996; Schushter et al. 1994; van Nimwegen et al. 1999). Following Eigen's quasi-species theory, it is perhaps the most influential idea to emerge from this body of work.

3.2.1. NEUTRAL NETWORK STRUCTURE. Consider a phenotype in a fitness landscape. The structure of its neutral network and its mutational connectivity to the neutral networks of other phenotypes determines the likelihood that it will evolve, and if so, whether it will give rise to other phenotypes. To understand constraints on phenotypic evolution, we must address questions like the following: Are neutral networks confined to small sections of sequence space or do they span the entire space? Do phenotypes have single contiguous neutral networks or several disjoint components? What patterns of adjacency exist between neutral networks for different phenotypes?

The first generality to emerge from neutral network studies is that "not all phenotypes are equal" (Fontana & Schushter 1998b, Schushter et al. 1994). Within an RNA fitness landscape, any given shape may be realized by many or only a few sequences. In other words, the sizes of the neutral networks vary considerably. Henceforth we use the term

phenotype abundance to refer to the number of genotypes that map to a particular phenotype. The distributions of phenotype abundances within RNA fitness landscapes have been shown to follow a generalized Zipf's law, a type of semiexponential distribution (Cowperthwaite et al., submitted; Fontana et al. 1993a,b; Schushter et al. 1994). The critical implication is that most RNA shapes are relatively rare while a few are quite abundant.

The neutral network of a particular phenotype may be composed of a single component or multiple disjoint components (Gruner et al. 1996a,b). A component is a set in which all genotypes are connected by paths of neutral mutations. If a neutral network is comprised of disjoint components, then it contains two or more components that are not connected to each other by neutral mutations. Surprisingly, the number of disjoint components in a phenotype's neutral network does not appear to correlate with its abundance (M.C. Cowperthwaite & L.A. Meyers, unpublished).

The neutral networks of highly abundant phenotypes have been shown typically to span entire fitness landscapes (Fontana et al. 1993b, Schushter et al. 1994). In other words, it is possible to mutate (in succession) every nucleotide in a sequence, all the while preserving its shape. Maynard Smith proposed a similar phenomena in protein fitness landscapes (Maynard Smith 1970). This suggests that neutral networks may facilitate evolution by allowing populations to explore vast expanses of genotype space (via mutation) while maintaining constant fitness (Kirschner & Gerhart 1998, Wagner 2005).

3.2.2. PHENOTYPE NETWORKS. As illustrated in **Figure 3**, mutational networks connecting genotypes give rise to mutational networks connecting phenotypes, or phenotype networks. In particular, we aggregate all genotypes that produce a particular phenotype into a single node and connect two phenotypes with an edge if there is at least one point mutation that converts one phenotype to the other. For RNA, we say that two shapes A and B are mutationally adjacent if there exists at least two sequences a and b that differ by exactly one mutation and produce A and B, respectively. Mutationally adjacent shapes are connected by edges in the corresponding phenotype network.

RNA phenotype networks appear to be highly irregular, with few nodes connected to many others and most nodes connected to few others (Cowperthwaite et al., submitted,

Schushter et al. 1994, Stadler et al. 2001). In contrast, classical population genetic models often assume that genotypes map one-to-one onto phenotypes, and that the mutational connectivity among phenotypes is fairly homogeneous. Thus the RNA model system can offer valuable insights into patterns of mutational connectivity and the evolutionary implications of such patterns (Cowperthwaite et al., submitted, Fontana & Schushter 1998a, Huynen et al. 1996).

One of the first studies to characterize the mutational adjacencies of RNA shapes found that almost any genotype is surrounded by a specific set of highly abundant phenotypes (Schushter et al. 1994). In other words, almost any genotype is within one or a few point mutations of the most common shapes in the landscape; and vice versa, these common shapes are mutationally close to most other phenotypes in the landscape. This hypothesis is called shape-space covering. In phenotype network terms, abundant shapes are connected to almost every other shape. We similarly found a positive correlation between shape abundance and the number of mutationally adjacent shapes for small RNA molecules (Cowperthwaite et al., submitted).

Fontana and colleagues developed a formal theory to describe the genetic accessibility among mutationally adjacent phenotypes and the implications of different mutational structures on evolutionary dynamics (Stadler et al. 2001). Mutationally adjacent shapes are those shapes for which there exists at least one point mutation that can cause a change between those two shapes. These efforts and earlier simulation studies suggest that the degree of mutational connectivity is not simply a binary property (connected or unconnected by point mutations) (Fontana & Schushter 1998a,b; Huynen et al. 1996). Rather some mutationally adjacent phenotypes are nearer to each other than other mutationally adjacent phenotypes, meaning that they are more likely to reach each other via mutation (Fontana & Schushter 1998b). Furthermore, this connectivity is always asymmetrical, resulting from the nonuniform boundaries among adjacent neutral networks (Fontana & Schushter 1998b, Stadler et al. 2001). For example, consider two phenotypes A and B: Asymmetry means that mutating from A frequently produces B, whereas mutating from B does not frequently produce A. In phenotype network terms, this variation in connectivity can be represented as weighted, directed edges between nodes. The weight on an edge pointing from A to B indicates the probability that any

given genotype in the neutral network for A will mutate to phenotype B, and, vice versa, the weight on the edge pointing in the opposite direction indicates the fraction of mutations to genotypes in the neutral network for B that produce phenotype A.

3.2.3. RUGGED NEUTRAL NETWORKS: AN IMPORTANT CAVEAT. Most RNA neutral network studies have assumed the simple model in which the fitness of a molecule is determined entirely by its mfe shape. The neutral networks in these studies are simply sets of RNA molecules that fold into the same mfe shape. In reality, however, the fitness of a molecule is determined by other factors, notably the kinetics and energetics of folding. Two molecules that share the same mfe shape may have very different thermodynamic properties and, consequently, different fitnesses. Thus, so-called neutral networks may not truly be neutral.

The plastic model, introduced by Ancel & Fontana (2000), inserts ruggedness into neutral networks. Recall that, in this model, the fitness of an RNA molecule is determined by its entire ensemble of energetically favorable shapes (the specific structures in the ensemble and their relative thermostabilities). Whereas the simple fitness function was discrete (only a finite set of possible values corresponding to a finite set of mfe shapes), the plastic fitness function is continuous (infinite possibilities). In general, any two molecules that share the same mfe shape will have different fitnesses under this model. Ancel & Fontana found that neutral networks have distinct patterns of heterogeneity, with the most thermodynamically stable molecules lying at the dense centers of neutral networks, where most mutations preserve the mfe shape. Thus, if fitness positively correlates with thermodynamic stability, then mfe neutral networks are no longer plateaus but rather mounds that may impede the neutral drift of a population toward alternative phenotypes.

Given that the plastic model is probably more realistic than the simple model, one might be tempted to reject the notion of a neutral network altogether. We argue, however, that the concept remains instructive. The mfe shape is the most likely structure and an important determinant of fitness. Although neutral networks may be more rugged than often assumed, they still contain expansive sets of mutationally connected molecules with roughly similar fitness.

4. EVOLUTIONARY DYNAMICS

4.1. Introduction

Intuitively, the structures of fitness landscapes fundamentally constrain evolution. In this section we review a number of theories linking mutational connectivity to evolutionary dynamics that originated in and/or have been tested using the RNA model system. First we focus specifically on the evolutionary consequences of mutational networks and then turn to more general studies of mutations and their interactions.

4.2. Evolutionary Dynamics on Mutational Networks

Natural selection acts on variation, and thus requires mutations to new phenotypes. The likelihood that a novel mutant phenotype will arise in a population, however, depends on the underlying mutational network, and can itself evolve as the population traverses this network.

4.2.1. EVOLVABILITY: NEUTRAL NETWORKS ENABLE EVOLUTION. There is a widely believed claim that neutral networks increase evolvability (Kirschner & Gerhart 1998, Stadler et al. 2001, Wagner 2005). The rationale is that populations evolving on neutral networks may undergo significant genetic change with only negligible phenotypic change, and can thereby explore fitness landscapes. In other words, neutral mutations can accumulate until a genetic background arises that is poised for beneficial change. Under this scenario, neutral mutations will be transient, ultimately facilitating adaptation by subsequent beneficial mutations (Wagner 2005).

Several RNA simulation studies have shown that populations evolving toward a target shape tend to experience long periods of phenotypic stasis, interspersed with short periods of rapid phenotypic change (Ancel & Fontana 2000; Cowperthwaite et al. 2006; Fontana & Schushter 1987, 1998a,b; Huynen et al. 1996). The last of these studies showed that the number of unique sequences in the population increased during periods of phenotypic stasis and used multidimensional scaling to illustrate the genetic dispersal of the population. The population typically subdivides into several genetically different

yet phenotypically equivalent subpopulations, each exploring a different region of the fitness landscape via mutation and natural selection.

According to this theory, the more expansive a neutral network, the more likely a population will be able to discover higher fitness phenotypes and thus evolve away from that network. In recent work, however, we systematically asked whether the abundance of a phenotype (the size of its neutral network) increases the likelihood of (*a*) evolving that particular phenotype and/or (*b*) evolving from that phenotype to other new phenotypes (Cowperthwaite et al., submitted). We found that phenotype abundance positively correlates with the number of mutationally adjacent phenotypes, which, on the surface, supports both claims. Yet, in silico simulations suggest that populations evolving on large neutral networks (of abundant phenotypes) did not adapt more quickly than those evolving smaller neutral networks. This stems from the fact that, as the abundance of a phenotype increases, the probability of locating adjacent phenotypes rapidly diminishes (Cowperthwaite et al., submitted). Populations that evolve abundant phenotypes may therefore face a "needle in the haystack" problem and be unlikely to further adapt even if superior phenotypes are just a single mutation away.

The size of a phenotype's neutral network does, however, affect the probability that the phenotype will arise (Cowperthwaite et al., submitted). Specifically, our simulated populations were more likely to evolve to abundant phenotypes than rare phenotypes. Thus, the structure of RNA mutational networks may bias evolution towards abundant shapes, whether or not those shapes are optimal.

In the same study, we turned to real RNA molecules to test this provocative hypothesis (Cowperthwaite et al., submitted). Unfortunately, we are far from having the computational resources necessary to characterize entire fitness landscapes for large molecules. We thus developed a new statistical shortcut for estimating shape abundance: the "contiguity statistic" measures the cohesiveness of a shape and significantly correlates with abundance (based on an exhaustive folding of all molecules of length 12 through 18). **Figure 4** details the calculation of the contiguity statistic. By calculating the contiguity statistic for thousands of naturally occurring functional RNA molecules in Rfam---a curated database of functional RNA genes (Griffiths-Jones et al. 2005)---it was found that natural phenotypes, indeed, have significantly higher contiguity values (and

thus higher abundance, perhaps) than expected for molecules of similar length and base composition (Cowperthwaite et al., submitted).
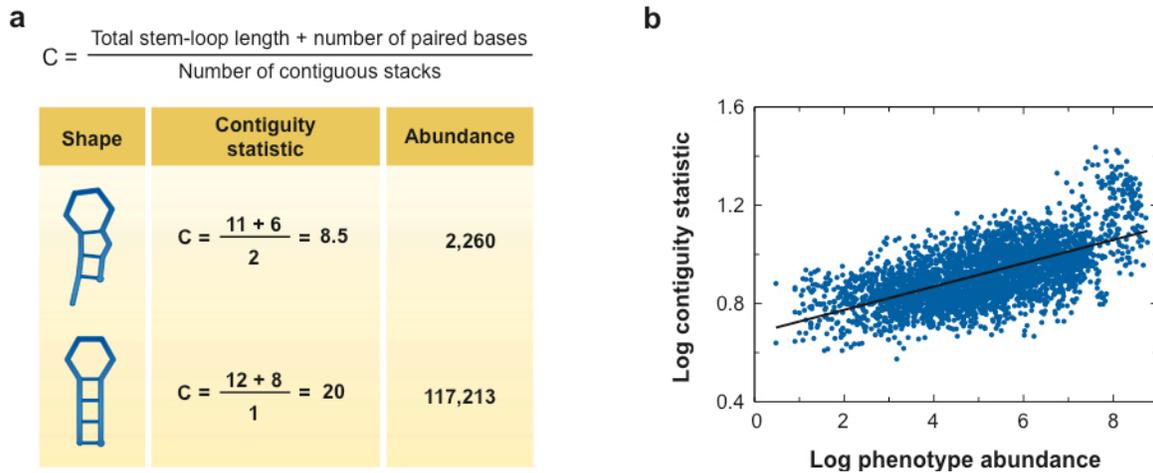

Figure 4. The contiguity statistic was developed to estimate phenotype abundance for large RNA molecules. This statistic came out of a study in which we folded all molecules of length 12 through 18 and directly measured abundances of all unique shapes (Cowperthwaite et al., submitted). (*b*) The contiguity statistic formula (C) captures the cohesiveness of the shape. We calculate this statistic on two simple shapes of length 12 and give the abundance of each shape. (*b*) The contiguity statistic strongly correlates ($R \approx 0.80$; $P < 2 \times 10^{-16}$) with phenotype abundance for RNA molecules of length 18. The graph shows the abundances and contiguity statistics for all 3211 unique shapes realized by molecules of this length. This correlation is equally strong for molecules of lengths 12 through 17.

RNA molecules may therefore be constrained by both functionality and mutational accessibility, a phenomena we termed ascent of the abundant (Cowperthwaite et al., submitted). This suggests not only that RNA shapes (and other phenotypes) may be suboptimal, but also that evolution may be more repeatable and predictable than previously thought by virtue of underlying mutational constraints.

4.2.2. PUNCTUATED EQUILIBRIA: CROSSING FROM ONE NEUTRAL NETWORK TO THE NEXT. One striking feature of the fossil record is the extensive discontinuity in forms (Eldredge et al. 2005), that is, periods of rapid phenotypic change are often separated by longer periods of relative stability. Although this may stem partly from observational biases (Eldredge et al. 2005), punctuated equilibria have also been observed in RNA models (Ancel & Fontana 2000, Cowperthwaite et al. 2006; Fontana & Schushter 1987,

1998a,b; Huynen et al. 1996), protein models (Chan & Bornberg-Bauer 2002), digital organisms (Wilke et al. 2001) and microorganisms (Burch & Chao 1999).

Figure 5 shows a typical simulation of RNA molecules evolving toward a target shape. As described earlier, populations disperse through neutral networks during the long periods of stasis. Fontana and colleagues set out to characterize the evolutionary transitions between these epochs (Fontana & Schushter 1998a). They claimed that there were two types of transitions---continuous and discontinuous---and proposed a simple criterion to distinguish them (Fontana & Schushter 1998b, Stadler et al. 2001). Recall that phenotypes differ greatly in their nearness, and a phenotype is said to be near any other phenotype that is likely to be produced by mutation. Continuous transitions are those that involve nearby phenotypes and discontinuous transitions are those that involve phenotypes that are relatively distant (unlikely to be realized by a single mutation). This study reconstructed the steps leading to each major transition. The initial period of rapid adaptation in the simulations occurred primarily through continuous phenotypic transitions; however, the transitions taking place during the subsequent punctuated dynamics were predominantly discontinuous. Thus, major adaptations are hypothesized to occur through fairly improbable jumps between barely adjacent neutral networks.

These jumps are thought to be mediated by extensive neutral drift (Fontana & Schushter 1998a, Huynen et al. 1996). Genotypes that produce one phenotype but are converted to a very different (but better) phenotype by a single mutation must precede these jumps. Such genotypes are likely to be very rare, and may only appear after long periods of evolutionary wandering through neutral networks (Fontana & Schushter 1998a, Schushter & Fontana 1999).

In a related in vitro RNA study, researchers synthesized a single RNA sequence that assumes two entirely different phenotypes, each of which catalyzes a distinct ribozyme reaction (Joyce 2000, Schultes & Bartel 2000). By making relatively few mutational changes to this sequence, these researchers could produce new ribozymes that were highly active for one or the other ribozyme reaction. Thus this single sequence lies at the intersection of the two neutral networks for each function. Schultes & Bartel (2000) suggest that intersection sequences (those that realize both phenotypes) may mediate discontinuous transitions between phenotypes.
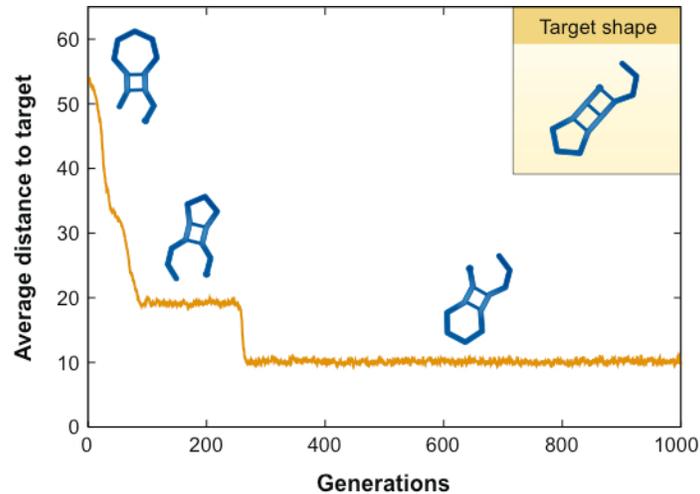
Figure 5. Typical evolutionary dynamics in the RNA model system. Evolving populations experience relatively long periods of phenotypic stasis interspersed with short periods of rapid phenotypic change. This figure is based on a simulation of a population containing 500 RNA molecules, in which selection favors molecules that resemble the target shape (*upper right*). The Y-axis gives the average phenotypic distance of the population to the target shape, and thus low values correspond to high fitness. Shapes that dominate the population are depicted above the curve.

4.2.3. GENETIC ROBUSTNESS: EVOLVING TO THE HEART OF A NEUTRAL NETWORK.

Organisms exist in an ever-changing world. They must evolve to withstand heterogeneous conditions, which include both environmental and genetic perturbations (Meyers & Bull 2002). Evolutionary biologists seek to identify the mechanisms for achieving environmental and genetic robustness as well as the evolutionary origins of those mechanisms.

Genotypes are genetically robust when mutations (or recombination) leave the resulting phenotype unchanged. In mutational network terms, genetically robust genotypes lie in the "dense" regions of neutral networks, where most mutations are likely to create genotypes within the same neutral network. In **Figure 3**, a genotype in the middle of a colored region would be completely robust because all of its mutations are neutral.

Although it is easy to envision natural selection favoring organisms that can cope with environmental variation (Meyers & Bull 2002), the origins of genetic robustness are less intuitive (de Visser et al. 2003). Because a deleterious germ-line mutation does not manifest itself until the next generation, there is no immediate natural selection to prevent

it. Under certain circumstances, however, natural selection can act over several generations to reduce the burden of such mutations (de Visser et al. 2003, van Nimwegen et al. 1999). There are several other theories for the origins of genetic robustness, some of which are nonevolutionary (de Visser et al. 2003, Gibson & Wagner 2000).

This discussion goes back to the founders of the modern synthesis---Haldane, Fisher, and Wright---who offered different theories for the evolution of dominance. Dominance is a simple mechanism for robustness by which potentially deleterious mutations at a diploid locus are silenced by the dominant allele. Evolutionary biologists have focused on three scenarios that could give rise to genetic robustness: (*a*) adaptive robustness---robustness evolves by natural selection, (*b*) intrinsic robustness---robustness is a correlated byproduct of character selection, and (*c*) congruent robustness---genetic robustness is a correlated byproduct of selection for environmental robustness (de Visser et al. 2003). These mechanisms are not necessarily mutually exclusive.

Natural RNA molecules and RNA viruses appear to be both environmentally (thermodynamically) and genetically robust (Meyers et al. 2004; Sanjuán et al. 2006a,b; Wagner & Stadler 1999). Studies using the RNA model system have contributed significantly to our understanding of genetic robustness, particularly scenarios *a* and *c* above. For scenario *a*, van Nimwegen and colleagues developed an elegant mathematical model to show that the trans-generational costs of deleterious mutations are enough to drive populations into the hearts of neutral networks, in other words, that adaptive robustness is possible (van Nimwegen et al. 1999). In particular, this model considers a population evolving on an arbitrary neutral network and assumes that all mutations off the network are lethal. They successfully tested the predictions of their model using RNA simulations. Genetic robustness only evolved in these models, however, under relatively high mutation rates.

Turning to scenario *c*, Wagner was the first to hypothesize that genetic robustness may evolve as a by-product of selection for environmental robustness (Wagner et al. 1997). The first semiempirical support for this hypothesis came somewhat accidentally from an RNA study (Ancel & Fontana 2000). Microenvironmental thermal fluctuations can cause an RNA molecule to wiggle between alternative low free energy shapes. An

environmentally robust molecule is one that will fold rapidly and reliably into its optimal shape despite these fluctuations.

To study the evolution of environmental robustness, Ancel & Fontana introduced the plastic model, which maps sequences to their ensemble of thermodynamically favorable shapes (described above). Selection for stable folding into a target shape indeed yielded populations of highly stable (environmentally robust) molecules. Surprisingly, the dominant shapes in the evolved populations looked nothing like the target shape. This was in dramatic contrast to natural selection under the simple (mfe shape) model, which almost always led populations to the target shape.

Why did selection for environmental robustness drive populations into apparent evolutionary dead ends? The evolved populations were also highly genetically robust, to the extent that mutations almost never produced phenotypic novelty, thus precluding further adaptation. The researchers eventually connected the dots when they discovered a correlation between the alternate shapes that a molecule produces under thermodynamic noise and the shapes it produces upon mutation. They called this general property of the map from genotype-to-phenotype "plastogenetic congruence" (Ancel & Fontana 2000). As a consequence, molecules that are insensitive to thermal noise are also insensitive to the effects of mutation. A similar correlation has been observed for proteins (Bornberg-Bauer & Chan 1999, Bussmaker et al. 1997, Vendruscolo et al. 1997). Extreme genetic robustness, to the point of an evolutionary standstill, thus evolved simply as a byproduct of environmental robustness.

Ancel & Fontana's study has other evolutionary implications. First, plastogenetic congruence may extend beyond biopolymers and be a general feature of genotype-to-phenotype maps. Phenocopies---epigenetic mimics of genetically based phenotypes---provide anecdotal evidence for plastogenetic congruence in other complex phenotypes (Queitsch et al. 2002; Rutherford & Lindquist 1998; True et al. 2004; Waddington 1950, 1959). This may shed new light on Waddington's theory of developmental canalization from the 1950s (Waddington 1950, 1959). He was among the first to argue that organisms have evolved developmental pathways that are robust to both environmental and genetic perturbations, and thus produce standard phenotypes in the face of variable environments and mutation. He does not, however, claim that these two forms of

robustness share a common evolutionary origin. If plastogenetic congruence holds for organismal phenotypes, then Ancel & Fontana's study suggests that genetic canalization may arise as a byproduct of environmental canalization.

Second, the extremely robust molecules found at the end of the evolutionary simulations were also extremely modular (Ancel & Fontana 2000). They can be easily partitioned into structural subunits that withstand thermodynamic perturbations or genetic changes elsewhere in the molecule. Modularity, as it shifts the syntax of genetic variation, opens new avenues for phenotypic innovation. Though this advantage is compelling, it does not explain the origins of modularity in the first place. We have a chicken-and-egg predicament: Until both the modules themselves and recombinational mechanisms are in place, it is not clear that natural selection would favor such organization. The RNA study suggests an origin of modularity that does not rely on the eventual evolutionary benefits modularity might provide. In particular, it arises as a (second) byproduct of selection for environmental robustness. Consider a rough analogy between RNA folding and organismal development. Interactions between nucleotides influence the kinetic pathway of the molecule and its robustness to both the environment and mutations. Similarly, interactions between genes determine the outcome and stability of developmental pathways. Perhaps natural selection for environmental stability similarly sets the stage for modularity in genetic networks.

4.2.4. SURVIVAL OF THE FLATTEST: QUASI-SPECIES AND ERROR THRESHOLDS IN COMPLEX MUTATIONAL NETWORKS. Recall that populations evolving under moderate mutation rates can form quasi-species---mutational clouds around a wild-type (optimal) genotype (Eigen 1971). Quasi-species have been observed in simulated populations of evolving RNA (Ancel & Fontana 2000), proteins (Wilke et al. 2001), and digital organisms (Wilke et al. 2001). Many RNA viruses are believed to exist as quasi-species, though there has been considerable debate over the utility of the term (Holmes & Moya 2002, Moya et al. 2000, Wilke 2005).

Recall further that error catastrophes occur when mutation swamps selection and a population is unable to maintain the wild type or its close relatives. Eigen originally discovered the error threshold (the critical mutation rate above which error catastrophes occur) in a model that assumes there is a single wild-type genotype and all other

genotypes have identical significantly lower fitnesses (Eigen & Schushter 1979). What happens when the wild-type phenotype is produced by an entire neutral network of genotypes and not just one? Roughly speaking, an error threshold still exists, but it increases with the breadth of the neutral network, that is, the number of and mutational connectivity among genotypes contained within it. The larger and more connected the neutral network, the more likely a mutation will preserve the wild type phenotype.

Similar reasoning suggests that neutral network breadth may influence the likelihood that a population will evolve one phenotype versus another. Imagine a population evolving in a complex mutational network where the topologies of neutral networks vary considerably among phenotypes. Under high mutation rates, phenotypes that have high fitness but small neutral networks may be easily displaced by less fit but more robust phenotypes. The extent to which neutral networks influence such competition among phenotypes depends on the mutation rate. Under very low mutation rates, fitness considerations alone dictate dynamics, whereas under high mutation rates, the breadth of neutral networks can be as or more important than fitness. This hypothesis has been called "survival of the flattest" (Wilke et al. 2001) and is a natural extension of Eigen's theory.

Survival of the flattest has been developed and tested in a series of mathematical models and simulations of evolving RNA and digital organisms (Bull et al. 2005, Wilke et al. 2001). In the Wilke et al. study, populations of digital organisms were evolved under two distinct mutation rates (high and low). When subsequently placed in competition under high mutation rates, populations that originally evolved under high mutation rates out-competed those that evolved under low mutation rates even though they had lower fitnesses. More recently, a plant virus competition experiment has suggested that similar tradeoffs may hold for plant viral pathogens (Codõner et al. 2006).

Although virologists have latched onto these ideas and harnessed them to develop effective antiviral strategies (Domingo 2003), Bull and colleagues have suggested that the theory may be widely misinterpreted (Bull et al. 2005). In particular, they distinguish between error catastrophes, in which high mutation rates lead to the complete loss of the wild type in favor of suboptimal genotypes, and extinction catastrophes, in which lethal mutations are so common that no viable genotype can persist. The use of mutation-

inducing drugs may not drive viral populations toward error catastrophes as has been claimed (reviewed in Anderson et al. 2004) but rather toward extinction catastrophes.

4.3. The Mutational Spectra of RNA

The phenotypic effects of mutations determine the rate and outcome of evolution. Evolutionary biologists have thus sought to characterize the distributions of fitness effects based on theoretical considerations (Gillespie 1984; Orr 2002, 2003) as well as laboratory mutation accumulation, knockout, and mutagenesis experiments (Estes et al.2004, Sanjuán et al. 2004, Rosen et al. 2002, Imhof & Schlotterer 2001). The RNA model system offers a pseudoexperimental compromise approach to estimating these distributions. It is more biologically grounded than the theoretical models yet yields vastly more information than experimental approaches. Here we review a series of RNA studies that offer new perspectives on local mutational structure, as opposed to global properties of entire mutational networks.

4.3.1. BENEFICIAL FITNESS EFFECTS: MANY SMALL MUTATIONS AND FEW LARGE ONES. Beneficial mutations are those that increase the fitness of individuals carrying them, and are the fuel of adaptation. Somewhat counterintuitively, recent theoretical work suggests that distributions of beneficial fitness effects are similar for many fitness landscapes (Gillespie 1984, 2003). This theory is based on Gillespie's mutational landscape model, which considers a high fitness wild type that has just experienced a minor environmental change (Gillespie 1984). The model assumes that the environmental perturbation was small, and thus the wild-type genotype remains reasonably fit, such that fit genotypes are rare in the fitness landscape and that the fitness of any given mutant is chosen at random from the distribution of all fitnesses. Gillespie claimed that the distribution of beneficial mutations could be predicted using —extreme value theory (EVT), and Orr subsequently derived the shape of this distribution (Orr 2003). EVT states that, for a large class of common distributions, the differences between the top few values in a large random sample will be exponentially distributed. According to Gillespie's assumptions, the wild type would be among the largest values in a random sample from the distribution of all fitnesses and thus the fitness effects of any beneficial mutations would fall within the purview of EVT (Gillespie 1984). Orr concluded that the fitness effects of beneficial

mutations should therefore be exponentially distributed regardless of biological system (Orr 2003).

Several groups have attempted to test this hypothesis experimentally, with most offering mixed support of the Orr-Gillespie theory (Rokyta et al. 2005, Sanjuán et al. 2004, Imhof & Schlotterer 2001). The most comprehensive of these studies used the RNA virus ϕX174 and supported a modified version of the model that incorporated a mutation bias, which could account for the higher frequency of transitions than transversions (Rokyta et al. 2005). An other study, in vesicular stomatitis virus (VSV), however, measured beneficial fitness effects that did not appear to be exponentially distributed (Sanjuán et al. 2004).

Recently, the Orr-Gillespie theory was tested in the RNA model system (Cowperthwaite et al. 2005). First, the researchers randomly chose two large sets of sequences and measured the fitness effects of every possible point mutation to each sequence in the set. These sets of genotypes differed in their average fitness---one set had relatively low fitness and the other set had relatively high fitness. The distributions of beneficial fitness effects in both low and high fitness regions of the landscape were decidedly nonexponential. There was a significant overabundance of small-effect mutations; and the distribution appeared exponential only upon truncation of the lower 99% of it.

The discrepancy between the theory and the RNA study rests on a fairly unbiological assumption of the Orr-Gillespie model---that the fitness of any given mutant is essentially a random draw from the distribution of all fitnesses (Cowperthwaite et al. 2005). Intuitively, the fitnesses of mutants are often highly correlated to the fitnesses of their parents, as has been demonstrated in RNA and proteins (Atchley et al. 2000, Fontana et al. 1993b, Parsch et al. 2000). The RNA study suggests that a predictive theory of beneficial fitness effects must consider fitness correlations. Orr recently extended his mathematical analysis to consider fitness correlations, and found that EVT does indeed break down under extreme correlations (Orr 2006).

4.3.2. EPISTASIS: MUTATIONAL EFFECTS VARY WITH GENETIC BACKGROUND. The RNA model system determines fitness from first principles of molecular folding. The shapes of molecules arise out of complex thermodynamic interactions among the nucleotides in the

primary sequence. The contribution of any particular nucleotide to the shape (and thus fitness) of the molecule often intricately depends on the nucleotides at several other sites. For example, see **Figure 6**. Epistasis---when the action of one gene is modified by one or more other genes---is thus a ubiquitous property of RNA fitness landscapes.
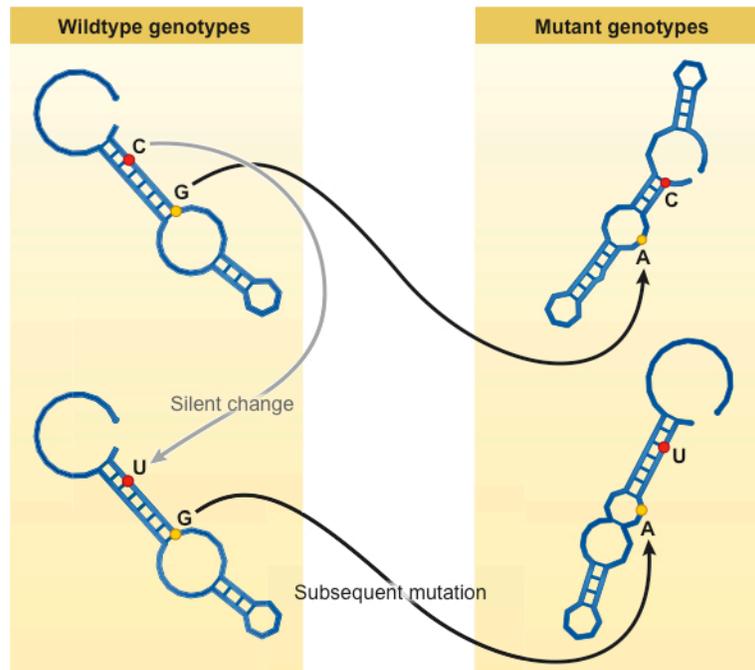

Figure 6. Epistasis in RNA results when the phenotypic effects of mutations depend on the surrounding nucleotides. The two molecules on the left differ at one position (*red*) but fold into the same shape. Mutations at the same site in each of these molecules (*blue*) produces very different shapes. Thus, through epistasis, a silent change in background (*gray arrow*) dramatically influences the fitness effect of the subsequent mutation (*black arrows*).

The presence, magnitude, and direction of epistasis are key inputs to many evolutionary theories, including those that seek to explain the evolution and maintenance of sexual reproduction and the rate of adaptation in asexual organisms (Peters & Otto 2003, Whitlock et al. 1995). Epistatic interactions are often divided into two classes: (*a*) antagonistic epistasis, which occurs when simultaneous mutations at interacting sites yield a smaller fitness effect than the sum (or product) of their individual effects, and (*b*) synergistic epistasis, which occurs when the combined effect of the mutations is greater than the sum (or product) of their individual effects. A third form of epistasis has recently

appeared in the literature: sign epistasis, which occurs when the direction of a fitness effect (deleterious or beneficial) is reversed by interactions with other mutations (Weinreich & Chao 2005). One study in the RNA system suggests that most interactions are antagonistic (Wilke et al. 2003). In particular, starting from a high fitness genotype, as deleterious mutations accumulate, the rate of fitness decline decreases, regardless of the order of those mutations.

4.3.3. COMPENSATORY EVOLUTION. Although beneficial mutations are essential for evolution, it is more likely that mutations entering a population will be neutral or deleterious. There is well-developed evolutionary theory that predicts the fates of deleterious mutations in evolving populations (Crow & Kimura 1970, Gillespie 2004). Deleterious mutations are likely to be eliminated by natural selection, but can occasionally reach fixation by chance (drift) alone, particularly in small populations, or by hitchhiking along with beneficial mutations elsewhere in the genome (Johnson & Barton 2002, Kim & Stephan 2000, Peck 1994). A recent RNA study has shown that, under high mutation rates, a third process, compensatory evolution, may lead to the fixation of deleterious mutations much more frequently than either of these other well-studied processes (Cowperthwaite et al. 2006).

Consider a new deleterious mutation. It is possible that, when combined with a subsequent mutation, the original mutation becomes less deleterious, or even beneficial. For example, a mutation to a paired base may break that pairing, to the detriment of the molecule. A subsequent mutation at the matching site may recover that pairing, or perhaps even strengthen (or weaken) the interaction, to the benefit of the molecule. The latter scenario is an example of compensatory evolution through sign epistasis in RNA molecules.

Prior studies of compensatory evolution have focused primarily on compensatory mutations that occur after initially deleterious mutations have fixed in the population, and thus do not contribute the fixation events themselves (Burch & Chao 1999, Escarmis et al. 1999, Poon & Chao 2005). In one of these studies, researchers grew an RNA virus at small population sizes to increase the strength of genetic drift and the likelihood of fixing deleterious mutations. They then allowed strains that had experienced a deleterious mutation to evolve at larger population sizes and found that compensatory mutations

generally afforded modest recoveries in viral fitness in comparison to the initial deleterious mutation (Burch & Chao 1999). A later study found that compensatory evolution mediated fitness recoveries in roughly three-quarters of populations in which deleterious mutations fixed (Poon & Chao 2005). There is further evidence for compensatory evolution across many natural and model systems (Poon et al. 2005).

Compensatory evolution may occur prior to fixation of the initial deleterious mutation and, consequently, make fixation of the mutation more likely. As recently illustrated in the RNA model system, this is common under relatively high mutation rates (like those found in RNA viruses) (Cowperthwaite et al. 2006). In evolutionary simulations, initially deleterious mutations fixed far more frequently than was expected by drift alone. Initially harmful mutations interacted with subsequent mutations to increase fitness beyond that of the ancestor and, thus, brought about fitness reversals. Such compensatory events explained as many as 70% of the deleterious fixation events.

Comparative genomic studies have identified possible fixed deleterious mutations in insect and human genomes (Kondrashov et al. 2002, Kulathinal et al. 2004). These observations must be interpreted with caution, however, because the order in which the mutations entered the genome is unknown, and currently deleterious mutations may not have been so when they first appeared. Nonetheless, these studies highlight the complicated nature of mutational interactions and suggest that deleterious mutations may be more than just temporary nuisances. Metaphorically speaking, they may provide stepping stones to distant adaptive peaks.

5. CONCLUSION

In the past two decades, a new generation of computationally intensive and biologically grounded models have changed our perspectives on evolutionary dynamics. We now have a more global understanding of mutational relationships and how they constrain evolution. Here we have reviewed a class of models that have been particularly fruitful. Detailed simulations of evolving RNA structures have inspired general predictive theories about the nature of adaptation, the determinants of evolvability, the origins and mechanisms of robustness, and more.

As volumes of biological data accumulate and computational power grows, these models will improve and continue to enrich comprehension of the natural world.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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