
THE FUTURE OF EVOLUTIONARY BIOLOGY

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INTRODUCTION

One of my grandfathers liked to say that he had experienced the most remarkable period of human history and progress, having been born before the automobile and lived to see a human walk on the moon. When my grandfather spoke of the advances he had witnessed during his lifetime, I wondered what further changes were left for my generation. Would there be another period so remarkable in terms of technological progress? The tone of my grandfather's voice certainly suggested that he viewed the scope of change in his lifetime as unique.

Ernst Mayr's one hundredth birthday provides us a wonderful opportunity to celebrate his contributions to evolutionary biology and to consider the tremendous advances in biological understanding that have occurred in that span. His own birth occurred around the time that modern genetics was born, and he has lived to see the entire human genome revealed. Along the way, there have been such milestones as the 'Evolutionary Synthesis'—including Mayr's contributions to the nature of biological species and the mechanisms of speciation—and the discovery of DNA as the material basis of heredity. At the same time, this celebration led me to wonder about what findings and discoveries lay in store for evolutionary biology in the decades and century ahead.

As evolutionary biologists, we are the intellectual grandchildren of Darwin, Mayr, and others who have made key discoveries in our field. And while we revere their contributions, we may reasonably wonder what more is left for us to discover. Could there ever be another advance in evolutionary biology as fundamental as Darwin's principle of adaptation by natural selection? No doubt many of us have day-dreamed that, had we lived in Darwin's time, we also could have conceived that idea and marshaled the evidence. (If we really believe that, then we are endorsing T. H. Huxley's self-criticism when he said: "How incredibly stupid not to have thought of that myself!") But I suspect most of us find it difficult even

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to imagine another discovery of such importance as the principle of adaptation by natural selection. Perhaps this reflects a failure of our imagination, and some future Darwin will prove us wrong. Or perhaps the most fundamental stepping stones on the path of scientific understanding are indeed finite, making it progressively more difficult to discover new ones.

What about the contributions of genetic processes and molecular data to evolutionary biology, with all the implications for understanding the mechanisms and history of evolutionary change? Has that infusion run its course, or at least been so widely recognized as to have lost its excitement? Is there anything else on the horizon, or that we can even conceive, that could have such a great impact on the way we think about and perform our science as the input of molecular genetics into evolutionary biology? In this article, I will attempt to support two points. First, I claim that the molecular-genetic revolution in evolution has just started, such that we can only now begin to see some of the most exciting ways it will advance our field. I will lay out a couple of directions where I see this research going over the next few years and decades. Second, I suggest that there is another revolution on the horizon, one that may basically influence not only the practice of our science, but might even substantially impact the future of our own species. In particular, I am referring to the emergence of artificial life, which results from the fusion of concepts from evolutionary biology with technological advances in computer science, engineering and robotics. If I am correct in even one of these claims, then the future of evolutionary biology will indeed be an exciting one.

Owing to space limitations, I highlight only a few examples in each section of the sort of current research that points towards these future directions. Of course, I have chosen examples that I am more familiar with (sometimes including my own work) but hope that the reader can see beyond the specific examples to the amazing breadth of possibilities that await the future of evolutionary biology.

THE MOLECULAR REVOLUTION IN EVOLUTION

Recent advances in molecular genetics have revolutionized evolutionary biology, especially our understanding of the history of life and the mechanisms of genetic change. DNA sequences, including entire genomes, are now widely used to trace phylogenetic relationships among organisms. We are moving ever closer to seeing the complete 'tree of life' and, along the way, finding some unexpected relationships and even new branches, most notably the discovery of the Archaea by Carl Woese. These data also allow us to make inferences about the past, including the properties of LUCA, the last universal common ancestor, and to discern key genetic

events that occurred in the history of life, including huge duplications (in some cases of entire genomes) and horizontal flow of genetic information between deep branches of life. The last mechanism complicates any representation of the tree of life, and may even compromise efforts to characterize LUCA. Molecular confirmation of Lynn Margulis' endosymbiotic theory of the bacterial origins of mitochondria and chloroplasts in eukaryotic cells provides the most dramatic example of these points. On a much finer temporal scale, burgeoning data on sequence variation within and between species provides information on rates of evolution and the roles of genetic drift, natural selection, and functional constraints.

Among all the research directions enabled by molecular approaches to evolutionary biology, I expect that case-by-case integrative analyses of the interconnections between molecular genetics, organismal development, and ecological phenotypes will produce some of the most interesting findings and increased understanding. In the next section, I review a few recent studies that have used this integrative approach to understand precisely how evolutionary adaptation has occurred and, sometimes, led to new species.

THE MECHANISTIC UNDERPINNINGS OF ADAPTATION

As an exemplar of this approach, let me summarize recent research by Doug Schemske and Toby Bradshaw (1999) on the genetic bases of adaptation and divergence of two closely related monkeyflowers. The authors generated a set of F1 and F2 hybrids between the bee-pollinated *Mimulus lewisii* and the hummingbird-pollinated *M. cardinalis*, which they placed in a field site where they could monitor pollinator behavior and selection on several floral traits. They also identified species-specific alleles for quantitative-trait loci that influenced two traits, carotenoid concentration in petals and nectar volume per flower, which they scored for each hybrid plant. High carotenoid levels were shown to be important in dissuading visits by bees, whereas high nectar volume was most important for attracting hummingbirds. Quite remarkably, variation at a single locus controlling pigment levels accounted for a five-fold shift in pollinator visitation, while variation at a locus involved in nectar production had a roughly two-fold effect. Bradshaw and Schemske (2003) also performed backcrosses for many generations to produce near isogenic lines in which the carotenoid-controlling alleles were swapped between the same two species. Experiments with the backcrossed lines provided further evidence that differences at a single locus substantially changed pollinator specificity. Although the precise numbers of mutations in the genes controlling carotenoid concentration and nectar production are not yet known, it is clear that mutations in a few genes of large effect were substantially responsible for the suite of traits that adapted each species to its own pollinators.

This example is a particularly appropriate one to highlight in a volume dedicated to Ernst Mayr, because it provides such a compelling demonstration of the close connection between adaptation and speciation. The pollinators are not only agents that contribute to the differential reproductive fitness of individual plants; they are also responsible for the reproductive isolation of these two species. In coming years, we can look forward to studies on a wide range of species pairs that will examine whether the same mutations responsible for adaptation of each species to its own environment are also those responsible for their reproductive isolation or, alternatively, whether different genetic changes are involved in adaptation and speciation. Thus, is allopatric speciation largely dependent on the magnitude of environmental differences leading to selective divergence, or does it instead reflect the mere passage of sufficient time to accumulate enough genetic differences, however inconsequential each may be, that reproductive incompatibility is the eventual outcome? As Mayr (2001, pp. 183-186) has recently emphasized, there is at present no strong consensus on whether the genetic differences responsible for reproductive isolation are driven primarily by selection or drift. I think that the on-going integration of molecular-genetic approaches and evolutionary hypotheses will shed much more light on this issue, especially for organisms that are well suited to both genetic and ecological experiments.

In my own group's research with bacteria, we also seek to identify the molecular genetic bases of phenotypic evolution. For more than a decade, we have propagated 12 replicate populations of *E. coli*, each founded from the same ancestral clone, in identical environments. The bacteria have undergone more than 20 000 generations of adaptation and divergence. We have observed substantial, and often strikingly parallel, evolution of such phenotypes as competitive fitness, cell size, catabolic niche breadth, and global patterns of gene expression (Lenski 2004). We are now in the midst of extending our analyses to the molecular level to quantify the overall extent of genomic change and, moreover, whether the tendency toward parallel phenotypic evolution is reflected in the pattern of genetic changes. A survey of DNA sequences at 36 randomly chosen loci finds almost a complete absence of any genetic changes, with none of the loci showing substitutions in more than one of the derived populations (Lenski *et al.* 2003b). These data remind us that 20 000 generations may be a long time for an experiment, but it is a mere 'drop in the bucket' in terms of genomic evolution. More importantly, these negative data provide a valuable control for patterns of sequence variation at candidate loci that we are investigating.

Much of our work on the molecular-genetic bases of adaptation is in progress, but two published cases illustrate what can be learned. All 12 populations lost the ability to use the sugar ribose, and in every case this

change occurred by a deletion of the *rbs* operon (Cooper *et al.* 2001). In all cases, one endpoint of the deletion coincided precisely with an insertion-sequence element that was present in the ancestral genome just upstream of the *rbs* operon. By contrast, the other endpoint of the deletions was different in every case. Further experiments and analyses showed that the high rate of substitution of these deletion mutations depended on both an unusually high mutation rate (attributable to the insertion sequence) and selection favoring loss of the ribose catabolic function. In the second case, changes in gene-expression profiles led us to *spoT*, a gene that encodes a global regulatory protein that allows *E. coli* to respond in a coordinated fashion to changes in its nutrient conditions (Cooper *et al.* 2003). Eight of the 12 populations had non-synonymous mutations in this gene, but no two affected the same codon. By moving one of the evolved alleles to the ancestral genetic background, we showed that this mutation gave a large fitness advantage under the conditions of the evolution experiment. However, moving the same allele to one of the lines that retained the ancestral *spoT* sequence provided no advantage at all. This derived line that did not benefit from the evolved *spoT* allele had itself undergone similar changes in gene expression to those that led us to find the *spoT* mutations in other lines. Taken together, these data indicate that most lines evolved functionally similar changes in *spoT*, but other lines must have achieved similar changes in gene expression by mutations in one or more other genes. Thus, even so simple a system as this one can show parallel phenotypic evolution that results from both parallel and divergent genetic changes.

Surely some of the most fascinating research on the genetic bases of adaptation and divergence will focus on finding those changes that make us human. I will mention just a few of the many exciting directions for this research. At the level of whole genomes, recent work indicates that there was a rapid rate of expansion of the size of the genome in the anthropoid primates (baboon, chimpanzee, and human) relative to prosimians (lemur), with a 15-20 per cent increase in genome size of the anthropoids in the last 50 million years (Liu *et al.* 2003). Most of this expansion appears to reflect retroposon insertions, including L1 and Alu elements. Even in comparison with the chimpanzee, the human genome appears to have expanded by some 30 Mb or so. By contrast, rates of substitution for point mutations appear to have been roughly constant across the primate lineages. Does the genome expansion represent merely the accumulation of junk DNA, perhaps correlated with changes in life-history and demography? Or has this genome expansion shaped important phenotypes that made us anthropoid and, ultimately, human?

At a finer scale of resolution, Rockman and Wray (2002) examined published data from the field of human genetics, and they found evidence of more than 100 upstream regulatory elements that are polymorphic and

contribute significantly to phenotypic variation among individual humans. For 21 of these polymorphisms, they were able to identify certain alleles as ancestral and others as derived, including seven cases in which the derived allele represented the majority type in at least one population. This finding suggested that “functional *cis*-regulatory polymorphisms are contributors not only to transient human variation but likely also to divergence of humans from our ancestors.” They also calculated that the average human is heterozygous at more upstream regulatory sites than amino-acid positions. Taken together, Rockman and Wray’s findings seem to support the view articulated by King and Wilson (1975) that changes in gene regulation, more so than in protein sequence, have shaped human evolution. As more primate genomes are fully sequenced, as more phenotypically relevant polymorphisms are discovered in humans and other species, and as new methods are developed to assess variation in gene expression and protein function, we will better understand the origin of our own species. Such research has important biomedical applications as well. For example, the *F7* gene encodes a protease essential for coagulation, the expression of which is a significant risk factor for heart attacks. Phylogenetic and population-genetic analyses of regulatory variants flanking this locus reveal an allele that has increased, apparently under positive selection, in certain populations (Hahn *et al.* 2004).

In a recent paper that has attracted considerable attention, Stedman *et al.* (2004) discovered that all humans, unlike other primates, have a frameshift mutation in a gene, *MYH16*, that encodes a myosin chain that is expressed in muscles controlling the more powerful jaws of other primates. Using the number of other mutations that accumulated in this inactivated gene as a molecular clock, the authors suggest that the frameshift mutation arose at about the same time that fossil hominid species began to show less robust jaws and cranial structures. Other studies have shown that reductions in muscle activity can have substantial effects on bone structure. Stedman *et al.* further suggest that the *MYH16* mutation, by reengineering craniofacial morphology, might have “removed an evolutionary constraint on encephalization” and thereby predisposed the evolution of larger brains. It is unclear what selective advantage was associated with this reduction of jaw musculature, but a change in diet and increasing use of hands in food processing have been suggested (Currie 2004). Regardless of how this story develops, we can look forward to many more studies that examine the interplay between genetic mutations and ecological pressures that led to modern humans.

More generally, I expect to see over the coming decades great strides in integrating data on rates and patterns of genomic and phenotypic evolution. Application of whole-genome sequences and expression arrays, coupled with rigorous analyses and experiments, should fuel these advances.

THE EVOLUTION OF EVOLVABILITY

A second area of growing interest at the interface of molecular biology and evolution concerns the mechanisms of genetic change at the sequence level, and how these mechanisms might have evolved to promote evolvability. We now know that evolutionary changes can involve not only simple point mutations but also transpositions of mobile elements and duplications of large regions of the genome. Such duplications allow subsequent functional divergence of initially identical copies of the same gene, with important consequences for the evolution of development and morphological complexity (Force *et al.* 1999). We also now recognize that organisms have evolved molecular mechanisms that enable them to repair incipient mutations, before the errors become established in both DNA strands. These new findings and perspectives raise fascinating questions about molecular mechanisms that influence evolvability. How can such mechanisms evolve? Are the consequences of mechanisms that enhance evolvability merely coincidental? Or have the mechanisms been shaped by selection and, if so, at what level has selection acted?

There is a large body of literature on the evolution of sexual reproduction that addresses related questions (Maynard Smith 1978; Kondrashov 1993; West *et al.* 1999; Rice 2002). However, in this section, I will discuss these questions in the context of mutational mechanisms, which are less familiar to most evolutionary biologists but have proven to be both interesting and tractable.

An appropriate place to begin is with the view that mutation is a passive process, one in which mistakes simply happen during replication of the genome. While such mistakes do indeed occur, in fact organisms have exquisite molecular machinery to find and correct such mistakes before they become mutations. An example is the methyl-directed mismatch repair process. Briefly, this system consists of enzymes that detect mismatches between the two DNA strands, then excise the region around the incipient mutation, and finally re-synthesize the excised region from the opposing template. The system is able, with high fidelity, to excise the incipient mutation (rather than the strand having the correct sequence) because the template strand of the DNA has had more time to become fully methylated, whereas the excision process is targeted to the newly synthesized strand that has not yet been methylated (Friedberg *et al.* 1995). It is important to realize that this system suppresses the mutation rate well below what it otherwise would be—in effect, it is a sort of anti-evolvability adaptation. Of course, organisms have no interest in impeding adaptive evolution. Rather, most mutations are detrimental, and so an anti-mutation function is beneficial because it tends to preserve the parental genotype and its associated high fitness. Such repair functions have undoubtedly been important for the evolution of larger genomes encoding more complex

organisms; such large genomes would otherwise decay in the absence of repair. The methyl-directed mismatch repair system has been highly conserved in evolution, such that humans and other eukaryotes have homologous repair genes to those found in bacteria, and defects in these genes are implicated in certain cancers (Friedberg *et al.* 1995).

Although mismatch repair does not promote evolvability, its existence indicates that organisms can exert some control over the mutational process, which raises the more general question of what other controls organisms might have evolved. Can organisms increase mutation rates in periods of stress? Can they target mutations to particular regions of the genome? Can they even somehow choose precisely which mutations to produce depending on their immediate needs?

The most extreme possibility—that some organisms might be able to direct the production of particular beneficial mutations in response to the present environment—was advanced by John Cairns and colleagues in a provocative and widely read paper (Cairns *et al.* 1988). Without going into all the details, Cairns and co-workers investigated mutations in *E. coli* that allowed cells to grow under certain conditions that would not support growth of their parental genotypes. Various observations suggested to these authors that the relevant mutation rates increased only under those specific conditions that provided the resulting phenotype with a selective advantage and, moreover, that this increased mutability was confined to those genetic sites that yielded the beneficial phenotype. In their words, “cells may have mechanisms for choosing which mutations will occur.” This phenomenon was dubbed ‘directed mutation’ and it received much attention, including reports of several other cases that appeared similar in nature. It received such attention because it challenged the modern Darwinian view that selection provides the directional force in evolution, whereas mutation is random. To make a long story short, various alternatives were proposed to account for the observed data without requiring such an extreme interpretation as directed mutation (Charlesworth *et al.* 1988; Lenski *et al.* 1989). In time, several purported cases of directed mutation were reexamined with new controls to account for potential artifacts, including two cases investigated by John Mittler and myself (Mittler and Lenski 1990, 1992). Although some individuals may have a different opinion, my own view is that these and other studies substantially refuted the hypothesis of directed mutation as it was originally put forward. But while bacteria might not be able to choose precisely which mutations will occur depending on their immediate needs, this controversy generated new interest in understanding what controls bacteria might be able to exert over their mutational processes and evolvability.

Following publication of a review article emphasizing that the phenomenon of directed mutation was not supported by further experiments

(Lenski and Mittler 1993), Ernst Mayr wrote me that "I could imagine that processes in prokaryotes could be of such immediate selective advantage that they would be incorporated into the variational mechanism of the genotype" (letter, 21 January 1993). Mayr elaborated on this idea by saying "I did not imagine that my proposed mechanism consists of the induction of the needed mutation, but rather of an increase in the mutability of the relevant locus" (letter, 22 February 1993). Although Mayr said "I do not have enough molecular know-how to know whether the pathway suggested by me is feasible" (letter, 21 January 1993), he was remarkably prescient about the possibility that bacteria could have evolved ways to bias the production of mutations toward certain loci.

Around the time of my correspondence with Mayr, I also received correspondence from two bacterial geneticists, Paul Rainey and Richard Moxon, in which they summarized evidence for the very sort of process that Mayr had suggested. Their letter was published (Rainey and Moxon 1993), as was a subsequent review of the hypothesis and evidence (Moxon *et al.* 1994). The gist of the hypothesis is that bacteria—especially pathogens that face changing host environments—have evolved 'contingency' genes that are far more mutable than typical 'housekeeping' genes. Importantly, this hypothesis is not merely about the extent of standing variation in a population, but rather it concerns the rate of production of new mutants. For example, homopolymeric tracts and other repetitive sequences are prone to strand slippage during DNA replication, which can produce changes in gene regulation or frameshift mutations depending on where the slippage occurs. These regions mutate at high frequency in both directions, allowing subsequent recovery of a previously lost function. This hypothesis does not suggest that bacteria are able to sense which mutation, if any, would be beneficial in a particular environment and produce that change. Instead, according to this hypothesis, bacteria that had evolved sequence motifs with localized hypermutability in genes subject to frequent changes in selection (such as those encoding surface structures recognized by the host immune system) would tend to be favored. By contrast, hypermutability in housekeeping genes would be deleterious, owing to the much higher ratio of deleterious to beneficial mutations in genes that encode functions that remain constant over long periods. This hypothesis raises interesting issues about the level at which such selection acts, but simple mathematical models show the plausibility that evolution could produce a hypermutable subset of the genome (Moxon *et al.* 1994). Compelling support for the hypothesis came from a systematic analysis of the first whole-genome sequence, that of the bacterial pathogen *Haemophilus influenzae* (Hood *et al.* 1996). The entire sequence was searched for tandem oligonucleotide repeats, which are prone to hypermutability via strand slippage. Far from being randomly distributed

throughout the genome, these motifs were substantially overrepresented in genes encoding virulence determinants for which variation is advantageous in navigating diverse host environments and evading immunity.

Several groups have sought to determine if organisms, including eukaryotes as well as bacteria, may have evolved mechanisms to increase their mutation rates during times of stress. Evidence has been provided that some mutation rates do indeed increase under certain stresses. However, it is not yet clear, in my opinion, whether such increased mutability is a pathological symptom of stress (such as cells losing control over DNA repair functions) that occasionally has fortuitous consequences, or whether it is an adaptive mechanism that promotes genetic change in a changed environment (Sniegowski and Lenski 1995). To address this issue, future researchers will need to manipulate systems in such a way as to allow quantification of the relative costs and benefits of stress-induced mutability, on the one hand, and resisting mutation and riding out unfavorable conditions, on the other hand.

In this and other future endeavors, the goal will be to understand just how 'clever' evolution has been, not only in the adaptations of organisms that fit them to their present circumstances, but also in their capacity to respond genetically to changing environments in ways that increase their chances of long-term success.

THE BIOLOGY OF ARTIFICIAL LIFE

Although the idea of artificial life may seem more like science-fiction than science, I think that artificial living systems will become an increasingly important focus for evolutionary biology in the future. The increasing interest in artificial life will stem from two distinct imperatives. First, artificial life can provide powerful experimental systems for testing complex hypotheses about evolutionary dynamics and mechanisms, with a precision and scope that are beyond what can be achieved with even the most tractable model organisms. Second, engineers working in a variety of different media are increasingly using concepts borrowed from genetics and evolution to find new solutions and implement new technologies, in ways that may substantially alter the future evolution of our own species.

Before giving some examples of recent efforts and future challenges in these two areas, let me explain what I mean by artificial life. Different criteria can be used to draw the line between living and non-living entities. The atoms that comprise DNA are certainly not alive, and I think most would agree that a DNA molecule in isolation is also not alive, although it is organic. An entire bacterial cell is clearly a living entity. To some, a troublesome intermediate case is that of a virus particle. Two objections are often raised against recognizing viruses as living entities. One is that

viruses cannot replicate except using a living system and its organic constituents as a host. I find that objection weak because many organisms that are clearly alive, such as bacterial pathogens, also require other organisms to reproduce. A second, and related, objection is that viruses are inert and lack metabolism. But viruses promote specific metabolic activities once they have infected a cell, and those activities are directed toward making copies of the virus. This reproductive potential is an essential component of a living system. Moreover, viruses, like other living entities, have genomes that encode functions necessary for their own reproduction (within the environment of the host), and those genomes can change by mutation. The resulting variants may differ in their phenotypic properties and, as a consequence, in their expected survival and reproductive success, thereby allowing adaptation by natural selection. In my view, something is alive if it has the capacity not only to reproduce but also to evolve and adapt by natural selection. The fact that its reproduction requires some 'assistance' from another entity is of interest, but does not disqualify it as living. How far such assistance might extend in an artificial system, before it was disqualified as living, could be debated on the specifics of any case. However, the most important criteria to me are that the system possesses a genome that encodes machinery for its own replication, its genome can undergo mutation, and the mutations can cause differences in phenotypes that determine survival and reproductive success.

To illustrate these points, it might be useful to consider a couple of familiar examples of artificial systems. First, consider a robot or computer built by humans that exhibits artificial intelligence, such as the famous fictional computer, HAL, in Arthur C. Clark's novel *2001: A Space Odyssey*. Such a machine could exhibit intelligence and even emotion, and it might have a sense of self-preservation. Yet, in the absence of any capacity for self-reproduction, such a machine would not be judged alive by the criteria above. Now consider the computer viruses that have become an increasing annoyance to anyone using e-mail. Are they alive? A computer virus contains a set of instructions for copying and propagating itself, which can be viewed as a genome that encodes machinery necessary for reproduction. As is the case for an organic virus, a computer virus also requires other machinery besides that which it encodes in its own genome—in one case various molecules inside a host cell, and in the other various functions inside the host computer. With respect to the second criterion, the capacity to evolve and adapt by natural selection, I think that computer viruses are a very interesting case because they are presently in a gray area, but show signs of making the transition from non-living to living. A few years ago, I would have said that computer viruses failed this second test. Although new viruses were appearing even then, and although many were variants of previously existing forms that allowed them to evade

computer defenses, the viruses did not evolve and adapt by natural selection. Instead, an extrinsic agent—a malicious hacker—had to intervene and modify the virus code in an intentional and directed manner. In other words, the population of computer viruses did not spontaneously mutate and adapt. But things have recently begun to change in a way that leaves this issue more ambiguous. In particular, many viruses now pick up new ‘subject lines’ and other content from computers that they have infected, and they transmit this changed information as they propagate. Not all of the virus code is subject to this mutagenic recombination, but the trajectory is clearly toward more intrinsic and spontaneous evolution, a fact that is unsettling, given the potential of computer viruses for nuisance and even destruction.

Although the examples in the previous paragraph emphasize computers, similar issues arise with some new research directions on evolving organic molecules. For example, a number of groups are evolving novel RNA and protein molecules *ex vivo*, removed from the organismal context in which such molecules would normally function (Salehi-Ashtiani and Szostak 2001; Glieder *et al.* 2002; Kuhne and Joyce 2004). The implementation of evolution in these experiments typically involves the following steps: produce a large population of molecules that contains variation due to mutation; select variants that possess altered properties of interest, such as enhanced ability to bind some other molecule or to catalyze some chemical reaction; make copies of the selected molecules with further mutation and, in some cases, recombination among the molecules; and so on through multiple cycles of replication, variation, and selection. Although the molecules are organic in origin, the processes involved in replication require assistance of the investigator, who provides a soup of other reagents that copy the selected molecules. My intent here is not to settle the issue of whether these or other systems are best described as living or non-living, but rather to show the ambiguity that can arise in certain contexts, especially those in which humans seek to harness evolutionary processes in order to manipulate the world in which we live.

In the next two sections, I will briefly discuss two distinct goals of research on artificial life. The first approach employs artificial life as a tool for studying evolutionary processes and dynamics, while the second approach employs biological principles to evolve new technological solutions. Of course, the distinction between basic and applied research is rarely as clean as this division would suggest, but nevertheless it is convenient for the points I wish to make.

ARTIFICIAL LIFE AS A MODEL SYSTEM FOR UNDERSTANDING EVOLUTION

The study of artificial systems as models for understanding biological evolution is fairly new, but it appears to be gaining interest. It is important

to emphasize at the outset that most research on artificial life does not seek to duplicate many details of organic systems (such as the DNA-based genetic code), but instead it aims to instantiate the most fundamental properties of life in order to examine general principles of evolution. As Daniel Dennett (2002) has stated, "It has long been clear that, in principle, the process of natural selection is *substrate-neutral*. That is, evolution will occur whenever and wherever three conditions are met: replication, variation (mutation), and differential fitness (competition)."

The study of evolution in artificial living systems has several attractions. First, there is interest in addressing the generality of evolutionary mechanisms when they are tested in a new medium. In commenting on an early study in this field, John Maynard Smith (1992) pointed out that "So far, we have been able to study only one evolving system and we cannot wait for interstellar flight to provide us with a second. If we want to discover generalizations about evolving systems, we will have to look at artificial ones."

Second, experiments with artificial life allow one to monitor and analyze evolutionary changes in extraordinary detail. In a recent paper with digital organisms (Lenski *et al.* 2003a), we identified 111 intermediate stages in an evolving lineage that yielded a complex new phenotype, out of more than ten-million genotypes that existed in the population. In other words, there were no missing links. We also characterized every mutation in the lineage with respect to its effects on all phenotypes of interest, including fitness.

Third, one can run replicated experiments to examine the statistical repeatability of evolutionary dynamics and outcomes. And coupled with the aforementioned ability to trace each intermediate stage in an evolving lineage, one can 'rewind' an experiment to any particular point in time and restart the experiment, with replication, from that precise moment. Charles Ofria and I are doing such experiments to explore why some digital populations achieved certain phenotypes whereas others did not. The ability to rewind and restart the tape is critical for putting hypotheses that invoke historical contingency into an experimental context—one that offers maximum power but is usually not available to evolutionary biologists.

Fourth, one can manipulate fundamental variables that cannot readily be manipulated in organic systems. For example, there is considerable interest in understanding the evolutionary effects of alternative genetic codes (Freeland and Hurst 1998; Knight *et al.* 2001), but manipulating the code is difficult with organic life. Using digital organisms, Ofria *et al.* (2002) experimentally tested how different underlying genetic codes impacted the evolvability of artificial life.

Fifth, and finally, one can perform certain experiments with artificial life that are inconceivable with organic forms. For example, some theore-

ticians emphasize the role of 'neutral networks' in promoting evolution; the idea is that a population may drift between states of equal fitness before encountering some region of genotypic space from which beneficial traits become accessible by single mutations (Huynen *et al.* 1996; Gavrillets 2003). In our own work, we found that some deleterious mutations occurred in lineages leading to advantageous new traits (Lenski *et al.* 2003a). Although most deleterious mutations are eliminated by selection, others are constantly produced and they represent the majority of all mutations; hence, even if only a small fraction of them have high-fitness neighbors, they may provide important stepping stones along pathways leading to adaptive traits. Also, deleterious mutations may tend to lead to regions of genotypic space that have a higher proportion of phenotypically interesting—and potentially adaptive—neighbors than do neutral mutations, which might be neutral precisely because they do nothing of interest and thus may interact with few other mutations that have selectable effects. Based on this reasoning, Ofria and I are performing experiments with digital organisms to see which class of mutations, neutral or deleterious, is more important in promoting adaptive evolution. We do so by 'pre-testing' the fitness effect of every mutation before it is placed in the population, an experiment that is beyond the realm of possibility in any organic system. In both cases, we allow beneficial mutations to occur, but in one case all neutral mutations are prevented and in the other case all deleterious mutations are prevented, such that we can compare which treatment has the greatest impact in slowing the rate of evolutionary adaptation.

It is also important to emphasize some differences between *experiments* with artificial life and *simulations* of the sort performed by theoretical population geneticists. First, the combinatorial possibilities for genotypes are vast in experiments with artificial life, typically exceeding the number of atoms in the universe. Therefore, it is impossible to perform an exhaustive search of the parameter space, as one could with, say, a two-allele, two-locus model in population genetics (which can already be surprisingly complex). Second, while population-genetic simulations can explore cases with infinite alleles and loci, the model must specify a priori the exact distribution of selection coefficients and epistatic interactions among mutations. By contrast, selection in experiments with artificial life acts at the level of the organismal phenotype, and all the selection coefficients and epistatic interactions emerge from whatever rules govern the development of the phenotype from the genotype. As with organic life, the rules are sufficiently complex as to defy any easy parameterization at the level of selection coefficients and epistatic interactions. Finally, and more philosophically, artificial life, as noted earlier, must at some level physically encode its own replication and any associated traits, whereas numerical simulations merely abstract all biological properties into a set of equations.

Let me conclude this section by mentioning three studies in which artificial life has already been used to study evolution. One of the pioneers of this field was Tom Ray, a tropical ecologist who became interested around 1990 in the potential to create self-replicating and evolving computer programs. While computer scientists had explored this possibility, they were having a hard time making it work because most self-replicating systems were so fragile, and most coding systems so rigid, that almost any mutation was lethal to the incipient artificial organisms (Adami 1998). Working alone, and with no formal training in computer science, Ray used biological insight to recognize that the fragility could be overcome by changing the underlying operating system from one in which instructions executed by organisms referenced fixed addresses in computer memory to one in which instructions referenced a complementary template. This insight, as well as other innovations, allowed Ray to produce software that he called *Tierra*, in which digital organisms replicated, competed (for space in the computer's memory), mutated, and evolved. In his initial experiments, a fascinating dynamic arose in which certain mutant programs evolved the capacity to exploit other programs by using a portion of the other program's code to replicate themselves; in time, yet other programs evolved the ability to resist this exploitation (Ray 1991). Thus, parasitism and coevolution spontaneously emerged within this artificial world.

Building on the foundation of *Tierra*, Chris Adami (a theoretical physicist) and Charles Ofria (a computer scientist) developed a new program, called *Avida*, that allows many different types of experiments and analyses to be performed on digital organisms. One innovative feature of *Avida* is that digital organisms can obtain resources, which supply them with the energy they need to replicate, by performing computations that solve problems presented by the environment (Wilke and Adami 2002). In one project, Wilke *et al.* (2001) allowed populations of digital organisms to evolve, all in the same computationally rich environment but under two different mutation rates. At the lower mutation rate the organisms evolved more productive computational metabolisms that allowed them to replicate faster than those that had evolved at the higher mutation rate. Yet, despite their lower replication rates, the organisms that evolved at the higher mutation rates were often superior competitors when competitions were performed at high mutation rates, because their genomes evolved to be more robust to the effects of mutation. The evolved robustness did not reduce the mutation rate *per se*, but rather the populations that evolved at high mutation rates ended up in relatively 'flat' regions of genotypic space where mutations tend to be less harmful.

Also using *Avida*, my colleagues and I studied the evolutionary origin of complex phenotypic traits (Lenski *et al.* 2003a). A long-standing chal-

lenge to Darwin's theory of evolution by natural selection has been to explain the origin of features, such as the vertebrate eye, that he called "organs of extreme perfection and complication" (Darwin 1859). Such features are too complex to appear *de novo*, and Darwin therefore argued that they must evolve by incremental transitions through many intermediates, often involving changes in function along the way. Substantial evidence in support of Darwin's explanation has accumulated for many complex traits, including eyes (Salvini-Plawen and Mayr 1977; Goldsmith 1990; Dawkins 1996). However, it remains difficult, if not impossible, to obtain a complete record of the evolution of any very complex feature owing to the extinction or modification of intermediate forms, the imperfection of the fossil record, and incomplete knowledge of the genetic and developmental processes that produce such features. By using digital organisms, however, we overcame these limitations. Starting from a simple ancestor that could replicate but not perform any computational function, we observed the step-by-step evolution of a complex computational metabolism in many independently evolving populations. Having evolved such complex traits, we performed genetic tests to show that the most complex phenotypes depended on the coordinated execution of dozens of derived genetic instructions that were not present in the ancestor. Such complexity has a vanishingly low probability of arising *de novo* and, consistent with the Darwinian expectation, we demonstrated that the most complex functions could evolve only by building on simpler components that served other functions that also provided the digital organisms with energy.

In my view, there is no shortage of theories in evolutionary biology that can be profitably tested by means of careful experiments using artificial life. For example, there are many theories about the evolution of sexual reproduction, but little evidence to favor one theory over another. Of course, experiments with artificial life could not prove how sex actually evolved in organic life, but they would allow more thorough analyses of various scenarios. In so doing, such research can help develop our intuition about complicated dynamics and feedbacks among evolutionary forces. But instead of presenting a detailed research agenda, I will close this paper by suggesting that artificial life may, over the coming decades, advance from being an interesting tool for studying evolution to become a challenging new component of the evolving world in which we live.

ARTIFICIAL LIFE AS A FUTURE COMPONENT OF OUR EVOLVING WORLD

My sense is that most biologists are aware only vaguely, if at all, of the extent to which concepts from evolutionary biology are being used by engineers to shape the world in which we live. The idea of artificial

intelligence has certainly reached the masses, despite being mostly unsuccessful in achieving its objectives to date. By contrast, artificial life as an area of research remains much more hidden from public awareness, despite arguably much more rapid progress. This difference might reflect the fact that our understanding of how brains manifest intelligence lags behind our understanding of how organisms evolve. In any case, a few examples will indicate how widely evolutionary concepts are already being used to develop new technologies, in both software and hardware. Let me emphasize that many of the systems being developed are not alive; some self-replicate but do not evolve, whereas others evolve but require an external agent to make copies. However, one can readily imagine how various systems might be combined, at least in principle, to produce artificial life.

In software development, some programmers and engineers are now using approaches in which a population of diverse programs is maintained. The programs are all individually evaluated for fitness using criteria relevant to the intended application. Those programs with higher fitness have a greater probability of being copied, and genetic operators are used to modify the programs by mutation, recombination, or both. This process is continued iteratively until a satisfactory solution is achieved. Various approaches go by such names as genetic algorithms, evolutionary computation, and so on, which differ in how the underlying programs are represented. But they all share the essential element of an iterative process with random variation and selection (Foster 2001). Such approaches are becoming increasingly powerful. As one set of concrete examples, John Koza and colleagues have used genetic programming to evolve representations of complex electronic circuits and controllers, including many designs that were recently patented by expert teams using traditional approaches as well as several other designs that are novel, useful, and apparently patentable inventions (Koza *et al.* 2003).

Unlike computer viruses or the digital organisms used in basic research, these evolving programs do not encode instructions for their own replication. Moreover, fitness criteria are often based on simulations or abstractions of physical processes, such that high-fitness solutions must be tested by building a physical version to ensure that the evolved solution works as well as it is supposed to work. For example, an evolved controller might not perform well in the real world if some simplifying assumption in the simulation was not adequately met in the physical world. On the other hand, this approach allows far more solutions to be tested than can be done mechanically, and the combination of random variation and selection allows the discovery of solutions that are novel, complex, and functional. Another frequent problem with this approach is that an evolving population of programs may become trapped in some local region of

genetic space. Because this engineering approach is consciously goal-directed, considerable effort is being put into devising strategies to avoid this problem, such as by actively preserving variation to prevent premature convergence on an unsatisfactory solution. Also, after a subroutine or some other component of a larger program has been judged to be acceptable or even optimal, a higher-level representation can be employed such that the desired component is no longer subject to mutation. [See Ayala (1999) for an interesting discussion of teleological explanations in biology, including similarities and differences between features designed by natural selection and by purposeful decisions.]

An intriguing example of artificial evolution at the interface of software and hardware involves visual art. Karl Sims has had several shows, including at the Centre Georges Pompidou in Paris, where he presented evolving art and the viewers acted as the agents of selection. Depending on which images attracted more or less attention (using sensors to determine where viewers were standing), some programs (genomes) encoding attractive images (phenotypes) reproduced while others perished. Reproduction of programs that encoded the attractive images involved mutation and recombination, generating an evolving population of images (<http://www.genarts.com/karl/>).

Prior to evolving art, Sims developed impressive evolutionary simulations of biological forms. Physical laws and the properties of real materials, such as water, were represented in programs inhabited by virtual creatures constructed from blocks (morphology) with sensors and effectors (neurology) to control their movements (behavior) in the simulated world (ecology). Starting from very simple creatures, evolving populations underwent mutation and recombination that produced new creatures with different morphology or neurology and, thus, with altered behavior. In various runs, populations of creatures were selected for their abilities to swim, walk, jump, or follow an object, with their relative performances judged as though they were physical entities in the corresponding medium (Sims 1994). It is quite amazing to see the virtual life that evolved, such as one creature that looks and swims like a sea snake, and others that look totally out of this world (<http://www.biota.org/ksims/>).

Could these virtual creatures ever be embodied in the physical world? The field of evolutionary robotics seeks to achieve precisely that aim, using components that are more appropriate to the intended functions of robots (Nolfi and Floreano 2001). In some cases, robots have fixed morphology, and it is only the 'brains' that control their behavior that are allowed to evolve. In other cases, body and brain are allowed to co-evolve. As one can imagine, however, robots are not cheap to build and destroy in large numbers. Because most mutations are deleterious, and because an improvement in one robot raises the bar for all others, the monetary costs of

imposing selection on a population of robots might seem prohibitive. But, as mentioned in the context of software used to design physical systems, there is a cheaper solution. In the field of evolutionary robotics, most evolution takes place in populations of virtual robots. Virtual worlds are built that simulate relevant physical laws (gravity, friction, etc.) and serve as home to virtual robots, whose components mimic those of physical robots. Mutation and selection occur within a virtual population, and the eventual winner is physically embodied and tested to ensure that it behaves as intended. A significant problem that must be overcome is avoiding an overly simple or uniform representation of the physical world. Subtle variation in lighting or friction, for example, might confuse or alter the sensory input or motor output of a robot. A solution to this problem has been to ensure that the virtual world contains heterogeneity and other ambiguities. It is admittedly rather strange to think of physical robots having evolved in a virtual world, yet the resulting machines exhibit some remarkably interesting and complex behaviors. Moreover, it is often difficult, even for an expert, to 'reverse engineer' the evolved robot—that is, to take the final product and understand its inner workings in sufficient detail that one can build something comparable from scratch—just as biologists have great difficulty in reverse engineering real organisms.

And, finally, what about reproduction? Will robots ever achieve the ability to reproduce without human intervention? A step toward this goal has been taken by Hod Lipson and Jordan Pollack (2000). They evolved virtual robots capable of moving across a surface, using only a few simple components that could be readily manufactured. Then, using a machine developed by engineers to build thermoplastic prototypes from encoded specifications, the virtual robots (including even ball-and-socket joints) were physically built without human handiwork. The only direct human interventions were to snap into place small electric motors and a micro-controller that had the co-evolved neural network to control the motors. Obviously, a great deal of work went into setting the stage for this process, and there remains more to be done to make robot reproduction fully autonomous even against this prepared backdrop. Nevertheless, one cannot help but be struck by how much progress has been made in such a brief period of human history.

The future is never certain, of course. But, in my view, there exists the strong possibility that we humans will eventually create artificial life that will have a major impact on the future course of our evolution. Humans have a creative impulse, and I see no fundamental obstacle to extending this creativity to produce novel and powerful forms of artificial life. Will artificial life ever become completely autonomous and perhaps even threatening to us? Or will artificial organisms live in a mutually beneficial

symbiosis with humans? My guess is that artificial life will become increasingly autonomous, yet it will remain domesticated. We humans will become increasingly dependent on functions performed by artificial life, and our interactions with artificial organisms will be those of a mutualism. Although feral forms of artificial life may evolve and cause some problems, artificial life will likely require the infrastructure that our civilization provides for a very long time. Whatever the future may bring, as a scientist I feel fortunate to be present at the dawn of these new creations. It is a bit like being transported back in time to study the earliest stirrings of organic life. And even if artificial life remains forever a mere curiosity, these systems provide us an intriguing platform for experiments that may lead us to new ideas about life and evolution.

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