Synthetic Biology: A Bridge Between Functional and Evolutionary Biology

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Abstract

The interests of synthetic biologists may appear to differ greatly from those of evolutionary biologists. The engineering of organisms must be distinguished from the tinkering action of evolution; the ambition of synthetic biologists is to overcome the limits of natural evolution. But the relations between synthetic biology and evolutionary biology are more complex than this abrupt opposition: Synthetic biology may play an important role in the increasing interactions between functional and evolutionary biology. In practice, synthetic biologists have learnt to submit the proteins and modules they construct to a Darwinian process of selection that optimizes their functioning. More importantly, synthetic biology can provide evolutionary biologists with decisive tools to test the scenarios they have elaborated by resurrecting some of the postulated intermediates in the evolutionary process, characterizing their properties, and experimentally testing the genetic changes supposed to be the source of new morphologies and functions. This synthetic, experimental evolution will renew and clarify many debates in evolutionary biology: It will lead to the explosion of some vague concepts as constraints, parallel evolution, and convergence, and replace them with precise mechanistic descriptions. In this way, synthetic biology resurrects the old philosophical debate about the relations between the real and the possible.

Keywords

constraints, directed evolution, leaps in evolution, modularity, the real and the possible, synthetic experimental evolution, tinkering

September 23, 2009; accepted November 12, 2009
Biological Theory 4(4) 2009, 368–377.

The distinction between two forms of biology—functional and evolutionary—was made explicit by Mayr (1961). Functional biologists look for mechanisms to answer the question "How?" whereas evolutionary biologists propose scenarios to answer the question "Why?" Theodosius Dobzhansky's famous assertion that nothing makes sense in biology except in the light of evolution does not apply to most studies performed in functional biology, where questions concerning the origin and historical transformations of the systems under study are not addressed.

This traditional separation is becoming less and less obvious, and there is a trend today to link evolutionary and functional explanations (Dean and Thornton 2007; Morange 2009b,c). One motivation—not the most significant one—is that the gap between the two approaches has been an Achilles' heel for evolutionary theory and a point of entrance for its opponents. Recent transformations in functional biology have probably been the most important trigger for this changing mind set. The development of genome sequencing programs has led to an accumulation of data. One way to exploit the information stored in these sequences is to compare them with sequences of other genomes. Comparison can be simply the way to attribute functions to the products of genes that so far have no identified functions. But it can also raise questions about the nature and significance of the events that have occurred since the time of divergence of these two genomes from their common ancestor (Koonin et al. 2000).

Pushed to their limit, molecular descriptions have also paved the way to investigating the evolution of the systems under study. Attempts to account for the structure and function of the molecular devices present in organisms in terms of physicochemical explanation soon reach their limits: Some characteristics have no physicochemical rationale but are only explained by the way these complex systems were progressively elaborated during evolution.

Finally, with the development of sequencing programs and the rise of systems biology and synthetic biology, a new population of researchers has invaded biology: computational biologists, physicists, mathematicians, etc. The separation between two forms of biology was not a part of their disciplinary training. It is striking how easily these "new" biologists can shift from modeling biological systems to work on the evolution of these systems and the selective pressures involved.

With this scientific context in mind, I will look for the specific place and role synthetic biology might play in this encounter between the two biologies. I will first consider the immediate and most obvious answer—"the objectives of synthetic and evolutionary biologists are diametrically opposed"—and progressively reveal more complex and more interesting relations between the two disciplines. Interactions between evolutionary biologists and synthetic biologists in practical terms already occur: *Evolutionary biology provides synthetic biology with tools, and synthetic biology provides evolutionary biology with tools as well*. By providing ways to test evolutionary scenarios, synthetic biology can help evolutionary biologists address and explore some of the most fundamental questions within their disciplines. Synthetic biology renews this questioning and extricates debates from the dead ends into which they have all too often strayed.

I need a provisional definition of synthetic biology for the purpose of this contribution, based on the opposition between synthetic biology and traditional genetic engineering approaches (Brent 2004): Instead of introducing one gene after another and looking for the effects, synthetic biologists adopt a rational approach in the spirit of engineers. The clearly defined objectives are more ambitious than older ones; the aim is to introduce new functional modules resulting from the combined action of different components. Modeling is systematically used to see how preexisting "biobricks" have to be modified and assembled to generate the functional modules (Morange 2009a). Synthetic biology aims to be *cumulative*, each accomplishment generating tools for future projects, in sharp opposition to past attempts to modify organisms by molecular biologists.

Synthetic biology has close relations to systems biology. The modules and networks characterized by systems biologists are the synthetic biologists' building blocks. In addition, systems biologists shed light on the principles of construction of organisms that will be used by synthetic biologists. One illustration of this relation is the considerable amount of work that has been invested in characterizing the origin and nature of *molecular noise*—stochastic variation in the concentrations of components and rates of reactions within organisms—and the way organisms deal with it. Synthetic biologists exploit the studies on molecular noise to generate stable, robust circuits, and networks (Chen et al. 2009). Synthetic biology can also be a tool allowing systems biologists to disentangle the complex architectures and organization of cellular systems (Kinkhabwala and Guet 2008).

The distinction between researchers working on the modification of (micro-)organisms and those more interested in generating simple "prebiotic" systems in order to synthesize, in a more or less distant future, "artificial" organisms is less important than the way the work is developed and the rational engineering approach is used. In this sense, the new synthetic biology owes an obvious debt to the work of "protein engineers." Although they focused on single macromolecules rather than functional modules, their efforts to rationalize the synthesis of new proteins have much in common with present endeavors in synthetic biology, a point to which I will return below.

A distinction (already) between different stages of synthetic biology, the earlier stage focused on modules and the new on systems, has recently been proposed (Purnick and

Weiss 2009), but appears to be more programmatic than a faithful description of the current situation.

Synthetic biology is not new, and historians and philosophers of science have reminded us of the efforts to synthesize life made in the early 20th century (Keller 2002, 2009; Peretó and Català 2007; Bensaude-Vincent, 2009). In terms of the question addressed here—the relations between functional and evolutionary biology—these early attempts can be neglected.

Synthetic Biology and Evolutionary Biology Have Diametrically Opposed Objectives

As a general rule, the chronology of events that interest functional biologists and evolutionary biologists are quite different. Evolutionary biologists study what happened in the last 500 million years, whereas the progressive construction of the functional devices studied by molecular and cell biologists took place in the previous 3.3 billion years, or even before the advent of the first living cell. An additional major difference between the work of evolutionary biologists and that of synthetic biologists is the time scale of the events under consideration. Evolutionary biologists look at modifications of organisms that have required thousands (and frequently much more) of generations, whereas synthetic biologists aim to modify organisms rapidly, in one or a few steps.

This difference is far from anecdotal. The long period needed for the evolutionary transformation of an organism is due to the conditions in which evolution takes place. What is selected each time in natural conditions is increased fitness, i.e., the relative number of progeny. At each step, the benefits from the modification must be greater than its cost; this is why small variations are favored by natural selection. No variation can be selected if it transiently decreases fitness, even if additional variation going in the same direction or complementary variation induces a later increase in fitness—natural selection always acts in the present. (Genetic drift effects—the perturbing effects that may originate from the small size of the populations under selection—will be ignored here.)

In contrast, when synthetic biologists modify or improve a system, they use massive mutagenesis. New technologies are developed to generate the maximum number of possible mutations in one or a few cycles (Wang et al. 2009). The reason is that the process of artificial selection used in these experiments has nothing in common with natural selection: rare events, even those leading to a decrease in fitness but going in the direction aimed at by the experimenter, can be selected.

The highly important notion—for evolutionists—of *the cost of a mutation* has no role in synthetic biology. Let us consider, for instance, the modification of the activity or specificity of an enzyme. What limits the success of the many experiments done to alter an enzyme is the destabilizing effect of the modifications made at the active site on the global structure of the protein. Some potentially favorable modifications cannot be introduced for this reason; or they have to be complemented by other mutations leading to a simultaneous stabilization of the protein. This antagonism between a selective alteration of the catalytic capacities and the decreased stability is a wonderful example of the tradeoff process that evolution permanently engages in (Tokuriki et al. 2008).

Recently, experiments have been done to overcome these limits by increasing the rate of synthesis of chaperonins, proteins that participate in protein folding and stabilize the structure of unstable proteins by permanently refolding them (Tokuriki and Tawfik 2009). The result was spectacular, and interesting mutations of residues in the active site were obtained that had never been obtained in the normal conditions of selection.

The important point for our discussion is that even when they use a Darwinian process to generate the proteins and modules they construct, the goal of synthetic biologists is to overcome the limits of Darwinian evolution in nature, and not to mimic it. Selection in nature and evolution directed by biochemists and molecular biologists increasingly differ as technological progress is made in synthetic biology. It is not obvious that "accelerated evolution" is still evolution. Such a transformation is not unusual: Many models of biological phenomena—say, the automata model of von Neumann for cell replication, neural networks, and genetic algorithms—have been progressively used for purposes other than to mimic the functions of organisms and have lost their value as models of the biological phenomena. It is important to keep this in mind: The way synthetic biologists proceed to obtain the functional devices they want to build has little in common with the way natural evolution proceeds.

The limits and complexity of the action of natural evolution are frequently outlined by synthetic biologists: "The principles of natural protein engineering are obscured by overlapping functions and complexity accumulated through natural selection and evolution" (Koder et al. 2009: 305). The same is true for the construction of synthetic promoters. The modular organization of promoters has been extensively studied. Transcriptional factors bind at different positions, and the transcriptional activity is the result of the combined action of these factors (Yuh et al. 1998). Nevertheless, in most cases, the transcriptional activity cannot be deduced from the structure of the promoter. A passage through synthetic promoters is necessary to extract the combinatorial rules (Alper et al. 2005; Hartner et al. 2008; Gertz et al. 2009). Evolutionary history is seen as a "burden" preventing further modification of the organisms and their components.

The metaphor of "bricolage" (tinkering) has been recurrently used, first by Darwin, then by Jacob (1977) to designate the limits and imperfections of the action of natural selection.

These limits are two characteristics of the action of a tinkerer. The first is that the same pieces are used again and again. Only a limited number of the range of possible organisms has been explored by evolution. Once an invention has been made, the invention of another process that performs the same function more efficiently becomes impossible—the reason being that the adoption of a new functional device would provoke a transient decrease in fitness, and fitness has to increase at every step of evolution. These limits are designated as the "frozen accidents" of evolution. For instance, the number of protein types is limited, whereas there are many more possibilities for stable folding of proteins. In contrast, some families of proteins contain a huge number of members. The sharp contrast between a tinkerer and an engineer would suggest that the latter creates de novo all the pieces he will use. Reality is quite different: Engineers also create new models by using what is at hand, which partially explains why technological choices rapidly become irreversible. The sharp and illuminating distinction between engineers and tinkerers holds only as far as one has an ideal view of the behavior of engineers.

A second limit, distinct from the previous one and referred to under the name of "complexity," is that evolution has not changed the pieces, but has created more and more links between them. These connections form the basis of regulation and homeostasis. But they have the paradoxical effect of limiting the possibility for systems to evolve. As pointed out by Denis Duboule (Duboule and Wilkins 1998) and Eric Davidson (Davidson and Erwin 2006), the gene networks controlling the development of complex organisms are so intensively connected that the number of possible variations has been highly reduced. Interconnections create regulations and increase the adaptation of organisms, but they make their future transformations more difficult.

In addition, by creating new links between previously independent functional devices, the blurring action of evolution masks the modular organization of organisms. Consider, for instance, the structure of proteins: There are plenty of arguments supporting the hypothesis that present-day proteins are the result of the assembly of smaller proteins, each having had one simple, unique function. This modular organization is still visible in some families of proteins, such as the transcription factors and the proteins of the extracellular matrix. But in most proteins, the modular ancestral organization has been blurred by the numerous mutations that have created new structural and functional relations between these original modules, which gave proteins their integrative capacities.

This blurring action of evolution is a nightmare for synthetic biologists. The modules that will be inserted into a recipient microorganism—considered as a chassis—must be as much insulated from the rest of the organism as possible, "orthogonal" in the words of synthetic biologists. It means that there must not be interferences between the new devices and the pre-existing machinery of the cell. And the eventuality that connections are created by mutations must be limited as much as possible.

This emphasis on orthogonality has been introduced by researchers who aimed at expanding the genetic code and the number of amino acids that can be incorporated into proteins (Wang et al. 2001; Cropp and Schultz 2004): A perfect orthogonality between the protein synthesis machinery of the host and the new system introduced is an absolute requirement for obtaining new proteins, having incorporated "nonclassic" amino acids (An and Chin 2009). But orthogonality became a standard for synthetic biologists in general. The absence of perturbing interference between the system introduced and the pre-existing machinery of the host is a guarantee that these kinds of experiments will not alter the normal functions of the microorganism. Such a possible alteration is an obvious source of concern for the opponents of these manipulations.

Nevertheless, the Lessons of Evolutionary Biology Are Highly Useful for Synthetic Biologists

Despite the oppositions that I have previously outlined, synthetic biologists still have much to learn from evolutionary biologists. This is the obvious conclusion that emerges from the efforts made by protein engineers. The strategy of protein modification initially favored in the early 1980s was a rational strategy: with the help of the 3D protein structure replacements of individual amino acids were proposed, and the possible results of these modifications were explored by modeling. If the results of these simulations were positive, the substitutions were made. Such a rational strategy was quite successful, but often for reasons that were understood afterwards only and were at odds with the experimenters' initial interpretations (see, e.g., Wilks et al. 1988). Very rapidly, however, the limits of this rational strategy appeared. The effects that the replacement of amino acids situated at a huge distance from the active site might have on the catalytic properties of the proteins could not be anticipated by the rational approach. But systematic stochastic variation of amino acid sequences with selection for function was in most cases impossible, the number of variants being too high.

Consider the case of abzymes, antibodies in which catalytic activity was introduced by stochastic mutations (Pollack et al. 1986; Tramontano et al. 1986). This approach worked because the variable domains in these proteins consist of only three short amino acid loops. This limited size of the protein domains likely to be subject to directed evolution was an advantage *and* a drawback—despite numerous efforts, the catalytic capacities of abzymes rapidly reached a limit, several orders of magnitude below that of natural enzymes.

These difficulties led to the strategy generally adopted today, which is a combination of stochastic variations on

limited portions of the proteins selected (thanks to the structural knowledge accumulated on these proteins) and selection for a function, combined with site-directed mutations leading to the replacement of well-chosen amino acids or segments of polypeptides.

The same, mixed strategy was adopted by synthetic biologists such as Frances Arnold for the design of new functional modules (Haseltine and Arnold 2007). The rational design of the module is complemented by a step of directed evolution that fine-tunes the functioning of the system. This strategy is now widely used. It is probably the only one likely to generate stably functional modules. Here artificial selection acts as natural selection to optimize a system (to drive it toward maximum efficiency).

Despite the limits of natural selection, it is nevertheless generally accepted—in particular by chemists—that organisms have explored a range of chemical possibilities *widely surpassing* the limited number accessible to chemists so far (Wender and Miller 2009). For instance, the diversity of volatile molecules produced by plants is astounding, and such diversity has been reached by using a small palette of chemical reactions (Pichersky et al. 2006). These metabolisms are peripheral to the central metabolism of the cell and therefore less constrained. Another interesting lesson from evolutionary biology allowed for the design of new specific enzymes (Yoshikuni et al. 2006). It is admitted that evolution progressively transformed promiscuous nonspecific enzymes into specific ones. Using this principle, Jay Keasling's group was able to create specific terpene synthases from a promiscuous enzyme. The lessons from evolution still have their place in the work of synthetic biologists!

Synthetic Biology Provides Tools for Evolutionary Biology

An important area of research in which synthetic biology can easily join the efforts of evolutionary biologists concerns the origin of life. The experiments by synthetic biologists are the only way to test scenarios in the absence of any remnants of prebiotic evolution. The only signs of prebiotic evolution are those still present in extant organisms: they are the palimpsests of these early steps whose traces have not been fully erased (Danchin 2007). The construction of these prebiotic systems is frequently done with a dual objective: first, to explore possible scenarios for the origin of life; but if this initial project fails, what has been learnt will eventually help to design artificial devices able to explore solutions that have not been explored by the evolution of life on Earth. The transfer can also occur in the reverse direction, and the characterization of rare, "abnormal" forms of life can be seen as the way to explore transient steps in the formation of extant organisms (Leaver et al. 2009). I will not describe this part of the work of synthetic biologists here, as it is discussed in Christophe Malaterre's paper in this issue.

Synthetic biology can help characterize the properties of extinct organisms and their macromolecules: molecular phylogenies enable the reconstruction of the sequences of ancestral proteins with plausible probability, and therefore to synthesize the protein and study its characteristics. One interesting application was to infer the environmental conditions in which early organisms were living. Steven Benner's group synthesized a protein synthesis elongation factor hypothesized to be present in early bacteria, and studied its properties, in particular its temperature dependence (Gaucher et al. 2003). Proteins present in organisms are optimally adapted to the ambient temperature in which these organisms live—this is the case of bacteria—or to the internal temperature of the organisms. Benner concluded that early bacteria were adapted to temperatures of 50 to 60°C. Such a result is important, since there is a debate among specialists about whether the first organisms were hyperthermophilic or not. As is often the case, the experimental answer was different from expectations: neither hyperthermophilic, nor similar to present organisms, but intermediate.

A similar reconstitution can be used to address other questions, say, to determine the characteristics of the first estrogen receptor and the hormone it bound (Thornton et al. 2003; Ortlund et al. 2007).

Many efforts are being made to reconstruct the way complex macromolecular machines, such as ribosomes (Bokov and Steinberg 2009) and flagella (Liu and Ochman 2007), were progressively elaborated. Synthetic biology can support these—often fragile—scenarios by synthesizing the hypothetical intermediates in these evolutionary processes, and checking their stability and properties.

Another contribution of synthetic biology to evolutionary biology is to extend the possibilities raised by experimental evolution. The limits in time and number of organisms produced by experimental evolution can be partially overcome by means of the tools of synthetic biology. I have already discussed the caution with which the results of this accelerated evolution has to be considered. Nevertheless, they can help to explore the robustness of biological systems as well as their evolvability (Isalan et al. 2008). The notion of evolvability is very vague, and a study in which perturbations are artificially introduced can help make it more precise.

The use of synthetic biology can be generalized to all the scenarios produced by evolutionary biologists as soon as they provide some clues to the nature of the genes and the genetic mechanisms involved in the evolutionary process. Tsong et al. (2006) compared the mechanisms used by different species of yeasts to determine their mating type. They elaborated a scenario to explain how the different extant alternative transcriptional circuits might have been generated from a unique ancestral mechanism. Such a scenario is ripe for confirmation by synthetic biology. In particular, it would be interesting

to synthesize the system suggested to have been the transition step from positive to negative regulation, and to check its functionality.

The domain where this approach can be most fruitful is EvoDevo. For historical reasons, obvious in this Darwin commemoration year, one interesting objective is to understand the genetic mechanisms that were involved in the evolution of the beak morphology of the different species of finches present on the Galapagos Islands. Some years ago, variations in the expression of the *BMP4* gene were proposed to have been responsible for this morphological transformation (Abzhanov et al. 2004; Wu et al. 2004). This hypothesis emerged from a careful description of the expression of the *BMP4* gene during the formation of the beaks in the different species of finches, and the demonstration that modifications in the expression of *BMP4* might alter the formation of their beaks.

More recently, Clifford Tabin and his group have described a new candidate gene for this morphological transformation of the beaks, a component of the calmodulin-dependent pathway (Abzhanov et al. 2006). They checked that the variations in the expression of this gene during the formation of the beaks was that expected if this pathway was involved in the morphological changes that took place during evolution.

Tabin and colleagues also demonstrated on chicken embryos that alterations in the expression of the *BMP4* gene and gene coding for a component of the calmodulin-dependent pathway could mimic the modifications of beak morphology observed in finches (Abzhanov et al. 2004, 2006). Their purpose was not to "replay" the evolutionary process but to reproduce evolutionary pathways, and to confirm the scenarios that have been proposed. What is required is an excellent molecular description of the system and that of the gene regulatory network involved in its ontogenesis. A model organism, related to the organism whose evolution is studied and able to be transformed, is also necessary. In this case, it was the chicken, a traditional model organism for embryologists. Finally, the experimenter must also master the techniques necessary for the introduction of the part of the gene regulatory network involved in this process (Erwin and Davidson 2009).

Another example concerns the lengthening of the forelimbs in bats as compared to other mammals. By introducing the *Prx1* gene in mice, it has been possible to demonstrate that the genetic variations responsible for this lengthening are located in the promoter of the *Prx1* gene (Crelekos et al. 2008).

Eric Davidson has recently stressed the need to develop a new approach to evolutionary questions, which he has called "synthetic experimental evolution" (Erwin and Davidson 2009). Davidson is well known for his precise description of the gene regulatory network controlling sea urchin development, in particular the development of the endomesodermal derivatives. He has shown the existence of a hierarchy in this gene regulatory network, with central and peripheral subsystems. The first, called the kernel subsystems, are made of highly interconnected regulatory genes. These subsystems are constrained, and most variations in the genes encoding their components are lethal. In contrast, the peripheral subsystems, such as the batteries of differentiation genes, can mutate without drastic effects on the development of organisms. From these observations Davidson concluded that the variations that occurred in the different subsystems generated events of a different nature: variations in the kernel subsystems were the source of new phyla, whereas mutations in the batteries of differentiation genes generated new varieties or new species (Davidson and Erwin 2006).

Synthetic experimental evolution complements experimental evolution. The latter has recently gained more importance, thanks to the new possibilities of characterizing with greater precision the molecular events underlying the phenotypic transformations observed in the test tube. It is, however, difficult to imagine how events such as the modification of beak morphology could be observed by such an approach. Synthetic experimental evolution will not *prove* that natural evolution has generated the morphological transformations that occurred in the past by modifying such or such gene. But it will support the scenarios that have been proposed, and help choose between different candidate genes and different scenarios. It will help reduce the gap between phenotypic and molecular descriptions of evolution (Dean and Thornton 2007). It is clear that such an experimental approach will often focus on one or a few genes and will neglect other genetic variations that contributed, even marginally, to the morphological transformation. In the future, this obvious bias of synthetic experimental evolution will probably be the basis of numerous misunderstandings and disagreements between synthetic biologists and evolutionary biologists.

Synthetic Biology Helps Address and Explore Some of the Most Fundamental Questions in Evolutionary Biology

The previous examples of what synthetic biology might bring to evolutionary biology show that this discipline will be important in reducing the gap between the two traditional approaches to biological phenomena (Morange 2009b).

But these new experimental tools can contribute more than simply fleshing out the too abstract models of evolutionary biology. They can help to directly address fundamental questions on the mechanisms of evolution, and maybe to provide some answers.

Since Darwin, there has been a permanent opposition between those who consider that the evolution of organisms was the consequence of the selection of the small variations that can be observed in populations at all times, and those who favor

the existence of rare mutations (in the sense given by Hugo de Vries), i.e., leaps required for the big steps of evolution. The debate was resurrected in the 1930s by the proponents of the Modern Synthesis, and on the other side by Goldschmidt (1940), who introduced the distinction between micro- and macromutation.

The years that followed might be seen as the victory of the supporters of the Modern Synthesis: Evolution is the result of the accumulation of small variations, affecting any gene in the genome. One reason for this success was that the distinction between micro- and macromutation was not clear: Was it a distinction between the immediate consequences of the mutations, or the later effects they might have on the evolution of organisms? Was it a distinction between different types of mutations? And if the differences were in the nature of mutations, what were these differences? A distinction was proposed between gene and chromosomal alterations, without great success.

The debate sprang up again with the progressive description of the different types of mutations by molecular biologists, but mostly from the characterization of the genes involved in the control of development. The distinction between structural and regulatory genes, and structural and regulatory mutations, was a decisive step (Morange 2007). Eric Davidson (Britten and Davidson 1971) and Allan Wilson (King and Wilson 1975) independently proposed that the leaps in evolution were the results of changes in gene regulation, not of the modification of structural genes. Wilson added to his hypothesis that these regulatory changes were the consequence of chromosomal rearrangements (Wilson et al. 1974).

Nevertheless, the decisive event was the characterization of homeotic genes, the discovery of their conservation, and the rise of EvoDevo. A taboo vanished, viz., that all genes have the same role in evolution: in EvoDevo, the genes involved in the control of development have a major place in the explanations of evolutionary events. The heterodox ideas of Wilson were progressively resurrected. Leaders of EvoDevo, such as Neil Shubin and Clifford Tabin (Shubin et al. 2009), Carroll (2008), and Davidson (Davidson and Erwin 2006) explicitly consider that it is necessary to distinguish morphological and adaptive evolution. Nonetheless, their visions are slightly different. Carroll believes that morphological evolution is due to a modification in the control of gene expression, most probably at the promoter level. Davidson considers that important morphological variations during evolution result from the modification of genes encoding the components of the most central kernel subsystems. In both cases, different mechanisms are involved in different forms of evolution.

Not all developmental biologists share these visions (Hoekstra and Coyne 2007). But what is important is that the time of sterile discussions is behind us. We now have tools, in particular those of synthetic biology, to test these hypotheses.

This is not a unique example. Synthetic biology, in conjunction with experimental evolution, might help, at least in some cases, to experimentally test some of the hypothetical evolutionary models that have been proposed. Consider, for instance, the hypothesis of genetic assimilation proposed by Conrad Waddington in the 1940s: faced with a sharp variation in its environment, an organism will first modify its physiology (Waddington 1941). The selection of favorable genetic variations will only occur later, to stabilize the new functional state that has been engendered by the previous physiological adaptation. Look at the experiment with chaperonins we have presented earlier: An increase in the amount of chaperonins can stabilize new protein variants likely to afford the organism new catalytic activities (Tokuriki and Tawfik 2009). Mutations occurring subsequently, and stabilizing the structure of the protein, would allow these proteins to remain folded even with low "normal" levels of chaperonins. As the level of chaperonins is known to increase in stress conditions, such a scenario has strong similarities with the one proposed by Waddington.

Similarities, but not identity: in the scenario concerning chaperonins, the first variations are genetic, and not physiological. So the proposed scenario is a wedding between the intuition of Waddington that late mutations can stabilize the phenotype, and not create it, and the more traditional vision of evolutionary biologists according to which mutations are the prime mover toward adaptation. Without losing their explanatory potential, many of the evolutionary models will probably be similarly transformed by this encounter with experimental reality. This example deserves an additional comment. In the previous description of these experiments I emphasized the non-natural conditions in which selection was effected by synthetic biologists. Here I show that these non-natural conditions might have naturally existed in some special (stress) conditions. This means that some of the extraordinary scenarios of evolution, designed by synthetic biologists, must be carefully considered by evolutionary biologists. Maybe the models provided by evolutionary biologists are too restrictive, leaving too much place to uniformitarianism, the hypothesis that the mechanisms of evolution have always remained the same. Another example of a hypothesis that might be efficiently tested through synthetic biology is the evolutionary role of partial penetrance of mutations (Eldar et al. 2009).

But the most significant role of synthetic biology will be to disentangle the different meanings of many concepts around which evolutionary biologists debate, allowing the questions to be addressed in a more precise way. Consider, for instance, the question of modularity (Schlosser and Wagner 2004). It is presently assumed by many biologists that modularity opened new avenues to evolution, and the road that led to modularity is actively explored (Wagner et al. 2007). But the word "module" designates very different objects, from complex genetic circuits to domains of proteins and short sequences in a promoter.

Is the advantage provided by modularity the same at these different levels of organization, and in different environments (Kreimer et al. 2008)? Synthetic biology can help answer these questions and in so doing explode a poorly defined category.

A similar case is the notion of "constraints," and the related question of the place of convergence in evolutionary biology. Constraints can be of a different nature. There are physical constraints, which, for instance, make the existence of flying elephants highly improbable. There are also functional constraints, which have been named "design" constraints or principles: they originate in the fact that there are not billions of solutions to produce a certain function. For instance, genetic circuits allowing an organism (a microorganism) to measure concentrations of molecules in the environment, and to orient the trajectory of the organism toward the source of these molecules if they are nutrients, or to avoid it if the molecules are harmful, must be built according to certain engineering principles to faithfully fulfill their task (Yi et al. 2000; Kollmann et al. 2005). These design constraints are linked to physical constraints, but they only apply when a certain functional purpose is required by the organism.

Finally, there are the historical constraints, which can be divided into two different categories. The first are internal, in a particular molecular, cellular, and tissular organization that is the result of previous evolutionary steps. The second category of constraints is ecological. A given organism is adapted to a specific environment and interacts with other organisms, which limits its possibilities of variation. The future is constrained by the position the organism presently occupies in the inhomogeneous space of the possible and in real ecological space. As we have seen before, many developmental biologists have insisted on the increasing place that these historical constraints, in particular the internal ones, would have in the evolution of higher organisms.

Linked with the previous question are the phenomena of convergence and parallel evolution (Conway Morris 2003). Both exist; the question is whether convergence and parallel evolution are preeminent phenomena in the evolution of organisms. There is an abundance of studies of characters at different levels of the hierarchical organization of organisms, supporting (Weinreich et al. 2006; Gross et al. 2009) or opposing (Beldade et al. 2002) the primacy of convergence and parallel evolution. It is necessary to distinguish functional convergence from structural convergence. The former can be reached without the latter. But even in the case of structural convergence, the mechanisms can be of a very different nature for apparently similar phenomena: A genetic convergence may be the result of the fixation of one specific allelic form already present in the population (Colosimo et al. 2005), or the repeated appearance of mutations affecting the same gene, or the same genetic pathway. In the case of microorganisms, experimental evolution has already helped to distinguish different phenomena put under the same banner of "convergence" and to show the complexity of the evolutionary processes (Lenski and Travisano 1994; Woods et al. 2006; Buckling et al. 2009). Synthetic biology can facilitate this process by creating new situations, and therefore allowing us to discriminate between historical constraints on one hand, and physical and design constraints on the other. Globally, it is probable that concepts such as convergence and constraints will lose a part of their apparent explanatory power through the clarifying action of synthetic biology.

Finally, synthetic biology readdresses the important, recurring philosophical question concerning the relation between the possible and the real. This long philosophical debate was initiated by Aristotle, pursued by Duns Scotus, Descartes, and Leibniz, and addressed by 20th-century philosophers such as Bergson. It was driven by theological motivations that are no longer present: What is the relation between the extant creation and other possible worlds? Did God also create the realm of the possible? But the fundamental question did not fully disappear after theology became less important. There are two different attitudes among synthetic biologists, reflecting different conceptions of the relations between the real and the possible that can now be driven to existence. This difference in attitude is not independent of disciplinary affiliations. The first attitude is to consider that the possible does in fact exist, and that the work of scientists only reveals it (or not, if it was falsely considered as possible). Synthetic biologists only explore a part of the reality that organisms had no time to explore, or from which they were rerouted by initial frozen accidents of evolution. The other attitude, more common among chemists, is to consider that synthetic biology will lead to the creation of new organisms as chemical synthesis generates new molecules. History permanently moulds reality. Evolution is open, and reality is to be constructed by humans.

In the first vision, synthetic biology is expanding our capacity to explore the world; in the second, it is a full participant in the shaping of future reality. In the first, synthetic biologists are taking evolution into their own hands; in the second, they are defining what evolution is. Both attitudes support an optimistic vision of the future successes of synthetic biology, which will overcome the constraints limiting the evolution of extant organisms. What is ethically at stake with synthetic biology is obviously not identical within these two different visions.

Acknowledgments

I am indebted to Dr. David Marsh for critically reading the manuscript version of this article. The TARPOL consortium (Seventh European Framework Programme) and IHPST supported this work.

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