CHALLENGE 5

EVOLUTIONARY SYSTEM BIOLOGY

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EXECUTIVE SUMMARY

"Nothing in Biology makes sense except in the light of Evolution"
Theodosius Dobzhansky (1973).

Evolution is a complex, multilevel process that operates at long time scales and can only be understood from a systems perspective. Though we have a considerable wealth of experimental data, the major challenge is to develop models and theoretical frameworks to understand empirical results and to pose better focused experimental questions. One of the first unsolved questions is how phenotypes arise from genotypes. The full picture is lacking, and modelling is limited to the dynamics of molecules, for instance RNA or protein folding, or to the emergence of simple molecular interactions —as regulatory motifs. A deeper understanding of the structure of genotype spaces might lead, among others, to a quantification of the relative roles played by neutral and adaptive mechanisms. This research program will have to investigate the effects of evolutionary innovation. Can complex biological functions be constructed from previous simpler modules? How do regulatory circuits emerge? What are the limits to the design of robust and portable functional modules? Answers to these questions will assess the validity of reductionist approaches, as opposed to viewing innovation as an emergent phenomenon, arising from network-like distributed properties. In a broader framework, we should be concerned about the mechanistic origin of evolutionary transitions, and on the role played by external forcing versus

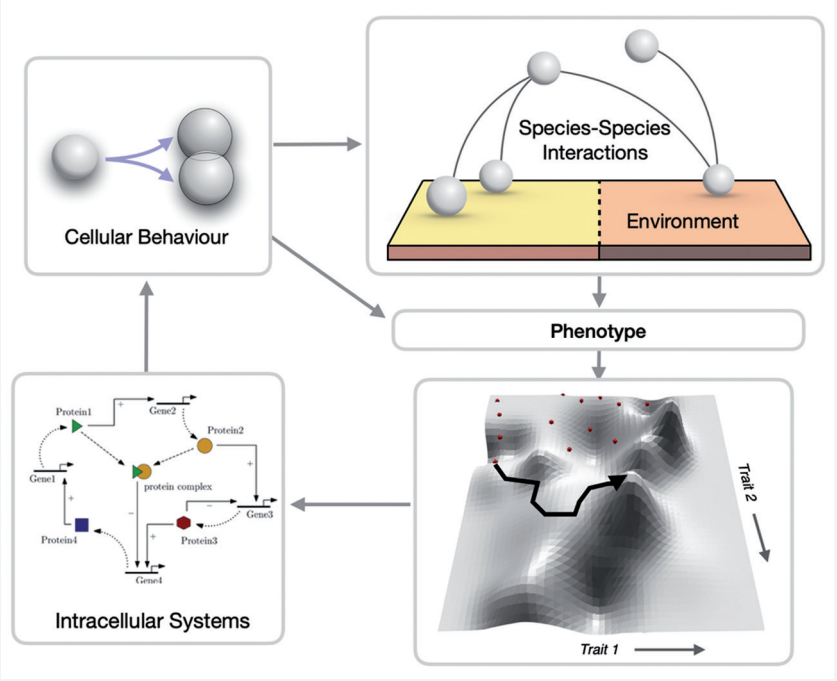
contingent or stochastic phenomena in their generation. Evolution itself can be viewed as a tool for Synthetic Biology, since directed evolutionary selection is a way to attain desired functions. A major goal is to integrate design with a selection-driven exploration of phenotypic spaces. Advances will be conditional on the construction of large evolutionary platforms where experimental evolution informed by design and theory can proceed *en masse*. The exploration of the possible phenotypes of engineered organisms at a large scale, as opposed to simple addition or deletion of a gene, calls for an urgent understanding of the plasticity and adaptability of new organisms and their traits of interest. An eventual commercial use of these organisms demands more research at the frontiers between evolutionary science, climate, and ecology: how will the ecosystem be affected by these organisms, but also, how will the organisms be affected by the ecosystem in a rapidly changing environment?

1. INTRODUCTION AND GENERAL DESCRIPTION

This question is at the core of Evolutionary Systems Biology (EvoSysBio), a new interdisciplinary research area that reconstructs fitness landscapes to predict (1) the fitness of individual organisms under state and environmental changes and (2) the evolutionary trajectories of populations across landscapes (Medina, 2005; Soyer and O'Malley, 2013; Loewe, 2016). EvoSysBio belongs to a broader trend in the biological sciences, i.e., the Modern Evolutionary Synthesis, which has been constructing a coherent view of evolution since the 1920s (Mayr and Provine, 1998). EvoSysBio is an emergent field that takes knowledge currently dispersed over many research fields, from population genetics and biochemistry to ecology. EvoSysBio recognises that evolution is a complex, multilevel process operating at long temporal scales, which can only be understood from a truly systemic perspective. Previous efforts attempted to explain evolutionary processes in the narrower context of individual genes and protein structures. However, complex organisms cannot be reduced to the workings of their components in isolation. EvoSysBio addresses this goal by modelling phenotypes as the outcome of evolving intracellular subsystems, e.g., signalling, regulation and metabolism, which are interacting with each other. EvoSysBio aims to synthesize and ultimately, predict, the complex multi-scale interactions between evolutionary processes and systemic properties (Fig. 1).

EvoSysBio has also been fuelled by the widespread adoption of quantitative and computational methods in the biological sciences. A clear exponent is

FIGURE. 1–The goal of EvoSysBio is to understand and predict genotype-phenotype maps in biological systems. Evolution is a multilevel process operating at a wide range of temporal scales. SysBio addressed this question by focusing on intracellular sub-systems (*e.g.* gene regulatory networks) while overlooking organism evolution. EvoSysBio extends the SysBio vision by including the ecological and evolutionary drivers of organismal complexity. Cellular networks determine species' interactions with their environment and other species. Ecological interactions among species are responsible for the fitness of organisms. Evolutionary processes (*e.g.* neutral drift and adaptation) move populations of organisms on dynamic fitness landscapes by changing the features of intracellular networks.



Systems Biology (SysBio), or the study of how organisms are organized by combining experimental data with mathematical modelling and computer-aided analysis techniques (Ideker et al., 2001; Kitano, 2002). SysBio has traditionally oriented towards the modelling aspects of biological systems ("how" systems are implemented at the molecular level, see Boogerd et al., 2007). Inevitably, overlooking the evolutionary component ("why" biological systems have those features) yields partial explanations of biological functions. The combination of evolutionary and systems biology leads to a better understanding of complex biological features. The evolutionary aspect is what unifies the high diversity of methods employed by EvoSysBio researchers. By

integrating the "how" and "why" questions under the same framework, we can better understand how biological systems work and why they function in that particular way.

EvoSysBio encompasses an integration of theoretical, empirical and computational approaches. A key component is the synthesis of universal "design principles" in biology (Poyatos, 2012; Katsnelson et al., 2018). Achieving this long-term goal crucially depends on identifying (and characterising) the universal principles that hold for large domains of life. For example, we have developed sophisticated empirical and computational tools that enable the detailed study of biological functions. It is however unknown to what extent the observed dynamics and organisation of any particular species, *e.g.*, how signalling networks enable chemotaxis *in Escherichia coli* or the dynamics of osmoregulation in *Saccharomyces cerevisiae* (Alon et al., 1999; Klipp et al., 2005), can also explain the physiological responses in other species. Ignoring intermediate states of population-level variation cannot fully explain the evolution of biological complexity (Lynch, 2007). Complex features in biological systems can be adaptive (Kashtan and Alon, 2005), neutral (Wagner, 2003; Solé and Valverde, 2006) or a mix between functional and non-adaptive processes (Wagner, 2008). In this context, evolutionary methods can support generalization of system properties, e.g., network patterns, beyond any specific biological model.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Evolution itself can be viewed as a tool for Synthetic Biology (SynBio) since directed evolutionary selection is a way to generate and optimize desired functions (Arnold, 2018). A major goal is to integrate design with a selection-driven exploration of phenotypic spaces. Advances will be conditional on the construction of large evolutionary platforms where experimental evolution informed by design and theory can proceed *en masse*. The exploration of the possible phenotypes of engineered organisms at a large scale, as opposed to simple addition or deletion of a gene, calls for a deep and reliable understanding of the plasticity and adaptability of new organisms and their traits of interest. An eventual commercial use of these organisms demands more research at the frontiers between evolutionary science, climatology, agriculture, and ecology: how will the ecosystem be affected by these organisms, but also, the analysis and prediction of the emergence of new pathogens and their potential epidemic spread.

2.1 Modelling and Computer-Aided Simulation of Biological Systems

The interplay of mathematical modelling with experiments is one of the central elements in SysBio. Model-building is therefore a key step, involving the reverse engineering (i.e., inference and identification) of equations that describe the biosystem, and their calibration to existing data (Klipp et al., 2005). Typically, these models can be either mechanistic (Stelling, 2004) or data-driven (as in machine learning and statistics; Kell & Oliver, 2004). Although the latter can be useful in many applications, mechanistic models generally provide a better framework to distil knowledge and understanding from data. Despite the many advances in model building in SysBio during the last two decades, the evolutionary perspective is absent in most of these models. Thus, a general theoretical and computational framework for multiscale modelling in EvoSysBio remains as a core objective.

The real power of mathematical models is unleashed when exploited via methods for their computer aided simulation, analysis, optimization and control (Wolkenhauser and Mesarović, 2005; Sontag, 2005). Although these tools have been widely used in areas like biosystems and bioprocess engineering (Park et al., 2008), we are still missing the evolutionary component integrated across different scales. Thus, another core objective would be to exploit EvoSysBio models as the kernel of process systems engineering in the bio-industries (e.g., agri-food and industrial biotechnology), particularly in areas like metabolic engineering. A possible route towards such objective could be through the integration of evolutionary game-theoretic approaches with multiscale modelling. The same approach can be adapted and extended to key problems in areas like environmental engineering (e.g., dynamics of microbial communities in bioremediation) and medicine (e.g., cancer evolution, or multiple microbial infections).

2.2 A first step towards understanding ecological complexity: modelling complex microbial communities

The last decades have witnessed the development of modelling approaches to describe cellular communities: from continuous to individual-based models of tissues and biofilms; from phenomenological ecological models to more mechanistic genome-scale approximations (Zomorrodi and Segrè, 2016). Still, their scope mostly restricts to "simple" systems (single or co-cultures), under steady-state or controlled extracellular environmental conditions. Also, evolutionary aspects are barely considered. The integration of multi-species multi-scale dynamic models incorporating both ecological and evolutionary

mechanisms (Valverde et al., 2020) are required to fully realize the potential of multicellular systems in answering biological questions and applications.

Model-based optimally designed microbial mixed-cultures will enable metabolically complex tasks by the division of labour and/or experimentally evolved cooperation (Harcombe, 2010; Hays et al., 2015; Thommes et al., 2019). As a result, bioprocesses will be more efficient, productive and stable than the current single-species bioprocesses and will allow for a broader range of products.

Similar approaches could be used to engineer the microbiome to generate potential therapies against metabolic, inflammatory, and immunological diseases, among others. The evolutionary dimension becomes fundamental to understand the dynamics of communities in which microbes and viruses coexist. In such systems, rapid co-evolution of viruses and microbial hosts (e.g. via acquisition of spacers in CRISPR arrays) cannot be disentangled from ecological processes, which has direct implications for the development of phage therapies and other virus-based microbiome engineering strategies.

2.3 Synthetic Biology: human design of non-biological constructs

In SynBio, we aim at using engineering principles of rational design to modify organisms, or to build new bio-artifacts (Purnick & Weiss, 2009; Cameron et al., 2014; Schwille et al., 2018). As in SysBio, SynBio can also exploit model-based approaches to guide the design, analysis, optimization and control of genetic systems (Marchisio et al., 2009). In addition to recent progress in genetic parts standardization and characterization (McLaughlin et al., 2018), in recent years we have also witnessed significant advances in the application of microfluidics, machine learning and automation to SynBio (Melin and Quake, 2007; Nielsen et al., 2016; Aoki et al., 2019; Carbonell et al., 2019). We are also already close to having programming languages to design computational circuits in living cells (Nielsen et al., 2016). These approaches open up new avenues for important applications, including biosensors (Gupta et al., 2019), biotherapeutics (Ozdemir et al., 2018), metabolic engineering (Keasling, 2012), biomanufacturing (Chen et al., 2020), and bioremediation (de Lorenzo et al., 2018).

However, with the exception of efforts in the field of directed evolution (Arnold, 2018), we are still lacking a truly evolutionary approach to SynBio. In particular, although computer-aided methods can help us in the design of synthetic parts in a similar way, as done in e.g. electronics, the unpredictability

and complexity created by the variability and evolvability of cell behaviour (Kwok, 2010) needs to be taken into consideration in order to achieve the envisioned engineering of biology. Finally, cell-free approaches (Hodgman and Jewett, 2012) will help us to improve our understanding of the design of evolved natural biosystems, and also to enable a novel kind of biomanufacturing with more freedom of design and improved control.

3. KEY CHALLENGING POINTS

3.1 Multiscale theoretical framework

Though we have a considerable wealth of experimental data, a main challenge is to develop models and theoretical frameworks explaining these results and pose better-focused experimental questions. We currently lack a multi-scale modelling framework that captures the essential features of biological systems, from genome to metabolism. At the same time, we need models that are simple enough to provide useful answers and insights. An essential part of future developments in EvoSysBio is the construction of a hierarchy of *in silico* tools and computational models that can guide experimental studies.

3.2 Evolution of Novelties

Ecosystems are highly nonlinear, complex dynamical systems (May and Leonard, 1975; Clark and Luis, 2020). Competition, cooperation, or victim-exploiter dynamics, are density-dependent interactions that induce non-linear effects in population dynamics. This is particularly relevant when dealing with ecosystem's responses to external perturbations, in particular, of anthropogenic origins (Lade et al., 2020). It has been conjectured these nonlinearities give rise to sharp shifts in the ecosystem composition, also known as "tipping points", which are becoming an important subject or research in ecology (Berdugo et al., 2020). Moreover, in SysBio, a tipping point driving population to extinction has been reported in yeast (Dai et al., 2012).

Sudden changes could be associated with large modifications. Interestingly, studies suggest that smooth alterations to the environment might also be responsible for such drastic shifts. Theoretical analyses of dynamical systems have interpreted these transitions as jumps along evolutionary optimization, where long periods without changes evidence the presence of high fitness barriers that the population cannot easily overcome (Huynen et al., 1996). This research program will have to investigate the causes of these sudden shifts in the genetic

composition of populations. Explaining the mechanistic origin of evolutionary transitions, and the role played by external forcing versus contingent or stochastic phenomena in their generation, will help understanding the effects of (and hopefully predict) evolutionary novelties (Wagner and Lynch, 2010).

3.3 Modelling and simulation of biological networks

Biological systems are composed of genes encoding the molecular machinery that provides the basic functions of life. Biological functions can be rarely reduced to specific components in isolation. Instead, they are the outcome of multiple interactions between different components. For example, networks of regulatory interactions specify how genes are expressed, with both operating on multiple, hierarchical levels of organization. Quantifying the structural features of biological and artificial systems has been the target of Network Science (a branch of Statistical Physics and Complex Systems developed during the last 25 years). SysBio has relied on the results of Network Science but we still do not understand the origin of structural regularities in biological networks, and how they shape function and evolution. This is particularly relevant in one of the main open problems in SysBio, namely, how to define a robust approach to reverse engineering and systems identification in biological systems (Villaverde and Banga, 2014). What is needed is the cooperation of network scientists with EvoSysBio researchers for developing a rigorous, biologically-realistic, evolutionary theory of biological complexity.

Genome-scale models and optimality principles have shown their potential for application in generating mappings genome-phenotype in EvoSysBio, for example in the prediction of phenotypic outcomes of short-term adaptive evolution or in the analysis of viability of mutant strains (Palsson, 2015). However, developing its full potential for EvoSysBio requires the predictive capabilities of these models to be improved in different ways. Clearly, the assumption of optimal growth in genome-scale metabolic models is not suitable for mutants and not valid in many (time-varying) environmental conditions. Thus, alternative evolutionary objectives or trade-offs or game-theoretical approaches are to be explored. Expansions including protein structures, integrated models of metabolism and protein expression (O'Brien et al., 2015) as well as hybrid modelling frameworks that incorporate explicit information on reaction rates are yet in progress. GSMs have also been expanded from single populations of cells to simple microbial communities (Harcombe et al., 2014), further developments are required to describe complex multi-species populations and changing environments (in time and space).

3.4. Structure of fitness landscapes

To predict the evolutionary path from one species to another, we need first to understand the underlying configuration space, *i.e.*, we need a 'map' where we can locate every possible intermediate species, for each environmental condition (see Appendix C). Unfortunately, fitness landscapes are huge, they live in spaces of very high dimensionality, and are difficult to visualize. The traditional metaphor of the fitness landscape is based on a gradualistic perspective about organismal change and evolution. Evolution has been described as the diffusion of populations on a relatively smooth landscape, always climbing towards regions of high fitness, which are eventually trapped in mountain peaks possibly separated by deep valleys of lower fitness (Wright, 1931). Due to conceptual advances in our understanding about the molecular structure of populations, we now know this picture is clearly incomplete and misses important ingredients. The structure of fitness landscapes is not a smooth and continuous surface, but rugged (Kauffman and Levin, 1987). Instead, the structure of the space of genotypes is a network of networks (or multilayer network) whose nodes (genomes) are mutually accessible through mutations. In the landscape, we can find regions with sharp discontinuities (signalling the presence of lethal and deleterious mutations) and long-range connections between distant regions of the landscape. EvoSysBio will help us understand (and characterise) landscape properties to obtain realistic models of adaptive fitness landscapes (in particular, through the development of models of genotype-phenotype maps at different levels of the biological hierarchy, see below), and validate these models with the reconstruction of empirical landscapes using network tools.

3.5. New constructions of genotype-phenotype (GP) maps

The mapping function between the instructions encoded in the genotypes and the structures and functions of the phenotypes is fundamental to every aspect of Biology. Given its importance, GP maps have attracted a lot of attention both from theoreticians and experimentalists (de Visser and Krug, 2014; Ahnert, 2017). As a result of these exercises, a number of universal properties shared by most GP maps have been derived. These properties are structural in the sense that they depend on the distribution of phenotypes across the network of genotypes. These properties include redundancy of genotypes (many encode for the same phenotype), a highly non-uniform distribution of the number of genotypes per phenotype resulting in a high phenotypic robustness, and the capacity to explore the landscape efficiently, reaching very distant phenotypes throughout a quite limited number of genotypic changes.

Despite these advances, we still miss a coherent theoretical description of the GP maps that explain why all these properties emerge (Manrubia et al., 2020). Novel approaches such as modelling GP maps as a network of networks in which different nodes in the genotypic network level correspond to networks of neutral genotypes and these nodes map into similar networks in the phenotypic space (Aguirre et al., 2018), or the seascape metaphor, in which the landscape topology fluctuates as a result of changes in the biotic and abiotic composition (Mustonen and Lässig, 2009) seems promising. Still open questions exist. To name two of the most relevant ones: a) whether the GP map as an object evolves (Manrubia et al., 2020) and whether it does so by natural selection or by neutral processes; b) how the GP map accommodates changes in genome size; in other words what are the consequences of making the genotypic network (of networks) growing or shrinking with evolutionary time? (see also Challenge 4).

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SUMMARY FOR EXPERTS AND GENERAL PUBLIC