Frontiers in Ecology, Evolution and Complexity

Mariana Benítez, Octavio Miramontes & Alfonso Valiente-Banuet (Editors)

Prologue by Stuart A. Kauffman

Authors
Julio Alcántara · Luis David Alcaraz · Maximinio Aldana · Elena R. Alvarez-Buylla
Eugenio Azpeitia · Jordi Bascompte · Mariana Benítez · Lorena Caballero · José Ángel Capitán
Eliane Ceccon · Álvaro Chaos · Germinal Cocho · Og DeSouza
José Davila-Velderrain · Ana E. Escalante · Carlos Espinosa-Soto · Juan Fornoni · Mónica García
Luis García Barrios · Mayra García · Sergio Hernández · Juan E. Keymer · Rafael López
Bartolome Luque · Susanna Manrubia · Pablo Marquet · Carlos Martorell · Pedro Miramontes
Octavio Miramontes · Alicia Montesinos · Silvia Pajares · Elia Ramírez · Thiago F. Rangel
Pedro J. Rey · Santiago Sandoval · Christian Torres · Alfonso Valiente-Banuet
Isabela Varassin · Miguel Verdú · Fabricio Villalobos

EditoraC3
CopIt-arXives
Publishing Open Access
with an Open Mind
2014

Electronically published in Mexico
First published in 2014 by Copit-arXives

Benítez, Mariana, Miramontes, Octavio, Valiente-Banuet, Alfonso (editors)
Frontiers in Ecology, Evolution and Complexity/
by Mariana Benítez, Octavio Miramontes and Alfonso Valiente-Banuet (editors)
Includes references and table of contents
Copit-arXives, México DF 2014
ISBN: 978-1-938128-05-9 ebook
1. Theoretical ecology. 2. Evolution 3. Social evolution 4. Ecosystem restoration
5. Complex networks 6. Complex Systems 7. Biocomplexity 8. Morphogenesis
6. Microbial ecology 6. Biodiversity 7. Biogeography 8. Pangenomics

ISBN: 978-1-938128-05-9 ebook
`http://scifunam.fisica.unam.mx/mir/copit/TS0012EN/TS0012EN.html`

Produced with Free Software including LaTeX and Linux. Composed in Adobe Palatino. Indexed in the Electronic Publications Catalog at the Universidad Nacional Autónoma de México and Google Books.

This book has been peer reviewed

CopIt-arXives
Cd. de México - Cuernavaca - Madrid - Curitiba
Viçosa - Washington DC - London - Stockholm

Kind support from
UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
Instituto de Física

Acknowledgments

All figures and pictures are courtesy of www.wikimedia.org or the authors unless explicitly stated.

Portrait image: Eliane Ceccon ©.

The editors deeply thank Edgardo Ugalde, Karo Michaelian, Antonio Neme, Alma Piñeyro, David Sanders, Rasmus Winther, Ek del Val, Wentian Li, León Martinez, Florian Greil, Mark Olson, José Luis Gutierrez, Consuelo Bonfil, Will Ratcliff, Eneas Aguirre, Carlos Villareal, Leticia Ribeiro de Paiva, Stuart Kauffman, Jorge Meave, Cecilia Gónzalez, Lucero Pacheco and Fabián Aguirre for their valuable support. Special thanks to Sven Siegmund (Leipzig) for developing and sharing the proceedings.cls used in this book.

The editores thank the support from DGAPA-UNAM PAPIIT Grant IN-101712.

Contents

Authors & Affiliations

Julio Alcántara Departamento de Biología Animal, Vegetal y Ecología, Área de Ecología, Universidad de Jaén, E-23071 Jaén, Spain

Luis David Alcaraz Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Maximinio Aldana Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México, Mexico.

Elena R. Alvarez-Buylla Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Eugenio Azpeitia Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Jordi Bascompte Estación Biológica de Doñana (CSIC), Sevilla, Spain.

Mariana Benítez Laboratorio Nacional de Ciencias de la Sostenibilidad (LANCIS), Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Lorena Caballero Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

José Ángel Capitán Centro de Astrobiología (CSIC), Spain.

Eliane Ceccon Centro de Investigaciones Multidisciplinarias, Universidad Nacional Autónoma de México, Mexico.

Álvaro Chaos Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Germinal Cocho Gil Instituto de Física and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Og DeSouza Termitology Lab at the Entomology Department of the Universidade Federal de Viçosa, Minas Gerais, Brazil.

José Davila-Velderrain Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Ana E. Escalante Laboratorio Nacional de Ciencias de la Sostenibilidad (LANCIS), Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.

Carlos Espinosa-Soto Instituto de Física, UASLP, Mexico.

Juan Fornoni Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico.
Mónica García Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Luis García Barrios El Colegio de la Frontera Sur (ECOSUR), Mexico.
Mayra García Alcalá Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México.
Sergio Hernández Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Stuart A. Kauffman University of Vermont, USA.
Juan E. Keymer Institute of Ecology and Biodiversity (IEB), Casilla 653, Santiago, Chile.
Rafael López Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Bartolome Luque. Departamento de Matemáticas Aplicadas de la ETSI-Aeronáuticos of the Universidad Politécnica de Madrid, Spain.
Susanna Manrubia Centro de Astrobiología (CSIC), Spain.
Pablo Marquet Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, C.P. 6513677, Santiago, Chile and Institute of Ecology and Biodiversity (IEB), Casilla 653, Santiago, Chile and The Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, New Mexico 87501, USA and Instituto de Sistemas Complejos de Valparaíso (ISCV), Subida Artillería 470, Valparaíso, Chile.
Carlos Martorell Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico.
Pedro Miramontes Facultad de Ciencias and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Octavio Miramontes. Instituto de Física and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Alicia Montesinos Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico.
Silvia Pajares Institute of Ecology and Evolution, University of Oregon, USA.
Elia Ramírez Instituto de Geología, Universidad Nacional Autónoma de México, Mexico.
Thiago F. Rangel Universidade Federal de Goiás, Brazil.
Pedro J. Rey Departamento de Biología Animal, Vegetal y Ecología, Área de Ecología, Universidad de Jaén, E-23071 Jaén, Spain
Santiago Sandoval Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México, Mexico.
Christian Torres Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México, Mexico.
Alfonso Valiente-Banuet Instituto de Ecología and Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico.
Isabela Galarda Varassin Universidade Federal do Paraná, Curitiba, Brazil.
Miguel Verdú Centro de Investigaciones sobre Desertificación, Valencia, Spain.
Fabricio Villalobos Universidade Federal de Goiás, Brazil

Prologue

Right now, the understanding of life on Earth, its origins and dynamics, is going trough a deep transformation rooted in the major breakthroughs of the sciences of complexity. This transformation is so important and transcendental that it is challenging most of what biologist knew just a few decades ago. At its center is the realization that order in life emerges spontaneously from the interaction processes of their components. Bio-molecules self-assemble to form new entities with properties that are not reducible to the separate components and the same happens at different levels of organization, from the genes, the cells, the individuals and the communities. This is a new fascinating paradigm that is being explored and whose frontiers are being pushed forwards by a generation of mostly young scientists. This book is an example of this. Here the authors are exploring and sharing their intellectual excitement on different fronts, about a new kind of science that explains how complexity and self-organization drives living phenomena. Ecology and Evolution are two topics where fertile ideas are flourishing. One is the science of interactions among individual living entities at its many levels of organization and the other, the science of the origins and changes of these entities and their interactions. Major breakthroughs in ecological theory include the understanding that interactions have a non-linear nature and that these interactions can be understood as complex networks; and that emergent spatial order are present in driving patterns of biodiversity and distribution. This new ecological vision is extremely important for a world who is in an urgent need of novel approaches for ecosystems and biosphere sustainability and restoration. Self-organization is an undisputed major principle of nature. At the present we see how complexity scientists are busy extending Darwin's theory of evolution to incorporate it as a major driving force. This requires a visionary thinking and certainly, the authors of this book are not intimidated on demonstrating it. A mixture of recent findings in biology, physics, chemistry and mathematics have been assembled together to give the readers a fascinating look on the problems and perspectives of the new emergent and cutting edge theory of biological evolution.

Stuart A. Kauffman
Vermont, 2014

Introduction

All in this book is about doing a new kind of science. It is the collective effort of 44 authors from five countries who have generously agreed in writing an authoritative book in an open access format. This is also a new form of communicating science where knowledge flows freely to the general audience without the barriers, restrictions and imprisoning of commercial contracts of the "free"-trade society. Readers world-wide are allowed to freely copy and distribute this book for their personal use.

The Sciences of Complexity are a new whole scientific paradigm where biological phenomena are viewed and explained as emergent properties of interconnected networks out of equilibrium. This is a highly interdisciplinary view of nature that requires the concurrent participation of biologists, mathematicians, physicists, chemists, etc. Over the last decades, a world-wide mesh of scientists with visionary attitudes have been challenging our current knowledge of how life originated and has evolved into the natural wonders we see today on this planet. All authors participating in this book are part of this challenging effort.

Self-organization is a natural phenomenon of major importance in Biology, it might be the source of many ordered patterns we see; however it has been largely overlooked, until now. Theories and concepts integrated in the Modern Evolutionary Synthesis have been central to explaining the changes and transformations that living forms have been going through since the origin of life on Earth. But it has been argued that essential components are missing in this tradition; it has become more and more clear that this framework has to be extended in order to fully understand the origination, development and evolution of organisms phenotypes and ecological structures. A key component to explain the emergence of biological order is self-organization. This idea, together with recent major breakthroughs in evolutionary biology, is shaping a new face of biological evolution.

Evolutionary and ecological processes and phenomena occur in a wide range of spatiotemporal scales, in which a variety of biological, geological and human agents interact with each other in a non-linear way. Adding to this complexity, over the past few decades, experimental and field data have shown that phenotypic plasticity might be central for the generation of inheritable phenotypic variation, and that the development and evolution of organisms largely shapes their own niche, placing the organism-environment interactions in a central position in contemporary evolutionary biology. Also, advances in molecular biology, phylogenetic inference, remote sensing, systems biology, bioinformatics, non-linear science and other fields have rendered a great amount of data that remain to be integrated into models and theories that are capable of accounting for the complexity of ecological systems. It is thus necessary to provide a solid basis to discuss and reflect on these and other challenges derived from the study of ecological systems and their evolution, both at the local and global scales.

Many different topics are addressed in this book, initiating from species diversity patterns, primarily regarded as the product of local, regional processes and historical events. In this sense, a macroecological approach pretends to fill the gap between local and regional processes to explain diversity patterns with different methodological approaches, as presented in their chapter by Villalobos and Rangel. A conceptual framework for plant community ecology is proposed considering both historical biogeographical processes and biotic interactions discussing the ways these two components evolve in mutual response to each other. Understanding the assembly of communities would be the only way to explain the sixth major extinction in the history of life and to transit towards sustainable practices, as proposed by Valiente-Banuet *et al.* Flower complexity, studied as the fractal dimension of the corolla outline, provides a useful and standardized way to understand the factors underlying plant-pollinator communication and mutualistic interaction networks. This shows that the highest number of pollinators visiting flowers occurs in the intermediate region of the range of flower complexity, suggesting that pollinators face a tradeoff when deciding the types of flowers they visit. This is the central idea in the chapter by Medel *et al.*

In their contribution, Alcántara and Rey discuss the importance of the temporal patterns of change in abundance and composition of natural assemblages of species, and the mechanisms behind these changes. They emphasize that these are fundamental aspects to understand the structure, function and stability of biodiversity. Their essay shows that some of the dynamic properties depend on the structure of the interaction matrix considering strongly connected components. In the same line, the relevance of restoration practices, as far as present disturbance human activities are not intensified, constitute a paramount for biological conservation. Therefore, it is possible to use plant-pollination interactions to evaluate the success of restoration practices, as discussed by Ceccon and Varassin. On the other hand and in the context of the present biodiversity and alimentary crises, it is necessary to develop and promote agricultural practices that contribute to food security and biodiversity conservation. The use of dynamical complex networks is presented by Benítez *et al.* as a way to study sustainable agricultural practices in the Mesoamerican polycrop known as *Milpa*.

Bacteria is by far the largest gene repository know on the planet and its importance pervades any process on earth. The possibilities of using pangenomics as a workhorse is central to describe both taxonomical and functional diversity within bacteria, being this the central idea in Alcaraz's contribution. But the study of the unseen majority of ca. 10^{30} bacterial cells affecting any biological process, such as biogeochemical cycles pose a number of difficulties, as discussed by Escalante and Pajares, who present them as potential new venues to overcome delays in the advance of microbial ecology.

The necessity of an interdisciplinary program on cancer research, from fields such as physics, ecology and evolution -assuming that the human body is inherently complex- is opening novel perspectives for effective therapeutic interventions and shows clearly that metaphors based in the generic properties of complex systems such as ecosystems and cell tissues are not only useful but imperative to develop new approaches to deal with complex diseases, as shown by Keymer and Marquet.

Ecological science has emerged into the XXI century as one of the most complete and formalized topics in life sciences. It has benefited from the pioneering long tradition of bio-mathematicians that started in the early XX Century with the Lotka-Volterra formalism and into the Chaos theory of the 70s of the last Century. But it has entered a new and dramatic change in the last two decades: Modern ecological science is based, as never before, on the concept of non-linear interactions among components. But this is also a commonly definition given when explaining the nature of complex systems. Therefore simple computational complex systems displaying spatiotemporal self-organization are very useful to put on test the emergent nature of ecosystems dynamics. On its chapter, Caballero *et al.* have used Conway's *Game of Life* model as a metaphor for studying simple ecological interactions.

Non-linearity pervades population ecology and complex phenomena such as chaos, self-organization, or criticality arise when deterministic population models are analyzed, as discussed by Martorell. Moreover the dichotomy between determinism and stochastic has been recently revisited and so the biological relevance of chance an its role in biology, and specifically in evolutionary biology, is under review in the light of advances on dynamical systems theory. This reevaluation of determinism and chance, and their role, provides new elements to perceive how biological phenomena may be operating in nature. This is the contribution of Pedro Miramontes.

The mapping of genotypes into phenotypes is a central challenge of current biological research, which historically has assumed a linear causation scheme in which the non-genetic character of developmental dynamics has been neglected, as discussed by Davila-Velderrain and Alvarez-Buylla. However, in the post-genomic era, a systems-view based on nonlinear (network) assumptions is increasingly adopted, showing that evolutionary dynamics can be studied using simple dynamical models of gene regulatory networks.

Under an evolutionary framework, most organisms cope with a huge spectrum of perturbations and the inherent disruptions such as genetic mutations. Therefore, they must be flexible enough as to develop new phenotypes in order to keep up with new environmental challenges. Under this scenario, the central question is to determine how organisms reach this equilibrium between phenotypic robustness and phenotypic innovation, leading to the concept of dynamical criticality, as discussed by Sandoval-Motta *et al.* Although phenomena at the molecular and cellular levels, as well as environment interactions during evolutionary processes, have been studied independently from each other. With the advent of new theoretical and technological approaches for biological system research, multi-scale models allow rapid progress in their study, as shown by García and Azpetia.

Despite that some morphological traits have been commonly interpreted as adaptations maintained and modified just by natural selection, self-organization processes are able to determine the characteristics of the basic building units of organisms, relegating natural selection to a secondary role, this the central idea explored by Álvaro Chaos in his contribution. On the other hand, modularity is a common feature of biological systems and there are several evolutionary paths to explain its evolution, as noted by Espinosa-Soto. Luque and Bascompte remark that self-organization processes play an important role as a source of evolutionary novelty and causes the emergence of complex structures, while natural selection operates on the existing ones.

In the opinion of Miramontes and DeSouza, cooperation –and not competition– has played a central role in social evolution. In order to elaborate a modern view and theory of social evolution, concepts such as group selection and those from the sciences of Complex Systems must be integrated together along with the Darwinian tradition.

Analogies between biological and linguistic evolution are deep to the point that both evolutionary systems can be studied by using models of biological evolution. This allows to explore a number of questions related to the origin, causes, development, interaction, and fate of languages, as discussed by Capitán and Manrubia.

All chapters in this volume aim to delineate an integrative and interdisciplinary view highlighting new avenues in research and teaching, critically discussing the scope of the diverse methods in the study of complex systems, and pointing at key open questions expanding the program of evolutionary ecological studies. This book arises with the aim to provide students and specialists with a collection of high quality essays that will contribute to integrate Ecology, Evolution and Complexity in the context of fundamental biological research and possible applications.

We would like to express our gratitude to the Physics Institute and the Center for Complexity Sciences at the Universidad Nacional Autónoma de México for trusting on the relevance and success of the present publishing initiative (CopIt-arXives and EditoraC3). We also appreciate the enthusiastic participation of all contributors and reviewers who have devoted their time to a task –writing books– that is less and less considered and valuated in our present academic evaluation practices.

Mariana Benítez, Octavio Miramontes and Alfonso Valiente-Banuet
México, D.F. 2014

Geographic patterns of biodiversity

F. Villalobos & T.F. Rangel, Universidade Federal de Goiás, Brazil

1 Abstract

Geographic variation in biodiversity is a well-documented natural phenomenon but no satisfactory explanations regarding its origins have yet been reached. The inherent complexity of ecological systems hampers the comprehensive study and understanding of biodiversity patterns. Recent advancements in theoretical and methodological approaches provide means to explicitly deal with such ecological complexity allowing developing and testing specific predictions about potential causal mechanisms. Macroecology is a relatively recent ecological discipline that has emerged as a synthetic research program focused on the emergent, statistical properties of complex ecological systems at broad spatial and temporal scales. Different approaches, from statistical correlations to computer simulations, are employed within macroecology in order to understand biodiversity patterns. Here we provide a brief overview of such macroecological approaches and their relevance for studying the complex phenomenon of biodiversity as expressed over the geography of our planet.

2 Resumen

La variación geográfica de la biodiversidad es un fenómeno natural bien documentado pero del cual aún no se tienen explicaciones satisfactorias. La inherente complejidad de los sistemas ecológicos impide el estudio y comprensión completos de los patrones de biodiversidad. Avances recientes en diferentes enfoques teóricos y metodológicos proveen la posibilidad de lidiar directamente con la complejidad ecológica, permitiendo desarrollar y evaluar predicciones específicas acerca de los potenciales mecanismos causales. La macroecología es una disciplina ecológica relativamente reciente que ha surgido como un programa de investigación sintético enfocado en las propiedades estadísticas y emergentes de sistemas ecológicos complejos en amplias escalas espaciales y temporales. Distintos enfoques, desde correlaciones estadísticas hasta simulaciones por computadora, son empleados por la macroecología para entender los patrones de biodiversidad. Aquí

presentamos una breve descripción y discusión de dichos enfoques macroecológicos y su importancia para estudiar el complejo fenómeno de la biodiversidad y su expresión a lo largo de la geografía de nuestro planeta.

3 Introduction

One of the most intriguing natural phenomena is the variety of species and their heterogeneous distribution over the planet. For instance, most species are distributed along the tropics whereas a smaller fraction of species is distributed over temperate regions, a pattern known as the Latitudinal Diversity Gradient. Such biodiversity gradient is well documented, yet explanations about its causes remain elusive. Explanations for this and other geographic patterns of biodiversity have changed through time and disciplines, with biogeography favoring regional, historical processes –speciation, extinction, dispersal– and ecology favoring local, contemporary processes –mainly biotic interactions. The ecological approach was heavily influenced by the experimental tests of ecological systems, which attempt to take ecological processes apart and study their components separately. However, it has been implicitly recognized that complex ecological systems are not amenable to simple experiments [1].

The processes underlying biodiversity patterns are complex and varied. At local spatial scales, ecological communities vary greatly, imposing challenges to the detection of general patterns and processes that may govern the origin and maintenance of biodiversity [2]. In an attempt to overcome this lack of generalization, a more comprehensive, broad-scale and statistical approach has been proposed to study biodiversity patterns. This approach has been defined as Macroecology [1, 3]. Macroecology introduces a historical and geographical perspective on local, ecological systems and an ecological perspective to understand regional and continental biotas [1]. It applies a statistical mechanics approach with an emphasis on the statistical regularities that emerge from studying large groups species, about which it makes the fewest possible assumptions [4]. The macroecological rationale is based on the assumption that macroscopic patterns of complex ecological systems in space and time are not epiphenomena, thus not reducible to a small set of local components and current processes [5].

The macroecological framework to understand the mechanistic basis of geographic patterns of biodiversity has changed since its original inception, even within the short time that the discipline has been around. From a purely descriptive and correlative methodology to a stochastic simulation and mechanistic modeling approach, macroecology has established itself as major biological research program [6]. Here we provide a brief overview of current macroecological approaches and discuss their relevance for understanding the complex phenomenon of biodiversity at geographic scales.

4 Description of patterns and correlations

Initially, macroecology was mostly occupied with the description of patterns and their explanation under a correlative approach. Studies relying on this approach were limited to the simple evaluation of the concordance between the geographic patterns, mainly species richness variation, and environmental factors, such as temperature, precipitation, and evapotranspiration [7] (Figure 1). The underlying assumption is that species are in equilibrium with climate –existing where climate is favorable– and species richness gradients are driven by contemporary climate. Similar, strong correlations have been described for a varied set of taxa (e.g. animals and plants) over the same or distinct regions, suggesting that such correlative patterns are widespread and, more importantly, underlying mechanisms may be general. For example, [8] found globally consistent strong correlations between angiosperm richness and mean annual temperature and potential evapotranspiration; whereas [9] identified water and energy-related variables (e.g. actual evapotranspiration and plant productivity) as important determinants of both animal and plant species richness. Notwithstanding the generality of such correlations, they fail to inform about the actual processes changing the number of species in an area.

5 Mechanistic theories of biodiversity

Statistical descriptions of ecological attributes represent the core of the macroecological endeavor and the focus of current biodiversity theories. Patterns such as the frequency distributions of abundance, body size, geographic range size, and the correlations between these attributes are of paramount importance to understand the processes underlying its emergence [4]. Frequency distributions of ecological attributes, namely those representing lognormal and power law distributions, are uncannily similar to patterns in physical, geological, economic, and cultural systems [10]. For instance, the frequency distribution of abundances among species in ecological communities has a remarkable similarity with the citation frequencies of scientific papers. Likewise, the relationship between species richness and area –one of the few general rules in ecology– resembles the number of unique words and total word length in texts [10]. These congruent patterns beg for a more universal explanation. Complexity science has explained such patterns as a result of general processes and the multiplicative, rather than additive, interaction of variables within complex systems (e.g. ecological communities) [10]. In ecology, such processes have been related to the dynamics of species at both local and broad spatial scales [5].

The neutral theory of biodiversity (NTB, [11]) provided the first model linking local scale population dynamics with broad scale biodiversity dynamics. This theory is based on a simple set of assumptions: that all organisms of all species have identical ecological properties and that local communities are saturated with no changes in the total number of individuals occurring over time (i.e. “zero-sum” dynamics). Under NTB, diversity

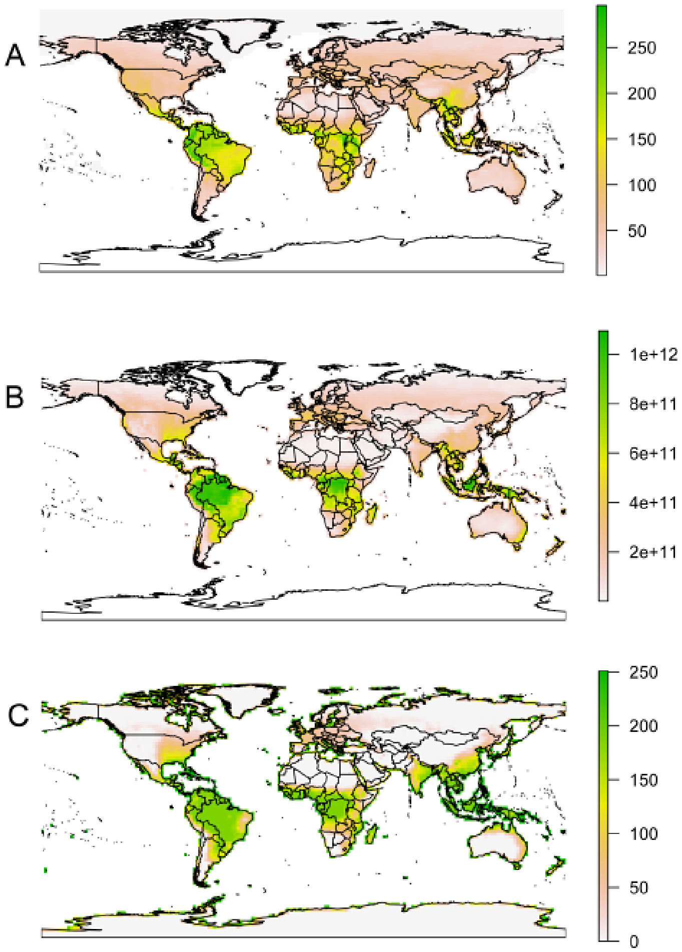
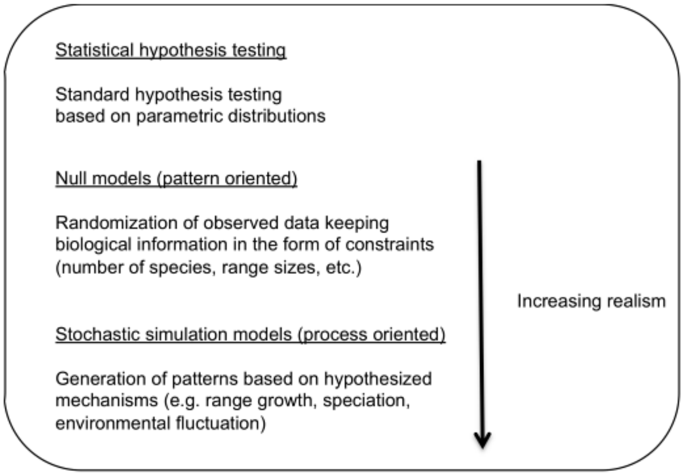


Figure 1: (A) Map depicting the species richness of mammals in the world. (B) Map showing the variation in Net Primary Productivity (NPP; units are in mass of carbon per unit area per year ($gCm^{-2}yr^{-1}$)). (C) Map showing the variation in Actual Evapotranspiration (AET; $mmyr^{-1}$). Note that all three variables (species richnes, NPP and AET) show higher values in the Tropics.

is generated and maintained by stochastic birth, death and immigration processes [11]. Neutral theory predictions have remarkably reproduced biodiversity patterns such as the species-abundance distribution in local communities. Furthermore, NTB makes additional testable predictions such as species-area relationships, population and community dynamics, and phylogenetic tree shape and branch lengths, thus linking locally and contemporary to broader and historical spatial and temporal scales. In this sense, NTB continue to play an important role in biodiversity research by providing quantitative and process-based null hypotheses against which macroecological patterns can be contrasted.

Another relevant biodiversity theory is the metabolic theory of ecology (MTE) [12]. This theory relies on first principles of physics, chemistry and biology to predict ecological properties of populations, communities and ecosystems from organisms' metabolic rates [12]. MTE predicts a mass-temperature dependence of biological processes that integrates cellular to global-level processes based on the well-known scaling law between metabolic rate and body size in which the whole-organism metabolic rate, I, scales as $I = I_o M^{3/4}$ (where I_o is a normalization constant and M is the body mass). For instance, a proposed MTE model predicts that when temperature scales as $1/kT$ (where T is temperature in Kelvin and k is the Boltzmann's constant of $8.62 \times 10^{-5} eV^{K-1}$) the logarithm of species richness should be linear with slope between -0.6 and -0.7, thus species richness increasing with temperature [13]. MTE has provided interesting explanations of biodiversity patterns linking temperature, metabolic biochemistry, physiology, and evolutionary rates. [14], for example, developed a model of kinetic energy that successfully predicted rates of genetic divergence and speciation in planktonic foraminifera as increasing toward tropical latitudes. Further improvements to the MTE are expected, including testing its foundations and assumptions. As such, MTE is far from complete but efforts are being conducted to formalize and test the essential roles of body size, metabolism, and temperature. In sum, MTE represents a fundamental advancement linking biodiversity patterns to basic biological principles influenced by the environment and should still prove useful in theoretical and applied terms for understanding biodiversity.

A more recent biodiversity theory aimed at predicting scaling metrics such as the species-area and species-abundance relationships, and the species-level occupancy distributions across space is the Maximum Entropy (MaxEnt) theory [15]. MaxEnt is based on maximizing information entropy –a measure of the lack of structure or detail in the probability distribution describing a knowledge system– to infer such macroecological metrics. MaxEnt aims to describe the central tendencies observed for the entire range of macroecological metrics of interest without adjusting parameters or prejudging what is driving the system [15]. This approach is based on state variables, which are properties of a system that comprise the conditions whose specification is necessary to implement theory but whose determination lies outside the theory. In macroecology, these state variables can be defined as the area of the system, the number of species in that area, the total number of individuals in those species, and the total rate of metabolic energy consumed by those individuals [15]. Contrasting with the neutral and metabolic theories, MaxEnt



Statistical hypothesis testing

Standard hypothesis testing based on parametric distributions

Null models (pattern oriented)

Randomization of observed data keeping biological information in the form of constraints (number of species, range sizes, etc.)

Stochastic simulation models (process oriented)

Generation of patterns based on hypothesized mechanisms (e.g. range growth, speciation, environmental fluctuation)

Figure 2: Conceptual description highlighting differences among statistical hypothesis testing, standard null models and stochastic simulation models (based on [19]).

theory has yet to be widely applied and tested.

The above theories rely on the statistical description of observed patterns without invoking the multiple interactions among different ecological mechanisms or, in the case of MTE and MaxEnt, without considering stochasticity and contingence of species spatial and temporal dynamics. Indeed, this is one of the great advantages of such null theories [16]. These theories can be informative when succeed as well as when they fail. For instance, success of the theory means that mechanisms incorporated into the parameters' values are sufficient to explain patterns. Conversely, when failure, the theory tells us that more mechanistic information than that captured by its parameters is needed to predict patterns [16]. Alternatively, the goal of including explicit mechanistic processes and tracking their dynamics over space and time has been the focus of recent theoretical developments based on computer simulation models.

6 Null and stochastic simulation models

Traditional approaches for understanding biodiversity patterns have relied on small-scale experiments or on fitting simple statistical models to macroecological data. More recently, computer simulation models have emerged as an important alternative to traditional macroecological approaches [17]. These simulation models can be considered as experimental systems and used to mimic biodiversity in a way that can be manipulated, thus helping to develop and test theories about its origin, maintenance and dynamics [18].

Early efforts in simulation modeling were based on the application of null models that include the action of stochastic processes and the possibility of no ecological effect.

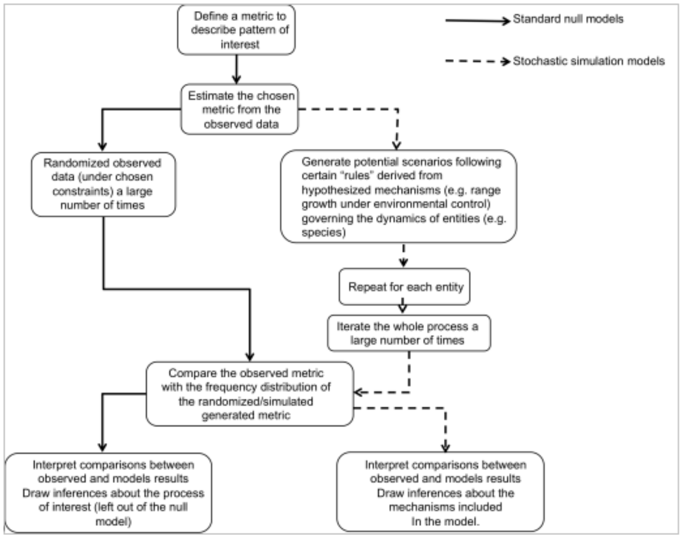


Figure 3: Flow chart illustrating the general protocol for conducting and testing null (solid line) and stochastic simulation models (dashed line) (based on [19]).

Such null models generate expected statistical distributions of the variable of interest from stochastic models based on biological theory and Monte Carlo methods (i.e. keeping biological information; species richness or distribution, while randomizing the data) [20] (Figure 2). The interpretation of null models based on their "falseability" and, thus, in the relevance of excluded variables has been and is still valid and important in biogeography, ecology, and macroecology. For instance, much has been learned from the application of null models in the fields of ecology, biogeography, and evolution, with its paramount influence in testing ecological theory in the debate of competition versus stochasticity in determining community structure as an example [20]. In macroecology, null models have been regularly applied with different procedures simulating null scenarios being implemented, especially when testing species richness gradients and coexistence patterns [21, 22]. Nevertheless, the growing interest in considering specific mechanisms beyond the randomization of data together with the availability of greater computational capacity has led to more sophisticated null and mechanistic models (Figures 2 and 3).

Geographic variation in species richness is ultimately determined by the differential coexistence of species in distinct regions of the globe, resulting from the overlap of species ranges differing in size, shape, and location. Thus, current macroecological approaches advocate the stochastic simulation of species' range building and placement to study geographic patterns of biodiversity [17]. These simulation models started with the now classic Mid-Domain Effect (MDE), which examined the effect of geometric constraints imposed by hard boundaries on the distribution of species, and consequently on emerging patterns of species richness [23]. Initial MDE models simulated ranges within a homogeneous, one-dimensional domain of regular shape [23] or resembling actual two-dimensional continents [24]. More recently, climatic and topographic gradients have been included in spatially explicit simulations as well as evolutionary dynamics such as speciation, extinction, dispersal, and niche conservatism/evolution [25], thus directly including ecological and evolutionary mechanisms into the modeling framework.

Simulation models differ from statistical models such as the correlative models or some of the mechanistic theories outlined above. These latter, parameter-fitting methods are usually developed to predict rather than explain biodiversity patterns. Conversely, simulation models are built to offer an explanation of biodiversity patterns [18]. Indeed, great conceptual advancements have been reached by applying simulation models to comprehend the causes of biodiversity patterns. For instance, the explicit consideration of first principles thought to govern the origin and geographic spread of species has highlighted the interplay among evolutionary dynamics, ecological processes, and environmental conditions in effectively driving biodiversity patterns [17]. In a seminal contribution, [25] developed a simulation model incorporating ecological and evolutionary "mechanisms" such as climatic fluctuation, species' environmental preferences and evolution, as well as formation of new species by speciation. They found that the species richness pattern for the Birds of South America could be closely reproduced by a combination of species origin within the Tropics and strong niche conservatism [25]. In a less dynamic framework but similarly informed model, [26] showed that climatic conditions along the geographic domain as well as inheritance of niche preferences among species are important but not enough to explain similarity among sites and species in the bat family Phyllostomidae. Instead, other, additional causes such as historical processes are needed to explain the observed patterns in that important bat family.

Stochastic simulation models represent one of the most important methods in biogeography and macroecology [27], allowing the exploration of specific hypotheses and predictions that otherwise would be impossible [18]. Further developments are still required to understand biodiversity patterns and evaluate the usefulness of simulation models, some of which are well on their way. For instance, applying a "virtual ecologist approach" [28] to evaluate simulation models against known virtual data can help to assess the model's capacity to discern underlying processes. Also, the consideration of composite response variables and patterns beyond species richness (e.g. co-diversity among sites in terms of shared species and co-distribution among species in terms of overlapping geographical distributions [26]) can be used to validate models at different hierarchical levels [29] and provide stronger tests of potential mechanisms.

Geographic patterns of biodiversity are a complex natural phenomenon requiring advanced methodologies to comprehend their causes. Macroecology has come a long way in providing a synthetic framework for broad-scale biodiversity patterns, linking different disciplines and introducing novel methods. More recently, it has expanded its focus and now routinely considers larger temporal scales, including phylogenetic and paleoclimatic information to allow a deep-time perspective on biodiversity patterns. Despite increasing documentation of empirical patterns and significant conceptual and methodological advances, we are still far from explaining biodiversity patterns. Several challenges remain to be solved in order to accomplish this objective. Among others, the paucity of good data (e.g. standardized sampling schemes at different spatial scales, information on species traits, phylogeny and behavior) continues to be a problem even for well-known taxa such as vertebrates. In addition, better methods are still needed to deal with data biases, uncertainty assessment, and correlations among hierarchical predictors [6], as well as procedures for model testing and dealing with more informative response variables or patterns [17]. This being recognized, the discipline of macroecology will certainly continue to expand and contribute towards the overarching goal of generating a unifying theory of biodiversity.

The macroecological programme currently integrates varied and sophisticated approaches aimed at understanding biodiversity patterns. Of these, simulation models hold great promise to represent and understand the inherent complexity of ecological systems as well as anticipating potential scenarios for biological conservation under natural and anthropogenic disturbances such as the current biodiversity crisis and climate change. Macroecological approaches thus offer promise and fruitful means to explicitly deal with ecological complexity and provide a more comprehensive understanding of biodiversity patterns at broad spatial and temporal scales.

7 Bibliography

[1] J. H. Brown, *Macroecology*. University of Chicago Press, 1995.

[2] J. H. Lawton, “Are there general laws in ecology?” *Oikos*, vol. 84, no. 2, pp. 177–192, 1999.

[3] J. H. Brown and B. A. Maurer, “Macroecology: the division of food and space among species on continents,” *Science*, vol. 243, no. 4895, pp. 1145–1150, 1989.

[4] P. A. Marquet, “Macroecological perspectives on communities and ecosystems,” *The Princeton guide to ecology*, p. 386, 2009.

[5] B. A. Maurer, *Untangling ecological complexity: the macroscopic perspective*. University of Chicago Press, 1999.

[6] J. Beck, L. Ballesteros-Mejia, C. M. Buchmann, J. Dengler, S. A. Fritz, B. Gruber, C. Hof, F. Jansen, S. Knapp, H. Kreft *et al.*, “What’s on the horizon for macroecology?” *Ecography*, vol. 35, no. 8, pp. 673–683, 2012.

[7] K. J. Gaston, “Global patterns in biodiversity,” *Nature*, vol. 405, no. 6783, pp. 220–227, 2000.

[8] A. P. Francis and D. J. Currie, "A globally consistent richness-climate relationship for angiosperms," *The American Naturalist*, vol. 161, no. 4, pp. 523–536, 2003.

[9] B. A. Hawkins, R. Field, H. V. Cornell, D. J. Currie, J.-F. Guégan, D. M. Kaufman, J. T. Kerr, G. G. Mittelbach, T. Oberdorff, E. M. O'Brien *et al.*, "Energy, water, and broad-scale geographic patterns of species richness," *Ecology*, vol. 84, no. 12, pp. 3105–3117, 2003.

[10] J. C. Nekola and J. H. Brown, "The wealth of species: ecological communities, complex systems and the legacy of frank preston," *Ecology Letters*, vol. 10, no. 3, pp. 188–196, 2007.

[11] S. P. Hubbell, *The unified neutral theory of biodiversity and biogeography (MPB-32)*. Princeton University Press, 2001.

[12] J. H. Brown, J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West, "Toward a metabolic theory of ecology," *Ecology*, vol. 85, no. 7, pp. 1771–1789, 2004.

[13] A. P. Allen, J. H. Brown, and J. F. Gillooly, "Global biodiversity, biochemical kinetics, and the energetic-equivalence rule," *Science*, vol. 297, no. 5586, pp. 1545–1548, 2002.

[14] A. P. Allen, J. F. Gillooly, V. M. Savage, and J. H. Brown, "Kinetic effects of temperature on rates of genetic divergence and speciation," *Proceedings of the National Academy of Sciences*, vol. 103, no. 24, pp. 9130–9135, 2006.

[15] J. Harte, *Maximum entropy and ecology: a theory of abundance, distribution, and energetics*. Oxford University Press, 2011.

[16] ——, "The value of null theories in ecology," *Ecology*, vol. 85, no. 7, pp. 1792–1794, 2004.

[17] N. J. Gotelli, M. J. Anderson, H. T. Arita, A. Chao, R. K. Colwell, S. R. Connolly, D. J. Currie, R. R. Dunn, G. R. Graves, J. L. Green *et al.*, "Patterns and causes of species richness: a general simulation model for macroecology," *Ecology Letters*, vol. 12, no. 9, pp. 873–886, 2009.

[18] S. L. Peck, "Simulation as experiment: a philosophical reassessment for biological modeling," *Trends in Ecology & Evolution*, vol. 19, no. 10, pp. 530–534, 2004.

[19] N. J. Gotelli and W. Ulrich, "Statistical challenges in null model analysis," *Oikos*, vol. 121, no. 2, pp. 171–180, 2012.

[20] N. J. Gotelli and G. R. Graves, *Null models in ecology*. Smithsonian Institution, 1996.

[21] H. T. Arita and E. Vázquez-Domínguez, "The tropics: cradle, museum or casino? a dynamic null model for latitudinal gradients of species diversity," *Ecology Letters*, vol. 11, no. 7, pp. 653–663, 2008.

[22] F. Villalobos and H. T. Arita, "The diversity field of new world leaf-nosed bats (phyllostomidae)," *Global Ecology and Biogeography*, vol. 19, no. 2, pp. 200–211, 2010.

[23] R. K. Colwell and D. C. Lees, "The mid-domain effect: geometric constraints on the geography of species richness," *Trends in Ecology & Evolution*, vol. 15, no. 2, pp. 70–76, 2000.

[24] W. Jetz and C. Rahbek, "Geometric constraints explain much of the species richness pattern in African birds," *Proceedings of the National Academy of Sciences*, vol. 98, no. 10, pp. 5661–5666, 2001.

[25] T. F. L. Rangel, J. A. F. Diniz-Filho, and R. K. Colwell, "Species richness and evolutionary niche dynamics: a spatial pattern–oriented simulation experiment," *The American Naturalist*, vol. 170, no. 4, pp. 602–616, 2007.

[26] F. Villalobos, A. Lira-Noriega, J. Soberon, and H. T. Arita, "Co-diversity and co-distribution in Phyllostomid bats: Evaluating the relative roles of climate and niche conservatism," *Basic and Applied Ecology*, vol. 15, no. 1, pp. 85–91, 2014.

[27] T. F. Rangel and J. A. F. Diniz-Filho, "Biogeographical models," in *Encyclopedia of Biodiversity 2nd ed.*, S. Levin, Ed. Waltham, MA: Academic Press, 2013, vol. 1, pp. 565–575.

[28] D. Zurell, U. Berger, J. S. Cabral, F. Jeltsch, C. N. Meynard, T. Münkemüller, N. Nehrbass, J. Pagel, B. Reineking, B. Schröder *et al.*, "The virtual ecologist approach: simulating data and observers," *Oikos*, vol. 119, no. 4, pp. 622–635, 2010.

[29] V. Grimm, E. Revilla, U. Berger, F. Jeltsch, W. M. Mooij, S. F. Railsback, H.-H. Thulke, J. Weiner, T. Wiegand, and D. L. DeAngelis, "Pattern-oriented modeling of agent-based complex systems: lessons from ecology," *Science*, vol. 310, no. 5750, pp. 987–991, 2005.

Plant community ecology

A. Valiente-B., E. Ramírez, M. Verdú & A. Montesinos, UNAM, Mexico and CIDE, Spain

1 Abstract

As long as human activities have continued to disturb Earth's climate, biota, and entire ecosystems at unprecedented rates, the science of ecology needs to provide realistic knowledge. We propose that this knowledge can only be provided under a community ecology approach. In this essay we propose a conceptual framework for plant community ecology considering both historical biogeographical processes and biotic interactions, and discuss the ways in which these two components evolve in mutual response to each other. Given the overwhelming complexity of multiple ecological processes we need to understand general patterns governing the assembly of communities to be able to face the sixth major extinction in the history of life and to transit towards sustainable practices in ecosystems.

2 Resumen

En tanto continúen las actividades humanas que están perturbando el clima global, a la biota y a ecosistemas completos a tasas sin precedente, la ciencia de la ecología debe proveer conocimiento realista. Nosotros proponemos que este conocimiento solo puede ser provisto bajo un enfoque de la ecología de comunidades. En este ensayo proponemos un enfoque conceptual para la ecología de comunidades de plantas considerando tanto procesos de biogeografía histórica como de interacciones bióticas, discutiendo la forma cómo ambos componentes evolucionan como respuesta de su interrelación. Dada la abrumadora complejidad de múltiples procesos ecológicos necesitamos entender de manera integral los procesos que gobiernan la organización de las comunidades para ser capaces de encarar la sexta mayor extinción de especies en la historia de la vida y poder transitar a prácticas sustentables en los ecosistemas.

3 Introduction

The vast number of species inhabiting the Earth are not randomly distributed, but are distributed differentially in ecosystems across different geographical areas. The central issue of community ecology is to determine those processes that determine that composition of species within a particular geographical area. A community is comprised of the entirety of the biotic elements in an ecosystem: an interactive assemblage of species occurring together within a particular geographical area, a set of species whose ecological function and dynamics are in some way interdependent [1]. In an ecological context, interdependence means that life is supported by life, in such a way that ecological interrelationships among species are central to the maintenance of species in communities. However, the mechanisms explaining which species form a community do not only depend on the biotic interactions but also on the historical events through the geological time (speciation, extinction, and immigration); this provides the source of species that eventually make up the community (i.e., the regional species pool). Consequently, a community is a subset of species from the regional species pool that are able to colonize an area and interact among them, a topic named "community assembly rules" [2]. Thus, although community ecology embraces the study of the mechanisms of biotic interactions that support life, it also depends upon the historical biogeographical processes that have molded regional species pools over geological time.

The overwhelming complexity of understanding multiple ecological processes occurring at different temporal scales has led ecologists to approach community assembly rules by breaking this complexity into small bits of research. However, although this approach has proven to be efficient in answering a few specific questions, in order to understand the general patterns governing the assembly of communities it is required to consider other conceptual framework and methodologies which consider the complexity of these processes. Firstly, ecologists have traditionally only emphasized local short-term ecological processes, ignoring evolutionary processes that can help to explain the patterns found in current communities. Secondly, when biotic interactions have been acknowledge in community ecology, the approach has been to study specific ecological interactions focusing on small subsets of species, isolating them from the rest of co-occurring ecological processes within their community context. The aim of this essay is to propose a conceptual framework and methodologies to analyze plant communities integrating both the historical and ecological processes, and to discuss the ways in which these two temporal components may evolve in mutual response to each other. To do this, we first discuss our personal view on the main methodological limitations of the historical background of community ecology; these have traditionally looked for explanations to diversity patterns as arising exclusively from the individual behavior of species or their subsets usually taken out of context. Then, we discuss a conceptual framework that highlights the relevance of considering large levels of temporal and spatial scales to understand the regional species pool. We show how the biogeographic properties of the regional species pool, linked with systematic biology, geology, and paleontology has led to the development of phylogeny-based approaches which have provided challenging results suggesting extremely fruitful hypotheses on the regional causes of species diversity [3], as well as the ecology underlying phylogenetic community structure [4]. Lastly, we integrate a series of examples for each topic trying to illustrate the ideas. In doing this, we hope to spark the interest of many students in the study of communities.

4 Historical background

The histories of ecology and biogeography are indissolubly tied as they emerged at the same time with overlapping explanations for species richness patterns from local to global scales. For example, Alexander von Humboldt is often credited with the first ideas about the influence of climate on plant distribution and the foundations of ecology and biogeography. Although true, the credit comes also initially from Carl Ludwig Willdenow [5], who recognized the dominant role of climate in governing plant geography and vegetation zonation [6], thus opening the emergence of ecology to explain biogeographical patterns. However, the emphasis on local, short-term ecological processes by ecologists on one hand and on historical long-term evolutionary processes by biogeographers on the other provoked an early separation of ecology and biogeography. This divergence was noticed by one of the earliest biogeographers, the Swiss botanist Alphonse De Candolle, as one of the main obstacles for the development of biogeography as well as of ecology [7]. Despite the recognition that local diversity patterns are affected by biogeographic, historical and evolutionary processes [8, 9], community ecologists disconnected rapidly from the historical aspects of natural systems and tried to understand communities just in terms of the individual properties of species in communities. For example, the most accepted idea on community organization assumed that communities are the result of the confluence of species adapted to a specific environment [10], and the outcome of competitive exclusion [11]. This axiomatic-like paradigm originated from Darwin's ideas connecting natural selection with the universal density-dependent Malthusian population theory [12]. Thus, competition was thought to affect the numerical processes in both populations and communities. Therefore, the species composition of a given area is determined by the physiological tolerances of species to the specific environment, and then by the omnipotent role of competition to finally determine the specific composition of communities [11].

Philosophically, Gleason's view about communities can be traced back to the seventeenth century philosophy of René Descartes's Discours in which phenomena are the consequences of the confluence of the individual atomistic bits, each with its own intrinsic properties, determining the behavior of the system as a whole [13]. From this perspective, parts of a whole (e.g., species) are ontologically prior, and thus lines of causality run from part to whole, and therefore the whole (e.g. community) is defined by the sum of its parts.

Cartesian reductionism therefore denies the possibility of emergent properties as a consequence of the interaction between the parts [13]. Actually, parts do acquire new properties by being together imparting new properties to the whole, but the true magnitude of said properties at the community level can only emerge when processes that drive diversity are analyzed under a complete framework without isolating the processes to a few levels. However, in practice and probably as a way to simplify the problem, biotic interactions have traditionally been analyzed as isolated pairwise interactions, an approach that also has been highly influenced by Lotka [14] and Volterra [15] models, in which biotic interactions are considered in pairs of species. Although successful in determining specific interaction mechanisms, such a reductionist approach has limited the search for emergent properties at the community level, being at the same time one of the main impediments to the development of community ecology. However, the development and the use of methodological tools by ecologists -such as complex networks theory- represent a great step for the analysis of complete sets of species and the search for emergent properties in communities.

5 The framework

Proper understanding of a community should be viewed as a contingent structure in reciprocal interaction with its own parts (i.e., species and their interactions at different trophic levels) and with the regional species pool of which it is a part [13]. Traditionally, a community has been considered to be a subset of species from the biogeographic region after the species have passed through an environmental filter (Figure 1a). However, biotic interactions may be also the responsible for the maintenance (Figure 1b) or the local extinction (Figure 1c) of some of those species. Once the regional species pool has been reduced to the community composition, as mentioned above, to study community ecology ecologists commonly have isolated interactions from the complex community context. For example, plants interact with other plants, pollinators, herbivores, etc, (Figure 1d), but this traditional approach has forgotten the unseen majority, namely soil microbes [16] (Figure 1e). Thus, ecological linkages between aboveground and belowground biota have acquired a growing recognition since the past decade [17], although both have traditionally been considered in isolation from one another. Numerous studies prove plant-mediated linkages between aboveground and belowground biota ([17], and references therein). For example, while herbivores may affect the function of soil communities, in turn soil microorganisms can change the morphology and chemical composition of plant tissues, altering the fecundity and activities of aboveground herbivores [18]. Moreover, the presence of belowground mutualists such as arbuscular mycorrhizal fungi in the roots increase pollinator visitation and seed set of plants [19]. In short, the microbes that live belowground directly and indirectly influence the productivity, diversity, and composition of plant communities.

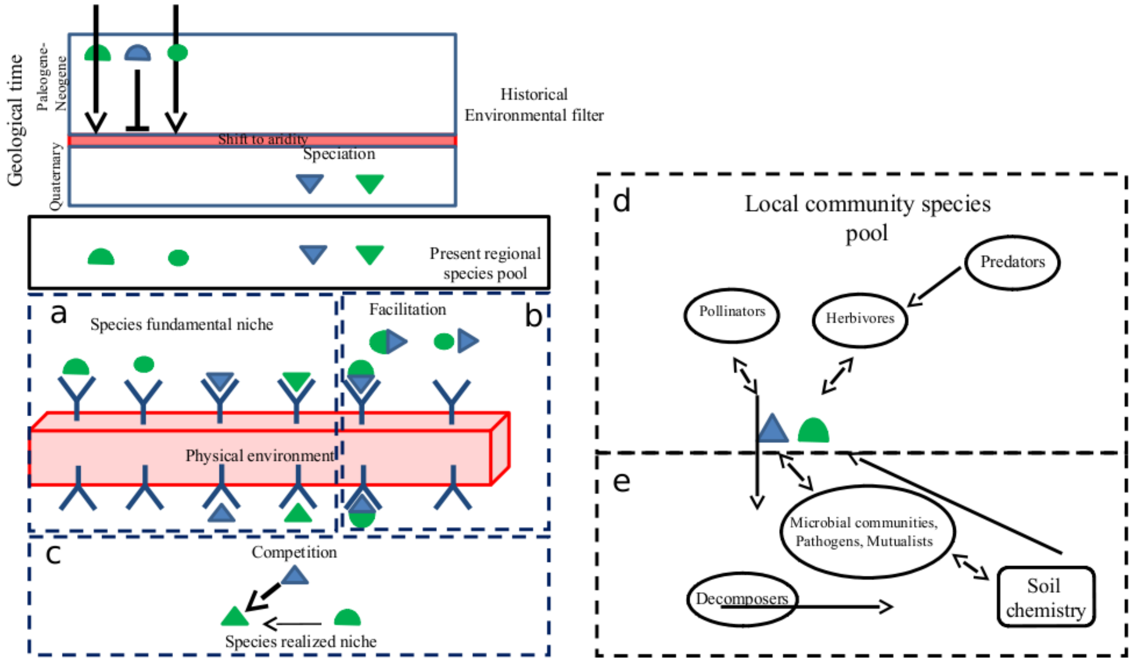


Figure 1: The regional species pool is originated from a mixture of different lineages or species (triangles, circles and semicircles) that originated in different geological times and have survived (←) or become extinct (⊢) in different historical environmental filters such as the shift to a more arid environment which occurred from the Paleogene/Neogene to the Quaternary. Paleogene/Neogene species (circles and semicircles) evolved under an environment different from current conditions and might not be by perfectly adapted to the current physical environment (species fundamental niche, a). However, some Paleogene/Neogene species can remain in present communities thanks to the buffered conditions provided by recent evolved drought tolerant species (Quaternary) (triangles) that recreated the Paleogene/Neogene environmental conditions (facilitation, b). Afterwards, the regional species pool is reduced to the local community species pool due to ecological processes such as competition that can lead some species to local extinction (e.g green triangle) (competition, c). The width of the arrows indicates the strength of competition of one species on another. In addition to competition, multiple ecological interactions, both above (d) and below ground (e) also act synergistically to define the local community species pool. For example, plants interact with other plants, pollinators, herbivores, and also with soil microbes. However, ecological linkages between aboveground and below ground biota have been traditionally considered in isolation from one another.

6 Regional species pool and ecological connections

In community ecology, the composition of local communities depends on the regional species pool, which in turn is determined by large-scale biogeographical processes [3]. However, to understand global patterns of biodiversity, it is necessary to know the ecological processes that have eliminated species from communities or allowed them to persist through time [20] (Figure 1b). Current plant communities are the product of historical sorting processes and, therefore, they include mixtures of floristic elements that evolved during different geological times. Thanks to paleoecological reconstructions of paleofloras using leaf characters [21] we know that many of those species evolved under different environmental conditions and inhabit plant communities different from those in which they originated [22]. The notion that interdependent processes among plant species have been insignificant over evolutionary time frames has been central to the Gleason's ideas, assuming that plant communities do not possess stable properties determined by plant-plant interactions. However, the idea that communities are constituted by adapted species to a specific environment [10] has lost terrain by showing that present-day species inhabit environments different to those where they originated, and that facilitative ecological interactions among plant species have been a crucial component of historical and sorting processes that allowed species to survive under dramatic changes of global climate [20]. Particularly, one of the most important floristic sorting periods worldwide to affect modern plant communities occurred during the shift from the wet Paleogene/Neogene periods to the unusually dry Quaternary, when most global deserts developed [20]. During this transition a wave of new plant species emerged, presumably in response to the new climate. In contrast, many Paleogene/Neogene species that have been tracked through the fossil record in different environments remained relatively abundant despite the development of a much more unfavourable climate for species adapted to moist conditions [20]. However, these old species remained thanks to the buffered conditions provided by recent evolved drought tolerant species (Quaternary) that recreated the Paleogene/Neogene environmental conditions [20]. Regeneration niches and recruitment life histories strategies of taxa belonging to old lineages match with the environment in which these taxa evolved and consequently nowadays, after an environmental shift to arid conditions, positive biotic interactions are crucial for the maintenance of old linages, constituting a key aspect to understand the maintenance of local and regional species pools. In other words, niche conservatism (i.e., related lineages tend to have similar niche requirements) offers a mechanism to explain large and local-scale species-richness patterns, thus reconciling ecological and evolutionary perspectives. In our framework, positive local ecological processes have acted by expanding the fundamental niche of species allowing old lineages to be part of communities quite different from those where those species evolved (Figure 1b).

At present megadiverse areas of the world such as Mexico are inhabited by very old lineages under conditions that are quite different from those where species originated, and therefore it is possible that similar processes have occurred worldwide affecting regional species pools. This fact emphasizes the importance of paleobotanical and paleoecological research. For example, biogeographical and paleobotanical evidence of megadiverse areas of tropical America such as in the Neotropics [23], including different parts of Mexico [24, 25] that harbor higher number of plant species than other continental areas with similar ecological conditions, indicate that most of the taxa evolved during the last 65 Ma of the Cenozoic era and have persisted through evolutionary time [24, 26, 27]. In particular, the Paleogene/Neogene transition constitutes a paramount to understand the evolution and mixing of taxa to explain the causes of megadiversity patterns in these areas [20, 24, 28]. For example, the fossil plant record has already shown that forests and savannas covered areas of the present subtropical and tropical deserts well into the Eocene, and that tropical forest, woodland and thorn forest covered the modern tropical deserts into the Middle and late Neogene [22, 29].

7 Niche conservatism and phylogenetic community structure

Species that evolved within a similar environment are expected to possess similar traits that might have provided them with fitness advantages in said environment. Traditionally, in an attempt to search for patterns in the species composition and dynamics of communities, species have been treated as equivalent units, with independent functional traits. However, as many species traits are evolutionarily conserved, it can be expected for lineages originated during different geological times, and environments, which now coexist in communities composed of a mixture of linages, to present different traits. Thus, closely related species, or lineages originated in a given environment, will tend to be more similar regarding their ecology and life-history strategies shaped by species traits [30, 31], and so to have similar requirements to survive and reproduce (i.e., niche conservatism).

The ecology and life-history strategies of a species can determine two main processes that have been traditionally thought to structure ecological communities: competition and habitat filtering (e.g., [32, 33]). Ecological similarity can result in more severe competition between closely related species than between distantly related species, as the former will share similar requirements and ways of exploiting the resources. However, it is also a general pattern that species inhabiting extreme habitat share similar traits, such as many desert plant communities which are dominated by spiny or succulent plant species, most of them taxonomically related (e.g. cacti).

The phylogenetic structure of a community can provide insight to the ecological processes that are taking place in that community. Many classical topics in community ecology have been enriched with the consideration of the phylogenetic relationships among species in the community compared to the regional pool. For example, regarding species diversity, it is essential to understand why similar habitats in different regions have different numbers of species. One potential explanation is that different regions are occupied by different clades (e.g. [34, 35]), which in turn can differ in their potential for diversification (e.g., [36, 37]). The availability of phylogenies, along with methods for the construction of supertrees, now allows community structure to be assessed phylogenetically. As an example, Phylomatic is a tool for attaching members of a list of taxa to a "master" phylogeny of the angiosperm, using the internal node names of the megatree [38]. Afterwards the resulting phylogeny can be used as an input in available software for the analyses of phylogenetic community structure such as PHYLOCOM [39] or several packages in R.

In the past few decades, a new conceptual framework has been developed in which phylogenetic information from co-occurring species is used as an indicator of two main assembly processes (competition and habitat filtering) [4]. This framework has provided a set of methods aimed at testing the structure of communities. Multiple metrics, null models and statistical testing have been developed that quantify the distribution of taxa in a community relative to the regional species pool [40]. A broadly used metric that quantifies the distribution of taxa in a sample relative to a regional pool is the Net Relatedness Index (NRI). This is a standardized measure of the mean pairwise phylogenetic distance of taxa in a sample (MPD), relative to the MPD of a similar sample selected randomly from the regional pool. The Nearest Taxon Index (NTI) is also a standardized measure of the phylogenetic distance to the nearest taxon for each taxon in the sample. In general terms, the objective of calculating these metrics is to detect patterns (e.g., clustering, overdispersion) from which it is possible to infer community processes (e.g., filtering, competition). Both NRI and NTI increase with increasing clustering and become negative with overdispersion. Clustering or overdispersion can be studied either on species traits (phenotypic clustering or overdispersion) or on the phylogenetic relationships of species (phylogenetic clustering or overdisperison) [41].

Phenotype-based methods aim to infer the assembly process from the distribution of species traits in the communities relative to the distribution of traits in the available species pool. Phenotypic clustering arises when co-occurring species are more similar (phenotypically) than expected from the distribution of traits in the regional species pool, whereas phenotypic overdispersion refers to co-occurring species which are less similar (phenotypically) than expected in the same species pool. In general, when environmental filtering is the main assembly process, species in the community tend to share the trait values which enable them to tolerate that environmental filter, leading to phenotypic clustering. However, when competition is the main assembly process, phenotypically similar species tend to exclude each other, leading to phenotypic overdispersion [32]. In addition, phenotypic information can be combined with co-occurrence or environmental information to test whether similar phenotypes co-occur in environments. For example,Verdú & Pausas [42], use the matrix correlation method to show that plant species sharing the same post-fire germination trait tend to co-occur (phenotypic clustering) in fire-prone communities.

However, there are several difficulties to completely characterize the phenotype of all the species in a community. The feasibility of measuring certain traits, or the selection of certain traits considered ecologically relevant, can influence the community patterns studied. Phylogenetic relatedness -given that phenotypes are likely to be conserved because of common ancestry- can be considered a proxy for the species similarity considering every phylogenetically conserved functional trait and not only the traits that are easy to measure or intuitively relevant. In addition, phenotypic and phylogenetic information can be combined to differentiate between environmental filtering and competitive exclusion [4]. For example, the implications of a phylogenetic clustering in a community structured by an environmental filter depend on whether the trait evolved in a conserved or convergent way. If the traits that confer tolerance to the environmental filter are phylogenetically conserved, the coexisting species will be both phylogenetically and phenotypically clustered. However, if distantly related species have more similar trait values than expected by chance (trait convergence), the coexisting species will be phenotypically clustered but phylogenetically overdispersed.

When traits are phylogenetically conserved, the implications of phylogenetic clustering or overdispersion in community structure can be understood in the same way as the implications of phenotypic clustering or overdispersion. Phylogenetic clustering implies that closely related species co-occur more often than expected according to a null model, while phylogenetic overdispersion indicates that closely related species co-occur less often than expected. Based on coexistence theory, when species compete for the same limiting resource, all but one species will be driven to extinction. Accordingly species can coexist by inhabiting different niches that partition the available resources (i.e phylogenetic overdispersion), and competitive exclusion will prevent coexistence of similar species [43]. More recently it has been reported other ecological processes, such as facilitation, complementary to competition which can also result in phylogenetic overdispersion.

8 The role of plant-plant interactions in structuring communities

Positive and negative interactions act simultaneously, and the balance between them ultimately determines coexistence. For example, positive interactions like plant facilitation can turn into negative interactions (competition along the ontogeny of a plant), and this can influence the phylogenetic community structure of the community [44]. Plant-plant facilitation is a key process structuring plant communities in semi-arid environments. In some plant communities, more than 90% of the species recruit successfully only beneath the canopies of nurse plants and therefore are maintained via facilitation [45, 46]. Facilitation, as well as competition, is an ecological process influenced by the phylogeny of the species involved. Within a community, approaches using null models have been used to test for the effects of the balance between facilitation and competition on the phylogenetic structure of the community. Valiente-Banuet and Verdú [44] characterized the facilitated-nurse pairwise interaction present in three different communities. They distinguished between the interactions in which the nurse and the seedlings of facilitated species persisted when facilitated become adults (remaining interactions; facilitation), and those interactions in which the facilitated species outcompete the nurse later in time (lost interactions; competition), and calculated the mean phylogenetic distance between the nurse and the facilitated species in those two types of interactions. They compared these values with the frequency distribution of the phylogenetic distance between a nurse and a facilitated species chosen randomly from the species pool in the community (null model). The mean phylogenetic distance between the nurse and the facilitated species was significantly lower and higher in the lost and remaining interactions respectively than expected by chance. This result shows that facilitation occurs among distantly related species, and only interactions above a threshold of phylogenetic distance remain later in time, potentially driving coexistence patterns and phylogenetic overdispersion in the community influenced by plant facilitation. In addition, the regeneration niche (i.e., if a species can regenerate in the open or only beneath another plant (facilitated) has been shown to be phylogenetically conserved [46], so that closely related species tend to be either nurse or facilitated species. In order to test for a phylogenetic signal in a trait, one may assess whether related species are more similar than expected by chance, based on the minimum number of evolutionary steps required to obtain the observed distribution of the traits in the phylogeny. The minimum number of evolutionary steps observed is compared with a null model, in which the taxa are reshuffled across the tips of the phylogeny several times and the distribution of the minimum steps required in each time is estimated.

9 From pair-wise interactions to community approaches: ecological networks

There is evidence, at different spatial and temporal scales, that plant facilitation is a species-specific process (i.e., non-random), and that nurse species tend to facilitate distantly related facilitated species. An interesting approach used to explore non-random patterns in multiple species interactions is network analyses. Bipartite ecological networks provide a framework to assess a wide variety of ecological processes in which nodes (species) of two different guilds (parties) are connected by links (interactions) between (but not within) guilds [47]. Historically, these networks have been described and analyzed by graph theory, and allow testing for non-random patterns in the interactions between two guilds. A few interesting characteristics, among many others, than can be extracted from a network are, for example, the species degree (i.e., the number of different species a certain species interacts with, distinguishing between generalist and specialist species), the nestedness (i.e., a pattern of interaction in which specialists interact with species that form perfect subsets of the species with which generalists interact, avoiding specialist-specialist interactions) or the modularity (i.e., a tendency of certain species (a module) to interact more between themselves than with species from other modules) [48].

One interesting contribution from complex networks is the recognition that several mutualistic networks, despite differences in the nature of their nodes, exhibit similar characteristics, such as nestedness. This has implications for the robustness of the system to the loss of species and the maintenance of biodiversity [47]. In the case of facilitation, although most of the knowledge is based on isolated pairwise species interactions, a network approach provides the potential to test for non- random patterns of associations between nurse and facilitated species in

complex ecological communities. The networks between nurse and facilitated species have been shown to behave as a mutualistic network, presenting a nested structure in which a few generalist nurses facilitate a large number of species while the rest of nurses facilitate only a subset of them [49].

10 Multiple ecological processes acting synergistically

Although the structure of plant facilitation networks indicates that nurse-facilitated interactions are specific and non-random, it is still unknown which mechanisms could be underlying the fact that facilitation is more prone to occur between distantly related species. It has been argued that multiple ecological relevant traits are phylogenetically conserved, and these traits will ultimately condition with which species (pollinators, dispersers, mutualistic fungi pathogens, predators) a particular plant can interact. It has been shown across the entire tree of life that closely related species tend to interact with similar species [50]. Thus, facilitation between distantly related species could imply that they provide different microbes to the rhizosphere. This will be beneficial in the case of pathogens, as avoiding plants that share similar pathogens could be a driver of coexistence, or also, in the case of positive interactions, increasing the richness or diversity of mutualists could be beneficial due to functional complementarity of the mutualistic species. Plant- arbuscular mycorrhizal fungi (AMF) symbiosis is one of the oldest positive associations on earth and the majority of plant species interact with AMF in all kinds of environments [51]. The richness and the phylogenetic diversity of AMF have been shown to increase plant biomass and promote plant coexistence [52–54]. Accordingly, the specificity in plant facilitation associations could be influenced by the AMF with which each plant species interacts, and provides to the common rhizosphere. Firstly, for plant-AMF interactions to condition plant-plant interactions, it has to be proven that plant-AMF interactions are non-random, so that different plant species can interact with different AMF. As mentioned before, one approach to test for non-random interactions within the whole community is network analyses. Montesinos-Navarro et al. [55] proved using network analyses that plant-AMF network show a non-random pattern of associations, presented a modular pattern. This means that there are certain plant and AMF that tend to interact more between them than with plants or AMF in other modules. Later on, using dissimilarity index they characterized the community of AMF with which each plant species tend to interact, and showed that the plant-plant associations that are more frequent in the community occur between pairs of plant species that have a more dissimilar community of AMF associated to their roots [56]. These approaches represent examples of how multiple and connected ecological processes can be understood by considering simultaneously the communities of several interacting guilds.

Finally, these multi-guild interactions occur within very limited local space. Plant facilitation generates a cluster distribution of plants (and associated species with which they interact) throughout space, resulting in discrete vegetation patches. This spatial distribution of ecological interactions in space leads the way to the consideration of multiple patches as a meta-community and allows applying analytical techniques to explore phylogenetic community structure developed for approaching multi-guild interactions in meta-communities frameworks [57].

11 Implications on biodiversity maintenance and sustainable development

As long as human activities have continued to disturb Earth's climate, biota, and entire ecosystems at unprecedented rates, the science of ecology has been subjected to considerable criticism, because of its incapacity to fill the gap between ecological theory and management/conservation practices [58]. One of the main tasks of this big challenge for community ecology is to determine the way in which plant communities are assembled [59], as well as what the effects of species overexploitation, habitat disturbance and biotic invasions on this assemblage will be. Particularly relevant will be to predict how ecological communities will respond to different rates of species loss and to determine the existence of a threshold for ecosystem collapse. The loss of biological diversity is one of the most pronounced changes to global environment which is able to impact biomass production and ecosystem services [60]. Of course, the design of management policies before humans continue disturbance practices is at the center of any discussion.

Given the interdependence among species in ecological communities, the loss of species can trigger a cascade of secondary extinctions with potentially dramatic effects on the functioning and stability of ecosystems upon which a growing human population depends [61, 62]. All species are embedded in networks of ecological interactions and the understanding of the robustness of these interaction networks to species loss is essential to forecast the effects of populations' decline and species extinctions [63]. Network theory has been a useful tool for simulating co-extinction cascades following the removal of particular species [64], but these simulations have typically been performed as part of theoretical scenarios, as opposed to realistic human-driven realistic scenarios [58]. Recently, multiple networks approaches have been proposed for agroecosystems [63] showing that interdependent networks do not strongly covary in their robustness and then the management or restoration practices benefiting one functional group will not inevitably benefit others.

A recent new approach has been used to assess co-extinction cascades into a realistic scenario by combining multiple ecological networks (facilitation, pollination, and seed dispersal networks) in central México (Los Reyes Metzontla, Puebla) in a Popoloca town whose subsistence economy is sustained by ceramic pottery production and agave extraction for mezcal production. Pottery production demands large volumes of wood for fuel for firing ceramics, thus impacting plant nurses and facilitated species. These human effects in one network (facilitation), may, through feedback loops, impact concomitant (pollination and dispersal) networks of interactions to produce co-extinction cascades leading to ecosystem collapse [58]. This study documents that ecosystems can be more vulnerable than they seem, when most of the species depends on others for their maintenance and how the system can reach a critical threshold of rapid and unexpected change.

Also given the high degree of habitat destruction, ecological restoration will necessarily be a key process for the conservation of biodiversity, which can benefit from the knowledge acquired among disciplines such as community ecology and evolutionary ecology [65]. As we have shown here, biotic interactions assembling plant communities can be positive (facilitation) and negative (competition) and operate simultaneously. The balance between these facilitative interactions and subsequent competition is one of the mechanisms triggering succession, thus providing a good scenario for ecological restoration. Despite the ubiquity of plant facilitation for ecological restoration of disturbed ecosystems, this interaction was not considered for restoration until very recently [66]. Such a gap is consistent with the traditional view that competition is the omnipresent force shaping ecological communities [67]. In contrast to competition-focused afforestation techniques, in which seedlings are planted after eliminating the pre-existing vegetation, restoration based on facilitation, consists of planting the plants spatially associated with other plants, which provides them with a favorable microhabitat [68]. In fact, nurse-based restoration experiments have been increasingly performed in different types of ecosystems worldwide, with varying success (see [69], for a review). Similarly, nurse-assisted planting may promote more rapid natural succession in disturbed habitats in tropical areas [70].

Through considering restoration studies worldwide and by means of a Bayesian meta-analysis of nurse-based restoration experiments, the importance of phylogenetic relatedness and life-form disparity in the survival, growth and density of facilitated plants was tested [65]. This study found that the more similar the life forms of neighboring plants are, the greater the positive effect of phylogenetic distance is on survival and density. This result suggests that other characteristics beyond life form are also contained in the phylogeny, and the larger the phylogenetic distance, the less is the niche overlap, and therefore the less intense is the competition.

12 Concluding remarks

Human alteration of the global environment is triggering ecologists to fill the gap between ecological theory and management and conservation practices, at a point where realistic knowledge is urgently needed. We propose that this approach can only be achieved under the community ecology framework in which all the processes maintaining biodiversity are linked as we have tried to exemplify here. Only until we modify our theoretical and methodological approaches will we be able to face the present sixth major extinction event in the history of life, transiting towards sustainable practices in ecosystems.

Acknowledgments

This work was funded by DGAPA-UNAM (Project IN-213414-3), and CYTED (Acción 409AC0369). AMN was supported by a DGAPA-UNAM postdoctoral fellowship and an Early Career Project Grant from the BES (3975-4849). We want to thank to two anonymous reviewers for valuable comments to the ms. Also to Sonia Helen Ponce Wainer for the revision of the English version.

13 Bibliography

[1] R. Putman, *Community ecology*. Springer, 1994.

[2] J. M. Diamond, “Assembly of species communities,” in *Ecology and Evolution of Communities*. Belknap Press, 1975, pp. 342–444.

[3] J. J. Wiens and M. J. Donoghue, “Historical biogeography, ecology and species richness,” *Trends in Ecology & Evolution*, vol. 19, no. 12, pp. 639–644, 2004.

[4] C. O. Webb, D. D. Ackerly, M. A. McPeek, and M. J. Donoghue, “Phylogenies and community ecology,” *Annual Review of Ecology and Systematics*, vol. 33, pp. 475–505, 2002.

[5] D. C. L. Willdenow, *The Principles of Botany, and of Vegetable Physiology: Tr. from the German*. Blackwood, 1811.

[6] S. T. Jackson, “Introduction: Humboldt, ecology, and the cosmos,” in *Essay on the Geography of Plants. Alexander von Humboldt and Aimé Bonpland*. The University of Chicago Press, 2009.

[7] P. Posadas, J. V. Crisci, and L. Katinas, “Historical biogeography: a review of its basic concepts and critical issues,” *Journal of Arid Environments*, vol. 66, no. 3, pp. 389–403, 2006.

[8] R. H. MacArthur and E. O. Wilson, *The theory of island biogeography*. Princeton University Press, 1967, vol. 1.

[9] R. E. Ricklefs, "A comprehensive framework for global patterns in biodiversity," *Ecology Letters*, vol. 7, no. 1, pp. 1–15, 2004.

[10] H. A. Gleason, "The individualistic concept of the plant association," *Bulletin of the Torrey Botanical Club*, vol. 53, no. 1, pp. 7–26, 1926.

[11] J. Silvertown, "Plant coexistence and the niche," *Trends in Ecology & Evolution*, vol. 19, no. 11, pp. 605–611, 2004.

[12] P. J. den Boer, "The present status of the competitive exclusion principle," *Trends in Ecology & Evolution*, vol. 1, no. 1, pp. 25–28, 1986.

[13] R. Levins and R. Lewontin, *The dialectical biologist*. Harvard University Press, 1985.

[14] A. J. Lotka, *Elements of physical biology*. Williams & Wilkins Co., Baltimore, 1925.

[15] V. Volterra, *Variazioni e fluttuazioni del numero d'individui in specie animali conviventi*. C. Ferrari, 1927.

[16] M. G. A. Van Der Heijden, R. D. Bardgett, and N. M. Van Straalen, "The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems," *Ecology Letters*, vol. 11, no. 3, pp. 296–310, 2008.

[17] D. A. Wardle, R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. Van Der Putten, and D. H. Wall, "Ecological linkages between aboveground and belowground biota," *Science*, vol. 304, no. 5677, pp. 1629–1633, 2004.

[18] R. D. Bardgett and D. A. Wardle, "Herbivore-mediated linkages between aboveground and belowground communities," *Ecology*, vol. 84, no. 9, pp. 2258–2268, 2003.

[19] B. E. Wolfe, B. C. Husband, and J. N. Klironomos, "Effects of a belowground mutualism on an aboveground mutualism," *Ecology Letters*, vol. 8, no. 2, pp. 218–223, 2005.

[20] A. Valiente-Banuet, A. V. Rumebe, M. Verdú, and R. M. Callaway, "Modern quaternary plant lineages promote diversity through facilitation of ancient tertiary lineages," *Proceedings of the National Academy of Sciences*, vol. 103, no. 45, pp. 16 812–16 817, 2006.

[21] J. A. Wolfe, "Paleoclimatic estimates from tertiary leaf assemblages," *Annual Review of Earth and Planetary Sciences*, vol. 23, pp. 119–142, 1995.

[22] D. I. Axelrod, *Age and origin of Sonoran Desert vegetation*. California Academy of Sciences, 1979.

[23] W. W. Thomas, "Conservation and monographic research on the flora of tropical america," *Biodiversity & Conservation*, vol. 8, no. 8, pp. 1007–1015, 1999.

[24] J. Rzedowski, *Vegetación de México*. Editorial LIMUSA, México, 1978.

[25] A. Graham, "Paleofloristic and paleoclimatic changes in the tertiary of northern latin america," *Review of Palaeobotany and Palynology*, vol. 60, no. 3, pp. 283–293, 1989.

[26] P. S. Herendeen, W. L. Crepet, and D. L. Dilcher, "The fossil history of the leguminosae: phylogenetic and biogeographic implications," *Advances in Legume Systematics*, vol. 4, pp. 303–316, 1992.

[27] R. T. Pennington, M. Lavin, D. E. Prado, C. A. Pendry, S. K. Pell, and C. A. Butterworth, "Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both tertiary and quaternary diversification," *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, vol. 359, no. 1443, pp. 515–538, 2004.

[28] J. X. Becerra, "Timing the origin and expansion of the mexican tropical dry forest," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 31, pp. 10 919–10 923, 2005.

[29] E. Ramírez-Arriaga, M. B. Prámparo, E. Martínez-Hernández, and A. Valiente-Baunet, "Palynology of the paleogene cuayuca formation (stratotype sections), southern mexico: Chronostratigraphical and palaeoecological implications," *Review of Palaeobotany and Palynology*, vol. 141, no. 3, pp. 259–275, 2006.

[30] D. R. Brooks, *Phylogeny, ecology, and behavior: a research program in comparative biology*. University of Chicago Press, 1991.

[31] J. Silvertown, M. Franco-Baquiero, and J. L. Harper, *Plant life histories: ecology, phylogeny and evolution*. Cambridge University Press, 1997.

[32] E. Weiher and P. A. Keddy, "The assembly of experimental wetland plant communities," *Oikos*, pp. 323–335, 1995.

[33] J. B. Wilson, "Assembly rules in plant communities," *Ecological assembly rules: perspectives, advances, retreats*, pp. 130–164, 1999.

[34] R. E. Latham and R. E. Ricklefs, "Continental comparisons of temperate-zone tree species diversity," *Species diversity in ecological communities: historical and geographical perspectives*, pp. 294–314, 1993.

[35] R. Ricklefs, E. Bermingham, C. Dick, C. Moritz *et al.*, "Phylogenetic perspectives on patterns of regional and local species richness." in *Tropical rainforests: past, present and future*. University of Chicago Press, 2005.

[36] B. D. Farrell, D. E. Dussourd, and C. Mitter, "Escalation of plant defense: do latex and resin canals spur plant diversification?" *American Naturalist*, pp. 881–900, 1991.

[37] T. Gardezi and J. da Silva, "Diversity in relation to body size in mammals: a comparative study," *The American Naturalist*, vol. 153, no. 1, pp. 110–123, 1999.

[38] C. O. Webb and M. J. Donoghue, "Phylomatic: tree assembly for applied phylogenetics," *Molecular Ecology Notes*, vol. 5, no. 1, pp. 181–183, 2005.

[39] C. O. Webb, D. D. Ackerly, and S. W. Kembel, "Phylocom: software for the analysis of phylogenetic community structure and trait evolution." *Bioinformatics*, vol. 24, no. 18, 2008.

[40] C. O. Webb, "Exploring the phylogenetic structure of ecological communities: an example for rain forest trees," *The American Naturalist*, vol. 156, no. 2, pp. 145–155, 2000.

[41] J. G. Pausas and M. Verdú, "The jungle of methods for evaluating phenotypic and phylogenetic structure of communities," *BioScience*, vol. 60, no. 8, pp. 614–625, 2010.

[42] M. Verdú and J. Pausas, "Fire drives phylogenetic clustering in mediterranean basin woody plant communities," *Journal of Ecology*, vol. 95, no. 6, pp. 1316–1323, 2007.

[43] M. Hutchinson, "Population studies, animal ecology and demography: concluding remarks," in *Cold Spring Harbour Symposia on quantitative biology: population studies: animal ecology and demography. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA*, 1957, pp. 415–427.

[44] A. Valiente-Banuet and M. Verdú, "Temporal shifts from facilitation to competition occur between closely related taxa," *Journal of Ecology*, vol. 96, no. 3, pp. 489–494, 2008.

[45] J. R. McAuliffe, "Markovian dynamics of simple and complex desert plant communities," *American Naturalist*, pp. 459–490, 1988.

[46] A. Valiente-Banuet and M. Verdú, "Facilitation can increase the phylogenetic diversity of plant communities," *Ecology Letters*, vol. 10, no. 11, pp. 1029–1036, 2007.

[47] J. Bascompte and P. Jordano, "Plant-animal mutualistic networks: the architecture of biodiversity," *Annual Review of Ecology, Evolution, and Systematics*, pp. 567–593, 2007.

[48] J. M. Olesen, J. Bascompte, Y. L. Dupont, and P. Jordano, "The modularity of pollination networks," *Proceedings of the National Academy of Sciences*, vol. 104, no. 50, pp. 19 891–19 896, 2007.

[49] M. Verdú and A. Valiente-Banuet, "The nested assembly of plant facilitation networks prevents species extinctions," *The American Naturalist*, vol. 172, no. 6, pp. 751–760, 2008.

[50] J. M. Gómez, M. Verdú, and F. Perfectti, "Ecological interactions are evolutionarily conserved across the entire tree of life," *Nature*, vol. 465, no. 7300, pp. 918–921, 2010.

[51] S. E. Smith and D. J. Read, *Mycorrhizal symbiosis*. Academic Press, London, 1977.

[52] M. G. A. van der Heijden, J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders, "Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity," *Nature*, vol. 396, no. 6706, pp. 69–72, 1998.

[53] H. Maherali and J. N. Klironomos, "Influence of phylogeny on fungal community assembly and ecosystem functioning," *Science*, vol. 316, no. 5832, pp. 1746–1748, 2007.

[54] C. Wagg, J. Jansa, M. Stadler, B. Schmid, and M. G. A. Van Der Heijden, "Mycorrhizal fungal identity and diversity relaxes plant-plant competition," *Ecology*, vol. 92, no. 6, pp. 1303–1313, 2011.

[55] A. Montesinos-Navarro, J. G. Segarra-Moragues, A. Valiente-Banuet, and M. Verdú, "The network structure of plant–arbuscular mycorrhizal fungi," *New Phytologist*, vol. 194, no. 2, pp. 536–547, 2012.

[56] ——, "Plant facilitation occurs between species differing in their associated arbuscular mycorrhizal fungi," *New Phytologist*, vol. 196, no. 3, pp. 835–844, 2012.

[57] V. D. Pillar and L. D. S. Duarte, "A framework for metacommunity analysis of phylogenetic structure," *Ecology Letters*, vol. 13, no. 5, pp. 587–596, 2010.

[58] A. Valiente-Banuet and M. Verdú, "Human impacts on multiple ecological networks act synergistically to drive ecosystem collapse," *Frontiers in Ecology and the Environment*, vol. 11, no. 8, pp. 408–413, 2013.

[59] J. M. Tylianakis, R. K. Didham, J. Bascompte, and D. A. Wardle, "Global change and species interactions in terrestrial ecosystems," *Ecology Letters*, vol. 11, no. 12, pp. 1351–1363, 2008.

[60] M. W. Cadotte, B. J. Cardinale, and T. H. Oakley, "Evolutionary history and the effect of biodiversity on plant productivity," *Proceedings of the National Academy of Sciences*, vol. 105, no. 44, pp. 17 012–17 017, 2008.

[61] B. Ebenman and T. Jonsson, "Using community viability analysis to identify fragile systems and keystone species," *Trends in Ecology & Evolution*, vol. 20, no. 10, pp. 568–575, 2005.

[62] B. Worm, E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. Jackson, H. K. Lotze, F. Micheli, S. R. Palumbi *et al.*, "Impacts of biodiversity loss on ocean ecosystem services," *Science*, vol. 314, no. 5800, pp. 787–790, 2006.

[63] M. J. Pocock, D. M. Evans, and J. Memmott, "The robustness and restoration of a network of ecological networks," *Science*, vol. 335, no. 6071, pp. 973–977, 2012.

[64] J. A. Dunne and R. J. Williams, "Cascading extinctions and community collapse in model food webs," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1524, pp. 1711–1723, 2009.

[65] M. Verdú, L. Gómez-Aparicio, and A. Valiente-Banuet, "Phylogenetic relatedness as a tool in restoration ecology: a meta-analysis," *Proceedings of the Royal Society B: Biological Sciences*, vol. 279, no. 1734, pp. 1761–1767, 2012.

[66] F. M. Padilla and F. I. Pugnaire, "The role of nurse plants in the restoration of degraded environments," *Frontiers in Ecology and the Environment*, vol. 4, no. 4, pp. 196–202, 2006.

[67] J. F. Bruno, J. J. Stachowicz, and M. D. Bertness, "Inclusion of facilitation into ecological theory," *Trends in Ecology & Evolution*, vol. 18, no. 3, pp. 119–125, 2003.

[68] J. Castro, R. Zamora, J. A. Hódar, and J. M. Gómez, "Use of shrubs as nurse plants: a new technique for reforestation in mediterranean mountains," *Restoration Ecology*, vol. 10, no. 2, pp. 297–305, 2002.

[69] L. Gómez-Aparicio, "The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems," *Journal of Ecology*, vol. 97, no. 6, pp. 1202–1214, 2009.

[70] S. P. Goosem and N. I. Tucker, *Repairing the rainforest: theory and practice of rainforest re-establishment in North Queensland's wet tropics*. Wet Tropics Management Authority, 1995.

Flower complexity and fractals

R. Medel, C. González-Browne & C. Botto-Mahan, Universidad de Chile, Chile

1 Abstract

We present an approach to the study of plant-pollinator interactions based on the analysis of flower complexity, understood as the fractal dimension of corolla dissection. Corolla complexity was examined regarding its ability to predict the number of pollinator species that visit 21 flowering plant species in a Chilean ecosystem. A second order polynomial function was the best descriptor of the flower complexity-pollinator relationship. In particular, flowers with intermediate complexity were those that received the highest diversity of pollinators. Flowers with rounded and highly dissected corollas were less visited, which suggests that pollinators face a behavioral tradeoff associated to landing in broad platforms or manipulation of dissected corollas when deciding the type of flowers they visit. This result suggests that emergentist perspectives, such as the used in this study, permit to predict flower attraction, one of the most important variables in pollination ecology and evolution. More studies including Mandelbrot's perspective to flower complexity are needed to complement reductionist approaches and have a more inclusive understanding of plant-pollinator interactions.

2 Resumen

Presentamos una aproximación al estudio de interacciones planta-polinizador que se basa en el análisis de complejidad floral, entendida como la dimensión fractal de la disección del contorno de la corola. Se examinó en qué medida la complejidad floral es útil para predecir el número de especies de polinizadores visitantes en 21 especies de plantas en un ecosistema de Chile. Una función polinomial de segundo grado fue el mejor descriptor de la relación. En particular, las flores con complejidad intermedia fueron las que recibieron una mayor diversidad de polinizadores. Las flores más redondas y con alta disección de sus corolas recibieron menos especies, lo cual sugiere que los polinizadores enfrentan un compromiso al momento de decidir cuales flores visitar. Este resultado sugiere que perspectivas emergentistas, tal como la usada en este estudio, permiten predecir la atracción floral, una de las variables más importantes en ecología y evolución de la polinización. Más estudios que incluyan la perspectiva de Mandelbrot a la complejidad floral son necesarios para complementar aproximaciones reduccionistas y lograr un entendimiento más inclusivo de las interacciones planta–polinizador.

3 Introduction

One of the most conspicuous patterns of angiosperm diversification is the enormous diversity in size, color, visual patterns, shapes, odors, and overall design shown by flowers at present times. This observation can be traced back to the first floral biologists, at least 250 years ago [1, 2], and was part of the empirical evidences used by Darwin to support his theory of evolution by natural selection (see [3, 4]). In spite of the time elapsed from such seminal contributions, the understanding of factors involved in such morphological diversity is still an important research area, in part due to the increasing awareness that flowers constitute complex structures that result from the combined action of genetic, developmental, and environmental processes.

Flowers are attractive and tractable models on which diverse hypotheses of adaptation can be tested. They are directly related to plant fitness, provide replicated units to examine the consistency of adaptation within and among populations, and represent an appropriate model to experimentally examine the relevance of different floral parts for reproductive success. Currently, there is ample correlative and experimental evidence that pollinators play an important role in floral evolution (reviewed in [5]). Indeed, diverse flower traits such as corolla color, nectar guides, and flower size, among others, have been extensively examined as relatively independent traits that participate in pollinator attraction. Likewise, recent statistical tools based on geometric morphometrics have permitted the inclusion of corolla shape in studies of pollination ecology and evolution (e.g., [6–11]).

Unlike the successful incorporation of shape analysis to pollination ecology, however, studies that measure flowers from the perspective of complex systems are almost absent in the literature (but see [12]). This omission is consistent with the historical overemphasis given to disintegrate the floral phenotype into relatively independent functional pieces. The basic assumption of reductionism is that complex systems can be understood by characterizing the properties and behaviors of their component parts. While this strategy has been obviously successful, emergent properties, by definition, do not fit into this paradigm but may instead contribute to a more complete understanding of plant-pollinator relationships. In this chapter we attempt to show that complexity, an emergent property of organisms, may represent a useful perspective to be adopted in pollination biology.

Most studies of floral preference have recorded bee's behavior (honeybees and bumblebees) in a range of flower traits such as flower color, shape, scent, and size under laboratory conditions (e.g., [13–15]). Under such circumstances the floral preferences seem to depend almost entirely on the pollinator cognitive abilities and the signals on which they rely on (e.g., [11, 16, 17]). Results from such studies indicate that the degree of corolla dissection is one of the main spatial parameters used by bees to discriminate flowers.

In principle, insects prefer highly disrupted over less disrupted corollas [13, 18]. However, Lehrer et al. [19] found that bees prefer less disrupted patterns to high ones. These two opposite views are not necessarily conflicting because preferences were measured under different conditions. In the first case, bee choices were evaluated at a very close distance, but the other one analyzed preference at a larger distance [13].

These foregoing findings suggest that pollinator floral preference depends on a higher number of variables than previously thought. This conclusion is supported by the study of Dafni and Kevan [16], where a relation between corolla complexity and size was detected in 42 plant species.

In general, pollinator choice differed depending on the plant species under assessment, but a clear pattern emerged: medium and large sized pollinators were associated with large flowers with simple shapes, while small pollinators were related to small flowers with disrupted outlines. The authors proposed that the high contour density in small flowers may increase their visibility hence representing a strategy to counterweigh their small size. Despite the fact that most studies were performed in bees under laboratory conditions, there are some studies performed in other pollinator groups under natural populations. For example, Johnson and Dafni [20] examined the response of bee flies to shape, size, color, and pattern separately. They found that dissected outlines were more prefered than simple ones. Similar results have been found for hawkmoths [12, 21] but an opposing trend was detected in beetles [22]. Although in general these studies have shown that pollinators respond to corolla dissection, no attempt has been made to quantify rigorously corolla complexity which precludes useful generalizations.

In this study we will examine the extent to which corolla complexity, influences the attraction of pollinators in a plant-pollinator community. In principle, plant species differing in the dissection of their corollas (i.e., the amount of edge per unit area) may differ in the amount of pollinators they attract, creating clusters of species associations around flowers. Even though there are many ways to define complexity, for our purposes, we will follow the proposed by Mitchell [23], who defines a complex system as one that exhibits at least some properties that cannot be explained as the linear sum of properties of the component elements. To measure complexity, we will use the approach devised by the French mathematician Benoit Mandelbrot. Under Mandelbrot's perspective, fractals represent the geometric shape of an object and the fractal dimension can be calculated as the number of copies of the self- similar object at different levels of magnification.

4 Methods

This study is based on data from a more inclusive study carried out in the austral spring and summer seasons of 2007 and 2008 in an area near Los Ruiles National Reserve (35° 85′ S, 72° 83′ W) in the coastal range of central Chile (see description in [24, 25]). The sampling procedure consisted on recording the identity and frequency of visitation of insect visitors to flowers of every plant species during 10-min observation periods. Observations were performed by 3.8 persons/day, on average, and always on sunny days. Observations on plant species were performed according to their abundance in the site. Visitors were considered legitimate pollinators when they contacted the anthers or stigma of flowers or entered the flower tube. We collected insects in the first year of the study for subsequent taxonomic identification in the laboratory.

Plant species satisfying the two following criteria were included in analysis: i) to be an herbaceous plant, and ii) to exhibit a planar corolla (i.e., with petals open in about 180° in anthesis). After applying these selection criteria to the species present at Los Ruiles National Reserve, we recovered 21 plant species, most of them (17 species) belonging to different plant genera. Corollas were then photographed from a perpendicular perspective to mimic the view of a pollinator approaching to the plane of the corolla. Pictures were transformed to black and white so that flowers and outlines were clearly distinguished from white backgrounds. We quantified corolla complexity as the fractal dimension of the corolla outline in Benoit 1.2.

The concept of fractal dimension D [26] can be used as a simple descriptor of complexity for any object. By using calculation methods for identification of self-similar patterns in 2D graphic objects, this method provides a useful approach to detect scale-invariant properties where traditional statistical approaches fail. We estimated the fractal dimension of the corolla outline through the mass dimension procedure. Briefly, this method consists in finding the mass radius (mr), that is, the number of points within a circle of radio r in the outline of interest and to estimate the changing mr data across circles of increasing radius from the center of the figure. If the figure of interest has fractal structure, plotting the logarithm of m versus the logarithm of r will result in a straight line with slope D_m, where D is the fractal dimension. In this way, low D-values will be observed in flowers with simple (non-dissected) corollas because the number of white points will not increase from the centre to the periphery of flowers. On the contrary, the number of white points interspersed in the corolla outline will increase from the centre to the periphery of flowers with complex (dissected) corollas. To reduce the measurement error attributable to variation in the initial values of parameter estimations (i.e., those coordinates corresponding to the center of corollas), we recorded 10 measures of fractal dimension corresponding to circles with radio 1.1 to 2.0 and calculated an average D-value per plant species. All analyses were performed in *Benoit 1.2* and R version 3.0.1 (The R Foundation for Statistical Computing 2013).

5 Results

Fractal dimension D-values ranged from 1.22 to 2.0 with a mean (SD) of 1.58 (0.29) in the plant community. The data come from a lognormal distribution (Kolmogorov's D test, $D = 0.137$, $P = 0.150$). As no abrupt decrease of D-values was evident (Figure 1a), the fractal dimension was homogeneously distributed in the plant community. GLM models were adjusted for each parameter (linear and quadratic) using Poisson-distributed errors and log link using D as predictor variable, and maximum likelihood as estimation method. While the linear model did not predict the number of pollinator species on flowers [estimate (SD) = -0.108 (0.227), $P = 0.633$, $AIC = 347.6$], the quadratic model predicted successfully the variation in the number of pollinator species [D estimate (SD) = 0.048 (0.262), $P = 0.854$; D_2 estimate = -532 (1.102), $P < 0.001$, $AIC = 325.82$). This result implies that interspecific variation in corolla complexity, estimated through the fractal dimension, accounts at least in part for differences in the number of pollinator species attracted to flowers. The negative coefficient of the quadratic term indicates a concave function, with a maximum degree-value around $D = 1.6$ (Figure 1b), that is, in the medium region of the fractal dimension scale.

This result indicates that flowers with intermediate complexity tend to receive the highest number of pollinator species, and plants with minimal and maximal complexity received a lower number of them. As most plant species belong to different genera (with the exception of two pairs of species, *Anagallis* (Primulaceae) and *Hypericum* (Clusiaceae), phylogenetic effects may have a minor importance in the resulting pattern. But why should pollinators be less attracted to flowers with high or low corolla complexity? One potential line of reasoning relates to pollinator specialization and attraction. The low number of pollinator species observed in the left side of Figure 1b may be a direct consequence of the low attraction provoked by simple and non-dissected corollas. On the other hand, it is likely that highly dissected and complex corollas tend to be visited by specialized pollinators with phenotypes that permit them to manipulate adequately the flower in order to obtain rewards (see [27]).

A second line of reasoning relates to the tradeoffs faced by pollinators when visiting and manipulating flowers. As flower shape is a character that provides attraction to pollinators and at the same time participates in the mechanical fit with pollinators [10, 11], there is a potential conflict for plants between attracting pollinators and providing an adequate phenotype that adjust the flower-pollinator interface. Evidence from pollinator preference provides support to such dichotomy. Many laboratory and field studies have demonstrated that pollinators tend to prefer flower models with narrow petals and dissected corollas (e.g., [8, 13, 14, 18, 20]). Flowers with dissected outlines are probably more detectable to moving insects because they create a greater on–off stimulus in the compound eye [28, 29], and provide a reliable informative cue about the quantity and quality of pollen and nectar reward. However, recent field evidence indicates that contrary to expectations, some pollinators tend to prefer flowers with rounded and simple corollas over

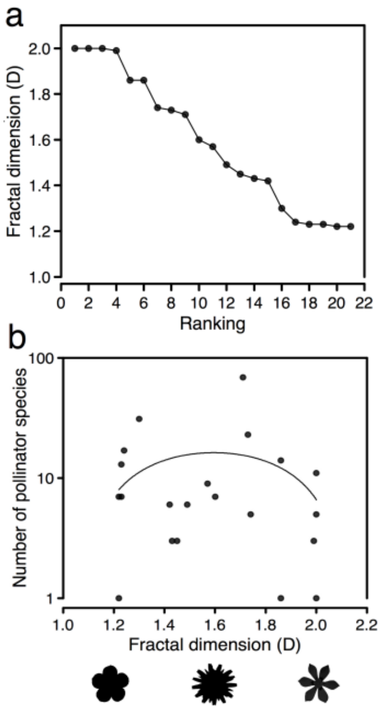


Figure 1: a) Distribution of D fractal values ranked from high to low values. Log-transformed values come from a normal distribution (Shapiro- Wilk W test, W = 0.943, P = 0.277). b) Relationship between the corolla fractal dimension and the number of pollinator species visiting flowers. The polynomial equation is Pollinators $= -133 + 188D - 59D_2$. The silhouettes depicted from left to right along the X-axis correspond to *Anagallis arvensis* (Primulaceae), *Chamaelemun mixtum* (Asteraceae), and *Sisyrinchium graminifolium* (Iridaceae).

complex outlines, apparently because rounded corollas provide suitable landing surfaces and higher handling effectiveness in reward retrieval [11, 27, 30]. Under such circumstances, pollinators may face an obvious conflict in their foraging decisions, that can be solved, at least in part, by preferring intermediate floral phenotypes that provide the best of two options.

6 Concluding remarks

Recent interest in the conceptualization of biological systems from a complexity perspective has stimulated the emergence of new programs in ecological and evolutionary research such as ecological networks, phenotypic integration, phenotypic plasticity, and genetic of developmental processes, among others. These perspectives have revealed new patterns of organization that often escape to the eye of mechanistic approaches. In this chapter we have shown that pollinator attraction, a critical element in pollination ecology, can be further understood under the perspective of complex systems and flower fractality. Even though flower complexity is frequently invoked as an organismic property detectable by pollinators, attempts to measure complexity are almost lacking in the literature, in part, because complexity means different things for different people. In this chapter, we argue that it is time for pollination ecologists to search for common definitions and move beyond the reductionist focus to examine how much we might be missing by not treating flower complexity on its own right. We think complexity is one of the major remaining frontiers in plant-animal interactions. This chapter has provided a simple example of the way an emergentist approach may suggest new avenues of research and complement previous findings in pollination ecology.

Acknowledgements

We thank Mariana Benitez and Alfonso Valiente-Banuet for their invitation to write this chapter. An anonymous reviewer made important comments that improved substantially the quality of this manuscript. This study was made possible by grants FONDECYT 1120155 and CONICYT-PBCT ACT 34/2006 to RM. CONAF provided important facilities during fieldwork at Los Ruiles National Reserve. We are indebted to many people that contributed to data acquisition in the field.

7 Bibliography

[1] J. G. Kölreuter, *Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen.-Leipzig, Gleditsch 1761-1766*. Gleditsch, 1761, vol. 4.

[2] C. Sprengel, *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen*. Verlag Wilhelm Engelmann, Leipzig, 1793.

[3] C. Darwin and E. Mayr, “On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. john murray, london.” *On the Origin of Species by Means of Natural Selection.*, 1859.

[4] D. Charles, “On the various contrivances by which british and foreign orchids are fertilised by insects, and on the good effects of intercrossing,” 1862.

[5] L. D. Harder and S. C. H. Barrett, *Ecology and evolution of flowers*. Oxford University Press Oxford, 2006.

[6] C. Herrera, “Selection on complexity of corolla outline in a hawkmoth-pollinated violet,” *Evolutionary Trends in Plants*, vol. 7, p. 1, 1993.

[7] R. Medel, C. Botto-Mahan, and M. Kalin-Arroyo, "Pollinator-mediated selection on the nectar guide phenotype in the andean monkey flower, mimulus luteus," *Ecology*, vol. 84, no. 7, pp. 1721–1732, 2003.

[8] Y. Yoshioka, K. Ohashi, A. Konuma, H. Iwata, R. Ohsawa, and S. Ninomiya, "Ability of bumblebees to discriminate differences in the shape of artificial flowers of primula sieboldii (primulaceae)," *Annals of Botany*, vol. 99, no. 6, pp. 1175–1182, 2007.

[9] M. Cuartas-Domínguez and R. Medel, "Pollinator-mediated selection and experimental manipulation of the flower phenotype in chloraea bletioides," *Functional Ecology*, vol. 24, no. 6, pp. 1219–1227, 2010.

[10] J. Nattero, A. Cocucci, and R. Medel, "Pollinator-mediated selection in a specialized pollination system: matches and mismatches across populations," *Journal of Evolutionary Biology*, vol. 23, no. 9, pp. 1957–1968, 2010.

[11] R. L. Kaczorowski, A. R. Seliger, A. C. Gaskett, S. K. Wigsten, and R. A. Raguso, "Corolla shape vs. size in flower choice by a nocturnal hawkmoth pollinator," *Functional Ecology*, vol. 26, no. 3, pp. 577–587, 2012.

[12] C. M. Herrera, "Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet," *Ecological Monographs*, pp. 251–275, 1993.

[13] M. Giurfa and M. Lehrer, "Honeybee vision and floral displays: from detection to close-up recognition," *Cognitive Ecology of Pollination*, pp. 61–82, 2001.

[14] L. Chittka and N. E. Raine, "Recognition of flowers by pollinators," *Current Opinion in Plant Biology*, vol. 9, no. 4, pp. 428–435, 2006.

[15] R. Wehner, "The generalization of directional visual stimuli in the honey bee, *Apis mellifera*," *Journal of Insect Physiology*, vol. 17, no. 8, pp. 1579–1591, 1971.

[16] A. Dafni and P. G. Kevan, "Flower size and shape: implications in pollination," *Israel Journal of Plant Sciences*, vol. 45, no. 2-3, pp. 201–211, 1997.

[17] J. Spaethe, J. Tautz, and L. Chittka, "Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior," *Proceedings of the National Academy of Sciences*, vol. 98, no. 7, pp. 3898–3903, 2001.

[18] A. Dafni, M. Lehrer, and P. Keyan, "Spatial flower parameters and insect spatial vision," *Biological Reviews*, vol. 72, no. 2, pp. 239–282, 1997.

[19] M. Lehrer, G. Horridge, S. Zhang, and R. Gadagkar, "Shape vision in bees: innate preference for flower-like patterns," *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, vol. 347, no. 1320, pp. 123–137, 1995.

[20] S. Johnson and A. Dafni, "Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a mediterranean herb," *Functional Ecology*, vol. 12, no. 2, pp. 289–297, 1998.

[21] A. Kelber and M. Pfaff, "Spontaneous and learned preferences for visual flower features in a diurnal hawkmoth," *Israel Journal of Plant Sciences*, vol. 45, no. 2-3, pp. 235–245, 1997.

[22] A. Dafni, "The response of amphicoma spp.(coleoptera; glaphyridae) beetles to red models differing in area, shape, and symmetry," *Israel Journal of Plant Sciences*, vol. 45, no. 2-3, pp. 247–254, 1997.

[23] M. Mitchell, *Complexity: A guided tour*. Oxford University Press, 2009.

[24] M. Murúa, C. Espinoza, R. Bustamante, V. H. Marín, and R. Medel, "Does human-induced habitat transformation modify pollinator-mediated selection? a case study in viola portalesia (violaceae)," *Oecologia*, vol. 163, no. 1, pp. 153–162, 2010.

[25] A. Rivera-Hutinel, R. Bustamante, V. Marín, and R. Medel, "Effects of sampling completeness on the structure of plant-pollinator networks," *Ecology*, vol. 93, no. 7, pp. 1593–1603, 2012.

[26] B. B. Mandelbrot, *The fractal geometry of nature*. Macmillan, 1983.

[27] T. Laverty, "The flower-visiting behaviour of bumble bees: floral complexity and learning," *Canadian Journal of Zoology*, vol. 58, no. 7, pp. 1324–1335, 1980.

[28] E. Wolf and G. Zerrahn-Wolf, "The effect of light intensity, area, and flicker frequency on the visual reactions of the honey bee," *The Journal of General Physiology*, vol. 18, no. 6, pp. 853–863, 1935.

[29] A. M. Anderson, "The influence of pointed regions on the shape preference of honey bees," *Animal Behaviour*, vol. 25, pp. 88–94, 1977.

[30] J. M. Gomez, J. Bosch, F. Perfectti, J. Fernández, M. Abdelaziz, and J. P. M. Camacho, "Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators," *Proceedings of the Royal Society B: Biological Sciences*, vol. 275, no. 1648, pp. 2241–2249, 2008.

Community dynamics: lessons from a skeleton

J.M. Alcántara & P.J. Rey, Universidad de Jaén, Spain

1 Abstract

Models describing the dynamics of complex ecological communities share one important component: a matrix describing which species interact with which others and how. The parameters of interaction matrices determine the dynamic properties of these models (e.g. species coexistence, temporal changes in abundance or responses to disturbance), but estimating these parameters from real communities is frequently daunting, if not impossible. Fortunately, some dynamic properties depend only on the structure, the skeleton, of the interaction matrix (i.e. on which species interact with which others). We illustrate how the concept of Strongly Connected Components (SCCs), that only requires knowledge of the structure of the interaction matrix, is useful to decipher relationships between structure, function and dynamics of complex systems. We focus our examples in models of plant communities driven by the replacement of individuals (replacement networks), but the framework can be applied to other ecological systems. Plant communities from Southern Europe, Northern Africa and North America share the same simple functional structure: a core of species benefiting each other and providing support for many other species. This structure can confer high long term persistence to the species in these communities, even after the eventual extinction of one of them.

2 Resumen

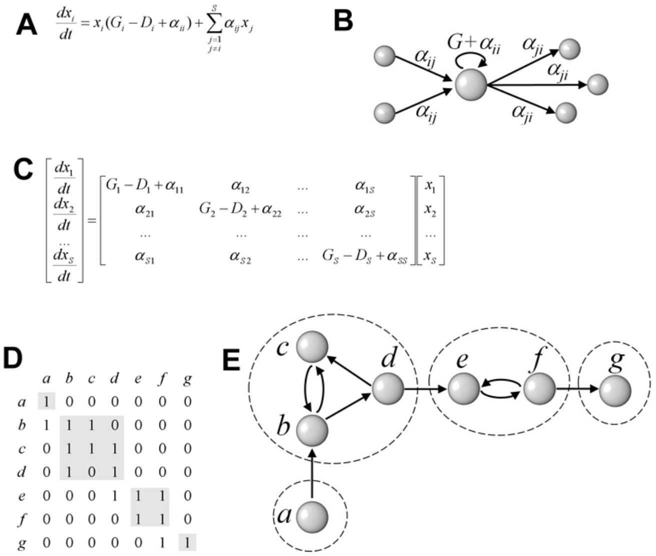
Los modelos de dinámica de sistemas ecológicos complejos incorporan una matriz que describe qué especies interactúan con cuales otras y cómo lo hacen. Los parámetros de esta matriz determinan las propiedades dinámicas del modelo (p. ej.: la coexistencia de especies, sus cambios de abundancia o su respuesta a perturbaciones), pero obtener estos parámetros en comunidades reales es extremadamente difícil. Afortunadamente, algunas propiedades dinámicas dependen solo de qué especies interactúan con cuales otras; es decir, de la estructura, o esqueleto, de la matriz. El concepto de Componentes Fuertemente Conectados (SCCs por sus siglas en inglés), que depende únicamente de la estructura de la matriz, permite relacionar estructura, función y dinámica de sistemas complejos. Como caso de estudio aplicamos el concepto de SCCs a modelos de comunidades de plantas basados en el reemplazamiento entre individuos (redes de reemplazamiento), aunque puede aplicarse a otros sistemas ecológicos. Nuestros análisis de redes de reemplazamiento de Europa, África y América sugieren que éstas poseen el mismo tipo de estructura funcional: un núcleo de especies que se benefician mutuamente y que proporciona soporte para muchas otras. Esta estructura permitiría la persistencia a largo plazo de la mayoría de especies, aún tras la extinción de alguna de ellas.

3 Community dynamics: lessons from a skeleton

In a broad sense, the study of community dynamics seeks to understand the temporal patterns of change in abundance and composition of natural assemblages of species, and the forces and mechanisms behind these changes. Thus, community dynamics addresses fundamental questions about the structure [1, 2], function [3, 4] and stability [5, 6] of biodiversity. Natural assemblages of species are inherently complex systems, as they are composed by a large number of elements (i.e. the species), each with different properties (i.e. different life history), that interact with each other in different ways (e.g. through competition, predation, facilitation or mutualism). Because of this complexity, many questions about the properties of these systems have only been addressed from theoretical, largely mathematical, approaches [7, 8].

One key component of mathematical models of complex systems is the interactions matrix (Figure 1A, B, C). Take for example the classical Lotka-Volterra models [9], Markov chain models [10], or bioenergetic consumer-resource models [11]. In these matrices, the diagonal elements describe intrinsic population properties of the species, such as their population growth rate, birth and death rates, biomass density, mean individual size and growth rate, the strength of intraspecific interactions, or the probability that the individuals take hold of the space they occupy. On the other hand, off-diagonal elements of the matrix indicate the strength or frequency of interactions between species (see Figure 1A, B and C). Depending on the model, the values of the elements in the interactions matrix can be constant, or they can vary deterministically with time, the environment or species density, and/or they can vary stochastically.

Obviously, many important dynamic properties of community models depend on the values of the elements of the matrix. For example, in the simple two-species competition Lotka-Volterra model, the stable coexistence of the species requires that the effect of intraspecific competition in each species (i.e. the main diagonal elements of the interactions matrix) is greater than the effect of interspecific competition with the other species (i.e. the off diagonal elements of the interactions matrix), otherwise one of the species



$$\frac{dx_i}{dt} = x_i(G_i - D_i + \alpha_{ii}) + \sum_{\substack{j=1 \\ j\neq i}}^{S} \alpha_{ij} x_j$$

$$\begin{bmatrix} \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \\ \dots \\ \frac{dx_S}{dt} \end{bmatrix} = \begin{bmatrix} G_1 - D_1 + \alpha_{11} & \alpha_{12} & \dots & \alpha_{1S} \\ \alpha_{21} & G_2 - D_2 + \alpha_{22} & \dots & \alpha_{2S} \\ \dots & \dots & \dots & \dots \\ \alpha_{S1} & \alpha_{S2} & \dots & G_S - D_S + \alpha_{SS} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ \dots \\ x_S \end{bmatrix}$$

	a	b	c	d	e	f	g
a	1	0	0	0	0	0	0
b	1	1	1	0	0	0	0
c	0	1	1	1	0	0	0
d	0	1	0	1	0	0	0
e	0	0	0	1	1	1	0
f	0	0	0	0	1	1	0
g	0	0	0	0	0	1	1

Figure 1: Different forms of expressing a mathematical model of community dynamics. A) Synthetic expression of a model of replacement dynamics in a community of competing species. The model contains a number S of species. The subindex i refers to the recruiting species, and the subindex j to the nurse species. The abundance (x) of each species varies with time as a function of its own abundance (x_i) and the balance between the vegetative growth rate of its individuals (G_i), their death rate (D_i), the rate of recruitment under conspecific individuals (α_{ii}), and the abundance of nurse species (x_j) and the rate of recruitment of species i under each nurse j (α_{ij}). B) The same synthetic expression can be represented as a simple graph of the inputs and outputs affecting a given component of the community. In this example, a focal species receives inputs of space from two nurse species and also gains some space through vegetative growth. When individuals of the focal species die, part of the space they occupied is passed to individuals of the same species or to individuals of other three species. If the system is at equilibrium, the death rate equals the sum of outputs from i. C) Representation of the model with explicit indication of the interactions matrix. D) Example of adjacency matrix showing the qualitative information of an interactions matrix with 7 species (a to g). The matrix is arranged in Lower Triangular Block Form. This arrangement is unique and places some non-zero elements of the matrix forming blocks along the main diagonal (shaded areas), and the rest of non-zero elements in the lower triangular side of the matrix. E) Replacement network derived from the adjacency matrix in D. The dashed circles indicate the Strongly Connected Components (SCCs) of the network. The SCCs of a directed network are groups of nodes in which resources, like biomass or space, can flow (directly or indirectly) from any node in the group to any other node in the same group, and back (i.e., resources can cycle between any pair of nodes within a SCC). In the example, there are two trivial SCCs (species a and g), one formed by two species ($e - f$) and one formed by three species ($b - c - d$). The SCCs correspond to the diagonal blocks of the adjacency matrix, and the links between SCCs are the non-zero elements in the lower triangular part of the matrix. Assuming that the SCC with three species is the "basic" SCC (see main text for a definition), then species b to g could coexist indefinitely because they belong to the basic SCC, or they recruit under some of its species directly (e recruits under d which belongs to the basic SCC) or indirectly (g recruits under f, which recruits under e, which recruits under d which belongs to the basic SCC). On the other hand, species a will eventually disappear because it does not recruit directly or indirectly under any species of the basic SCC. The interested reader can find further details in Alcántara and Rey [12].

becomes extinct. In this simple model, the community could finally reach three possible sets of stable species compositions depending on the relative values of the elements in the interaction matrix: species 1 only, species 2 only or the two species present. The more species there are in the model, the wider the set of stable community compositions possible. For example, with 10 species there are 1023 theoretically possible stable community compositions; the ones that actually occur in natural communities can only be ascertained through knowledge of the interaction matrix.

It remains an enormous challenge to obtain the information necessary to parameterize theoretical models with values from real communities [13, 14]. Setting aside the difficulties inherent to obtaining interaction parameters through field studies, the complexity of any community model grows exponentially with the number of species (S) because the number of parameters (i.e. interactions) in the matrix is S^2 (e.g. a model with 32 species involves a matrix with, at least, 1024 parameters). In fact, the few studies that have faced the challenge of parameterizing theoretical models with real world data have used the strategy of reducing the number of species by collapsing them through some type of aggregation, like grouping species by their trophic habit, size or any other relevant life history traits (e.g. [9], [15]).

Fortunately, some properties of the structure, function and stability of complex systems (e.g. biological communities) do not depend entirely on the exact values of the parameters of the interaction matrix. Interaction matrices contain two sources of information: quantitative and qualitative. Quantitative information refers to the exact values of the parameters in the matrix or the functions describing their variation, while qualitative information refers to the presence/absence of interactions (because not all possible pair-wise interactions do occur in real communities), their sign, and how they are arranged within the matrix. An interaction matrix parameterized with quantitative information contains also the qualitative information. Some aspects of the dynamics of a model depend more on the quantitative information but other properties depend more on the qualitative information contained in the interactions matrix [16, 17]. To fully understand the dynamics of the system we need to be aware of the contributions of each source of information.

The skeleton of the interaction matrix

To illustrate the utility of the qualitative analysis of complex systems, we will show how knowledge of the qualitative information of interactions matrices can be used to understand some important properties of the community. We will focus on replacement models of woody plant communities (e.g. shrublands and forests). These models consider that the change in species abundance in the community is driven by the replacement of dead individuals of a given species by individuals of the same or a different species growing beneath (see for example [18, 19]). This conceptualization of plant community dynamics as replacement systems can be traced back at least to [20], and has been recently advocated by [21]. We have presented a detailed account of the theory and its application to replacement networks and food webs in [12].

The qualitative information, the skeleton, of a replacement model is a replacement network (Figure 1D and E) composed by populations of species forming a local assemblage. Each species is represented by a node in the network. There is an arrow from species j pointing to species i, indicating that individuals of species i recruit under individuals of species j (the nurse species) so if the individual of species j dies, the space it occupied can now be gained by individuals of species i. This convention makes replacement networks comparable to food webs where the arrows point from prey to predator, indicating the direction of biomass flow. In replacement networks the space (and the resources it contains) flows from the nurse to the recruiting species. A special node represents space not occupied by any plant (open space; e.g. a clearing in the forest), so some species can also recruit without the assistance (or without the interference or competition) of nurse plants. The graph representing the interaction network can be derived from its adjacency matrix, which is simply a binary version of the interaction matrix of the community model with the non-zero entries replaced by ones (ones in the adjacency matrix correspond to arrows in the network). Transforming the interactions matrix into an adjacency matrix means that we lose the quantitative information from the matrix but we retain the qualitative one. Nevertheless, it is far easier to obtain the information necessary to build the adjacency matrix of a real community than obtaining the information to parameterize the whole interaction matrix for the same community. In fact, the study of ecological networks based on the analysis of adjacency matrices has a long tradition and has flourished especially in the last decades [22].

Understanding the dynamical properties from the skeleton

Knowing the network's structure is not necessarily the same as understanding such structure. A key example of this problem is the issue of whether the species of an ecological network form compartments or modules. Identification of compartments in ecological networks is not merely a descriptive task: theoretically, compartmented systems have particular stability properties ([5, 23, 24]). Paradoxically, the search for compartments in ecological networks remains disconnected from the study of their dynamical properties, so even after finding compartments through some ordination procedure or modularity maximization algorithm [25], we would still be wondering whether such structures affect network dynamics. As stated by Dunne (2006): "There are likely innumerable ways for chopping up networks into clusters, but that does not mean that such clusters are necessarily meaningful for ecological function or dynamics."

To find out structures within the network that provide information about community dynamics we need first to realize that the network is part of a mathematical model of the community (Figure 1). In replacement models, like in any flow model that quantifies the interchanges of resources (e.g. biomass or energy flow in food web models), the interaction matrix contains only zero or positive values (they are called "non-negative" matrices), and the corresponding networks are directed (i.e. the links between species indicate directions of flows so they are depicted as single headed arrows). The species in any non-negative directed network can be uniquely arranged into groups known as Strongly Connected Components (SCCs; Figure 1D,E). When applied to replacement networks, SCCs can be defined as groups of species such that the space occupied by individuals of one of the species can be occupied in the future by individuals of any of the species in the group. A SCC with a single species is called "trivial". Note that finding the SCCs requires only the qualitative information of the interaction matrix.

Since SCCs are groupings of species internally connected, they are useful to address questions related to the compartmentalization or modularity of ecological networks. Differently to most algorithms used to define modules in a network [25], the concept of SCCs has the advantage that there is only one way of partitioning a network into SCCs. In the replacement networks we have previously analyzed (10 from Southern Spain, 2 from Northern Africa and 3 from Mexico), the number of SCCs was very variable, but all the networks had a single large SCC and many trivial ones (Figure 2). So, according to their division into SCCs, replacement networks from three continents seem to share a common structure: a "core" of species recruiting directly or indirectly under each other, and a large set of "satellite" species linked to it. This structure indicates that woody plant communities have very low modularity (single species cannot be considered a compartment or module). Although it is still premature, it is tempting to ask why this particular structure arises in such different communities.

To begin with, this structure is different from the configuration expected if the recruitment interactions were randomly determined: at the density of interactions found in replacement networks, a randomly assembled network would form a single SCC grouping all the species [27]. Our next step seeks a functional interpretation of the network structure. We will start by assuming, as it is common in community dynamic modeling and ecological network analysis, that the qualitative structure of the replacement network remains constant (but see [28]): the zero elements of the matrix remain zero (i.e. some species will never recruit under others) and the positive elements remain non-zero although their value may not be constant. We also need to define the "basic" SCC of a network: mathematically, an SCC is called basic if its largest eigenvalue is the largest eigenvalue of the matrix. Although we would need quantitative information to ascertain which one is the basic SCC, in replacement networks it will usually be the one with the largest number of interactions (which will likely be the one formed by more species). According to matrix theory, when a system contains several SCCs, the species that can coexist are those forming the basic SCC and those that can recruit directly or indirectly under the species of the basic SCC [29]. This simple theorem explains the importance of SCCs, and how they are arranged, for the persistence of species in a replacement network [12]. Based on this theorem we can see that the structure we have found in replacement networks can allow a large proportion of species (83% on average) to coexist in the long term

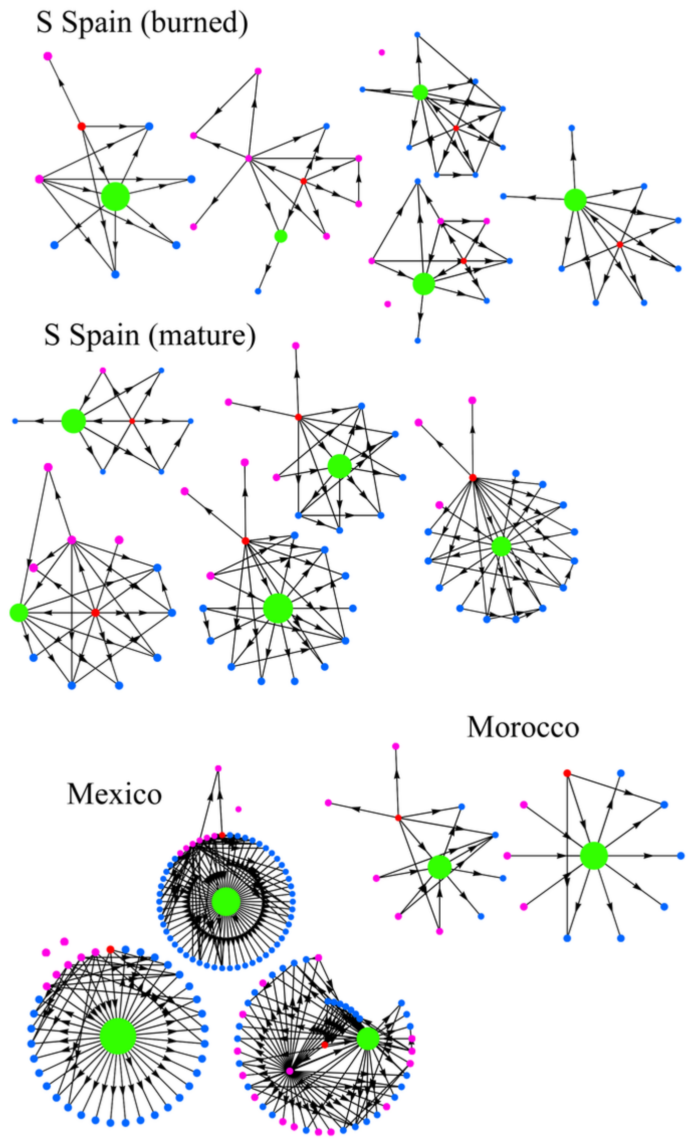


Figure 2: Examples of replacement networks from Southern Spain [12], Southern Morocco (Rey and Alcántara unpublished data) and Mexico [26]. Each node represents a SCC. The green SCC is the only non-trivial one in each network, and its size is proportional to the number of species it contains; it forms the "core" of species in the network. The blue and purple nodes are trivial SCCs (i.e. SCCs formed by a single species each) that can be considered as "satellite" species, related but not included in the core. The red node represents space not covered by any nurse species. Assuming that the green node is a basic SCC (see main text for a definition), the species in the green and blue nodes could coexist, but the species represented by purple nodes would eventually disappear from the local community.

because most of them are integrated in the core SCC and those that are not, are nevertheless able to recruit under some species of the core. Still, an average 17% of species in these communities seem deemed to local extinction. Moreover, we can also infer that replacement networks are quite robust after the extinction of one species. On average, the extinction of one species would lead to the subsequent extinction of no more than 5% of species. Noticeably, this high robustness to species extinction seems to emerge from the low modularity of these species interaction matrices.

In conclusion, the skeleton of the interaction matrix, when considered as part of a community dynamics model, can provide important insights about community structure, function and stability. Although many more study cases are required to conclude about general patterns, the scarce replacement networks available suggest that local woody plant communities may have a simple functional structure with most species forming a core of interactions that supports most of the rest of species. This structure can confer the species in these communities a high probability of persistence in the long term, even after the eventual extinction of one of them.

4 Bibliography

[1] S. L. Pimm and J. H. Lawton, "Are food webs divided into compartments?" *The Journal of Animal Ecology*, pp. 879–898, 1980.

[2] D. Kenny and C. Loehle, "Are food webs randomly connected?" *Ecology*, pp. 1794–1799, 1991.

[3] F. Jordán, I. Scheuring, and G. Vida, "Species positions and extinction dynamics in simple food webs," *Journal of Theoretical Biology*, vol. 215, no. 4, pp. 441–448, 2002.

[4] T. Säterberg, S. Sellman, and B. Ebenman, "High frequency of functional extinctions in ecological networks," *Nature*, 2013.

[5] R. M. May, "Will a large complex system be stable?" *Nature*, vol. 238, pp. 413–414, 1972.

[6] S. Allesina and S. Tang, "Stability criteria for complex ecosystems," *Nature*, vol. 483, no. 7388, pp. 205–208, 2012.

[7] R. M. May, *Stability and complexity in model ecosystems*. Princeton University Press, 1973a.

[8] S. P. Hubbell, *The unified neutral theory of biodiversity and biogeography (MPB-32)*. Princeton University Press, 2001, vol. 32.

[9] M. Spencer and J. E. Tanner, "Lotka-Volterra competition models for sessile organisms," *Ecology*, vol. 89, no. 4, pp. 1134–1143, 2008.

[10] M. F. Hill, J. D. Witman, and H. Caswell, "Spatio-temporal variation in Markov chain models of subtidal community succession," *Ecology Letters*, vol. 5, no. 5, pp. 665–675, 2002.

[11] R. J. Williams and N. D. Martinez, "Stabilization of chaotic and non-permanent food-web dynamics," *The European Physical Journal B-Condensed Matter and Complex Systems*, vol. 38, no. 2, pp. 297–303, 2004.

[12] J. M. Alcántara and P. J. Rey, "Linking topological structure and dynamics in ecological networks," *The American Naturalist*, vol. 180, no. 2, pp. 186–199, 2012.

[13] P. Yodzis and S. Innes, "Body size and consumer-resource dynamics," *American Naturalist*, pp. 1151–1175, 1992.

[14] R. P. Freckleton and A. R. Watkinson, "Nonmanipulative determination of plant community dynamics," *Trends in Ecology & Evolution*, vol. 16, no. 6, pp. 301–307, 2001.

[15] A. Boit, N. D. Martinez, R. J. Williams, and U. Gaedke, "Mechanistic theory and modelling of complex food-web dynamics in lake Constance," *Ecology Letters*, vol. 15, no. 6, pp. 594–602, 2012.

[16] R. M. May, "Qualitative stability in model ecosystems," *Ecology*, pp. 638–641, 1973b.

[17] J. M. Dambacher, H.-K. Luh, H. W. Li, and P. A. Rossignol, "Qualitative stability and ambiguity in model ecosystems," *The American Naturalist*, vol. 161, no. 6, pp. 876–888, 2003.

[18] H. Horn, "Markovian properties of forest succession," in *Ecology and evolution of communities*, M. L. Cody and J. Diamond, Eds. Cambridge, MA.: Harvard University Press, 1975.

[19] G. Siles, P. Rey, J. Alcántara, and J. Ramírez, "Assessing the long-term contribution of nurse plants to restoration of Mediterranean forests through markovian models," *Journal of Applied Ecology*, vol. 45, no. 6, pp. 1790–1798, 2008.

[20] P. J. Grubb, "The maintenance of species-richness in plant communities: the importance of the regeneration niche," *Biological Reviews*, vol. 52, no. 1, pp. 107–145, 1977.

[21] R. W. Myster, "Plants replacing plants: the future of community modeling and research," *The Botanical Review*, vol. 78, no. 1, pp. 2–9, 2012.

[22] R. M. May, "Network structure and the biology of populations," *Trends in Ecology & Evolution*, vol. 21, no. 7, pp. 394–399, 2006.

[23] I. D. Rozdilsky, L. Stone, and A. Solow, "The effects of interaction compartments on stability for competitive systems," *Journal of Theoretical Biology*, vol. 227, no. 2, pp. 277–282, 2004.

[24] D. B. Stouffer and J. Bascompte, "Compartmentalization increases food-web persistence," *Proceedings of the National Academy of Sciences*, vol. 108, no. 9, pp. 3648–3652, 2011.

[25] S. Fortunato, "Community detection in graphs," *Physics Reports*, vol. 486, no. 3, pp. 75–174, 2010.

[26] A. Valiente-Banuet and M. Verdú, "Facilitation can increase the phylogenetic diversity of plant communities," *Ecology Letters*, vol. 10, no. 11, pp. 1029–1036, 2007.

[27] V. C. Barbosa, R. Donangelo, and S. R. Souza, "Directed cycles and related structures in random graphs: I—Static properties," *Physica A: Statistical Mechanics and its Applications*, vol. 321, no. 3, pp. 381–397, 2003.

[28] P. Staniczenko, O. T. Lewis, N. S. Jones, and F. Reed-Tsochas, "Structural dynamics and robustness of food webs," *Ecology Letters*, vol. 13, no. 7, pp. 891–899, 2010.

[29] F. Gantmacher, "Matrix theory," *Chelsea, New York*, p. 21, 1959.

Plant-pollinator interactions in ecosystems restoration

E. Ceccon & I.G. Varassin, CRIM-UNAM, Mexico and UFPR, Brazil

1 Abstract

Measuring ecological restoration success is not simple, because the structure and composition of communities are very variable due to considerable fluctuations regarding soil nutrient levels, hydrology, and landscape, among others. Pollination is a process related to system sustainability and may be independent of the structural variation. Therefore, a failure to manage and promote pollinators could lead to decline or collapse in ecological restoration. Under any type of perturbation, plant–pollinator interactions disruption will depend on the level of specialization between a plant and its pollinators, on their abundance, and their sensitivity to land-use change. The proximity to natural landscapes can serve as an important support to pollinator communities in restoration activities. There is a vast amount of studies in pollination ecology but only a few addressed the question of plant-pollinator interactions as a tool to evaluate the success of restoration. Practical options aimed at restoring functional complementarity as resetting the maximum number of different functional groups or functional redundancy and resource use overlap can lead to different successional trajectories in the restored areas. Since different plant-pollinator communities might be regulated by different assembly rules there is still a vast amount of work to be done to understand the build-up of plant-pollinator communities in restored areas.

2 Resumen

Medir el éxito de la restauración ecológica no es sencillo, ya que la estructura y composición de las comunidades son muy variables debido a una considerable fluctuación en relación a los niveles de nutrientes del suelo, la hidrología, y el paisaje, entre otros. La polinización es un proceso relacionado con la sostenibilidad del sistema y puede ser independiente de la variación estructural. Por lo tanto, una falla en el manejo y promoción

de los polinizadores podría conducir a un fracaso en la restauración ecológica. Una perturbación significa una interrupción en las interacciones planta-polinizador y sus consecuencias dependerán del grado de especialización entre una planta y sus polinizadores, de su abundancia y de su sensibilidad a los cambios de uso del suelo. La cercanía a los paisajes naturales puede servir como un importante soporte para las comunidades de polinizadores en las actividades de restauración. Aunque hay una gran cantidad de estudios en ecología de la polinización, sólo unos pocos abordan la cuestión de las interacciones planta-polinizador como una herramienta para evaluar el éxito de la restauración. Opciones prácticas encaminadas a restablecer la complementariedad funcional, como el restablecimiento del número máximo de los diferentes grupos funcionales, o la redundancia funcional y la superposición de tareas en el uso de recursos, pueden dar lugar a diferentes trayectorias sucesionales en las zonas restauradas. Dado que las diferentes comunidades de plantas y sus polinizadores pueden ser reguladas por normas diferentes de ensamblaje, todavía hay una gran cantidad de trabajos por hacer para entender la construcción de estas interacciones en las zonas restauradas.

3 Introduction

The science of restoration ecology has experienced a major advance in the last 20 years, and numerous techniques have been proposed as tools to improve the biotic and abiotic properties of degraded systems [1–3]. Restoration goals usually emphasize structural aspects of biodiversity, such as species richness and abundance. Although several studies have found that structure influences function (e.g., [4, 5]), some processes related to system sustainability may be independent of the structural variation in healthy communities. These processes include trophic interactions, disturbance regimes, pollination and seed dispersal [6, 7]. Indeed, there is a growing concern about restoration dynamics [8] and the reintegration of interactions and processes in restoration programs [9, 10]. Moreover, there may be a considerable variation of the structural diversity of restored sites due to variations in soil nutrient levels, hydrology, and landscape context among others [11] and there are few studies bridging structural changes to processes [12, 13]. Hence, one of the challenges restorationists face is to maintain the self-sustainability of restored systems and to develop tools for assessing acceptable levels of variability among restored ecosystems.

Ecological science has devoted over the recent years a large effort to understand some aspects of ecosystem processes such as nutrients cycling and climate regulation. Interactions among species, including its effects on other species' populations, are less well known. The pollination of flowering plants is an emblematic example: approximately 90% of flowering plant species rely on biotic pollination for reproduction and genetic viability maintenance [14]. Although important for population maintenance, relatively few plant-pollinator interactions are absolutely obligate and most are more generalized [15].

These interactions also face a high level of variability in time and space [16, 17]. This is an important point because some researchers have suggested that pollination restoration may be independent of the taxa of pollinator involved [10, 18], but long-term data to address this is lacking. Due to their effect on plant reproduction and genetic flow, the failure to manage and promote pollinators could lead to a decline or failure of ecological restoration efforts [19]. Besides that, plant–pollinator interactions may not re-establish automatically themselves in communities undergoing restoration management, because pollinators establish populations only once their habitat requirements have been met. For example, in addition to food resources, bees require nesting sites and nesting materials (e.g. [20, 21]). These features make pollination a useful functional bio-indicator for comparing restored communities to reference communities. However, ecological restoration of plant–pollinator interactions has had few recent experimental studies [10, 12, 18, 22]. This deficiency in the knowledge to restore pollinator capability represents a major drawback in restoration programs, particularly in regions where specialist invertebrate and vertebrate pollinators exist, such as in global biodiversity hotspots [23]. In this chapter we will first review the effects of human disturbances in pollination and the role of landscape in the restoration of plant-pollinator webs. Then we will review recent experimental studies on plant-pollinator interactions in restoration and finally we will address some steps in ecological restoration that may improve the relation plant-pollinator.

4 The role of anthropogenic perturbations in pollination

Under any type of perturbation, plant–pollinator interactions disruption will depend on the level of specialization between a plant and its pollinators, on their abundance, and their sensitivity to land-use change [24–26]. Generalist plants tend to be more protected against the loss of any particular pollinator than highly specialized plants due to the risk of a reproductive failure [25, 27]. These asymmetries may buffer against species loss in mutualistic networks [28] and appear to be the norm in plant-pollinator networks [29]. Even considering pollinator redundancy in a network, shared traits by pollinators may imply shared sensitivity to anthopogenic changes, as it was reported to some functional groups of bees whose abundance declines with climate changes [30]. Plant attractiveness and rewards for pollinators may be potentially influenced by perturbation because it alters the amount of light, water and nutrients received by plants [31]. These environmental modifications may alter the number and size of flowers or the amounts and qualities of pollen and nectar produced by them. These changes in turn may affect the behavior of pollinators and the pollen transfer and plant reproductive success (reviewed in [32]).

Landscape fragmentation

The interactions in fragmented habitats are mainly affected by changes in the abundance of populations [33]. However, relatively few studies have directly measured changes in species interactions in fragmented landscapes and have consistently found that species interactions involving predators, parasitoids and pollinators are frequently more severely affected by fragmentation than host–herbivore interactions [26, 34–41]. Actually, the increase in fragmentation is expected to reduce the functional diversity [42] and to promote the erosion of reproductive traits in small fragments as observed in tropical rain forest remnants [43]. Evidence of nonrandom loss of interactions with decreasing fragment size was found in 12 pollination webs from isolated fragments in Argentina, ranging from tens to thousands of hectares [26]. Species with low interaction frequency and more specialized are subject to a higher risk. Besides that, there are structural changes in the networks associated to fragmentation, as for example changes in the central role of generalist species depending on fragment size.

Climate change

Climate change is responsible for a variety of responses in natural systems, including changes in species distribution, abundance and phenology [44]. Because these changes may affect both partners in pollination interactions, phenological changes may not be concordant [30, 45]. There is a reported advance in bee emergence [30], hummingbird immigration [45] and flowering [46] in temperate areas associated with global warming. Despite that, Memmot et al. [47, 48] found few research papers that specifically investigate pollination networks and persistence facing climate change. Climate change also may lead to partial or total asynchrony between pollinator life cycles and flowering phenologies that may result in a breakdown of pollination mutualisms in the case of obligate pollination systems [49, 50]. Less seasonal systems might be expected to support a higher asynchrony due to the longer growing season and longer phenological cycles than more seasonal systems. This can be expected because population-level flowering asynchrony results in higher plant reproductive success due to a reduction in competition for pollinators, an increase in the number of mates due to temporal changes in mate availability and a reduction on the effective population size [51]. Changes in abundance and asynchrony may be especially critical to short-lived species, but long-lived or migrating species can be very sensitive to climatic changes since they depend for longer on their partners' abundance [50, 52].

5 The role of landscape in pollination

The proximity to natural landscapes that may support pollinator communities is an important component of pollinator activity [53]. Agro-ecosystems with more natural or semi-natural habitats are often more pollinator-species rich [41, 54, 55]. On the other hand, restoration of pollinator communities may have the potential added benefit that pollinators deliver service to crops and native plants beyond the restored site, adding a direct value to the restored habitat [55, 56]. The presence of natural landscapes in fragmented habitat matrices is expected to affect the ability of pollinators to migrate and establish and, as a result, it is expected also to affect restoration capability [23]. Some taxa, less agile or less distributed, may be especially susceptible [23].

It is not known how ecological corridors may adequately support pollinator migration to restored sites. The responses to corridors are taxa-dependent [57]. Eventually a landscape intervention may require the establishment of corridors [57] or pollinator-friendly agri-environments [58] involving key resources: (i) species that provide a major nectar or pollen source, (ii) bridging species (plants that provide resources over resource-limited times) and (iii) magnet species (plants with attractive flowers associated with species with unattractive or small flowers; [59].

6 Experimental studies on plant-pollinator interactions in restoration

Even that there is a vast amount of studies in pollination ecology, only a few have addressed the question of plant-pollinator interactions as a tool to evaluate the success of restoration [60]. In the English heathlands, it was showed that although two ancient and two restored meadows were structurally very different, there were no significant differences between restored and reference meadows in plant or insect species richness, in the proportion of flower species visited by insects, in the numbers of pollen grains being moved by flower visitors, or in the number of links per species [10]. In these heathlands, Forup et al. [22] compared the complex network structure between restored heaths and ancient heaths. They found that four restoration projects established successfully heathland plants and pollinator communities and was stable after 14 years. The key pollinators were the same on ancient and restored sites after 11 years and were also the most abundant flower visitors 14 years after restoration. Another important result was that heathland restoration sites may not need to be immediately adjacent to intact habitat to be successful, since species composition was not related to distance from ancient sites. This may be due to the fact that in their study, the functional important pollinators are mobile, abundant and able to traverse other habitat than heathland (honeybees, *A. mellifera*, and bumblebees, mainly *B. terrestris/lucorum*), which are traits related to a higher regional abundance and so they may buffer against local loss [61, 62].

Bee and plant communities at restored at mid-successional stage riparian sites along the Sacramento River in California, United States were compared to remnants of riparian habitat within the same region [18]. Restored riparian habitats presented richness and abundance of native bees equal to that found in close remnants of riparian habitat. Connectance of bee–plant interaction networks in restored riparian habitats was similar to that of remnant riparian habitats and the proportion of native plants receiving visits by bees at restored sites. However the compositions of the bee communities at restored sites were different from those at remnant riparian habitats, with a lower redundancy of

Figure 1: *Placidina euryanassa* (Nymphalidae: Lepidoptera) visiting flowers of *Acnistus arborescens* (Solanaceae) in a restored area of Southern Atlantic Forest, Paraná, Brazil. Photo kindly provided by Jana M. Tesserolli de Souza.

pollinators in restored areas. The author argues that restoration of pollination may be achieved with a different species composition from those of reference sites, but the lack of pollinator redundancy may result in a lower robustness in restored areas.

In Mauritius island, restored areas may benefit from controlled removal of alien species, with an increase of native plant abundance, pollinator richness, floral abundance and pollinator visitation rates, resulting in a higher redundancy in pollinators [63]. Much less is known for tropical forests. Phenological coupling among plants and pollinators had a major effect on interactions establishment in plant-pollinator networks in restored sites of a tropical rain forest in Southern Brazil [13]. Concordant to that, at restored areas with four different ages (4, 5, 6, 7 years) in tropical rain forest in Southern Brazil, herbs and shrubs were very important food resources at the beginning of the restoration because they increase the supply of resources in areas where pioneer tree species take two to ten years to start the reproductive phase [64]. Herbs and shrubs were generalists plants able to establish interactions with specialists or rare insects (see Figures 1 and 2) [64]. Besides that, the structure of plant-pollinator networks in restored sites of this tropical rain forest was related to structural changes, canopy height, tree diameter variation, basal area and understory density [13]. Restoration of pollination network structure for a temperate forest was also affected by structural changes, such as tree diameter variation and tree density [12]. These structural effects on pollination networks may be directly related to flower resource availability to pollinators (as in [63]), as well as other non-food resources [12, 13].

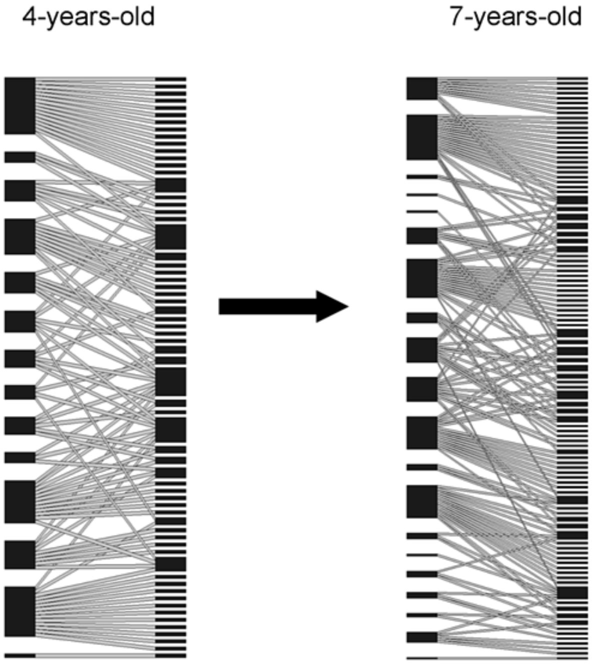


Figure 2: Plant-pollinator networks in four-years-old (left) and seven-years-old (right) restored areas of Southern Atlantic Forest, Paraná, Brazil. Data kindly provided by Ana Júlia Donatti

7 How to restore the pollination interactions in a degraded area?

Increase in network interaction diversity and interaction evenness seems to be good indicators of a healthy successional process including those in restored areas [12, 13, 65]. Some authors argue for a higher redundancy to increase the stability of restored areas which may be achieved in species-rich communities [18, 63]. Practical choices aiming to restore functional complementarity, the maximal number of different functional groups, or functional redundancy, overlap in resource use, may result in different successional trajectories in restored areas [12]. More studies are also needed on restored systems involving butterfly and bird pollination networks. Functional traits can be used to access the process involved in the assembly of plant-pollinator communities [66]. The balance between neutral or niche-based processes changes along the successional process [67] with a growing importance of niche-based factors in older forests. At least in successional areas, the structure and interaction frequency of tropical hummingbird networks are better explained by neutral factors in early successional sites and by niche-based factors in late successional areas [68]. This calls for attention for the need to understand how functional traits are related to community assembly rules in restored areas. At the same time, there are recent developments in ecological theory [69] that would impact many aspects of ecosystem restoration. These developments include concepts and methodologies from complex systems and network theory. Many important questions in restoration such as how to measure its success can be now reviewed from the point of view of non-linear dynamics where an important conclusion is that even under almost identical initial conditions, the outcome of the restoration could lead to a new ecosystem different from the previous undisturbed one [70]. However, this is in no way discouraging. Better understanding of ecological interactions such as pollinator-plant relationships seen as complex networks would help in unprecedented ways the practical goals of restoration.

Acknowledgements

Eliane Ceccon very much appreciates PAPIIT-UNAM grants IN300112, IN101712. Isabela Galarda Varassin was funded by Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES) grant BEX 8971/11-0.

8 Bibliography

[1] M. R. Perrow and A. J. Davy, *Handbook of ecological restoration: Restoration in practice*. Cambridge University Press, 2002, vol. 2.

[2] J. van Andel and J. Aronson, *Restoration ecology: the new frontier*. John Wiley & Sons, 2012.

[3] E. Ceccon, *Restauración en bosques tropicales: fundamentos ecológicos, prácticos y sociales.* Díaz de Santos/UNAM-CRIM, Mexico, 2013.

[4] M. Cadenasso and S. Pickett, “Linking forest edge structure to edge function: mediation of herbivore damage,” *Journal of Ecology*, vol. 88, no. 1, pp. 31–44, 2000.

[5] E. M. Spehn, J. Joshi, B. Schmid, J. Alphei, and C. Körner, “Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems,” *Plant and Soil*, vol. 224, no. 2, pp. 217–230, 2000.

[6] A. M. Montalvo, S. L. Williams, K. J. Rice, S. L. Buchmann, C. Cory, S. N. Handel, G. P. Nabhan, R. Primack, and R. H. Robichaux, “Restoration biology: a population biology perspective,” *Restoration Ecology*, vol. 5, no. 4, pp. 277–290, 1997.

[7] E. Ceccon and P. Hernández, “Seed rain dynamics following disturbance exclusion in a secondary tropical dry forest in Morelos, Mexico,” *Revista de Biología Tropical*, vol. 57, no. 1-2, pp. 257–269, 2009.

[8] V. T. Parker, “The scale of successional models and restoration objectives,” *Restoration Ecology*, vol. 5, no. 4, pp. 301–306, 1997.

[9] R. J. Hobbs and D. A. Norton, "Towards a conceptual framework for restoration ecology," *Restoration Ecology*, vol. 4, no. 2, pp. 93–110, 1996.

[10] M. L. Forup and J. Memmott, "The restoration of plant–pollinator interactions in hay meadows," *Restoration Ecology*, vol. 13, no. 2, pp. 265–274, 2005.

[11] T. Tscharntke, I. Steffan-Dewenter, A. Kruess, and C. Thies, "Characteristics of insect populations on habitat fragments: a mini review," *Ecological Research*, vol. 17, no. 2, pp. 229–239, 2002.

[12] M. Devoto, S. Bailey, P. Craze, and J. Memmott, "Understanding and planning ecological restoration of plant–pollinator networks," *Ecology Letters*, vol. 15, no. 4, pp. 319–328, 2012.

[13] J. M. T. de Souza, "Redes de polinização em áreas restauradas de Floresta Atlântica do Sul do Brasil," Ph.D. dissertation, Universidade Federal do Paraná, Brazil, 2013.

[14] J. Friedman and S. C. Barrett, "Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants," *Annals of Botany*, vol. 103, no. 9, pp. 1515–1527, 2009.

[15] N. M. Waser, L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton, "Generalization in pollination systems, and why it matters," *Ecology*, vol. 77, no. 4, pp. 1043–1060, 1996.

[16] R. Alarcón, N. M. Waser, and J. Ollerton, "Year-to-year variation in the topology of a plant–pollinator interaction network," *Oikos*, vol. 117, no. 12, pp. 1796–1807, 2008.

[17] T. Petanidou, A. S. Kallimanis, J. Tzanopoulos, S. P. Sgardelis, and J. D. Pantis, "Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization," *Ecology Letters*, vol. 11, no. 6, pp. 564–575, 2008.

[18] N. M. Williams, "Restoration of nontarget species: bee communities and pollination function in riparian forests," *Restoration Ecology*, vol. 19, no. 4, pp. 450–459, 2011.

[19] S. N. Handel, "The role of plant-animal mutualisms in the design and restoration of natural communities," *Restoration Ecology and Sustainable Development*, pp. 111–132, 1997.

[20] C. A. Kearns, D. W. Inouye, and N. M. Waser, "Endangered mutualisms: the conservation of plant-pollinator interactions," *Annual Review of Ecology and Systematics*, vol. 29, no. 1, pp. 83–112, 1998.

[21] A. Gathmann and T. Tscharntke, “Foraging ranges of solitary bees,” *Journal of Animal Ecology*, vol. 71, no. 5, pp. 757–764, 2002.

[22] M. L. Forup, K. S. Henson, P. G. Craze, and J. Memmott, “The restoration of ecological interactions: plant–pollinator networks on ancient and restored heathlands,” *Journal of Applied Ecology*, vol. 45, no. 3, pp. 742–752, 2008.

[23] K. W. Dixon *et al.*, “Pollination and restoration,” *Science*, vol. 325, no. 5940, p. 571, 2009.

[24] M. A. Aizen, L. Ashworth, and L. Galetto, “Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter?” *Journal of Vegetation Science*, vol. 13, no. 6, pp. 885–892, 2002.

[25] D. P. Vázquez and D. Simberloff, “Changes in interaction biodiversity induced by an introduced ungulate,” *Ecology Letters*, vol. 6, no. 12, pp. 1077–1083, 2003.

[26] M. A. Aizen, M. Sabatino, and J. M. Tylianakis, “Specialization and rarity predict nonrandom loss of interactions from mutualist networks,” *Science*, vol. 335, no. 6075, pp. 1486–1489, 2012.

[27] T.-L. Ashman, T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell *et al.*, “Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences,” *Ecology*, vol. 85, no. 9, pp. 2408–2421, 2004.

[28] J. Bascompte, P. Jordano, and J. M. Olesen, “Asymmetric coevolutionary networks facilitate biodiversity maintenance,” *Science*, vol. 312, no. 5772, pp. 431–433, 2006.

[29] D. P. Vázquez and M. A. Aizen, “Asymmetric specialization: a pervasive feature of plant-pollinator interactions,” *Ecology*, vol. 85, no. 5, pp. 1251–1257, 2004.

[30] I. Bartomeus, J. S. Ascher, D. Wagner, B. N. Danforth, S. Colla, S. Kornbluth, and R. Winfree, “Climate-associated phenological advances in bee pollinators and bee-pollinated plants,” *Proceedings of the National Academy of Sciences*, vol. 108, no. 51, pp. 20 645–20 649, 2011.

[31] D. Goulson, S. A. Hawson, and J. C. Stout, “Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species,” *Animal Behaviour*, vol. 55, no. 1, pp. 199–206, 1998.

[32] M. Aizen and D. Vázquez, “Flower ecology and evolution in human-altered environments,” in *The Ecology and Evolution of Flowers*, L. Harder and S. Barrett, Eds. Oxford University Press, 2006, pp. 357–366.

[33] W. F. Fagan, R. S. Cantrell, and C. Cosner, “How habitat edges change species interactions,” *The American Naturalist*, vol. 153, no. 2, pp. 165–182, 1999.

[34] A. Kruess and T. Tscharntke, “Habitat fragmentation, species loss, and biological control,” *Science*, vol. 264, no. 5165, pp. 1581–1584, 1994.

[35] I. Steffan-Dewenter and T. Tscharntke, “Resource overlap and possible competition between honey bees and wild bees in central Europe,” *Oecologia*, vol. 122, no. 2, pp. 288–296, 2000.

[36] D. M. Golden and T. O. Crist, “Experimental effects of habitat fragmentation on rove beetles and ants: patch area or edge?” *Oikos*, vol. 90, no. 3, pp. 525–538, 2000.

[37] A. Kruess and T. Tscharntke, “Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*,” *Oecologia*, vol. 122, no. 1, pp. 129–137, 2000.

[38] B. Schulke and N. M. Waser, “Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*,” *Oecologia*, vol. 127, no. 2, pp. 239–245, 2001.

[39] C. Denys and T. Tscharntke, “Plant-insect communities and predator-prey ratios in field margin strips, adjacent crop fields, and fallows,” *Oecologia*, vol. 130, no. 2, pp. 315–324, 2002.

[40] I. Steffan-Dewenter, U. Münzenberg, and T. Tscharntke, “Pollination, seed set and seed predation on a landscape scale,” *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 268, no. 1477, pp. 1685–1690, 2001.

[41] I. Steffan-Dewenter, U. Münzenberg, C. Bürger, C. Thies, and T. Tscharntke, “Scale-dependent effects of landscape context on three pollinator guilds,” *Ecology*, vol. 83, no. 5, pp. 1421–1432, 2002.

[42] L. C. Girão, A. V. Lopes, M. Tabarelli, and E. M. Bruna, “Changes in tree reproductive traits reduce functional diversity in a fragmented atlantic forest landscape,” *PLoS One*, vol. 2, no. 9, p. e908, 2007.

[43] A. V. Lopes, L. C. Girão, B. A. Santos, C. A. Peres, and M. Tabarelli, “Long-term erosion of tree reproductive trait diversity in edge-dominated Atlantic Forest fragments,” *Biological Conservation*, vol. 142, no. 6, pp. 1154–1165, 2009.

[44] C. Parmesan and G. Yohe, “A globally coherent fingerprint of climate change impacts across natural systems,” *Nature*, vol. 421, no. 6918, pp. 37–42, 2003.

[45] A. M. McKinney, P. J. CaraDonna, D. W. Inouye, B. Barr, C. D. Bertelsen, and N. M. Waser, “Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources,” *Ecology*, vol. 93, no. 9, pp. 1987–1993, 2012.

[46] A. Fitter and R. Fitter, “Rapid changes in flowering time in british plants,” *Science*, vol. 296, no. 5573, pp. 1689–1691, 2002.

[47] J. Memmott, N. M. Waser, and M. V. Price, “Tolerance of pollination networks to species extinctions,” *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 271, no. 1557, pp. 2605–2611, 2004.

[48] J. Memmott, P. G. Craze, N. M. Waser, and M. V. Price, “Global warming and the disruption of plant–pollinator interactions,” *Ecology Letters*, vol. 10, no. 8, pp. 710–717, 2007.

[49] S. J. Hegland, A. Nielsen, A. Lázaro, A.-L. Bjerknes, and Ø. Totland, “How does climate warming affect plant-pollinator interactions?” *Ecology Letters*, vol. 12, no. 2, pp. 184–195, 2009.

[50] W. F. Fagan, S. Bewick, S. Cantrell, C. Cosner, I. G. Varassin, and D. W. Inouye, “Phenologically explicit models for studying plant–pollinator interactions under climate change,” *Theoretical Ecology*, pp. 1–9, 2014.

[51] B. Rathcke and E. P. Lacey, “Phenological patterns of terrestrial plants,” *Annual Review of Ecology and Systematics*, pp. 179–214, 1985.

[52] G. Aldridge, D. W. Inouye, J. R. Forrest, W. A. Barr, and A. J. Miller-Rushing, “Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change,” *Journal of Ecology*, vol. 99, no. 4, pp. 905–913, 2011.

[53] E. Lonsdorf, C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf, “Modelling pollination services across agricultural landscapes,” *Annals of Botany*, vol. 103, no. 9, pp. 1589–1600, 2009.

[54] R. Blanche and S. A. Cunningham, “Rain forest provides pollinating beetles for atemoya crops,” *Journal of Economic Entomology*, vol. 98, no. 4, pp. 1193–1201, 2005.

[55] C. Kremen, N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, I. Steffan-Dewenter, D. P. Vazquez *et al.*, “Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change,” *Ecology Letters*, vol. 10, no. 4, pp. 299–314, 2007.

[56] C. Kremen, N. M. Williams, and R. W. Thorp, "Crop pollination from native bees at risk from agricultural intensification," *Proceedings of the National Academy of Sciences*, vol. 99, no. 26, pp. 16 812–16 816, 2002.

[57] P. A. Townsend and D. J. Levey, "An experimental test of whether habitat corridors affect pollen transfer," *Ecology*, vol. 86, no. 2, pp. 466–475, 2005.

[58] I. Perfecto and J. Vandermeer, "The agroecological matrix as alternative to the land-sparing/agriculture intensification model," *Proceedings of the National Academy of Sciences*, vol. 107, no. 13, pp. 5786–5791, 2010.

[59] M. A. Molina-Montenegro, E. I. Badano, and L. A. Cavieres, "Positive interactions among plant species for pollinator service: assessing the 'magnet species' concept with invasive species," *Oikos*, vol. 117, no. 12, pp. 1833–1839, 2008.

[60] M. H. Menz, R. D. Phillips, R. Winfree, C. Kremen, M. A. Aizen, S. D. Johnson, and K. W. Dixon, "Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms," *Trends in Plant Science*, vol. 16, no. 1, pp. 4–12, 2011.

[61] D. H. Wright, B. D. Patterson, G. M. Mikkelson, A. Cutler, and W. Atmar, "A comparative analysis of nested subset patterns of species composition," *Oecologia*, vol. 113, pp. 1–20, 1998.

[62] K. J. Gaston, T. M. Blackburn, J. J. Greenwood, R. D. Gregory, R. M. Quinn, and J. H. Lawton, "Abundance–occupancy relationships," *Journal of Applied Ecology*, vol. 37, no. s1, pp. 39–59, 2000.

[63] C. N. Kaiser-Bunbury, J. Memmott, and C. B. Müller, "Community structure of pollination webs of mauritian heathland habitats," *Perspectives in Plant Ecology, Evolution and Systematics*, vol. 11, no. 4, pp. 241–254, 2009.

[64] A. J. Donatti, "Como estão organizadas as redes de interações de visitação floral em áreas de restauração de Floresta Atlântica?" Master's thesis, Universidade Federal do Paraná, Brazil, 2011.

[65] M. Albrecht, M. Riesen, and B. Schmid, "Plant–pollinator network assembly along the chronosequence of a glacier foreland," *Oikos*, vol. 119, no. 10, pp. 1610–1624, 2010.

[66] R. D. Sargent and D. D. Ackerly, "Plant–pollinator interactions and the assembly of plant communities," *Trends in Ecology & Evolution*, vol. 23, no. 3, pp. 123–130, 2008.

[67] C.-J. Chu, Y.-S. Wang, G.-Z. Du, F. T. Maestre, Y.-J. Luo, and G. Wang, "On the balance between niche and neutral processes as drivers of community structure along a successional gradient: insights from alpine and sub-alpine meadow communities," *Annals of Botany*, vol. 100, no. 4, pp. 807–812, 2007.

[68] T. Malucelli, "Fatores envolvidos na estruturação das redes de polinização beija-flor-planta em um gradiente sucessional," Master's thesis, Universidade Federal do Paraná, Brazil, 2014.

[69] U. Bastolla, M. A. Fortuna, A. Pascual-García, A. Ferrera, B. Luque, and J. Bascompte, "The architecture of mutualistic networks minimizes competition and increases biodiversity," *Nature*, vol. 458, no. 7241, pp. 1018–1020, 2009.

[70] M. Anand and R. E. Desrochers, "Quantification of restoration success using complex systems concepts and models," *Restoration Ecology*, vol. 12, no. 1, pp. 117–123, 2004.

Networks in agroecology

M. Benítez, J. Fornoni, L. García-Barrios & R. López, UNAM and ECOSUR, Mexico

1 Abstract

In the context of biodiversity and alimentary crises, it is necessary to develop and promote agricultural practices that both contribute to food security and biodiversity conservation. The Mesoamerican polycrop known as milpa is a diverse and complex agroecosystem that, given its ample diversification and adaptation to local conditions, provides an excellent model system for Agroecology. In this chapter we argue that such a system can be studied with the conceptual and technical tools of Theoretical Ecology and Complex Systems Sciences —dynamical network models in particular–- in order to advance in the understanding and design of sustainable agricultural practices, and to render analytical tools that can inform farmers and other social actors by enabling the assessment of contrasting scenarios and management schemes.

2 Resumen

En el contexto de las crisis alimentarias y de biodiversidad, es necesario desarrollar y promover prácticas agrícolas que al mismo tiempo contribuyan a garantizar la seguridad alimentaria y a conservar la biodiversidad. El policultivo mesoamericano conocido como milpa es un agroecosistema diverso y complejo que, dada su amplia diversificación y adaptación a ambientes locales, provee de un excelente sistema modelo para la agroecología. En este capítulo, argumentamos que dicho sistema puede ser estudiado con las herramientas técnicas y conceptuales de la ecología teórica y las ciencias de la complejidad —los modelos dinámicos de redes en particular–- para avanzar en la comprensión y diseño de prácticas agrícolas sustentables, así como para generar herramientas analíticas que permitan a los campesinos y a otros actores sociales evaluar distintos escenarios y esquemas de manejo.

3 Agroecological communities: multiscale nonlinear interactions

Agroecological systems are inherently complex, as they comprise non-additive or non-linear interactions established among cultivated plants, associated native species of plants and animals, soil micro- and meso- communities, and the human agency. The prevalence of non-linear interactions among and within populations often give rise to unpredictable or non-propotional effects in the face of community alterations. Moreover, species in these ensembles are tightly intertwined, which makes them an ecological unit that, due to the human management, are subjected to natural and artificial processes of selection and evolution at the species and the community level. For example, artificial selection driven by Mesoamerican peasants has led to the domestication and semi-domestication of thousands of maize, bean, squash and other plant races and varieties, many of them generated and locally adapted in close association with each other. The study of agrocommunities could thus help address the question of how selection on species and community traits shapes ecosystem structure and services; for instance, how selection on species and community traits shapes ecosystem structure, and how this translates to enhancement of farmers life quality.

With this integrative view, the growing field of Agroecology postulates that agricultural systems may be studied and modeled by using tools and concepts similar to those that have proven useful in the study of "natural" communities and ecosystems. For instance, agricultural production can be seen from the perspective of population and community ecology; then, yield and other properties become a consequence of the growth and survival rates of each plant population and the effect of species interactions. Similarly, the transit from one community state to another (e.g. from high to low biodiversity) is also largely due to the collective plant-plant and animal-plant interactions [1, 2]. Hence we revise the use of particular ecological and mathematical tools to study agroecosystems. There are, however, some specific aspects of agroecological systems that should be taken in consideration when being studied as ecological units, such as the forcing of the system by producers (farmers), which establishes initial conditions of the system and may change ecological interactions between crops and the associated biota. Indeed, research of simple agroecosystems such as monocrops and two-crop mixtures pioneered the study of intra and interspecific plant competition, in man-made and natural communities [3, 4], and it is likely that the study of well-characterized and relatively controlled ecological communities such as diversified agroecosystems may lead to a better understanding of ecological systems in general. For example, the study of agrocommunities could help address the question of how selection on species and community traits shapes ecosystem structure.

As complex systems, agroecological systems exhibit global properties that arise from the interactions among their components, this is, among species and abiotic factors. These properties, often referred to as emergent properties, are thus defined for the whole web of interactions and cannot be understood only from the isolated study of species or pairs of species. Among them are resilience, productivity and diversity, which in turn appear to affect each other in non-trivial ways [5, 6]. A property that has been less studied as an emergent and community-level feature is tolerance. At the population level tolerance has been defined in a broad sense as the capacity to buffer individual fitness consequences of physical and biotic stresses [7]. Tolerance is usually estimated as the relationship between fitness and a gradient of damage (intensity of stressful conditions) for individual genotypes [8]. The rationale behind this concept could be easily escalated at the community level to better understand the connection between complexity and stability. Communities are also known to be negatively affected by natural and human induced perturbations [9]. However, attributes like species composition and diversity can increase the speed of recovery (resilience) of communities after disturbance [10] (a component of community tolerance). Further examination of emergent properties of communities is required to focus management efforts to warrant community stability in agroecosystems. In this sense, identification of population and community attributes that increase tolerance, for example, to disturbance pressures can help to design better management programs.

The milpa as a model system in agroecology

The milpa is a polyculture originated and still practiced in Mexico and the rest of Mesoamerica (see Figure 1). In this system maize, common beans and squash are typically grown in association, sometimes along with tomatoes, multiple varieties of chilies, semi-domesticated herbs (*quelites*), etc. This method of polyculture has been improved over thousands of years, and is now adapted to a variety of climatic, edaphic and cultural conditions and, despite the current tendency towards its extreme simplification, it has been the foundation of food security in many Latin American rural communities for centuries [11]. The milpa's value extends far-beyond the calories it provides or the simple combination of species; being based on locally adapted and diversified plant varieties, the system provides a reliable source of diverse and complementary foodstuffs that meet nutritional needs and local cultural preferences [11, 12]. In addition, domesticated species used in the milpa were selected in the presence of natural consumers (in the absence of agrochemicals), thus human selection has favored crop varieties, genotypes and species associations that are tolerant and resilient in an ecological scenario were pests, competition, and resource limitation were the most frequent conditions. The milpa thus represents a valuable source of genetic and ecological resources to face the negative effect of diverse factors affecting plant performance and productivity. For example, theoretical and empirical studies support the expectation that because plant tolerance does not affect negatively natural consumers, it reduces the coevolutionary response usually observed between resistant traits of plant and their natural enemies [13]. Thus, studying crop tolerance in agroecosystems can help reduce the need of artificially defending plants through agrochemicals while reducing the opportunities for pest adaptation to the plant resistance.

Figure 1: The milpa as a complex agroecosystem. A) The milpa is a polyculture based on maize that usually also includes bean, squash, semidomesticated herbs, and other plant and animal species. Picture taken at the milpa plots located at Vicente Guerrero, Tlaxcala, Mexico. B) Schematic network representation of some of components and interactions comprised in the milpa agroecosystem: 1. *Zea mays L.* (corn), 2. *Cucurbita* ssp (squash), 3. *Phaseolus vulgaris* (bean), 4. *Spodoptera frugiperda* (army worm), 5. *Baculovirus SfVPN*, 6. *Homo sapiens* (farmer).

Moreover, it has been suggested that biodiverse agroecological systems such as the milpa play a key role in the maintenance and regeneration of biological diversity. In contrast to conventional –species poor– agricultural systems, the milpa constitutes a heterogeneous system based on combinations of locally adapted species, often using little or no agrochemicals, which enables the coexistence of the cultured plants with numerous associated species, which frequently includes shrubs, columnar cacti, and trees in the borders or dispersed within the plot [14]. Agro- and biodiversity sustained in systems like the milpa make it a matrix permeable to native animal and plant species, thus facilitating migration, dispersal and, in consequence, the conservation of primary vegetation [15, 16]. Then, the milpa offers a valuable resource to meet ecological and socioeconomic challenges, such as climate change, changes in water availability, disease and pest incidence, and the alimentary crisis currently faced by Mexico and other countries.

Some systems have been established as model ecological systems; their components and interactions have been thoroughly characterized, enabling the postulation and analysis of particular ecological networks. Among these systems are for example, some lake and grass communities [6]. The consolidation of these ecological systems as models for field, experimental and theoretical work has provided ecologists with a catalog of ecological networks that can now be used to point at common structural and dynamical properties of ecological communities. Similarly, the milpa constitutes a model system in agroecology, from which the collective dynamics of cultivated plants and the associated diversity can be uncovered. Additionally, this system has been studied in detail, although not necessarily with an integrative nor an evolutionary approach, mainly by farmers – who have crystallized in traditional practices their profound ecological understanding of the milpa – and also by agronomists. There is therefore a large amount of data that can inform the evolutionary patterns and specification of a network model for the milpa (e.g. [17]).

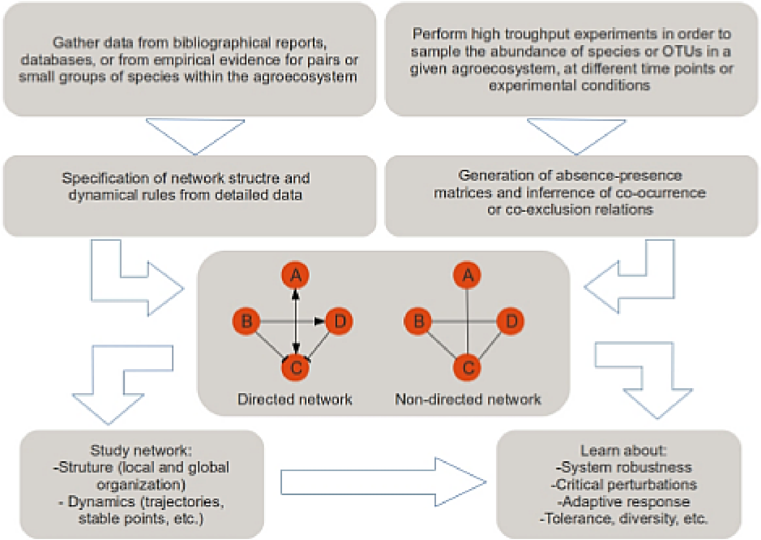


Figure 2: Diagram showing the basic steps to the inference of ecological networks from field, genetic, physiological an other types of data.

Dynamic network models in the study of agroecosystems

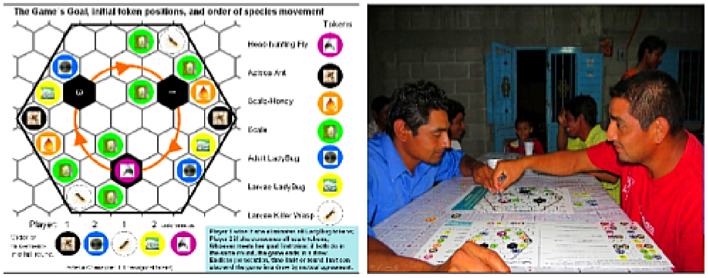
Networks have been widely used to represent the skeleton of complex systems, as they provide a rather natural way to integrate diverse interacting elements. Network modeling has been extensively used during the last century in the ecosystems study, initially mostly with a trophic-energy flux focus [18, 19], but it was not until Robert May's work [2] that new formal aspects about the ecological networks (e.g. topology, stability) began to be considered. Since the mid-twentieth century, theoretical ecologists posed questions regarding the chaotic dynamics that could arise in communities that involved two or more populations. For example, a long standing question in ecology concerns the relationship between the complexity of a community and its stability, and recent studies on this topic have been motivated by conservational and ecosystem management issues [20]. In the last decades, other studies have developed analytic criteria to evaluate and compare the stability of relatively large competitive, mutualistic and predator-prey communities [21]. Indeed, the use of network models has helped address this and other central questions in ecology (e.g. [22–24]). We, as well as other authors [25], argue that agrocommunities can be modeled with the set of tools developed to study networks and that the quantitative and qualitative data generated by the agronomists and farmers will help in the model building and validation.

Network models consist of nodes that represent the elements conforming a web of interactions, and edges that correspond to such interactions. In directed networks, such interactions can be positive or negative depending on the effect that a node has on another one. These models have been used to study not only the structure but also the collective dynamics of biological systems [26]. In a dynamic network model, any given node can take a quantitative value, which is calculated for every time unit according to a function that considers the state of the nodes regulating each node. In ecological networks nodes often correspond to species (taxonomic or functional), while the edges stand for trophic, competitive, mutualistic, and other types of ecological interactions.

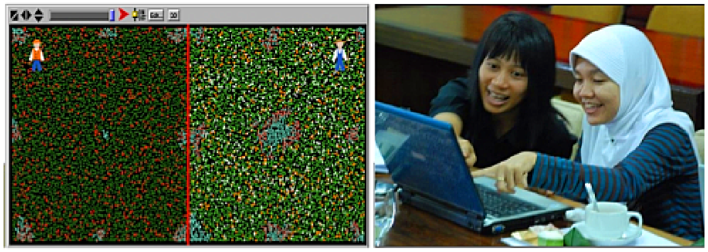
There are several theoretical approaches that have been proposed to model network dynamics, two of which are: the continuous and the discrete. Continuous network models are built as a system of coupled differential equations that quantitatively describe the change of a node state in time. In the context of ecological systems, this description is particularly suitable when the systems under study is small – or can be simplified to consider few nodes – and the interactions among species have been thoroughly characterized (e.g. [4, 32]). However, at the ecological level, tens or even hundreds of species are required for global ecological processes to occur, such as the completion of biogeochemical cycles. In that case, the continuous approach can become intractable due to the great number of variables and unknown parameters involved. The discrete approach to model ecological networks describes in a qualitative way the ecological interactions occurring in a community or ecosystem, and can be used to understand the overall regulatory logic of ecological processes. This approach focuses on the persistence of a population or species, rather than on its abundance. Thus, the state of a given node is represented by a discrete variable that usually takes the values 1 if the species is present and 0 if is not. However, there are also multivalued discrete systems in which the abundance of a species can be approximated by a set of discrete values (0,1,2,...). The use of network models in various fields, such as genetics and development, has shown that discrete models can incorporate a large number of components and interactions, and that in spite of their simplicity, they are able to capture key aspects of biological complexity [33].

When using a network approach for modeling the ecological dynamics of a particular agrocommunity, one aims at representing the taxonomical or functional groups as nodes and their ecological interactions or energy fluxes as directed edges [34, 35]. Several issues emerge from this approach. It is possible to aggregate or collapse some of the components

Azteca Chess : Enacting Trophic Networks & Pest Control at the Coffee Bush Level



Agrodiversity v.2 : Managing Eco-Networks & Functional Biodiversity at the Plot Level



Sierra Springs : Negotiating Eco-Networks & Cooperation Dilemmas at the Watershed Level.

Figure 3: Examples of Educational Resources for Navigating the complexity of Ecological and Socio-Ecological Networks. (1) Azteca Chess [27] captures in a stylized form some of the cascading interactions that occur among a number of insects and fungi species in the Mesoamerican Shade Coffee Agroecosystem. The complexity of this network and its practical implication for autonomous pest control has been unveiled by the seminal work of a large international team of researchers and postgraduate students led by world leaders in agroecology (see for example [28]). Agrodiversity v.2 [29] is an upgrade of our previous Agent Based Model in Netlogo Language that challanges users to find the biological and management parameters with which functional biodiversity can selforganize to produce ecological and economic sustainability far from equilibrium (for details see [30]. (3) Sierra Springs [31] is a four player board game that can be played under a number of different sets of social norms, and that challenges participants to make a livelihood without collapsing critical ecological functions at the watershed level. It exposes participants to coordination, cooperation and solidarity dilemmas that emerge from the interaction of environmental constraints, social norms and public policies.

of the network by considering their ecological role, but there is a risk of oversimplification of the network if partially redundant paths are lost or disregarded, as different types of redundancy has been shown to confer robustness to networks [36]. Also, in order to simplify their study, ecological networks have often been separated into subnetworks including only trophic, mutualistic, or other types of interactions, yet it is central to keep in mind that these networks are part of larger ones and to develop integral models as data become available.

The detailed evidence to build data-based ecological networks and specify the functions that determine their dynamics can be obtained through at least two different approaches. The first one is based on gathering direct evidence for interactions between pairs or groups of species. Such data can be obtained in the field, common garden experiments, or in the laboratory, as well as from scientific reports and databases. Several ecological network models have been successfully built and analyzed following this strategy (e.g. [37–39]). Current high-throughput technology has opened a second avenue to infer interspecific interactions and ecological networks, specially in the case of microbial communities. Data generated by next-generation sequencing – for example, a microbial soil community – provides information about the abundance of the different microbial operational taxonomic units (OTUs) in different experimental conditions. The pattern of co-ocurrence and co-exclusion among these OTUs has been used to predict ecological interactions among species (e.g. mutualism, competition, predation, etc.), which may then be summarized in networks where the nodes correspond to OTUs and the edges to the interactions among them (for an excellent review regarding this type of network inference see [40], see also Figure 2).

Inferring ecological networks by either method allows for structural and sometimes also dynamical analysis of ecological systems, as well as for the identification of the features that are key to their resilience, tolerance and productivity, and the study of their behavior in different scenarios. For instance, structural analyses reveal how connectivity patterns affects the networks overall stability; this helps predict system responses to local extinction or species' introductions. Additionally, specifying the dynamical rules of these networks, be it with a discrete or continuous formalism, helps to identify the nodes and interactions that are sufficient and necessary for global properties such as resilience to emerge [41–43]. Importantly, network approaches to the study of agroecosystems can lead to the development of software and other tools that help farmers and other social actors in the assessment of different scenarios, as well as to attain a better understanding of complex socio-ecological systems. The complexity, relevance, challenges, and beauty of many ecological and socio-ecological processes are not easily grasped by social actors collectively involved in their (mis)management. At El Colegio de la Frontera Sur, Mexico, L.G.B and coworkers have drawn inspiration from the recent and increasing efforts world-wide to model such networks, and have developed a number of original stylized board games and interactive agent-based models that allow participants coming from the most contrasting social backgrounds to experience and understand the non-linear processes and the surprising properties, challenges and opportunities that emerge at different scales in socio-ecological systems. Figure 3 depicts three examples of such network-inspired board games.

We have outlined a general framework that could be used to build integrative and dynamical models of the milpa agroecosystem on the basis of currently available and continuously emerging data. As in other areas of Biology, and in Ecology in particular, such models could help uncover and understand complex processes of different variants of the milpa. In turn, this analysis would provide novel insights about key elements or interactions leading to successful management in this and other agroecosystems. Recent advances in agroecology jointly with complex systems theory can learn from ancestral and more recent practices to suggest model systems of sustainable production and management. We advocate that studying the milpa in this context can represent a feasible alternative strategy to warrant food security and production in Mexico and, at the same time preserve both agrobiodiversity and biodiversity in general.

Acknowledgements

The authors would like to thank Ana E. Escalante for valuable discussions, and the organization for rural development 'Vicente Guerrero' at Tlaxcala, Mexico, for their lessons and for allowing us to learn from their plots. M. Benítez thanks UC-Mexus (CN 13-636) for financial support.

4 Box 1. Glossary

Complex system: a set of entities interacting usually in a non-linear manner, that can become auto-organized and exhibit spatiotemporal heterogeneities or patterns [44].

Diversity: at the community level, it is the quantity of species in relation to their abundances in a location [9].

Emergent property: it is a system's property that can only be observed when its parts are interacting, and cannot be understood only from the study or observation of the isolated parts [44].

Matrix: in the context of Agroecology, it is the set of the different types of fragments from a landscape that characterizes a particular region. Even if organisms usually inhabit within one or a few of these particular fragments, populations are often maintained because of the migration dynamics generated between them. In this way, it is important to define and evaluate the quality of a matrix, as this could affect the migration rates of subpopulations and, consequently, their conservation [15].

Non-linear interaction: given a dynamical system, its dynamic is said to be non-linear if the relationship between its subsequent states is not determined by a proportional quantity that remains constant during time, that is, the rate of change is not constant [45].

Productivity: the biomass quantity per unit area that is produced by the organisms of a particular locality [9].

Redundancy: in information theory, it is the property exhibited by a system when two or more of its components are identical and have the same effects over an output of the system. In biological systems, the identity of the components is almost impossible to satisfy, mostly because of the ambiguity that emerges when distinguishing between their structure and function and also because some components may perform differently in different contexts. So degeneracy, the capability of structurally different components to affect an output in a similar way is a complementary concept that accounts for the evaluation of the redundancy in a system [46].

Resilience: the velocity in which a system returns to an equilibrium state after a perturbation, understanding an equilibrium state as in the context of dynamical systems [20].

Robustness: a type of stability that focuses on the response of certain features when a system is perturbed. In ecology, the notion of robustness is useful when thinking about the maintenance of certain properties of a system in the face of species lost [20].

Stability (local and global): local asymptotic stability is the property of a dynamical system to response to perturbations, applied around a small neighborhood of an equilibrium state, making its effect decay during time in an asymptotic way (i.e. observing long term behaviors). Qualitative global stability, is also a characteristic of the way in which a system responds to perturbations, it is qualitative in the way that its analysis is based just on the signs and not on the magnitudes of the interactions, it is global because there are no constraints about the initial conditions (i.e. no small neighborhood around an equilibrium state is required). This last one has been widely used in the ecological network analysis [20].

Tolerance: capacity of biological systems (genotype, population, community) to buffer the negative effects of natural or human induced disturbances on their functioning and performance [8].

5 Bibliography

[1] S. Allesina and S. Tang, "Stability criteria for complex ecosystems," *Nature*, vol. 483, no. 7388, pp. 205–208, 2012.

[2] R. M. May, "Qualitative stability in model ecosystems," *Ecology*, vol. 54, pp. 638–641, 1973.

[3] C. deWit, "On competition," *Verslagen van Landbouwkundige Onderzoekingen*, vol. 66, 1960.

[4] J. Vandermeer *et al.*, *The ecology of intercropping.* Cambridge University Press, 1989.

[5] F. Isbell, P. B. Reich, D. Tilman, S. E. Hobbie, S. Polasky, and S. Binder, "Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity," *Proceedings of the National Academy of Sciences*, vol. 110, no. 29, pp. 11 911–11 916, 2013.

[6] D. Tilman, P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman, "Diversity and productivity in a long-term grassland experiment," *Science*, vol. 294, no. 5543, pp. 843–845, 2001.

[7] E. L. Simms, "Defining tolerance as a norm of reaction," *Evolutionary Ecology*, vol. 14, no. 4-6, pp. 563–570, 2000.

[8] J. Fornoni, "Ecological and evolutionary implications of plant tolerance to herbivory," *Functional Ecology*, vol. 25, no. 2, pp. 399–407, 2011.

[9] M. Begon, C. R. Townsend, and J. L. Harper, *Ecology: From individuals to ecosystems.* Blackwell Publishing., 2006.

[10] P. Carvalho, S. Thomaz, J. T. Kobayashi, and L. Bini, "Species richness increases the resilience of wetland plant communities in a tropical floodplain," *Austral Ecology*, vol. 38, no. 5, pp. 592–598, 2013.

[11] M. A. Altieri, F. R. Funes-Monzote, and P. Petersen, "Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty," *Agronomy for Sustainable Development*, vol. 32, no. 1, pp. 1–13, 2012.

[12] S. R. Isakson, "No hay ganancia en la milpa: the agrarian question, food sovereignty, and the on-farm conservation of agrobiodiversity in the Guatemalan highlands," *The Journal of Peasant Studies*, vol. 36, no. 4, pp. 725–759, 2009.

[13] M. D. Rausher, "Co-evolution and plant resistance to natural enemies," *Nature*, vol. 411, no. 6839, pp. 857–864, 2001.

[14] A. Moreno-Calles, A. Casas, J. Blancas, I. Torres, O. Masera, J. Caballero, L. Garcia-Barrios, E. Pérez-Negrón, and S. Rangel-Landa, "Agroforestry systems and biodiversity conservation in arid zones: the case of the Tehuacán Valley, Central México," *Agroforestry Systems*, vol. 80, no. 3, pp. 315–331, 2010.

[15] I. Perfecto and J. Vandermeer, "The agroecological matrix as alternative to the land-sparing/agriculture intensification model," *Proceedings of the National Academy of Sciences*, vol. 107, no. 13, pp. 5786–5791, 2010.

[16] L. García-Barrios, Y. M. Galván-Miyoshi, I. A. Valsieso-Pérez, O. R. Masera, G. Bocco, and J. Vandermeer, "Neotropical forest conservation, agricultural intensification, and rural out-migration: the Mexican experience," *Bioscience*, vol. 59, no. 10, pp. 863–873, 2009.

[17] M. A. Altieri and J. Trujillo, "The agroecology of corn production in Tlaxcala, Mexico," *Human Ecology*, vol. 15, no. 2, pp. 189–220, 1987.

[18] H. T. Odum, "Primary production in flowing waters," *Limnol. Oceanogr*, vol. 1, no. 2, pp. 102–117, 1956.

[19] R. L. Lindeman, "The trophic-dynamic aspect of ecology," *Ecology*, vol. 23, no. 4, pp. 399–417, 1942.

[20] M. Pascual and J. A. Dunne, *Ecological networks: linking structure to dynamics in food webs*. Oxford University Press, 2005.

[21] M. Loreau, S. Naeem, P. Inchausti, J. Bengtsson, J. Grime, A. Hector, D. Hooper, M. Huston, D. Raffaelli, B. Schmid *et al.*, "Biodiversity and ecosystem functioning: current knowledge and future challenges," *Science*, vol. 294, no. 5543, pp. 804–808, 2001.

[22] B. Kartascheff, C. Guill, and B. Drossel, "Positive complexity–stability relations in food web models without foraging adaptation," *Journal of Theoretical Biology*, vol. 259, no. 1, pp. 12–23, 2009.

[23] I. D. Rozdilsky and L. Stone, "Complexity can enhance stability in competitive systems," *Ecology Letters*, vol. 4, no. 5, pp. 397–400, 2001.

[24] S. J. Plitzko, B. Drossel, and C. Guill, "Complexity–stability relations in generalized food-web models with realistic parameters," *Journal of theoretical biology*, vol. 306, pp. 7–14, 2012.

[25] P. Tixier, P.-F. Duyck, F.-X. Côte, G. Caron-Lormier, and E. Malézieux, "Food web-based simulation for agroecology," *Agronomy for Sustainable Development*, vol. 33, no. 4, pp. 663–670, 2013.

[26] M. Newman, *Networks: an introduction*. Oxford University Press, 2010.

[27] L. Garcia-Barrios, P. I., and J. Vandermeer, "Azteca chess: an educational board game to explore complex network interactions and autonomous pest control in the shade-coffee agroecosystem," *Unpublished*, 2013.

[28] J. Vandermeer, I. Perfecto, and S. Philpott, "Ecological complexity and pest control in organic coffee production: uncovering an autonomous ecosystem service," *BioScience*, vol. 60, no. 7, pp. 527–537, 2010.

[29] E. Speelman, L. García-Barrios, J. Groot, and P. Tittonell, "Gaming for smallholder participation in the design of more sustainable agricultural landscapes," *Agricultural Systems*, 2013.

[30] E. Speelman and L. García-Barrios, "Agrodiversity v. 2: An educational simulation tool to address some challenges for sustaining functional agrodiversity in agro-ecosystems," *Ecological Modelling*, vol. 221, no. 6, pp. 911–918, 2010.

[31] L. García-Barrios, R. García-Barrios, A. Waterman, and J. Cruz-Morales, "Social dilemmas and individual/group coordination strategies in a complex rural land-use game." *International Journal of the Commons*, vol. 5, no. 2, 2011.

[32] L. García-Barrios, D. Mayer-Foulkes, M. Franco, G. Urquijo-Vásquez, and J. Franco-Pérez, "Development and validation of a spatially explicit individual-based mixed crop growth model," *Bulletin of Mathematical Biology*, vol. 63, no. 3, pp. 507–526, 2001.

[33] E. R. Alvarez-Buylla, M. Benítez, E. B. Dávila, A. Chaos, C. Espinosa-Soto, and P. Padilla-Longoria, "Gene regulatory network models for plant development," *Current Opinion in Plant Biology*, vol. 10, no. 1, pp. 83–91, 2007.

[34] S. E. Jørgensen, *A New Ecology: Systems Perspective*. Elsevier Inc, 2007.

[35] R. Margalef, *Teoría de los sistemas ecológicos*. Universitat de Barcelona, 1991.

[36] M. Benítez and E. R. Alvarez-Buylla, "Dynamic-module redundancy confers robustness to the gene regulatory network involved in hair patterning of *Arabidopsis* epidermis," *Biosystems*, vol. 102, no. 1, pp. 11–15, 2010.

[37] D. M. Evans, M. J. Pocock, and J. Memmott, "The robustness of a network of ecological networks to habitat loss," *Ecology Letters*, vol. 16, no. 7, pp. 844–852, 2013.

[38] E. L. Rezende, J. E. Lavabre, P. R. Guimarães, P. Jordano, and J. Bascompte, "Non-random coextinctions in phylogenetically structured mutualistic networks," *Nature*, vol. 448, no. 7156, pp. 925–928, 2007.

[39] M. Verdú and A. Valiente-Banuet, "The nested assembly of plant facilitation networks prevents species extinctions," *The American Naturalist*, vol. 172, no. 6, pp. 751–760, 2008.

[40] K. Faust and J. Raes, "Microbial interactions: from networks to models," *Nature Reviews Microbiology*, vol. 10, no. 8, pp. 538–550, 2012.

[41] J. Bascompte *et al.*, "Structure and dynamics of ecological networks," *Science*, vol. 329, no. 5993, pp. 765–766, 2010.

[42] J. A. Dunne, R. J. Williams, and N. D. Martinez, "Network structure and biodiversity loss in food webs: robustness increases with connectance," *Ecology Letters*, vol. 5, no. 4, pp. 558–567, 2002.

[43] E. Thébault and C. Fontaine, "Stability of ecological communities and the architecture of mutualistic and trophic networks," *Science*, vol. 329, no. 5993, pp. 853–856, 2010.

[44] M. Mitchell, *Complexity: A guided tour*. Oxford University Press, 2009.

[45] S. Smale, M. W. Hirsch, and R. L. Devaney, *Differential equations, dynamical systems, and an introduction to chaos*. Academic Press, 2003, vol. 60.

[46] G. Tononi, O. Sporns, and G. M. Edelman, "Measures of degeneracy and redundancy in biological networks," *Proceedings of the National Academy of Sciences*, vol. 96, no. 6, pp. 3257–3262, 1999.

Pan-genomics: unmasking hidden gene diversity in bacteria

L.D. Alcaraz, Instituto de Ecología, UNAM, Mexico

1 Abstract

The dramatic increase in genome sequencing during the last years has changed our ideas about bacterial diversity, from single gene to whole community DNA surveys; we have learned that nature's largest gene repository resides in bacteria. Comparison of bacteria genomes has contributed to understand the flexibility in size and gene content as well as the gene movement due to gene family expansions and Horizontal Gene Transfer. Bacteria species are currently defined by means of 16S rRNA sequence comparisons and some limited phenotypic traits. There is an ongoing debate about the biological and evolutive significance of the bacteria species, and thus the need to refine the definition of it using the most of the genomic shared information across any taxonomic range. When comparing multiple genomes of related strains we can distinguish a set of common shared features which are known as the core genome. In the other hand, the set of strain specific genes are known as accessory genome. The accessory and core genome conform the total of the genetic composition, and are known as pan-genome. Here we present the possibilities using pan-genomics as a workhorse to describe both taxonomical and functional diversity within bacteria.

2 Resumen

El abrumador aumento en la cantidad de genomas secuenciados de los últimos años ha cambiado las ideas que teníamos sobre la diversidad en bacterias. Desde las exploraciones de genes hasta la secuenciación del DNA de una comunidad hemos aprendido que la vida en este planeta almacena a sus genes en las bacterias. La genómica comparativa ha permitido entender la flexibilidad en el tamaño y contenido de los genomas bacterianos, expansiones particulares de familias génicas y su movilización mediante Transferencia Horizontal de Genes. En estos momentos, las especies de bacterias se definen comparando

secuencias del gen 16S rRNA, así como de algunas características fenotípicas limitadas. Existe en la actualidad un debate sobre el significado evolutivo y biológico del concepto de especie en bacterias, con lo cual surge la necesidad de evaluar dicho concepto utilizando la mayor cantidad de información genómica compartida posible. Cuando comparamos distintos genomas bacterianos podemos analizar todos los genes compartidos entre todas las cepas analizadas, el genoma núcleo, así como los genes cepa-específicos conocidos como genoma accesorio. El genoma núcleo y accesorio de una especie en su conjunto se conocen como pangenoma. Aquí presentamos la posibilidad de utilizar la pan-genómica como un caballo de batalla para describir, tanto la diversidad taxonómica como la funcional, dentro de las bacterias.

3 Bacterial Pan-genomics

The largest amount of life's gene functions diversity resides in bacteria. This affirmation was possible in the last decade due to rapid development of sequencing technologies, also known as Next Generation Sequencing (NGS) [1]. NGS has aided to describe huge amounts of new species at the genomic level. Despite a bias toward sequencing of human pathogens there are up to 7,411 complete sequenced bacteria genomes up to date. As well as, thousands of Whole Genome Shotgun (WGS) sequencing projects, which when taken into account they gave an approximate of $\geq$ 30,000 ongoing and available genome sequences [2]. Our current knowledge at the genes level could be summarized as genes in this planet are the ones kept by bacteria, and its exceptions (including us within exceptions). Current criteria for naming a bacterium species rely mostly on comparison of 16S rRNA gene (16S) sequences and evaluation of some phenotypic traits like fatty acid profiles, sugar uptake and assimilation, etc. The 16S threshold for delimiting a species is 97% identity of sequence conservation, lower identity values stands for different species. This cut-off value was derived from an old fashion metric when comparing genomes of different species (like *Escherichia coli* and *Salmonella* sp.) and showing a 70% whole DNA-DNA hybridization along phenotypic shared traits, when 16S arose like the gold standard for molecular phylogenetics the equivalent for a 70% DNA-DNA was correlated with a 97% identity at 16S level [3]. There are several criticims to define bacteria species through this arbitrary cut-off criteria and its biological meaning, nonetheless the value of 16S comparisons to determine large scale evolutionary relationships is accepted universally, what is questioned is to rely only in 16S sequence comparisons for defining bacteria species [4] (see Figure 1).

Further complications with the use of 16S as a tool to define species are that current species conceptual frame was intended for sexual organisms, inheriting their genomes in a vertical direction. With bacteria and their promiscuity things go complicated, bacteria have capabilities to perform Horizontal Gene Transfer (HGT) and recombination of genes varies from clonal lineages to highly recombinant even named panmictical (highly

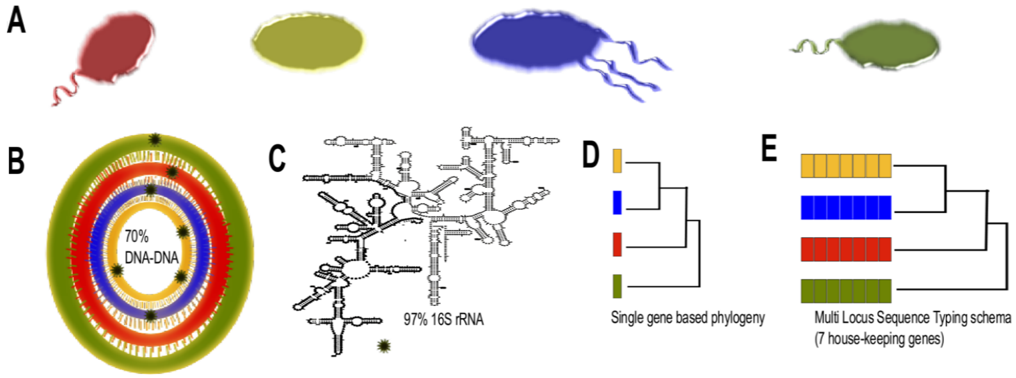


Figure 1: The current systematics of Bacteria. (A) The possible morphological traits evaluated in bacteria are limited, as the range of biochemical tests performed to analyze if two strains are part of the same species. (B) First taxonomic approaches using whole genome comparisons (1970s) rely on DNA-DNA hybridization of different strains, using an arbitrary 70% hybridization cut-off value to define a same species. (C) The use of universally conserved 16S rRNA sequence comparison has a cut-off value of 97% identity when aligned to other sequences, note the secondary structure of the molecule, in bold is shown current average output of NGS sequencing for describing bacteria diversity (∼ 400 bp). The current 97% identity cut-off was intended originally for a whole length 16S (∼ 1600 bp), and it corresponded to the identity for the sequence comparison of two organisms with a whole genomic DNA-DNA hybridization of 70%. The asterisks shown in (B) and (C) denote the location of 16S sequences within the genome, showing that some genomes hosts multiple copies of the very same gene. (D) After PCR amplifying, which happens to be another source of posible biases, and sequencing of the 16S a single gene phylogenetic analysis is performed to define the bacteria species. (E) Current Multi Locus Sequence Typing (MLST) schema uses information of multiple (∼7) coding gene sequences, each homologue gene is aligned and then concatenated to construct a phylogenetic tree based on the evolution of multiple genes which in turn has better resolution to define close related strains, and is used in molecular epidemiology studies to solve the evolutionary emergence of pathogens. Item C created by the SSU-ALIGN package (http://selab.janelia.org/software.html) and structure diagram derived from CRW database (http://www.rna.ccbb.utexas.edu/).

promiscuous) strains [5]. HGT can operate from single genes to whole genomic islands which are a plus in highly selective environments (think about antibiotic resistance mechanisms, etc.). The difficulties come to a dead-end when it comes to define species in bacteria, if the plasticity of gene movement in these organisms is forgotten. We have to cope with Taxonomic Operational Units (OTUs) as our closest proxy to define the bacteria species, more with a need of a working unit rather than following its biological or evolutionary significance. The OTUs are defined as clusters of aligned 16S rRNA sequences having at least 97% identity amongst them [6]. One of the currently finest strategies, without genome sequencing is to define close related bacteria strains by means multiple gene alignments and compare to define close related strains, this is called Multi Locus Sequence Typing and Multi Locus Sequence Analysis (MLST and MLSA, respectively). The rationale behind MLST is to use several ($\sim$7) conserved genes interdispersed in the genomes to avoid the chance of genetic linkage, by amplifying, sequencing, aligning and concatenating the sequences, put them in a single artificial sequence to maximize the amount of genetic information parsed into the substitution model and thus propose a phylogenetic hypothesis that helps to discriminate between close related strains [7].

Adding up complexity layers, we are just recently noticing that we were missing huge amounts of bacteria diversity out there, for some environments we only knew about 1% of the estimated diversity in part because of the difficulties to culture bacteria in Petri dishes [8]. Parallel to the advancement of sequencing technologies the sequencing of environmental DNA and thus the genomes of uncultivated bacteria are being developed, which is known as metagenomics. Metagenomics can be performed on virtually any environment to study both functional and taxonomical diversities [9]. The metagenomes taxonomical diversity is mostly conducted via Polymerase Chain Reaction (PCR) amplification of 16S rRNA libraries of the studied community. Whole Genome Shotgun Metagenomics has also been developed and with this insight we can know about both community taxonomic and metabolic diversity. Metagenomics has been applied to a wide range of environments to know the microbes associated to them, and which is named the microbiome. The studied microbiomes and their environments are diverse and go from acid mine drainages, soils, oil spills, sea water, plants, and animals [9–13].

The vast majority of current microbial diversity studies rely only on 16S rRNA amplicon sequencing and comparison. At the end of the study one ends up with a large dataset of OTUs and bunches of multivariate analysis. The final goal is trying to correlate, and in the best cases associate one particular trait (i.e. disease, pollution resistance, etc.) with a particular set of OTUs. One major source of error for these studies has to do with the sampling, which usually lacks direct replicas and studies across time; this is getting better due to the reducing costs of mass sequencing. Additional experiment complications with 16S involves variable copy number across different genomes [14] biased PCR due to the primer design template [15], varying sequence lengths result of the current technologies (100 - 1,000 base pairs) and using the same threshold as if the sequence was full length 16S, etc.

The sequencing technologies are getting cheaper and increasingly accurate, facts that had allowed analysis of whole genomic variation within the very same bacterium species. The pan-genome concept arose when comparing *Streptococcus agalactiae* strains who accomplished all the current taxonomical and clinical criteria to be part of the very same species, producing the same symptomatic illness and hosting the very same 16S rRNA sequences [16]. But, when comparing the genome sequences of the *S. agalactiae* isolated from different patients against the reference genome it was totally unexpected to find out that each strain shared about ±20% of the genes. In bacteria genomes, there is a high density of coding genes with small intergenic spaces and a lower amount of repetitive DNA when comparing with eukaryotes. So, in bacteria, differences in genome size correlate directly with coding sequences, the larger the bacterial genome, the more functions it can potentially perform with the genes coded in the genome. *S. agalactiae*'s variation in conservancy of genes are huge, when comparing different eukaryote species, for instance divergence in shared coding sequences across different species like chimpanzees and humans does not go further than 1.23% [17]. Thinking about the differences of ±20% in a single bacterium, supossed to be the very same species and finding this difference within the same species is astonishing. As stated above, the sum of the shared and strain unique genes across all the compared genomes is called pan-genome, which in turn can be divided in core genome and accessory genome. In some cases, like the *S. agalactiae*, there is a predicted chance to get new genes for each new sequenced strain, this is called an open pan-genome. For other groups the dynamic is different and there are not new genes predicted for new sequenced strains like in the case of *Bacillus cereus*; this is called a closed pan-genome [16, 18, 19].

Core genome phylogenetic analysis are the next level of the MLST schema, comparing whole shared genome information coded in the genomes of interest strains. Core genome phylogenomics is done by comparing all the shared (orthologous) genes amongst all the compared species, then aligning each one of the genes and concatenating the alignments to build a supermatrix, which in turns feeds a phylogenetic reconstruction; this approach is known as core genome phylogenomics [20] (see Figure 2). The usual parameters of classic molecular evolution, like nucleotide diversity and synonymous/non-synonymous ratios, could be inferred from the core genome alignments. The core genomes could be defined at varying taxonomical depths and could be used to analyze shared gene features from species, genus, family, order, class, and phylum. The upper level of taxonomical resolution (*i.e.* phylum), has the fewer shared genes expected and the lower taxonomical hierarchy (*i.e.* species) is expected to have the larger amount of shared genes. The extra bonus of getting core genomes is that we are able to build molecular functions profiles with the conserved genes across a taxonomic range and find out gene functions responsible for the group cohesion. For example, core genome analysis can aid to find for the expected genes for a enterobacteria like *E. coli* or a sporulating genus like *Bacillus* [19, 21]. Core genome analysis can be helpful to analyze particular phenotypic features like the core genome for any shared trait (i.e. sporulation, heat resistance, antibiotic degradation, etc.) when comparing the shared genes conservancy profile for multiple species dealing with the same environmental challenges [19].

The in-depth study of the core genome sheds light over relevant evolutionary questions, like what are the conserved genes across a taxonomic range, its cut-off similarity values and what are the functional gene constrains of this conservancy. Based on the central dogma of molecular biology one would expect that genes coding for the core machinery of replication (DNA), transcription (RNA) and translation (proteins) would be universally conserved as well as some other house-keeping genes. Phylogenetic reconstructions relying in the whole genome are maybe closer to explain the organism evolutionary history, rather than individual gene genealogies. Important applications also arise, such as the development of strain specific vaccines based on the knowledge of the variation within the conserved genes of a species [22].

The presence and absence patterns of the accessory genome observed throughout a set of bacteria being compared could be the result of gene loss or gene acquisition through Horizontal Gene Transfer (Figure 2). The importance of gene acquisition/loss ratio is yet to be investigated but there are some examples like the one examining the Achaea *Sulfolobus ilsandicus* and the importance of analyzing its pan-genome to determine strain and even location specific genes and their dynamics [23]. The building of the pan-genome is helpful to have a full inventory of the metabolic capabilities of a given group of organisms. Differences in the unique genes of close related bacteria could be a partial answer of local adaptation to particular life styles or niches (i.e. free-living, host-associate, virulence, etc.).

The main goal of the 16S amplicon studies is to have a diversity inventory of a particular environment and try to associate OTUs with particular functions, using the 16S OTU as a proxy for the metabolic diversity. Predictions trying to connect a particular OTU with metabolic functions, for example a gut environment is dominated by say *E. coli* related OTUs, then taking a single reference genome of a single strain would be an naive guess if it fails to consider that we can have up to ± 30% in presence-absence of coding genes, and that particular genes in the accessory genomes are the probable environment restricted genes. However, several attempts are being performed now trying to use the large amount of 16S massive sequencing from virtually any environment and infer metabolic diversity and link it to phylogenetic distances [24, 25]. Another approach in trying to gain insights into the metabolic diversity, when only having 16S sequences, could be to develop pan-genomes catalogues for all the known groups of bacteria with sequenced genomes, generating confidence intervals based on gene presence-absence within particular bacteria.

The 16S gene databases are among the most prolific ones. The understanding of what we know actually about bacteria diversity is mostly in debt with 16S sequence analysis. For sure 16S analysis has been useful and will continue that way when studying unknown environments and when the goal is to have a first glimpse about the complexity of the community structure. For pathogens, diagnosis and management 16S analysis was out-

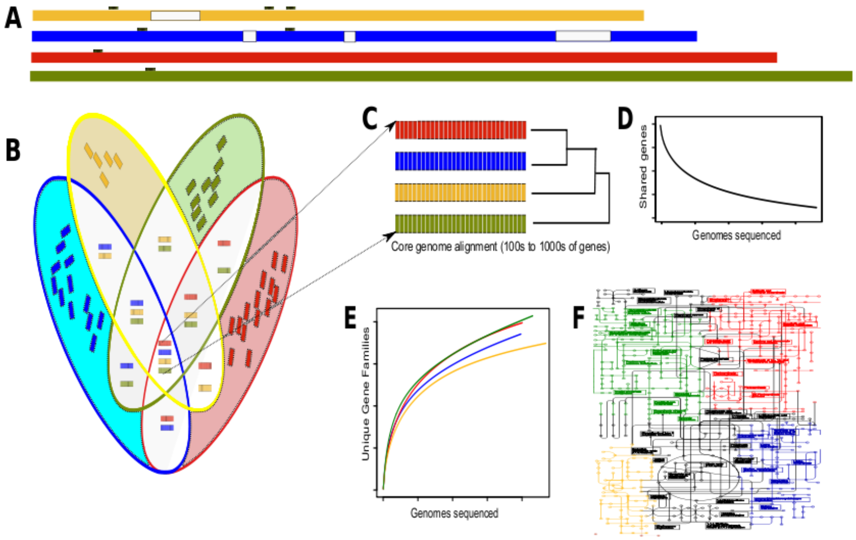


Figure 2: Taxonomical and functional pan-genomics uses. (A) Whole genome alignments make possible to find out gene and operon order conservation across analyzed strains. Each line represents a linearized genome. Blank boxes represents genes not present in a strain compared with its relatives, asterisks represent the 16S variation in copy number and location. (B) Venn diagram representation of the different shared orthologous genes, for four genomes. The intersection, the sub-set of ortholgous genes shared by all analyzed bacteria represents the so called core genome. The sum of core genome and the strain specific genes, which are also called accesory genome, compose the pan-genome. Accesory genome's genes give hints about environment-specific adaptations and functional constrains. (C) Individual core genome's genes could be used to build individual alignments, which in turn are concatenated to build a supermatrix and then perform thorough phylogenetic analysis with all the shared information across a taxonomic range, this increases the resolution of phylogenetic analysis. Core genome's phylogenetic analysis power resides in the fact that whole gene set responsible for the taxonomic range analyzed is taken into account. (D) The core genome's tends to diminish when more genomes are sequenced, due to the amount of shared genes across all the individuals in the analysis tend to decrease with larger samples. (E) The pan-genomes could be plotted as a collector's curve which shows the amount of new genes added to the pan-genome with each new sequenced bacterium added to the analysis. Pan-genomes are told to be open if there is new gene appeareance when adding new strains to the analysis. Closed pan-genomes reffer to the lack of new genes within a taxonimic range when new individuals are added to the analysis and the collector's curve has reached a plateau. (F) Both core genome and pan-genome are prone to be functional described. Then it is easier to pin-out responsible genes for environmental responses (i.e. pathogenesis, symbiosis, nutrient deprivation etc.) as well as predict metabolic profiles from their sequences. Item F, original metabolic path modified from http://www.genome.jp/kegg/

dated some time ago and the need to develop rapid and accurate methods for resolving close relative type strains derived into the MLST analysis. With the current pace of sequencing technologies development is urgent to redefine the minimum standards when defining bacteria diversity. The new bacteria diversity standards are likely to require core and pan-genomics analysis to define the bacteria taxa, as well as understanding local dynamics for pan-genomics at each taxonomic unit.

The beauty of not knowing a precise way to describe a species should not be taken as a pitfall for microbiology. With bacteria, we are dealing with the main repository of genes and biological functions that have allowed microbes to be the major players in our world, from biogeochemical cycles, energy harvesting and cycling and thus making life for all the other being forms possible. The species concept, developed for when you are well behaved and transmit your genes in vertical form seems a little rigid when dealing with bacteria and their tremendous capabilities of transforming and sharing genes in a happy and promiscuous way. We just need to refine our vision and take into account the internal variability for genes and thus functions of each related bacteria, and praise it, develop new indexes (like Genome Similarity Score [19]) that take into account the whole set of shared features when comparing bacteria, along sides phylogenetic traditional ways. If we are aware of the current utilitarian bacterium species concept and that we understand that some of the major traits of a bacterium, like pathogenesis, are likely to occur in close related working units, call them species or OTUs, we can cope with that. But being unaware of the huge functional diversity connected to what we already call a bacterium species is nonsense nowadays.

Acknowledgements

The author is grateful to an anonymous reviewer for the insight and comments to the manuscript. This work has been supported by the Instituto de Ecología, UNAM.

4 Bibliography

[1] M. L. Metzker, “Sequencing technologies — the next generation,” *Nature Reviews Genetics*, vol. 11, no. 1, pp. 31–46, Dec. 2009.

[2] I. Pagani, K. Liolios, J. Jansson, I.-M. A. Chen, T. Smirnova, B. Nosrat, V. M. Markowitz, and N. C. Kyrpides, “The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata.” *Nucleic Acids Research*, vol. 40, no. Database issue, pp. D571–D579, Jan. 2012.

[3] J. Goris, K. T. Konstantinidis, J. a. Klappenbach, T. Coenye, P. Vandamme, and J. M. Tiedje, “DNA-DNA hybridization values and their relationship to whole-genome sequence similarities.” *International Journal of Systematic and Evolutionary Microbiology*, vol. 57, no. Pt 1, pp. 81–91, Jan. 2007.

[4] C. Fraser, E. J. Alm, M. F. Polz, B. G. Spratt, and W. P. Hanage, "The bacterial species challenge: making sense of genetic and ecological diversity," *Science*, vol. 323, no. 5915, pp. 741–746, 2009.

[5] J. M. Smith, N. H. Smith, M. O. Rourke, B. G. Spratt, and M. O'Rourke, "How Clonal are Bacteria?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 10, pp. 4384–4388, 1993.

[6] K. T. Konstantinidis and J. M. Tiedje, "Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead," *Current Opinion in Microbiology*, vol. 10, no. 5, pp. 504–509, 2007.

[7] D. Gevers, F. M. Cohan, J. G. Lawrence, B. G. Spratt, T. Coenye, E. J. Feil, E. Stackebrandt, Y. V. Peer, P. Vandamme, F. L. Thompson, and J. Swings, "Re-evaluating prokaryotic species," *Microbiology*, vol. 3, no. September, pp. 733–739, 2005.

[8] S. R. Vartoukian, R. M. Palmer, and W. G. Wade, "Strategies for culture of 'unculturable' bacteria." *FEMS Microbiology Letters*, vol. 309, no. 1, pp. 1–7, Aug. 2010.

[9] D. S. Lundberg, S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. D. Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl, "Defining the core *Arabidopsis thaliana* root microbiome," *Nature*, vol. 488, no. 7409, pp. 86–90, Aug. 2012.

[10] P. Belda-Ferre, L. D. Alcaraz, R. Cabrera-Rubio, H. Romero, A. Simón-Soro, M. Pignatelli, and A. Mira, "The oral metagenome in health and disease," *The ISME Journal*, vol. 1, no. 11, pp. 46–56, Jun. 2011.

[11] D. B. Rusch, A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, Falc, L. I. N, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier, and J. C. Venter, "The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific," *PLoS Biology*, vol. 5, no. 3, pp. 399–431, 2007.

[12] R. Mendes, M. Kruijt, I. de Bruijn, E. Dekkers, M. van der Voort, J. H. M. Schneider, Y. M. Piceno, T. Z. DeSantis, G. L. Andersen, P. a. H. M. Bakker, and J. M. Raaijmakers, "Deciphering the rhizosphere microbiome for disease-suppressive bacteria." *Science (New York, N.Y.)*, vol. 332, no. 6033, pp. 1097–100, May 2011.

[13] M. J. Beazley, R. J. Martinez, S. Rajan, J. Powell, Y. M. Piceno, L. M. Tom, G. L. Andersen, T. C. Hazen, J. D. Van Nostrand, J. Zhou, B. Mortazavi, and P. a. Sobecky, “Microbial community analysis of a coastal salt marsh affected by the Deepwater Horizon oil spill.” *PloS one*, vol. 7, no. 7, p. e41305, Jan. 2012.

[14] J. a. Klappenbach, P. R. Saxman, J. R. Cole, and T. M. Schmidt, “rrndb: the Ribosomal RNA Operon Copy Number Database.” *Nucleic Acids Research*, vol. 29, no. 1, pp. 181–4, Jan. 2001.

[15] A. Engelbrektson, V. Kunin, K. C. Wrighton, N. Zvenigorodsky, F. Chen, H. Ochman, and P. Hugenholtz, “Experimental factors affecting PCR-based estimates of microbial species richness and evenness.” *The ISME journal*, vol. 4, no. 5, pp. 642–647, May 2010.

[16] H. Tettelin, V. Masignani, M. J. Cieslewicz, C. Donati, D. Medini, N. L. Ward, S. V. Angiuoli, J. Crabtree, A. L. Jones, A. S. Durkin, R. T. DeBoy, T. M. Davidsen, M. Mora, M. Scarselli, I. Margarit y Ros, J. D. Peterson, C. R. Hauser, J. P. Sundaram, W. C. Nelson, R. Madupu, L. M. Brinkac, R. J. Dodson, M. J. Rosovitz, S. A. Sullivan, S. C. Daugherty, D. H. Haft, J. Selengut, M. L. Gwinn, L. Zhou, N. Zafar, H. Khouri, D. Radune, G. Dimitrov, K. Watkins, K. J. O’connor, S. Smith, T. R. Utterback, O. White, C. E. Rubens, G. Grandi, L. C. Madoff, D. L. Kasper, J. L. Telford, M. R. Wessels, R. Rappuoli, and C. M. Fraser, “Genome analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial ”pan-genome”,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 39, pp. 13 950–13 955, 2005.

[17] The Chimpanzee Sequencing and Analysis Consortium, “Initial sequence of the chimpanzee genome and comparison with the human genome.” *Nature*, vol. 437, no. 7055, pp. 69–87, Sep. 2005.

[18] A. Mira and A. Martín-Cuadrado, “The bacterial pan-genome: a new paradigm in microbiology,” *International Microbiology*, vol. 10, pp. 45–57, 2010.

[19] L. Alcaraz, G. Moreno-Hagelsieb, L. E. Eguiarte, V. Souza, L. Herrera-Estrella, and G. Olmedo, “Understanding the evolutionary relationships and major traits of Bacillus through comparative genomics,” *BMC Genomics*, vol. 11, no. 1, p. 332, 2010.

[20] L. Alcaraz, V. López-Ramirez, A. Moreno-Letelier, L. Herrera-Estrella, V. Souza, and G. Olmedo-Alvarez, “Genomics of bacteria from an ancient marine origin: Clues to survival in an oligotrophic environment,” in *Earth and Environmental Sciences*, 1st ed., I. A. Dar and A. D. Mithas, Eds. Croatia: Intech, 2011, pp. 609–630.

[21] G. E. Sims and S.-H. Kim, “Whole-genome phylogeny of *Escherichia coli/Shigella* group by feature frequency profiles (FFPs).” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 20, pp. 8329–8334, May 2011.

[22] F. Luciani, R. Bull, and A. R. Lloyd, "Next generation deep sequencing and vaccine design: today and tomorrow." *Trends in Biotechnology*, vol. 30, no. 9, pp. 443–452, Jun. 2012.

[23] M. Reno and N. Held, "Biogeography of the *Sulfolobus islandicus* pan-genome," *Proceedings of the National Academy of Sciencies of the United States of America*, vol. 106, no. 21, 2009.

[24] M. G. I. Langille, J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. a. Reyes, J. C. Clemente, D. E. Burkepile, R. L. Vega Thurber, R. Knight, R. G. Beiko, and C. Huttenhower, "Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences." *Nature Biotechnology*, vol. 31, no. 9, pp. 814–21, Sep. 2013.

[25] S. Okuda, Y. Tsuchiya, C. Kiriyama, M. Itoh, and H. Morisaki, "Virtual metagenome reconstruction from 16S rRNA gene sequences," *Nature Communications*, vol. 3, p. 1203, Nov. 2012.

The coming of age of microbial ecology

A.E. Escalante & S. Pajares, IE UNAM, Mexico and IEE, University of Oregon, USA

1 Abstract

Research in microbial ecology is challenging due to its inherent complexity and, at the same time, is extremely important, because the relationships between microorganisms and their environments have a crucial role in the health of the planet and all of its inhabitants. Despite major challenges, new technological advances in genomic sciences have prompted microbial ecology into a revolution in data generation that has allowed us to move beyond studies of single isolates to the study of entire microbial communities without reliance on culture-dependent methods. Besides the data generation using these molecular tools, the adequate use of a theoretical framework is needed to test specific hypothesis and develop mechanistic models of microbial diversity. The development of a theory-based and hypothesis-driven research, along with a complex systems analytical approach, will result in appropriate models required to predict and possibly control the effect of environmental impacts on microbial diversity.

2 Resumen

La investigación en ecología microbiana es un gran reto debido a la complejidad inherente de su estudio y es, al mismo tiempo, extremadamente importante porque la relación entre los microorganismos y sus ambientes tiene un papel crucial en la salud del planeta y de todos sus habitantes. A pesar de los grandes retos, nuevos avances tecnológicos en las ciencias genómicas han impulsado a la ecología microbiana hacia una revolución en cuanto a la generación de datos, que han permitido movernos de estudios de cepas únicas, aisladas en cultivo, hacia el estudio de comunidades microbianas completas y sin necesidad de su aislamiento en cultivo. Además de la generación de datos con herramientas moleculares, es necesario el uso adecuado de un marco teórico que permita probar hipótesis específicas y desarrollar modelos mecanísticos sobre la diversidad mi-

crobiana. El desarrollo de investigaciones enmarcadas en la teoría y que pongan a prueba hipótesis, junto con aproximaciones analíticas de sistemas complejos, resultará en modelos apropiados necesarios para predecir y posiblemente controlar el efecto de los impactos ambientales en la diversidad microbiana.

3 Introduction

Microorganisms play a primary role in a vast array of ecosystems, from soils to hydrothermal vents to the human body, in which microbes drive key processes, as the heart of ecosystem function and the keystone of global health. Despite the clear ecological importance of microbes, understanding of underlying mechanisms of microbial behavior, community assembly and functional consequences of diversity shifts is still limited.

The emergence of microbial ecology as an independent field of investigation in the 1960s was promoted by both the increasing public interest in environmental issues and the recognition of the essential role of microbes in the biosphere. Microbial ecology links those areas in which microbiologists are well trained (biochemistry, microbiology, molecular biology) with ecology or the study of the interactions between living organisms and their biotic and abiotic environment. Partly due to this dichotomy of disciplinary training (microbiologists vs ecologists), the field of microbial ecology has suffered a delay in its development as a scientific discipline, but other factors have contributed as well. In this essay we will review three main avenues that have delayed the advance of microbial ecology as a mechanistic and predictive discipline: (i) practical impediments related to observation and isolation of microorganisms from natural environments, (ii) problematic definition of species, and (iii) limited use of a theoretical framework. Nonetheless, in the recent years these challenges have been overcome or at least addressed, allowing microbial ecology to enter a new phase in which, beyond conducting important natural history descriptions, clear and well-defined hypothesis are now being posed and investigated.

4 Overcoming difficulties in the study of microbial communities

It has been estimated that the Earth hosts $> 10^{30}$ microbial cells [1], a figure that exceeds the number of stars in the universe by nine orders of magnitude [2]. Besides their great abundance, microorganisms are also immensely diverse and constitute about 60% of the Earth's biomass [1]. But this diversity is literally unseen under the naked eye, and even if seen, the morphological diversity of microorganisms is unrepresentative of their phylogenetic, metabolic and functional diversity.

As a consequence of these limitations, and until not so long ago, the only way to study and characterize microorganisms and their functions was through culture and isolation in selective or enrichment media in the laboratory. However, this approach introduces important biases, as well as recovers a very small fraction of the naturally occurring microorganisms in any given environment, and loses the ecological context of the organisms under study [3]. Thus, the pure culture paradigm has not only limited what microbiologists have studied, it has also limited how they have thought about microbes.

In recent years, breakthrough advances in molecular biology and bioinformatics have permitted access to the uncultured microbial diversity of any given environment through the development of culture-independent methods (Figure 1), resulting in a vast literature on shotgun metagenomics [4, 5] and SSU rRNA gene-based sequencing surveys [6–8]. Furthermore, with the dramatic decline in cost and increase in output, high- throughput sequencing technologies, such as 454 pyrosequencing and Illumina platforms, have changed the scale of microbial ecological studies and have made deep metagenomic sequencing much more affordable, and have enabled robust statistically replicated designs even for highly diverse communities [2, 9, 10]. However, some limitations remain a challenge, such as intrinsic sequencing errors, PCR primers biases, overestimation of taxon abundance or distinguishing metagenomic signatures of uncultured taxa from computational artifacts.

The analysis of microbial communities through genomic approaches, either with targeted genes (e.g. 16S rRNA as taxonomic standard) or by sequencing all the genomic content of a sample, has allowed a gene-based exploration of complex evolutionary processes and ecological interactions in microbial communities [11–13]. Furthermore, these technologies have revolutionized our understanding of the microbial diversity in our bodies [14, 15] and on our planet [16–18]. Specifically, culture independent approaches have permitted to unveil the existence of biogeographical patterns in microbes [19–21], which was a matter of great discussion during a good part of the last decade. The success of these first discoveries of the spatial distribution of microbial diversity prompted, in part, the evolution of culture independent approaches from giving data of only gene sequences to providing with both information on the taxonomic composition (metagenomes) as well as description of the metabolic routes (metabolomes) and functional capabilities (transcriptomes and proteomes) of a given community without losing the ecological context. Moreover, these new technical advances are opening the door to sophisticated studies for testing the functional responses of microbial communities to environmental stresses [22].

Nonetheless, the promise of genomic studies as a way to build a mechanistic knowledge that links microbial diversity with ecosystem function is challenging, given the extent of microbial diversity and the uncontrolled character of natural environments that complicate the ecological interpretation of genomic data [23]. A way to manage this challenge is to follow the example of classical ecological studies, where systematic experiments have been pursued under natural or laboratory controlled conditions, aimed to test specific hypotheses and simplify interpretations [24]. Despite the fact that examples of experimental approaches with microbes are still few, they are promising [25, 26], as microbial ecologists are aware of the need for a robust experimental design (e.g. replication, ecosystem characterization, controlled manipulations) across temporal and spatial scales

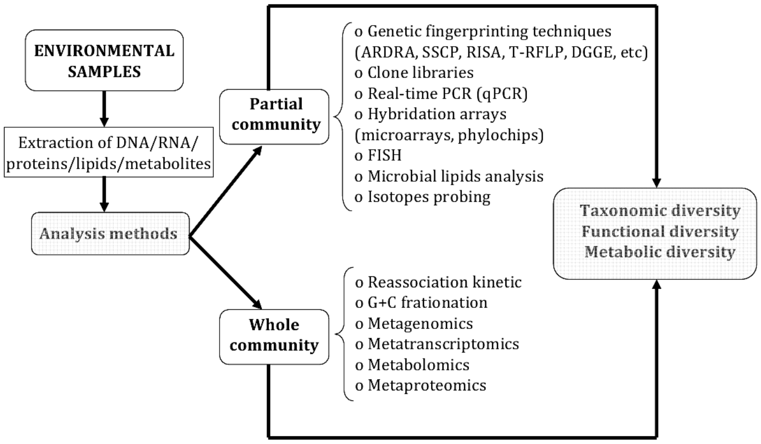


Figure 1: Culture-independent molecular methods to characterize microbial community diversity in the environment.

[2]. Moreover, given the great metabolic plasticity, functional redundancy and adaptive potential of microorganisms [12, 22, 26], integrative multidisciplinary approaches including genomics, ecological and evolutionary theory will help develop stronger conclusions and better predictions in microbial ecology.

5 Problematic definition of species

Species is the fundamental unit of biological classification and is critical for describing, understanding and comparing biological diversities at different levels among ecological niches. However, what constitute a species is still controversial, especially in microbiology. This is because most ecological theory heavily relies on Mayr´s biological concept of species [27] or groups with barriers to recombination. It is thus clear that microorganisms, particularly prokaryotes, with asexual reproduction do not fit to the biological species definition and make conceptually difficult the analysis of "species" diversity within the ecological theory.

Discussion around this conundrum has provided some insightful ideas and concepts that try to reconcile microorganisms with the species concept. One of these ideas is the alternative ecological species concept, which defines species as populations that are genetically cohesive and ecologically distinct. Frederick Cohan [28] has argued that this concept is appropriate for bacteria and has named bacterial ecological species as "ecotypes". The postulates of this proposal include that bacteria occupy discrete niches and that periodic selection purges genetic variation within each niche without preventing divergence between members of the different niches. Thus, genetically and ecologically distinct species can arise (given little recombination or clonality), permitting the application of the ecological theory of populations, communities or macroecology to prokaryotes.

Nonetheless, lack of recombination is an important assumption of prokaryotes that might not be absolute, given the overwhelming evidence of horizontal gene transfer in all prokaryotic phyla studied to date. A consequence of gene transfer is that prokaryotic genomes are thought to be the sum of the core genome and the accessory genome, called pan-genome [29][1]. The core genome includes all genes that encode essential metabolic housekeeping functions and can be regarded as the biological species, which maintains coherence of species in the face of homologous recombination. The accessory genome encodes especial ecological adaptations in genes that can be readily interchanged. Microbes that belong to the same species, as defined by the core genome, can differ substantially in the accessory genome, and thus have different ecological capabilities or be different ecotypes, which makes it incongruent with the ecological species concept.

Thus, when reconciling species concepts, we face a pragmatic problem: how do we define the unit of study in microbial ecology? The current solution has opted for looking at genes of the core genome (e.g. 16S rRNA gene) that are indicators of what we understand as a coherent evolutionary unit that is robust in the face of homologous recombination. These evolutionary units, called Operational Taxonomic Units (OTUs), have been a practical solution that, however, is far from being a solution to the conceptual problem. Consequently, much research is required to form a coherent body of theory that relates the fluid nature of prokaryotic genomes to the ecology of their populations and communities [30].

6 Use of a theoretical framework

Without a theoretical framework, knowledge can be merely seen as a collection of observations and anecdotes with very little potential for generalizations and little predictive power. Theory helps understanding through the construction of a mechanistic knowledge of the world. If we do not understand how something works, it is impossible to predict its behavior or intervene to recover from perturbations.

The comprehension of the principles that govern the communities' dynamics is a central goal of the general science of ecology [30]. Ecology has its origins in natural history, and early publications tended to be very descriptive and site-specific [31]. Although many questions remain unanswered, modern ecology has progressed enormously from an almost anecdotal discipline to a very sophisticated model-based and hypothesis-driven science [31]. However, this theory has been developed mainly from plant and animal studies. On the other hand, traditionally, microorganisms have been considered "different" from macroscopic organisms, which implies that different rules, even different theories apply to them, thus limiting microbiology to separate and disconnected laboratory and ecology studies. Some of the arguments in favor of considering microbes different are their

---

[1] see also Alcaraz's Chapter in this book.

huge abundance, total biomass and diversity, their high rate of dispersal and global activities [30]. Moreover, the particular biology of microorganisms regarding sex and genetic isolation has limited the incorporation of a theoretical framework in microbial ecology research. These scenarios pose the challenge of investigating the applicability of ecological theory developed from macroorganisms to microbes.

In the course of the development of ecological theory, microbial model systems have played and important role in the evolution of more rigorous theory, which is also relevant for all types of organisms [25]. Despite the intrinsic value of bringing theory into the study of microbial ecology and the great potential of theoretical enrichment through controlled experiments using microbial systems, there has been a delay in the development of theory in microbial ecology. One of the most important reasons for this is strongly related with the challenges described above (technical difficulties in studying microbes from natural environments and conflicting species concepts).

In recent years, however, microbial ecologists have begun applying macro-ecological theory into experimental design of hypothesis-driven studies, providing a reference point for the development of new theories. Specifically, works conducted at the community scale by Martiny and collaborators [22, 32] and by Fierer and collaborators [26, 33] are paradigmatic in the use and construction of ecological theory with microbial systems. Particularly, questions regarding the functional role of such vast microbial diversity and the drivers of dispersion and spatial distribution of microorganisms have guided their most recent investigations [7, 16, 34]. In line with these works, Bohannan and collaborators have also applied ecological theory in the study of spatial patterns in microbial diversity [35] and ecological dynamics of human microbiome [36]. These studies have provided important glimpses into the underlying mechanisms that structure ecological communities.

7 The coming of age, towards a predictive science

The development of bioinformatics and molecular genomics tools has replaced methodological barriers with conceptual barriers in the progress of microbial ecology. To date, microbial ecologists recognize a lack of unifying theories and concepts that can serve analysing and interpreting the vast amount of information and scattered results derived from metagenomic approaches [34, 37, 38]. Thus, despite many fundamental questions that remain to be answered (see Box 1), current avenues of research in microbial ecology are aimed in the direction to fill this conceptual gap.

The revolution in sequencing and advanced computational tools offers affordable access to functional and taxonomical inventories of microbial communities in any given space and time. On the other hand, despite unresolved debates on microbial species definition, practicality has prevailed by using 16S rDNA sequences as a proxy for microbial taxonomic diversity [17, 19]. However, recent studies in microbial ecology have been conducted to go beyond taxonomic or functional descriptions, using massive inventory information (i.e. metagenomics, metatranscriptomics, metabolomics) within a complex ecological and evolutionary framework to unveil mechanistic aspects of the microbial world through predictive models [11, 39].

Box 1. Challenges for microbial ecologists.

- The large fraction of microbial life that remains uncultivated.
- Accurate modification of natural microbial communities for study in the laboratory.
- Predicting the effects of disturbance in natural microbial communities.
- Unravelling the functions of unknown microbial genes.
- The importance of microbial community assembly in ecosystems.
- Spatial scale of microbial community functions and interactions.
- Co-evolution in interacting microbial species.
- Study of unexplored genetic and physiological diversity of the rare biosphere.
- Testing ecological theory in microbial model systems.
- Applying the principles of population biology and evolution to the study of microbial communities.

The transition towards a predictive science is not of minor importance, as it must consider the impact that microbial community modelling can have in almost every biological realm, from disease to ecosystem management. Examples of current avenues of research that are filling the technological-conceptual gap in microbial ecology come from studies of human and soil microbiomes [14, 40]. In both, we find notable efforts to develop general theories and models that aim to understand the relationship between community structure and ecosystem function in order to predict microbiome behaviour and its consequences [13, 34, 41].

Microbial ecologists are also conducting research around a key debate regarding the relevance of microbial taxonomic composition in the functioning of ecosystems. In other words, is it important to know who is there to predict what they will do? [22, 38]. To address this question, different approaches are being followed, from field samplings to laboratory experiments, most using new technologies available for sequencing, but more importantly with a theoretical ground for the study of microbial diversity patterns (see “use of a theoretical framework”). However, the full potential of massive sequencing technologies in the study of ecosystem functions may come also from other study approaches. Ecosystem functions (e.g. mineralization rates in soils) or system properties, such as resilience and robustness, may not be information readily accessible from inventory or multivariate analyses. Thus other approaches, such as network analyses, promise to advance our understanding of microbial systems properties and dynamics.

Recently, microbial ecology studies have started to incorporate analytical tools derived from complexity theory, among which network analysis coupled with dynamic modelling offer great promise in the search for a more predictive science [13, 34, 39, 42]. The reason, we believe, is because the relationship between microbial diversity and ecosystem functioning may lie greatly in the complexity of interactions that exists within any microbial consortia or community [43], as well as with abiotic parameters. The consequences of these interactions are nearly impossible to account for with standard multivariate analyses. Network theory approaches coupled with dynamic modeling of the interactions may aid in both experimental design, to test specific hypothesis, and visualizing consequences in scenarios where the structure of the network is altered.

Given current scenarios of environmental change and the major influence that microorganisms play in biogeochemical cycles, it is not only scientifically important to transcend a descriptive discipline towards a theory-based predictive science. Understanding and predicting microbial diversity responses facing environmental challenges is of major relevance because of the ecosystem consequences that microbial communities perturbations may have. Today, challenges are in the theoretical and analytical approaches to be taken and in the collective efforts of the scientific community to gather information and make it publicly available for its analysis. The massive data collection and analysis within a solid and systems-based theoretical framework in the study of microbial diversity will allow us to develop predictive models for improved strategies of study and concrete actions in ecosystem restoration and management, as well as in public health policies.

8 Acknowledgements

Ana E. Escalante acknowledges financial support from Universidad Nacional Autónoma de México [UNAM-PAPIIT grant IA200814]

9 Bibliography

[1] W. B. Whitman, D. C. Coleman, and W. J. Wiebe, "Prokaryotes: the unseen majority," *Proceedings of the National Academy of Sciences*, vol. 95, no. 12, pp. 6578–6583, 1998.

[2] R. Knight, J. Jansson, D. Field, N. Fierer, N. Desai, J. A. Fuhrman, P. Hugenholtz, D. van der Lelie, F. Meyer, R. Stevens *et al.*, "Unlocking the potential of metagenomics through replicated experimental design," *Nature Biotechnology*, vol. 30, no. 6, pp. 513–520, 2012.

[3] E. J. Stewart, "Growing unculturable bacteria," *Journal of Bacteriology*, vol. 194, no. 16, pp. 4151–4160, 2012.

[4] J. C. Venter, K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson *et al.*, "Environmental genome shotgun sequencing of the sargasso sea," *Science*, vol. 304, no. 5667, pp. 66–74, 2004.

[5] S. R. Gill, M. Pop, R. T. DeBoy, P. B. Eckburg, P. J. Turnbaugh, B. S. Samuel, J. I. Gordon, D. A. Relman, C. M. Fraser-Liggett, and K. E. Nelson, "Metagenomic analysis of the human distal gut microbiome," *science*, vol. 312, no. 5778, pp. 1355–1359, 2006.

[6] M. D. Lage, H. E. Reed, C. Weihe, C. M. Crain, and J. B. Martiny, "Nitrogen and phosphorus enrichment alter the composition of ammonia-oxidizing bacteria in salt marsh sediments," *The ISME journal*, vol. 4, no. 7, pp. 933–944, 2010.

[7] H. E. Reed and J. B. Martiny, "Microbial composition affects the functioning of estuarine sediments," *The ISME journal*, vol. 7, no. 4, pp. 868–879, 2013.

[8] J. G. Caporaso, C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight, "Global patterns of 16s rrna diversity at a depth of millions of sequences per sample," *Proceedings of the National Academy of Sciences*, vol. 108, no. Supplement 1, pp. 4516–4522, 2011.

[9] J. I. Prosser, "Replicate or lie," *Environmental Microbiology*, vol. 12, no. 7, pp. 1806–1810, 2010.

[10] J. Zhou, L. Wu, Y. Deng, X. Zhi, Y.-H. Jiang, Q. Tu, J. Xie, J. D. Van Nostrand, Z. He, and Y. Yang, "Reproducibility and quantitation of amplicon sequencing-based detection," *The ISME journal*, vol. 5, no. 8, pp. 1303–1313, 2011.

[11] A. Barberán, S. T. Bates, E. O. Casamayor, and N. Fierer, "Using network analysis to explore co-occurrence patterns in soil microbial communities," *The ISME journal*, vol. 6, no. 2, pp. 343–351, 2012.

[12] J. Comte, L. Fauteux, and P. A. del Giorgio, "Links between metabolic plasticity and functional redundancy in freshwater bacterioplankton communities," *Frontiers in Microbiology*, vol. 4, 2013.

[13] R. Levy and E. Borenstein, "Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules," *Proceedings of the National Academy of Sciences*, vol. 110, no. 31, pp. 12 804–12 809, 2013.

[14] H. M. P. Consortium *et al.*, "Structure, function and diversity of the healthy human microbiome," *Nature*, vol. 486, no. 7402, pp. 207–214, 2012.

[15] E. K. Costello, C. L. Lauber, M. Hamady, N. Fierer, J. I. Gordon, and R. Knight, "Bacterial community variation in human body habitats across space and time," *Science*, vol. 326, no. 5960, pp. 1694–1697, 2009.

[16] J. B. Martiny, J. A. Eisen, K. Penn, S. D. Allison, and M. C. Horner-Devine, "Drivers of bacterial β-diversity depend on spatial scale," *Proceedings of the National Academy of Sciences*, vol. 108, no. 19, pp. 7850–7854, 2011.

[17] M. L. Sogin, H. G. Morrison, J. A. Huber, D. M. Welch, S. M. Huse, P. R. Neal, J. M. Arrieta, and G. J. Herndl, "Microbial diversity in the deep sea and the underexplored "rare biosphere"," *Proceedings of the National Academy of Sciences*, vol. 103, no. 32, pp. 12 115–12 120, 2006.

[18] C. L. Lauber, M. Hamady, R. Knight, and N. Fierer, "Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale," *Applied and Environmental Microbiology*, vol. 75, no. 15, pp. 5111–5120, 2009.

[19] N. Fierer and R. B. Jackson, "The diversity and biogeography of soil bacterial communities," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 3, pp. 626–631, 2006.

[20] J. B. H. Martiny, B. J. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske *et al.*, "Microbial biogeography: putting microorganisms on the map," *Nature Reviews Microbiology*, vol. 4, no. 2, pp. 102–112, 2006.

[21] A. M. Noguez, H. T. Arita, A. E. Escalante, L. J. Forney, F. García-Oliva, and V. Souza, "Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest," *Global Ecology and Biogeography*, vol. 14, no. 3, pp. 241–248, 2005.

[22] S. D. Allison and J. B. Martiny, "Resistance, resilience, and redundancy in microbial communities," *Proceedings of the National Academy of Sciences*, vol. 105, no. Supplement 1, pp. 11 512–11 519, 2008.

[23] J. A. Fuhrman, "Microbial community structure and its functional implications," *Nature*, vol. 459, no. 7244, pp. 193–199, 2009.

[24] T. Bell, M. O. Gessner, R. I. Griffiths, J. R. McLaren, P. J. Morin, M. van der Heijden, and W. van der Putten, "Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild," in *Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective. Oxford University Press, Oxford, United Kingdom*, 2009, pp. 121–133.

[25] C. M. Jessup, R. Kassen, S. E. Forde, B. Kerr, A. Buckling, P. B. Rainey, and B. J. Bohannan, "Big questions, small worlds: microbial model systems in ecology," *Trends in Ecology & Evolution*, vol. 19, no. 4, pp. 189–197, 2004.

[26] M. S. Strickland, C. Lauber, N. Fierer, and M. A. Bradford, "Testing the functional significance of microbial community composition," *Ecology*, vol. 90, no. 2, pp. 441–451, 2009.

[27] E. Mayr, Ed., *The species problem*, no. 50. The American Association for the Advancement of Science, Atlanta meeting, 1957.

[28] F. M. Cohan, "What are bacterial species?" *Annual Reviews in Microbiology*, vol. 56, no. 1, pp. 457–487, 2002.

[29] J. P. W. Young, L. C. Crossman, A. W. Johnston, N. R. Thomson, Z. F. Ghazoui, K. H. Hull, M. Wexler, A. R. Curson, J. D. Todd, P. S. Poole *et al.*, "The genome of rhizobium leguminosarum has recognizable core and accessory components," *Genome Biology*, vol. 7, no. 4, p. R34, 2006.

[30] J. I. Prosser, B. J. Bohannan, T. P. Curtis, R. J. Ellis, M. K. Firestone, R. P. Freckleton, J. L. Green, L. E. Green, K. Killham, J. J. Lennon *et al.*, "The role of ecological theory in microbial ecology," *Nature Reviews Microbiology*, vol. 5, no. 5, pp. 384–392, 2007.

[31] W. J. Sutherland, R. P. Freckleton, H. C. J. Godfray, S. R. Beissinger, T. Benton, D. D. Cameron, Y. Carmel, D. A. Coomes, T. Coulson, M. C. Emmerson *et al.*, "Identification of 100 fundamental ecological questions," *Journal of Ecology*, vol. 101, no. 1, pp. 58–67, 2013.

[32] H. E. Reed and J. B. Martiny, "Testing the functional significance of microbial composition in natural communities," *FEMS Microbiology Ecology*, vol. 62, no. 2, pp. 161–170, 2007.

[33] N. Fierer and J. T. Lennon, "The generation and maintenance of diversity in microbial communities," *American Journal of Botany*, vol. 98, no. 3, pp. 439–448, 2011.

[34] M. A. Bradford and N. Fierer, "The biogeography of microbial communities and ecosystem processes: implications for soil and ecosystem models," *Soil Ecology and Ecosystem Services*, pp. 189–200, 2012.

[35] J. Green and B. J. Bohannan, "Spatial scaling of microbial biodiversity," *Trends in Ecology & Evolution*, vol. 21, no. 9, pp. 501–507, 2006.

[36] E. K. Costello, K. Stagaman, L. Dethlefsen, B. J. Bohannan, and D. A. Relman, "The application of ecological theory toward an understanding of the human microbiome," *Science*, vol. 336, no. 6086, pp. 1255–1262, 2012.

[37] N. Fierer, M. A. Bradford, and R. B. Jackson, "Toward an ecological classification of soil bacteria," *Ecology*, vol. 88, no. 6, pp. 1354–1364, 2007.

[38] D. R. Nemergut, S. K. Schmidt, T. Fukami, S. P. O'Neill, T. M. Bilinski, L. F. Stanish, J. E. Knelman, J. L. Darcy, R. C. Lynch, P. Wickey *et al.*, "Patterns and processes of microbial community assembly," *Microbiology and Molecular Biology Reviews*, vol. 77, no. 3, pp. 342–356, 2013.

[39] K. Faust and J. Raes, "Microbial interactions: from networks to models," *Nature Reviews Microbiology*, vol. 10, no. 8, pp. 538–550, 2012.

[40] P. J. Jansson J, "Microbiology: the life beneath your feet," *Nature*, vol. 494, pp. 40–41, 2013.

[41] P. E. Larsen, D. Field, and J. A. Gilbert, "Predicting bacterial community assemblages using an artificial neural network approach," *Nature methods*, vol. 9, no. 6, pp. 621–625, 2012.

[42] A. Shade, H. Peter, S. D. Allison, D. L. Baho, M. Berga, H. Bürgmann, D. H. Huber, S. Langenheder, J. T. Lennon, J. B. Martiny *et al.*, "Fundamentals of microbial community resistance and resilience," *Frontiers in Microbiology*, vol. 3, 2012.

[43] A. E. Little, C. J. Robinson, S. B. Peterson, K. F. Raffa, and J. Handelsman, "Rules of engagement: Interspecies interactions that regulate microbial communities," *Annual Review of Microbiology*, vol. 62, pp. 375–401, 2008.

The complexity of cancer ecosystems

J.E. Keymer & P.A. Marquet, IEB and PUC de Chile

1 Abstract

The tumorigenic process shares many similarities with the evolution of ecosystems. Different processes promote heterogeneity in the population of neoplastic cells, this in turn is manifested in differential rates of proliferation and the emergence of selection, whereby tumor cells with the highest survival and proliferative advantage are selected for in the face of environmental filters. This model of clonal selection, is of wide acceptance and represents core knowledge regarding cancer progression and tumor evolution. However, according to it, tumor evolution is associated to a series of clonal expansion, linked to driver mutations that confer fitness gains such that one clone competitively exclude less fit ones. The end result of this process will be the eventual domination by one clone (clonal homogenization). However, heterogeneity is the rule. The issue we address in this contribution is what prevents clonal homogenization and what is the impact of this upon metastatic progression. We do that by developing two separate ecological models to understand neoplastic progression and invasion of secondary organs (metastasis) respectively. In particular, we propose that after its initial appearance, populations of malignant cells can further fine-tune their local fitness by internal Darwinian selection creating new malignant strategies which are more efficient at exploiting the growth opportunities within the local tissue. This initiates an evolutionary progression of clone replacements. After a period of such microscopic directional evolution, the local ecology of the tissue undergoes a transition into a neutral ecology. Such ecology then generates malignant clones with a range of proliferation strategies (neoplastic biodiversity) which then venture into the circulatory system reaching out secondary organs. Subsequently, at a secondary organ, the malignant cell remain in a latent state until opportunities for invasion show up due to the disappearance of resident normal cell linages that prevented their invasion. A process akin to invasion in metacommunities.

2 Resumen

El proceso de crecimiento tumoral comparte muchas similitudes con la evolución dentro de ecosistemas. Distintos procesos promueven la emergencia de heterogeneidad en la población de células neoplasicas, esto a su vez se manifiesta en tasas diferenciales de proliferación celular y la emergencia de selección, tal que las células tumorales con la mayor sobrevivencia y ventaja proliferativa son seleccionadas. Este modelo de selección clonal es ampliamente aceptado. Sin embargo, de acuerdo a él, la evolución del tumor se asociaría a una serie de expansiones clonales asociadas a mutaciones tipo ”driver” que confieren ganancias en adecuación tal que un clon excluiría a los menos competitivos. Este proceso terminaría con la eventual dominación por un único clon (homogeneización clonal). Sin embargo la heterogeneidad es la regla. El problema que nos ocupa en este trabajo dice relación con entender qué es lo que previene la homogeneización clonal y cúal es el impacto de este proceso sobre la progresión metastática. Para hacer esto desarrollamos dos modelos para entender la progresión neoplásica y la invasión de otros órganos secundarios (metástasis) respectivamente. En lo particular, proponemos que las poblaciones de células malignas, en el órgano primario, atraviesan por un proceso de selección clonal que genera clones con potencial de crecimiento e invasión cada vez mayor y que este proceso termina con una ecología neutral. Los clones que caracterizan este ecosistema neutral poseen un rango de estrategias proliferativas (biodiversidad neoplásica) algunos de los cuales se dispersan y llegan a otros órganos. Una vez en el órgano secundario, las células malignas esperan en un estado de latencia, la emergencia de una oportunidad que les permita invadir, lo que asociamos a la desaparición de ciertos linajes que previenen la invasión. Un proceso similar a la invasión en metacomunidades.

3 Introduction

Complexity science is increasingly gaining importance in biomedicine [1] as a result of the realization that the human body, as any other living system, is inherently complex and that to fix its malfunction requires an interdisciplinary approach. This trend is particularly apparent in cancer research, where new perspectives coming from fields such as physics [2–5], ecology [6–9] and evolution [10–13] are becoming popular to deal with the challenges that the complexity of cancer poses. The complexity of neoplastic disease progression is manifested somehow in the hallmarks of cancer [14]; six biological capabilities acquired during the multistep development of tumors (sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis) that, as we will discuss more in detail, manifest in the emergence of a complex cellular ecosystem. The existence of these hallmarks, however, does not imply that all cancers are equal, for another quintessential characteristic of cancer is heterogeneity. This is manifested in changes in the identity of genes that drive the development of tumorigenesis across different cancers, in the diversity of clones that coexist within tumors and through out cancer progression within each cancer type, and in the range of potential non-exclusive processes that underlie this diversity, including genomic instabilities, drift, selection, stochasticity in gene expression, and non-genetic causes [15–18]. Further, understanding the functional roles associated to this diversity, which is sustained and sustains a complex web of intracellular and extracellular networks known as the ”tumor ecosystem” [19], may be a key to harness cancer progression and its robustness [20].

There is limited knowledge on the structure of the tumor ecosystem and the kind of interactions that different neoplastic clones can sustain, both among themselves and with recruited normal cells [21, 22]. Available evidence suggests, however, that at least competition, commensalism and cooperation are important [3, 7, 23]. Similarly, the dispersal of cells from the primary tumor during metastatic progression also represents a research challenge. We know that those migrant cells that can survive in the circulation and adapt to the new environment of a distant organ are the ones that will prevail and proliferate but it looks like describing metastasis as a simple one-way migration of cells from the primary tumor to the target organ may not do justice to the complexity of the phenomenon and may miss important mechanisms that can be therapeutic targets [24]. Thus to achieve understanding of the complexity of tumor ecosystems and the suite of adaptive strategies that cancerous cells can exhibit in different host environments is of paramount importance for todays cancer research [25] and may be key for the development of effective therapeutic interventions.

The tumorigenic process shares many similarities with the evolution of ecosystems; there are factors within tumors and in the surrounding healthy tissue that promote the emergence of heterogeneity in the population of neoplastic cells, this in turn is manifested in differential rates of proliferation and the emergence of selection, whereby tumor cells with the highest survival and proliferative advantage are selected for in the face of environmental filters, which could be a therapeutic treatment or associated with the process of cancer progression itself, such as hypoxia that occurs as a consequence of growing further and further apart from servicing blood vessels [26, 27]. This model of clonal selection, first proposed by Peter Nowell in a seminal contribution [17], has become a well established core knowledge of cancer progression and tumor evolution. However, according to it, tumor evolution is associated to a series of clonal expansion, linked to driver mutations that confer fitness gains such that one clone competitively exclude less fit ones much alike periodic selection in stressed bacteria [28]. The end result of this process will be the eventual domination by one clone (clonal homogenization). However, heterogeneity is the rule [21]. The issue then is what prevents clonal homogenization and what is the impact of this upon metastatic progression. In a recent review [18] has pointed out that tumor homogenization could be constrained by driver mutations having a small fitness effects, by spatial variability and by microenvironmental variability, which may tend to equalize fitness and promote coexistence of clones.

In this chapter we develop two separate ecological models to understand neoplastic progression and invasion of secondary organs (metastasis) respectively. We aim at generating a simple mathematical framework that will increase our understanding of the linkages between tumor progression and subsequent metastasis. To achieve this we strongly argue for an ecology of cancer ecosystems bringing together ecological approaches to oncology. In particular, we propose that within the tissue located in the primary organ, after its initial appearance, populations of malignant cells can further fine-tune their local fitness by internal Darwinian selection [13, 16, 17] creating new malignant strategies which are more efficient at exploiting the growth opportunities within the local tissue. This initiates an evolutionary progression of clone replacements. After a period of such microscopic directional evolution, the local ecology of the tissue undergoes a transition into a neutral ecology [29]. Such ecology then generates malignant clones with a range of proliferation strategies (neoplastic biodiversity) which then venture into the circulatory system reaching out secondary organs. Subsequently, at a secondary organ, a transition in tissue status from resistant to permissive ecologies could characterize the latent *versus* metastatic transition.

Before delving into our model for the emergence of clonal selection and diversity in tumor ecosystems we need to make some consideration regarding our view of multicellular organisms and in particular, to introduce a conceptual view of organisms as cellular ecosystems.

4 Metazoa as coherent multicellular ecosystems

Life cycles span a continuum of cellular ecosystems exhibiting multiple levels of integration, cooperation, degrees of physical attachment, as well as other adaptations for viscous selective assortment. Along this continuum we find several life history strategies ranging from: quorum sensing bacteria like *Vibrio harveyi* that exhibit high degree of functional coherence; loose aggregates of cells with little degree of differentiation, coordination and integration such as the case of *Trichoplax ahaerens*; or like *Dictyostelium discoideum*, which behaves as a population of single-celled amebas as well as a multicellular slug and fruiting body. Higher order metazoa are highly integrated and aggregated life cycles that lie at the complex end of the continuum of integration levels and therefore posses highly structured regulatory systems. These regulatory structures exist at multiple scales within the individual host and have been structured by thousands of years of evolution. Such macroevolutionary process has produced a large degree of modularity, with bodies being organized into organs and organs into tissues and where numerous cell types are continuously being produced and destroyed allowing for the dynamic emergence of a multicellular individual. The evolutionary trajectory of metazoa, however, endowed each cell with a hidden repertoire of modular ancient regulatory structures. This repertoire or toolkit corresponds to pre-existing adaptations, of an earlier layer of genes that controlled loose-knit colonies of only partially differentiated cells, similar to tumors, and characteristic of proto-metazoan or transitional forms between unicellular and multicellular organisms. These hidden modules (*i.e.* Metazoa 1.0s *toolkits*) can overrun the current modes of operation upon environmental insult [3]. Metazoa organisms are monopolies of niche construction [30] on which cells transiently generate an autopoietic machine [31] operating far from equilibrium [32]. In homage to Davis and Lineweaver [3], we label this machine as if running a "Metazoa 2.0" as its *operating system*.

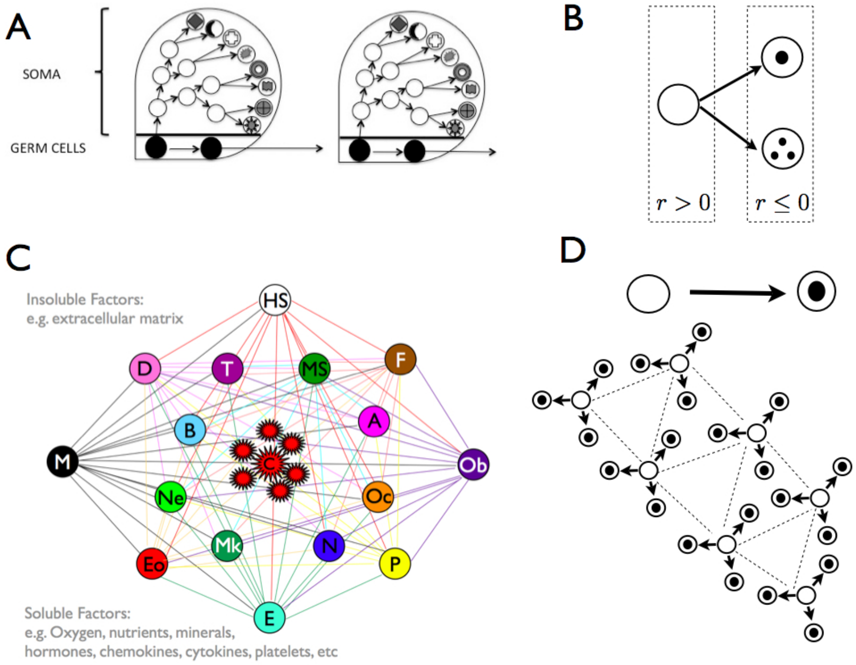


Figure 1: Metazoa as cellular ecosystems and the organ's tissues as a lattice. (A) At different time scales tissues are generated from somatic stem cell lines (white circles). Filled circles represent the germ line, and white circles filled with symbols represent several terminal somatic cell lines. Reproduced from [33]; (B) Stem cell populations are capable of longterm proliferation and persistence while local populations of differentiated cells represent sink populations, whose persistence depends upon the continuous recruitment from stem cell differentiation. (C) A network of ecological interactions. Adapted from [8]; (D) Stem cell (white circles) differentiate into specific somatic cell type populations (patterned white circle), they do it in specific locations that are the basic units of tissue physiology. We represent such spatial landscape as the organ lattice $\mathcal{L} = \mathcal{L}_{\mathrm{SC}} \cup \mathcal{L}_{\mathrm{Diff}}$: a network of stem cell niches (white circles, sub-lattice $\mathcal{L}_{\mathrm{SC}}$) connected by dispersal (dashed edges) to other stem niches and by differentiation-migration (black arrows) to specific somatic niches (patterned white circles, sub-lattice $\mathcal{L}_{\mathrm{Diff}}$).

Organ's tissues as cellular networks building networks of patches

Coherent supra cellular structures such as tissues and organs from metazoa form a body (see Figure 1A) which is for the reproduction of the germinal line through a monopoly of niche construction [30] enforced by control systems which are nested and modular. At temporal scales larger than the lifespan of the host, only the germinal line has reproductive potential while bodies are transient structures. As ecosystems, bodies are also modular systems where proliferation, differentiation, cell migration, and cell attachment are highly structured by a combination of global and local control factors. Persistent somatic stem cell lines (open white circles in Fig. 1A,B) supply all differentiated cell types (patterned white circles in Fig. 1A,B) by differentiation, migration (black headed arrow) and recruitment.

Inspired by the work of Pienta and collaborators [8], we describe an organ (such as the bone marrow for example) as an ecological community of different cells types interacting in a complex network embedded together within a landscape of extracellular components. In Figure 1C we depict such scenario as an interaction graph. In this graph, each node (colored circles) represent a cell of a given cell type (species) such as: hematopoietic stem cells (HS), mesenchymal stem cells (MS), endothelial cells (E), pericytes (P), fibroblasts (F), macrophages (M), T lymphocytes (T), B lymphocytes (B), dendritic cells (D), and other cell types interacting in several manners (colored edges) while co-constructing the organ (bone marrow) in a coherent fashion. Each of these types in the network, has cell populations in precisely regulated anatomic locations around stem cell micro environments known as stem cell niches (SCN, [34]) forming a landscape ecology determined by the histology of the tissue. Local stem cell populations inhabit such locations and from these stem cell micro-patches, differentiated cells migrate to replenish nearby locations hosting sink-populations of terminally differentiated cells. In Figure 1B we represent such differentiation and dispersal process where a persistent ($r > 0$) stem cell population at a given location, supply with new cells a nearby sink population ($r \leq 0$) of differentiated cells. Such landscape can be abstracted as a lattice $\mathcal{L}$ consisting of discrete locations (Figure 1D), referred here as ecological micro-scale patches, which have the potential to host local populations of a given cell type. This *lattice organ* is composed of a sub-lattice $\mathcal{L}_{\text{SC}}$ of SCNs and a sub-lattice $\mathcal{L}_{\text{Diff}}$ of patches with the potential of hosting local populations of terminally differentiated cells.

5 Neoplastic progression and the adaptive phases of cancer

The control of proliferation within microscopic patches

Tissue architecture is represented here by a lattice of local patches of opportunity (local niches) for clonal expansion at given anatomical locations $x \in \mathcal{L}$. We can think of the dynamics of cell densities within a single patch. The local environment is limited from the *top-down* by physical factors such as insoluble factors patterning the extra-cellular matrix and pressure from the nearby tissue. Thus, the patch has its maximum carrying capacity (in terms of local cell density of a given type). A patch is also regulated *bottom-up* by the host by providing local soluble factors which act as nutrients. We imagine a local population of cells with density $\phi \in [0, 1]$ following density dependent growth,

$$\frac{1}{1-\phi}\frac{1}{\phi}\frac{d}{dt}\phi = r(\vec{s}, \vec{\omega}) \tag{1}$$

and where $r(\vec{s}, \vec{\omega})$ is the local per-capita per-niche population growth rate. The growth rate depends on the cell proliferation strategy $\vec{s} = (\beta, \delta)$ and the control field from the host $\vec{\omega} = (\omega_+, \omega_-)$ operating in the local tissue. Thus we define,

$$r(\vec{s}, \vec{\omega}) \equiv \vec{s} \cdot \vec{\omega} = \beta\omega_+ + \delta\omega_- \tag{2}$$

to represent local growth (here $\omega_+ \in [0, 1]$ and $\omega_- \in [-1, 0]$). Notice that growth is regulated by both, a cell's strategy $\vec{s}$ as well as by the location dependent factor $\vec{\omega}$ representing the host's tissue renewal processes operating as complex spatial fields.

For simplicity let's imagine a location $x \in \mathcal{L}_{\text{Diff}}$ within some terminally differentiated somatic tissue such that $\omega_+ = \omega$ $(0 \leq \omega \leq 1)$ and $\omega_- = -1$. In such location x, the rate of cell death is δ and it sets up the lifespan δ^{-1} for a cell of a specific somatic type. Host's homeostasis and self regulation regenerates levels of habitat quality ω in the location at a net rate $F = \lambda(1-\omega)$, where λ is the overall rate at which the components of habitat quality (i.e. nutrients, oxygen) are provided,and working against a local habitat degradation rate $C = \epsilon\phi\omega\beta$ (where ϵ takes care of units). With such representation of a cell population and its local ecology of habitat renewal we get,

$$\begin{aligned} \frac{d}{dt}\phi &= (\beta\omega - \delta)\phi(1-\phi) & (3) \\ \frac{d}{dt}\omega &= \lambda(1-\omega) - \epsilon\phi\omega\beta & (4) \end{aligned}$$

which corresponds to the system studied by Keymer and collaborators [29, 35]. For terminally differentiated cell populations, proliferation rates $(\beta\omega)$ have to be small compared to apoptotic processes (δ) such that long-term persistence is not possible in the system described by eqs. 3-4. Regeneration of such local terminally differentiated populations is only by differentiation and migration from a near-by SCNs. In this manner, terminally differentiated cell populations are controlled so they cannot persist in the long-run. Thus, all somatic terminally differentiated populations are represented by the "extinction" solution $(\hat{\omega}_0, \hat{\phi}_0) = (1, 0)$ of equations 3-4 which corresponds to the "normal" phase of healthy tissue.

Normalizing the timescale by the scale of the maximum cell proliferation rate (β^{-1}), we can represent a cell proliferation strategy $\vec{s}$ by the scalar $\omega^* \equiv \delta/\beta$ which corresponds to a dimensionless niche utilization parameter space (ecological aspect space). With these rescaled parameters, the system is,

$$
\begin{aligned}
\frac{d}{d\tau}\phi &= (\omega - \omega^*)\phi(1-\phi) \qquad (5)\\
\frac{d}{d\tau}\omega &= \frac{\lambda}{\beta}(1-\omega) - \epsilon\phi\omega. \qquad (6)
\end{aligned}
$$

The emergence of Neoplastic progression

Here, the *extinction* solution $(\hat{\omega}_0, \hat{\phi}_0) = (1, 0)$ of equations 5-6 corresponding to the healthy state is stable for proliferation strategies satisfying $\omega^* \geq 1$ (see left panel in Figure 2). We denote these collection of healthy strategies $\Omega_0 = \{\omega^* : \omega^* \geq 1\}$. However, due to genomic instabilities one cell can mutate and change its strategy to a new one in the strategy space defined $\Omega_{\neq 0} = \{\omega^* : 0 \leq \omega^* < 1\}$ leading to the stability of the *bottom up* $(\hat{\omega}_{1/2}, \hat{\phi}_{1/2})$ and *top-down* $(\hat{\omega}_1, \hat{\phi}_1)$ solutions of equations 5-6 (see [29]. If this happens we have the emergence of neoplastic progression, which starts with a cell changing its healthy strategy $\omega^*_r \in \Omega_0$ into a malignant one $\omega^*_\mu \in \Omega_{\neq 0}$. Genetic instability, environmental insults as well as genetic predisposition and non-genetic factors [18] can trigger such change but the origin of the first malignant cell population is not at focus here. What it is, is what follows once this mutant malignant clone has appeared within a tissue.

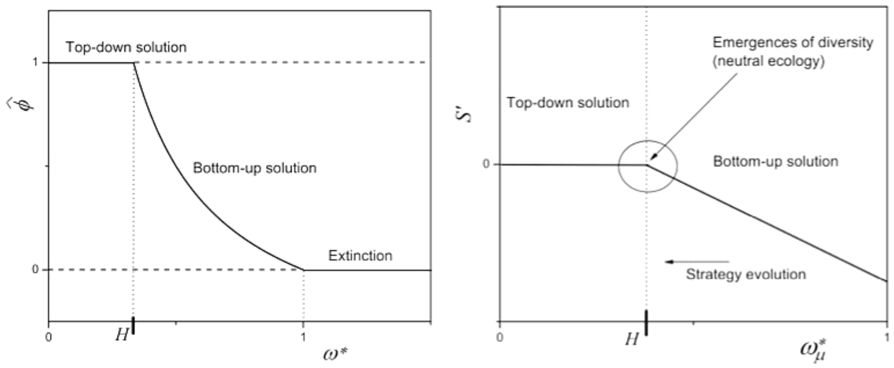


Figure 2: Solutions and fitness landscape for the adaptive dynamics of eqs. 5-6. Left is the stable solution $\hat{\phi}$ as a function of parameter ω^*. Right, is the fitness gradient S' for the range of cell proliferation strategy parameter. The value H identify the transition between the periodic selection and the neutral regime. Adapted from [29].

The aftermath of cell's disobedience

The neoplastic progression starts when a somatic cell stops listening to the social contract ruling its host and instead opts for the non-trivial solutions ($\hat{\phi} > 0$) of equations 5-6. Once a malignant cell line exists, it starts evolving its strategy ω^* by internal darwinian mechanism outlined by [17]. Here there is directional selection towards more efficient types with more aggressive growth rates and smaller values of ω^* than their ancestor linages. Ecological replacements ensure low biodiversity of clones exhibiting unregulated growth rates and causing a persistent accumulation of biomass (pressure) which create the tissue anomalies characteristic of hyperplasia. The topology of the fitness landscape characterizing the adaptive dynamics [29], induces a succession of evolutionary replacements $\omega^*_r \to \omega^*_\mu$ driven by driver mutations that confer fitness advantages, increasing the growth rate or fitness of the mutant in the environmental condition set by the resident. This is manifested in increasingly larger invasion exponents calculated as

$$S \equiv S_{\omega^*_r}(\omega^*_\mu) \equiv \frac{1}{\phi_r}\frac{d}{d\tau}\phi_\mu = (\omega^*_r - \omega^*_\mu)(1 - \hat{\phi}_r) \qquad (7)$$

that dictate that a malignant clone will take over the patch from the healthy resident and the fate of that mutant clone once a new one arises. At each replacement event $\omega^*_r \to \omega^*_\mu$, the mutant becomes a resident and due to its smaller value $\omega^* \in \Omega_{\neq 0}$ it will establish itself. As shown in the right panel of Figure 2, we can see that the fitness gradient S' rules the adaptive dynamics so that that every new mutant who's strategy is to the left (smaller in value) of the resident clone will invade the patch. Under this regime, malignant clones evolve towards smaller values of ω^* thus becoming incresingly aggresive in terms of growth advantage.

The development of heterogeneous tumors

The serial replacement of clones proceeds until a critical value $H = \lambda/(\lambda + \epsilon\beta)$ is reached; a point where the ecology of the tissue transitions into a neutral regime, that is the fitness landscape becomes flat. At this critical value of a cell strategy $\omega^* = H$, an heterogenous neoplasia begins to develop as the local ecology saturates and biodiversity emerges [29] and thus the tumor ecosystem begins to accumulates biodiversity and developing heterogeneity. The emergence of invasive neoplasia, corresponds to the emergence of neutrality in the local ecology of the tissue. At this point in neoplastic progression the malignant population has a strategy $\omega^* \leq H$ and therefore has no competitive advantage towards any other mutant on the left of H (with small enough ω^*). Here multiple clones coexists in a neutral ecology consisting of a diverse cellular metacommunity of cell proliferation strategies $\omega^*_{r_1}, \ldots, \omega^*_{r_N}$.

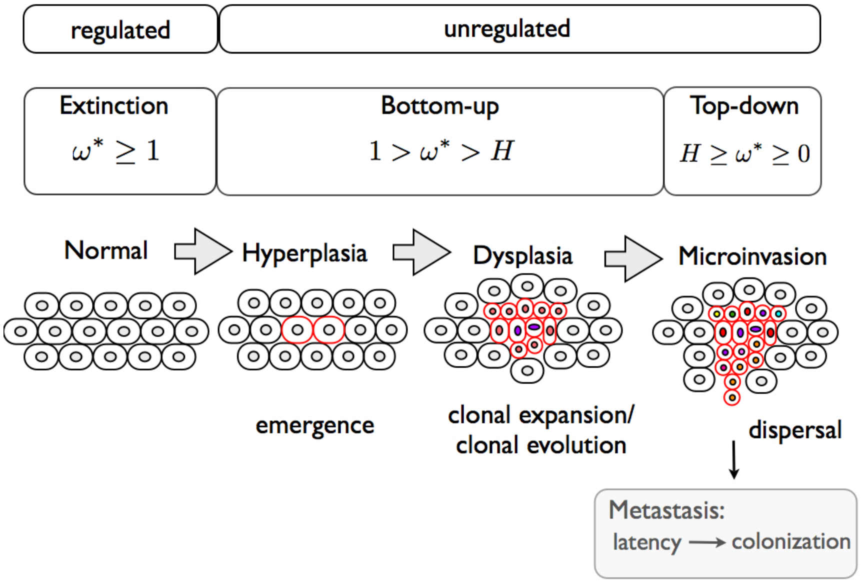


Figure 3: The progression of Neoplasia and the emergence of biodiversity

Biodiversity emerging as the onset of neoplasia

The adaptive dynamics studied by [29] can be made to correspond to the continuum of tissue phenotypes characterizing neoplastic progression: (0) normal tissue, (1) hyperplasia (precursor to neoplasia), (2) dysplasia (intra-epithelial neoplasia), (3) micro invasive (invasive neoplasia), (4) metastasis. We can think these phenotypes as caused by cell proliferation strategies which are distributed along an ecological aspect-space defining a cell's capacity to exploit system disobedience. In Figure 3, we map the neoplastic progression to the three possible regimes of the adaptive dynamics:

- *extinction regime*, where a population of healthy cells running "Metazoa 2.0" is maintained by the supply of differentiated cells from SCNs dispersing into local niches hosting differentiated sink-populations (where $r \leq 0$). Here the extinction solution $\hat{\omega} = 1$ and $\hat{\phi} = 0$ represents the state of the tissue
- *bottom-up regime*, where an unregulated population of differentiated cells is growing at rate $r > 0$ by avoiding the control mechanisms of the host. These cells, running some broken version of a "Metazoa 1.0" toolkit start generating new variants. Thus, the malignant biomass begins to evolve a better proliferation strategy ω^*. New more efficient clones take over and expand the malignant biomass. Clonal replacements

continues until the evolving value of proliferation strategy reaches a critical value $\omega = H$.

- *top-down regime* is when the local ecology has become neutral due to saturation. An invasive neoplasia consists of a community of multiple neutral strategies. Such neutral ecology produces a diverse spectrum of aggresive malignant cell types which go around trying to disperse to a differnt community or secondary organ, and initiating metastasis progression.

In a linear and order sequence the affected tissue progresses from the bottom-up regime towards the top-down regime where it will produce malignant cells that disperse into other organs. The issue then is would these cells invade this new communities of cells and colonize the secondary organ or not?

6 Metastasis, dispersal and invasion of secondary cell communities

So far we developed a simple model to understand neoplastic progression but does not include metastasis. Neoplasias, however, produce invasive malignant cell populations that will reach far away organs where after some time in dormancy (latency) these cancer cells invade and colonize the secondary organ [36]. Metastasis is the final stage of neoplastic progression and associated to the spread, and colonization of a distant organ by cell originated from a primary tumor. This process can be conceptualized as an invasive ecology problem [8]. In what follows we describe a simple model, originally used in metapopulation dynamics, to understand this process.

The conditions that allow for the colonization and invasion of a secondary organ is the critical question underlying metastasis formation. This process is complex as cells arriving in secondary organs can be hidden in small numbers without invading the tissue suggesting that successful invasions by metastatic propagules could be facilitated or prevented by the ecological status of the cell community at the secondary organ. This can be shown by developing models of organogenesis based on schemes of cell community assembly using metapopulation models. By extending on the work of Chen and collaborators [9], We suggest that changes in the patterns of species packing could determine the susceptibility to invasions by metastatic cells.

To motivate our model we will focus in a particular tissue, the bone marrow. Like in many tissues, in the bone marrow, differentiation from stem cells occurs in patches know as Hematopoietic Stem Cell (HSC) niches. At these locations, persistent populations of HSC can be found. Since there are multiple of these locations within the tissue, at a large enough spatial scale (a landscape metapopulation scale), following Chen and collaborators [9], we can represent the tissue by a patch occupancy model of a HSC metapopulation.

In this view, several locations (stem cell niche patches) can be empty or occupied by a local population of HSC at any given moment. Thus, we can use the following model [37],

$$dp/dt = fp(1-p) - mp, \tag{8}$$

to represent the proportion of occupied patches the metapopulation of HSC holds in the organ. A particular strategy characterizing the metapopulation is given by the pair of colonization and extinction rates (f and m respectively). A persistent HSC metapopulation in endured as long as its reproductive number (R) satisfy $R = f/m > 1$.

The question we will now try to address is Under which conditions would this system be invaded by a malignant cell? In the metapopulation scenario of Chen and collaborators [9], the invasion of a secondary organ (i.e. the bone marrow) can be understood by applying the corresponding two-species (cancer clone vs. HSC) model representing a competitive hierarchy between a superior competitor (HSCs, type 1) and an inferior one (cancer clones, type 2) but which nonetheless has a better colonization strength ($f_2 > f_1$). Under a constant extinction rate m we represent such system by,

$$\frac{d}{dt}p_1 = f_1p_1(1-p_1) - mp_1 \tag{9}$$

$$\frac{d}{dt}p_2 = f_2p_2(1-p_1-p_2) - (f_1p_1+m)p_2. \tag{10}$$

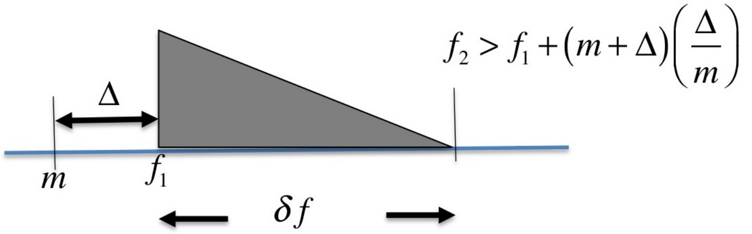


Figure 4: The limiting similarity and packing in secondary organs (see text for details). Fig. Adapted from [38]

As noticed by [9], the consequences of this trade-off (Figure 4) is associated with the emergence of a “competitive shadow” (depicted in black), which imposes a limit to how similar the two species can be in aspect space. For the sake of simplicity let us consider the mortality constant case where the HSCs that are the top competitor which have the lowest colonization rate f_1. At equilibrium, it is clear that $f_1 > m$ is needed for the viability of the HSCs. Note then that now the cancer cells are represented as another value $f_2 > f_1$, since they are better colonizers but poorer competitors for the niche-lattice (representing the 2-cell-type model of the organ—bone marrow). A cancer strategies with a parameter value f_2 lying within a zone of aspect space shadowed by strategy f_1 cannot invade. Thus for cancer colonization we have $\delta f \equiv f_2 - f_1 = (m + \Delta)(\Delta/m)$, where $\Delta = f_1 - m$ and we have,

$$f_2 > f_1 + \delta f \tag{11}$$

A cancer cell can only coexist (invade the organ) if its fecundity is greater than that of the HSC by amount δf. Notice that the long-term occupancy of HSC-niches by HSCs (vertical hight) determines the size of the shadow and this is an organ property, not a cancer cell property. If another sub-type of HSCs is created which would have a lower fecundity it would achieve a lower site-occupancy and therefore it will cast a smaller shadow.

An organogenesis model of a diverse bone marrow

Inspired by the diversity that has been described in adult tissue SCs [39], we imagine the same principle as discussed above (between cancer and one type of HSC) but now we apply a multi-cell type model of the organ (bone marrow) where HSCs are not only a single type with a specific colonization capability $R = f/m$ but rather a diverse collection of values $R_i = f_i/m$ forming an organ community with the same competitive trade-off.

A simple model for organogenesis can be simulated by using a community assembly model consisting of serial introductions of different cells types with random values for their relevant parameters (see refs [40] and [41]). Taken the constant mortality case, we get that our bone-marrow-organ-lattice would look now like a multi-type competitively hierarchical community of different stem cells,

$$\frac{1}{p_i}\frac{d}{dt}p = f_i\left(1 - \sum_{j \le i} p_j\right) - \sum_{j<i} f_j p_j - m. \tag{12}$$

The important point is that organogenesis is the process by which an equilibrium community is achieved. And this involves the assembly of different SCs that fill up physical space (patches/stem cell niches) as well as aspect space while being serially introduced. These serial introductions of types (see [40]) acts here as a model for bone marrow organogenesis. In this view, as organs (communities) assemble, shadows in aspect space emerge. These shadows protect the local organ from cancer invasion. Even though there might be dormant cancer cells waking up everywhere, since the community is packed with cell types whose aspect space is covered with their competitive shadows, the invaders (the dormant cancer cells) cannot invade.

Invasion and extinction cascades

If any of the cell-types in the assembled organ goes extinct, it will trigger a domino effect since its shadow will go away with it and then a hole in aspect space is created. As these holes develop, the protected polymorphism of cell types making the organ looses protection from invasion by the same cancer cells which where not able to invade before. If such scenario is true there are immediate consequence for cancer metastasis. The organ side is important. Sometimes recurrence of a tumor that was cured can be due to changes in the native cells of an organ. If changes due to host aging or other stresses change the patterns of ecosystem packing in host organs they could become vulnerable to invasion by awakening dormant cancer cells already present on the organ which nevertheless have always the same intrinsic properties. As organs change their composition in terms of cell strategy parameters (aging), otherwise healthy organs could become vulnerable to metastatic invasion due to intrinsic changes on their constituent cells rather than changes in the cancer cells themselves.

7 Final remarks

In this contribution we have presented our views on the phenomenon known as cancer. We see it as an ecological and evolutionary process that can be understood using simple models of ecological interaction and evolution. In particular, we have shown that the model introduced by us to account for the emergence of diversity in microbial ecosystems [29] can be applied to understand the emergence of diversity in tumor ecosystems and in particular the existence of two adaptive regimes; one of competitive replacement or clonal selection, and one of neutral coexistence. This result helps to reconcile the existence of tumors with different amounts of heterogeneity and suggest that clonal diversity should increase through time. Interestingly, in a recent study of Barret´s esophagus, a premalignant condition in which the lining of the esophagus is damaged due to chronic stomach acid exposure, Carlo Maley and collaborators [42] showed that clonal diversity increases through time, as the disease progresses and asserts that "*Progression to cancer through accumulation of clonal diversity, on which natural selection acts, may be a fundamental principle of neoplasia with important clinical implications*." We could not agree more. However, how much time is required to attain diversity may differ among tumor ecosystems. In [43, 44] it is reported on the early emergence of diversity in colorectal cancer progression. It would be particularly illuminating to compare the trajectories of clonal diversification in different types of tumors to get a better understanding on how variable is clonal succession in tumor ecosystems.

Ours is not the only model that attempts to understand clonal evolution and interaction. Among the first models to explicitly cue in on the interaction between clones in a tumor is the model of cellular competition by [45] on clones found in Ehrlich ascites carcinoma. Their model, and subsequent elaborations upon it [46] are purely ecological and do not includes evolution or progression, but they highlight the importance of competition among clones. An interesting step forward is [47] who models the interaction dynamics of normal and cancer cell populations to derive the conditions under which a cancer cell population would invade, and concludes "*the importance of increased efficiency in substrate* *absorption as a mechanism enabling tumor cells to (a) proliferate despite inefficient energy production and (b) compete successfully for resources with the numerically superior host cells. As with many biological invasions observed in nature, success of the invaders can be enhanced by disruption of the local ecology..."* Although the model by Gatenby does not include evolution, it does point out to a plausible mechanism by which the progression of cancer could get started, emphasizing the importance of the up regulation of glycolysis observed in cancer cells (or Warburg's effect) and the increase in acidity that this ensues, as fundamental for cancer invasion. In subsequent models Gatenby have included evolution explicitly by using game theoretical arguments to understand the emergence of the glycolytic phenotype [48, 49] but do not explicitly reproduce cancer progression and the coexistence of multiple clones in the tumor ecosystem. We see our model as similar, though more general and less mechanistic, that the one introduced by [49]. Both models point out that ecological theory and evolutionary dynamics may hold the clue to crack open the tumor ecosystem and advance in both treatment and understanding of cancer complexity.

Space is recognized as an important factor in ecological dynamics and in explaining coexistence in interacting populations (e.g. [50, 51]). In cancer research, the existence of spatial heterogeneity in tumor ecosystems has long been recognized (e.g. [6, 52, 53]) but only recently became under mathematical analysis, after the seminal work by [6] on the role of spatial heterogeneity in maintaining clonal diversity. Stochastic spatial models have shown that space may affect both cancer initiation and progression [54] as well as the emergence of diversity [55]. The latter work in particular use ecological and life history theory to assess the role of competition-colonization tradeoffs, typically associated to the spatial dynamics of species invasion and persistence to model tumor ecosystems. In particular, the authors aim at testing the notion that clonal diversity may result from the existence of different and spatially predictable selection regimes that select for different phenotypes; an invasive one at the front of the tumor and a maintenance one associated to promote tumor infrastructure inside the tumor. Their model support the existence of spatially variable selection regimes that promote the existence of different phenotypes, we argue that this may be one of the process involved in the competitive replacement that we observe in our model, but it cannot account for the coexistence of clonal diversity, which in our case is associated with the emergence of neutrality.

Acknowledgements

This research was partially supported by Award Number U54CA143803 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. We also acknowledge funding from ICM P05-002 and PFB-23.

8 Bibliography

[1] D. S. Coffey, “Self-organization, complexity and chaos: The new biology for medicine,” *Nature Medicine*, vol. 4, pp. 882–885, 1998.

[2] R. H. Austin and B. S. Gerstman, “Preface: Physics of cancer,” *AIP Advances*, vol. 2, no. 1, p. 010901, 2012.

[3] P. C. W. Davies and C. H. Lineweaver, “Cancer tumors as metazoa 1.0: tapping genes of ancient ancestors,” *Physical Biology*, vol. 8, no. 1, p. 015001, 2011.

[4] A. B. Herman, V. M. Savage, and G. B. West, “A quantitative theory of solid tumor growth, metabolic rate and vascularization,” *PLoS One*, vol. 6, 2011.

[5] D. Wirtz, K. Konstantopoulos, and P. C. Searson, “The physics of cancer: The role of physical interactions and mechanical forces in metastasis,” *Nature Reviews Cancer*, vol. 11, pp. 512–522, 2011.

[6] I. Gonzalez-Garcia, R. V. Solé, and J. Costa, “Metapopulation dynamics and spatial heterogeneity in cancer,” *Proceedings of the National Academy of Sciences USA*, vol. 99, no. 19, pp. 3085–1308, 2002.

[7] B. Crespi and K. Summers, “Evolutionary biology of cancer,” *Trends in Ecology & Evolution*, vol. 20, no. 10, pp. 545–552, 2005.

[8] K. J. Pienta, N. McGregor, R. Axelrod, and D. E. Axelrod, “Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments,” *Translational Oncology*, vol. 1, no. 4, p. 158, 2008.

[9] K.-W. Chen and K. J. Pienta, “Modeling invasion of metastasizing cancer cells to bone marrow utilizing ecological principles,” *Theor Biol Med Model*, vol. 8, pp. 36–36, 2011.

[10] D. Basanta, R. A. Gatenby, and A. R. Anderson, “Exploiting evolution to treat drug resistance: combination therapy and the double bind,” *Molecular Pharmaceutics*, vol. 9, no. 4, pp. 914–921, 2012.

[11] S. A. Frank, *Dynamics of cancer: incidence, inheritance, and evolution*. Princeton University Press, 2007.

[12] R. A. Gatenby and T. L. Vincent, “An evolutionary model of carcinogenesis,” *Cancer Research*, vol. 63, no. 19, pp. 6212–6220, 2003.

[13] L. M. Merlo, J. W. Pepper, B. J. Reid, and C. C. Maley, “Cancer as an evolutionary and ecological process,” *Nature Reviews Cancer*, vol. 6, no. 12, pp. 924–935, 2006.

[14] D. Hanahan and R. A. Weinberg, “Hallmarks of cancer: the next generation,” *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[15] J. Cairns, “Mutation selection and the natural history of cancer,” *Science of Aging Knowledge Environment*, vol. 2006, no. 10, p. cp1, 2006.

[16] M. Greaves and C. C. Maley, “Clonal evolution in cancer,” *Nature*, vol. 481, no. 7381, pp. 306–313, 2012.

[17] P. C. Nowell, “The clonal evolution of tumor cell populations,” *Science*, vol. 194, no. 4260, pp. 23–28, 1976.

[18] A. Marusyk, V. Almendro, and K. Polyak, “Intra-tumour heterogeneity: a looking glass for cancer?” *Nature Reviews Cancer*, vol. 12, no. 5, pp. 323–334, 2012.

[19] D. F. Camacho and K. J. Pienta, “Disrupting the networks of cancer,” *Clinical Cancer Research*, vol. 18, no. 10, pp. 2801–2808, 2012.

[20] H. Kitano, “Biological robustness,” *Nature Reviews Genetics*, vol. 5, no. 11, pp. 826–837, 2004.

[21] G. H. Heppner, “Tumor heterogeneity,” *Cancer research*, vol. 44, no. 6, pp. 2259–2265, 1984.

[22] D. Hanahan and L. M. Coussens, “Accessories to the crime: functions of cells recruited to the tumor microenvironment,” *Cancer cell*, vol. 21, no. 3, pp. 309–322, 2012.

[23] E. Ben-Jacob, D. S Coffey, and H. Levine, “Bacterial survival strategies suggest rethinking cancer cooperativity,” *Trends in microbiology*, vol. 20, no. 9, pp. 403–410, 2012.

[24] K. J. Pienta, B. A. Robertson, D. S. Coffey, and R. S. Taichman, “The cancer diaspora: Metastasis beyond the seed and soil hypothesis,” *Clinical Cancer Research*, vol. 19, no. 21, pp. 5849–5855, 2013.

[25] B. Alberts, “The challenge of cancer,” *Science*, vol. 331, no. 6024, pp. 1491–1491, 2011.

[26] R. A. Gatenby and R. J. Gillies, “Why do cancers have high aerobic glycolysis?” *Nature Reviews Cancer*, vol. 4, no. 11, pp. 891–899, 2004.

[27] K. Polyak, I. Haviv, and I. G. Campbell, “Co-evolution of tumor cells and their microenvironment,” *Trends in Genetics*, vol. 25, no. 1, pp. 30–38, 2009.

[28] S. E. Finkel and R. Kolter, “Evolution of microbial diversity during prolonged starvation,” *Proceedings of the National Academy of Sciences*, vol. 96, no. 7, pp. 4023–4027, 1999.

[29] J. E. Keymer, M. A. Fuentes, and P. A. Marquet, "Diversity emerging: from competitive exclusion to neutral coexistence in ecosystems," *Theoretical Ecology*, vol. 5, no. 3, pp. 457–463, 2012.

[30] D. C. Krakauer, K. M. Page, and D. H. Erwin, "Diversity, dilemmas, and monopolies of niche construction," *The American Naturalist*, vol. 173, no. 1, pp. 26–40, 2009.

[31] F. G. Varela, H. R. Maturana, and R. Uribe, "Autopoiesis: the organization of living systems, its characterization and a model," *Biosystems*, vol. 5, no. 4, pp. 187–196, 1974.

[32] I. Prigogine, *From Being To Becoming*. Freeman, USA, 1980.

[33] L. W. Buss, *The Evolution of Individuality*. Princeton University Press, USA, 1987.

[34] D. T. Scadden, "The stem-cell niche as an entity of action," *Nature*, vol. 441, no. 7097, pp. 1075–1079, 2006.

[35] J. E. Keymer, P. Galajda, C. Muldoon, S. Park, and R. H. Austin, "Bacterial metapopulations in nanofabricated landscapes," *Proceedings of the National Academy of Sciences*, pp. 7290–1729, 2006.

[36] D. X. Nguyen, P. D. Bos, and J. Massagué, "Metastasis: from dissemination to organ-specific colonization," *Nature Reviews Cancer*, vol. 9, no. 4, pp. 274–284, 2009.

[37] R. Levins, "Some demographic and genetic consequences of environmental heterogeneity for biological control," *Bulletin of the Entomological Society of America*, vol. 15, no. 3, pp. 237–240, 1969.

[38] A. Kinzig, S. A. Levin, J. Dushoff, and S. Pacala, "Limits to similarity, and spacies packing in competition-colonization models," *American Naturalist 153*, pp. 371–383, 1999.

[39] M. Grompe, "Tissue stem cells: new tools and functional diversity," *Cell Stem Cell*, vol. 10, no. 6, pp. 685–689, 2012.

[40] J. Dushoff, L. Worden, J. Keymer, and S. A. Levin, "Metapopulations, community assembly, and scale invariance in aspect space," *Theoretical Population Ecology*, pp. 329–338, 2002.

[41] S. A. Levin, J. Dushoff, and J. E. Keymer, "Community assembly and the emergence of ecosystem pattern," *Scientia Marina*, vol. 65, no. S2, pp. 171–179, 2001.

[42] C. C. Maley, P. C. Galipeau, J. C. Finley, V. J. Wongsurawat, X. Li, C. A. Sanchez, T. G. Paulson, P. L. Blount, R.-A. Risques, P. S. Rabinovitch *et al.*, "Genetic clonal diversity predicts progression to esophageal adenocarcinoma," *Nature Genetics*, vol. 38, no. 4, pp. 468–473, 2006.

[43] J.-L. Tsao, S. Tavaré, R. Salovaara, J. R. Jass, L. A. Aaltonen, and D. Shibata, "Colorectal adenoma and cancer divergence: evidence of multilineage progression," *The American Journal of Pathology*, vol. 154, no. 6, pp. 1815–1824, 1999.

[44] J.-L. Tsao, Y. Yatabe, R. Salovaara, H. J. Järvinen, J.-P. Mecklin, L. A. Aaltonen, S. Tavaré, and D. Shibata, "Genetic reconstruction of individual colorectal tumor histories," *Proceedings of the National Academy of Sciences*, vol. 97, no. 3, pp. 1236–1241, 2000.

[45] B. Jansson and L. Révész, "Analysis of the growth of tumor cell populations," *Mathematical Biosciences*, vol. 19, no. 1, pp. 131–154, 1974.

[46] S. Michelson, B. Miller, A. Glicksman, and J. Leith, "Tumor micro-ecology and competitive interactions," *Journal of Theoretical Biology 128*, pp. 233–246, 1987.

[47] R. A. Gatenby, "Population ecology issues in tumor growth," *Cancer Research*, vol. 51, pp. 2542–2547, 1991.

[48] R. A. Gatenby and T. L. Vincent, "An evolutionary model of carcinogenesis," *Cancer Research 63*, pp. 6212–6220, 2003.

[49] T. L. Vincent and R. A. Gatenby, "An evolutionary model for initiation, promotion, and progression in carcinogenesis," *International Journal of Oncology 32*, pp. 729–737, 2008.

[50] R. Durrett and S. Levin, "The importance of being discrete (and spatial)," *Theoretical population biology 46*, pp. 363–394, 1994.

[51] J. E. Keymer, P. A. Marquet, and A. R. Johnson, "Pattern formation in a patch occupancy metapopulation model: A cellular automata approach," *Journal of Theoretical Biology 194*, pp. 79–90, 1998.

[52] S. Yachida, S. Jones, I. Bozic, T. Antal, R. Leary, B. Fu, M. Kamiyama, R. H. Hruban, J. R. Eshleman, M. A. Nowak *et al.*, "Distant metastasis occurs late during the genetic evolution of pancreatic cancer," *Nature*, vol. 467, no. 7319, pp. 1114–1117, 2010.

[53] R. A. Gatenby, O. Grove, and R. J. Gillies, "Quantitative imaging in cancer evolution and ecology," *Radiology 269*, pp. 8–15, 2013.

[54] N. L. Komarova, "Spatial stochastic models for cancer initiation and progression," *Bulletin of Mathematical Biology*, vol. 68, pp. 1573–1599, 2006.

[55] P. A. Orlando, R. A. Gatenby, and J. S. Brown, "Tumor evolution in space: The effects of competition colonization tradeoffs on tumor invasion dynamics," *Front Oncol*, vol. 3, no. 45, 2013.

Game of Life: simple interactions ecology

L. Caballero, G. Cocho & S. Hernández, IF and C3, UNAM, Mexico

1 Abstract

Alan Turing in his work of 1952 stated the importance of the analysis of pattern formation in biological systems focusing in color patterns and subject to chemical mechanisms, but his work was limited because of the mathematical complications of the model. However he had the clarity to propose the need of a new paradigm to face such biological problems. It was until the late seventies that John Conway proposed a mathematical game, based on cellular automata, with a clear biological similarity, where the most basic entities, called cells, can be in one of two possible states, conceptualized as dead or alive. The proposed rules give the game a critical trait, this is, within a specific threshold the cells can stay alive otherwise they will become dead. This game is known as Conway's Game of Life. The emergence of new patterns is one of the properties of Conway's Game of Life, another is the great diversity of forms that it can span. All of this depend on the initial conditions of the game. Along this chapter we propose Conway's Game of Life as an alternative model to study ecological systems.

2 Resumen

Alan Turing en su trabajo de 1952 planteó la importancia del análisis de la formación de patrones en los sistemas biológicos enfocándose en los patrones de color y restringiéndose a mecanismos principalmente químicos, pero su trabajo quedó acotado por la complicación derivada del modelo matemático propuesto. Sin embargo, tuvo la claridad de establecer la necesidad de plantear un nuevo paradigma para abordar este tipo de problemas biológicos. Fue hasta los setentas que John Conway propuso un juego matemático, basado en autómatas celulares, con un claro símil biológico, donde las entidades más básicas son celdas con dos estados posibles, conceptualizadas como vivas o muertas. Las reglas propuestas le dan un un caracter crítico al juego, esto es, dentro de un umbral es-

pecífico las células pueden seguir viviendo y fuera de este umbral la supervivencia no es posible. A este juego se le conoce como el Juego de la Vida de Conway. La emergencia de patrones no preestablecidos es una de las propiedades de este juego, otra característica importante es la gran variedad de formas que pueden generarse. Todas estas dependientes de las condiciones iniciales con las que comience la evolución del Juego de la Vida. A lo largo del capítulo proponemos al Juego de la Vida como una alternativa de modelo para estudiar a los sistemas ecológicos.

3 Introduction

Since the publication of the article *The chemical basis of morphogenesis* written by Alan Mathinson Turing in 1952, the study of morphogenetic patterns has taken a surprising turn. Turing proposed a model to explain how patterns emerge during development. The purpose of his work was to discuss a possible mechanism by which the genes of a zygote can determine the anatomical structure of the resulting body. To do this he proposed that by using certain known physical laws it is possible to explain many of the phenomena of morphogenesis. His proposal suggests that a system of chemicals called morphogens (for qualities in the generation of shapes) react together and diffuse through the tissue, which can help to account for the main phenomena of morphogenesis. Such a system, although originally it can start from a quite homogeneous state, after a while may develop a pattern or structure due to instability of homogeneous equilibrium, which is triggered by some random disturbances [1, 2].

This model describes the evolution of the system in two parts: the mechanical and the chemical. The mechanical part of the system describes positions, masses, speeds and elastic properties of the cells, and also the forces interacting among them. The continuous form of the model generates essentially the same information but in the form of tension, speed, density and the elasticity of the material.

The chemical part of the system is given by the chemical composition of an individual cell and the rate of diffusion of each substance between two adjacent cells. In the continuous form of the model, the concentrations and diffusion rates of each substance must be determined for each point in the system and for every moment.

Although Turing did not consider all of the aspects proposed in his reaction-diffusion model (hereafter RD), he postulated that in order to compute the system's evolution one must take into account changes in the position and changes and velocity which are given by Newton's laws of motion. He also considered osmotic pressure, tensions generated by the system's elasticity and movement as given by chemical information, dissemination of chemicals. Regions where such diffusion is possible are given by mechanical information.

Having in mind some of these postulates Turing presented a mathematical model based on nonlinear partial differential equations with constant coefficients. In it he established the basis for the development of models, now known as reaction-diffusion (RD) models (see review in [2–5]).

Since the development of Turing's RD model, the theoretical study of the emergence of patterns in morphogenesis has received a lot of attention including research on color patterns studied by dynamic generic RD models [1, 3]. But oftne RD models do not consider the cellular and tissue environment in which these patterns emerge (see [6–9]), it is therefore important and useful to integrate in the same model the mechanical and chemical aspects considered in the Turing model, aiming at an explanation supported by experimental biological evidence, about the mechanisms involved in the emergence and maintenance of patterns in morphogenesis.

One of the main interests driving Turing in his work was the need for a deep understanding of the formation of patterns, but as he himself states it, when many different properties are included in this problem, such as electrical and mechanical properties, not only the chemical aspects originally considered, the complexity of the problem escalates rapidly. Thus he pointed out the need to develop a paradigm where the exploration of different environments, and conditions over these environments, could be stated in a more natural and biological fashion, setting the basis for further exploration and analysis on a very complex and widely misunderstood topic.

Following this reasoning, we propose to describe a framework where we can include aspects like tissue and physical mechanisms involved in the study of cellular interactions during morphogenesis which were put aside in Turing's original proposal, although they were mentioned as important for understanding the general problem of morphogenesis. In contrast with classical mathematical techniques the framework that we are seeking needs to be able map the different properties of the living systems in a more natural way.

In the 1970's, John Conway described a mathematical game named The Game of Life or Conway's Life that makes an analogy with living cells. That is, if a living cell is overcrowded or extremely isolated it will die, and it will survive only if it has the right number of neighbors around it. This game has many interesting properties and it will be described in the next section.

Given that Ecology is the science that studies the interactions between sets of biotic and abiotic factors we can establish an analogy with Conway's Life through the Ecology that emerges through a basic and simple set of rules that make a diverse zoology of patterns (see [10]) which coexist and interact giving rise to a set of complex interactions in a self-organized way. This contrasts with the general perception that every system requires a different history and that a completely different set of rules is needed to describe such a system. It is very important to specify that the simple rules we are talking about are not the ones that give rise to different patterns in the skins of animals or at least not only that set of rules (which can be interesting in itself) but include those rules that apply to any kind of system that is known to show emergent properties through the interaction of autonomous entities within it.

Historically biology has understood biological mechanisms as objects that are restricted by matter in a physical and tangible way. Modeling in biology is usually carried out in a canonical fashion incorporating all of the interacting aspects of the modeled phenomenon in an ad-hoc way, but it is often forgotten that restrictions interacting with the systems are an intrinsic part of them. This meaning that such restrictions are not just a different dimension added on to a biological one, but are an active part of this biological dimension.

In contrast with this historical vision of how modeling is carried out in biology, the proposed alternative framework seeks to rethink this modeling process by starting with a set of simple and representative rules for all of the different entities interacting in the phenomenon selected to be modeled and letting the devised rules make the system evolve long enough for all of the entities that cannot coexist gets eliminated through such evolution.

As we will see in the following sections, the Game of Life provides an excellent example of how a set of simple rules can span a diverse zoology, not only designing the final form that each member of this zoology should have but also defining the interactions among them, therefore providing a framework where we can really talk, experiment with and analyze an ecology of biological patterns.

4 Computation, Cellular Automata and Conway's Game of Life

One of the most interesting and profound debates in Biology concerns form and function. Which one determines the other? Throughout time from ancient Greek philosophers to the present day scientists, such as biologists have given much attention to this specific question.

This question has received a lot of attention from a great and diverse community of scientists but at this point we are going to make reference to two who which have made landmark contributions in the field of Mathematics and Computer Science.

The first is Alan M. Turing, part of whose work was described in the first part of the present chapter. The second is the Hungarian John von Neumann, a prolific mathematician, who inspired by the work of Alan Turing, worked in the 1940s on the technical and philosophical problem of self replication. His main aim was to determine the specific set of rules by which a machine could replicate itself. He devised an automaton D structured in the following way: One part is the functional structure A. A second part is the structure that copies the set of instructions I_A while a third part C inserts the copy of the instructions into the new structure [11]. This is what was later known as a cellular automaton.

In the late 60s John Conway worked on a form derived from than in Neumann's work. He and his students devised an automaton with a simpler set of rules than Neumann's but with some important mathematical inclusions, such as the capacity universal computation [12]. He then came up with a recreational mathematical construction named by Conway himself as *Life*, or as the Game of Life as we know it, which was published in [13].

As said in the previous paragraph one of the most important characteristics of Conway's Game of Life is its capacity to perform universal computation, meaning that what every other computer can do, can be done with this specific cellular automaton, given the proper initial conditions.

The usual concept of computation sees it as a synonym of calculation. This is not wrong only incomplete. The concept of computation in cellular automata (CA) can be understood in the following two ways [14]:

- If the CA is understood as the program, then the mapping between the initial configuration (input) and the final state (output) is what we can call computation.
- A more theoretical approach would be to select a very specific input that can emulate any other computer (universal computation). However this interpretation is extremely difficult to go into in depth and its valuable results are restricted to a computational sciences theoretical frame.

The biological and physical importance of the concept of computation resides in the relationship between discrete and dynamical systems, and the utility of different models designed to understand natural phenomena. Bearing this in mind we now can describe the general aspects of Conway's Game of Life as follows. A group of cellular automata will be arranged in a bidimensional lattice in which every cell can be in two states, 0 to resemble the *dead* state and 1 to resemble *alive* state. The value of every cell will be determined by the state of its neighbors by the following rules:

- Every cell remains dead if its surroundings are dead. Meaning that if no more than 2 two cells are alive, the present cell will remain dead.
- Every cell will die from overcrowding or solitude. Meaning that a cell which is alive will die if it has more than three living or less than two living cells adjacent.
- Every cell will come to life if it has three living neighbors.
- Every new cell stays alive if it has either two or three living neighbors. This establishes that life can exist in a small and fair well determined range of eight possible values.

All this rules are updated in a synchronous way throughout the whole lattice [15, 16].

A great diversity of different patterns emerged from this small set of rules, which can be classified into three main groups: a set of static objects, a set of objects with periodic forms but static in space, and a set of moving objects. Some examples can be seen in figures 1, 2, 3 and 4.

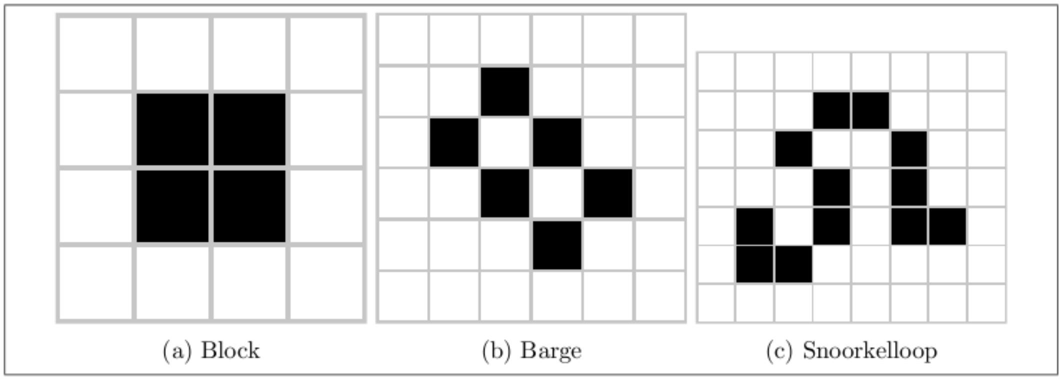

(a) Block (b) Barge (c) Snoorkelloop

Figure 1: Three still forms of life which can serve as memory.

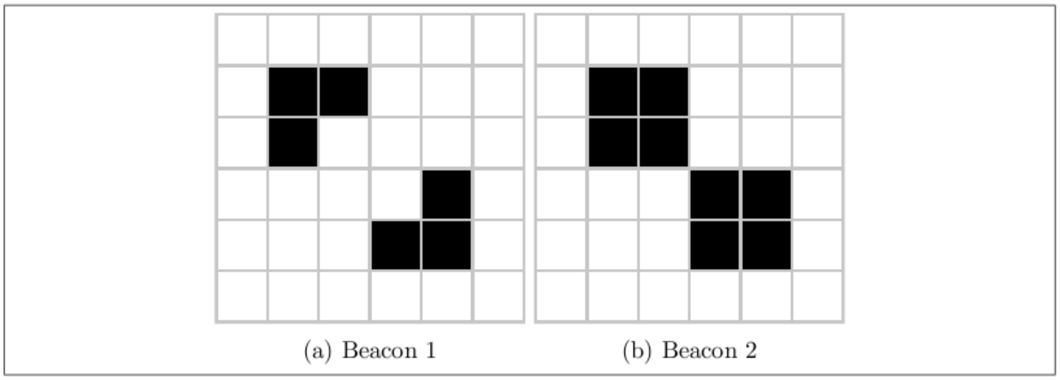
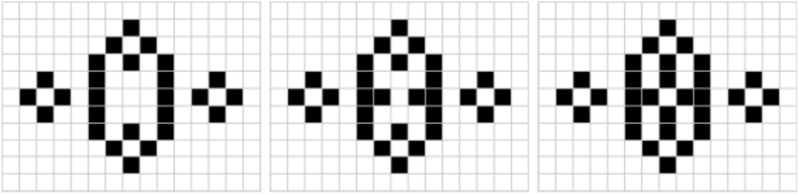

(a) Beacon 1 (b) Beacon 2

Figure 2: Both steps of a period-2 oscillator named Beacon.

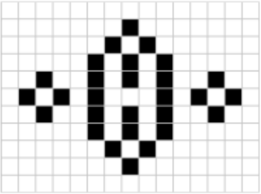
The importance of these types of objects formed in Conway's Game of Life comes from the fact that to carry out universal computation the combination of the former elements is needed in the following way.

In order to compute anything it is easy to realize that the ability to count and to store information is required. This is accomplished by the periodic and static classes respectively. That is, the still elements work as memory elements and the periodic elements can aid as counting entities. The third class, namely the class of moving objects class, carries out the task of transporting information. This particular aspect is of great importance, since our main interest is in the mechanism to communicate information in different scales.

These three types of elements are used to prove that Conway's Game of Life can perform universal computation. A sketch of universality of computation can be seen in [16].

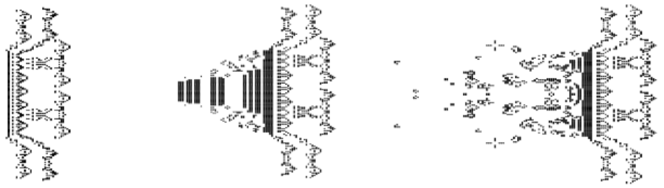


(a) Gray Counter 1 (b) Gray Counter 2 (c) Gray Counter 3



(d) Gray Counter 4

Figure 3: A period-4 oscillator named Gray Counter.



(a) Line Puffer 1 (b) Line Puffer 2 (c) Line Puffer 3

Figure 4: A puffer that acts like a *train*, except only that it leaves debris behind

5 Simple Rules and Pattern Formation

The Game of Life is a particular case of cellular automaton but the cellular automata model is widely used to model patterns using simple interactions. For an extensive review you can check [9].

During the 1980s and early 1990s Stephen Wolfram did an extensive research into a simplified form of a cellular automaton, one that can evolve in only one spatial dimension and one temporal dimension. This automaton consists only two possible states to describe and a radius. This is the so called Elemental Cellular Automata which is an automata whose actual state can be affected by its present state and the one of its nearest neighbors. That is:

$$\sigma_n^{t+1} = f(\sigma_{n-1}^t, \sigma_n^t, \sigma_{n+1}^t) \tag{1}$$

Wolfram's work is an extensive qualitative and quantitative exploration of all the possible elemental cellular automata that can be formed with this elemental configuration. From this research he came up with a widely used classification of all possible cellular automata formed with this configuration and based upon statistical properties of different configurations. His work can be read in [17] and with more technical detail in [18]. The classification of the different types of CA is shown in the following table.

Class	Description
1	Evolution rapidly leads to a unique homogeneous state, that is they evolve to a fixed point pattern.
2	Evolution, after some iterations that depends on some appropriate initial sequence, leads to a set of values that are either stable or periodic.
3	Almost all initial states lead to chaotic patterns with such statistical properties that makes it difficult to extract structure out of them.
4	The most interesting class of them all since even when they seem to reach a quiescent state, some of them show persisting spatial structures that repeat in time, that is, structures that can propagate indefinitely through the evolution of the cellular automata. The Game of Life is known to be in this class, also the Elementary Cellular Automata rule 110.

Table 1: Classification of different rules derived from Wolfram's work

Our main interest in the Game of Life is precisely the fact that there is speculation that it is a Class 4 CA and that out of very simple and biological-like rules a varied and rich range of patterns can be formed. Bak et al showed in [19] that this game can be in a critical regime, meaning that structures of all scales are present at one given time. Also, as stated before, the Game of Life is a self-organized system, meaning that it is driven by evolution of the system. Thus, as shown by Per Bak et al., this simple set of rules can produce a self-organized criticality.

The importance of this fact is that when the system is in a critical state it can provide a mechanism for the emergence of scale-free structures [19] and therefore resembles at least in a way the mechanisms used by Nature to form all sorts of structures and patterns. Some examples of recent findings can be seen in [20, 21].

6 Conclusions

As we have mentioned, Conway's Game of Lfe is a versatile mathematical and computational tool with some interesting particularities. The first is the limited and simple set of rules describing it. The second is the fact that it is a zero player game, that is, it is a game driven by the evolution of the initial condition, a set of initial living and dead cells, with successive iterations of the simple set of rules previously described. The third is the apparently abundant zoology that can be produced with them. It is important to note that the Game of Life is reported to be, not without debate [14, 22], to be a class 4 cellular automaton, suggesting that the ability to create all of this ecology of living creatures (in a broad sense of "living" and "creatures") is partly related to the fact that a set of rules operating in a critical regime is necessary in order to create this diverse set of patterns.

Another interesting aspect that needs to be remarked is the fact that in this computational game, unlike many canonical mathematical structures, form is function, meaning that specific forms induce specific functions. For example, if we alter just one neighbor in some of the objects presented like the oscillators (figure 2) or the puffer (figure 4) we may completely alter its function. This is observable even in the static forms in the Game of Life like the still objects (figure 1). All these aspects taken in consideration imply that synchronization and specific structural issues need to be taken into account in order to present a functional Game of Life that can carry universal computation.

This small computational self-organized game is a good start to model and comprehend the importance of the rules and the interactions between the forms they generated through them in order to rise produce great ecological diversity. It is an alternative way to understand such a system instead of the usual approach of constructing different histories and sets of working rules for every system. In order to create new and possibly more complex forms the rules can be used in a recursive fashion with multiple iterations. It is important to note that if the chosen rules work well with the formation of small scale structures then there is no apparent need to choose some other rules until they stop working. All the forms created under this framework can be tested together, forming bigger ensembles at each time step, and the persistence in time of such ensembles will depend entirely of their surroundings.

In this context it is important to note that in the biological world we are surrounded by "historical contingencies" wherever we look. These contingencies include the evolution of genetic information coding phenotypical characteristics and the fact that such genetic information is subject to changes in the environment. Another example is the way biological diversity is reshaped by the different mass extinctions registered in biological history. All these examples can be modeled through a CA with a layered model in which the upper layer interacts with the bottom layer in a stochastic fashion. But the central point about the effects of historical contingencies is that evolution in biology is not determined by the initial conditions of the system. This is not the case in the Game of Life, which functions by a deterministic set of rules, since every final state is determined by a specific initial condition which the game starts with. We believe that further research needs to done on this topic.

Another topic for further research is the fact that the structure of biological organisms is modular and hierarchical. Could it be possible that dynamics determined by a Conway's Game of Life set of rules can show this specific aspect?

If we take Conway's Game of Life as our paradigm, then we can say that patterns emerge under diverse circumstances subject to ecological interactions. These seems as very sophisticated patterns however it also seems that a great diversity of patterns can emerge under the restrictions of simple rules: that is, that the interactions among different biological factors can span a great number of different form of life but we can only account for just a small group of conserved patterns.

And extending this particular subject the reader could ask whether only celullar automata defined in Conway's Game of Life are capable of spanning such a diverse interacting zoology with such properties. Could another type of automaton give rise to such a rich environment? So, a further research topic can be precisely this: what is the simplest automaton capable of spanning this diverse ecosystem? The reasons why we chose the Game of Life is because of its natural and biology-like set of rules which we think makes it easier to understand and reinterpretate for this purpose.

As we have seen in the Game of Life we only have a small set of rules which that nevertheless can span complex patterns which maintain a close resemblance to biological life as we know it.

As we know, in biological life we can have basal states and modifications of these basal states generated throughout their evolution. So we can ask ourselves whether it is the diversity of the forms of life which must be accounted for as a possible outcome, even when life itself strike us as limited.

7 Bibliography

[1] A. M. Turing, "The chemical basis of morphogenesis," *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, vol. 237, no. 641, pp. 37–72, 1952.

[2] A. Koch and H. Meinhardt, "Biological pattern formation: from basic mechanisms to complex structures," *Reviews of Modern Physics*, vol. 66, no. 4, p. 1481, 1994.

[3] A. Gierer and H. Meinhardt, "A theory of biological pattern formation," *Kybernetik*, vol. 12, no. 1, pp. 30–39, 1972.

[4] S. Kondo, "The reaction-diffusion system: A mechanism for autonomous pattern formation in the animal skin," *Genes to Cells*, vol. 7, no. 6, pp. 535–541, 2002.

[5] S. Kondo and H. Shirota, "Theoretical analysis of mechanisms that generate the pigmentation pattern of animals," in *Seminars in Cell & Developmental Biology*, vol. 20. Elsevier, 2009, pp. 82–89.

[6] G. Cocho, R. Pérez-Pascual, and J. L. Rius, "Discrete systems, cell-cell interactions and color pattern of animals. i. conflicting dynamics and pattern formation," *Journal of Theoretical Biology*, vol. 125, no. 4, pp. 419–435, 1987.

[7] G. Cocho, R. Pérez-Pascual, J. Rius, and F. Soto, "Discrete systems, cell-cell interactions and color pattern of animals ii. clonal theory and cellular automata," *Journal of Theoretical Biology*, vol. 125, no. 4, pp. 437–447, 1987.

[8] J. Moreira and A. Deutsch, "Cellular automaton models of tumor development: a critical review," *Advances in Complex Systems*, vol. 5, no. 02n03, pp. 247–267, 2002.

[9] A. Deutsch and S. Dormann, *Cellular automaton modeling of biological pattern formation: characterization, applications, and analysis*. Springer, 2005.

[10] "Lifewiki." [Online]: http://www.conwaylife.com/

[11] R. Herken, *The universal Turing machine: a half-century survey*. Springer, 1995, vol. 2.

[12] E. R. Berlekamp, J. H. Conway, and R. K. Guy, *Winning ways for your mathematical plays*. AK Peters, 1982.

[13] M. Gardner, "Mathematical games: The fantastic combinations of John Conway's new solitaire game "Life"," *Scientific American*, vol. 223, no. 4, pp. 120–123, 1970.

[14] M. Mitchell, P. Hraber, and J. P. Crutchfield, "Revisiting the edge of chaos: Evolving cellular automata to perform computations," *arXiv preprint adap-org/9303003*, 1993.

[15] G. W. Flake, *The Computational Beauty of Nature: Computer Explorations of Fractals, Chaos, Complex Systems, and Adaptations*. The MIT Press, 1998.

[16] A. Ilachinski, *Cellular automata: a discrete universe*. World Scientific, 2001.

[17] S. Wolfram, *A new kind of science*. Wolfram media Champaign, 2002, vol. 5.

[18] ——, "Universality and complexity in cellular automata," *Physica D*, 1984.

[19] P. Bak, K. Chen, and M. Creutz, "Self-organized criticality in the 'game of life'," *Nature*, vol. 342, p. 14, 1989.

[20] "Fastest 90-degree stable reflector," 2012. [Online]: http://tinyurl.com/lskbzww

[21] "Recent discoveries, part II," 2013. [Online]: http://tinyurl.com/n7op6sh

[22] H. J. Blok, "Life without bounds: Does the game of life exhibit self-organized criticality in the thermodynamic limit?" Ph.D. dissertation, Citeseer, 1995.

Non-Linearity in population ecology

C. Martorell, Facultad de Ciencias, UNAM, Mexico

1 Abstract

Non-linearity pervades population ecology at all levels, from the vital rates of individuals to multispecies interactions. Here, I exemplify some of such non-linear processes, and how the models that we use to describe them suggest that a wide variety of complex phenomena may arise, e.g., chaos, self-organization, or critical thresholds. In some cases I also point out how the usage of different non-linear functions determines the behavior of the models. Thus, it is important to know which functions describe real processes appropriately, parametrize them with actual data, and assess their effects on population dynamics that is modeled. This is critical to understand the causes of complexity in nature.

2 Resumen

Los procesos no lineales caracterizan a la ecología de poblaciones en diferentes niveles, desde las tasas vitales de los individuos que las componen hasta las interacciones con numerosas especies. Aquí se ejemplifican algunos de estos procesos no lineales, y cómo los modelos que empleamos para describirlos sugieren la existencia de una gran variedad de fenómenos complejos, e.g., el caos, la autoorganización o los umbrales críticos. En algunos casos se señala cómo el uso de diferentes funciones no lineales determina el comportamiento de los modelos. En consecuencia, es importante conocer cuáles de dichas funciones describen más adecuadamente los procesos reales, parametrizarlas con datos, y evaluar sus consecuencias sobre la dinámica poblacional modelada. Esto es fundamental para comprender cuáles son las causas de la complejidad en la naturaleza.

3 Non-Linearity in population ecology

Non-linearity permeates biology, and populations are no exception. In fact, populations have played a central role in the development of non-linear sciences. They have also ignited the interest of many scientists in complexity, self-organization, critical transitions, and chaos, perhaps because non-linearity arises so naturally in population ecology. The causes for non-linearity in populations span different levels of organization, from the attributes of individuals to interactions with other species.

Demography integrates the vital rates (survival, growth, and fecundity) of individuals in order to understand and model population growth. These vital rates are intrinsically non-linear, frequently as a result of bounds on parameter values; for instance, survival probability is bounded at zero and one, and only non-negative fecundities are biologically feasible. Several models based on different assumptions have been proposed to describe the growth of individuals, but none of them is linear. Our everyday experience confirms that we animals do not grow indefinitely, but undergo a series of changes in growth rate and ultimately reach a maximum size that changes very little as we grow older. Perhaps because linearity in vital rates is so biologically absurd we know little about how demography would change if linearity were assumed. However, the specific form of non-linearity chosen when modeling vital rates has a significant impact on our inferences about population dynamics [1].

Intraspecific competition has received much attention from population ecologists. The earliest models for population dynamics, i.e., the changes in population numbers over time, already incorporated the effect of diminishing resource availability as population density increases. The well-known logistic curve dates from 1838. Subsequent development of the discipline has resulted in a true bestiary of functions that describe population growth, both in continuous and discrete time. These models are known by the names of their authors: Ricker, Gompertz, Hassell, Beverton-Holt, Maynard-Smith and so on. All of them are non-linear, as changes in competition over time modify the population growth rate, which eventually becomes zero (or shows a more or less complex dynamics). Each model is based on different mechanistic assumptions, and appears to describe accurately the dynamics of different taxa. For instance, the Ricker model

$$N_{(t+1)} = \lambda N_t e^{-\alpha N_t} \tag{1}$$

(where N_t is the size of the population at time t, λ is the intrinsic growth rate, and α is the per-capita competitive effect) has been used to model animal populations successfully [2], while the Hassell model

$$N_{(t+1)} = \frac{\lambda N_t}{(1 + \alpha N_t)^{\beta}} \tag{2}$$

almost universally provides the best fit to plant data. It must be noted that β frequently equals one, so the Hassell model becomes the Beverton-Holt model in most plant species [3].

Population growth models have played a prominent role in the development of the theory of complex systems. In a groundbreaking paper, Robert May (who at the time was studying population growth models with George Oster) popularized the notions of bifurcations and chaos by showing that, as the intrinsic population growth rate increases, the population does not reach a fixed size but instead cycles between two points. Further increments in the intrinsic growth rate induce new doubling (or bifurcations) of the number of points in the cycle until the modeled behavior of the population becomes extremely complex, i.e., chaotic [4]. The bifurcation diagram of the model analyzed by May has since become one of the visual stereotypes of complexity and chaos.

In population models, complex dynamics can only occur if the population does not approach the carrying capacity monotonically, but "overshoots" it [5]; hence the name overcompensation for such behavior. The mechanism is easy to envisage: assume a population of annual insects below its carrying capacity. Because resources are abundant, the number of eggs laid can be extremely large, leading to a population that is well above the carrying capacity in the next year. Then, calamitous competition may preclude reproduction, again sending the population back to a negligible density. In simple continuous-time models, where the population regulates itself instantaneously in response to changes in density, such behavior in unlikely. Time lags may nevertheless occur. As I write this, children in the Mexican population are less numerous than teenagers as a happy result of a reduction in birth and population growth rates. However, when the current demographic wave of teenagers becomes reproductive, Mexico's population growth is expected to accelerate again as a time-lagged echo of larger birth rates in the past. Thus, time lags may induce models that resemble overcompensation and thus result in very complex dynamics [6]. Overcompensation can only arise if the function relating N_{t+1} to N_t reaches a maximum for some value of N_t [2]. Nevertheless, it must be noted that density-dependence in one of the vital rates of a demographic model may result in very complex dynamics regardless of whether it is over- or undercompensating [7].

Allee effects are ubiquitous in nature and result necessarily in a non-linear behavior. Allee effects occur when populations perform poorly when density is low because of inbreeding, low probability of finding mates, collapse of social systems, lowered capability to deter predators, etc. This means that the population growth rate may increase with density, but, because of intraspecific competition, growth must eventually decline if density is high enough. In models, the interaction between these opposing forces may result in chaos [8]. Such models also show another trait of complex systems: critical transitions. If the population is large enough it may persist indefinitely, but if its density falls below a critical threshold, the population becomes trapped in an "extinction vortex" and disappears [9].

Interspecific competition is similar to intraspecific competition in many respects, and the mathematical models used to study both of them are frequently the same, displaying the same range of complex responses. However, things become complicated when many species are considered. For instance, the Lotka-Volterra model with two species converges to a unique stable point, or else to one of two stable attractors depending on the initial conditions. However, if four species interact, chaos may appear, and with five species any complex behavior is possible [10].

The coexistence of several competing species has been the subject of a long-lasting debate in ecology. As early mathematical and experimental research demonstrated, in simple systems one or a few species outcompete the others. Temporal or spatial variability in environmental conditions have been proposed to preclude such extinctions, and may thus explain the high diversity observed in natural communities. Peter Chesson has shown that variability can only promote coexistence among competitors through two mechanisms: storage effects and relative non-linearities. The former occur if the effects of competition and environment are non-additive. Life-cycle stages that endure adverse conditions also promote storage effects by establishing strongly non-linear relationships between environmental conditions and population growth. Relative non-linearities permit coexistence in models when a species that would be displaced competitively under average environmental conditions has the most concave response to the environment. If so, this species becomes the strongest competitor under extreme events, which, if occur frequently enough, may rescue the species from imminent extinction [11]. Despite the (biological) simplicity and generality of Chesson's theory, storage effects and relative nonlinearities have seldom been tested in nature.

The population dynamics of predator-prey systems also relies heavily in non-linear phenomena. Perhaps the most important of these is the functional response of the predator, i.e., the number of prey consumed as a function of prey density. On one hand, increased prey density reduces the time that the predator invests in searching for food, thus increasing the consumption rate. On the other hand, there is a minimum time required to handle and assimilate food items, setting an upper limit to the number of prey that the predator may eat per time unit. The balance between these opposing trends imposes a strong non-linearity on functional responses. The specific form of these functions depends on the biological attributes of the system such as the capability of many predators to switch between different prey items. Several models have been proposed to describe functional responses, such as the popular Holling equations in all their variants [12, 13].

The form of the functional responses determines the dynamics of predation models in several ways. It affects coexistence: it is easier for animals preying on the same items to coexist if their functional responses are different. Also, models with type-III functional responses (in which predators ignore specific prey items when their density is low) may easily result in the growth of the prey population when it has a low density, allowing it to recover when it becomes scarce. This does not happen when the functional response is type II, making coexistence more difficult. Functional responses also determine whether the model's dynamics shows damped oscillations, cycle limits, or is unstable [12, 13].

Traditionally, models assume that the vital rates or the density-dependent interactions are averaged across all the individuals in the population. More sophisticated approaches that simulate the behavior of each organism are becoming increasingly popular [15]. One of the reasons why individual-based models have a remarkable predictive power in plant ecology is that they explicitly incorporate space. Interactions between plants in a population depend non-linearly on the space between them, and the dispersal kernels of seeds are frequently non-monotonic functions of distance [16]. Limited dispersal causes the ag-

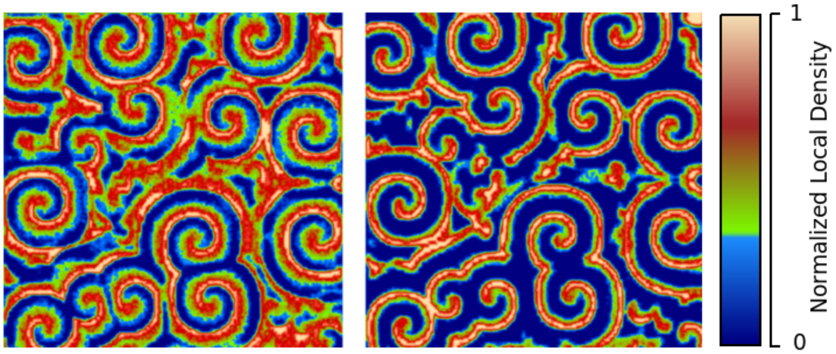


Figure 1: Self-organized spiral spatial patterns that emerge in a predator-prey system. Colors correspond to different predator (in this case a parasitoid; right panel) and prey (host; left panel) local densities. Modified from Rohani & Miramontes 1995 [14]. Image courtesy of the authors.

gregation of individuals, increasing the demographic importance of intraspecific interactions inordinately relative to their per-capita intensity [17]. Spatial patterns also determine the population-level outcome interspecific competition, which may not be proportional to the competitive ability of species [18].

One of the most appealing emergent properties of non-linear, spatially-explicit models is self-organization. Mosaics of areas dominated by different competitors permit coexistence in models that otherwise predict extinction. Beautiful spirals characterize the landscape when predation drives population dynamics [19](see Figure 1). At least that is what theory says: the empirical evidence for such phenomena in real populations is still weak. Nevertheless, some intriguing results have arisen from these models: for instance, a minimal area is required for self-organization to permit coexistence. Such insights are most relevant when designing natural preserves or assessing the conservation value of vegetation remnants .

4 Concluding remarks

By now it must be obvious that non-linearity is omnipresent in population ecology, and that perhaps every conceivable form of complexity may arise from it. I would like to finish stressing some points that hopefully are also apparent by now. 1) The outcome of non-linearity is heavily dependent on its functional form. Theoretical ecology is ripe with non-linear models, but such corpus is rarely confronted with the data in a direct, quantitative manner: empirical studies are full of proxies and indirect indexes that preclude direct tests of the theory. It is encouraging that this panorama has been changing in the last couple of decades thanks to potent statistical techniques and computational tools. It is critical to know which non-linear models are appropriate and under which circumstances.

2) Perhaps because of the hypnotic beauty of complexity, much emphasis has been placed on searching for complexity in our models. However, we need to test if the observed complexity in real populations actually arises from the mechanisms assumed in our models. Lets assume that we observe a complex population-dynamics: does this complexity arise from specific parameters in a simple model such as May's? Or is it the result of interactions between several populations as in the Lotka-Volterra model? We need to build models that comprise the intricacies of nature [20], parametrize them with real data, and then analyze if the dynamics of the model is truly complex and why. 3) That deterministic systems may be inherently complex must not blind us from the fact that populations are also driven by external factors that ecologists envision as stochastic (e.g., climate, disturbances). In practice, stochasticity and chaos produce similar patterns that are difficult to tell without very large data sets. Methods such as the analysis of non-linear time series are becoming available for such task, but are still cumbersome or unable to deal with high-dimensionality problems. We need to understand how complexity interacts with environmental noise in model and real populations. This interaction may itself be complex, as it happens with resonance [21]. Population biology needs to link the complexities in the mathematical and the real worlds –and exemplar efforts are currently under way.

5 Bibliography

[1] J. P. Dahlgren, M. B. García, and J. Ehrlén, "Nonlinear relationships between vital rates and state variables in demographic models," *Ecology*, vol. 92, no. 5, pp. 1181–1187, 2011.

[2] B. K. Williams, J. D. Nichols, and M. J. Conroy, *Analysis and management of animal populations: modeling, estimation, and decision making*. Academic Press, 2002.

[3] R. P. Freckleton and A. R. Watkinson, "Nonmanipulative determination of plant community dynamics," *Trends in Ecology & Evolution*, vol. 16, no. 6, pp. 301–307, 2001.

[4] R. M. May *et al.*, "Simple mathematical models with very complicated dynamics," *Nature*, vol. 261, no. 5560, pp. 459–467, 1976.

[5] W. Gurney and R. M. Nisbet, *Ecological dynamics*. Oxford University Press, Oxford, 1998.

[6] O. N. Bjørnstad and B. T. Grenfell, "Noisy clockwork: time series analysis of population fluctuations in animals," *Science*, vol. 293, no. 5530, pp. 638–643, 2001.

[7] H. Caswell, *Matrix population models*. Wiley Online Library, 1989.

[8] S. J. Schreiber, "Allee effects, extinctions, and chaotic transients in simple population models," *Theoretical Population Biology*, vol. 64, no. 2, pp. 201–209, 2003.

[9] M. E. Gilpin, "Minimum viable populations: processes of species extinction," in *Conservation biology: the science of scarcity and diversity*, M. E. Soule, Ed. Sinauer, MA, USA, 1986, pp. 19–34.

[10] S. Smale, "On the differential equations of species in competition," *Journal of Mathematical Biology*, vol. 3, no. 1, pp. 5–7, 1976.

[11] P. Chesson, "Mechanisms of maintenance of species diversity," *Annual Review of Ecology and Systematics*, vol. 31, no. 1, pp. 343–366, 2000.

[12] T. J. Case, *An illustrated guide to theoretical ecology*. Oxford University Press, 2000.

[13] M. Kot, *Elements of mathematical ecology*. Cambridge University Press, 2001.

[14] P. Rohani and O. Miramontes, "Host-parasitoid metapopulations: the consequences of parasitoid aggregation on spatial dynamics and searching efficiency," *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 260, no. 1359, pp. 335–342, 1995.

[15] V. Grimm and S. F. Railsback, *Individual-based Modeling and Ecology*. Princeton University Press, 2005.

[16] R. Nathan, "Dispersal biogeography," *Encyclopedia of biodiversity*, vol. 2, pp. 127–152, 2001.

[17] L. A. Turnbull, D. A. Coomes, D. W. Purves, and M. Rees, "How spatial structure alters population and community dynamics in a natural plant community," *Journal of Ecology*, vol. 95, no. 1, pp. 79–89, 2007.

[18] J. Silvertown and J. B. Wilson, "Spatial interactions among grassland plant populations," *The Geometry of Ecological Interactions: Simplifying Spatial Complexity. Cambridge University Press, Cambridge*, pp. 28–47, 2000.

[19] R. V. Solé and J. Bascompte, *Self-Organization in Complex Ecosystems.(MPB-42)*. Princeton University Press, 2006, vol. 42.

[20] M. R. Evans, V. Grimm, K. Johst, T. Knuuttila, R. de Langhe, C. M. Lessells, M. Merz, M. A. O'Malley, S. H. Orzack, M. Weisberg *et al.*, "Do simple models lead to generality in ecology?" *Trends in Ecology & Evolution*, vol. 28, no. 10, pp. 578–583, 2013.

[21] N. B. Janson, "Non-linear dynamics of biological systems," *Contemporary Physics*, vol. 53, no. 2, pp. 137–168, 2012.

Randomness in Biology

P. Miramontes, Facultad de Ciencias UNAM, Mexico

1 Abstract

Using concepts of dynamical systems theory a formal framework of randomness is advanced. Given the fact that chance plays an important role in biology and specifically in evolutionary biology, the purpose of this essay is to reevaluate, under this framework, the way we perceive how biological phenomena may be operating.

2 Resumen

El azar juega un papel muy relevante en la biología. Aquí se propone un nuevo enfoque para lo que se debe de entender como aleatorio y se espera que dicha propuesta modifique la manera como percibimos los fenómenos biológicos.

3 Introduction

The concept of randomness is deeply ingrained in biology. Specifically in evolutionary biology where it plays a major role in the Neodarwinian scheme where the major, or even unique, source of genoptypic variation is random mutations. As a matter of fact, in the 1940s and 1950s, the Modern Synthesis fused the Darwin's principle of natural selection with Mendelian inheritance to create the Synthesis where the gradual and random allelic substitution is the only source of evolutionary change [1]

We now know that this is far from being true because there are some mechanisms contributing to the genotypic diversity that are not either random or small, in the Darwinian sense. Just for example: gene and genome duplication, horizontal gene transfer or symbiogenesis [2].

As of now, it seems to be that there is a vast majority of scholars accepting that the source of biological variation are random but its products are not and that the origins of biological order is natural selection. Then the lack of a clear definition of what should be understood by random is somewhat shocking. Most of the people feel comfortable with the dictionary definition of randomness as "Having no definite aim or purpose; not sent or guided in a particular direction; made, done, occurring, etc." Phrased this way (Oxford Dictionary) it seems tailored in a circular way to fulfill the Neodarwinian needs.

4 Chaos and Time Series

Irregular, uncorrelated behavior in biology used to be conceived as random without any further inquiry and with no analysis of the meaning of randomness. Most of the times it is modeled by adding white noise to a deterministic signal. The sharp separation between determinism and randomness was originated by a wrong identification of determinism with predictability. This scheme is being abandoned as our knowledge of chaos is spreading. The possibility of finding unpredictable behavior in a deterministic system was a shock for the advocates of the dichotomy determinism–randomness. Thanks to the seminal papers of Edward Lorenz [3] and Robert May [4] [1] chaotic systems have today a legitimate place in science.

Chaotic behavior is deterministic and unpredictable at the same time and notwithstanding it has a number of hidden regularities that allow the researcher to measure the degree of correlation (or lack of), the predictability horizon and the structure of the long-term behavior, the so-called *Attractor*. Before the advent of chaos theory, an attractor could be a point attractor or a periodic cycle. At present, it is well known that chaotic regimes give birth to attractors having fractal structure (the opposite is not always true). Ruelle and Takens christened them as *strange attractors*.

When collecting data from the field or the lab, a biologist usually gets it organized as a sequence of one or many variables taken at uniform time intervals. This is a *Time Series* and mathematically is expressed as a sequence

$$\{x_i\}_{i=1}^{n} \tag{1}$$

An actual time series from an experiment or census is hardly random [2]. Then it should be the outcome of a dynamical system, if this is the case, it could be an iterated system $x_{i+1} = f(x_i)$ or the discretization of a differential equation $x_{i+1} = hf(x_i) + x_i = g(x_i)$.

5 Attractor Reconstruction

A very important and yet unsolved, in general, task is to find the dynamical system that produces a given the time series. A celebrated advance in this direction is the embedding

---

[1] The chaotic behavior was already seen by Henri Poincaré by the end of the XIX century. See [5]

[2] In any sense of the term. Accepting the opposite would mean that there are no natural laws

theorem by F. Takens [6]. In 1980 he demonstrated that to find the dimension (the number of state variables) of a dynamical systems it is enough to know the output of one of them to reconstruct the attractor.

Let us assume that we have a scalar (one dependent variable) time series and that the underlying dynamics has an strange attractor embedded in an space whose dimension is yet to be determined (point or periodic attractors are easily detected by many ways so they are excluded from now on). The procedure is as follows:

- Construct a vector time series from the original one by pairing consecutive values allowing a time gap

  $$x_i \mapsto (x_i, x_{i+\tau}) \tag{2}$$

  The τ parameter is chosen by trial and error. If τ is too small the points will be too close to each other and the main traits of the reconstructed attractor will be hidden in a flatten figure over the identity line in $\mathbb{R}^2$. If, on the other hand, τ is large the points will be uncorrelated and the plot will be a shapeless cloud. Now, repeat the procedure increasing the embedding dimension:

  $$x_i \mapsto (x_i, x_{i+\tau}, x_{i+2\tau}, \ldots, x_{i+n\tau}) \tag{3}$$

  until the attractor geometrically fully develops.

  Figure 1 shows the Lorenz Attractor. It is the destination set of a system of three differential equations. The left figure is the phase space of the solution trajectories of the numerically integrated systems. On the right, the reconstructed attractor using only the data of one state variable following the procedure above outlined with $\tau = 8$. The issue is that having just a one-variable time-series it is not clear to know in advance how many degrees of freedom has the still unknown system. To overcome this problem the following procedure is recommended:

- Measure the fractal dimension of the vector set in $\mathbb{R}^2$. There is a number of methods to numerically estimate the fractal dimension of a set in $\mathbb{R}^n$. One of the faster and more accurate ones is the *correlation dimension* [7]:

  $$C(\epsilon) = \lim_{\substack{n\to\infty \\ \epsilon\to 0}} \frac{g(\epsilon)}{N^2} \tag{4}$$

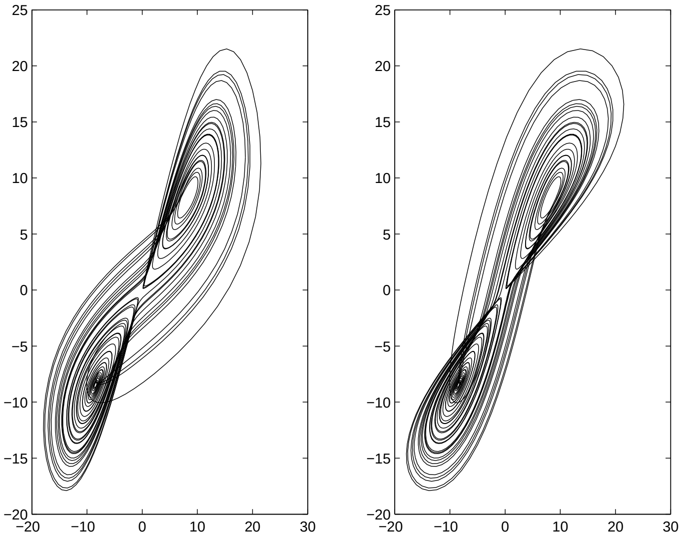


Figure 1: The Lorenz Attractor. The left figure Lorenz's System numerical solution. On the right, the attractor reconstruction after Takens' Theorem.

Where $g(\epsilon)$ stands for the number of points that are far from each other in less than ϵ. It is accepted that the correlation function scales as:

$$C(\epsilon) \sim \epsilon^d \tag{5}$$

where d is the *fractal (correlation) dimension*.

It is then straightforward to estimate the fractal dimension d as the slope of a line in a $\log(C(\epsilon))$ versus $\log(\epsilon)$. Once it is done, repeat the procedure and reevaluate the fractal dimension increasing the dimension of the embedding space. Plot the results in a diagram of the correlation dimension as function of the embedding dimension. The asymptote of the points (Figure 2) is the fractal dimension of the attractor and the integer number greater than it is the number of effective degrees of freedom of the putative dynamical system that produced the time series.

6 Discussion

Given a time series, it is then straightforward to follow the steps outlined above to reconstruct the putative attractor and to determine the number of state variables that are enough to engender it.

A white noise time series does not finds ever an asymptote; the straight line keeps going upwards as the embedding dimension growths. This is the signature of randomness. What we call chance, haphazard or stochastic is nothing more than high dimension

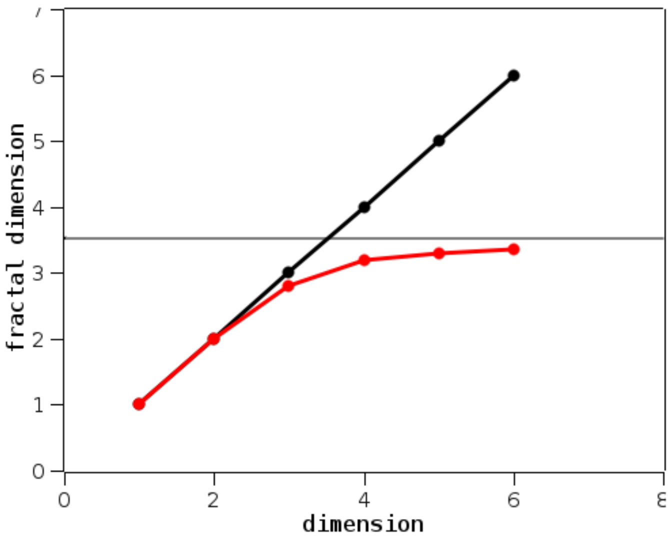


Figure 2: The asymptote of the red line is the dimension of the space that embedding the attractor. A white noise time series never bends down (black line)

chaos. It could be argued (mostly by mathematicians) that in any case randomness could be reached in the limit when the dimension approaches infinity and that those limits are never attained. This argument is easily treated when one reasons that the whole building of physics is founded in the model of the continuum of real numbers and that there seems to be no contradiction with the fact that physical objects are not continuous. Taking limits when one approaches zero or infinity should be thought as metaphors in Natural Sciences. So far I have shown that what we call randomness is nothing more that high–dimensional chaos[3].

Creationism often recur to the thought experiment of a typing monkey to claim the supposedly impossibility of Biological Evolution. Of course they are right of the monkey acts randomly. What they do not say is that evolution is restricted by Physical constraints that reduce the possible outcomes over which selections acts. Under constraints it is like the monkey typing over a doctored typing machine that does not allow forbidden dimers (in English the dimers “ww”, “qq”, etc. do not exist), trimers, tetramers, and having also limits over the word length. After all these restrictions the typing machine is not random anymore and it could be shown that it is colored noise and their reconstructed phase portraits are low dimensional. Nonetheless, there is no formal studies about the relationship of colored noise and chaos.

---

[3] An anonymous reviewer called my attention to the paper: “Chaos and Deterministic versus Stochastic Nonlinear Modeling” (Santa Fe Institute Working Paper, where Martin Casdagli advanced similar ideas years ago.)

There is fear to accept a deterministic Nature. This fear is understandable because "biological determinism" is a well characterized ideological posture that is frequently identified with political conservatism. There is nothing wrong with accepting a deterministic biology in the sense outlined in this essay even acknowledging that the ultimate components of biological systems should obey Quantum Mechanics where the notion of determinism gets blurred. The realm of Biology is far from the weird phenomena occurring in Quantum Mechanics; the Theory of Complex Systems teaches us that as we move up in the hierarchical ladder of the organization of matter, the laws governing the lower levels become irrelevant to describe the upper ones. To identify determinism with teleology is also a mistake since we understand the laws of chaos. Chaotical phenomena have no purpose and are unpredictable and, notwithstanding, are deterministic. Is there a real randomness? The kind of randomness illustrated by a Casino roulette?. The aim of this essay is to invite the community to view even this case as a case of ultimate determinism. In this framework, there is no point to treat as separate concepts randomness and high-dimensional chaos.

Randomness should be admitted as the mask we use to cover our lack of knowledge. Biology would gain a lot of understanding in the real meaning of its object of study if it accepts that behind what we call Randomness there are natural laws acting.

7 Bibliography

[1] S. Gould, "Is a New and General Theory of Evolution Emerging?" in *Self-Organizing Systems*, ser. Life Science Monographs, F. Yates, A. Garfinkel, D. Walter, and G. Yates, Eds. Springer US, 1987, p. 113–130.

[2] E. V. Koonin, "Darwinian evolution in the light of genomics," *Nucleic Acids Research*, vol. 37, no. 4, pp. 1011–1034, 2009.

[3] E. N. Lorenz, "Deterministic nonperiodic flow," *Journal of the Atmospheric Sciences*, vol. 20, no. 2, pp. 130–141, 1963.

[4] R. M. May, "Simple mathematical models with very complicated dynamics," *Nature*, vol. 261, no. 5560, pp. 459–467, 1976.

[5] F. Diacu and P. Holmes, *Celestial encounters: the origins of chaos and stability*. Princeton, NJ: Princeton Univ. Press, 1996.

[6] F. Takens, "Detecting Strange Attractors in Turbulence," in *Dynamical systems and turbulence*, D. A. Rand and L. S. Young, Eds. New York: Spinger-Verlag, 1980, p. 366–381.

[7] P. Grassberger and I. Procaccia, "Measuring the strangeness of strange attractors," *Physica D*, vol. 9, p. 189, 1983.

Bridging genotype and phenotype

J. Davila-Velderrain & E.R. Alvarez-Buylla, IE and C3, UNAM, Mexico

1 Abstract

Understanding the mapping of genotypes into phenotypes is a central challenge of current biological research. Such mapping conceptually represents a developmental mechanism through which phenotypic variation can be generated. Given the nongenetic character of developmental dynamics, phenotypic variation to a great extent has been neglected in the study of evolution. What is the relevance of considering this generative process in the study of evolution? How can we study its evolutionary consequences? Despite an historical systematic bias towards linear causation schemes in biology; in the post-genomic era, a systems-view to biology based on nonlinear (network) thinking is increasingly being adopted. Within this view, evolutionary dynamics can be studied using simple dynamical models of gene regulatory networks (GRNs). Through the study of GRN dynamics, genotypes and phenotypes can be unambiguously defined. The orchestrating role of GRNs constitutes an operational *non-linear* genotype-phenotype map. Further extension of these GRN models in order to explore and characterize an associated Epigenetic Landscape enables the study of the evolutionary consequences of both genetic and non-genetic sources of phenotypic variation within the same coherent theoretical framework. The merging of conceptually clear theories, computational/mathematical tools, and molecular/genomic data into coherent frameworks could be the basis for a transformation of biological research from mainly a descriptive exercise into a truly mechanistic, explanatory endeavor.

2 Resumen

Entender el mapeo de genotipo a fenotipo es un problema central en la investigación biológica moderna. Este mapeo representa conceptualmente un mecanismo de desarrollo capaz de generar variación fenotípica. Dado el carácter no genético de la dinámica del desarrollo, la variación fenotípica en gran medida ha sido ignorada en el estudio de la evolución. Cual es la relevancia de considerar este proceso generativo en el estudio de evolución? Como podemos estudiar sus consecuencias evolutivas? A pesar de una tendencia histórica hacia los esquemas lineales de causalidad en biología, en la actualidad la adopción de un enfoque de sistemas basado en razonamiento no lineal y de redes es cada vez más común. Dentro de este enfoque, la dinámica evolutiva puede ser estudiada mediante el uso de modelos dinámicos simples de redes regulatorias genéticas (RRGs). Mediante el estudio de la dinámica de RRGs, es posible definir genotipos y fenotipos. El rol coordinador de las RRGs constituye un modelo operacional de mapeo de genotipo a fenotipo. La extensión de estos modelos de RRGs con el objetivo de explorar y caracterizar un Paisaje Epigenético asociado permite el estudio de las consecuencias evolutivas de fuentes de variación fenotípica –tanto genéticas como no genéticas– en un mismo marco teórico coherente. La fusión de teorías conceptuales claras, herramientas matemático/computacionales y datos moleculares/genómicos en modelos coherentes podría ser la base de una transformación de la investigación en biología: pasando de ser un ejercicio principalmente descriptivo hacia un verdadero esfuerzo mecanístico y explicativo.

3 Introduction

The mechanistic understanding of the mapping of genotypes into phenotypes is at the core of modern biological research. During the lifetime of an individual, a developmental process unfolds, and the observed phenotypic characteristics are consequently established. As an example, a given individual may or may not develop a disease. Can we explain the observed outcome exclusively in terms of genetic differences and an unidirectional linear relationship between genotype and phenotype? Researchers in biology have mostly assumed so. Over the last decades, scientists under the guidance of such genetic-causal assumption have struggled with inconsistent empirical observations. The biological relevance of the phenotypic variability produced during the developmental process itself, and not as the consequence of genetic mutations, has only recently started to be acknowledged [1–5].

Understanding the unfolding of the individuals phenotype is the ultimate goal of developmental biology. Evolutionary biology, on the other hand, is largely concerned with the heritable phenotypic variation within populations and its change during long time periods, as well as the eventual emergence of new species. Historically, population-level models seek to characterize the distribution of genotypic variants over a population, considering that the phenotypic variation is a direct indicator of genetic change. Certain assumptions are implicit to such reasoning. Are those assumptions justifiable in light of the now available molecular data and the recently uncovered molecular regulatory mechanisms? What is the relevance of considering the generative developmental sources of phenotypic variation in the study of evolution? The aim of this paper is to highlight how a systems view to biology is starting to give insights into these fundamental questions. The overall conclusion is clear: an unilateral genocentric approach is not enough. Evolution and development should be integrated through experimentally supported mechanistic dynamical models [6–13].

In the sections that follow, we first present a brief historical overview of evolutionary biology and the roots of a systematic bias towards linear causation schemes in biology. Then, we discuss the assumptions implicit in the so-called neo-Darwinian Synthesis of Evolutionary Biology – the conventional view of evolution. In the last section, we briefly describe an emerging research program which aims to go beyond the conventional theory of evolution, focusing on a nonlinear mapping from genotype to phenotype through the restrictions imposed by the interactions in gene regulatory networks (GRNs) and its associated epigenetic landscape (EL). Overall, this contribution attempts to outline how the orchestrating role of GRNs during developmental dynamics imposes restrictions and enables generative properties that shape phenotypic variation.

4 Darwin's Legacy

Darwin eliminated the need for supernatural explanations for the origin and adaptations of organisms when he put evolution firmly on natural grounds [14]. In the mid-19th century, Darwin published his theory of natural selection [15]. He proposed a natural process, the gradual accumulation of variations sorted out by natural selection, as an explanation for the shaping and diversity of organisms. This insight was what put the study of evolution within the realms of science in the first place [14]. Although it has had its ups and downs [16], the Darwinian research tradition predominates in modern evolutionary biology. Much of its success is due to a new (gene-centric) interpretation, the so-called neo-Darwinian modern synthesis [17]: the merging of mendelian genetics and Darwin's theory of natural selection due to prominent early 20th century statisticians. In this framework, development was left outside, and evolution is seen as a change in the genotypic constitution of a population over time. Genes map directly into phenotypes (see Figure 1a), implicitly assuming that genetic mutation is the prime cause of phenotypic variation. Observed traits are generally assumed to be the result of adaptation, the process whereby differential fitness (the product of the probability of reproduction and survival) due to genetic variation in a particular environment, leads to individuals better able to live in such an environment.

From Natural Selection to Natural Variation

Natural selection -a force emanating from outside the organism itself- is the conceptual core of the Darwinian research tradition. Conceptually, the general process is as follows. *Random* mutations occur during reproduction; these mutations are responsible for generating different (genetic) types of individuals. The selection process then results from the fact that each type has certain survival probability and/or is able to achieve certain reproductive performance given the environment. Through this differential rate, some types are maintained while others are dismissed. It is said that, in this way, selection makes a "choice" [18]. From a wider perspective, it is generally accepted that selection is a generic process not restricted to biological evolution [19]. Any error-prone communication process in which information is consequently transmitted at different rates leads itself to a selection mechanism. However, despite the appealing conceptual clarity of the selection mechanism, it is not generally appreciated that the complexity inherent to biological systems hinders the mechanistic understanding of biological evolution. Because the reproductive performance of a given type of variant is, mainly, a function of its phenotype; the paradigmatic selection process described above is plausible when one assumes a straightforward causation of phenotype by genotype [10]. A more faithful model of biological evolution should explicitly consider a genotype-phenotype (GP) map [20, 21], a developmental mechanism which specifies how phenotypic variation is generated (Figure 1b). The generated variation is then what triggers selection [22]. Importantly, a deviation from a linear causation view of development would potentially impact the rate and direction of evolution [8, 23, 24].

Although not always discussed, Darwin himself devoted much more attention to variation than to natural selection, presumably because he knew that a satisfactory theory of evolutionary change requires the elucidation of the causes and properties of variation [25]. After all, natural selection would be meaningless without variation. Ironically, given the success of the neo-Darwinian framework, phenotypic variation to a great extent has been neglected in the study of evolution [26]. The mechanistic understanding of the sources of phenotypic variation constitutes a fundamental gap in conventional evolutionary theory. Neither Darwin, nor the founders of the neo-Darwinian modern synthesis were able to address this problem given the biological knowledge available at the time. Moreover, deviations from the basic assumptions of the conventional theory were not always generally appreciated [27].

Implicit Assumptions in Evolution

Being the development of science an evolutionary process itself, it is reasonable to expect that social-historical contingency has profoundly biased the pathways of scientific inquiry. This seems to be the case in the history of biology. For example, (1) Darwin's war against divine explanations for biological complexity caused within the scientific community an automatic rejection for any goal-oriented activity within organisms. This situation favored the adoption of the idea of random (uniform) variation [28, 29]. (2) The mainstream focus of neo-Dawinism on optimizing reproductive success (fitness) by natural selection of random variants; on the other hand, implicitly neglected the relevance of gene interactions (see Figure 1a) [30]. Finally, (3) the establishment of the central dogma of molecular biology (gene → mRNA → protein) further cemented a linear, unidirectional scheme of causation of molecular traits (one gene - one protein, one trait) [10]. These events are thought to be associated with a deeply rooted systematic bias towards linear causation schemes in biology [10, 31]. They also favored the adoption of three major implicit assumptions upon which the neo-Darwinian tradition was developed, namely: (1) mutational events occur randomly (e.g. unstructured) along the genome; (2) given that the phenotypic effects of successive mutations in evolution are of additive nature, gene interactions and their phenotypic influence can be, to a large extent, ignored; and (3) the phenotypic distribution of mutational effects mirrors the genetic distribution of mutations [30].

Scientists are now re-examining the most basic assumptions about evolution in light of post-genomic, systems biology [28, 32]. Compelling evidence has been presented even against assumption (1) above. For example, Shapiro has shown how a truly random (unstructured) nature of mutational events is empirically unsustainable. He has coined the term "natural genetic engineering", referring to the known operators that produce genomic changes and which are subjected to cellular regulatory regimes of epigenetic character [29]. It seems that the generative properties of genetic variation are nonuniform, and thus, biased as well. Assumptions (2) and (3) above are, instead, mainly concerned with how phenotypic variation is generated given a genetic background; or in other words, with the mechanistic understanding of the GP map. Here, we are concerned with this developmental process and its evolutionary relevance.

5 From Genes to Networks

At the beginning of the 21th century, biology confronted an uncomfortable fact: despite the increasing availability of whole-genome sequence data, it was not possible to predict, or even clarify, phenotypic observations. In fact, we now know that there is not sufficient information in the linear DNA sequences of the complete genomes to recover and/or understand the diverse phenotypic states of an organism. It was clear that cell behavior was much more complex than anticipated. Since then, biological research has increasingly been oriented towards a systems-level approach that goes beyond obtaining and describing large data sets at the genomic, transcriptomic, proteomic or metabolomic levels. An assumption of such *systems* approach to biology is that cell behavior can be understood in terms of the dynamical properties of the involved molecular regulatory networks. Modern molecular evolutionary studies are starting to incorporate this network thinking: genes are not individual entities upon which evolutionary forces act independently. Evolutionary forces, functional constraints, and molecular interactions are conditionally dependent on the systems level [33]. How a systems-view impacts our understanding of the GP map?

Fundamental Sources of Natural Variation

Although the concepts of genotype and phenotype are fundamental to evolution, it is not straightforward to operationally define them: In practice genotype and phenotype dis-

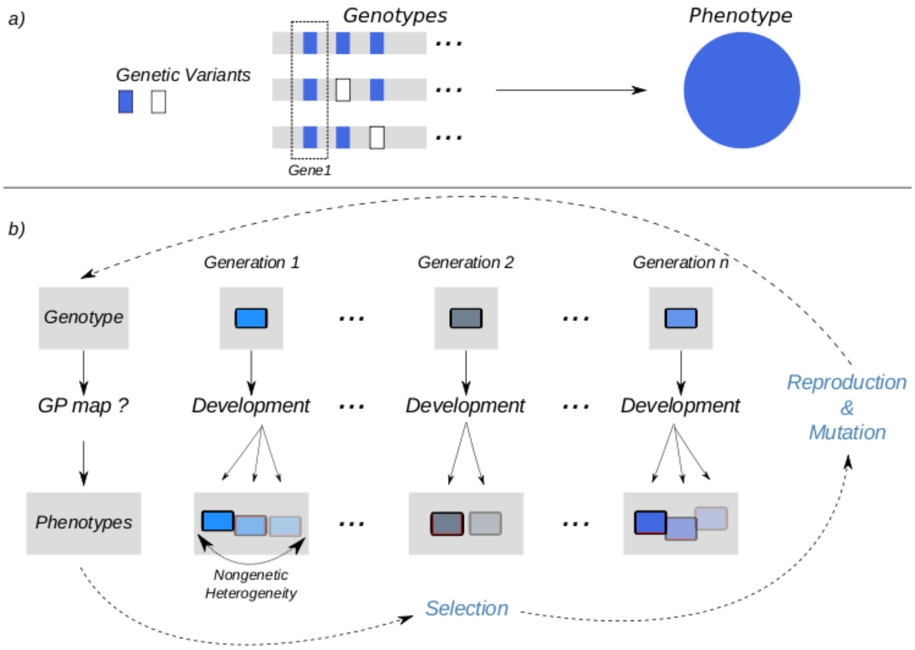


Figure 1: a) A straightforward genotype-phenotype relationship: the genetic distribution of the observed locus would completely mirror the phenotypic distribution; gene interactions are ignored; as a result, three different genotypes would correspond to the same phenotype given the locus under observation. b) A developmental process from genotype to phenotype, a GP map: through the development of an individual nongenetic phenotypic variation is generated each generation; in an evolutionary time-scale, evolution operations (blue) produce genetic variation. Selection acts on phenotypes; phenotypic variation is the product of both genetic mutational operations and epigenetic developmental processes.

tinctions are just partial [34]. This is part of the reason why simple theoretical models are so important for the epistemology of evolution. A common working model in systems biology is that in which the phenotypic state is defined at the cellular level. The cellular phenotype is represented by the activity of each of its genes, its expression pattern. Since the regulatory interactions among the genes within the cell constitute a network, the network effectively represents the genotype of the cell, while its associated expression profile represents its phenotype (Figure 2). The structure of the former derives directly from the genome, while the latter changes through development. In practice, we just observe certain expression patterns (e.g cell-types) - with small deviations - and not others. Why is that?

GRN developmental dynamics generates phenotypic nongenetic (epigenetic) heterogeneity

When thinking in terms of a genotype-phenotype distinction based on GRN dynamics, it is natural to consider an abstract space where all the virtually possible phenotypes reside. We call this space the *state-space*. Empirical observations suggest that something should be maintaining cells within specific, restricted regions of this space. The structured nature of the underlying GRN determines a trajectory in this state-space: given the state of the genes regulating a gene i, and the functional form of the regulation, the gene i is canalized to take specific future states. Eventually, this self-organizing process would inevitably lead to the establishment of those states which are logically consistent with the underlying regulatory logic. In this way, the GRN imposes constraints to the behavior of the cell. The resultant states are denominated *attractors* and correspond to observable cell-types. These are the basis of the well developed dynamical-systems theory of cell biology (for a review, see [35, 36]). This theory was first applied to propose a GRN grounded on experimental data for understanding how cell-fate specification occurs during early flower development (see, [37, 38] and update in [39]). Originally, the approach was inspired by theoretical work in randomly assembled networks by Stuart Kaufman [40]. In the last decades, the theory has been supported by a wealth of consolidated theoretical and experimental work (see, for example [7, 13, 41]).

Through GRN dynamics, development generates cellular phenotypes. The general acceptance of this generative role necessarily implies deviations from the neo-Dawinian framework. Importantly, (1) the effect of a perturbation (mutational or otherwise) on the manifested phenotype is not uniformly distributed (truly random) across all the genes in the network, and (2) the interactions in the network are fundamental to the establishment of the phenotype. The orchestrating role of GRNs constitutes a *non-linear* GP map: phenotypic variation does not scale proportionally to genotypic variation; it is not linear (Figure 2). Two important consequences of these mechanistic view of developmental dynamics have been eloquently pointed out recently. First, the nonlinear character of this mapping ensures that the exact same genotype (network) is able to produce several phenotypes (attractors) [40]. Second, given that molecular regulatory events are stochastic in nature, a cell is able to explore the state-space by both attracting and dispersing forces - forces that slightly deviate the dynamics from the determined trajectory. Any phenotype of a cellular population at any given time is statistically distributed: even seemingly homogeneous, isogenic populations present molecular phenotypic heterogeneity at the single-cell level [10]. These sources of variation are the natural product of developmental dynamics. Consequently, at any given time, a population can manifest phenotypic variation that is relevant to evolution (heritable) in the absence of genetic variation. How can we study evolution without ignoring the fundamental role of developmental dynamics?

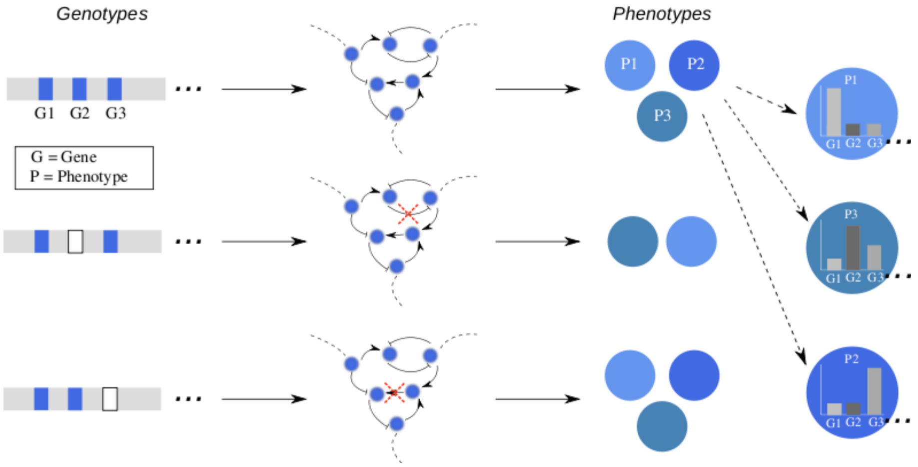


Figure 2: The orchestrating role of GRNs constitutes a *non-linear* GP map. Through the restrictions imposed by the interactions in GRNs, cellular phenotypes (represented by expression profiles) are generated. Due to the nonlinear character of GRN dynamics, the GP map is one-to-many. The effect of mutations in the phenotype is not uniformly distributed over the genes, but depends on the interactions: mutations can or cannot result in different phenotypes depending on the genetic background and the location of the affected genes in the network.

6 Evolutionary Systems Biology Approaches

A systems view to evolutionary biology, in which network models as GP mappings are considered explicitly, is under development (see, for example [9, 11, 42]). Within this general framework, several specific approaches are proposed in order to study the evolutionary consequences of considering developmental sources of phenotypic variation. In this section, we briefly present a preview of an emerging complementary approach.

Epigenetic (Attractors) Landscape Evolution

In 1950s, C.H. Waddington proposed the conceptual model of the epigenetic landscape (EL), a visionary attempt to synthesize a framework that would enable an intuitive discussion about the relationship between genetics, development, and evolution [43]. His reasoning was based on the consideration of a fact: the physical realization of the information coded in the genes - and their interactions - imposes developmental constraints while forming an organism. Now, in the post-genomic era, a formal basis for this metaphorical EL is being developed in the context of GRNs [10, 44, 45]. The key for this formalization is an emergent ordered structure embedded in the state-space, the attractors landscape (AL). As well as generating the cellular phenotypic sates (attractors), the GRN dynamics also partitions the whole state-space in specific regions and restricts the trajectories from one state to another one. Each region groups the cellular states that would eventually end up in a single, specific attractor. These sub-spaces are denominated the attractor's *basin* of attraction. Given this (second) generative property of GRN dynamics, the formalization of the EL in this context is conceptually straightforward: the number, depth, width, and relative position of these basins would correspond to the hills and valleys of the metaphorical EL. We refer to this structured order of the basins in state-space as the AL (see Figure 3). The characterization of an AL would correspond, in practical terms, to the characterization of an EL. Is this formalized EL useful for the mechanistic understanding of phenotype generation?

Multicellular morphogenetic processes unfold naturally in the EL

The structured EL is a generative property of the GRN dynamics, but at the same time, it also constrains the behavior of a developing system. While a developing system is following its dynamically constrained trajectory in state-space, developmental perturbations from internal or external origin can deviate it. In a cellular population, then, the probability of one phenotypic transition or another during development, as well as the stationary distribution of phenotypes, would be conditioned on both the localization of the individual cells in the EL and on the landscape's structure. As a general result of this interplay, determinism and stochasticity are reconciled, and robust morphogenetic patterns can be established by a hierarchy of cellular phenotypic transitions (see, for example [44, 45]). In this way, morphogenetic processes effectively unfold on ELs. How could this theoretical framework improve the understanding of evolutionary dynamics?

We have an effective nonlinear GP map from GRN to EL. Given an experimentally characterized GRN, the EL associated to real, specific developmental processes can be analyzed [13, 44, 45]. Both cellular phenotypes (attractors) and morphogenetic patterns are linked to the structure of the EL. Can we describe this structure quantitatively? How robust is the structure to genetic (network) mutation? Can we describe quantitatively the change in structure in response to both mutational and developmental perturbations? How slower is this rate of change in comparison to the time-scale of developmental dynamics (landscape explorations)? What are the phenotypic consequences of different relative rates of change? Does the resultant evolutionary trajectory of the reshaped EL structure subjected to mutations predicts the probability of phenotypic change (innovation) - based, for example, in the appearance of new cellular phenotypes or morphogenetic patterns? (Figure 3). Insight into these and similar questions could enhance the mechanistic understanding of the evolution of morphogenetic processes.

7 Conclusion and Challenges

A modern systems view to biology enables tackling foundational questions in evolutionary biology from new angles and with unprecedented molecular empirical support. Little

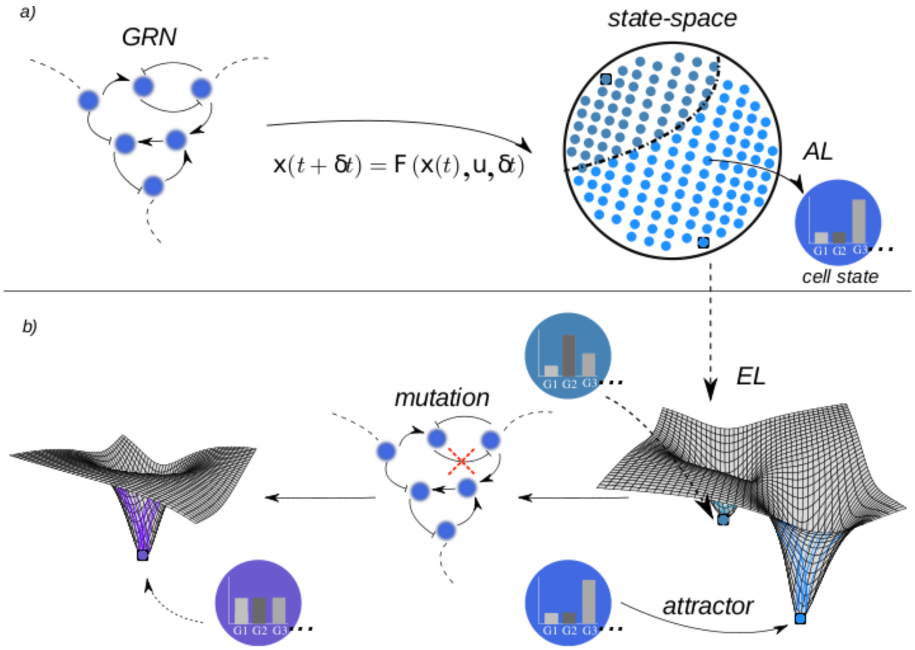


Figure 3: The Epigenetic (Attractors) Landscape. a) Through a dynamical mapping - a mathematical representation of the gene regulatory logic - GRNs generate both the cellular phenotypes (attractors) and the ordered structure of the state space - the AL. Through the structure of the AL, the EL is formalized in the context of GRNs. b) The number, depth, width, and relative position of attractors correspond to the hills and valleys of the EL. The topography of the landscape can change in response to perturbations. Mutations could eventually reshape the EL and consequently eliminate and/or generate novel phenotypes.

is known about the mechanistic sources of phenotypic variation and its impact on evolutionary dynamics. The explicit consideration of these processes in evolutionary models directly impacts our thinking about evolution. Simple, generic dynamical models of GRNs, where genotypes and phenotypes can be unambiguously defined, are well-suited to rigorously explore the problem. Further extension of these models in order to explore and characterize the associated EL enables the study of the evolutionary consequences of both genetic and non-genetic sources of phenotypic variation within the same coherent theoretical framework.

The network-EL approach to evolutionary dynamics is promising, as it directly manifests the multipotency associated with a given genotype - the capacity of a GRN to generate multiple, stable cellular phenotypes. Although conceptually clear and well-founded, its practical implementation implies several difficulties, nonetheless; specially in the case of large networks. Work has been done in which the landscape associated with a specific, experimentally characterized GRN is described quantitatively in terms of robustness and state transition rates [46], for example. However, neither the methodology to derive ELs from GRNs, nor the quantitative description of ELs are standard procedures. Most approaches require approximations and are technically challenging for the case of networks with more than 2 nodes. Further research in the quantitative description of experimentally grounded GRNs is still needed in order to explore the constraints and the plasticity of ELs associated with a genotypic (network) space. In this regard, discrete dynamical models are promising tools for the exhaustive characterization of the EL, and for the study of multicellular development [45]. A second major challenge is the generalization of GRN dynamical models in order to include additional sources of constraint during development. Tissue-level patterning mechanisms such as cell-cell interactions; chemical signaling; cellular growth, proliferation, and senescence; inevitably impose physical limitations in terms of mechanical forces which in turn affect cellular behavior. Although some progress has been presented in this direction [47, 48], the problem certainly remains open.

The post-genomic era of biology is starting to show that old metaphors such as Waddington's EL are not just frameworks for the conceptual discussion of complex problems. The merging of conceptually clear theories, computational/mathematical tools, and molecular/genomic data into coherent frameworks could be the basis for a much needed transformation of biological research from mainly a descriptive exercise into a truly mechanistic, explanatory and predictive endeavor - EL models associated with GRNs being a salient example.

8 Bibliography

[1] A. P. Feinberg and R. A. Irizarry, "Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease," *Proceedings of the National Academy of Sciences*, vol. 107, no. suppl 1, pp. 1757–1764, 2010.

[2] S. A. Frank and M. R. Rosner, "Nonheritable cellular variability accelerates the evolutionary processes of cancer," *PLoS Biology*, vol. 10, no. 4, p. e1001296, 2012.

[3] J. Freund, A. M. Brandmaier, L. Lewejohann, I. Kirste, M. Kritzler, A. Krüger, N. Sachser, U. Lindenberger, and G. Kempermann, "Emergence of individuality in genetically identical mice," *Science*, vol. 340, no. 6133, pp. 756–759, 2013.

[4] S. Huang, "Non-genetic heterogeneity of cells in development: more than just noise," *Development*, vol. 136, no. 23, pp. 3853–3862, 2009.

[5] A. O. Pisco, A. Brock, J. Zhou, A. Moor, M. Mojtahedi, D. Jackson, and S. Huang, "Non-Darwinian dynamics in therapy-induced cancer drug resistance," *Nature Communications*, vol. 4, 2013.

[6] E. R. Alvarez-Buylla, E. Azpeitia, R. Barrio, M. Benítez, and P. Padilla-Longoria, "From ABC genes to regulatory networks, epigenetic landscapes and flower morphogenesis: making biological sense of theoretical approaches," *Seminars in Cell & Developmental Biology*, vol. 21, no. 1, pp. 108–117, 2010.

[7] J. Jaeger and A. Crombach, "Life's attractors," in *Evolutionary Systems Biology*. Springer, 2012, pp. 93–119.

[8] J. Jaeger, D. Irons, and N. Monk, "The inheritance of process: a dynamical systems approach," *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, vol. 318, no. 8, pp. 591–612, 2012.

[9] A. Wagner, *The origins of evolutionary innovations*. Oxford University Press, Oxford, 2011.

[10] S. Huang, "The molecular and mathematical basis of Waddington's epigenetic landscape: A framework for post-Darwinian biology?" *Bioessays*, vol. 34, no. 2, pp. 149–157, 2012.

[11] O. S. Soyer, *Evolutionary Systems Biology*. Springer, 2012, vol. 751.

[12] M. Benítez, E. Azpeitia, and E. R. Alvarez-Buylla, "Dynamic models of epidermal patterning as an approach to plant eco-evo-devo," *Current Opinion in Plant Biology*, vol. 16, no. 1, pp. 11–18, 2013.

[13] E. Azpeitia, J. Davila-Velderrain, C. Villarreal, and E. R. Alvarez-Buylla, "Gene regulatory network models for floral organ determination," in *Flower Development*. Springer, 2014, pp. 441–469.

[14] F. J. Ayala, "Darwin's greatest discovery: design without designer," *Proceedings of the National Academy of Sciences*, vol. 104, no. Suppl 1, pp. 8567–8573, 2007.

[15] C. Darwin, *On the origins of species by means of natural selection*. London: Murray, 1859.

[16] D. J. Depew and B. H. Weber, *Darwinism evolving: Systems dynamics and the genealogy of natural selection*. Bradford Books/MIT Press, 1995.

[17] J. Huxley, *Evolution. The Modern Synthesis.* London: George Alien & Unwin Ltd., 1942.

[18] M. A. Nowak, *Evolutionary dynamics: exploring the equations of life*. Harvard University Press, 2006.

[19] P. Schuster, "Boltzmann and evolution: some basic questions of biology seen with atomistic glasses," *Boltzmann's Legacy*, pp. 217–241, 2008.

[20] R. C. Lewontin, *The genetic basis of evolutionary change*. Columbia University Press New York, 1974, vol. 560.

[21] P. Alberch, "From genes to phenotype: dynamical systems and evolvability," *Genetica*, vol. 84, no. 1, pp. 5–11, 1991.

[22] S. Schaper and A. Louis, "The arrival of the frequent: How bias in genotype-phenotype maps can steer populations to local optima," *PloS One*, vol. 9, no. 2, p. e86635, 2014.

[23] S. J. Gould and R. C. Lewontin, "The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme," *Proceedings of the Royal Society of London. Series B. Biological Sciences*, vol. 205, no. 1161, pp. 581–598, 1979.

[24] E. R. Alvarez-Buylla, M. Benítez, C. Espinosa-Soto *et al.*, "Phenotypic evolution is restrained by complex developmental processes." *HFSP Journal*, vol. 1, no. 2, pp. 99–103, 2007.

[25] S. J. Gould, *Hen's teeth and horse's toes*. WW Norton & Company, 1983.

[26] B. Hallgrímsson and B. K. Hall, *Variation: a central concept in biology*. Academic Press, 2011.

[27] R. G. Reid, *Biological emergences: Evolution by natural experiment*. MIT Press, 2007.

[28] J. A. Shapiro, *Evolution: a view from the 21st century*. Pearson Education, 2011.

[29] ——, "Rethinking the (im)possible in evolution," *Progress in Biophysics and Molecular Biology*, 2012.

[30] A. S. Wilkins, "Waddington's unfinished critique of Neo-Darwinian genetics: Then and now," *Biological Theory*, vol. 3, no. 3, pp. 224–232, 2008.

[31] S. Huang, "Systems biology of stem cells: three useful perspectives to help overcome the paradigm of linear pathways," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 366, no. 1575, pp. 2247–2259, 2011.

[32] E. V. Koonin, *The logic of chance: the nature and origin of biological evolution*. FT press, 2011.

[33] J. Davila-Velderrain, A. Servin-Marquez, and E. R. Alvarez-Buylla, "Molecular evolution constraints in the floral organ specification gene regulatory network module across 18 angiosperm genomes," *Molecular Biology and Evolution*, p. mst223, 2013.

[34] R. Lewontin, *The genotype/phenotype distinction*. Stanford Encyclopedia of Philosophy, 2011.

[35] S. Huang and S. Kauffman, "Complex gene regulatory networks-from structure to biological observables: cell fate determination," *Encyclopedia of Complexity and Systems Science. Meyers RA, editors. Springer*, pp. 1180–1293, 2009.

[36] K. Kaneko, "Characterization of stem cells and cancer cells on the basis of gene expression profile stability, plasticity, and robustness," *Bioessays*, vol. 33, no. 6, pp. 403–413, 2011.

[37] L. Mendoza and E. R. Alvarez-Buylla, "Dynamics of the genetic regulatory network for arabidopsis thaliana flower morphogenesis," *Journal of Theoretical Biology*, vol. 193, no. 2, pp. 307–319, 1998.

[38] C. Espinosa-Soto, P. Padilla-Longoria, and E. R. Alvarez-Buylla, "A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles," *The Plant Cell Online*, vol. 16, no. 11, pp. 2923–2939, 2004.

[39] Y.-E. Sanchez-Corrales, E. R. Alvarez-Buylla, and L. Mendoza, "The *Arabidopsis thaliana* flower organ specification gene regulatory network determines a robust differentiation process," *Journal of Theoretical Biology*, vol. 264, no. 3, pp. 971–983, 2010.

[40] S. A. Kauffman, *The origins of order: Self-organization and selection in evolution*. Oxford University Press, 1993.

[41] S. Huang, G. Eichler, Y. Bar-Yam, and D. E. Ingber, "Cell fates as high-dimensional attractor states of a complex gene regulatory network," *Physical Review Letters*, vol. 94, no. 12, p. 128701, 2005.

[42] J. Cotterell and J. Sharpe, "Mechanistic explanations for restricted evolutionary paths that emerge from gene regulatory networks," *PloS One*, vol. 8, no. 4, p. e61178, 2013.

[43] C. H. Waddington, *The strategy of genes*. London: George Allen & Unwin, Ltd., 1957.

[44] E. R. Alvarez-Buylla, A. Chaos, M. Aldana, M. Benítez, Y. Cortes-Poza, C. Espinosa-Soto, D. A. Hartasánchez, R. B. Lotto, D. Malkin, G. J. E. Santos *et al.*, "Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape," *Plos One*, vol. 3, no. 11, p. e3626, 2008.

[45] J. X. Zhou, X. Qiu, A. F. d'Herouel, and S. Huang, "Discrete gene network models for understanding multicellularity and cell reprogramming: From network structure to attractor landscapes landscape," *In: Computational Systems Biology. Second Edition. Elsevier*, pp. 241–276, 2014.

[46] C. Li and J. Wang, “Quantifying Waddington landscapes and paths of non-adiabatic cell fate decisions for differentiation, reprogramming and transdifferentiation,” *Journal of The Royal Society Interface*, vol. 10, no. 89, p. 20130787, 2013.

[47] R. A. Barrio, A. Hernandez-Machado, C. Varea, J. R. Romero-Arias, and E. R. Alvarez-Buylla, “Flower development as an interplay between dynamical physical fields and genetic networks,” *PloS One*, vol. 5, no. 10, p. e13523, 2010.

[48] R. A. Barrio, J. R. Romero-Arias, M. A. Noguez, E. Azpeitia, E. Ortiz-Gutiérrez, V. Hernández-Hernández, Y. Cortes-Poza, and E. R. Alvarez-Buylla, “Cell patterns emerge from coupled chemical and physical fields with cell proliferation dynamics: the arabidopsis thaliana root as a study system,” *PLoS Computational Biology*, vol. 9, no. 5, p. e1003026, 2013.

Criticality in gene networks

S. Sandoval, C. Torres, M.P. García & M. Aldana, UNAM, Mexico

1 Abstract

Along evolution, living systems have prevailed in different and constantly changing environments, each one demanding a distinctive set of phenotypic traits in order to survive. Over their lifetime, most organisms need to cope with a huge spectrum of perturbations ranging from external temperature and pressure changes to inherent disruptions such as genetic mutations. Life in these circumstances have forced organisms to be phenotypically robust, in the sense that their phenotypes have to maintain functionality under many conditions. At the same time, living organisms must be flexible enough as to develop new phenotypes in order to keep up with new environmental challenges. At the heart of the problem of how organisms reach this equilibrium between phenotypic robustness and phenotypic innovation, we find the concept of dynamical criticality. For it is at criticality, namely at the brink of a phase transition between ordered and chaotic dynamics, where phenotypic robustness and innovation can coexist. Here we present a theoretical framework for the evolution of genetic regulatory networks that provides a very likely explanation of how criticality emerges in evolution. Under this framework, we evolve populations of networks subjected to mutations and demand the fulfilment of two selection criteria that are common to the evolution of all living organisms: (i) at each evolutionary step the already acquired phenotypic traits must be conserved, and (ii) in the long term new phenotypic traits must emerge. (We will refer to "phenotypic traits" as the dynamical attractors of the network). Surprisingly, these two selection criteria are enough to rapidly produce populations of networks operating at criticality. Additionally, by demanding a non-trivial information content in the phenotypic traits of the network, we obtain topologies similar to the ones observed in real organisms, characterized by the presence of global regulators or "hubs" (i.e. nodes that regulate the expression of a great number of other genes). This last point is a clear example where restrictions imposed on the dynamical properties of the network can shape its topological structure.

2 Resumen

Durante su desarrollo, los organismos vivos tienen que contender con una gran variedad de perturbaciones que van desde cambios de temperatura y humedad en el medio ambiente, hasta alteraciones permanentes en su metabolismo y material genético. Por lo tanto, a lo largo de la evolución han tenido que generar dos características importantes para su supervivencia. Por un lado, el fenotipo de los organismos vivos tiene que ser lo suficientemente robusto para seguir funcionando adecuadamente en presencia de perturbaciones. Por otro lado, dicho fenotipo tiene que ser lo suficientemente flexible para eventualmente generar nuevas características que le permitan al organismo contender con nuevos retos ambientales. Existe evidencia teórica y experimental de que este balance entre robustez e innovación fenotípicas se logra al "borde del caos", es decir, cuando las redes genéticas de los organismos vivos operan en el punto crítico de una transición de fase entre dinámicas ordenadas y dinámicas caóticas. Sin embargo, aún no se sabe cómo es que a lo largo de la evolución se generaron redes genéticas operando con dinámicas críticas. En este capítulo presentamos un modelo evolutivo de redes genéticas que se basa en un principio muy sencillo pero fundamental de la evolución: La emergencia de nuevos fenotipos, necesarios para adaptarse a nuevos entornos, ocurre sin destruir las características fenotípicas que ya se habían adquirido antes. Esto se puede resumir coloquialmente diciendo: "la mosca no perdió las patas cuando le salieron las alas". Veremos como este principio es el responsable de generar redes operando con dinámicas críticas.

3 Introduction

One of the most challenging problems in Systems Biology is to understand the relationship of the structure and dynamics of the genome with the collection of phenotypes of the organism. There are two different approaches to this problem. One of them is to understand how gene expression patterns derive in distinct phenotypes [1–3]. This approach is of particular interest because it may lead to important therapeutic applications. For instance, one would like to predict the existence or absence of certain diseases (e.g. cancer or diabetes) from a particular set of gene expression patterns [4, 5]. On the other hand, we could also tackle this question from an evolutionary point of view, where one would aim to understand how adaptive constrains on the phenotypes influence the structure and dynamics of an underlying genetic network. It is clear that both the structure and dynamics of the genome have been crafted through evolution to determine the phenotypic traits of the organism. Indeed, experimental studies have shown that modifying the topology of a regulatory network directly alters its dynamics, which in turn affects the phenotypic traits of the organism. As an example of the above, in Ref. [6] it is shown that gene expression patterns resembling logic gates (AND, OR, NOR, etc.) arise by synthetically shuffling the regulatory architecture of a bacterial promoter. Depending on the logic gate coded in a particular promoter, the bacterium can codify (or not) a fluorescent protein. Analogously, constraining the dynamics of a system during its evolution results in the appearance of important topological properties. For example, in Ref. [7] Parotti and his co-workers showed that imposing some stability constraints on the dynamics of a growing network, results in the emergence of complex topological properties, such as the ubiquitous scale-free topology.

However, understanding how the genomes acquired their observed structural and dynamical properties, and how such properties are intertwined to determine the organism's phenotypic traits, is not a trivial matter. The main limitations lie in understanding how evolution has molded phenotypes, as we do not know the exact series of environmental changes that each species underwent throughout its evolutionary history. Additionally, we know neither the particular constraints that each environment imposes, nor the effect that such restrictions would have in the genome's structure and dynamics. Nonetheless, despite the particularities of the evolutionary history of each species, there are two common characteristics in the evolution of every organism:

(a) evolution occurs in changing environments, and

(b) the new phenotypic traits, needed to cope with new environmental challenges, emerge on top of the already existing ones.

Property (a) states the widely accepted fact that evolution towards different (and perhaps more complex) forms of life is necessary, only because the environment changes and confronts organisms with new survival challenges. If the environment, as perceived by the organism, stayed always the same, evolution would be unnecessary. This does not rule out the existence of diversity, as even in the absence of selective pressure genetic drift is known to occur. Property (b) states that when new phenotypic traits are developed, previous ones do not disappear. Instead they are most likely conserved or slightly transformed. Thus, changing environments represent the driving force that generates new phenotypic traits across evolution.

So, the fact that organisms develop and survive in changing environments has two important consequences: *phenotypic robustness* and *phenotypic innovation* [8–10]. This essentially means that living systems must be able to maintain certain functionality in the face of perturbations, imposed by the changing environments, and at the same time be able to transform their phenotypes, and consequently their gene expression patterns, when the new environmental challenges become so demanding that the emergence of new functionalities is required. In this context, phenotypic robustness is a measure of how resilient the organism's phenotypes are when faced with a wide variety of perturbations [11, 12]. Highly robust systems would be those that preserve their phenotypes and functionality under perturbations; while lesser robust systems would lose functionality and drastically transform their phenotypes, even in the presence of small perturbations. We will discuss later how a system can gain or lose phenotypes.

Very importantly, two broad types of perturbations must be differentiated. First, we have transient perturbations, which range from environmental noise or brief chemical exposures, to even some epigenetic modifications. These perturbations affect the patterns of gene expression and the corresponding phenotypes only in the short-term. Second, there are much more stable changes such as point mutations, DNA recombinations, gene duplications, deletions etc. These perturbations are of particular interest, since they may affect the evolutionary path of a living organism, and its descendants, in an almost permanent manner. Although it is true that most of the genetic mutations are either neutral or unfavorable, eventually a set of those changes could derive in a new and fitter phenotype. This is exactly what phenotypic innovation refers to. Therefore, phenotypic innovation will be defined as the capability of an organism to generate new phenotypes, in order to successfully adapt to new conditions.

Later on we will give a precise definition of phenotypic robustness and innovation in terms of the dynamical properties of genetic network models. Here, suffices it to mention that evolving in changing environments will have repercussions in the ability of the genetic network to innovate and be robust; properties which in turn are dictated by the topology and dynamics of the network. In fact, previous work in this direction shows that changing environments, each one demanding new abilities or posing new constraints, can significantly speed up evolution [13]. Interestingly, the highest speedup was found in environments that changed gradually and therefore shared some requirements with the previous ones. Such environments changed the network topology through the spontaneous modularization of the system [14].

The ability of living systems to generate new phenotypes while preserving the previous ones is called *evolvability* [15–18]. This term can be summarized in colloquial terms by saying that “the fly got the wings without losing the legs”. In other words, at the core of evolvability is the fact that, in the presence of new environmental challenges, new phenotypes emerge on top of the already existing ones. This requirement demands a delicate balance between forces of opposite nature: phenotypic robustness, in which organisms do not respond to perturbations (transient or permanent); and phenotypic innovation, which entails the generation of new phenotypes as a response to permanent mutations.

In physical systems, a very similar balance between robustness and responsiveness is often attained close to a critical point, namely at the brink of a phase transition, between ordered and chaotic dynamics [19]. Systems operating in the ordered regime are impervious to change, as they are able to gradually vanish every perturbation. On the contrary, chaotic systems are extremely sensitive to perturbations and their behavior is often unpredictable, with small initial perturbations propagating rapidly throughout the entire system. The delicate balance between robustness and sensitivity to perturbations is achieved close to criticality, where perturbations neither disappear nor propagate indefinitely, but typically remain confined to a small subset of elements. Because of this particularity of critical systems, it is natural to ask whether the juxtaposition in living systems of phenotypic robustness and phenotypic innovation could also be understood in terms of critical dynamics.

In 1969 Stuart Kauffman empirically found a phase transition between ordered and chaotic dynamics, with a non-trivial critical point, in simple genetic regulatory network models [20]. His studies led him to propose that real genetic networks should operate at (or close to) criticality, as it is exactly at this point where the system exhibits the aforementioned balance [21]. Since then, his idea has been known as the *life at the edge of chaos hypothesis*. Nonetheless, in that pioneering work, Kauffman analyzed the network response exclusively under *transient perturbations*. Despite this analysis is useful to describe the behavior of an organism under perturbations that occur during its life time, evolution also requires living systems to be phenotypically evolvable under genetic mutations, which occur at much longer time scales and across generations.

Unfortunately, the life at the edge of chaos hypothesis, as insightful and appealing as it is, was formulated on not very solid grounds. This is because for many years, the implications of the dynamical phase in which the network operates, to the evolution of such network under permanent mutations, were unclear. Here we discuss a model of network evolution, and show that when genetic mutations are properly considered, Kauffman's hypothesis still holds. We will see that the two properties (a) and (b) mentioned above, although simple, have profound implications in the evolution of genetic networks. These two properties constitute the main ingredients that generate critical dynamics, and consequently, phenotypic robustness and innovation. As a prototype model for genetic regulatory networks we will use the Boolean network model proposed by Kauffman [20], as there is now plenty of evidence showing that it effectively captures the essential aspects of the gene regulatory process [22–27]. So, in the next section we will describe the Kauffman model of gene regulation and its three dynamical phases: ordered, critical and chaotic.

4 Boolean networks and criticality

In the Boolean approach proposed by Kauffman, the dynamical state of the genetic network is encoded in a set of N boolean variables, $\sigma_1, \sigma_2, \ldots, \sigma_N$, each representing the state of expression of a given gene. Thus, $\sigma_n(t) = 1$ or $\sigma_n(t) = 0$ according to whether the n^{th} gene is expressed or not at time t, respectively. The state of expression σ_n of the n^{th} gene changes in time and is determined by the state of expression of its regulators according to the equation

$$\sigma_n(t+1) = F_n(\sigma_1^n(t), \sigma_2^n(t), \ldots, \sigma_{k_n}^n(t)) \tag{1}$$

where $\{\sigma_1^n, \sigma_2^n, \ldots, \sigma_{k_n}^n\}$ are the k_n regulators of σ_n and $F_n(\cdot)$ is a Boolean function of k_n arguments that is constructed according to the activatory or inhibitory nature of the regulations. For networks of real organisms, the regulators of each gene and the corresponding Boolean functions are constructed with base on the biological knowledge of the system. Nowadays there is solid evidence showing that the Boolean approach is able to reproduce the gene expression patterns observed experimentally in several organisms.

Since we are not interested in a particular network of any specific organism, in the initial population we use random networks in which the k_n regulators of a given gene σ_n are chosen randomly from anywhere in the system. The Boolean functions are also assigned randomly in a way such that for each of the 2^{k_n} configurations of the k_n regulators, the Boolean function evaluates to 1 with probability p and to 0 with probability $1-p$. This is just the standard Kauffman model whose dynamical properties have been extensively studied. In particular, it is known that this simple model exhibits a continuous phase transition between ordered and chaotic dynamics [28–31]. In the ordered phase, any perturbation in the initial condition eventually disappears whereas in the chaotic phase any such perturbation propagates to a large fraction of the network. The parameter that determines in which dynamical phase the network operates is the so called *network sensitivity* S defined as

$$S = 2p(1-p)K, \tag{2}$$

where K is the average number of regulators per gene. If $S < 1$ the network will be in the ordered phase, and if $S > 1$ it will be in the chaotic phase. The critical phase is attained for $S = 1$, where the dynamics are not extremely sensitive to perturbations in the initial conditions (as in the chaotic phase), but the perturbations will not always disappear (as in the ordered phase). Fig. 1 illustrates the dynamical behavior of the network in the three different phases.

Because the network has a finite number of genes, there is also a finite number $\Omega = 2^N$ of possible dynamical states, ranging from $000\ldots0$ where all the genes are inactive, to the state $111\ldots1$ where all the genes are active, including all the intermediate states. This does not mean that, starting from a given initial condition, the network will necessarily explore all the $\Omega = 2^N$ possible states. In fact, before the network can go through all the possible states, it gets trapped in a *dynamical attractor*. Since the dynamics given by Eq. (1) are deterministic, starting out from one initial state, the network will go through a series of transients until a previously visited state is reached. At this point the network enters into a periodic pattern of expression that repeats itself over and over again. These periodic patterns are exactly the dynamical attractors mentioned above. Usually, several attractors may exist for the same network. All the states that converge to the same attractor constitute its particular basin of attraction. Networks operating in the ordered phase typically have a small number of attractors, whereas networks in the chaotic phase have a really large number of them [29]. Thus, the dynamical rule given in Eq. (1) partitions the state space into disjoint sets consisting of the attractors and their corresponding basins of attraction. The set of all the attractors (and their basins of attraction) is known as the *attractor landscape* of the network. The biological relevance of the dynamical attractors was first pointed out by Kauffman, who formulated the hypothesis that the attractors correspond to the stable patterns of expression of the genetic network, which in turn correspond to the different cell types or, more accurately, to the different functional states of the organism (its phenotypic traits). This hypothesis has been firmly demonstrated for

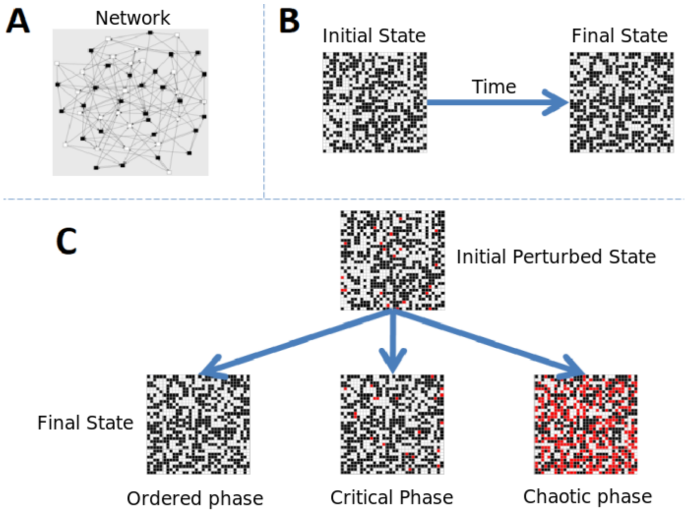


Figure 1: Network dynamics in the three different phases. (A) Graphic representation of the network state at a given time point. The color of the nodes represent their activity: white if the node is active and black if it is not active. (B) The N genes of the network have been placed on a square lattice just for visualization purposes. Starting with one initial state (on the left) the system develops in time until a stable state is reached (on the right). (C) The initial state is perturbed so that a few genes (less than 1%) are forced to change its activity. The perturbed genes are represented in red. If the network were operating in the ordered regime, the initial perturbation would disappear after some time and the network would reach the same stable state as without the perturbation. By contrast, in the chaotic regime the initial perturbation amplifies and propagates to a very large portion of the network, which ends up in a completely different state. In the critical phase, typically what happens is that the perturbation neither disappears nor propagates to the entire network, but remains confined to a small subset of genes.

several cases [22, 32, 33].

5 Waddington Epigenetic landscape

The fact that a given network has multiple attractors solves an important problem posed by Conrad H. Waddington with respect to the Modern Evolutionary Synthesis (MES), where it is proposed that a genotype (a given set of genes) corresponds to exactly one phenotype. In the 1950s Waddington noted that MES could not explain multicellularity, as this theory assumes that genetic mutation is the only source of phenotypic variation. But then, how could cell differentiation occur without any inheritable genetic mutation? Waddington proposed that throughout the development of an organism, intrinsic restrictions, imposed by gene expression and shaped by evolution, would occur. This idea led

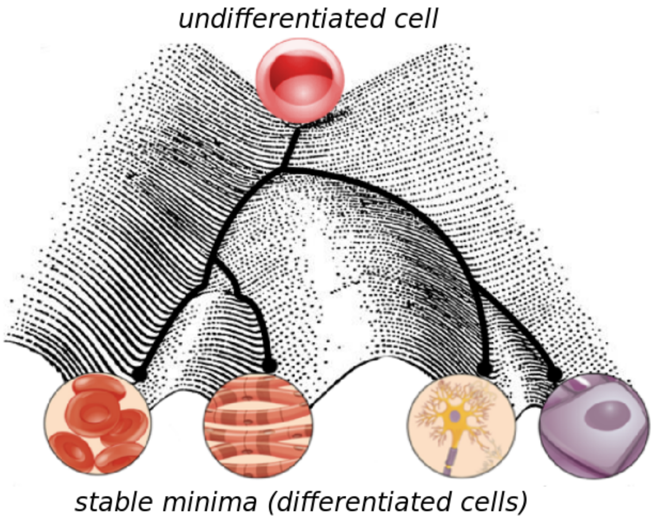


Figure 2: Waddington epigenetic landscape. In this metaphor, the undifferentiated embryonic cell is imagined like a ball that can roll down the hill on a surface that represents all possible states of expression in the genome. The stable minima in this surface would correspond to the stable phenotypes of the organism. The different bifurcation points represent alternative differentiation pathways.

him to formulate the concept of the *epigenetic landscape* as a metaphor of such restrictions. Waddington's epigenetic landscape consists of an hypothetical surface, with crests and valleys, over which a ball rolls down from the highest point of the surface, and ends into any of the possible lower minima (see Fig. 2). Along the way, there are ramification points where the ball can take different paths that lead to different minima. In this metaphor, the ball represents an embryonic undifferentiated cell whereas the surface represent all the possible states of expression of the genome. Thus, the undifferentiated cell "rolls down the hill" searching for the stable expression minima. In each ramification point of the development, the embryonic cell could take one path or another, depending on the presence of certain inductors, homeotic genes or even stochastic fluctuations. This mechanism was interpreted by Waddington as the effect of the environment over gene expression. The stable minima of the surface would then correspond to the stable phenotypes which the cell can get to.

Waddington's epigenetic landscape was considered for many years as a metaphor that could not be proved experimentally. However, after Kauffman's work, it was clear that the attractor landscape of a genetic network represents the formal materialization of Waddington's metaphor. The dynamical attractors correspond to the stable minima of Waddington's surface (stable phenotypes), whereas the basins of attraction correspond to the grooves and furrows (developmental pathways) that lead to these minima. This correspondence was first proved experimentally by S. Huang et al. and subsequently corroborated by other research groups [32]. Thus, the attractor landscape resolves the question of how the same genotype can give rise to a variety of different phenotypes.

6 Criticality of the attractor landscape

It is important to stress that the definition of the ordered, critical and chaotic phases given before, is closely related to the dynamical response of the network to transient perturbations (see Fig. 1). However, there is a much more profound manifestation of these dynamical phases, in relation to the way in which the attractor landscape changes when the network is permanently mutated [34]. Indeed, since the attractor landscape is determined by the network topology and the Boolean functions, one would expect that changing either of these properties consequently modifies the attractor landscape. One can also expect that the magnitude of this change will depend on the dynamical phase in which the network operates. In our group we have investigated the relationship between the dynamical regime of the network and the evolvability of its attractor landscape. To do this, we first formulate an operational definition of phenotypic robustness and phenotypic innovation as follows:

- A network is phenotypically robust, under a given mutation, if its dynamical attractors do not change as a result that mutation.
- A network is phenotypically innovative, under a given mutation, if new attractors appear as a result of that mutation.
- A network is evolvable under a given mutation if it is both phenotypically robust and innovative. In other words, if all the attractors it had before the mutation are conserved and also new attractors appear.

According to the previous definitions, it is also important to define the mutations under which a network is going to be considered evolvable. We have implemented a particular type of mutation that is the main cause of genome growth and evolution: gene duplication followed by divergence [34]. We start from a Boolean network with N genes, $\{\sigma_1, \sigma_2, \ldots, \sigma_N\}$, which we will call the *original network*, and randomly chose one of its genes for duplication. Let σ_i be the randomly chosen gene. We duplicate this gene and form a new network with $N+1$ genes, in which $\sigma_i = \sigma_{N+1}$. This means that immediately after the duplication event σ_{N+1} has the exact same regulators (inputs), the same regulated genes (outputs), and the same Boolean functions as σ_i. Afterwards, we mutate some of these properties in the duplicated gene σ_{N+1}, making it different from the parent gene σ_i. This process, called genetic divergence, is known to occur very rapidly after the duplication event [35]. We will call the network resulting from this duplication-divergence event the *mutated network*. As a result of the duplication-divergence event, the attractors of the mutated network may change, or they may even disappear whereas new attractors may appear. Robustness will be then quantified as the fraction of attractors of the original network that are conserved in the mutated network after the duplication-divergence event. If all the original attractors are conserved, then the network has robustness $R = 1$, whereas if none of the attractors are conserved, the network has no robustness: $R = 0$.

Fig. 3 shows the probability $P(R)$ that a random Boolean network has robustness R at each of the three different phases; ordered ($S = 0.5$), critical ($S = 1$) and chaotic ($S = 1.5$ and $S = 2$). Clearly, the network robustness decreases as the dynamics transit from the ordered to the chaotic regime. This can be observed as the probability $P(1)$ that the network conserves all of its original attractors rapidly decreases, whereas the probability $P(0)$ to conserve none of them increases. These results show that networks operating in the ordered regime are very robust, as with high probability their attractors do not change under mutations. But precisely because of this, ordered networks cannot evolve since their attractor landscape is "frozen", which makes them incapable of generating new attractors. On the contrary, chaotic networks are very innovative. In such networks, there is a very high probability that the attractor landscape completely changes after the duplication-divergence event. These chaotic network are innovative but they are not robust, so they cannot evolve either. Critical networks are peculiar in the sense that they are robust and innovative at the same time. This can be observed in Fig. 4, which shows the probability $P_e(S)$ that after a gene duplication-divergence event, a network with sensitivity S conserves all of its attractors and generates at least a new one. Note that this probability is maximum for critical networks ($S = 1$).

Thus, from the theoretical point of view, criticality is a desirable property that confers the phenotypic robustness and innovation the network needs to evolve. Two important questions arise from this conclusion: Are the genetic networks of real organisms critical? And if so, how did criticality emerge throughout evolution? The first question was answered affirmatively by several groups, who reported experimental evidence showing that the networks of real organisms exhibit dynamics compatible with criticality [36–40]. As for the second question, our group has investigated the evolutionary mechanisms that generate critical dynamics. In particular, we arre interested in knowing whether ordered or chaotic networks can evolve towards criticality, or if critical networks need to be born being critical. In the next section we present a simple evolutionary model, rooted on biological grounds, that gives a general answer to these questions.

7 Evolution towards criticality

In the previous section we assumed that the network is already operating in a given dynamical regime (ordered, critical or chaotic) and then proceed to determine the effect of mutations on the evolvability of the attractor landscape. The main result was that, under a gene duplication-divergence event, critical networks exhibited the highest evolvability,

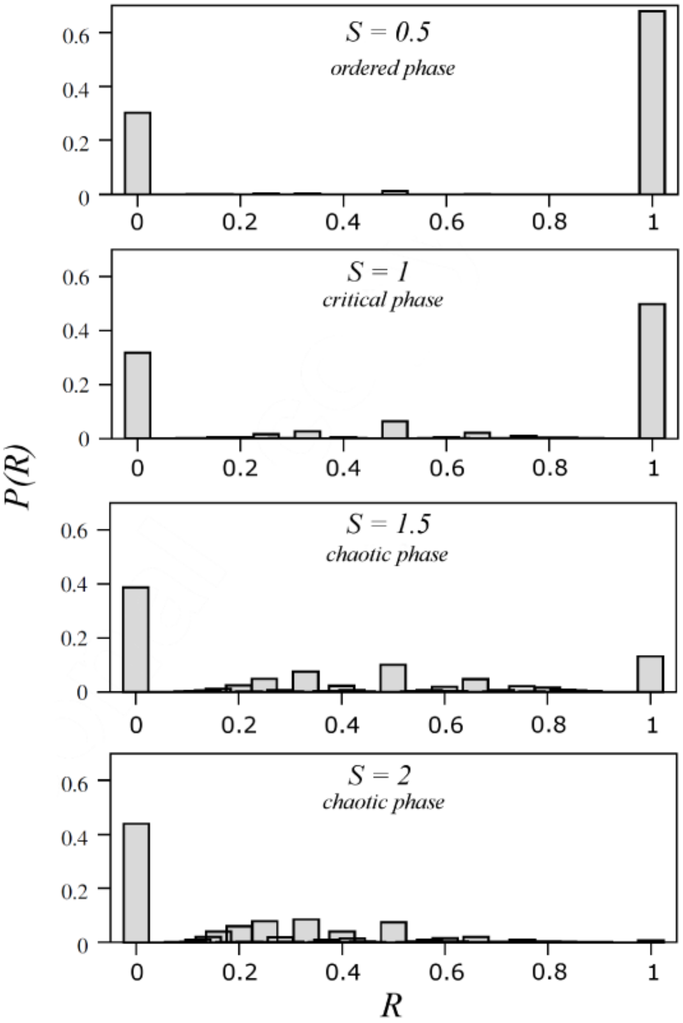


Figure 3: Probability $P(R)$ for the network to have phenotypic robustness R under gene duplication-divergence events. The different graphs correspond to networks operating in the three different dynamical phases: ordered ($S = 0.5$), critical ($S = 1$), chaotic ($S = 1.5$) and super chaotic ($S = 2$). Note that as the dynamical regime passes from ordered to chaotic, the network becomes less robust, as the probability $P(1)$ for the network to conserve all of its original attractors decreases and the probability $P(0)$ to conserve none of its attractors increases.

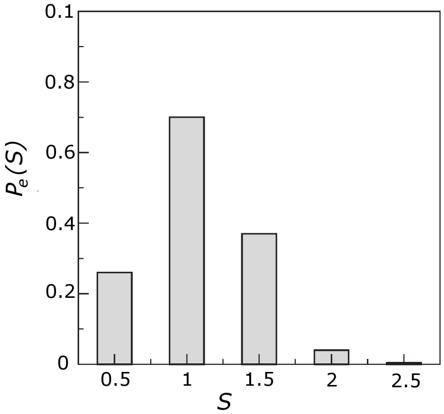


Figure 4: Probability $P_e(S)$ for a network with sensitivity S to be evolvable, namely, to conserve all its attractors and generate at least a new one, after a gene duplication-divergence event. Note that this probability is maximum for critical networks.

as they conserved all the original attractors (phenotypic robustness) and were able to generate new ones (phenotypic innovation). In this section we will proceed in the opposite direction, starting with random networks operating in arbitrary dynamical phases and evolving them through mutations and gene duplication-divergence events. It is throughout this process that we will demand evolvability. This means that only the networks that conserve their already acquired phenotypes (attractors) and also generate new ones, will be the ones selected to survive and continue further trough the simulation. Our goal is to determine if the requirement of evolvability across the evolutionary process will favor a particular dynamical regime.

We start with a population of $M_0 = 1000$ random Boolean networks (all different), each with $N = 20$ genes. At this point, all the genes have exactly K regulators and the Boolean functions have a bias of $p = 0.5$. Hence, the sensitivity of the networks in the initial population is entirely determined by the network connectivity K as $S_0 = 2p(1-p)K = K/2$. Through evolution, we mutate the networks in the population by adding or removing connections between the genes, changing the Boolean functions that regulate the expression of the genes, and adding new genes to the network. Although for each network we perform these mutations randomly, the way in which we implement them is deeply rooted on the biological phenomenology of genome growth and evolution. More specifically, we assume that each gene is composed of two parts, a regulatory region and a coding region, and that mutations can occur in any of these two parts with equal probability. Mutations in the regulatory region consist in the addition or deletion of binding sites to DNA, which in turn change the way in which the gene is regulated. In Ref. [41] the mutagenic algorithm is described in detail. Here we briefly mention that mutations in the regulatory region of a given gene σ_n will cause the loss or gain of regulators, as well as changes in its Boolean function. On the other hand, mutations in the coding region of σ_n change how this gene regulates its targets, which translates into the gain or loss of targets, as well as modifications of the Boolean functions of such gained or lost targets. Finally, the network growth is implemented through gene duplication followed by divergence up to a maximum size $N = 100$.

In each generation and for each network in the population, there is a probability μ for each gene to be mutated in either its regulatory or coding region. After the mutations, we check whether or not the mutated networks conserve the same attractors they had before the mutations, and eliminate from the population those networks which do not conserve *all* their attractors. Thus, only the phenotypically robust networks can go through the next generation. We will call this selection process the *phenotypic robustness criterion* (PRC). The elimination of the networks that do not satisfy this criterion reduces the population size to a new value, and therefore we have to replicate each of the surviving networks to restore the population to its original size. This replication is carried out with a certain bias (or fitness) α per network that will be discussed later.

Every two thousands generations all the networks in the population simultaneously undergo a duplication-divergence event, after which the only networks that survive and pass to the next generation will be the ones that in addition to fulfilling the PRC, also generate at least one new attractor. Thus, every two thousands generations we are demanding evolvability. Therefore, we will call this selection process the *phenotypic evolvability criterion* (PEC). Under this criterion we eliminate from the population all the networks which do not satisfy the PRC or do not generate new attractors (even if some of these latter networks do fulfill the PRC).

There are two important points to be considered when new attractors emerge. First, every time a new attractor is found, it is added to the set of attractors that must be conserved to fulfill the PRC. We will call this growing set of attractors, which will be under selective pressure, the *phenotypic attractors*. Each network has its own set of phenotypic attractors. Second, the genes in the phenotypic attractors must do something. More precisely, networks whose phenotypic attractors have all the genes in the same state (active or inactive) will have a low fitness and consequently a lower replication rate. We define the average genetic expression variability of the network as $\alpha = 1 - |\psi_1 - \psi_0|$, where ψ_0 and ψ_1 are the average fractions of 0's and 1's in all the states of all the attractors of the network (clearly, $\psi_0 + \psi_1 = 1$). Thus, $\alpha \approx 0$ if almost all the genes in the attractors are in only one state (either 0 or 1), whereas $\alpha \approx 1$ if more or less half of the genes in the attractors are in the state 1 and the other half in the state 0. In each generation, we replicate each surviving network in a quantity proportional to its average genetic activity α, which introduces competition in the replication of the surviving networks, being more favored the ones with an average genetic variability close to $\alpha = 1$.

Fig. 5A shows the evolution of the average network sensitivity $\langle S \rangle$, where the average is taken over all the networks in the population. The different curves depicted in Fig. 5A

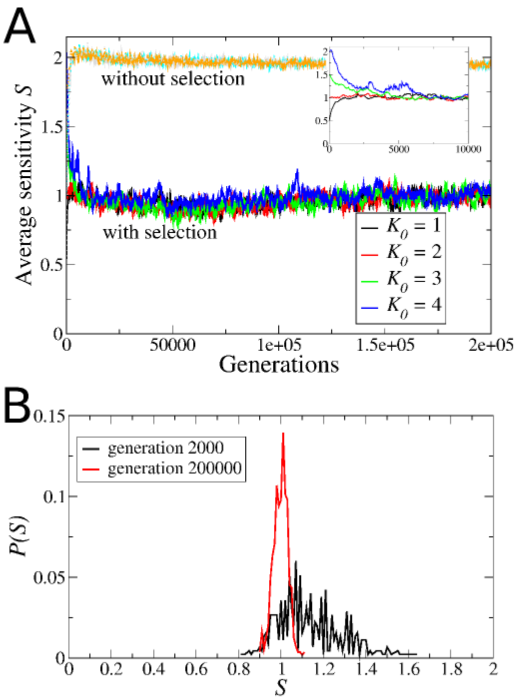


Figure 5: Evolution towards criticality. (A) Evolution of the average network sensitivity for four different populations, each initially composed of networks in one of the three dynamical regimes: ordered ($K = 1$, $S = 0.5$, black), critical ($K = 2$, $S = 1$, red), and chaotic ($K = 3$, $S = 1.5$, green; $K = 4$, $S = 2$, blue). Under the Darwinian selection given by the PRC and PEC, all the populations become critical ($\langle S \rangle \to 1$) in less than 5000 generations (see inset) regardless of their initial dynamical regime. The control curves (in light gray) were obtained evolving populations without selection, and show that the mutagenic method alone drives the networks into the chaotic regime ($\langle S \rangle \to 2$). Therefore, in our simulations evolution towards criticality is not an artifact of the mutagenic algorithm. (B) Distribution of sensitivities at two different generations for the population that started with $K = 3$ (chaotic networks). Early in the simulation, at generation $g = 2 \times 10^3$, $P(S)$ is quite broad (black line), reflecting a great diversity of networks. However, through evolution, all the surviving networks become critical and the distribution $P(S)$ narrows down (red line). The distribution shown here at generation $g = 2 \times 10^5$ has $\langle S \rangle = 0.998 \pm 0.035$.

correspond to four different populations that started with networks in the ordered, critical, and chaotic regimes. The curves in light gray that converge to $\langle S \rangle = 2$ show the effect of the mutagenic algorithm only, as they correspond to populations evolving with mutation but without selection (all the networks survive in each generation). Clearly, the mutagenic algorithm alone produces chaotic networks. Contrary to this, when the evolution takes place with selection, i.e. implementing the PRC and PEC, the sensitivity of the

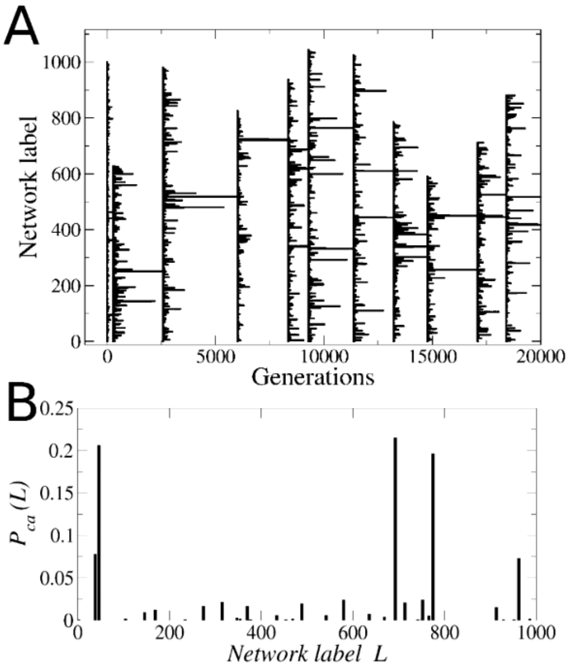


Figure 6: Evolutionary bottlenecks. (A) This plot shows the evolution of the lineages (network labels) across generations. Each horizontal line indicates the survival time of a particular lineage. The vertical lines indicate the fixation events in which all the networks in the population are relabelled again after only one lineage was left in the entire population. (B) Probability $P_{ca}(L)$ that a network with label L in the original population becomes the common ancestor (this is the lineage that goes through the first bottleneck, giving rise to the first fixation event). Note that only very few networks (less than 4%) in the original population can become common ancestors. Among this 4%, only 5 networks are selected in about 80% of the realizations.

networks in all the populations converge, on average, to the critical value $\langle S \rangle \approx 1$. This demonstrates that the Darwinian selection given by the PRC and PEC indeed makes the networks evolve towards criticality. Furthermore, Fig. 5B shows the distribution of sensitivities $P(S)$ in one of the populations that started with chaotic networks ($S = 1.5$), and for two distinct generation times: Very early in the simulation, at generation $g = 2 \times 10^3$ (black curve); and at the end of the simulation, at generation $g = 2 \times 10^5$ (red curve). It is clear that at the beginning of the evolutionary process a great diversity of networks is present, which is reflected in the broad distribution $P(S)$. Nonetheless, throughout evolution, the networks become critical and the final distribution of sensitivities $P(S)$ becomes very narrow, with mean $\langle S \rangle = 0.998$ and standard deviation $\Delta S = 0.035$. This shows that each network in the population is converging towards criticality.

As we mentioned before, the networks that do not satisfy the PRC in each generation, or the PEC after the gene duplication events every two thousands generations, are removed from the population. In order to determine how restrictive these selection criteria are, it is important to measure the survival times of the networks in the population. To do this, at generation $g = 0$ we label all the networks in the population with an integer ranging from 1 to 1000. Throughout generations, each network conserves its original label. Furthermore, when one network is replicated into several copies, the "daughter" networks acquire the same label from the "mother". Therefore, the labels are inherited from mother to daughters, which makes it possible to identify different "lineages" through the evolutionary process. Each network in the initial population gives rise to a different lineage and therefore, at the beginning of the process there are 1000 different lineages. However, since the networks that fail the selection criteria are removed from the population, some lineages might disappear. If at generation g only one lineage is left in the entire population, we relabel the networks in that particular lineage from 1 to M_g, being M_g the number of networks in the population. This can be considered as the "fixation" of that lineage in the population. (Note that the existence of only one lineage in the population does not mean that there is only one network. Rather, it means that all the M_g networks have the same label, and therefore, all of them share a common ancestor.) Fig. 6A shows the evolution of lineages throughout generations. The vertical lines show the fixation events, and the horizontal lines the survival time of a particular lineage. It is clear that the majority of lineages disappear from the population very quickly, and only very few lineages survive for long times. These results indicate that evolution towards criticality via the PRC and PEC confronts the population against a series of selective filters (bottlenecks) which only very few networks are able to go through.

A very important consequence of these bottlenecks is that the final population comes entirely from only one common ancestor. This rises the question of how reproducible is obtaining the same common ancestor in different realizations of the evolutionary process. In other words, if we perform one million different simulations, always starting with the same initial population of networks but with a different history of mutations and duplications in each realization, how many times the same network in the original population would be selected as the common ancestor? Since the networks in the original population were constructed randomly, one might expect that all of them have the same probability of making it through the bottlenecks imposed by the PRC and PEC. If this were the case, the probability $P_{ca}(L)$ that the initial network with label L becomes the common ancestor would be the same for all values of L. Nonetheless, Fig. 6B shows that this is not the case, as only very few networks are selected as common ancestors.

Another remarkable result is the topological structure of the networks in the final population. We start the simulation with homogeneous random networks for which all the nodes have the same number of inputs K and a number of outputs drawn from a Poisson distribution. However, at the end of the simulation the networks have global regulators (hubs), namely, nodes with a great number of output connections as it is illustrated in Fig. 7A. This topological structure is known to occur in the genetic networks of real or-

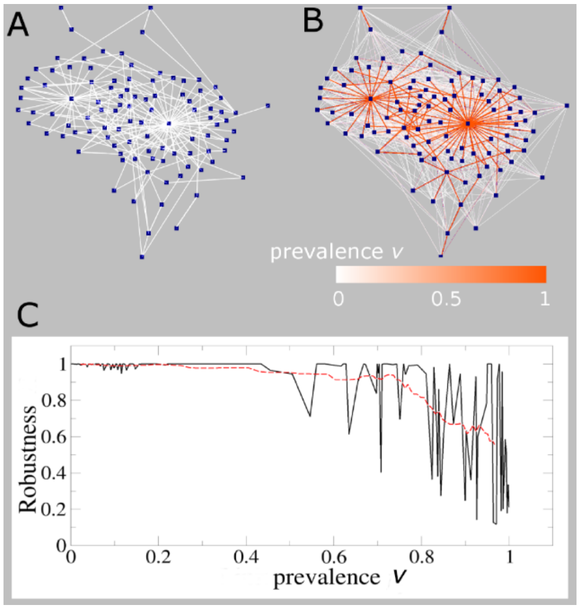


Figure 7: (A) Structure of a randomly chosen network in the final population. Note the existence of highly connected nodes (global regulators or "hubs"). (B) Diagram showing the superposition of all the networks in the final population. The color of a given link indicates its prevalence in the population, which is the fraction of final networks in which that link occurred. (C) Robustness of the network when a link with prevalence v is removed. The black curve corresponds to one randomly chosen network and the red dashed line is the average over the population. Note that on average, the robustness of the network decreases as the prevalence of the removed link increases.

ganisms, such a *E. coli*, *S. cerevisiae*, *S. pombe* and *B. subtilis* [34, 36, 42]. The existence of global regulators in the final networks was a very unexpected result for two reasons. First, the topological structure of the network was never considered in the selection mechanism. Second, and more importantly, global regulators introduce strong correlations in the network dynamics, and it is not obvious that these correlations can survive to the selection pressure imposed by the PRC and PEC. Interestingly, when the α-fitness criterion is not enforced, i.e. when we allow the possibility for all the genes in the attractors to be "frozen" in the same state (either 0 or 1), the networks never develop hubs. This strongly suggests that the existence or absence of global regulators is related to the information content of the attractor landscape but further investigation is necessary in this matter.

Fig. 7A shows a representative network of the final population. It is important to mention that the final networks, although similar, are not identical even though they all have the same set of phenotypic attractors. Fig. 7B shows a superposition of all the networks in the final population and the color code indicates the *prevalence of the links* in such networks. This prevalence is measured as $v_{ij} = m_{ij}/M_f$, where m_{ij} is the number of networks in the final population in which the nodes σ_i and σ_j are connected, and M_f is the total number of final networks. It is clear from Fig. 7B that the most prevalent links are the ones connecting the global regulators. This suggests that these hubs play an important role in the evolvability of the attractor landscape. Indeed, Fig. 7C shows the robustness of the network (the number of attractors that are conserved) when we remove links with different prevalence. It is clear that on average, the robustness greatly decreases when we remove the most prevalent links.

8 Discussion

Phenotypic robustness and innovation are two central properties common to all living organisms. These two properties are closely related to the dynamical regime in which the underlying genetic network operates. This is because networks that are dynamically critical are also robust and innovative not only under transient changes in the environment, but also under permanent mutations either in the topological structure of the network or in its regulatory interactions (the Boolean functions). Therefore, evolution towards criticality stems out as a fundamental process that can help us understand how living organisms are robust and at the same time have the ability to generate adaptable diversity. In this work we have shown that dynamical criticality can indeed emerge by means of a simple and biologically meaningful Darwinian selection process, that imposes two main constraints on the attractor landscape. First, the networks must conserve the attractors they have acquired through evolution and second, networks that generate new attractors as a consequence of mutations, are preferred over the networks that do not generate new attractors. In this sense, the balance between conservation and innovation of the attractor landscape plays an important role in the selection process. We should note that innovation of phenotypes occurs in two distinct ways. On the one hand, the emergence of new attractors can be considered as the generation of new phenotypes. On the other hand, the addition of new genes to the network also adds new information to the already existing attractors (the attractor states grow). In either case, for this information to be useful, the new genes must have some activity that changes from one attractor to another. Therefore, a third selection constraint comes up naturally, and consists in that the the genes in the attractors should not be “frozen”. This important biological constraint is not fundamental for the evolution towards criticality, as the populations become critical even without the fulfillment of the α-fitness criterion. But it is essential for the existence of global regulators in the final networks, which suggests a strong relationship between the network structure and the information content of the attractor landscape.

It is also important to mention that in our simulations the attractor conservation and innovation criteria are not as stringent as one may think. The reason is that, due to computer limitations, the attractor landscape can be known in full only for small networks. Thus, we completely determine the attractor landscape for all the networks in the population only for the first generation where the networks are small. After that, in order to find new attractors we just sampled a small fraction of the state space (we sampled about 10^4 states for each network). Clearly, we can apply the PRC and the PEC only to the attractors that are found by means of this under sampling (the set of phenotypic attractors). However, there can be "hidden" attractors that do not come out through this under sampling process. It is quite remarkable that even when many attractors may not be taken into account, the PRC and the PEC make the population evolve towards criticality. The under sampling in our numerical simulations has a biological counterpart, which is that for an organism like *E. coli*, with $N \approx 300$ regulatory genes, it is very unlikely that all the 2^{300} possible configurations could be explored throughout evolution in order to reach all the possible existing phenotypes.

Even though there is a great genotypic and phenotypic diversity in the initial population (because all the networks are structurally different and have different attractor landscapes), throughout generations the population passes through a series of selective filters which decrease this diversity by eliminating from the population the majority of lineages. At the end of the simulation all the networks have the same set of phenotypes (the same set of phenotypic attractors), but slightly different genotypes (different topological structures). Additionally, as we have mentioned before, the existence of highly connected nodes in the final networks seems to be a consequence of restrictions imposed on the information content of the dynamical attractors. Thus, our results are consistent with the idea that restrictions on the dynamics of the network can play an important role in shaping its topology, as it has been suggested for other types of networks [43, 44].

In conclusion, although dynamical criticality is not a necessary condition in the functioning of living organisms, it can be a consequence of evolution. For it naturally emerges from the very same forces that allow living organisms to evolve in changing environments: phenotypic robustness and phenotypic innovation.

9 Bibliography

[1] A. H. Brand and N. Perrimon, "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes," *Development*, vol. 118, pp. 401–415, 1993.

[2] M. Kaern, T. C. Elston, W. J. Blake, and J. J. Collins, "Stochasticity in gene expression: from theories to phenotypes," *Nature Reviews Genetics*, vol. 6, pp. 451–464, 2005.

[3] N. E. Lewis, H. Nagarajan, and B. O. Palsson, "Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods," *Nature Reviews Microbiology*, vol. 10, pp. 291–305, 2012.

[4] D. T. Ross, U. Scherf, M. B. Eisen, C. M. Perou, C. Rees, P. Spellman, V. Iyer, S. S. Jeffrey, M. Van de Rijn, M. Waltham, A. Pergamenschikov, J. C. Lee, D. Lashkari,

D. Shalon, T. G. Myers, J. N. Weinstein, D. Botstein, and P. O. Brown, “Systematic variation in gene expression patterns in human cancer cell lines,” *Nature Genetics*, vol. 24, pp. 227–235, 2000.

[5] J. E. Mickle and G. R. Cutting, “Genotype-phenotype relationships in cystic fibrosis,” *Medical Clinics of North America*, vol. 84, pp. 597–607, 2000.

[6] K. A. and G. C.C., “Uncovering *cis* regulatory codes using synthetic promoter shuffling,” *PLoS ONE*, vol. 3, no. 4, p. e2030, 2008.

[7] J. I. Perotti, O. V. Billoni, F. A. Tamarit, D. R. Chialvo, and S. A. Cannas, “Emergent self-organized complex network topology out of stability constraints,” *Physical Review Letters*, vol. 103, p. 108701, 2009.

[8] S. Ciliberti, O. C. Martin, and A. Wagner, “Innovation and robustness in complex regulatory gene networks,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, pp. 13 591–13 596, 2007.

[9] ——, “The role of robustness in phenotypic adaptation and innovation,” *Proceedings of the Royal Society B: Biological Sciences*, vol. 279, pp. 1249–1258, 2012.

[10] C. Espinosa-Soto, O. C. Martin, and A. Wagner, “Phenotypic robustness can increase phenotypic variability after nongenetic perturbations in gene regulatory circuits,” *Journal of Evolutionary Biology*, vol. 24, pp. 1284–1297, 2011.

[11] J. de Visser, J. Hermisson, G. P. Wagner, L. Meyers, H. Bagheri-Chaichian, J. L. Blanchard, and L. Chao, “Perspective: evolution and detection of genetic robustness,” *Evolution*, vol. 57, pp. 1959–1972, 2003.

[12] J. de Visser, J. Hermisson, G. P. Wagner, L. A. Meyers, H. Bagheri-Chaichian, J. L. Blanchard, and L. Chao, “Biological robustness,” *Nature Reviews Genetics*, vol. 5, pp. 826–837, 2004.

[13] N. Kashtan, E. Noor, and U. Alon, “Varying environments can speed up evolution,” *Proceedings of the National Academy of Sciences*, vol. 104, no. 34, pp. 13 711–13 716, 2007.

[14] N. Kashtan and U. Alon, “Spontaneous evolution of modularity and network motifs,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 39, pp. 13 773–13 778, 2005.

[15] S. Ciliberti, O. C. Martin, and A. Wagner, “Robustness can evolve gradually in complex regulatory gene networks with varying topology,” *PLoS Computational Biology*, vol. 3, p. e15, 2007.

[16] A. Crombach and P. Hogeweg, "Evolution of evolvability in gene regulatory networks," *PLoS Computational Biology*, vol. 4, p. 1000112, 2008.

[17] D. J. Earl and M. W. Deem, "Evolvability is a selectable trait," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, pp. 11 531–11 536, 2004.

[18] M. Pigliucci, "Is evolvability evolvable?" *Nature Reviews Genetics*, vol. 9, pp. 75–82, 2008.

[19] D. Sornette, *Critical Phenomena in Natural Sciences: Chaos, Fractals, Self Organization and Disorder*. New York, USA: Springer, 2006.

[20] S. A. Kauffman, "Metabolic stability and epigenesis in randomly constructed genetic nets," *Journal of Theoretical Biology*, vol. 22, pp. 437–467, 1969.

[21] ——, *Origins of Order: Self-organization and Selection in Evolution*. New York, USA: Oxford University Press, 1993.

[22] R. Albert, "Boolean modeling of genetic regulatory networks," *Lecture Notes in Physics*, vol. 650, pp. 459–481, 2004.

[23] F. Li, T. Long, Y. Lu, Q. Ouyang, and C. Tang, "The yeast cell-cycle network is robustly designed," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, pp. 4781–4786, 2004.

[24] C. E. Giacomantonio and G. J. Goodhill, "A boolean model of the gene regulatory network underlying mammalian cortical area development," *PLoS Computational Biology*, vol. 6, pp. 4781–4786, 2010.

[25] D. MacLea and D. J. Studholme, "A boolean model of the pseudomonas syringae hrp regulon predicts a tightly regulated system," *PLoS One*, vol. 5, pp. 101–114, 2010.

[26] R. Schlatter, K. Schmich, I. Avalos-Vizcarra, P. Scheurich, T. Sauter, C. Borner, M. Ederer, I. Merfort, and O. Sawodny, "On/off and beyond - a boolean model of apoptosis," *PLoS Computational Biology*, vol. 5, p. e1000595, 2009.

[27] A. Nakajima, T. Isshiki, K. Kaneko, and S. Ishihara, "Robustness under functional constraint: the genetic network for temporal expression in drosophila neurogenesis," *PLoS Computational Biology*, vol. 6, no. 4, p. e1000760, 2010.

[28] B. Derrida and Y. Pomeau, "Random networks of automata: a simple annealed approximation," *Europhysics Letters*, vol. 1, pp. 45–49, 1986.

[29] M. Aldana, "Boolean dynamics of networks with scale-free topology," *Physica D*, vol. 185, pp. 45–66, 2003.

[30] M. Aldana and P. Cluzel, "A natural class of robust networks," *Proceedings of the National Academy of Sciences*, vol. 100, no. 15, pp. 8710–8714, 2003.

[31] I. Shmulevich and S. A. Kauffman, "Activities and sensitivities in boolean network models," *Physical Review Letters*, vol. 93, no. 4, p. 048701, 2004.

[32] S. Huang, G. Eichler, Y. Bar-Yam, and D. E. Ingber, "Cell fates as high-dimensional attractor states of a complex gene regulatory network," *Physical Review Letters*, vol. 94, p. 128701, 2005.

[33] E. R. Alvarez-Buylla, E. Azpeitia, R. Barrio, M. Benítez, and P. Padilla-Longoria, "From abc genes to regulatory networks, epigenetic landscapes and flower morphogenesis: Making biological sense of theoretical approaches," *Seminars in Cell & Developmental Biology*, vol. 21, pp. 108–117, 2010.

[34] M. Aldana, E. Balleza, S. A. Kauffman, and O. Resendiz, "Robustness and evolvability in genetic regulatory networks," *Journal of Theoretical Biology*, vol. 245, pp. 433–448, 2007.

[35] S. A. Teichmann and M. Madan-Babu, "Gene regulatory network growth by duplication," *Nature Genetics*, vol. 36, pp. 492–496, 2004.

[36] E. Balleza, E. Alvarez-Buylla, A. Chaos, S. A. Kauffman, I. Shmulevich, and M. Aldana, "Critical dynamics in genetic regulatory networks: Examples from four kingdoms," *PLoS ONE*, vol. 3, p. e2456, 2008.

[37] R. Serra, M. Villani, and A. Semeria, "Genetic network models and statistical properties of gene expression data in knock-out experiments," *Journal of Theoretical Biology*, vol. 227, pp. 149–157, 2004.

[38] R. Serra, M. Villani, A. Graudenzi, and S. A. Kauffman, "Why a simple model of genetic regulatory network describes the distribution of avalanches in gene expression data," *Journal of Theoretical Biology*, vol. 246, pp. 449–460, 2007.

[39] I. Shmulevich, S. A. Kauffman, and M. Aldana, "Eukaryotic cells are dynamically ordered or critical but not chaotic," *Proceedings of the National Academy of Sciences*, vol. 102, pp. 13 439–13 444, 2005.

[40] M. Nykter, N. D. Price, M. Aldana, S. A. Ramsey, S. A. Kauffman, L. E. Hood, O. Yli-Harja, and I. Shmulevich, "Gene expression dynamics in the macrophage exhibit criticality," *Proceedings of the National Academy of Sciences*, vol. 105, pp. 1897–1900, 2008.

[41] C. Torres-Sosa, S. Huang, and M. Aldana, "Criticality is an emergent property of genetic networks that exhibit evolvability," *PLoS Computational Biology*, vol. 8, p. e1002669, 2012.

[42] R. Albert, "Scale-free networks in cell biology," *Journal of Cell Science*, vol. 118, pp. 4947–4957, 2005.

[43] B. D. MacArthur, R. J. Sánchez-García, and A. Ma'ayan, "Microdynamics and criticality of adaptive regulatory networks," *Physical Review Letters*, vol. 104, p. 168701, 2010.

[44] V. M. Eguiluz, D. R. Chialvo, G. A. Cecchi, M. Baliki, and A. V. Apkarian, "Scale-free brain functional networks," *Physical Review Letters*, vol. 94, p. 018102, 2005.

Multi-scale modeling of dynamic systems for evolution

M. García & E. Azpeitia, Instituto de Ecología and C3, UNAM, Mexico

1 Abstract

It has long been recognized that processes occurring at different scales of organization affect the evolution of organisms. Molecular, cellular and environmental sub-processes, among others, are involved in settling the basis where evolutionary processes will take place. Changes at the genetic level can affect gene expression patterns, protein biochemical properties, and regulatory interactions, which in turn can modify the structure and dynamic of regulatory networks. Changes at the cellular level can affect cell properties, which modify the cell microenvironment, communication and interaction with the surroundings. Finally, environmental changes modify the morphology, physiology and behavior of the organisms. Until now, most of these levels of organization have been studied as independent from each other. However, all of them interact, constraining and allowing the evolutionary possibilities at the same time. In such a scenario, the sub-processes are not just passive players during evolutionary processes, instead they help to determine the variety of possibilities. With the advent of new theoretical and technological approaches for biological research, especially from the computational and mathematical fields, multi-scale models that study the interaction of these sub-processes in evolutionary processes are starting to appear. Here we highlight some of the results obtained by these studies in order to gain a better view of their importance and utility in the areas of development, evolution and biological complexity.

2 Resumen

Desde hace mucho tiempo se sabe que procesos que ocurren en diferentes niveles de organización afectan la evolución de los organismos. Hay varios subprocesos, como los moleculares, los celulares y los ambientales, entre otros, involucrados en los procesos evolutivos. Cambios a nivel de la secuencia genética pueden afectar los patrones de expresión genético, las propiedades bioquímicas de las proteínas, e interacciones de regulación, que a su vez pueden modificar la estructura y dinámica de las redes de regulación. Cambios a nivel celular pueden alterar propiedades celulares, modificando el microambiente celular y el modo en que la célula se comunica e interactúa con su vecindario. Finalmente, cambios ambientales modifican la morfología, fisiología y el comportamiento de los organismos. Hasta ahora, los distintos niveles de organización han sido estudiados de forma independiente. Sin embargo, todos actúan de forma conjunta, interactuando entre si, para restringir y posibilitar los procesos evolutivos. En este escenario, los subprocesos no son actores pasivos durante la evolución, sino que participan en la determinación de las posibilidades evolutivas. Con la llegada de nuevas herramientas tecnológicas y teóricas para la investigación biológica, especialmente del campo de las matemáticas y la computación, comienzan a aparecer modelos multi-escala que permiten estudiar la acción conjunta de los subprocesos durante la evolución. En este trabajo describimos algunos de los principales resultados obtenidos con estos modelos, con la finalidad de mostrar su utilidad e importancia en las áreas de desarrollo, evolución y complejidad biológica.

3 Introduction: Changing the paradigm, multi-scale modeling approaches

Most of evolutionary research has been focused on the molecular basis of evolution, as genetic changes can result in beneficial or deleterious phenotypic changes. Based on the idea that differences in genes and allele frequency can reflect and determine the evolutionary history of any organism, population genetics has flourished arguably as the most important evolutionary research field in biology. The proliferation and advances in the tools and methodologies available for population genetics research have been marvelous (e.g., [1]), but underestimate the role of development and ecological influences in evolutionary processes. For example, the genotype-phenotype mapping that occurs during development, which is fundamental to validate current assumptions and understand evolution, is just starting to be understood. Nowadays, we know that the phenotype is the result of development, which is influenced by diverse processes performed by genes, proteins and other molecules, as well as cell interactions, morphogen fluxes, environmental conditions, physical forces, among other things, and not genes alone.

Importantly, in part due to the technological improvements, during the last decades the mechanisms behind biological complexity have become more evident, including developmental and evolutionary ones. There are a large number of elements involved in such processes, all of which interact non-linearly producing non-intuitive behaviors. Therefore, these processes are difficult to predict and understand. Given this complexity, the challenge to understand biological phenomena is largely about the interpretation and integration of experimental results of a sole process studied in pieces. Because of this, modeling approaches that allow the integration of different sorts of data have become indispensable for biological research.

Biological processes can be studied at different temporal and spatial scales. Spatially, biological research ranges from molecular, to cell, tissue, organ, organism and ecological scales. The temporal scale of biological processes also has a broad range, some processes occurring in microseconds, like the molecular interactions, while other take years, like many organisms life cycles, and other centuries or more, like evolutionary processes [2](see Figure 1).

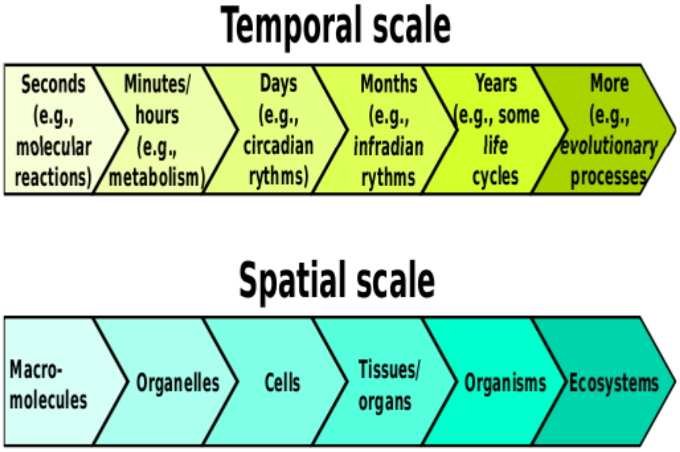


Figure 1: Different temporal and spatial scales of biological organization.

Different tools and methodologies have been developed to study processes at each scale. For example, network theory is useful to structurally and dynamically study processes at the molecular, cellular and ecological scale [3]. However, many biological processes are the product of processes acting together at different scales. For example, during developmental processes, while cells are differentiating via the action of their own gene regulatory network, they might influence the differentiation status of neighboring cells through the movement of molecules, leading to coherent cellular patterns, which will form different tissues and organs. The geometrical and mechanical properties of the tissue can influence morphogen distribution, cellular arrangement and behavior [4–6] which could modify gene activity. Thus, studying how these processes act together is fundamental for a better understanding of biological processes.

Consequently, multi-scale models are starting to appear more frequently. Similarly, software to analyze multi-scale models is being constantly developed (e.g., [7–15], see also the Supplementary Information section at the end of this chapter). In particular, multi-scale modeling has become a useful tool for the study of development.

The software for multi-scale modeling usually relies in different methodologies and formalisms that need to be coupled. For example, CompuCell3D (CC3D) is a software that implements the Cellular Potts Model (CPM) formalism and enables the user to develop multi-scale models [7]. Such models can take into account intracellular and extracellular dynamics, that in a population of cells can lead to the formation of tissues or complex organs. In CPM cells are modeled as joint positions in a lattice with the same identifier, and their behavior with a potential energy H. We provide an example of H in the next equation:

$$H = \sum_{(i,j)} \sum_{(i',j')} J_{\tau(\sigma_{i,j}),\tau(\sigma_{i^{`},j^{`}})} + \sum_{\sigma} (P_{\sigma} - P_{T})^{2} \tag{1}$$

where (i, j) is a position of the lattice, J is the contact energy between cells, τ is the cell type, σ is the cell identifier and P is a certain cellular property. The first term includes two summations over the lattice positions (i, j) and over its neighboring positions (i', j'). The parameter $J_{\tau(\sigma_{i,j}),\tau(\sigma_{i^{`},j^{`}})}$ quantifies the contact energy between the neighboring lattice points $(i, j), (i', j')$ and serves to model adhesive and cohesive interactions between cells. Such interactions underlie the formation of cell clusters by cell-cell contacts. The second term is a summation over all cells to quantify the deviation between the actual (P_{σ}) and the target value (P_{T}) of a cellular property. Using this term, the user can define different cellular properties, such as the cell perimeter or area. A CPM simulation uses a Monte-Carlo algorithm to select cellular configurations that minimize the potential energy.

At the same time, CC3D allows the use of different modeling techniques for other biological processes not considered in Equation (1). For example, regulatory networks can be introduced through different formalisms according to the specific requirements of a model. It is possible to use Boolean and ordinary differential equations formalisms, among others. Importantly, cellular properties like the cell adhesion parameters can be defined by the state of an intracellular network. Moreover, using partial differential equations (PDE), elements of a network can move between cells leading to communication circuits that help to coordinate a population of neighboring cells. Additionally, using PDE it can be modeled environmental inputs as nutrient availability.

Therefore, with CC3D it is possible to build models of complex process encompassing dynamics at the intracellular (regulatory network), cellular (cell adhesion) and tissular (molecule gradient) scale, which altogether regulate a certain biological phenomena (see Figure 2).

Multi-scale models of morphogenesis have been used with great results to give insights into how processes are spatio-temporally coupled and which is the role of each of them in the resulting macroscopical pattern. Anyhow, the use of multi-scale models to study evolution has remained scarce. Now we review how multi-scale modeling approaches have been successful in different contexts, with an emphasis for development. Then we look at a few examples of how they have been used in evolution. Finally, we discuss their utility and necessity to extend their use for the study of evolution.

4 Multi-scale models of non-evolutionary biological processes

Multi-scale models have been used in many biological contexts with successful results. For example, due to global climate change, the demand for models that can predict and understand the response of organisms to environmental changes has become a main issue for planning conservation strategies. However, in an ever-changing world, the number of variables that can affect the organism responses is huge and come from different organizational scales. First attempts to include multiple variables from different scales, like demography, gene flow and heterogeneous environments, among others, are starting to appear. These studies have provided projections of species distribution and community structure [16].

Other studies have focused on the interaction between organisms and environment. A methodology commonly used for this kind of models is the so-called functional-structural models [8]. One of the most employed functional-structural models are L-systems [17]. For example, using L-systems, Leitner and collaborators [18], developed a model of plant root growth that integrates internal cues that in turn responded to changes in the environmental conditions. Using this model, they were able to understand the impact of root and rizhosphere on plant resource efficiency.

Developmental studies have been the focus of multi-scale modeling. For example, when nutrients are scarce and the growing conditions are not adequate, *Dyctiostelium discoideum* aggregate in a multicellular slug that will develop into a fruiting body. Its formation involves some cells periodically secreting cAMP and others periodically moving chemotactically towards a cAMP gradient. This process was studied in a two-dimensional model considering intracellular excitable cAMP dynamics, cAMP secretion and cellular migration [19]. Because the model studies a population of cells, each of them with an intracellular dynamic and a gradient of cAMP that guides cell chemotaxis, it constitutes a multi-scale model. The model was able to reproduce the cellular movements observed *in vivo*, and was useful to understand how some sub-processes are spatio-temporally coupled for the morphogenesis of the fruiting body. Moreover the model predicted that chemotactic movements produce pressure waves displacing non-responding cells downwards and responding cells upwards of the fruiting body.

Another example of multi-scale models for the study of developmental processes is one of somitogenesis proposed by Hester and collaborators [20]. The formation of somites is an important event in the embryonic development of vertebrates as they form different body segments in organisms as varied as chicken, mice, zebrafish and snakes. Hester and collaborators [20] proposed a multi-scale model of somitogenesis that takes into account an oscillatory regulatory network, a growth factor gradient, differential cell adhesion and cell proliferation. Through multi-scale modeling it was possible to describe how these different sub-processes are concerted and establish the spatio-temporal dynamic observed during somite formation. The model makes predictions about some sub-processes and the somitogenesis process as a whole. For example, it provides hints of the changes in the

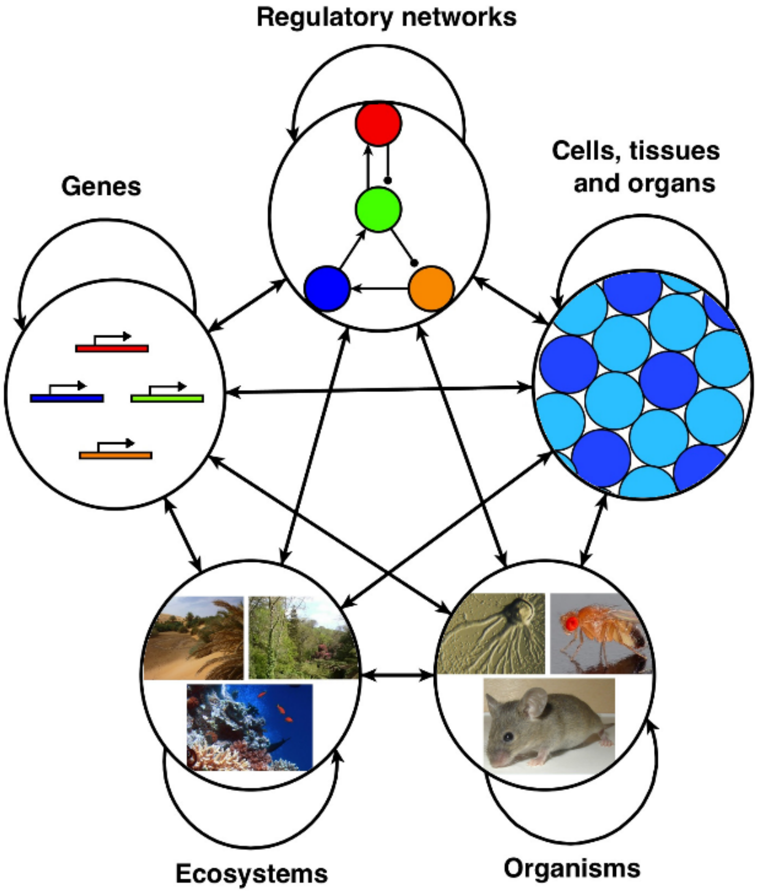


Figure 2: Scales of organization that can been considered in multi-scale models.

parameters of the model that lead to the different number and length of somites observed in different vertebrate organisms.

This is only a small sample of multi-scale modeling in biology. However, multi-scale models have been used also to study other developmental processes like gastrulation, stem cell differentiation, vasculogenesis, diseases like cancer [21] and tuberculosis [22], to combine physical and molecular processes [23, 24], among many other issues. Now, let us briefly review some of the multi-scale models of evolution published until now.

5 Multi-scale models of evolutionary processes

In one of the most interesting multi-scale models of evolution generated until now, the genotype was translated into a dynamical network in a multicellular space to study tooth morphogenesis [25]. Depending on the dynamic of the network, specific morphologies were observed, which corresponded with observed ones in different mammalian populations. The morphology was then translated into a fitness value, which determined the individual chances of contributing to the next generation and the model reproduced actual evolutionary transitions. Thus, the model allowed the study of the complex genotype-phenotype mapping and the identification of the changes in the parameters (mutations) that give rise to a variety of morphologies actually observed in nature. This is a pioneering work that considers a genotype translated into a realistic evolving phenotype.

Hogeweg [26] proposed a multi-scale model in which cells had a gene regulatory network that defined its differentiation status and adhesion properties. Cells stretch due to their adhesion properties and proliferate whenever their volume surpassed a threshold. Evolution was incorporated in the model by allowing random mutation in the gene regulatory network. Using this model it was possible to simulate and analyze the evolution of complex morphologies as engulfing, budding and elongation, intercalation and elongation, among others. Thus, by using a multi-scale approach, Hogeweg studied the concerted action of cell growth, cell differentiation and cell biophysical properties during evolution of multicellular morphologies.

Moving forward with the evolutionary multi-scale modeling approach, Ten Tusscher and Hogeweg [27] studied how body a pattern with segments and different cell types could evolve. In order to do this, they generated a population of organisms, each one composed of a hundred cells. The identity of the cells and the appearance of segments were determined by the stable states of a gene regulatory network, which perceived the concentration of a morphogen wavefront. The network was allowed to evolve, by changing the gene interactions and update functions. Finally, organisms were able to reproduce and they were selected according to the body pattern that they generated, following a genetic algorithm approach. This work not only showed how a network able to produce segments and different cell types could evolve, but also challenged some generic network features theoretically proposed. For example, it has been proposed that organisms produce different morphological traits (segments and cell types in this case) as a consequence of the modular structure of gene networks. However, the networks produced in this study were not structurally modular, but functionally modular.

6 Discussion and conclusions

Some biological processes contain sub-processes that can be studied independently from each other. Different mathematical tools can be used to model these sub-processes according to the level of description necessary to describe them dynamically. For example, gene regulatory networks can be modeled with boolean networks, cell signaling with differential equations, transport of molecules between cells with logic rules, and diffusion of morphogens with partial differential equations, etc. As useful as it is to separate spatio-temporal scales to simplify any research, it is extremely difficult to explain biological processes by studying independently each sub-process. Thus, it is often not possible to fully understand them if we only study their sub-processes in isolation. Consequently, multi-scale modeling is a necessary tool to understand many current questions in many different biological fields.

Evolution is not the exception. The evolution of organisms is the result of ongoing complex processes at different scales. Currently, with the advent of modeling tools and the increase in available biological data, it is expected a rise in the limited amount of work done for the study of evolution with multi-scale modeling approaches. Efforts in this direction would be important in order to understand and gain better insight about evolutionary processes. For example, it would be useful to understand the genotype-phenotype map and to consider the role of developmental processes to functionally understand how genetic mutations change the fitness of an organism. Such an approach could explain how phenotypic novelties arise and are subject to natural selection.

However, multi-scale models present some complications, constrains and limitations. For instance, as multi-scale modeling deals with spatio-temporal processes, one critical step is to carefully couple such processes in time and space, which can be methodologically challenging. Also, multi-scale modeling contain many elements and data. This complicates modeling, is computationally expensive and prevents analytical analyses of the models.

It is important to notice, that multi-scale models do not need to consider each and all sub-processes. Instead, it is an approach that opens the possibility to study how some subprocesses, important by themselves, are part of a sole process.

As briefly reviewed in this article, multi-scale modeling is a useful tool to discern the entanglement of processes regulating complex morphological traits, the environmental-organism interactions, and the effect of environmental change. Importantly, in the studies mentioned, some of the results could not be obtained without the use of multi-scale modeling since they are the outcome of the interplay between micro- and macroscale dynamics over evolutionary time. Evolutionary studies could take advantage of the great integrative and analytical capacity of the multi-scale models.

For the reasons exposed above, we think that the use of a multi-scale modeling approach could improve our predictability and understanding of evolutionary processes under an integrative framework. We hope that this article will convince the reader of the utility of multi-scale modeling for the study of biological processes, specially the evolutionary ones.

Supplemenary Information Online

For a table compiling a list with examples of software designed for multi-scale modeling, visit: `http://miro.fisica.unam.mx/SI-01.html`

7 Bibliography

[1] H. A. Orr, “The genetic theory of adaptation: a brief history,” *Nature Reviews Genetics*, vol. 6, no. 2, pp. 119–27, February 2005.

[2] J. O. Dada and P. Mendes, “Multi-scale modelling and simulation in systems biology.” *Integrative Biology*, vol. 3, no. 2, pp. 86–96, February 2011.

[3] H. de Jong, “Modeling and simulation of genetic regulatory systems: a literature review.” *Journal of Computational Biology*, vol. 9, no. 1, pp. 67–103, 2002.

[4] T. Mammoto and D. E. Ingber, “Mechanical control of tissue and organ development.” *Development*, vol. 137, no. 9, pp. 1407–20, May 2010.

[5] J. Traas and O. Hamant, “From genes to shape: understanding the control of morphogenesis at the shoot meristem in higher plants using systems biology.” *Comptes Rendus Biologies*, vol. 332, no. 11, pp. 974–85, November 2009.

[6] P. M. Lintilhac, “The problem of morphogenesis: unscripted biophysical control systems in plants.” *Protoplasma*, July 2013.

[7] M. H. Swat, G. L. Thomas, J. M. Belmonte, A. Shirinifard, D. Hmeljak, and J. A. Glazier, *Computational Methods in Cell Biology*, ser. Methods in Cell Biology. Academic Press, 2012, vol. 110, ch. Chapter 13 - Multi-Scale Modeling of Tissues Using CompuCell3D, pp. 325–366.

[8] T. M. DeJong, D. Da Silva, J. Vos, and A. J. Escobar-Gutiérrez, “Using functional–structural plant models to study, understand and integrate plant development and ecophysiology,” *Annals of Botany*, vol. 108, no. 6, pp. 987–9, October 2011.

[9] P. Joe, P. Pathmanathan, M. Bernabeu, R. Bordas, J. Cooper, A. Fletcher, G. Mirams, P. Murray, J. Osborne, A. Walter, S. Chapman, A. Garny, I. M. van Leeuwen, P. Maini, B. Rodriguez, S. L. Waters, J. Whiteley, H. Byrne, and D. J. Havaghan, “Chaste: a test-driven approach to software development for biological modelling,” *Computer Physics Communications*, vol. 180, no. 12, 2009.

[10] S. Chaudhary, S. Shin, D. Lee, J. Song, and K. Cho, “Elecans- and integrated model development environment for multiscale cancer systems biology,” *Bioinformatics*, vol. 29, no. 7, 2013.

[11] C. Pradal, S. Dufour-Kowalski, F. Boudon, C. Fournier, and C. Godin, “Openalea: a visual programming and component-based software platform for plant modelling,” *Functional Plant Biology*, vol. 35, pp. 751–760, 2008.

[12] T. Eissing, L. Kuepfer, C. Becker, M. Block, K. Coboeken, T. Gaub, L. Goerlitz, J. Jaeger, R. Loosen, B. Ludewig, M. Meyer, C. Niederalt, M. Sevestre, H.-U. Siegmund, J. Solodenko, K. Thelen, U. Telle, W. Weiss, T. Wendl, S. Willmann, and J. Lippert, "A computational systems biology software platform for multiscale modeling and simulation: Integrating whole-body physiology, disease biology, and molecular reaction networks," *Frontiers in Physiology*, vol. 2, no. 4, 2011.

[13] F. Faure, C. C. Duriez, H. Delingette, J. Allard, B. Gilles, S. Marchesseau, H. Talbot, H. Courtecuisse, G. Bousquet, I. Peterlik, and S. Cotin, "Sofa: A multi-model framework for interactive physical simulation," –, –.

[14] L. M. Loew and J. Schaff, "The virtual cell: a software environment for computational cell biology," *Trends in Biotechnology*, vol. 19, no. 10, 2001.

[15] R. M. Merks, M. Guravage, D. Inzé, and G. T. Beemster, "Virtualleaf: an open-source framework for cell-based modeling of plant tissue growth and development," *Plant Physiology*, vol. 155, no. 2, pp. 656–66, 2011.

[16] W. Thuiller, T. Münkemüller, S. Lavergne, D. Mouillot, N. Mouquet, K. Schiffers, and D. Gravel, "A road map for integrating eco-evolutionary processes into biodiversity models." *Ecology Letters*, vol. 1, pp. 94–105, May 2013.

[17] P. Prusinkiewicz and A. Lindenmayer, *The Algorithmic Beauty of Plants*. Springer-Verlag, 1990.

[18] D. Leitner, S. Klepsch, M. Ptashnyk, A. Marchant, G. J. Kirk, A. Schnepf, and R. T., "A dynamic model of nutrient uptake by root hairs," *New Phytologist*, vol. 185, no. 3, pp. 792–802, February 2010.

[19] A. F. Marée and P. Hogeweg, "How amoeboids self-organize into a fruiting body: multicellular coordination in *Dyctiostelium discoideum*," *PNAS*, vol. 98, no. 7, pp. 3879–3883, March 2001.

[20] S. D. Hester, J. M. Belmonte, J. S. Gens, S. G. Clendenon, and J. A. Glazier, "A multi-cell, multi-scale model of vertebrate segmentation and somite formation," *PLoS Computational Biology*, vol. 7, no. 10, October 2012.

[21] J. Wang, L. Zhang, C. Jing, G. Ye, H. Wu, H. Miao, Y. Wu, and X. Zhow, "Multi-scale agent-based modelling on melanoma and its related angiogenesis analysis," *Theoretical Biology and Medical Modelling*, vol. 10, 2013.

[22] M. Fallahi-Sichani, M. El-Kebir, M. Marino, D. E. Kirschner, and J. Linderman, "Multiscale computational modeling reveals a critical role for tnf-α receptor 1 dynamics in tuberculosis granuloma formation," *The Journal of Immunology*, vol. 186, pp. 3472–3483, 2011.

[23] R. A. Barrio, J. R. Romero-Arias, M. A. Noguez, E. Azpeitia, E. Ortiz-Gutiérrez, V. Hernández-Hernández, Y. Cortes-Poza, and E. R. Álvarez-Buylla, "Cell patterns emerge from coupled chemical and physical fields with cell proliferation dynamics: The *Arabidopsis thaliana* root as a study system," *PLoS Computational Biology*, vol. 9, no. 5, May 2013.

[24] R. Zubler, A. Hauri, S. Pfister, R. Bauer, J. C. Anderson, A. M. Whatley, and R. J. Douglas, "Simulating cortical development as a self constructing process: a novel multi-scale approach combining molecular and physical aspects," *PLoS Computational Biology*, vol. 9, no. 8, August 2013.

[25] I. Salazar-Ciudad and M. Marín-Riera, "Adaptive dynamics under development-based genotype-phenotype maps," *Nature*, vol. 497, pp. 361–364, 2013.

[26] P. Hogeweg, "Shapes in shadow: evolutionary dynamics of morphogenesis," *Artifical Life*, vol. 6, no. 1, pp. 85–101, 2000.

[27] K. H. Ten Tusscher and P. Hogeweg, "Evolution of networks for body plan patterning; interplay of modularity, robustness and evolvability," *PLoS Computational Biology*, vol. 7, no. 10, October 2011.

A sound of flower: evolutionary teachings from complex systems

A. Chaos, Facultad de Ciencias and C3, UNAM, Mexico

Embedded in the mud, glistening green and gold and black,
was a butterfly, very beautiful and very dead.
R. Bradbury

El especialista ≪sabe≫ muy bien su mínimo rincón de universo;
pero ignora de raíz todo el resto.
J. Ortega y Gasset

1 Abstract

The new synthesis, mainstream theory of biological evolution, has serious problems when explaining certain biological phenomena. Reasons of this incapacity lie in Aristotelian functionalism, in implicit or explicit reductionism and in a disproportionate protagonist role of natural selection as the principal source of order in nature. The physical form of living creatures, according with functionalism, has a secondary, even negligible role in establishing natural prototypes. Form follows function. Thus existing structures are seeing as adaptations maintained and modified just by natural selection, they are exclusively the product of natural selection, the basic idea of the famous adaptationist program. Natural selection is a process of election which lead organisms to have a higher fitness; however, it depends on the existence of characters to choose from. Therefore, other process responsible for the creation of such traits must exist. Self-organization is presented as the previous force who determines the characteristics of the basic building units, relegating natural selection to a secondary role. A new evolutionary theory must be formulated that incorporates the teachings of Boolean genetic networks, self- organization, complex systems, chaos, with the recoverable traditional elements of the new synthesis.

2 Resumen

La síntesis nueva, teoría dominante sobre la evolución biológica, presenta serios problemas al tratar de explicar ciertos fenómenos biológicos. La raíz de esta incapacidad se debe principalmente a que se basa en un funcionalismo aristotélico, en un reduccionismo explícito o implícito y en darle un papel protagonista a la selección natural como fuerza ordenadora de la naturaleza. Si se analiza a los seres vivos desde una perspectiva funcionalista, la forma cobra un papel secundario, hasta despreciable, en el establecimiento de los prototipos naturales. La forma sigue a la función. Por ello las estructuras se perciben como adaptaciones mantenidas y modificadas sólo por la selección natural, son un producto exclusivo de ella, idea fundamental del famoso programa adaptacionista. La selección natural es un proceso de elección que encamina a los seres vivos a aumentar su eficacia; sin embargo, depende de que las características estén disponibles para elegirlas. Por lo tanto, debe de existir otro proceso responsable de crear las unidades de construcción naturales sobre las cuales, posteriormente, la selección natural actuará. Se presenta a la autoorganización como la fuerza previa que determina las características de las unidades de construcción, desplazando a la selección natural a un plano subordinado. Urge crear una teoría evolutiva nueva que incorpore las enseñanzas de las redes genéticas binarias, de la autoorganización, de los sistemas complejos, del caos, así como los elementos tradicionales recuperables de la nueva síntesis.

3 Introduction

According to Darwin's theory of natural selection, biological evolution must proceed in infinitesimal steps [1]. This is known as gradual evolution. Under these circumstances, the species concept has no reality. It is just an abstraction of the human mind. All recent species can be traced back by intermediate forms to the universal common ancestor of all life on Earth. With this perspective, the importance of the fossil record is overwhelming. The search of intermediate forms, the famous missing links, became an obsession because several groups of organisms did not have a connecting fossil with any known group. Man, birds and flowering plants, were the most remarkable. The apparently sudden origin of the flower, a conspicuous reproductive structure within the botanic world, was called by Darwin as an "abominable mystery" in a letter written to botanist Joseph Dalton Hooker in 1881. Nowadays, the fossil record is more complete, thus according with a gradualist point of view, the evolutionary history of the flower can be linked by a series of steps. However, it is not clear which is the common ancestor of flowering plants.

The new synthesis, the mainstream theory of biological evolution, born in the middle of the 20th century, was conformed with a great amount of facts from different biological disciplines: systematics, biogeography, development, paleontology, genetics, etc. [2]. The main ideas sustaining the new synthesis are natural selection, Mendelian inheritance, population genetics, and germoplasm theory. Other ideas, as the use and disuse of characters and the inheritance of acquired characters, life's tendency to complexity, orthogenesis, and macroevolution as a different process from microevolution, were denied. The theory can be resumed as: biological evolution is gradual, it occurs mainly by natural selection operating on variation among individuals within a population.

More than half a century has past since the new synthesis hatched, but still some fundamental questions have remained unanswered[1]. Despite the fact that some wrong ideas about life have been abandoned, such as Platonic essentialism, others, such as Aristotelian functionalism and reductionism continue contaminating the interpretations of the evolutionary process. The former postulates that form follows function. Characters of a living being are the result of a purpose, in that sense, functionalists are teleologists. As such, all parts of an organism respond to a task, and thus can be construed as being adaptations. The structure of any character depends on its purpose, its role. The latter, supposes that an entity is just the sum of its parts. It is possible to study a creature by dividing its body into pieces, examine each one separately, and finally, put them back together. The organism is the sum of its parts. The inception of the well criticized adaptationist program lies in these two philosophical positions [3]. Once a living being is finally constructed, natural selection explains how well it is adapted, but the new synthesis remains mute about the origin of evolutionary novelties [4]. Final causes (*telos*) may be studied with a functionalist vision, but not proximal causes (pera); moreover, final causes depend on proximal ones [5].

4 A case for *Shellock* Holmes

We live in a planet with an overflowing biodiversity. Sometimes the spectrum of different shapes of flowers, leaves, eyes, shells, seems infinite. Is nature really so prolific? Where are the limits to the biodiversity of forms, of nature's creativeness? Among the animal kingdom, only four groups have shells. They are the Phylum Brachiopoda, and three classes within the Phylum Mollusca: cephalopods, bivalves, and gastropods. The richness of shell forms is astonishing. Ranging from the baroque beauty of the delicate comb of Venus (*Murex pecten*), through a resemblance of a blade as in sword razor (*Ensis ensis*), to the mystic proportions of nautilus (*Nautilius pompilius*), creativity of nature looks endless. Hardly can we imagine new forms. At a first glance, the vast majority of possible shells forms have been created. Or have they? The new synthesis cannot respond this question. We need to know how shells are constructed, the secret rules behind them.

Raup [6] made a mathematical model in which the shape of a shell can be described with three parameters. The first quantity (W) affects the rate at which the diameter of the tube grows. The opening of the shell becomes wider with each rotation with higher values. The second (D), affects the distance of the center of the tube from the axis coiling.

---

[1] The main reason of this situation involves the divorce and conflict between two plans of investigation: the study of development and the study of biological evolution, which occurred in 19th century.

With higher values the tube of the shell will form further from the coiling axis. Finally, the last variable (T), affects the distance of the center of the tube from the previous rotation along the axis of rotation. The shell will be taller with higher values. Any combination of these three parameters can be plotted in a cube, each dimension will represent one parameter. Inside this cube, each point is a combination of these parameters and represents a potential form of a shell. Any shell constructed by nature will be inside this cube. This space is called morphospace: the set of all possible morphs of shells (Figure 1). When we plot all known forms of these shields, only a small region of the cube is occupied. An enormous part of it remains empty. With this evidence, the new synthesis explains that natural selection is the responsible for the order found in the occupied zone. Shells are mainly, almost enterily, a product of this natural force. The explanation given from an adaptationist perspective is that there are no shells in the huge void of the cube because they are not adaptive, or some kinds of constraints avoid their existence. Even with Raup's model, these answers are extremely general. Much ado about nothing.

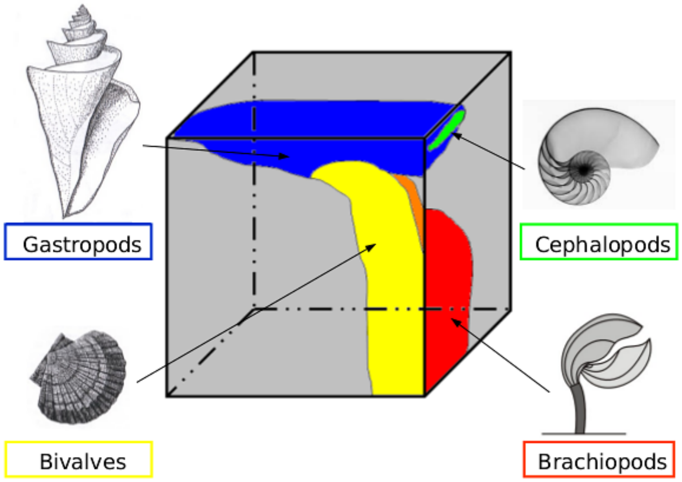


Figure 1: Shell morphospace. According to Raup's model, a shell can be constructed with three parameters, each point in the cube represents a particular combination of these measures, therefore a particular shell. Four groups of animals have shells, the zone they occupy is marked inside the cube with different colors.

5 Spices: self-organization, complex systems, and chaos

Some aggregations of organisms show extraordinary orderly behaviors. Shoals and flocks are typical examples. Starlings form a vast array of rich and complicated conformations as they fly over European skies. All birds fly seem to fly according to a general program, but there is no such plan. There is no leader who commands all birds to follow him. Order emerges spontaneously. Local rules between each starling (going near other individuals, avoiding crash, etc.), generate the global pattern. This property present in some systems is self-organization.

Reductionist science has been very successful; however, its limits become evident when it deals with certain kinds of systems and phenomena. Certain groupings are not the sum of parts, thus, to study them we need to take into account the connections between their elements. For instance, studying an individual ant or a neuron will not reveal that a group of them, under specific conditions, and as a property of a particular number and the way they are connected, can generate an anthill or consciousness. These properties emerge from the interaction of the elements and cannot be anticipated with a reductionist point of view. A system whose dynamical properties cannot be understood without acknowledging its connections is a complex system.

Certain processes show a messy relation between their inputs and their outputs. Sometimes this apparent absence of cause or rules is called chance. Moreover, little variations on the inputs can produce radically different results. But if the initial conditions are exactly the same, the final outcomes will be identical. A deterministic dynamic system with high sensibility to initial conditions is chaotic.

6 A, B, C, D, E, F, Genes

A wealth of data on the molecular genetic mechanisms underlying different aspects of flower development have accumulated during the last two decades. The genes controlling cell-fate determination during floral organ specification have yielded the so-called ABC model of flower development that was derived from genetic analysis of floral organ homeotic mutants in two plant species used as study systems: *Antirrhinum majus* L. and *Arabidopsis thaliana* (L.) Heynh. [7]. These species have the stereotypical floral arrangement of almost all eudicotyledoneous flora[2], with sepals in the outermost whorl, then petals, stamens, and carpels in the flower center. The ABC model states that the identities of the floral organ types are established by combinations of genes grouped in three main classes, A, B, and C. A genes alone determine sepal identity; A plus B, petal identity; B plus C, stamen identity; and C alone, carpel identity (Figure 2). Additionally, A and C genes are mutually exclusive. The ABC model has been gradually expanded to include D class and E class genes, which are necessary to produce ovules and floral whorls, respectively [8].

However, the ABC model does not provide an explanation for how such combinatorial selection of gene activity is established during floral organ primordia specification, and how the spatio-temporal pattern of ABC and non- ABC gene expression is estab-

---

[2] Among 257,000 species of flowering plants there are just two that have an inverted order of its reproductive whorls: sepals, petals, carpels and stamens. These plants are *Lacandonia schismatica* E. Martínez & Ramos, and *L. brasiliana* A. Melo & M. Alves.

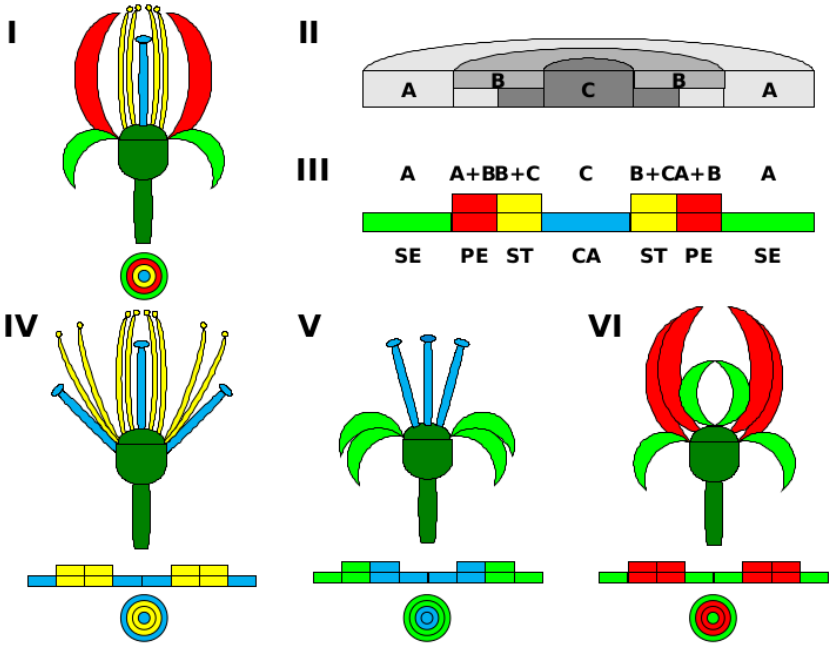


Figure 2: ABC model. I) Diagram of a wild flower with its four floral organs: sepals in light green, petals in red, stamens in yellow, and carpels in blue. Below: Transverse cut of the stem showing the concentric influence zones of ABC genes colored according to the floral organ produced. II) Influence zones of the ABC genes in the stem. III) Influence zones of ABC genes depicting which kind of floral organ they produce SE- sepal (light green), PE-petal (red), ST-stamen (yellow), CA-carpel (blue). IV) An *A* mutant produces stamens instead of petals, and carpels rather then sepals. V) A *B* mutant produces sepals instead of petals, and carpels instead of stamens. VI) A *C* mutant produces petals instead of stamens, and sepals rather than carpels.

lished. Furthermore, the conserved pattern of floral organ determination and the overall conservation of the ABC gene patterns of expression among eudicotyledoneous species suggest a robust mechanism underlying such combinatorial selection of gene activities. The ABC model by itself does not provide an explanation for such robustness either. The ABC model is purely narrative.

7 The oracle speaks

Inspired by the works of Jacob and Monod [9] about genetic regulation in prokaryotes, Kauffman [10] proposed that genetic systems can be modeled using Boolean or binary networks. Nodes represent genes. Links designate the interactions between them and symbolize how the products of a gene can activate or suppress the activity of other genes.

If a gene is active, it will be represented by a number one, otherwise it will be a zero. The state of a gene at any time will be determined by the combination of the states of all genes from which it receives information according to the equation:

$$G_N(t+1) = f_N(G_{N1}(t), G_{N2}(t), ..., G_{NK}(t)), \tag{1}$$

where $G_N(t+1)$ is the state of gene N at time $t+1$, $(G_{N1}(t), G_{N2}(t), ..., G_{NK}(t))$ are the states of the K genes at time t that regulate the activity of gene N, and f_N is the logical rule associated with gene N. The total number of combinations is equal to 2^N, where N is the number of genes. Each of these combinations is called a configuration, and the set of all possible configurations of a network conforms the Ω space.

At $t+1$, each configuration has two possible destinies according to logical rules: it may transform into another configuration or rest unchanged. At long term, the system dynamics will attain one or more configurations. These stable configurations are attractors. An attractor composed by just one configuration is called a point attractor, otherwise it is a cyclic attractor, and the number of configurations that form a part of it are its period. The set of all configurations that end in the same attractor, either point or cyclic, are its basin of attraction. Basin attraction sizes can range from just one configuration to the entire Ω space (See example in Figure 3) [11]. Point attractors can be interpreted as cell types or characters. All other configurations are transient, fugacious, thereby, natural selection is unable to detect and choose them.

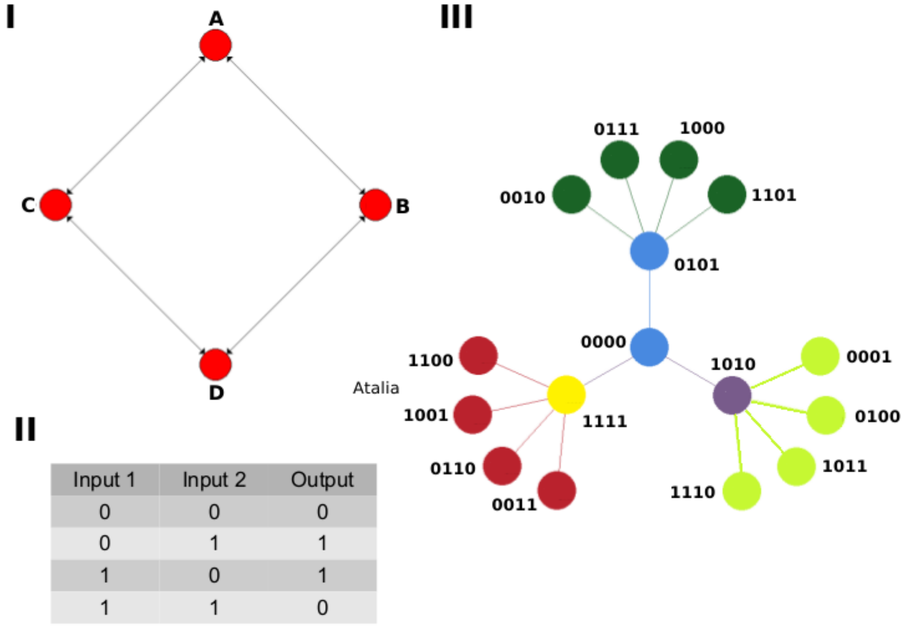
8 The symphony of flowers

According to the ABC model, certain genes are necessary to build a flower. If these genes are knocked out in the inflorescence, the plant will not produce this structure, rather, it will just develop an aggregate of leaves. Additionally, if the ABC genes are ectopically expressed in a leaf, no flower is generated; therefore, there must be more genes involved in flower formation. Based on experimental data Álvarez-Buylla and collaborators have delimited these genes, inferred their logical rules and modeled dynamics of the system. On a first approach [13] the network was not totally binary; 7 of 13 genes had three states. Finally, the network was translated to a full binary genetic network [14]. Of the total number of initial combinations ($2^13 = 8192$), the network attained 10 attractors, which coincide with the gene-expression profiles documented experimentally in cells of *A. thaliana* inflorescence meristems and floral organ primordia (Figure 4), and it also reproduces the gene-expression patterns documented in mutants[3].

When all outputs of the logical rules are systematically mutated, the vast majority of these point mutations (83.76%) conserved exactly the same 10 attractors originally obtained (we call this kind of change a neutral mutation). The other fraction obtained differ-

---

[3] The last update of the floral network has subtle variations, but they do not alter the number of attractors or the overall behavior [15]



Input 1	Input 2	Output
0	0	0
0	1	1
1	0	1
1	1	0

Figure 3: I) Network topology. II) Logical rule for all nodes. Every node has two inputs (ie. A receives information from B and D). The state of node A depends of the states of nodes B and D, and the logical rule of A. For example, if $B = 0$ (input 1) and $D = 1$ (input 2) then $A = 1$. III) System dynamics. The omega space is conformed by 16 configurations. Finally, all of them reach the same attractor at the center (0000), which is a point attractor. Colors are meaningless. Simulations and graphics were made using Atalia software [12].

ent combinations of attractors: 8.76% produced the same 10, plus new different attractors (an opportunistic mutation); 3.87% generated not all the original attractors, that is less than the original 10 (a reactionary mutation); and 3.60% created less than the 10 original attractors, but new attractors were obtained (an intrepid mutation) [16].

This behavior supports the idea that biological networks must be very robust in order to contend with most mutations, but at the same time they require to be a little flexible so that they can have the possibility to change. An absolutely robust network cannot evolve, it is frozen. In the other extreme, the dynamics of a completely flexible network is as a *tabula rasa* where with each alteration, the system cannot retain beneficial mutations or characters. Ii is like living in a perpetual revolution. This intermediate behavior lands in the zone called the edge of chaos [17] and exhibits criticality. Derrida's curve is another way to test this out [18].

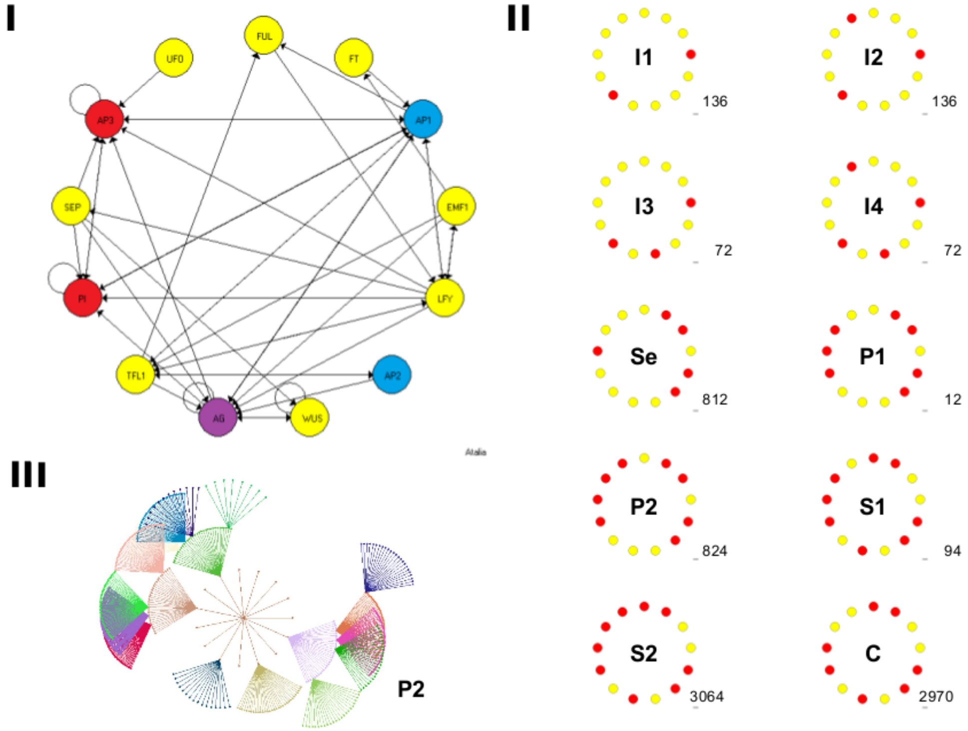


Figure 4: I) *A. thaliana* floral network. *A* genes are colored in blue, *B* genes in violet, *C* genes in red. II) From 8,192 total configurations just 10 are attractors. The figure shows each attractor configuration, nodes in red are active, nodes in yellow are inactive. Letters inside the circle of nodes are the floral organ which corresponds to that attractor: I- inflorescence, S-sepal, P-petal, St-stamen, C-Carpel. The number at the lower right corner is the size of the corresponding basin of attraction. Note the huge basins of attraction for the reproductive structures. III) The basin of attraction of Petal-2 attractor comprised 824 configurations. Each dot represents a configuration. The central node is the attractor. Simulations and graphics were made using *Atalia* software [12].

9 Coda

The Ω space is analogous to morphospace. Comprises all possible genetic configurations: the "geneconspace". Genetic networks model answers for phenomena that the new synthesis cannot account for. It explains why immense parts of the morphospace is empty, where most configurations are unstable, it identifies clearly which are the forbidden zones of this space. It gives priority to form over function, organization over selection.

Binary genetic networks help us understand some fundamental evolutionary properties. First, there is a preceding force of order to natural selection which dictates what is stable, what can exist. Natural selection is subjugated by it. Self-organization creates, natural selection shades. This is consistent with developmental studies in which natural selection is removed, but order prevails [19]. Nevertheless, natural selection retain its combinatorial power. Second, several biological systems cannot be understood under the assumption that they are just the sum of their parts. Third, in spite of the robustness of genetic networks and their deterministic behavior, they change chaotically, thus minor alterations to genomes can produce large and unexpected modifications of the organisms and their environments [11]. This is a warning to the indiscriminate and irresponsible use of genetic engineering and transgenic plants and animals.

Key elements and properties of self-organization, complex systems, and chaos, must be taken into account to construct a new evolutionary theory, in which adaptive landscapes, fitness, morphospaces, geneconfspaces, attractors, and system dynamics, are combined together into a new paradigm of biological evolution [20].

Furthermore, this interpretation has connotations of ontological ilk about causality in nature. Life´s contingency may be no more than chaos with camouflage (but see [21]).

10 Bibliography

[1] C. Darwin, *The origin of species by means of natural selection: or, the preservation of favored races in the struggle for life.* Culture et Civilisation, Bruxelles, 1969.

[2] J. Huxley, *Evolution. The Modern Synthesis.* London: George Alien & Unwin Ltd., 1963.

[3] S. J. Gould and R. C. Lewontin, “The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme,” *Proceedings of the Royal Society of London. Series B. Biological Sciences*, vol. 205, no. 1161, pp. 581–598, 1979.

[4] S. Løvtrup, *Darwinism: the refutation of a myth.* Croom Helm London, 1987.

[5] Á. Chaos, “La evolución biológica y el desarrollo,” in *Biología orgánica.* Facultad de Ciencias, UNAM, Mexico, in press.

[6] D. M. Raup, “Geometric analysis of shell coiling: general problems,” *Journal of Paleontology*, pp. 1178–1190, 1966.

[7] E. S. Coen and E. M. Meyerowitz, “The war of the whorls: genetic interactions controlling flower development,” *Nature*, vol. 353, no. 6339, pp. 31–37, 1991.

[8] M. C. Dornelas and O. Dornelas, “From leaf to flower: revisiting goethe’s concepts on the¨ metamorphosis¨ of plants,” *Brazilian Journal of Plant Physiology*, vol. 17, no. 4, pp. 335–344, 2005.

[9] F. Jacob and J. Monod, "Genetic regulatory mechanisms in the synthesis of proteins," *Journal of Molecular Biology*, vol. 3, no. 3, pp. 318–356, 1961.

[10] S. A. Kauffman, "Metabolic stability and epigenesis in randomly constructed genetic nets," *Journal of Theoretical Biology*, vol. 22, no. 3, pp. 437–467, 1969.

[11] ——, *The origins of order: Self-organization and selection in evolution*. Oxford university press, 1993.

[12] Á. Chaos, "Atalia. version 2. software for binary network analysis." 2011. [Online]: http://web.ecologia.unam.mx/achaos/Atalia/atalia.htm

[13] C. Espinosa-Soto, P. Padilla-Longoria, and E. R. Alvarez-Buylla, "A gene regulatory network model for cell-fate determination during arabidopsis thaliana flower development that is robust and recovers experimental gene expression profiles," *The Plant Cell Online*, vol. 16, no. 11, pp. 2923–2939, 2004.

[14] A. Chaos, M. Aldana, C. Espinosa-Soto, B. G. P. de León, A. G. Arroyo, and E. R. Alvarez-Buylla, "From genes to flower patterns and evolution: dynamic models of gene regulatory networks," *Journal of Plant Growth Regulation*, vol. 25, no. 4, pp. 278–289, 2006.

[15] E. R. Alvarez-Buylla, M. Benítez, A. Corvera-Poiré, Á. C. Cador, S. de Folter, A. G. de Buen, A. Garay-Arroyo, B. García-Ponce, F. Jaimes-Miranda, R. V. Pérez-Ruiz *et al.*, "Flower development," in *The Arabidopsis Book*. American Society of Plant Biologists, 2010, vol. 8.

[16] Á. Chaos, "Importancia biológica y evolutiva de la estructura y de la dinámica de redas genéticas pequeñas: modelos de simulación inpsirados en redes reales," Ph.D. dissertation, UNAM. Mexico, 2010.

[17] S. A. Kauffman, "Antichaos and adaptation," *Scientific American*, vol. 265, no. 2, pp. 78–84, 1991.

[18] E. Balleza, E. R. Alvarez-Buylla, A. Chaos, S. Kauffman, I. Shmulevich, and M. Aldana, "Critical dynamics in genetic regulatory networks: examples from four kingdoms," *PLoS One*, vol. 3, no. 6, p. e2456, 2008.

[19] P. Alberch, "The logic of monsters: evidence for internal constraint in development and evolution," *Geobios*, vol. 22, pp. 21–57, 1989.

[20] Á. Chaos, *Cazadores de monstruos. Monstruos esperanzados y sistemas complejos. Evolución y autoorganización*. UACM, Mexico, 2012.

[21] E. R. Álvarez-Buylla, Á. Chaos, M. Aldana, M. Benítez, Y. Cortes-Poza, C. Espinosa-Soto, D. A. Hartasánchez, R. B. Lotto, D. Malkin, G. J. E. Santos *et al.*, "Floral morphogenesis: stochastic explorations of a gene network epigenetic landscape," *Plos One*, vol. 3, no. 11, p. e3626, 2008.

Evolution of modularity

C. Espinosa-Soto, Instituto de Física, UASLP, Mexico

1 Abstract

Modularity is a widespread property in biological systems. In a modular system there are sets of densely interacting components, with sparse interactions between sets. Because of this arrangement, the behavior of elements inside a module depends little on factors external to the module. Modularity is very important in adaptive evolution as it allows the adjustment of one part of the organism without affecting previously adapted traits. It is thus a major determinant of evolvability. Despite its importance, the evolutionary origins of modularity are still not clear. Because modularity, by itself, does not confer an immediate fitness advantage to an organism, explaining its evolution is not as straightforward as it is for many other phenotypic traits. However, computational studies that simulate the evolution and development of simple phenotypic traits have recently allowed the proposal of several evolutionary scenarios that increase the modularity of different kinds of biological systems. Here, I review some of these studies to show that there are many possible evolutionary paths to modularity. This observation may help to explain the prevalence of modular arrangements in living beings.

2 Resumen

La modularidad es una propiedad común en los sistemas biológicos. En un sistema modular existen conjuntos de elementos con muchas interaccioness, con pocas interacciones entre elementos de conjuntos distintos. Esta disposición causa que la conducta de los componentes de un módulo dependa poco de factores externos al módulo. La modularidad es muy importante en la evolución adaptativa, ya que permite el ajuste de una parte del organismo sin afectar otros rasgos. Por lo tanto, es un componente importante del potencial evolutivo. A pesar de su importancia, el origen evolutivo de la modularidad aun no se ha aclarado. Debido a que la modularidad no confiere una ventaja inmediata en la adecuación, explicar su evolución no es tan sencillo como lo es para otros rasgos fenotípicos. Sin embargo, distintos estudios computacionales en los que se simula la

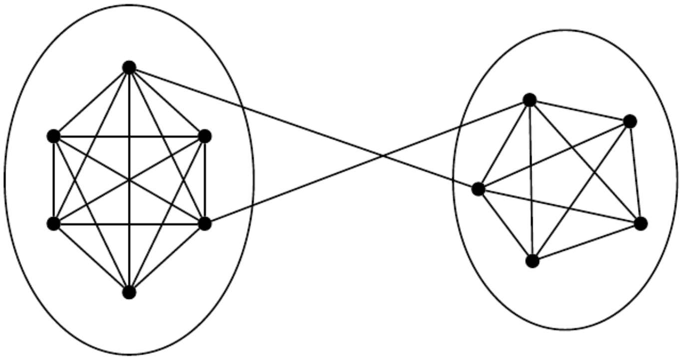


Figure 1: Modules are sets of densely interacting elements, with few interactions between elements in different sets. The figure shows a system in which elements (nodes) have interactions (straight lines) with other elements in the system. Two modules, surrounded by ellipses, are easily recognized.

evolución y el desarrollo de rasgos fenotípicos simples han permitido el planteamiento de varios escenarios evolutivos que incrementan la modularidad de distintas clases de sistemas biológicos. Aquí, yo reviso algunos de estos estudios para mostrar que existen muchos posibles caminos hacia la modularidad. Esta observación puede ser útil para explicar la alta frecuencia de estructuras modulares en los seres vivos.

3 The role of modularity in adaptive evolution

Biological systems are frequently arranged in a modular manner. This means that it is easy to recognize sets of densely interacting elements, with few interactions between elements in different sets (Figure 1). Modules exist in many different kinds of traits: from the structure of single macromolecules to complex organs and tissues. In the case of single macromolecules, a module corresponds to a structural element with a high number of bonds between monomers. In biological molecular or cellular networks, a module comprises a set of molecules, or cells, in which cross-regulatory or other kinds of interactions are significantly abundant. Such networks include metabolic networks, signaling pathways, gene regulatory circuits or neuron nets. At a higher scale, the development of a morphological structure may be modular, if morphogenetic interactions between cells and tissues occur mostly within a structure.

Elements inside a module behave semi-independently from elements outside the module because there are few interactions between them. For example, a structural element in a modular macromolecule can fold or unfold with little influence from other parts of the molecule. Thus, a stem-loop in the secondary structure of a modular RNA molecule may be preserved after other stem-loops in the molecule fold or unfold. Accordingly, the dynamic behavior of nodes inside a module of a biological network are only weakly affected by nodes in other modules. As for the development of a module-like body structure, it would occur semi-independently from adjacent tissues and organs.

Modularity has important effects on evolution. The reason is that genetic changes that affect one module are frequently constrained to that module. In a hypothetical modular RNA molecule, a mutation that changes the sequence that produces one stem-loop would only rarely affect a different stem-loop in the same molecule. Thus, distinct traits, such as stem-loops in the secondary structures of an RNA molecule, can be tuned independently by mutation and selection, without affecting those traits that are already adapted. For example, the catalytic function of one such stem-loop may be modified without altering interactions to other cell components mediated by other parts of the RNA molecule. Modularity affects the evolution of biological networks and macroscopic organismal traits in a similar manner. Consider, for instance, Darwin's finches. In these birds, the beak depth depends on a module of interacting genes and proteins that includes the protein BMP4. At the same time, the beak length depends on a different module including the protein calmodulin. Because of the modular structure of these interaction networks, there are mutations that change beak depth but leave length untouched, and mutations that alter beak length without changing depth [1]. Beak depth and length can be adjusted independently, thus allowing mutational access to a wide diversity of beak shapes. The increased access to many different combinations of trait variants makes modularity an essential component of evolvability, the potential to produce novel beneficial variation through random mutations [2, 3]. In fact, that modularity facilitates adaptive evolution is also supported by a study that links modularity of developmental stages to rates of diversification and adaptive radiations in insects [4]. In the case of Darwin's finches, modularity of beak development has apparently been paramount in adaptation to a wide diversity of foods, and hence to the finches' adaptive radiation [5].

That modularity increases evolvability is well established. However, how modularity itself evolves is not so easily explained. Since modularity only refers to the organization of interactions among a system's components, it does not increase fitness by itself [6]. Hence, the evolution of modularity cannot be explained in the same manner as the evolution of body structures, metabolic abilities, or of many other traits that, if altered, have immediate effects on organismal fitness. To study the origins of modules we must understand how modularity interacts with other properties of biological systems [6]. An additional complication in the understanding of the origins of modularity is that, among random structures, non-modular configurations far outnumber modular ones. Because of the importance of modularity for adaptive evolution, how modularity evolves in different kinds of biological systems, from single molecules to molecular and cellular networks to body parts, is a central question in evolutionary biology. Answering it would be a major advance in the understanding of the mechanisms that make organisms evolvable.

Despite the difficulties, there have been many recent advances in the study of the evolution of modularity. These advances are largely due to studies where the evolution and development of phenotypic traits are simulated *in silico*. Although limited by the necessary simplifications, such computational studies have many important advantages. First, a model of a developmental mechanism permits the analysis of how random genotypic change affects the production of the phenotypic properties that we study. Thus, we can study variational properties of a genotype, like its robustness to mutations [7] or its potential to access new phenotypes [8]. Implementing random genetic changes and selection we can also study the effects of different evolutionary scenarios on an evolving population of 'simulated organisms'. Because we can "re-run the tape of life" by repeating these simulations as many times as wanted, we can distinguish real evolutionary trends from historical accidents. Moreover, we can trace all ancestors of an 'evolved' population to study the genetic changes that occurred across such a lineage. In the following section I review some of the recent studies that have importantly enhanced our understanding of the evolution of modularity by following a modeling approach.

4 Evolutionary scenarios for the origin of modularity

Understanding how RNA molecules fold to attain their final structures, and how different structures evolve is an interesting topic in biology. One reason why the study of RNA structures is pertinent is because the catalytic activities that an RNA molecule performs depend on the shape that the molecule adopts. Many of the reactions that RNA molecules catalyze, like protein synthesis, are crucial to the cell. Indeed, RNA molecules may have been critical players in the earliest stages of the evolution of life. The reason is that, unlike DNA or proteins, RNA molecules can both carry genetic information and catalyze the chemical reactions that a primordial metabolism would require. In addition, we can use biophysically grounded algorithms to determine the secondary structure that an RNA sequence adopts. Thus, the relationship between genotype (RNA sequence) and phenotype (structure) can be assessed.

Modularity in RNA structures may allow tuning different parts of the molecule involved in different sub-functions. Indeed, RNA stem-loops that are conserved across lineages tend to tolerate changes in adjacent sequences [9]. In other words, such stem-loops may be considered modules, since they are little affected by neighboring monomers.

Ancel and Fontana simulated the evolution of populations of RNA molecules by implementing random mutations on RNA sequences. They used computational tools to determine the secondary structure adopted by each RNA molecule in an evolving population [10]. The authors designed their simulations so that selection favored structural similarity to a predetermined RNA secondary structure but also robustness of the minimum free energy secondary structure to thermal fluctuations. These conditions result in RNA molecules for which an increased fraction of mutations do not change the minimum free energy secondary structure. Importantly, this selection regime also leads to modularity: In a typical molecule from the evolved population, individual stem-loops fold an unfold independently from other structures in the same molecule [10]. Thus, scenarios that increase robustness to thermal fluctuations and mutations produce modular secondary structures in RNA molecules.

At a higher level of organization we find molecular and cellular networks. These networks include signal-transduction pathways that coordinate cellular functions and communicate cells, gene regulatory circuits, that direct changes in gene activity across development, or neuron nets that link nerve cells to orchestrate neural activity. Molecular and cellular networks also exhibit modularity. For example, that gene regulatory circuits are modular is sustained on several independent observations: i) Measures of clustering in large-scale maps of transcriptional regulation networks indicate highly connected sets of genes with sparse connections between sets [11]; ii) the existence of modularity in morphological traits, as in the finches' beaks, suggests an underlying modularity of the regulatory networks that produce those traits [1, 6]; and iii) many experimentally grounded computational models of gene regulatory circuits successfully reproduce specific developmental processes by considering only a handful of genes [12, 13]. This last observation also suggests modularity, as it shows that the influence of other factors in the processes under study is negligible.

One may think that the observations on the modularity of RNA structures could be extrapolated to molecular and cellular networks. However, this is not the case. While increased robustness to mutations results in modularity of RNA secondary structures [10], evolution of robustness in these networks does not produce modular configurations [14, 15]. Thus, there must be other mechanisms behind the evolution of modularity in these networks.

Kashtan and Alon used computer simulations to evolve networks while selecting them for their ability to perform a task, which is to compute a specific boolean function. Modular networks can evolve when selection oscillates so that it sometimes favors systems that perform one task A and sometimes favors those networks that perform a different task B [14]. A crucial additional requirement is that each of the alternative tasks A and B must be decomposable into sub-tasks, so that B contains the same sub-tasks as A, but combined in a different manner. Hence, this scenario demands that the goals that the environment imposes fluctuate in a modular manner. Modularity arises because, among systems that perform one task (e.g. A), those that are modular are more easily modified to produce the other task (B). Therefore, modular networks have higher chances to survive fluctuations. This scenario is currently our best explanation for modularity of traits for which the direction of selection fluctuates along time. Notwithstanding, while it is true that many environmental demands fluctuate, whether they do it in a modular manner is still an open question. Moreover, in this scenario modularity decays rapidly once fluctuations stop. Thus, this scenario cannot explain modularity where environmental demands do not fluctuate. This may be the case for gene regulatory circuits that perform the same function in the face of different kinds of perturbations in a wide range of species [12, 13].

Modularly-varying environmental demands are not essential for the evolution of modularity in molecular and cellular networks. Andreas Wagner and I studied a simple model of gene regulatory circuit dynamics that has been valuable to address different questions in evolutionary biology, like the relationship between sexual reproduction and robustness [16] or the role of plasticity in evolution [8]. Despite the necessary simplifications, this model is useful to study how cross-regulation produces the gene activity patterns that distinguish different parts of an organism. Moreover, because of its simplicity, the model allows the analysis of thousands or millions of gene regulatory circuit 'genotypes' and the gene activity phenotypes that they produce.

In our setup, a circuit 'genotype' specifies how a gene changes its activity state in response to the activity of other genes in the circuit. The genotype is summarized in a matrix W, in which non-zero entries w_{ij} indicate regulatory interactions. Specifically, gene j promotes (obstructs) the activity of gene i whenever w_{ij} is positive (negative). Given a matrix W, and an initial gene activity pattern, the model allows to follow the changes in gene activity until the system attains either a steady or an oscillatory gene activity pattern. We can consider such a final activity pattern as the output of the circuit's developmental dynamics, and thus, it defines the system's gene activity phenotype. Details of the model may be consulted in [15].

We asked what happens to the structure of gene regulatory circuits when organisms acquire the ability to produce new gene activity patterns. The evolution of such new activity patterns is very frequent across the history of life. It precedes the evolutionary appearance of new cell types, organs or body structures. We found that gene regulatory circuits that have evolved under selection to produce a single gene activity pattern I increase their modularity after selection for both the ancestral activity pattern I and, from a different initial condition, a new *additional* gene activity pattern II [15].

The vast majority of pairs of gene activity patterns I and II picked at random comprise two sets of genes: i) a first set S where selection requires that each gene has the same activity state (active or inactive) in the two gene activity patterns that the circuit produces, and ii) a second set of genes D where selection promotes different activity states in the gene activity phenotypes (Figure 2A). In this scenario, modularity evolves because interactions between genes in S and genes in D obstruct adaptation. Assume a circuit in which genes in the first set S indeed comply with selection, so that each of the genes in S has the same activity state in the two gene activity patterns that a circuit produces. In this case, genes whose activity depends mainly on genes in S are prone to also have the same activity state in the two patterns that the circuit produces. Thus, selection would not favor that genes in D were under control of genes in S. Along the same lines, if genes in D fulfill selection demands, genes regulated mainly by genes in D would likely have different activity states in the two patterns. Therefore, regulation of genes in S by genes in D is selected against. The result is the appearance of two densely connected sets of genes with only few regulatory interactions between sets. Modularity increases further when more new activity patterns evolve, and under a wide range of parameter values [15].

In contrast to gene regulatory circuits, in neuron nets there are clear connection costs:

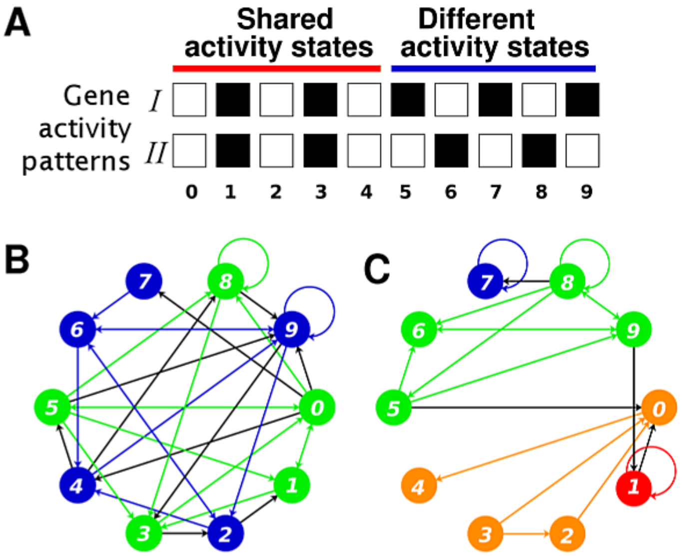


Figure 2: Evolution of modularity after selection to produce an additional gene activity pattern. A) In this evolutionary scenario, circuits that have evolved under selection for a single gene activity pattern I start being selected for an additional activity pattern II. B) A typical non-modular circuit after selection for a single gene activity pattern I. C) A typical modular circuit after selection for both gene activity patterns I and II. Panels taken from [15].

an organism must spend resources (proteins, membranes, ATP molecules) to create more connections. Clune and collaborators have recently analyzed the evolution of modularity in evolving populations of networks in a scenario where, like in neuron nets, there are connection costs. The authors found that modularity does not evolve when selection favors the efficient performance of one task. However, modularity increases if, in addition, selection promotes minimization of connection costs. This regime produces modular networks when selection disfavors the appearance of new connections and also when selection punishes an increased length of the summed length of all connections [17].

5 Conclusion

Here I have reviewed some of the evolutionary scenarios that increase modularity in different kinds of systems. The list is forcefully incomplete, as many other scenarios that lead to modularity have arisen in recent years [6, 18]. Is there a ‘winner’ among the several plausible explanations for the origin of modularity in living organisms? An open possibility is that several evolutionary mechanisms lead to the appearance of modularity, under different circumstances. Perhaps this is the reason why modularity is so widespread and why biological systems, across all levels of organization, are evolvable.

Advocating for a pluralistic perspective on the evolution of modularity does not mean considering that the issue is settled. The many roads to modularity may not be equally transited. It is necessary to define precisely the conditions under which each evolutionary scenario produces modularity. This will allow us to develop a consensus on the most relevant mechanisms for the appearance of modules in biological systems. It is also necessary to deepen in the consequences that modularity has in the evolution and development of phenotypic traits. Simulation studies will also have an important role in this endeavor. For example, this approach has already suggested that modularity favors the recurrent co-option of some sets of genes [15], and it has allowed deepening on how modularity facilitates adaptation [19]. New and exciting advances are expected from the study of the modular organization of biological systems and its impact on evolution.

6 Bibliography

[1] A. Abzhanov, W. P. Kuo, C. Hartmann, B. R. Grant, P. R. Grant, and C. J. Tabin, "The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches," *Nature*, vol. 442, no. 7102, pp. 563–567, 2006.

[2] G. P. Wagner and L. Altenberg, "Perspective: Complex adaptations and the evolution of evolvability," *Evolution*, no. 50, pp. 967–976, 1996.

[3] M. Kirschner and J. Gerhart, *The plausibility of life: Resolving Darwin's dilemma*. Yale University Press, 2005.

[4] A. S. Yang, "Modularity, evolvability, and adaptive radiations: a comparison of the hemi-and holometabolous insects," *Evolution & Development*, vol. 3, no. 2, pp. 59–72, 2001.

[5] P. R. Grant and B. R. Grant, "How and why species multiply: the radiation of Darwin's finches," *Princeton series in evolutionary biology*, 2008.

[6] G. P. Wagner, M. Pavlicev, and J. M. Cheverud, "The road to modularity," *Nature Reviews Genetics*, vol. 8, no. 12, pp. 921–931, 2007.

[7] S. Ciliberti, O. C. Martin, and A. Wagner, "Robustness can evolve gradually in complex regulatory gene networks with varying topology," *PLoS Computational Biology*, vol. 3, no. 2, p. e15, 2007.

[8] C. Espinosa-Soto, O. C. Martin, and A. Wagner, "Phenotypic robustness can increase phenotypic variability after nongenetic perturbations in gene regulatory circuits," *Journal of Evolutionary Biology*, vol. 24, no. 6, pp. 1284–1297, 2011.

[9] A. Wagner and P. F. Stadler, "Viral RNA and evolved mutational robustness," *Journal of Experimental Zoology*, vol. 285, no. 2, pp. 119–127, 1999.

[10] L. W. Ancel and W. Fontana, "Plasticity, evolvability, and modularity in RNA," *Journal of Experimental Zoology*, vol. 288, no. 3, pp. 242–283, 2000.

[11] N. Guelzim, S. Bottani, P. Bourgine, and F. Képès, "Topological and causal structure of the yeast transcriptional regulatory network," *Nature Genetics*, vol. 31, no. 1, pp. 60–63, 2002.

[12] G. Von Dassow, E. Meir, E. M. Munro, and G. M. Odell, "The segment polarity network is a robust developmental module," *Nature*, vol. 406, no. 6792, pp. 188–192, 2000.

[13] C. Espinosa-Soto, P. Padilla-Longoria, and E. R. Alvarez-Buylla, "A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles," *The Plant Cell Online*, vol. 16, no. 11, pp. 2923–2939, 2004.

[14] N. Kashtan and U. Alon, "Spontaneous evolution of modularity and network motifs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 39, pp. 13 773–13 778, 2005.

[15] C. Espinosa-Soto and A. Wagner, "Specialization can drive the evolution of modularity," *PLoS Computational Biology*, vol. 6, no. 3, p. e1000719, 2010.

[16] R. B. R. Azevedo, R. Lohaus, S. Srinivasan, K. K. Dang, and C. L. Burch, "Sexual reproduction selects for robustness and negative epistasis in artificial gene networks," *Nature*, vol. 440, no. 7080, pp. 87–90, 2006.

[17] J. Clune, J.-B. Mouret, and H. Lipson, "The evolutionary origins of modularity," *Proceedings of the Royal Society b: Biological sciences*, vol. 280, no. 1755, p. 20122863, 2013.

[18] D. M. Lorenz, A. Jeng, and M. W. Deem, "The emergence of modularity in biological systems," *Physics of Life Reviews*, vol. 8, no. 2, pp. 129–160, 2011.

[19] M. Parter, N. Kashtan, and U. Alon, "Facilitated variation: how evolution learns from past environments to generalize to new environments," *PLoS Computational Biology*, vol. 4, no. 11, p. e1000206, 2008.

Evolution and complexity: developmental constrains

B. Luque & J. Bascompte, UPM, Madrid and Estación Biológica de Doñana, CSIC, Spain

1 Abstract

In recent decades Complexity Sciences have added an important complement to the understanding of biological phenomena: the processes of self-organization that play an important role as a source of evolutionary novelty. In this view, the origin of the biological complexity is not due to natural selection alone but to a self-organized process. It is then when this new order will be modified in one direction or another by natural selection. Self-organization acts allowing the emergence of complex structures while natural selection operates on the existing ones.

2 Resumen

En décadas recientes, las Ciencias de la Complejidad han contribuido con novedosas ideas para complementar nuestro entendimiento de los fenómenos biológicos, particularmente con el concepto de auto-organización, el cual juega un papel muy importante como fuente de innovación evolutiva. Bajo esta visión, el origen de la complejidad biológica no se debe solamente a la selección natural sino a los procesos auto-organizados. Luego es que este nuevo orden será modificado en una u otra dirección por la selección natural. La auto-organización actúa permitiendo la emergencia de estructuras complejas mientras que la selección natural actúa sobre las ya existentes.

3 Introduction

It was the British naturalists Charles Robert Darwin and Alfred Russell Wallace who, independently of each other, advanced natural selection as the explaining mechanism for the evolution of species. In 1859 Darwin himself described and defended this mechanism in an encyclopedic way in his "The origin of species". Nowadays it can be described as: (1) There exists an intrinsic variability in the different populations as a consequence of mutations and, specifically, of genetic recombination in sexual organisms. (2) New emerging features are transmitted to the offspring. (3) Some variations have higher fitness than others to survive. Individuals owing those features will have a higher mean rate of survival and/or reproduction. As a consequence, these new features will extend and standardize along the successive generations until fixation.

The theory of evolution is nowadays one of the soundest theories in science. As the geneticist Theodosius Dobzhansky (1900-1975) used to say [1]: "Nothing in Biology makes sense except in the light of evolution". Its scientific success has reached far beyond Biology. Evolutionary Psychology and Evolutionary Medicine, Anthropology has gained insight on basic human emotions, innate language capacities, emergence of consciousness, biological basis of Moral and Ethics, Memetics, our knowledge about how the brain or the immune system works, genetic algorithms and evolutionary programming are just some examples of the large impact the Darwinian paradigm has reached out of its original field.

The idea on the book "Evolución y Complejidad" [2], by the authors of the present chapter, was to bring closer two perspectives of evolution which are usually introduced as confronted to each other. On one side, the vision which emphasizes contingency, frozen accidents and irreversibility and which determines an eminently historic science. On the other, a vision based on the comprehension of self-organized processes, analogous to physical systems far from thermodynamic equilibrium, which determines a structuralist science. This dialog between contingency and structuralism could well stand for the dialog between Biology and Physics the two authors represent. Multiple are the possible fronts: generality of the competition process, extinction of the less fit species, the concept of progress, the preeminent role of the gene in the evolutionary process, etc. We invite the reader to consult our book in order to gain further insight on these topics. Here, for reasons of space, we have decided to focus on a single topic: developmental constrains. The reason is twofold: the clarity of intellectual opposing positions and offer homage to the work of two great scholars of evolution as were Brian Carey Goodwin (1931-2009) and Pere Alberch (1954-1998).

Delimiting "the possible" in Nature

"Fauna secreta" [3] was an exhibition by Joan Fontcuberta and Pere Formiguera we were fortunate to enjoy in the late 90's in Barcelona. It described the heterodox scientific work of the naturalist Peter Ameisenhaufen. It was an installation of old-style "cabinets of wonders" where you could see the collection of strange beings professor Ameisenhaufen had been able to collect and study throughout his life. The sample consisted of photographs, field drawings, films, videos, sound recordings, maps, laboratory instruments, correspondence, etc. that Fontcuberta and Formiguera had been recovering from oblivion in a casual and miraculous way.

Figure 1: Left: *Solenoglypha polipodida* in attack position. In its behavior card we read: "Unlike the known reptiles, the *Solenoglypha* never rests after eating, on the contrary, it launches into a frantic race that stops only at the time of defecation". Right: *Centaurus neandertalensis* in the throes of communication with Aaru-1. In the professor's observations we read: "Every time I hear the recording of its voice calling my name (albeit with difficulty), I am possessed by a sense of unease". Photographs from [3].

Upon entering, the public thought they were going to attend a scientific exhibition proper of a Science Museum. They ran into the typical avalanche of facts and details presented with the apparent rigor and the distinctive style in which scientific information usually is presented. The prestige of science has made that. Even when we do not understand something, we suppose that its discourse is true. The exhibition attacked the voluntary suspension of our critical sense by means of a parody of the scientific discourse which was taken to absurdity (Figure 1).

Formiguera describes how they liked to walk incognito by the exhibition and to listen to the comments of the public. He recounts the following conversation between an excited father and his child: "Son, do you realize the amount of natural wonders yet unknown?". And the boy with the patience of a Buddhist said, "Dad, don't you realize that everything is a lie?" Outraged, the parent snorted: "Don't be silly, boy. Would it be exposed in a museum if it were a lie?". The two pictures below are two examples of what we could find there.

Fontcuberta's work [4] aims to stimulate our critical thinking and our imagination. Throughout his career, he has repeated similar formulas to "Fauna", where photography seeks to undermine the authority of the techno-scientific discourse. Let us recall "Herbarium" [5], a criptobotanical parody described as a "visual vaccine" by its author or "Sirens", devoted to the recently discovered fossil of an aquatic ancestor of hominids, the *Hydropithecus* (Figure 2).

"Fauna" was inspired by mythology and medieval bestiaries. But it was more than a

Figure 2: Left: Photography of a *Gilandria escoliforcia*, from the exhibition "Herbarium" [5], composed by "pseudoplants" made from detritus, plastics, animal bones or parts of various kinds. Right: Photography of a complete fossil of a *Hidropithecus tanaron*. This is an adult of 33 years and indeterminate sex, who was in good health but suffered food shortages, as evidenced by their dentition.

scientist bestiary, alternative worlds by Joan Fontcuberta invite us to reflect on why there are certain forms in nature and not others. The theory of evolution has shown us that the fauna today is only a subset of the possible (just remember Burgess Shale [6]). As Formiguera and Fontcuberta wrote: "We are pleased to work on zoology because it gives a very wide margin for uncertainty". Are we able to define what it is possible in nature (and what is not)?

A textbook example of functionalism: the limbs of tetrapods

Evolutionary Biology has been traditionally focused on the study of the function of the various structures of a living being. In this context, we can consider it a predominantly functionalist science. Structures, such as a limb, a flower or an eye, appear throughout history and, if they provide some biological effectiveness to their possessors, they are fixed by natural selection. So, typically, you tend to search for a functional reason to each organic structure and attention to its origin is relegated to a fortuitous occurrence, then selected by the advantage that gives its possessor. Today, most evolutionary biologists would agree that functionalist explanations have been abused. As an example of functionalist description, we will focus on the work of natural selection operating on the adaptive radiation of animal limb of tetrapods (four-legged animals: birds, mammals, amphibians and reptiles). The functionalist arguments will seem loud and clear but we will see that they do not tell us the whole substance of the matter.

The conquest of the land environment by vertebrates marked an important milestone

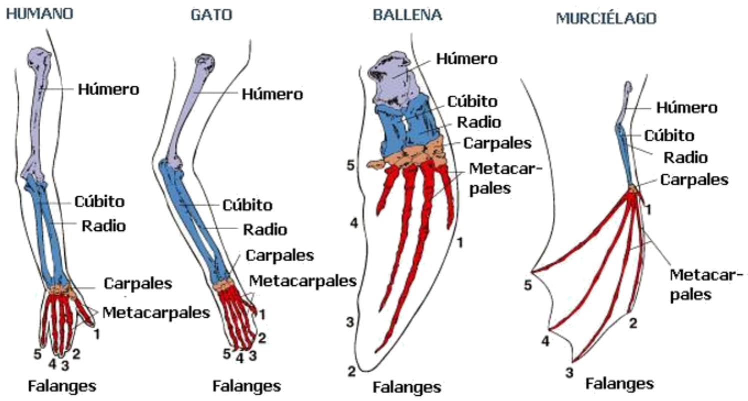


Figure 3: Scheme of the limbs of a human, a cat, a whale and a bat adapted form [7].

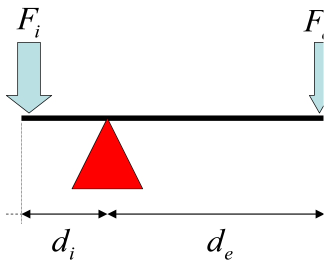


Figure 4: Schematic of a lever.

in the history of evolution. It imposed conspicuous morphological changes. Primarily, the development of four limbs on which supporting and moving the body. In parallel, changes occurred also in the structure of the spine, which was subjected to greater stresses, and the development of pelvic and pectoral girdles, which would serve to attach the limbs to the column.

The first limb derived from a primitive sarcopterygian fin, i.e., from a fin that had a central skeletal shaft coated with muscle, unlike the actinopterygian-type fin, characterized by having radius and be typical of the majority of fish. This primitive limb had the following structure: a first axial element, the stylopod, the closest to the trunk which in the front limb is called the humerus and in the rear limb is called femur. The zeugopod comes next: structure formed by two parallel bones (radius and ulna/tibia and fibula) and autopod finally, the structure corresponding to the hand or the foot (carpal/tarsal elements and metacarpal/metatarsal along with the fingers phalanges).

From this primitive pattern, we can see obvious changes driven by natural selection in order to achieve the adaptation to different forms of life as we see in Figure 3. In this regard, the adaptive value of the extremities is unquestionable. From a physical point of view, a limb is just a lever with his proximal limb, distal limb and its fulcrum. Statics would says that, when a limb is at equilibrium, the torque is the same at both ends, that is, the following equality holds:

$$F_i \cdot d_i = F_e \cdot d_e \tag{1}$$

Where d_i and d_e represent, respectively, the distance of the proximal and distal limbs and F_i and F_e are the forces applied on the proximal and distal limbs (see Figure 4). Given the physical laws of a lever, we can predict the morphological changes that have been wrought in the molding of a tetrapod limb depending on their lifestyle. Consider a couple of examples.

A digging animal such as the mole needs to develop large strength at the end of the distal limb. His limbs are true shovels. In a lever, this distal force can be increased by reducing the distal distance (d_e) and elongating the proximal distance (d_i), as obtained from the expression:

$$F_e = F_i \cdot \frac{d_i}{d_e} \tag{2}$$

This is precisely the solution seen in digger animals such as the mole. In these animals we observe the presence of a short, flat and very strong humerus with many crests where the muscles can insert. The triceps muscle is highly built up. The surface of the hand is also very large, to act as an actual shovel. Even a carpal bone appears, mimicking the presence of six fingers, to maximize the digging surface. They also possess a very elongated olecranon (an evolution of the ulna giving rise to the elbow, the proximal limb).

By contrast, a runner animal is subjected to radically different selection pressures. What is needed here is high speed delivered at the end of the distal limb. It is easy to deduce the relationship between the speeds at the two ends of the lever, since the angular velocities must be identical. The angular velocity is equal to the linear speed divided by the length of the associated limb end. So that:

$$\frac{v_i}{d_i} = \cdot \frac{v_e}{d_e} \tag{3}$$

Where v_i and v_e stand for the speed of the proximal and distal limb respectively. If what is needed is a high speed for the distal limb (as in this case), we can derive the following expression by substitution in the former one:

$$v_e = v_i \cdot \frac{d_e}{d_i} \tag{4}$$

Therefore the speed of the distal limb will be higher when the length of the distal limb is increased or when the length of the proximal limb is decreased. What happens to runner animals like the horse? They have very long distal limbs. The bones have been elongated along evolution (humerus/femur and ulna-radius/tibia-fibula). But this is not all. To maximize the length of the limb, runner animals tend to stand, not on the hands and the feet as humans and bears do but on the fingers (like dogs and lions) and even on the nails (like horses or gazelles). In parallel, due to the frequent physical stresses that occur during a high speed race, that in turn may lead to dislocations and even breakups, it tends to be fusion between different skeletal elements, such as the fusion of the ulna with the radius and the tibia with the fibula.

4 Structuralism in Biology

There is no doubt on the remarkable differences between the limbs of a mole, a horse, a dolphin, a bat and a bird. The physical interpretation of these variations in terms of adaptation is elegant and clear. The divergence is often emphasized from a functionalist perspective. This theoretical framework, that of natural selection modifying a structure in a particular direction is, however, only part of the whole story. It provides no information about the origin of the structure, explanation which has only been postponed back in time along our historical description. How does a limb, or any other structure like an eye, a leaf or a flower, originate? In all these cases we have a lot of information like “this structure develops this function” but, what are the rational laws that allow us to understand the physical process by which these complex structures emerge? How does complexity appear and to which restrictions is it subjected?

It is clear that natural selection is the main source of biological complexity and the historical component is of most relevance to understand the evolutionary phenomenon. But lets not forget that natural selection is not almighty, it is subjected to physical and chemical laws.

In recent decades Complexity Sciences have added an important complement to the understanding of biological phenomena: the processes of self-organization that play an important role as a source of evolutionary novelty. In this view, the origin of the complexity is not due to natural selection itself but to a self-organized process. It is then when this new feature will be modified in one direction or another by natural selection. Self-organization acts allowing the emergence of complex structures and natural selection operates on the existing ones. The theoretical biologist Brian Goodwin, recently deceased, was one of the main drivers of this new way of thinking in Biology, which could be called structuralist [8, 9]. Structuralism attempts to find laws that account, in a simple and consistent way, for the emergence of organic structures. Its core idea lies in the belief that the structure precedes the function.

Let’s return then to our question: we discussed the differences between different tetra-

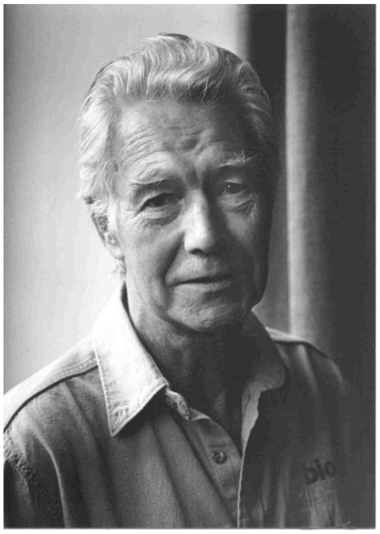


Figure 5: Brian Carey Goodwin (1931-2009), the poet of evolution. Great scientist and excellent person. Goodwin was a pioneer in considering that self-organization play an important role as a source of evolutionary novelty. He wrote on the preface of his book *How the Leopard changed its spots* [8]: "Here we face another curious consequence of Darwin's way of looking at life: despite the power of molecular genetics to reveal the hereditary essences of organisms, the large-scale aspects of evolution remain unexplained, including the origin of species. New types of organism simply appear on the evolutionary scene, persist for various periods of time, and then become extinct. So Darwin's assumption that the tree of life is a consequence of the gradual accumulation of small hereditary differences appears to be without significant support. Clearly something is missing from biology. It appears that Darwin's theory works for the small-scale aspects of evolution: it can explain the variations and the adaptations within species that produce fine-tuning of varieties to different habitats. The large-scale differences of form between types of organism that are the foundation of biological classification systems seem to require a principle other than natural selection operating on small variations, some process that gives rise to distinctly different forms of organism. This is the problem of emergent order in evolution, the origins of novel structures in organisms that has always been a primary interest in biology."

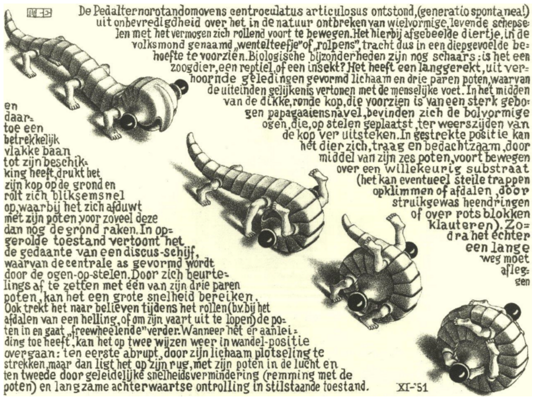


Figure 6: "Curl-up" by M. C. Escher. We all heard in school that nature had not invented the wheel, but it is not true. Howard Berg and colleagues found a wonderful example in the 60's: the flagella of bacteria. An appendix driven by a rotary engine which can spin up to 17,000 revolutions per minute, although it usually reaches no further than 200 to 1000 revolutions per minute. Although the details of its operation are still unclear, this discovery turned the unresolved question from "why nature did not invent the wheel?" into "why this design has not been extended to higher scales?".

pod limbs but, similarly, the presence of the same construction pattern becomes clear. There exist an invariance in the basic model that, although transformed over time, remains the same. What has made it possible for the above pattern to remain constant after millions of years of evolution?

The utility of monsters and the origins of order

We live in a world characterized by a great diversity of organic forms but, this is obvious, we can classify them because they are finite. However, structural plans different from the observed ones might exist, as evidenced by the artistic imagination of the next Escher lithograph (Figure 6). This ascertainment, discussed by the blighted theoretician of development and experimental embryologist Pere Alberch (Figure 7), can be the starting point to wonder about the source of order in the morphologies of nature. In other words, which factors make the existing configurations possible and, at the same time, prevent all other imaginary possibilities?

There are two major intellectual positions when answering this question. One is externalist, based on natural selection and the other is the structuralist or internalist. According to the first, natural selection is the main factor of generation of order, it avoids the unheard-of morphologies by removing them because of their dysfunctionality. The other position, the internalist, is to be developed in detail. In essence, it states that there are internal constraints, that the development process operates as a dynamical system and

Figure 7: Photograph of Pere Alberch (1954-1998) extracted from the poster of a Congress organized in his memory by the Cavanilles Institute of Biodiversity and Evolutionary Biology at the University of Valencia, Spain.

some solutions are more likely to occur than others.

The problem is how to distinguish between the two sources of order? Pere Alberch proposed a wildly imaginative approach . It is about understanding the "logic of monsters", the study of teratologies, a fully developed during the nineteenth century discipline [10]. Such abnormalities are often deleterious and exhibit no biological efficacy. All of them are eliminated by natural selection. However, there is some order in the generation of such monsters. This order can be seen in the classification of teratologies by I. Geoffroy St. Hilaire in their book "Histoire générale et particulière des anomalies de l'homme et l'organization chez les animaux". Malformations are not only limited in number but can be classified hierarchically. Actually, from this type of classification we can conclude that not all teratologies are possible. So there is a source of order. Hence the advantage of studying the monsters: natural selection can not be the engine of that order, there is no point in talking about monsters better adapted than others, since they are all nonviable. It is clear that, at least in this case, the source of order is solely driven by internal factors.

In particular, it is interesting to note that the two-headed monsters are very common along the different animal groups. In contrast, as Alberch emphasizes, there are no more three-headed monsters than the ones the human imagination has produced (Figure 8). The reason must be sought in the dynamical mechanism of embryonic formation, a dynamical system for which some solutions are stable and others not. It is the dynamics of the generating system which introduces the asymmetry of the solutions, these constrains on the possible. Let us see then if we can narrow these claims.

Figure 8: Photograph of the Sisters Salon, a case of a woman with two heads. Interestingly there is no news of similar cases of three heads. Is there some kind of impediment? Is there a hidden order in teratologies?

Pattern formation in tetrapod limbs

One of the most fascinating current problems in biology is the understanding in physicochemical terms of the embryonic development. The process leading from a fertilized egg (a zygote) to an adult individual is a paradigmatic example of self-organization, emergence of order and pattern formation. Until a few decades ago, it lacked a theoretical framework for understanding this self-organized system. Physics, specifically thermodynamics, was centered on the notion of equilibrium in isolated systems, there the Second Law of Thermodynamics unquestionably holds. Within this conceptual framework, life sciences seemed divorced from physical laws, surrounding with mystery the phenomenon of life. Nowadays, we know that biological systems do not violate the laws of thermodynamics, it is just that classical thermodynamics is not the appropriate framework for them. Biological systems are open systems and they must be addressed by modern nonlinear thermodynamics of irreversible processes, a discipline founded by the Nobel laureated Ilya Prigogine.

Dynamical processes involved in the pattern formation of a limb are highly stable spatial transformations for a wide range of initial conditions. And that is the main reason for its constancy along the evolutionary process. To support this claim, we will describe how the limb originates by summarizing the work, both experimental and theoretical, developed by P. Alberch, N. Shubin, G. Oster and J.D. Murray.

The limb of a tetrapod originates as an small expansion of the body in which there exists a uniform field of mesenchymal cells. These are surrounded by the extracellular

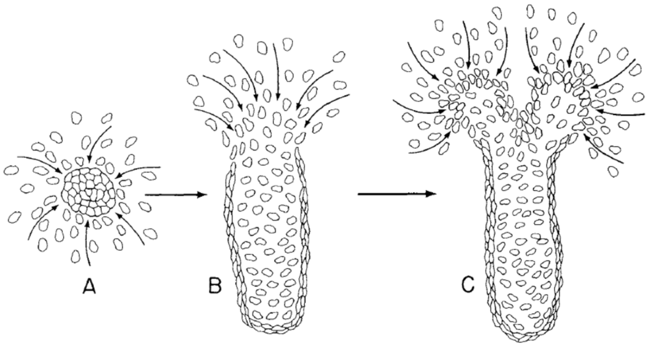


Figure 9: Diagram illustrating the formation and growth of cartilage by condensation (adapted from [11]).

matrix, consisting mainly of protein (Figure 9A). Although there are still many details of this process which remain unknown, we can form a picture clear enough for the present purposes (in [11] a more detailed discussion can be found). Once the first condensation has appeared, as the cartilaginous element develops the cells that form it begin to split into two groups (Figure 9B). The internal ones have a rounded morphology, while those that are located in the outer part are flattened. These latter ones differentiate giving rise to the perichondrium which encases the bone that is being developed. Its most direct action at this stage is to restrict the lateral growth of the cartilage which can only grows along its major axis because the subsequent recruitment of cells takes place exclusively in the distal end of the condensation. This causes its elongation.

In this pattern formation process, the size of the spatial domain is critical. Different foci of condensation compete for the cells. The size increases up to a threshold value for which a bifurcation occurs (Figure 9C). In this case, two different foci may appear, sufficiently distant from each other as to not interfere with one another. Thus, as the foci compete just for the neighboring cells, a completely heterogeneous distribution of them is formed, giving rise to zones between the foci which are substantially free of cells.

The model proposed by Oster et al. generates only three different patterns of condensation of cartilage, that is, three types of skeletal elements of the limb, shown in 10. The first is the focal condensation (Figure 10A), the second type corresponds to the branching of a pre-existing condensation (Figure 10B) and the third, called segmentation, is nothing but the fragmentation of a longitudinal element into two sub-segments (Figure 10C). These patterns have been experimentally confirmed and it should be noted that the whole process of formation of a limb, from its beginning to its final stages, is a process in which only those three patterns appear and always sequentially, never in parallel.

In the case of the tetrapod, the sequence would be as follows: the first focal condensation occurs and it elongates by recruitment of new cells at the distal extreme. The first

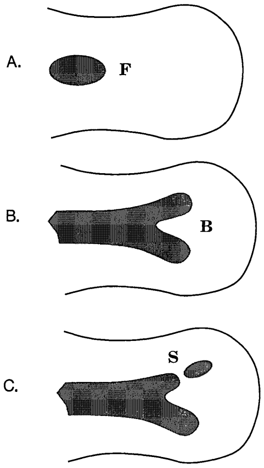


Figure 10: There are three types of cartilage by condensation: (A) focal condensation. (B) branching from a pre-existing condensation. (C) Segmentation, fragmentation of a longitudinal element S into two sub-segments (adapted from [11]).

element will lead to the humerus or femur, depending on whether we are considering an anterior limb or a posterior limb. When the length of this focus of aggregation exceeds a certain threshold, further growth by recruiting additional cells produces a branch, forming a Y-shaped pattern. Each of these branches will result in the following bone element: the pair ulna-radius in the forelimb and the pair tibia-fibula in the hind limb. In turn, these two bone elements begin to elongate and to repeat the branching process when reaching a certain size. For example, the ulna develop a new branch. The number of centers of condensation depends on the size of the limb bud, as different centers compete for a specific area of attraction. In this stage of development, this fact determines the next step in the process of sequential formation of the bone elements. The bifurcation of the ulna causes that one of the new branches is located very close to the radius. Due to the aforementioned inhibitory effect, the radius is prevented from branching itself. As a result of this competitive effect, the radius is forced to fragment longitudinally (this is the third type of cartilage condensation mentioned before). Such longitudinal fragmentation takes place when the length of the aggregate exceeds a certain critical value and the two ends become separate aggregation foci, dividing the segment in two parts. The process proceeds sequentially by repeating the three types of condensation described. The distal elongation progresses to the formation of carpal (or tarsal) proximal elements. At this stage, the development which, from the beginning, had taken place along the proximo-distal axis is interrupted and begins the sequential development along the anteroposterior axis. By means of the same processes of bifurcation and symmetry breaking, the phalanges of the fingers and the carpal (or tarsal) distal elements will be formed.

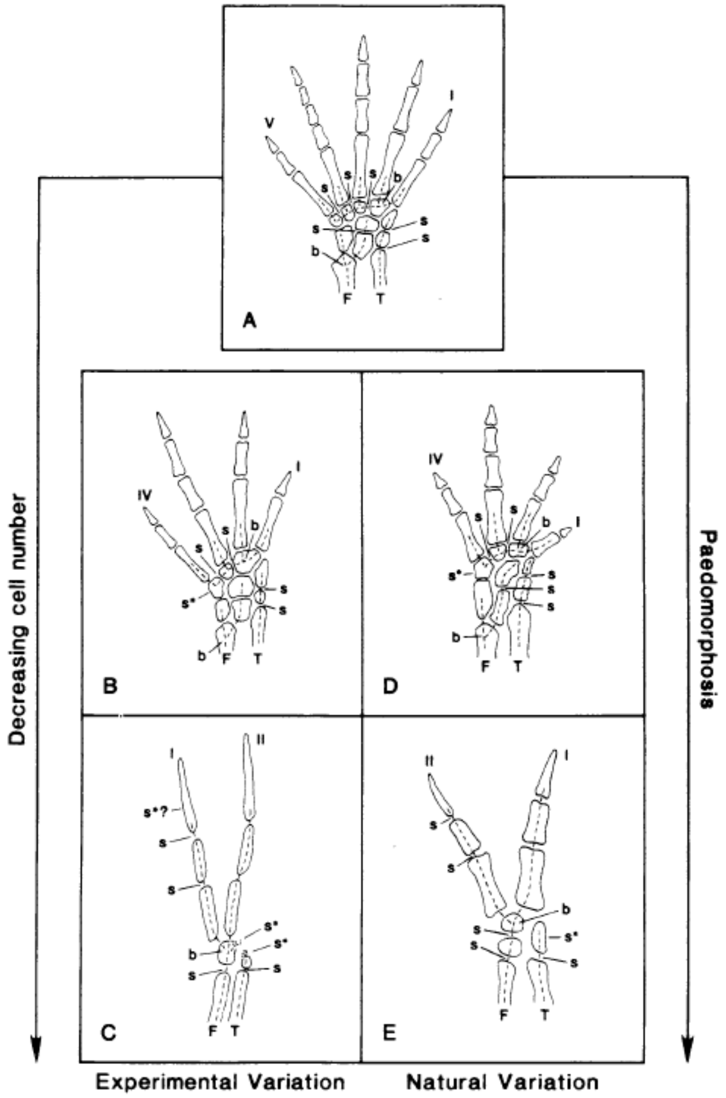


Figure 11: At the top: natural morphology of the hand of the salamander *Ambystoma mexicanum*. Series on the left (Figures B and C): two teratological forms of 4 and 2 fingers respectively, obtained experimentally. Each of these morphologies perfectly corresponds to a form observed in nature: to the species *Hemidactylium scutatum* and to the species *Proteus anguinus* respectively (adapted from [11]).

From a dynamics point of view, the three possible patterns represent robust attractors of the evolution in the embryonic field. This explains why the limbs of all tetrapods share such a well-defined and conserved pattern. As Oster et al. pointed out, other morphogenetic processes, even when they might take place, are extremely unlikely because they correspond to unstable physicochemical solutions. An example of this would be a trifurcation pattern, i.e., a three-branch condensation, as we had already mentioned when talking about teratologies or monstrosities.

Some cases of modification of the typical pattern of the limb corroborate the effect of the space in this cascade of bifurcations. Alberch and Gale [12–14] made a detailed study of the influence of the process of limb development in the genesis of an evolutionary trend developed in some amphibian species: the reduction in the number of fingers. This work is particularly illustrative of how evolution works restricting itself to the type of morphological variation compatible with the self-organizing process of forming a limb. And this is evidenced by comparison of the natural morphologies with those experimental patterns obtained by manipulation of some development parameters. Let us look at this in more detail.

The set of sequential rules described above for the formation of the skeletal elements that constitute the limb of a tetrapod establishes what may be called a development program. Minor variations will be obtained, not by changing the whole development program but through specific modifications of it. Because the size of the spatial domain has been so important for the development of this cascade of bifurcations, Alberch and Gale [12–14] tried experimentally to reduce the spatial domain of the developing limb and observed the type of change that was produced. To this aim, they made use of a mitotic inhibitor called colchicine which causes the cells not to divide for a certain period of time, therefore, at the end of the development period there should be a smaller amount of cells than usual, implying a smaller size of the spacial domain. Thus, depending on the model, one would expect that some of the branches did not show up because, due to their proximity, two centers of aggregation would interfere with each other and one of them would be absorbed by the other. Thus the number of skeletal elements should be reduced with the amount of colchicine provided. Once the embryo is developed, it will have experienced the loss of various phalanges, even of complete fingers. This is indeed the case in the experiments.

However, as Alberch and Gale emphasized, experimentally generated variations are not produced randomly, but there is a certain sequential order in which the bone elements disappear. Stated differently, some of these elements are more susceptible than others of disappearing. This ordering is a consequence of the fact that the limb does not originate synchronously but sequential. The most important thing is that a clear and manifest parallelism between the experimental variation and natural one can be observed as it is dramatically reflected in Figure 11. At the top (Figure 11A), the morphology of the hand of the salamander *Ambystoma mexicanum*, an example of a primitive hand, can be seen untransformed. In the series of the left (Figure 11B and Figure 11C), two experimentally obtained teratological forms with a progressive reduction in the number of fingers (they have 4 and 2 fingers respectively) can be observed. Each of these two morphologies perfectly correspond to those observed in nature. Thus, we find the stage of four fingers in the species *Hemidactylium scutatum* and the stage of two fingers is represented by the species *Proteus anguinus*. The qualitative similarity between the experimental and the natural series suggests that the mechanism by which the reduction in the number of fingers has occurred along the evolutionary line has been by reducing the spatial domain over which the process of self-organization operates. The order in the loss of fingers does not correspond to the order in the loss of phalanges in the fingers. The first sequence occurs along the anterior-posterior axis, while the second one occurs along the proximo-distal axis.

5 Towards a structuralist biology

We have seen an example of self-organization process where the interaction between the dynamics and the changing morphology (boundary conditions) can be stabilized in a finite number of structures. Other *a priori* valid solutions do not materialize because they are unstable against perturbations or because they are attractors with a very reduced basin of attraction. It is in this generative order, rather than in the historical inertia and natural selection, where we must seek the reason for the invariance of the structure of a limb. The structure can also be understood as the result of a self-organizing process, something that is eliminated from the classical evolutionary point of view. It is not a question of weighing both approaches, but of noting their complementarity, because natural selection is always at the end of the road.

The main lesson to be drawn from the studies of developmental constraints is that the structure cannot be separated from the process. In the words of Pere Alberch: “It is not enough to describe the form as a static entity, defined by the arrangement of its components in space, we must understand the dynamical process that controls its genesis”. When we build machines, function precedes and determines the shape of the artifact. In Biology, the shape is generated by internal processes that do not strictly depend on the function. So the form and the function engage in a dialectic in biological evolution.

Regarding the role played in this process by the genes, it is clear that these are necessary but not sufficient in understanding the formation of such structures. In addition, under this structuralist perspective, the relationship between genetic and morphological change is richer. A reduction in the final number of skeletal elements occurred when the size of the spatial domain was reduced. This can be achieved by various genetic mechanisms such as reducing the rate of cell replication or by modifying the diffusion properties of mesodermal cells that migrate into the limb in formation. Changes in many genes that determine different parameters of development can lead to the same morphological change. Moreover, as Pere Alberch *et al.* proposed, there is a nonlinear relationship between genetic and phenotypic change, where a small genetic change can cause a huge morphological change.

The ideas developed in this chapter are an example of the substantial increase in our understanding of the process of generation of morphological order along evolution. The structuralist approach represents a paradigm shift as opposed to the reductionist program. Under the influence of the latter, the gene was the fundamental unit and the origin of all complexity. The underlying principle can be summarized roughly as follows: a good knowledge of the genes will give us a good understanding of the resulting organism and its organizational characteristics. But in the example described herein, a reductionist approach is insufficient, many properties are emergent, i.e., can not be explained by the behavior of its individual parts.

Reductionism operates through a unidirectional, bottom-up causality, from the parts to the whole. Structuralism, by contrast, emphasizes the existence of a loop, a bidirectional causality between different levels: the genes generate a morphological structure that, in turn, modifies the boundary conditions on which these genes operate. The parts generate the whole which, in turn, modifies the behavior of the parts. In this sense, the limb of the tetrapods is formed from a cylindrical expansion. This morphology conditioned the orientation in which the system began to bifurcate as a result of the subsequent dynamical process. The first condensation will form a single bone element that will elongate in anteroposterior direction as a result of the cylindrical morphology upon which is acting. But the first bifurcations are also conditioning the resulting morphology as well which, in turn, will cause the symmetry breaking to be more probable in certain directions than in others.

Genes continue to have a great importance because they determine factors such as diffusion rates of certain morphogens or the size of the embryo from which the cascade of bifurcations will take place. And based on these values different morphologies can emerge. But genes –or they regulatory networks– alone do not explain fully the process by which morphological order originates. The work of Pere Alberch on teratologies is an exceptional example of this fact. Genes tell us nothing about why two-headed monsters exist but abnormalities with three heads never show up. Simply put, the greater stability of a bifurcation versus a trifurcation introduces a bias in the range of possibilities *a priori*.

6 Bibliography

[1] T. Dobzhansky, F. J. Ayala, G. L. Stebbins, and J. W. Valentine, *Evolution*. WH Freeman and Company, 1977.

[2] J. Bascompte and B. L. Serrano, *Evolución y complejidad*. Ediciones PUV, Colección Sin Fronteras, Valencia, 2012.

[3] J. Fontcuberta, *Fauna*. European Photography, Göttingen, 1987.

[4] ——, *Ciencia y fricción: fotografía, naturaleza, artificio*. Mestizo A. C., 1998.

[5] ——, "Herbarium," 1984.

[6] S. J. Gould, *Wonderful life: the Burgess shale and the nature of history*. W.W. Norton, New York, 1989.

[7] C. Colby, "Introduction to evolutionary biology," *The TalkOrigins Archive*, vol. 7, 1996.

[8] B. C. Goodwin, *How the leopard changed its spots: The evolution of complexity*. Charles Scribner's Sons, New York, 1994.

[9] B. Goodwin and P. T. Saunders, *Theoretical biology: epigenetic and evolutionary order from complex systems*. Edinburgh University Press, Edinburgh, 1989.

[10] P. Alberch, "The logic of monsters: evidence for internal constraint in development and evolution," *Geobios*, vol. 22, pp. 21–57, 1989.

[11] G. F. Oster, N. Shubin, J. D. Murray, and P. Alberch, "Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny," *Evolution*, pp. 862–884, 1988.

[12] P. Alberch and E. A. Gale, "Size dependence during the development of the amphibian foot. Colchicine-induced digital loss and reduction," *Journal of Embryology and Experimental Morphology*, vol. 76, no. 1, pp. 177–197, 1983.

[13] ——, "A developmental analysis of an evolutionary trend: digital reduction in amphibians," *Evolution*, pp. 8–23, 1985.

[14] P. Alberch, "Development and the evolution of amphibian metamorphosis," *Fortschritte der Zoologie*, vol. 35, pp. 163–173, 1989.

Social evolution: new horizons

O. Miramontes & O. DeSouza, IFUNAM, Mexico and Entomologia-UFV, Brazil

1 Abstract

Cooperation is a widespread natural phenomenon yet current evolutionary thinking is dominated by the paradigm of selfish competition. Recent advances in many fronts of Biology and Non-linear Physics are helping to bring cooperation to its proper place. In this contribution, the most important controversies and open research avenues in the field of social evolution are reviewed. It is argued that a novel theory of social evolution must integrate the concepts of the science of Complex Systems with those of the Darwinian tradition. Current gene-centric approaches should be reviewed and complemented with evidence from multilevel phenomena (group selection), the constrains given by the non-linear nature of biological dynamical systems and the emergent nature of dissipative phenomena.

2 Resumen

La cooperación es un fenómeno muy extendido en la naturaleza; sin embargo el pensamiento dominante en biología evolutiva se ha basado tradicionalmente en el paradigma de la competencia egoísta. Avances recientes en muchas áreas de la Biología y la Física no Lineal están contribuyendo a colocar al fenómeno cooperativo en su lugar correcto. En este trabajo se revisan las controversias más importantes, así como las oportunidades de investigación más promisorias en el campo de la evolución social. Se argumenta a favor de integrar, en una nueva teoría de la evolución social, a los conceptos de las ciencias de los Sistemas Complejos con aquellos más relevantes de la tradición Darwinista. Los actuales enfoques genecentristas deben ser revisados y complementados con la evidencia de fenómenos slectivos en múltiples niveles (selección grupal), las constricciones impuestas por la no linealidad de los sistemas dinámicos biológicos y la naturaleza emergente de los fenómenos disipativos.

3 Introduction

Cooperation[1] is everywhere but ecological and evolutionary theories are firmly grounded on competition. Cooperation is so common and overwhelming in nature that a simple turn of our head will spot it around immediately, appearing in multiple ways and forms. It is so widespread, so much widespread, that it is puzzling why scientists were not willing to easily acknowledge its ubiquitousness and importance. Cooperation and social phenomena are present in humans and in primates and in social insects –the common examples usually given– but it is also present in unexpected places such as in plants [1, 2] or bacteria [3, 4] or even as emergent phenomena in artificial societies of robots or other creatures of the cyberspace [5]. Why are we so late in acknowledging this fact? What is the reason of so many years in which biology has lacked a good evolutionary theory of cooperation and social emergence? Charles Darwin was already aware that social behaviour among animals was "the dirt under the carpet" for his hypothesis of evolution through natural selection. For him it was so obvious that there was a fundamental and worrisome contradiction in the mere fact of the existence of ant colonies. How can natural selection favour the worker ant that has given up its individuality in the name of the public, anonymous ways of the commune? [6]

It was short after the publication of *On the Origin of the Species* that Herbert Spencer first used the phrase "survival of the fittest". The phrase was quickly incorporated into the Darwinian views of biological evolution alongside another masterpiece of ideology uncritically converted into science:"the struggle for survival". Since these days of the newly-born social Darwinism to the present, little has changed in the mainstream view that social life is a sort of abomination and that the ultimate goal for the living is that egoist, strong and best-fitted individuals be passing their genetic material to future generations, leading to extreme gene-centric views.

But it needed not to be this way. As lucidly stated by S.J. Gould: [7] "struggle is often a metaphorical description and need not be viewed as overt combat, guns blazing. Tactics of reproductive success include a variety of nonmartial activities such as [...] better cooperation with partners in raising offspring." In fact, to cooperate rather than defect has been proven the best long-run strategy, even mathematically: game theory predicts that for interactions happening more than once, cooperation is the stable strategy. In order to profit from defection a player has to count on total mindlessness of its partner because an attentive partner will not tolerate recurrent defection. Since memory and cognition are ubiquitous among living beings –at least in rudimentary forms– mindless partners will not be easily found. The alternative for the compulsive defector is, therefore, to interact with naïve partners, but these become experient right after the first interaction! Since the amount of partners is finite, there will come a time when cooperation is the only option. Other mechanisms, besides memory, may stabilize cooperation, even in the face of defec-

---

[1] Cooperation here is understood as a nonlinear collective action that results in the benefit of a group.

tors, such as the system spatial structure [8, 9].

4 Cooperation at the dawn of life

It is unknown how the very first living organisms and their ecosystems on earth looked like. However it is known that the most ancient fossilized organisms were cooperative and social. This is the case of the 3.45 billion years-old Cyanobacteria estromatolites (see Figure 1). Cyanobacteria is perhaps the best example of how cooperative behaviour has driven biological evolution. They are suspected to have transformed the initial oxygen-free earth atmosphere into an oxygen-rich one triggering the emergence of aerobic metabolism and ultimately animals. Cyanobacteria are also implicated in the emergence of chloroplasts through a mechanism of endosymbiotic mutualism, a similar cooperative mechanism thought to have originated mitochondria and the eukaryote cell.

Stromatolites aside, the most ancient remains of an ecosystem activity currently known have an estimated age of 3.48 billion years-old. These are mineral structures known as Microbially Induced Sedimentary Structures (MISS) and are thought to have been formed by biofilms of single-cell organisms, likely bacteria [10]. It is worth noting that present day biofilms are well-known paradises for the emergence of complex social interactions and cooperative phenomena among microorganisms (Figure 2). In fact, it is remarkable that Horizontal Gene Transfer (HGT) was initially discovered in bacteria and that this mechanism of gene transferring is now regarded as a whole new paradigm in evolutionary biology[2] [11–16]. In order for HGT to work there are at least one essential requirement: that the cells involved in the process come and stay together for a while, socially interacting. This is the reason why biofilms are so important in the early evolution of social life and cooperation. The finding of HGT has triggered many fundamental questions [17, 18], for example, is the prokaryote diversity and evolution the result of the horizontal exchange of genetic material that allows for the sharing and incorporation of new encoding possibilities, i.e. genetic novelties that are more accessible to selection? or should we stick to the old idea of speciation through random mutations alone [19]? Let's remember just one thing: HGT is a gene mixing process that occurs between different prokaryotic species and even genera [20].

Closely related prokaryote species show individual genomes that are highly diverse in terms of gene content. As Cordero and Polz reviewed [21], much of this variation is

---

[2] This paradigm can be properly named *Post-Darwinian Collective Evolution*. Here we have a mesh of interconnected individual cells that transfer genetic material from cells to cells or that incorporate genetic pieces dispersed in the surrounding environment. This is a socio-environmental scenario where the dynamics of a large interactions network drives evolution without random mutations. Following what Escalante and Pajares have said in their chapter (this book), the picture that emerges is of the hugest living network ever faced by biologist before. It is the interconnected world of more than $> 10^{30}$ cells (much more than the number of stars in the visible universe) creating dynamically the largest genetic variability collective mechanism ever imagined.

Figure 1: Fossilized cooperation. One of the most ancient lifeforms on earth is the gregarious filamentous prokaryote known as Cyanobacteria (left). This is a photosynthetic organism that produces oxygen as a by-product of photosynthesis and is worldwide distributed in every ecosystem. Cyanobacteria may form massive aggregations of individuals in shallow waters known as stromatolites. These are layered accretionary structures formed by benthic microbial communities (BMC) where Cyanobacteria are dominant. Stromatolites are the product of dissipative, self-organized systems involving the BMC and its interactions with the environment. Fossilized stromatolites were first described and interpreted circa 1825 as biotic-induced geostructures in the late-Cambrian examples seen near Saratoga Springs, New York, USA (upper-right picture, courtesy of Michael C. Rygel). While declining in number since the Cambrian, stromatolites still can be found in present days as those seen in a Bahamas beach (bottom-right picture, courtesy of Vincent Poirier). Present-day living stromatolites are found in several places around the world, with notable examples at Shark Bay, Australia and at Cuatro Ciénegas, Mexico.

associated with social and ecological interactions, which have an important role in the biology of wild populations of bacteria and archaea. Genetic diversity requires the delineation of populations according to cohesive gene flows (social interactions) and ecological factors, as micro-evolutionary changes arise in response to local selection pressures and population dynamics.

In the evolutionary history, shortly after the emergence of the prokaryote, single-cell and multi-cell eukaryote emerged but not outside of a cooperative scenario. At a stage somewhere between grouped-individuals and complex multicellular organisms, the Colonial Invertebrates emerged. This is the case of the *siphonophores*, among others, that are highly cooperative forms that integrate multiple individuals, the zooids, into a metazoa that behaves as a single super-organism. Colonial Invertebrates are at the boundary between colonially-grouped and complex multicellular organisms. These are very inter-

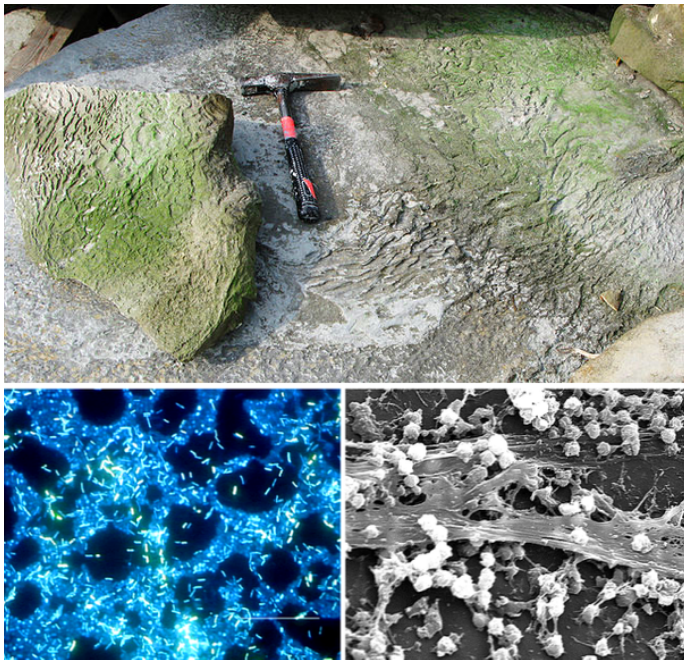


Figure 2: The oldest evidence of presumably cooperative life on earth are the Microbially Induced Sedimentary Structures (MISS). These are formed by the activity of microbial mats and biofilms (comprised mainly by bacteria). The first MISS described in the literature correspond to those at the Burgsvik Beds in Sweden (upper picture). However the most ancient vestige of biofilm activity in the planet is that at the Dresser Formation, Australia [10]. Biofilms and microbial mats are common among present day social bacteria such as those depicted in the lower photographs, corresponding to a polymicrobic biofilm epifluorescence (left) and an *Staphylococcus aureus* biofilm that has growth at the surface of a medical catheter. Biofilms act as spatio-temporal substrates for the assembly of micro-ecological conditions and social interactions among prokaryotic multi-species.

esting organisms for the study of social evolution but have been traditionally disregarded since current gene-centric theories of the origin of sociality offers no satisfactory explanations for their evolutionary pathways. *Volvox*, a colonial organism of green-algae is in a similar place regarding its evolutionary origins despite being a model organism for the study of multicelularity evolution (see Figure 3).

Cooperation played a crucial role in the emergence of multicellularity [27], regarded by John Maynard-Smith and colleges as one of the major transitions in the evolution of life [28]. It is also interesting to notice that Maynard-Smith regarded the origin of social groups (for example ants, bees, wasps and termites) as another major transition in evolution. He has, however, failed to remark that cooperation is implicated in most of his

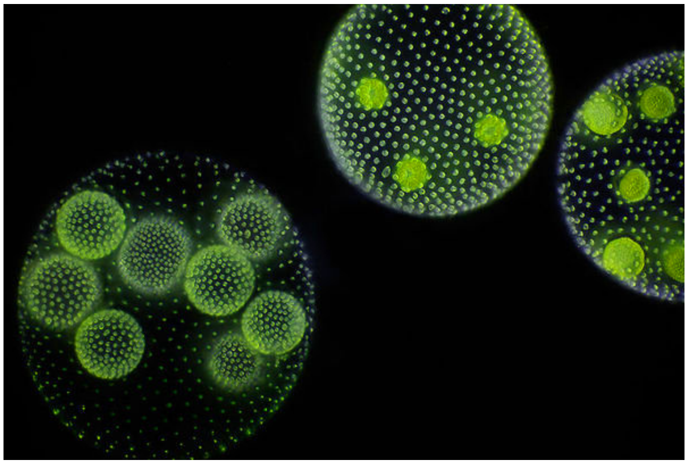


Figure 3: *Volvox*: a social green evolutionary road. Current evolutionary theory suggests that a photosynthetic cyanobacterium-like prokaryote was endosymbiotically engulfed by a eukaryotic cell giving rise, eventually, to the entire green plant clade but to green algae in the first place [22, 23]. Later in the evolution of sociality, about 200 million years ago, green algae would assemble into spherical colonies of up to 50,000 cells to form the Chlorophyta. This taxa has an extremely interesting genus called *Volvox* that was among the very first microscopic organisms seen in Antonie van Leeuwenhoek's microscope, circa 1700. Despite all the years that have passed since, there is not a clear idea of how this evolutionary road of cooperation has led to multicelularity, although some recent works point towards environmentally-induced factors as important mechanisms [24]. A typical *Volvox* colony includes both an asexual cell colony and a sexual one producing microgametes and would also include strong cell differentiation, for example the cells have phototropic eye-spots, which enable the colony to swim towards light. The swimming of the organism occurs in collective coordinated fashion, with many cells being flagellated. In the picture above, tree *Volvox* individuals. Images like this have given rise to the repeated question of, how does a cell group evolve into a multicellular individual? [25]. A fundamental question that still remains open; however emphasis on cooperative mechanisms is increasingly common [26].

identified major evolutionary transitions to the point that the opportunity to visualize cooperation as an extremely important force that drives biological evolution, was missed.

Modern post-Darwinian evolutionary theory sees natural selection and randomness as important mechanisms in evolution but argues that these are not the only sources of the extraordinary creativity of nature that we see around [7, 29–32], something else is missing[3]. Biological evolution did not strictly begin when the first life forms appeared on earth billions of years ago. It is part of a continuum unstopped evolution of matter that started with the Big Bang and where atoms, molecules and abiotic complex molecules have been

---

[3] See the Chapter by B. Luque & J. Bascompte and the one by P. Miramontes in this same book.

built up under the action of non-equilibrium thermodynamics and the physics of complex systems that inspire modern Systems Biology and explains its manifestations: self-organization, emergence, pattern formation, complex networks, dissipative structures, criticality, etc. In what follows we will review where we stand in the construction of post-Darwinian social evolutionary ideas and what we can devise for the future once an integrative view takes into consideration the missing factors of the social evolution of living matter.

5 Social evolution: the past

The old uncrossed frontier for the ideas on sociality

In contrast to HGT, Vertical Gene Transfer (VGT) is the mechanism where transmission of genes occurs from the parental generation to offspring via sexual or asexual reproduction. It is under this mechanism that most of the genetic hypothesis for the evolution of sociality and cooperation have emerged in the past, especially as an attempt to describe the emergence of the social life of insects.

As mentioned previously, social insects and their eusociality have always been a challenge for the theory of evolution in Biology. Social colonies are composed of cooperative individuals, most of them subfertile or even sterile, which would not succeed in a world "red in tooth and claw" where only the strongest and selfish merciless can prevail. Cooperation and, most notably, reproductive self-denial should have no place in this world. Both traits, however, are too frequent among animals to be simply considered as an insignificant exception. And indeed it is not, as deep analyses of this issue concern scientists, since Darwin himself. To consider recent hot debate on the matter [33, 34] this is far from settled, being perhaps the highest mountain pass, a formidable barrier we still need to cross in order to fully understand not simply sociality in insects but the very heart of the theory of evolution.

Examples abound of organisms exhibiting a behaviour in which sterile offspring cohabits with and cooperatively helps their parents to raise fertile offspring, the so-called "eusociality". It is found among bees, wasps, ants, termites [35], aphids [36], thrips [37], ambrosia beetles [38], shrimp [39] and naked mole rats [40]. If this definition is relaxed a bit, allowing senile sterility of parents (as opposed to offspring sterility) and keeping the idea of *group members containing multiple generations and prone to perform altruistic acts as part of their division of labor* [41, p. 22], then we may well add even humans to the list of eusocial animals [42].

The past: puzzles, solutions, and more doubts

Darwin himself, dedicated a whole chapter to this subject in "On the Origin of Species" [43]. Describing the puzzle of the existence of cooperative, sterile individuals in social insects, Darwin comments that they represent *one special difficulty, which at first appeared to me insuperable, and actually fatal to the whole theory.* He circumvented this doubt proposing that queens which are able to produce altruistic (cooperative sterile) offspring in addition to "normal" fertile ones, would succeed better than those producing only selfish (non-cooperative fertile) offspring, because these latter would not profit from the synergism inherent to cooperative work. In essence, Darwin shifted the problem back to the "selfish" scenario, in which the mother queen would be the target of selection. In this sense, the sterile cooperative individuals are a kind of "extended phenotype" of the queen, as a fruit is an extension of the mother plant.

Darwin's solution for the evolution of cooperation prevailed for nearly one hundred years, until 1964 when William D. Hamilton advanced an elegant mathematical formalism aimed as an attempt to solve the riddle [44]. It consisted of the so-called "kin selection", which differs from –but does not conflict with– Darwin's proposition by establishing that each member of the colony is targeted by selection individually, rather than together with its parents and siblings. Kin selection predicts that individuals cooperate with family members and hence enhance the spread of their genes, indirectly, when their kin reproduce. Cooperating within a colony would warrant transmission of genes even for steriles. Such a theoretical construct is sometimes referred to as "inclusive fitness".

Box 1. Relatedness in haplo-diploid systems: suppose a fully heterozygous haplodiploid cross:

	B
A	AB
a	aB

In Hymenoptera, all offspring produced from this cross are female (males are produced parthenogenetically). Let's take a look at the degree of relatedness between these sisters:

sisters	AB	aB
AB	1.0	0.5
aB	0.5	1.0

That is, on average, sisters are related to each other by:
$1.0 + 0.5 + 0.5 + 1.0 = 3/4 = 75\%$

Haplo-diploidy in Hymenoptera (bees, ants, wasps and sawflies), where males are haploid and females are diploid, was proposed by Hamilton to be the key to the puzzle (see Box 1). A hymenopteran female, by virtue of haplo-diploidy, can share 75% of its genes with her sisters. Haplo-diploidy, therefore, secures higher levels of kinship between females, which, by abstaining reproduction and helping their mother to raise reproductive sisters, would transfer a load of their own genes which is higher than the load transmitted by their direct reproduction. In a sense, by helping the queen, sterile hymenopteran females almost clone themselves.

Haplo-diploidy, however, is not sufficient to explain the evolution of eusociality: a significant portion of hymenopteran species, while haplo-diploid, are solitary. Maybe more striking, there are many diplo-diploid organisms (having both, males and females, diploid) which are eusocial (Box 2): all the nearly 3 thousand termite species plus aphids, beetles, shrimp, naked mole rats, and humans. Fully diploid organisms do not present kinship asymmetry among siblings, being at most 50% akin and hence profiting more from their own reproduction than from that of their parents.

It was indeed eusociality in termites –consistently overlooked by texts focusing kin selection– that always kept alive the challenge, and even more now when the list of eusocial diplo-diploids is frequently updated. Much effort has been made to conciliate termites with kinship selection [45–47] but, as noted by Thorne *et al.* [48], a convincing explanation on why they are eusocial despite their full diploidy is still needed . An important step in this direction was taken by Korb and collaborators [49], who presented a broad overview of the ecology of social evolution across large parts of the animal kingdom, including termites [50] and other diplo-diploids, thereby expanding the study beyond haplo-diploids.

Box 2. Relatedness in diplo-diploid systems: in a fully heterozygous diplo-diploid cross we would observe the following offspring:

	B	b
A	AB	Ab
a	aB	ab

This will imply in the following degree of relatedness between each of the siblings:

siblings	AB	Ab	aB	ab
AB	1.0	0.5	0.5	0.0
Ab	0.5	1.0	0.0	0.5
aB	0.5	0.0	1.0	0.5
ab	0.0	0.5	0.5	1.0

In such case, the average relatedness between siblings is:
$(1 * 4) + (0.5 * 8) = 4 + 4 = 8/16 = 50\%$

Contempts

Meanwhile, it has been argued that the right question has been not posed! In his heavy criticism of the way research has been conducted on kin selection, E.O. Wilson [41] claims to have spotted a philosophical fault in such studies: we have been busy trying to accommodate exceptions to the theory, rather than searching for a better theory that accommodates it all. That is, rather than asking how to conform termites and other diploids to kin selection theory, we should have kept Darwin's first doubt, namely, why are there social –cooperative– animals in a world apparently ruled by relentless "struggle for life" where only the best competitors would survive? Wilson states that we failed to consider multiple competing hypotheses, ignoring well established principles of science philosophy [51]. In Wilson's (2011, pag. 166) own words:

> "[...] unwarranted faith in the central role of kinship in social evolution has led to the reversal of the usual order in which biological research is conducted. The proven best way in evolutionary biology, as in most of science, is to define a problem arising during empirical research, then select or devise the theory that is needed to solve it. Almost all research in inclusive-fitness theory has been the opposite: hypothesize the key roles of kinship and kin selection, then look for evidence to test that hypothesis."

Stating that Hamilton's rule *"almost never holds"*, Martin Nowak and collaborators [34] brought recent upheaval to the community of scientists supporting kin selection. It attracted immediate reaction in the form of contentious papers [33, 52–55], readily counteracted by supporting ones [56–58]. In an attempt to perform neutral analysis of the debate Birch [59] identifies ambiguities in Hamilton's defenders and supporters and offers a common vocabulary to help their communication. In short, he states that while kin selection supporters' argument is based on a general form of Hamilton's rule, its opponents construe this rule in a particularly narrow sense. He continues to argue that the current state of deadlock attained by this acrimonious debate will only be broken if both sides agree on common terms [59].

As an urgent alternative to kin selection as an explanation for the emergence of sociality, Nowak *et al.* [34], followed by Wilson [41], proposed that the full theory of eusocial evolution would include the following stages (taken almost *ipsis litteris* from [34]):

1. The formation of groups.
2. The occurrence of a combination of pre-adaptive traits causing the groups to be tightly formed. Such a combination would include a valuable and defensive nest, they stress.
3. The appearance of mutations that prescribe the persistence of the group, most likely by the silencing of dispersal behaviour.
4. Emergent traits caused by the interaction of group members are shaped through natural selection by environmental forces.
5. Multilevel selection drives changes in the colony life cycle and social structures, often to elaborate extremes.

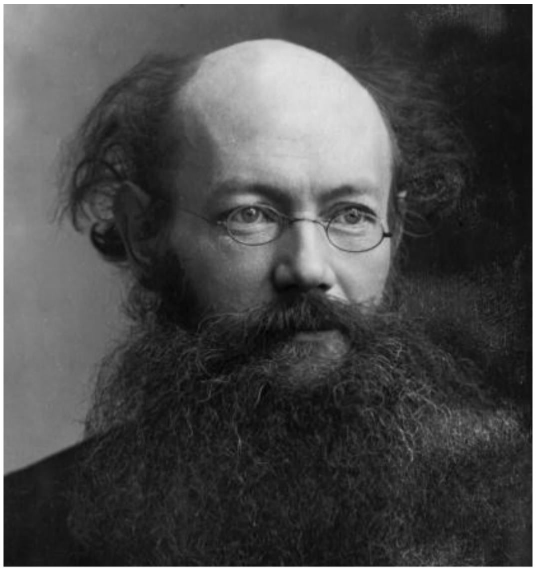


Figure 4: Forgotten evolutionary thinker. For many years Pyotr Kropotkin (1842–1921) was deliberately ignored by the mainstream of the evolutionary thought until recently when Group Selection was brought forwards again into the modern evolutionary biology school. Kropotkin was a young Russian scientist when he first read "The Origin of Species" and felt immediately persuaded by Darwin ideas. Inspired at the age of twenty by the voyages of Alexander von Humboldt, he embarked himself in a long five years exploration of the Siberian lands. While still a Darwinian, Kropotkin had developed his own views on how nature may work. At the time, evolutionary theory developed quickly in England under the conception that the natural world was a brutal place where competition and survival of strongest individuals was the driving force. However, after studying closely flocks of migrating birds, gregarious mammals, fish schools and insect societies, he concluded rightly that competition was almost absent there and that cooperation was indeed common and extended. "He advocated that natural selection was the driving force that shaped life, but that Darwin's ideas had been perverted and misrepresented by British scientists. Natural selection, Kropotkin argued, led to *mutual aid*, not competition. Natural selection favoured societies in which mutual aid thrived, and individuals in these societies had an innate predisposition to mutual aid because natural selection had favoured such actions" [60]. Kropotkin moved beyond into considering that the mechanism underlying human cooperation was also the altruistic mutual aid [61]. This observation led him to regard cooperative human societies as self-organized entities that do not need central ruling, being this the essence of anarchism. Due to this, Kropotkin ideas on the evolutionary mechanisms of cooperation were quickly dismissed and regarded as politically unacceptable for the competitive "free-world", until today since many current topics on the nature of cooperation were first advanced and investigated by him.

In essence, these authors consider groups as an additional unit of selection with selection simultaneously occurring at different levels, e.g., between individuals in the group and between groups. They also remark the importance of spatio-temporal mechanisms that cause individuals to come and stay together. Such ideas are not totally new: they've been long ago hinted by Kropotkin [61] (Figure 4).

6 Social evolution: the future

Emergent properties of grouping behaviour

Interestingly, some authors view Nowak and colleagues' proposition as *complementary* rather than *alternative* to kin selection theory. Better stated, they would claim that this "new" group-selection theory is in fact a more general proposition of kinship selection [62], despite strong refutation by Wilson [41]. Based on the empirical evidence compiled in the various chapters of their book for a broad range of animals (both vertebrates and invertebrates; full diploids or haplo-diploids), Korb and Heinze [49] agree with Wilson [41] that the newly re-discovered group-selection framework is a promising way to investigate the evolution of social phenomena.

This view would sustain that while in kin selection models relatedness is paramount, the new group-selection models emphasize between-group versus within-group selection, thereby opening an avenue for studies of group level phenomena. Group level phenomena, in which simple repeated interactions between individuals can produce complex adaptive patterns at the level of the group [63] are not new in the study of social insects [64–67]. What is new is the explicit recognition, within the biologists mainstream, that they may hold one important key to help fully understand eusociality. In fact, the awareness of the other (empathy) has been proposed to be one of the traits helping organisms to cross the barrier to sociality and eusociality [41, 68]. In humans this would be accomplished by language; in insects by chemical, tactile and visual communication that enhance their ability to interact hence forming cohesive grouping. Interactivity, in fact, seems to be a primary trait underlying grouping in social insects. Depending on the intensity of one-to-one interactions among individuals, complex behavioural patterns can arise at the group level, but these patterns are not hard-wired in these interactions. This should be in fact the next frontier in the studies of eusocial behaviour.

Non-randomness and interaction dynamics

Random mutations are at the centre of current evolutionary paradigm. While it is true that bacteria, for example, adapt and develop resistance to almost every antibiotic that is developed, not a single new species has been observed to arise after decades (hence, thousands or millions of generations) of laboratory experiments in which bacteria are exposed to mutagenic forces. Most of mutations seems to be neutral and do not provoke major

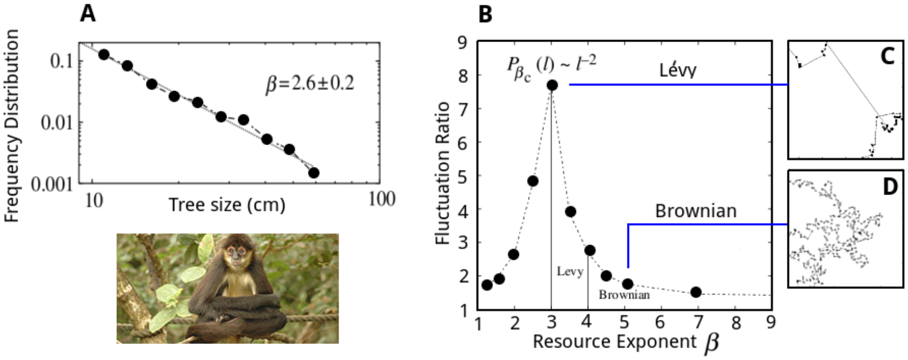


Figure 5: Emergent anomalous diffusion in social primates. Spider monkeys (*Ateles geoffroyi*) are highly social and forage in groups. (A) In the tropical forest of the Yucatan Peninsula in Mexico, a study of the tree-size frequency distribution showed that this distribution follows a power-law with an scaling exponent value $\beta = 2.6$. This fact was used in a foraging model where monkeys move to fruiting trees following a simple optimization rule of "move to the richest but nearest" tree (B). The model predicts that the monkey mobility is emergent as anomalous diffusion (Lévy-like) for β values close to the observed true value $\beta = 2.6$ (C) and is normal diffusive (Brownian) for other values of β (D). The emergent nature of the anomalous diffusion is due to nothing else but to the forager-environment interaction [69, 70]. It has been also noted that Lévy displacement distribution may bring optimal efficiency to the foraging process [71].

inheritable changes that could trigger observable speciation. In fact long-term studies on observable bacterial adaptation suggest that fitness changes in bacteria may occur primarily by the accumulation of neutral mutations [74].

Are random mutations a real mechanism for genetic variation and evolutionary change? or are they part of a limited working hypothesis that must me revisited and complemented with, for example, mutationless evolution [75, 76] or evolution by means of horizontal recombination mechanisms [77]? An illustrative example regarding random mechanism in theoretical ecology is useful at this point. For years it was thought that random climatic variations were responsible for driving population dynamics. However after the pioneering work of Robert May and others [78], it became clear that variations in population numbers may be due to the intrinsic changes of the ecosystems and the nonlinear universe of interactions on it. These erratic fluctuations are not random but chaotic and the difference between these concepts is not trivial. One is the outcome of stochastic casino-like events while the second is the outcome of a dynamical complex system with determinism embedded. Is it time to start looking for signs of deterministic dynamical systems as sources of genetic variation?[4]

---

[4] See the Chapter by Pedro Miramontes in this same book.

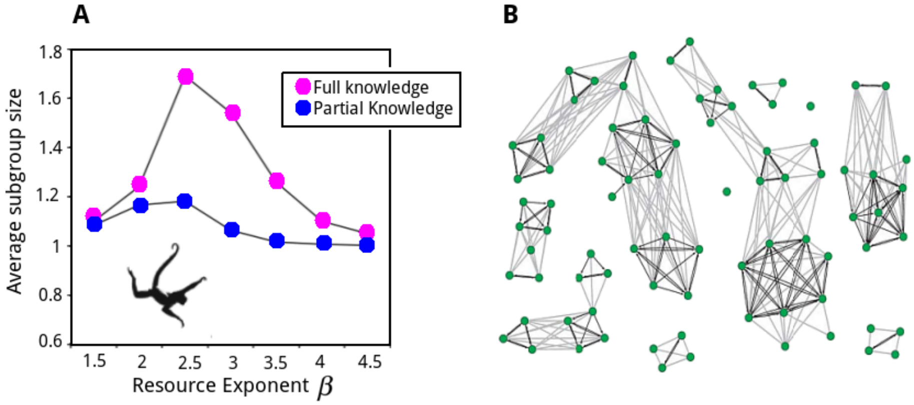


Figure 6: Spider monkeys emergent social networks. (A) The same foraging model described in Figure 5 is now used in a multi-agent environment [72]. It has been found that monkeys foraging in the Lévy anomalous diffusion regime (resource exponent $\beta = 2.6$) spontaneously form social ties with the largest average group size. These ties transform into emergent social networks (B). These complex networks would only exist because the monkey mobility is Lévy distributed [72]. The transition to an anomalous diffusion in this foraging model may be interpreted as an order-disorder phase transition [73]. Images adapted from [72].

Mobility: come and stay together

Another common widespread idea in biology is that individual social and ecological interactions follow essentially random patterns. Take for example mobility and dispersal. Since the 70s of the last century, it became theoretically obvious that a simplified agent would explore its surrounding space efficiently when moving in a fractal pattern that result in what is known as anomalous diffusion (Figure 5). Such an efficient pattern would result in increased encounter with prey or con-specifics. This would ultimately lead to increased reproduction rates (genetic diversity), either by obvious positive effects of increased food intake or by the less evident consequences of increased social interaction rates. Because such interactions translate in maximized information flow and processing, they promote efficient cooperation and hence social facilitation (Figure 6) leading to maximized survival under strong stresses (Miramontes and DeSouza 1996, Rosengaus et al. 1998, Desouza et al. 2001) and hence extending opportunities for reproduction.

There is growing evidence that biological organisms perform anomalous diffusion in their mobility patterns in the form of Lévy flights (scale-free probability distributions in the lengths of travelled distances). When efficient social interactions occur in the context of density-dependent ecosystems then another interesting phenomena emerges: the so called order-disorder phase transitions that are becoming a new paradigm in evolution [29, 30, 82].

Cyanobacteria, as said before, are intriguing social organisms that have been protago-

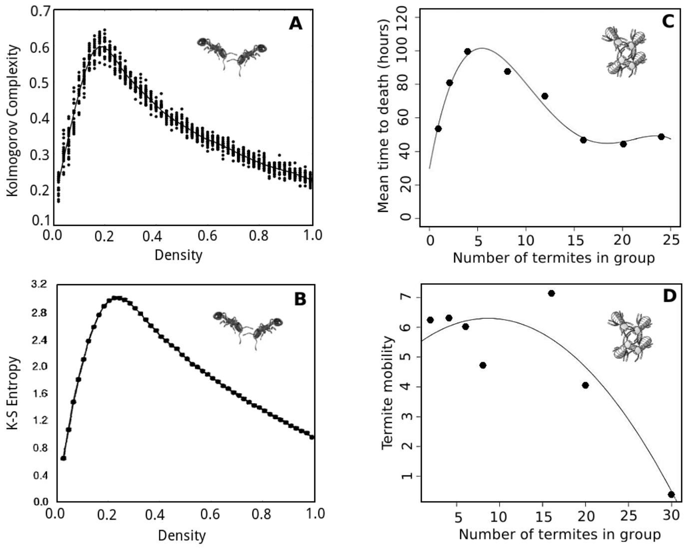


Figure 7: Social phase transitions in ants and termites. Social insects are good examples of self-organized societies exhibiting a range of group-size complex behaviours involving the criticality properties normally associated with order-disorder phase transitions. Ants of the genus *Leptho-torax* are known for displaying periodic pulses of activity in the colony when measured as movement inside the nest. However the individuals present low-dimensional chaotic movement activity. Then, as the density on the nest increases there is an order-disorder (edge of chaos) phase transition that can be explored when modelled with agent-based formalisms. In such ant models the phase transition occurs at a density that maximize the information transfer and the diversity of observable behaviours of the individuals as measured by (A) Kolmogorov complexity and (B) by a KS-Entropy [79, 80]. Experimental procedures reveal the presence of phase transitions in termite social behaviour. (C) Social-facilitated survival in size-dependent groups of termites show a peak at low densities (C) that is also in agreement with individual mobility (D) [81].

nists of important evolutionary changes in the history of life. Despite its apparent simplicity, they are known to have very complex patterns of non-random mobility, cell-to-cell interactions and communication [83]. Cyanobacteria do form pairwise ensembles of interaction and mobility. It would not be surprising at all that their mobility patterns are anomalous diffusion and so their social engagements may respond to optimized encounter rates. It will be also very interesting to know how and when these patterns have emerged in the evolution of these ancient organisms. Have these mechanisms been also present in the mobility and dispersal of interacting proto-cells or self-replicating biomolecules?

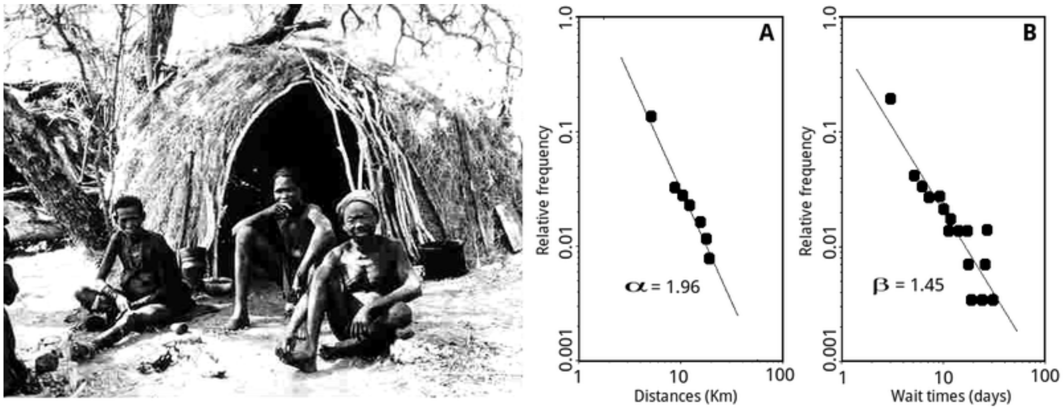


Figure 8: On human mobility and cooperation. No other aspect of the evolutionary biology is so impregnated by ideology as that of the nature of human cooperation. Fortunately scientific evidence is starting to prevail showing that mutual aid (reciprocity), as advanced by Kropotkin, is an extremely important factor that has shaped human evolution. Most of the evidence come from Game Theoretical results but most importantly from direct observation of human societies. Anthropological evidence has also provided great examples of human-environment interactions that clearly evince that human mobility patterns are landscape-driven [84, 85] and that this may enhance social coherence and genetic diversity. One paradigmatic example was the hunter-gatherers San people of the southern Africa of the early twenty century (left picture) whose mobility patterns revealed power-law distributions in travelling distances [86] (A) and waiting times (B). These mobility patterns help explain why these human groups have the most genetic diversity of all the people on the planet [87]. Graphs adapted from [86].

Stay together then interact

Another front that must be included in a more comprehensive evolutionary theory of cooperation is the evolution of social interactions. It has been clear since the last two decades that social interactions obey a scale-free network pattern and that it seems to be ubiquitous in nature. Gene regulatory networks, metabolic networks, mutualistic networks, communications networks, etc. all of them seem to have long-tailed distributions, corresponding for instance to scale-free topologies. The reason for this is robustness and flux efficiency. Are scale-free networks a physical constraint in the origin and evolution of life? Can life-related networks, including social networks, have other topologies? One is tempted to answer no and the reason is simple. It is becoming apparent that an scale-free topology would facilitate the emergence of criticality in the dynamics running on them. This seems to suggest a bridge between the criticality of a phase transition and social dynamics. Examples of this are starting to emerge [88].

It was shown in models of ant-to-ant interactions, that a colony is posed at an order-disorder phase transition where sociality emerges and information capacity is at its best (Figure 7). In models of spider monkey foraging, it was found that the individual interactions with a given forest structure pose the ecosystem in a state where complex social networks emerge facilitated by the anomalous-diffusion nature of the animal displacements (Figures 5 and 6). A similar phenomenon has been recently revealed in ancient nomad human groups in Africa (Figure 8).

A novel theory of social evolution must integrate the concepts of the science of Complex Systems with those of the Darwinian tradition. Gene-centric concepts should be reviewed and complemented with evidence from multilevel phenomena (group selection), the constrains given by the non-linear nature of biological dynamical systems and the emergent nature of dissipative phenomena. Cooperation only emerges in come-and-stay-together scenarios, because of this, exploration of the properties of anomalous diffusion and the topological evolution of scale-free networks is very important. On the positive side, this research roadmap is on its way right now.

Acknowledgements

We thank the late Dr. A. Chopps for many years of convivial and inspiring discussion and Dr. Karo Michaelian, Dr. Alessandra Marins and Dr. Paulo Cristaldo who kindly reviewed previous versions of this text providing invaluable insights. OM thanks DGAPA-PAPIIT Grant IN101712 and the Brazilian Ciência Sem Fronteiras (CSF-CAPES) program 0148/2012 for a Special Visiting Researcher position at the Universidade Federal de Viçosa, Brazil, during (2013-2015). ODS is supported by CNPq-Brasil, fellowship 305736/2013-2.

7 Bibliography

[1] R. M. Callaway and L. R. Walker, “Competition and facilitation: a synthetic approach to interactions in plant communities,” *Ecology*, vol. 78, no. 7, pp. 1958–1965, 1997.

[2] J. M. Biernaskie, “Evidence for competition and cooperation among climbing plants,” *Proceedings of the Royal Society B: Biological Sciences*, vol. 278, no. 1714, pp. 1989–1996, 2011.

[3] A. S. Griffin, S. A. West, and A. Buckling, “Cooperation and competition in pathogenic bacteria,” *Nature*, vol. 430, no. 7003, pp. 1024–1027, 2004.

[4] O. X. Cordero, H. Wildschutte, B. Kirkup, S. Proehl, L. Ngo, F. Hussain, F. Le Roux, T. Mincer, and M. F. Polz, “Ecological populations of bacteria act as socially cohesive units of antibiotic production and resistance,” *Science*, vol. 337, no. 6099, pp. 1228–1231, 2012.

[5] C. G. Langton, *Artificial life: An overview*. MIT Press, 1997.

[6] H. Cronin, *The ant and the peacock: Altruism and sexual selection from Darwin to today*. Cambridge University Press, 1993.

[7] S. J. Gould, "The evolution of life on the earth," *Revista Lusófona de Humanidades e Tecnologias*, vol. 4/5, pp. 8–13, 2001.

[8] M. A. Nowak, "Five rules for the evolution of cooperation," *science*, vol. 314, no. 5805, pp. 1560–1563, 2006.

[9] F. J. Hol, P. Galajda, K. Nagy, R. G. Woolthuis, C. Dekker, and J. E. Keymer, "Spatial structure facilitates cooperation in a social dilemma: Empirical evidence from a bacterial community," *PloS one*, vol. 8, no. 10, p. e77042, 2013.

[10] N. Noffke, D. Christian, D. Wacey, and R. M. Hazen, "Microbially induced sedimentary structures recording an ancient ecosystem in the ca. 3.48 billion-year-old Dresser formation, Pilbara, Western Australia," *Astrobiology*, vol. 13, no. 12, pp. 1103–1124, 2013.

[11] K. Vetsigian, C. Woese, and N. Goldenfeld, "Collective evolution and the genetic code," *Proceedings of the National Academy of Sciences*, vol. 103, no. 28, pp. 10 696–10 701, 2006.

[12] N. Goldenfeld and C. Woese, "Biology's next revolution," *Nature*, vol. 445, no. 7126, pp. 369–369, 2007.

[13] ——, "Life is physics: evolution as a collective phenomenon far from equilibrium," *arXiv preprint arXiv:1011.4125*, 2010.

[14] M. Buchanan, "Collectivist revolution in evolution," *Nature Physics*, vol. 5, no. 8, pp. 531–531, 2009.

[15] B. J. Shapiro, J. Friedman, O. X. Cordero, S. P. Preheim, S. C. Timberlake, G. Szabó, M. F. Polz, and E. J. Alm, "Population genomics of early events in the ecological differentiation of bacteria," *Science*, vol. 336, no. 6077, pp. 48–51, 2012.

[16] R. T. Papke and J. P. Gogarten, "How bacterial lineages emerge," *Science*, vol. 336, no. 6077, pp. 45–46, 2012.

[17] F. de la Cruz and J. Davies, "Horizontal gene transfer and the origin of species: lessons from bacteria," *Trends in Microbiology*, vol. 8, no. 3, pp. 128–133, 2000.

[18] J. G. Lawrence, "Gene transfer in bacteria: speciation without species?" *Theoretical Population Biology*, vol. 61, no. 4, pp. 449–460, 2002.

[19] L. Boto, "Horizontal gene transfer in evolution: facts and challenges," *Proceedings of the Royal Society B: Biological Sciences*, vol. 277, no. 1683, pp. 819–827, 2010.

[20] M. Z. DeMaere, T. J. Williams, M. A. Allen, M. V. Brown, J. A. Gibson, J. Rich, F. M. Lauro, M. Dyall Smith, K. W. Davenport, T. Woyke *et al.*, "High level of intergenera gene exchange shapes the evolution of haloarchaea in an isolated Antarctic lake," *Proceedings of the National Academy of Sciences*, vol. 110, no. 42, pp. 16 939–16 944, 2013.

[21] O. X. Cordero and M. F. Polz, "Explaining microbial genomic diversity in light of evolutionary ecology," *Nature Reviews Microbiology*, vol. 12, p. 263–273, 2014.

[22] P. J. Keeling, "The endosymbiotic origin, diversification and fate of plastids," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1541, pp. 729–748, 2010.

[23] O. De Clerck, K. A. Bogaert, and F. Leliaert, "Diversity and evolution of algae: primary endosymbiosis," *Advances in Botanical Research*, vol. 64, pp. 55–86, 2012.

[24] A. M. Nedelcu, "Environmentally induced responses co-opted for reproductive altruism," *Biology letters*, vol. 5, no. 6, pp. 805–808, 2009.

[25] R. E. Michod, "Evolution of individuality during the transition from unicellular to multicellular life," *Proceedings of the National Academy of Sciences*, vol. 104, no. Suppl 1, pp. 8613–8618, 2007.

[26] D. C. Queller and J. E. Strassmann, "Beyond society: the evolution of organismality," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1533, pp. 3143–3155, 2009.

[27] M. E. Hochberg, D. J. Rankin, and M. Taborsky, "The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity," *BMC Evolutionary Biology*, vol. 8, no. 1, p. 238, 2008.

[28] J. M. Smith and E. Szathmary, *The major transitions in evolution*. Oxford University Press, 1997.

[29] Stuart A. Kauffman, *The origins of order: Self-organization and selection in evolution*. Oxford University Press, 1993.

[30] B. C. Goodwin, *How the leopard changed its spots: The evolution of complexity*. Princeton University Press, 1994.

[31] O. Miramontes, "Evolución, autoorganización y otros números del montón," *Miscelánea Matemática*, vol. 49, 2009.

[32] J. Bascompte and Luque Bartolome, *Evolución y complejidad*. Universitat de València, 2012.

[33] P. Abbot, J. Abe, J. Alcock, S. Alizon, J. A. Alpedrinha, M. Andersson, J.-B. Andre, M. van Baalen, F. Balloux, S. Balshine *et al.*, "Inclusive fitness theory and eusociality," *Nature*, vol. 471, no. 7339, pp. E1–E4, 2011.

[34] M. A. Nowak, C. E. Tarnita, and E. O. Wilson, "The evolution of eusociality," *Nature*, vol. 466, no. 7310, pp. 1057–1062, 2010.

[35] E. O. Wilson, *The Insect Societies*. Massachusetts: Harvard University Press, 1971.

[36] D. L. Stern and W. A. Foster, "The evolution of sociality in aphids: a clone's-eye view," in *Caste and ecology in social insects*, J. C. Choe and B. J. Crespi, Eds. Cambridge, UK: Cambridge Univ Press, 1997, pp. 150–165.

[37] B. J. Crespi, "Eusociality in Australian gall thrips," *Nature*, 1992.

[38] D. Kent and J. Simpson, "Eusociality in the beetle *Austroplatypus incompertus* (Coleoptera: Curculionidae)," *Naturwissenschaften*, vol. 79, no. 2, pp. 86–87, 1992.

[39] J. E. Duffy, C. L. Morrison *et al.*, "Multiple origins of eusociality among sponge-dwelling shrimps (*Synalpheus*)," *Evolution*, vol. 54, no. 2, pp. 503–516, 2000.

[40] P. W. Sherman, J. U. M. Jarvis, and R. D. Alexander, *The Biology of the Naked Mole-Rat*. Princeton University Press, 1991.

[41] E. O. Wilson, *The social conquest of earth*. WW Norton & Company, 2012.

[42] K. R. Foster and F. L. Ratnieks, "A new eusocial vertebrate?" *Trends in Ecology & Evolution*, vol. 20, no. 7, pp. 363–364, 2005.

[43] C. Darwin, *On the origin of the species by natural selection*. Murray, 1859.

[44] W. D. Hamilton, "The genetical evolution of social behaviour. I," *Journal of Theoretical Biology*, vol. 7, pp. 1–16, 1964.

[45] S. Bartz, "Corretion: Evolution of eusociability in termites," *Procedures of the National Academy of Sciences USA*, vol. 77, p. 3070, 1980.

[46] S. H. Bartz, "Evolution of eusociality in termites," *Procedures of the National Academy of Sciences USA*, vol. 76, pp. 5764–5768, 1979.

[47] B. L. Thorne, "Evolution of eusociality in termites," *Annual Review of Ecology and Systematics*, vol. 28, no. 1, pp. 27–54, 1997.

[48] B. Thorne, N. Breisch, and M. Muscedere, "Evolution of eusociality and the soldier caste in termites : Influence of intraspecific competition and accelerated inheritance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 22, pp. 12 808–12 813, 2003.

[49] J. Korb and J. Heinze, *Ecology of social evolution*. Springer, 2008.

[50] J. Korb, "The ecology of social evolution in termites," in *Ecology of social evolution*, J. Korb and J. Heinze, Eds. Springer, 2008, pp. 151–174.

[51] T. Chamberlin, "The method of multiple working hypotheses," *The Journal of Geology*, vol. 5, pp. 837–848, 1897.

[52] J. J. Boomsma, M. Beekman, C. K. Cornwallis, A. S. Griffin, L. Holman, W. O. H. Hughes, L. Keller, B. P. Oldroyd, and F. L. W. Ratnieks, "Only full-sibling families evolved eusociality," *Nature*, vol. 471, no. 7339, pp. E4–E5, Mar. 2011.

[53] A. F. Bourke, "The validity and value of inclusive fitness theory," *Proceedings of the Royal Society B: Biological Sciences*, vol. 278, no. 1723, pp. 3313–3320, 2011.

[54] E. A. Herre and W. T. Wcislo, "In defence of inclusive fitness theory," *Nature*, vol. 471, no. 7339, pp. E8–E9, 2011.

[55] J. E. Strassmann, R. E. Page Jr, G. E. Robinson, and T. D. Seeley, "Kin selection and eusociality," *Nature*, vol. 471, no. 7339, pp. E5–E6, 2011.

[56] M. Doebeli, "Inclusive fitness is just bookkeeping," *Nature*, vol. 467, no. 7316, pp. 661–661, 2010.

[57] R. Gadagkar, "Sociobiology in turmoil again." *Current Science*, vol. 99, no. 8, 2010.

[58] M. van Veelen, J. García, M. W. Sabelis, and M. Egas, "Call for a return to rigour in models," *Nature*, vol. 466, no. 7316, pp. 661–661, 2010.

[59] J. Birch, "Hamilton's rule and its discontents," *The British Journal for the Philosophy of Science*, p. axt016, 2013.

[60] L. A. Dugatkin, "The Prince of Evolution: Peter Kropotkin's Adventures in Science and Politics," http://www.scientificamerican.com/article/the-prince-of-evolution-peter-kropotkin/, 2011.

[61] P. Kropotkin, *Mutual aid: A factor of evolution*. Courier Dover Publications, 2012.

[62] J. Korb and J. Heinze, "The ecology of social life: a synthesis," in *Ecology of social evolution*. Springer, 2008, ch. 12, pp. 245–259.

[63] D. J. T. Sumpter, "The principles of collective animal behaviour," *Philosophical Transactions Of The Royal Society B Biological Sciences*, vol. 361, no. 1465, pp. 5–22, Jan. 2006.

[64] O. DeSouza, O. Miramontes, C. Santos, and D. Bernardo, "Social facilitation affecting tolerance to poisoning in termites (Insecta, Isoptera)," *Insectes Sociaux*, vol. 48, no. 1, pp. 21–24, Mar. 2001.

[65] O. Miramontes and O. DeSouza, "Individual basis for collective behaviour in the termite, *Cornitermes cumulans*," *Journal of Insect Science*, vol. 8, p. 24, 2008.

[66] ——, "The nonlinear dynamics of survival and social facilitation in termites," *Journal of Theoretical Biology*, vol. 181, no. 4, pp. 373–380, 1996.

[67] R. V. Solé, O. Miramontes, and B. C. Goodwin, "Collective oscillations and chaos in the dynamics of ant societies," *Journal of Theoretical Biology*, pp. 161–343, 1993.

[68] F. De Waal, *The age of empathy: Nature's lessons for a kinder society*. Random House LLC, 2010.

[69] D. Boyer, G. Ramos Fernández, O. Miramontes, J. L. Mateos, G. Cocho, H. Larralde, H. Ramos, and F. Rojas, "Scale-free foraging by primates emerges from their interaction with a complex environment," *Proceedings of the Royal Society B: Biological Sciences*, vol. 273, no. 1595, pp. 1743–1750, 2006.

[70] D. Boyer, O. Miramontes, and H. Larralde, "Lévy-like behaviour in deterministic models of intelligent agents exploring heterogeneous environments," *Journal of Physics A: Mathematical and Theoretical*, vol. 42, no. 43, p. 434015, 2009.

[71] G. Ramos Fernandez, J. L. Mateos, O. Miramontes, G. Cocho, H. Larralde, and B. Ayala Orozco, "Lévy walk patterns in the foraging movements of spider monkeys (*Ateles geoffroyi*)," *Behavioral Ecology and Sociobiology*, vol. 55, no. 3, pp. 223–230, 2004.

[72] G. Ramos Fernández, D. Boyer, and V. P. Gómez, "A complex social structure with fission–fusion properties can emerge from a simple foraging model," *Behavioral Ecology And Sociobiology*, vol. 60, no. 4, pp. 536–549, 2006.

[73] R. Solé, *Phase Transitions*. Princeton University Press, 2011.

[74] N. Leiby and C. J. Marx, "Metabolic erosion primarily through mutation accumulation, and not tradeoffs, drives limited evolution of substrate specificity in *Escherichia coli*," *PLoS Biology*, vol. 12, no. 2, p. e1001789, 2014.

[75] S. Huang, "Tumor progression: chance and necessity in Darwinian and Lamarckian somatic (mutationless) evolution," *Progress In Biophysics And Molecular Biology*, vol. 110, no. 1, pp. 69–86, 2012.

[76] A. O. Pisco, A. Brock, J. Zhou, A. Moor, M. Mojtahedi, D. Jackson, and S. Huang, "Non-Darwinian dynamics in therapy-induced cancer drug resistance," *Nature Communications*, vol. 4, 2013.

[77] C. Fraser, W. P. Hanage, and B. G. Spratt, "Recombination and the nature of bacterial speciation," *Science*, vol. 315, no. 5811, pp. 476–480, 2007.

[78] R. M. May *et al.*, "Simple mathematical models with very complicated dynamics," *Nature*, vol. 261, no. 5560, pp. 459–467, 1976.

[79] O. Miramontes, "Order-disorder transitions in the behavior of ant societies," *Complexity*, vol. 1, no. 3, pp. 56–60, 1995.

[80] O. Miramontes, R. V. Sole, and B. Goodwin, "Neural networks as sources of chaotic motor activity in ants and how complexity develops at the social scale," *International Journal of Bifurcation and Chaos*, vol. 11, no. 06, pp. 1655–1664, 2001.

[81] O. DeSouza and O. Miramontes, "Non-asymptotic trends in the social facilitated survival of termites (Isoptera)," *Sociobiology*, vol. 44, no. 3, pp. 527–538, 2004.

[82] C. Torres Sosa, S. Huang, and M. Aldana, "Criticality is an emergent property of genetic networks that exhibit evolvability," *PLoS Computational Biology*, vol. 8, no. 9, p. e1002669, 2012.

[83] A. Galante, S. Wisen, D. Bhaya, and D. Levy, "Modeling local interactions during the motion of cyanobacteria," *Journal of Theoretical Biology*, vol. 309, pp. 147–158, 2012.

[84] O. Miramontes, O. DeSouza, D. Hernández, and E. Ceccon, "Non-Lévy Mobility Patterns of Mexican Me'Phaa Peasants Searching for Fuel Wood," *Human Ecology*, vol. 40, no. 2, pp. 167–174, 2012.

[85] D. Boyer, M. C. Crofoot, and P. D. Walsh, "Non-random walks in monkeys and humans," *Journal of The Royal Society Interface*, vol. 9, no. 70, pp. 842–847, 2012.

[86] C. T. Brown, L. S. Liebovitch, and R. Glendon, "Lévy flights in Dobe Ju/'hoansi foraging patterns," *Human Ecology*, vol. 35, no. 1, pp. 129–138, 2007.

[87] S. A. Tishkoff, F. A. Reed, F. R. Friedlaender, C. Ehret, A. Ranciaro, A. Froment, J. B. Hirbo, A. A. Awomoyi, J.-M. Bodo, O. Doumbo *et al.*, "The genetic structure and history of Africans and African Americans," *Science*, vol. 324, no. 5930, pp. 1035–1044, 2009.

[88] B. Luque, O. Miramontes, and L. Lacasa, "Number theoretic example of scale-free topology inducing self-organized criticality," *Physical Review Letters*, vol. 101, no. 15, p. 158702, 2008.

The ecology of human linguistic groups

J.A. Capitán and S. Manrubia, Centro de Astrobiología, CSIC-INTA, Madrid, Spain

1 Abstract

Similarities between linguistic and biological diversity were identified long ago. As research on both fields has advanced, qualitative parallelisms have turned into quantitatively comparable patterns. Remarkable examples are the statistical properties of taxonomy, the decline of diversity with latitude, or the allometric relationship between population abundaces and range sizes. Though multiple factors may underlie these remarkable patterns, the similarites uncovered between linguistic and biological diversity point to a relevant role of environment in shaping them. Eventually, the study of a human macroecology may lead to the discovery of generic mechanisms behind the evolution and interaction of populations.

2 Resumen

Hace mucho tiempo que ciertas semejanzas entre la diversidad lingüística y la diversidad biológica fueron identificadas. A medida que la investigación en ambos campos ha avanzado, lo que en principio fueron paralelismos cualitativos se han convertido en patrones cuantitativamente comparables. Algunos ejemplos destacados son las propiedades estadísticas de la taxonomía, la disminución de la diversidad con la latitud o la relación alométrica entre la abundancia y el área ocupada por una población. Aunque son múltiples los factores que subyacen a estos patrones, las semejanzas entre la diversidad lingüística y la biológica sugieren que el ambiente debe desempeñar un papel relevante en su emergencia. Finalmente, el estudio de una macroecología humana puede llevar al hallazgo de mecanismos genéricos tras la evolución e interacción de poblaciones.

3 Introduction

One of the strongest evidences for evolution is the observation of resemblances between separated entities, since similarity may speak for shared ancestry. As early as in the sixteenth century, it was independently proposed that species, as well as languages, presented intriguing commonalities that were far from trivial. At the time, the first European visitors of Asiatic regions noted similarities between Indian, Iranian, and European languages, while on the biological side systematic comparisons between the anatomy of organisms began to be carried out.

The hypothesis that linguistic similarities could be due to a common origin was put forward in the eighteenth century. In 1786, Sir William Jones, founder of the Asiatic Society of Calcutta, demonstrated the presence of fundamental similarities among Latin, Greek, Persian, Sanskrit, and, with less confidence, Celtic languages and Gothic. In his view, these similarities could only be explained if those languages arose from a common ancestor through descent with modification. Later, that ancestral language became known as Proto-Indo-European. Sir William Jones settled the basis for what is nowadays termed comparative linguistics and introduced important elements of evolution in linguistics – without natural selection, which is not applicable to languages.

Comparative linguistics had its biological counterpart in comparative anatomy, a discipline that, after the pioneering work of Edward Tyson on mammals, became established also in the eighteenth century. Studies carried out by anatomists like George Cuvier, Richard Owen or Thomas Henry Huxley represented a breakthrough in our understanding of the relatedness among vertebrates. Comparative anatomy and embryology have been the major tools to understand phylogeny until quite recently, when they have been complemented and even displaced by genomic knowledge. Though techniques other than comparative linguistics are currently used to establish the relatedness of languages, a revolution tantamount to that brought by sequencing techniques has not been produced in linguistics.

Studies on the origin of languages were severely impeded shortly after the publication of Darwin's book *On the Origin of Species by Natural Selection*, at a time when evolution was becoming a most fashionable concept. Actually, most absurd theories on the origin of language and on the nature of the "primitive language" were sprouting like weeds to the point that, in 1866, the Linguistic Society of Paris included in its founding statutes the following statement: "The Society does not accept papers on either the origin of language or the invention of a universal language". This scholarly disapproval continued well into the twentieth century, when advances in human evolution and comparisons between human and animal communication systems turned the origin of language into a respectful topic [1].

At present, the analogies between biological and linguistic evolution are much deeper than previously suspected [2], and relevant to the point that some models of evolution are applicable to both systems. An interesting advance has been to realize that many features of the distribution of biological populations can arise in neutral scenarios, where selection plays an insignificant role. In many respects, a human language is equivalent to a biological species, and this similarity applies to qualitative as well as quantitative aspects. The comparison of those two evolutionary systems has been mediated by the ever increasing amount of data describing both biodiversity and languages. Ecology has a long tradition of cataloging species, their locations and their interactions. Nowadays, information can be easily downloaded by any interested user from databases such as The Global Biodiversity Information Facility[1] or the Web of Life[2]. As for languages, detailed information can be obtained from The Linguasphere Register[3] or from The Ethnologue[4] which is the most comprehensive catalog to date, with information on over 7,000 living languages. All these databases are being continuously amended and enlarged, and their reliability depends on the work and criteria of expert ecologists and linguists. Though these extensive datasets might contain errors that should affect predictions at the level of specific species or languages, the overall, statistical patterns that we are going to discuss should not be qualitatively affected by present mistakes or future improvements.

4 Linguistic and biological taxonomy

The Indoeuropean family of languages is formed by several hundred related languages, about half of them now extinct. It was the first linguistic family to be recognized and accepted, at the beginning of the nineteenth century. The identification of other major families was more difficult and not devoid of controversy. Between 1940 and 1960 Joseph Greenberg made significant progress when he convincingly demonstrated that about two thousand aboriginal African languages could be grouped into only four families. Towards the end of the 1980's, Merritt Ruhlen, one of his disciples, suggested that all human languages can be grouped together, a claim that implied the existence of an ancestral language from which seventeen families, in his classification, should have branched [3].

The current classification of languages into families is congruent with knowledge gathered from anthropology and genomics. That is to say, when two populations are close from a genomic viewpoint, they tend to speak languages belonging to the same family. The tree that compares linguistic families and genetic similarity is coherent in this respect with three exceptions: Lapps, Ethiopians, and Tibetans [4]. Congruence of the two data sets, but differences as well contribute to disentangle the patterns of divergence and dispersion of human populations. Interestingly, new independent data are continuously added to those studies, as in an investigation where linguistic phylogeny was complemented with the genetic analysis of human gastric bacterial parasites, leading to a reliable

---

[1] http://www.gbif.org

[2] http://www.web-of-life.es

[3] http://www.linguasphere.info

[4] https://www.ethnologue.com

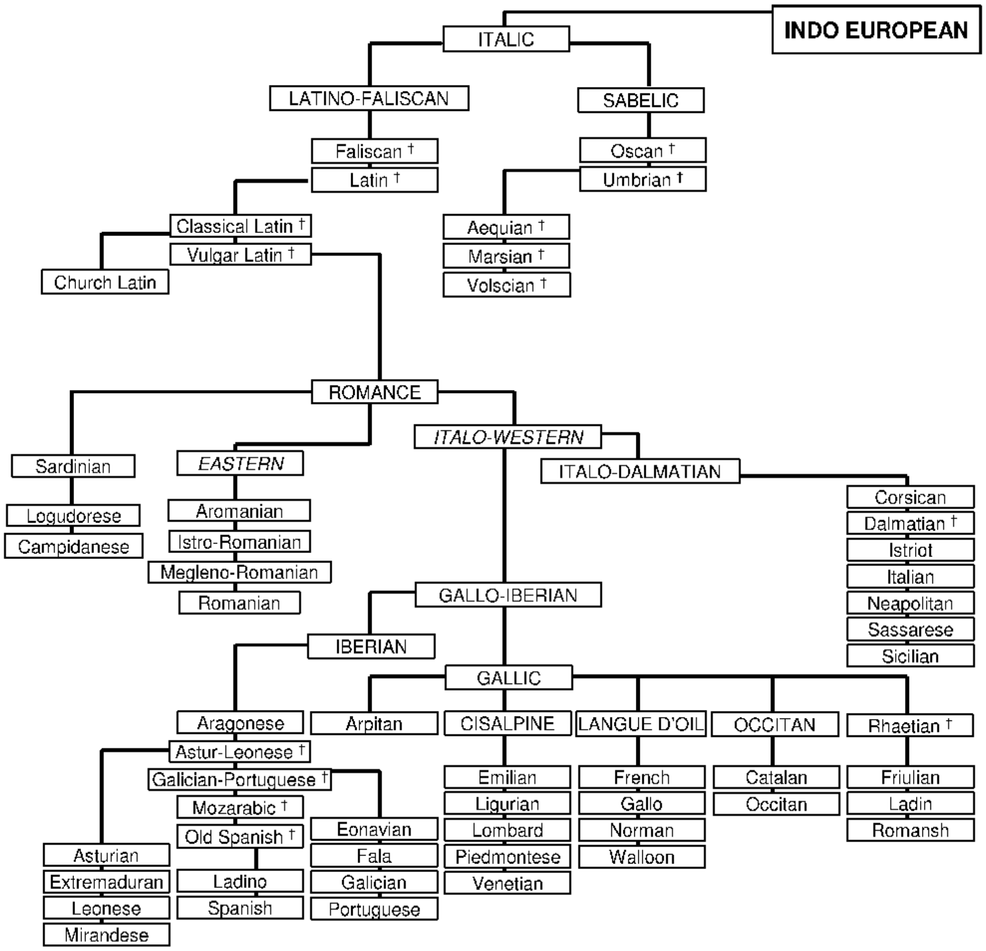


Figure 1: Languages can be hierarchically grouped in taxonomic levels, as it is done for species. In this example we observe several taxonomic levels that link present languages (leaves of the tree) in the Indo-European family to a hypothetical ancestor through a significant number of now extinct languages (indicated with a † sign). This is a partial tree that only represents the Italic group. The complete Indo-European family can be found in open places such as the Wikipedia.

reconstruction of Pacific population history [5]. This is an extreme (and rare to date) example of how similar biological and linguistic phylogenies might be.

The classification of languages includes a variable number of taxonomic levels in addition to that of family. Particularly rich linguistic groups, as that of Bantu, in Africa, may entail up to seventeen hierarchical levels. These levels are conceptually similar to biological taxa in that new similarities among groups of related languages appear every time we go down one level in the taxonomy (languages occupy the lowest level). As it happens with hierarchical groupings of species, the branching of languages in their reconstructed phylogenies is highly uneven: most groups are small, while a few are composed of many languages, the pattern repeating as one climbs up taxonomic levels. This was one of the first observations regarding the quantitative properties of biological phylogeny. The processes behind such regularities are thought to be of multiplicative nature, analogous to branching processes.

In this kind of processes, the essential mechanism is the branching of a variable number of subtaxa from a given taxon, independently of the taxonomic level. In simple representations of the process, the probability that the taxon has no subtaxa, or 1, 2, 3 or more "daughter" branches is assumed to be independent of the "parental" taxon. Figure 1 contains several cases of branching. For instance, Gallo-Iberian is the ancestor of a single taxon one level below, Iberian, from which Aragonese, Astur-Leonese, Galician-Portuguese, Mozarabic, and Old Spanish, branched. In its turn, Old Spanish splitted into Ladino and Spanish. The first model of this kind aimed at explaining the structure of biological taxonomy was proposed in 1924 [6]. Much later, the statistical properties of the classification of human languages were analyzed [7] to reveal that the distribution of the number of subtaxa within a given taxon follows a power-law distribution, with an exponent that increases in absolute value with the taxonomic level. This scaling is fully analogous to the self-similarity that had been described about ten years earlier for biological taxonomy [8]. The invariance of the functional form describing both systems supports its robustness against different possible classification schemes that coherently assign subtaxa to the taxon from where they originated, and plausibly establishes its emergence from an underlying stochastic branching process.

Linguistic phylogenies reflect highly contingent historical processes of language change, diversification, and extinction. Several such processes are known, though the time scales involved and the depth of the modifications caused are not easy to quantify. Words modify their prevalence in a population through time, change their meaning, are borrowed from other languages, or disappear when speakers stop using them. It suffices to pay attention to different regions where the mother tongue of any of us is spoken to realize how often names of plants or food change, and how particular idioms characterize subpopulations of speakers. These modifications at the local scale do not alter languages in any major way and resemble minor, neutral mutations in genotypes. More severe changes have occurred historically and can be identified in languages with a written record. An example is English, which incorporated a huge amount of lexicon and some grammar

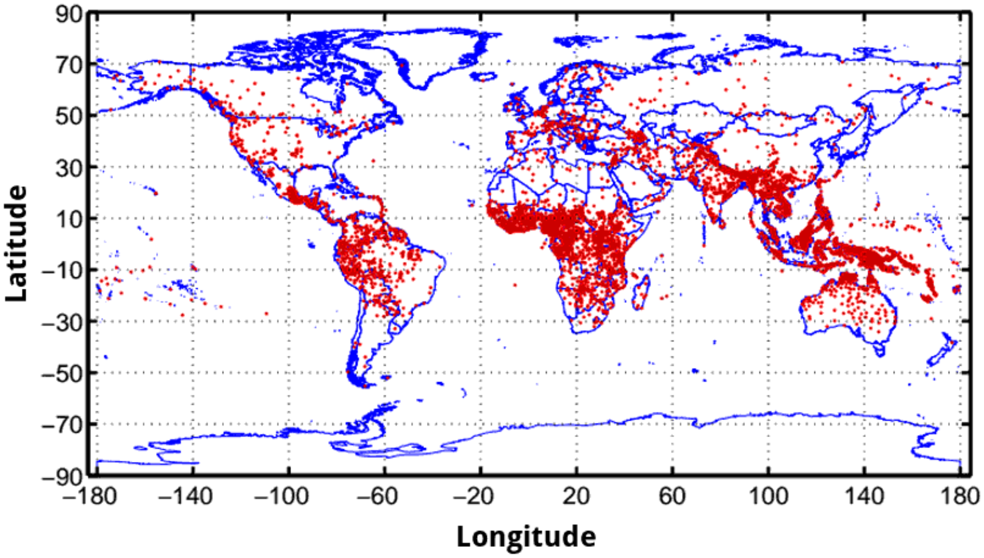


Figure 2: Distribution of linguistic diversity as compiled in the Ethnologue. Each point represents the centroid of the area covered by each language, as reported in the database. As it happens with biodiversity, linguistic diversity diminishes with increasing latitude.

from different languages in successive waves, as from Norman French or later from Latin. These processes are reminiscent of what is known as horizontal gene transfer in biology. A more dramatic influence of one language over another is the case of Creoles, full fledged natural languages that emerge from two parent languages in a time as short as two generations. Haitian Creole has been described as a West African language with French words, since it took the grammar from the former and the lexicon from the latter [9]. Cases as this one are, now metaphorically speaking, evocative of hybridization or genomic admixtures, where the two "parent" languages contribute in similar amounts to the emerging language, or of symbiotic associations, where one language provides the structure for interactions (e.g. the grammar) and another one the molecular elements (the lexicon).

5 Diversity and latitude

The spatial distribution of species over the Earth's surface develops several regularities that are far from trivial. Among them, the most prominent pattern relates biological diversity and latitude. Ecological communities in the tropics are fundamentally more diverse, and biodiversity declines as latitude increases. Though this observation was already known at Darwin's time, we still lack a convincing explanation of why ecological communities are more diverse near the equator [10].

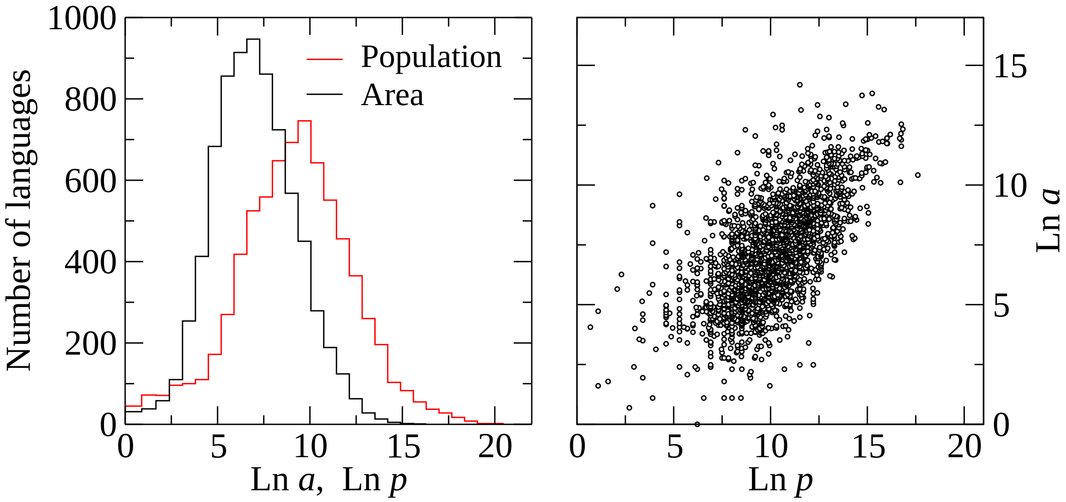


Figure 3: Left: Histograms of the number of linguistic groups occupying an area a or formed by p speakers. Note the Gaussian shape of the distributions, which can be well fitted by log-normal functions since the $x-$axis is the logarithm of the relevant variable. Right: Correlation between the number of speakers of a language (population) and the area over which they spread. The plot contains 2314 African languages. In this case, $z = 0.94$. Modified from [15].

In studying mammals, Eduardo Rapoport observed that home ranges, that is, the area spanned by a given species, were generally smaller at lower latitudes [11]. One may conclude that narrower ranges at lower latitudes would facilitate the coexistence of a larger number of species, and this may provide a partial explanation for this pattern. But it was later shown that there are many exceptions to this rule, which seems to be applicable only to high latitudes and for a subset of the species living there. Thus, it has been argued that the rule simply describes a local phenomenon, and that it can not be used to explain the latitudinal decline of biodiversity [12]. It has been put forward [13] that the latitudinal pattern of biodiversity could be a simple, statistical consequence of the wide distribution of species ranges via the so-called mid-range effect, which means that if species within a bounded geographical domain were randomly shuffled, their ranges would overlap towards the center of the domain. Another hypothesis stresses that ecological phenomena, such as climatic variability, act as selection pressures driving species to acquire high climatic tolerances, thus favoring adaptation to wider latitudinal ranges [14].

Setting aside the multiple mechanisms devised for explaining the decline of diversity with latitude, the same pattern is found in the distribution of human linguistic domains [16]. Figure 2 shows the geographical distribution of linguistic diversity. Apparently, language richness concentrates in a latitudinal band at both sides of the equator.

Is this pattern caused by the same phenomena that determine the geographical distribution of species richness? We do not yet have a fully conclusive answer to this puzzling question, though we might guess that the dominant processes should not rely solely on strictly ecological or cultural factors: whatever determines the diversity-latitude pattern should be affecting species and human linguistic groups in a similar manner. The role of the physical environment in determining this pattern might be essential, despite the unsolved controversy on its precise origin. It has been demonstrated that up to 80% of the linguistic diversity measured in relatively small regions of 200×200 km^2 can be explained on the basis of few environmental variables, among which river density and landscape roughness are those with the higher explanatory power [17]. It is likely that a better understanding of the commonalities between biodiversity and linguistic diversity can discriminate between candidate mechanisms to explain the latitude-diversity pattern.

6 Population abundance and range sizes

There are two important quantities revealing the nature of population dynamics. These are the home range and the abundance of individuals within a given group. The distribution of these two quantities, and their mutual dependence –leading to what can be called a population-area relationship– are essential signatures to unveil relevant mechanisms shaping large-scale diversity patterns.

The probability that a language is spoken by a certain number of humans follows a log-normal distribution [18]. This pattern can be easily explained on the basis of demographic dynamics. An important assumption in this context is that linguistic change can be essentially discarded [19], since demography is the dominant process on historically short time scales (several centuries). A log-normal distribution of abundances has been also reported for some biological groups, as birds and insects [20], though this pattern is not universal in biology. Regarding the abundance distributions of species and languages, we may confidently state that the same pattern speaks for the same process, which in this case is the intrinsic dynamics of population growth. If demography can be represented as the result of a certain (variable) growth rate at each year or generation –that is, the population one step later is the original one times its growth rate that year or that generation–, then the abundance distribution takes a log-normal shape. When other processes affect demography (for instance shortage of resources or space limitation), the distribution might change.

The number of individuals a species hosts has been correlated with the size of the range it spans, yielding an allometric (power-law) relationship in which the exponent varies along different taxa and habitats [21]. Several mechanisms have been proposed to explain this scaling, such as self-similarity in the spatial distribution of individuals [22] or stochasticity [23]. As of today there is no agreement on the mechanism that explain the species abundance-species range relationship. Interestingly enough, a similar functional dependence has been described for human linguistic groups [15]. Since both variables, language size p and area a, are log-normally distributed, it is natural to assume a mutual dependence with the same functional form as observed for species abundances and ranges, $a \sim p^z$. This relationship is indeed observed for all languages reported in the Ethnologue database, as well as for the languages spoken in the five largest continental landmasses separately (Europe, Asia, North and South America, and Africa). Different continental regions are characterized by different values of the exponent z, a fact that suggests that particular historical processes may have quantitatively influenced the current distributions of areas and populations.

Are the abundances of linguistic groups and the areas they span shaped by the same processes? Actually, though the distribution of domain areas is also log-normal, a multiplicative mechanism analogous to demographical dynamics –which explains the distribution of the number of speakers per language– does not appear as natural for the case of the areas. However, the strong correlations observed between both variables support the existence of a process that couples demographic growth to area occupied. It seems reasonable to assume that growing populations tend to expand their ranges, and that neighboring populations might clash if they both grow and thus compete for the same territory. Following this idea, it has been suggested [15] that the addition of a form of conflict between neighboring human groups might be the ingredient explaining the variations in the value of z. It remains to be seen whether a similar competitive scenario could be translated to the case of species.

7 Prospects

The multiple quantitative patters shared by biodiversity and human linguistic groups pose a number of questions related to their origin, causes, development, interaction, and fate. While some models shed light on the mechanisms behind some of the observations and occasionally reproduce them, others, such as the decrease of diversity with latitude, remain puzzling. It may well be that some patterns result from multiple causes, and in this sense be intrinsically more difficult to explain. Also, we cannot discard that some others which are apparently repeated in species and human groups happen to be due to chance or trivially result from external factors –as the two-dimensional space where they are bound to happen. The use and integration of independent data sets (from genetics, archeology, or history) will permit further advances in the characterization and eventual understanding of the ecological processes behind human cultural diversity, and likely of the relevant differences, if any, between human groups and biological species at the large scale.

8 Bibliography

[1] J. Aitchinson, *The Seeds of Speech. Language Origin and Evolution*. Cambridge: Cambridge Univ. Press, 2000.

[2] J. McWorther, *The Power of Babel*. New York: Times Books, 2001.

[3] M. Ruhlen, *The Origin of Language*. New York: John Wiley & Sons, 1994.

[4] L. L. Cavalli-Sforza, "Genes, peoples, and languages," *Proceedings of the National Academy of Sciences USA*, vol. 94, pp. 7719–7724, 1997.

[5] R. D. Gray, A. J. Drummond, and S. J. Greenhill, "Language phylogenies reveal expansion pulses and pauses in pacific settlement," *Science*, vol. 323, pp. 479–483, 2009.

[6] G. U. Yule, "A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis," *Proc. R. Soc. London Ser. B*, vol. 213, pp. 21–87, 1924.

[7] D. H. Zanette, "Self-similarity in the taxonomic classification of human languages," *Adv. Compl. Syst.*, vol. 4, pp. 281–286, 2001.

[8] B. Burlando, "The fractal dimension of taxonomic systems," *J. Theor. Biol.*, vol. 146, pp. 99–114, 1990.

[9] D. Bickerton, *Bastard Tongues*. New York: Hill and Wang, 2008.

[10] S. L. Pimm and J. H. Brown, "Domains of diversity," *Science*, vol. 304, pp. 831–833, 2004.

[11] E. H. Rapoport, *Areography. Geographical strategies of species. Trad. B. Drausal*. Oxford: Pergamon Press, 1982.

[12] K. Rohde, "Rapoport's rule is a local phenomenon and cannot explainlatitudinal gradients in species diversity," *Biodoversity Letters*, vol. 3, pp. 10–13, 1996.

[13] R. K. Colwell, C. Rahbek, and N. Gotelli, "The mid-domain effect and species richness patterns: What have we learned so far?" *American Naturalist*, vol. 163, pp. E1–E23, 2004.

[14] M. H. Fernandez and E. S. Vrba, "Rapoport effect and biomic specialization in african mammals: revisiting the climatic variability hypothesis," *Journal of Biogeography*, vol. 32, pp. 903–918, 2005.

[15] S. C. Manrubia, J. B. Axelsen, and D. H. Zanette, "Role of demographic dynamics and conflict in the population-area relationship for human languages," *PLoS ONE*, vol. 7, p. e40137, 2012.

[16] R. Mace and M. Pagel, "A latitudinal gradient in the density of human languages in north america," *Proc. R. Soc. Lond. B*, vol. 261, pp. 117–121, 1995.

[17] J. B. Axelsen and S. Manrubia, "River density and landscape roughness are universal determinants of linguistic diversity," *Proceedings of the Royal Society of London B*, 2014.

[18] W. J. Sutherland, "Parallel extinction risk and global distribution of languages and species," *Nature*, vol. 423, pp. 276–279, 2003.

[19] D. H. Zanette, "Demographic growth and the distribution of language sizes," *Int. J. Mod. Phys. C*, vol. 19, pp. 237–247, 2008.

[20] F. W. Preston, "The commonness, and rarity, of species," *Ecology*, vol. 29, pp. 254–283, 1948.

[21] T. M. Blackburn, K. J. Gaston, R. M. Quinn, H. Arnold, and R. D. Gregory, "Of mice and wrens: The relation between abundance and geographic range size in british mammals and birds," *Philosophical Transactions of the Royal Society of London B, Biological Sciences*, vol. 352, pp. 419–427, 1997.

[22] J. Harte, T. Blackburn, and A. Ostling, "Self-similarity and the relationship between abundance and range size," *American Naturalist*, vol. 157, pp. 374–386, 2001.

[23] K. J. Gaston and F. He, "The distribution of species range size: a stochastic process," *Proc. R. Soc. Lond. B*, vol. 269, pp. 1079–1086, 2002.